Quantitative genetic analysis of ascites in broilers

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Abstract

The aim of this thesis was to estimate genetic parameters and identify QTL that are involved in the complex multi-factorial metabolic disorder ascites, by a combination of genetic and genomics techniques.

Blood gas parameters have been suggested as indicator traits for ascites. Therefore we estimated genetic and phenotypic relationships between heart ratio (RATIO – a postmortem indicator for ascites) and blood gas parameters. Heritabilities for blood gas parameters and genetic correlations between blood gas parameters and heart ratio were low. Therefore, the results from this study do not support the suggestion that blood gas parameters measured during week 3 or 4 are useful indicator traits for ascites.

Male broilers have a higher body weight than females and might consequently be more prone to develop ascites. We therefore estimated the genetic correlation between RATIO and body weight separately for male and female broilers. The results show that genetic correlations differed between male and female broilers and therefore under circumstances with ascites, data from male and female broilers should be analyzed separately.

Alternatively, ascites data can be analyzed using a liability normal mixture (LNM) model. An LNM model can account for differences in (co)variance components between healthy and diseased chickens. Results show that the genetic correlation between RATIO in healthy and in diseased chickens is 0.75, indicating that RATIO is a different trait in healthy and diseased chickens. In addition the genetic correlation between RATIO and liability differed between healthy and diseased chickens.

Finally, a genome wide association study was performed for RATIO. Significant associations were detected on chromosome 1, 2, 3, 7, 8, 10, 11 and 20. The most significant SNPs were found on chromosome 1, 8 and on 22. A number of the SNPs associated with RATIO were also associated with fluid in the abdomen and body weight.

Contents

1

General Introduction

1.1 Introduction

Developments in Poultry Production

The global consumption of meat has increased during the last century. The world production of meat alone for broilers has increased from 58,017 metric tons in 1999 – 2001 to 79,596 metric tons in 2009 (FAO stat, 2010). Meat consumption from broilers is expected to increase at an average annual rate of 1.6% in developed countries and 2.7% in developing economy countries (Executive Guide to World Poultry Trends, 2010). In recent years there has been increasing attention for welfare issues in western countries (Moynagh, 2000). The animal production sector has responded to this demand by increased attention to animal welfare in breeding programs.

While animal health is an important component of animal welfare, the concern with welfare of the broiler has led to increased focus on health and liveability traits in breeding programs. According to Arthur and Albers (2003) relative selection pressure for production, fertility and liveability traits has changed over the last decades (1975 to 2002), and especially liveability traits have gained importance in breeding program. The attention for individual diseases in breeding programs has also resulted in increased research on the genetic background of diseases and relation between diseases and other relevant traits.

Realized Genetic Improvement of Efficiency in Broilers

The genetic improvement of the commercial broiler has been focused on increased growth and high muscle mass. Therefore, the commercial broiler is a fast growing bird with a low feed conversion rate and a high breast meat yield. At present, broilers are slaughtered when the chicken is between 35 and 40 days old at a live weight of approximately 2 kg. Alone genetically improved for the growth rate of modern broilers has been fourfold during the last century (Arthur and Albers, 2003). Focusing on the years 1925 to 1998 the time to reach a body weight of 1.5 kg has decreased from 120 days to 33 days (Arthur and Albers, 2003). The improvement of the commercial broilers is not just a result of genetic selection, but a combination of genetic selection, improve nutrition and better management. However, the genetic selection by commercial breeding companies contributed to about 85 to 90% of the change that has occurred in broiler growth rate the past decades. Nutrition has provided 10 to 15% of the improvements (Havenstein et al., 1994; Havenstein et al., 2003).

Havenstein et al. (2003) compared male and female broilers from a 1957 line with a modern line from 2001, and both lines were fed on feed formulas from 2001. Between three and twelve weeks of age the males from 2001 line increased body weight from 0.79 kg to 5.96 kg, whereas males from the 1957 line increased body weight from 0.21 kg to 1.90kg. The females from the same 2001 line increased body weight from 0.70 kg to 5.08 kg and the 1957 line increased body weight from 0.18 kg to 1.32 kg. However, the focus on selection for improved growth did not result in a comparable increase in organ size (Julian, 2000), for example the pulmonary and cardiac capacity of modern broilers is very similar to that of older broiler lines (Schmidt et al., 2009). Schmidt et al. (2009) compared relative growth of the breast, heart, liver, and intestine, and suggested that selection for increased breast muscle has resulted in relative lower weight of the heart muscle. Therefore, the muscle mass of the modern broiler increased (Table 1.1), but the relative size of the heart muscle has decreased.

Table 1.1 Average body weight (g) of line 1950s and modern line 2009 by day

Day	Line 1950s	Modern line 2009
2	38	36
7	92	149
14	234	432
21	450	856
28	699	1,411
35	1,047	1,804

(Schmidt et al., 2009)

Pulmonary Hypertension Syndrome (PHS or Ascites)

The reduced relative heart size in the modern broilers might imply insufficient cardiac capacity, and this might play a role in the increased susceptibility of modern broilers to heart failure and ascites (Maxwell and Robertson, 1998; Olkowski et al., 2007; Schmidt et al., 2009). Ascites is a cascade of events that results in physically abnormality related to the heart including enlarged and loose heart especially the right ventricular, and exuded of non-inflammatory fluid in lungs, pericardium and abdominal cavity (Balog, 2003). Originally ascites was associated with broilers raised at high altitude (Cueva et al., 1974). However, at present ascites is also found in populations raised at sea level (Scheele et al., 2005; Decuypere et al., 2005; Bahadoran and Hassanzadeh, 2010). Ascites is considered to be the result of a combination of unfavourable environmental conditions and physiology of the chicken, e.g. high growth rate, high feed intake, exposure to high altitude, high CO2 levels or low temperatures, relative small heart size and obstruction of the airways (figure 1.1). A combination of environmental and physiological factors either

increases the production or decreases the removal of peritoneal lymph fluid (Julian, 1993). Ascites is also known as pulmonary hypertension syndrome, and occurs when the heart does not have capability to pump adequate blood through the lungs (pulmonary) to meet the oxygen requirement of the body, which leads to an increase of the blood pressure (hypertension). High oxygen requirement is the most critical trigger for ascites and in broilers under cold conditions oxygen requirement is increased (Julian, 1998). Broilers that grow faster also have a higher oxygen requirement than slower growing broilers (Julian, 1993). For that reason, cold conditions are expected to especially increase ascites incidence in broilers with a high genetic potential for growth.

The development of pulmonary hypertension syndrome or ascites occurs through a progression of contributing factors and physically events (figure 1.1) caused by increased pressure in the pulmonary arteries (Baghbanzadeh and Decuypere, 2008). Usually the development of ascites start with pressure overload on the right ventricle causing hypertrophy of the right ventricular wall, valvular insufficiency, right ventricular failure and finally the chicken have ascites. Pulmonary hypertension syndrome is caused when an increased oxygen requirement in the chicken forces the heart to pump additional blood compared to normal through the lungs increases and then increase the pressure of the pulmonary arteries.

When venous blood in the chicken enters the right atrium of the heart and then passes through the valve into the right ventricle, the right ventricle normally pumps at a low pressure that is just sufficient to push all of the returning venous blood through the blood vessels of the lungs. However, the lungs of birds are inflexible and fixed in the thoracic cavity (figure 1.2). The small capillaries can expand only very little if they have to contain increased blood pressure and blood flow. The lungs are only capable to expand a little in the birds, because the air goes through the lungs into the air sacs and back through the lungs on expiration. The unique series of air sacs in birds (figure 1.3) are thin walled pouches connected to their lungs (figure 1.2). Therefore, the right ventricle of the heart responds very rapidly to the increased workload by enlargement (figure 1.1). If the enlargement workload of the heart continues, the right ventricle has to pump harder to meet the increased pressure, and the wall of ventricular thickens and enlarges. The increase in the weight of the right ventricle is associated with increases in blood pressure in the arteries leading to the lungs (pulmonary arteries).

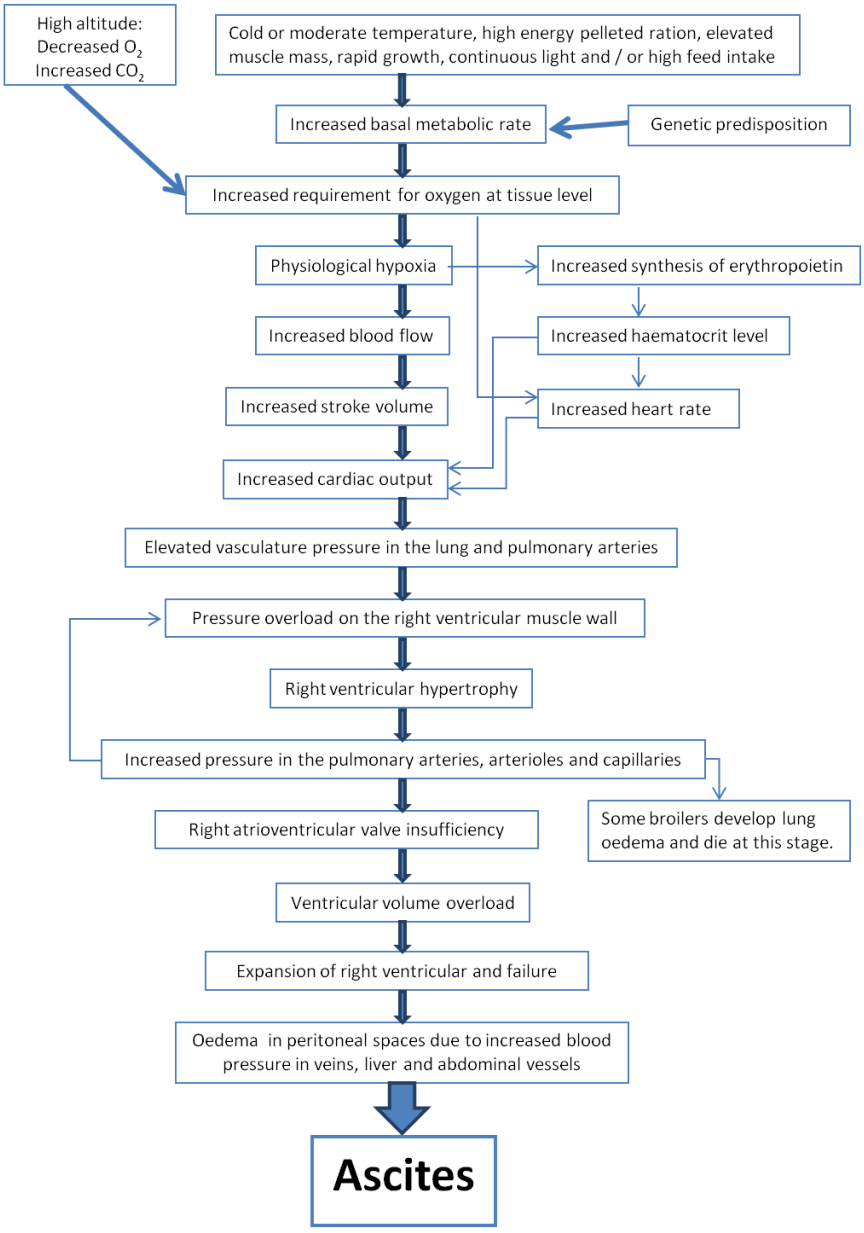

Figure 1.1. Flow chart showing the development of the ascites syndrome in broilers (Baghbanzadeh and Decuypere, 2008).

In order to compensate for the insufficient oxygen supply, there will be an increasing pulmonary vascular resistance and pulmonary arterial pressure. The right ventricle of the heart responds very rapidly to the increased workload by enlargement (figure 1.1). If the pulmonary hypertension continues, the right ventricle has to pump harder to meet the increased pressure, and the wall of ventricular thickens and enlarges. The increase in the weight of the right ventricle is associated with increases in blood pressure in the arteries leading to the lungs (pulmonary arteries). After the thickening of the wall of the right ventricle has started, the ventricle then begins to stretch and enlarge. The volume within the right ventricle has to increase when the increased amount of blood remains within the pumping chamber. The enlargement of the right ventricle physically reduces the pumping efficiency, and extensive enlargement seems to prevent the valve between the right atrium and right ventricle from closing correctly which permits blood to flow back into the right atrium during each ventricular contraction.

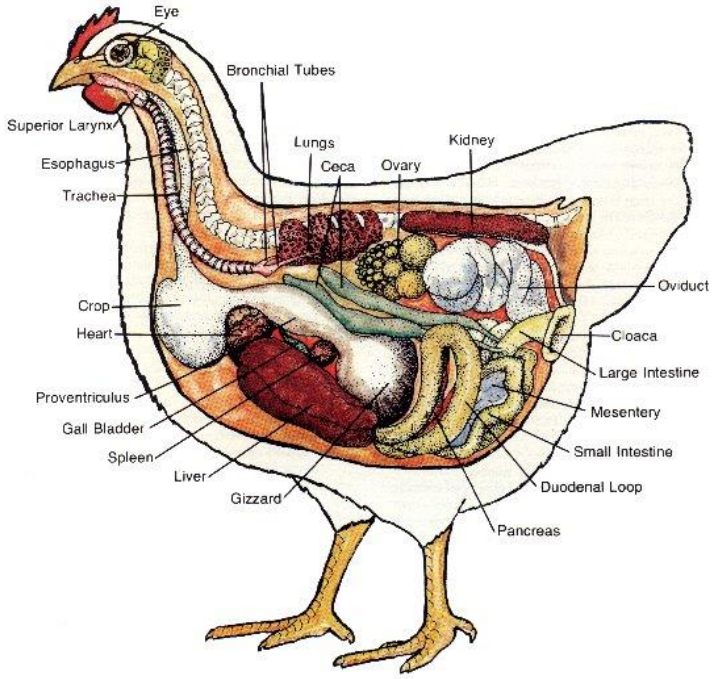

Figure 1.2 Internal Anatomy of chicken. The avian respiratory system delivers O_2 from the air to the tissues, removes CO_2, and maintains the body temperature. Chickens have relative small lungs and nine airs sacs, which play an important role in respiration. The air sacs permit a unidirectional flow of air through the lungs.
http://studentvet.files.wordpress.com/2011/03/chicken_turkey_anatomy-3332254071.jpg

Consequently, increased blood flow can force even mild penetration of fluid and to lead to the development of ascites at an early age (Julian, 1993). The leakage of ascites fluid will increase the vascular permeability and increases the susceptibility of the chicken to finally develop ascites (Julian, 2005). Ascitic fluid is a combination of lymph and blood plasma which has leaked from the liver and leaking from the blood vessels. The accumulation of ascites fluid is most frequently in the ventral hepatic, peritoneal, or pericardial spaces, may contain yellow protein clots. The leakage of ascites fluid will increase the vascular permeability and increases the susceptibility of the chicken to finally develop ascites (Julian, 2005). Many of the lesions observed in chickens with ascites are direct consequences of right ventricular failure and/or chronic hypoxemia, and often reflect inadequate adaptive responses or secondary tissue dysfunction and damage (Julian, 2005).

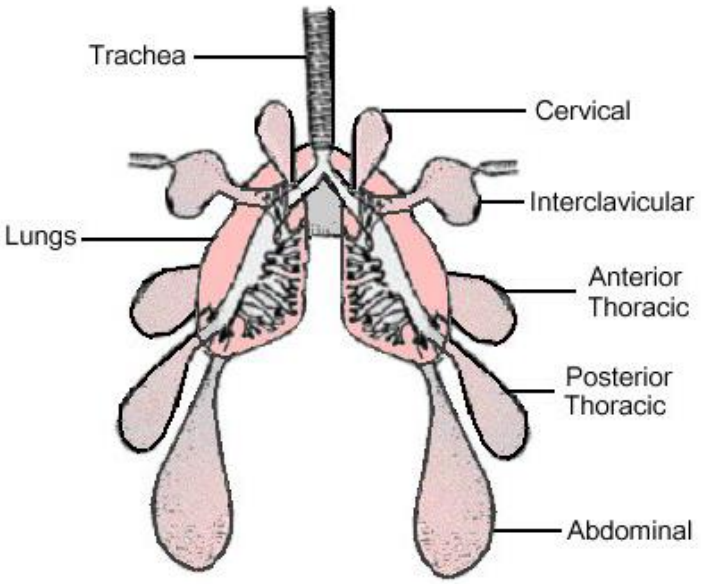

Figure 1.3 Air sac system of the chicken. http://www.fsis.usda.gov/pdf/psit_anatomy.pdf

Genetic Parameters for Ascites Indicator Traits

Several studies have evaluated traits that could serve as indicators for ascites susceptibility. Some of the most common clinical signs associated with ascites are right ventricular hypertrophy, fluid accumulation in the abdomen and increased haematocrit value (Decuypere et al., 2000; Moghadam et al., 2001; Pakdel et al.,

2005a; Zerehdaran et al., 2006). The ratio of right to total ventricular weight (RATIO), which measures right ventricular hypertrophy, has been suggested as a good indicator for ascites (Julian, 1993; McGovern et al., 1999; Pakdel et al., 2005b). The estimated heritabilities for ascites indicator traits, e.g. RATIO and haematocrit value are moderate to high (Lubritz et al., 1995; Pakdel et al., 2005b). It, therefore, seems reasonable to expect that genetic selection against ascites susceptibility can decrease ascites incidence in broilers.

The heritability estimates for indicator traits measured under normal temperature conditions have been found to be lower than under cold conditions, for example, the heritability estimate for haematocrit values under normal conditions 0.17 as compared to 0.46 under cold conditions, and for RATIO the estimated heritability under normal conditions was 0.12 compared to 0.45 under cold conditions (Pakdel et al., 2005b). The reason for this difference in heritability under different environmental conditions might be that genetic differences in ascites susceptibility are expressed to a larger extends under cold conditions. This might imply that estimated genetic parameters depend upon the incidence of ascites.

Several studies show that both genetic and maternal factors play a role in the development of ascites (Lubritz et al., 1995; Pakdel et al., 2002). Not accounting for maternal effects in the statistical model has been found to result in overestimated heritabilities (van Kaam et al., 1998; Pakdel et al., 2002).

Genetic Correlation between Ascites Related Traits and Production Traits

Genetic correlations between indicator traits and body weight have been shown to shift to more positive values under normal conditions as compared to the estimates under cold conditions. Pakdel et al (2005b) estimated the genetic correlation between body weight and heart characteristics like right ventricular weight and total ventricular weight for broilers that were challenged under ascites inducing conditions and under normal conditions. Under normal commercial environmental contentions the broilers with higher genetic growth potential also had higher genetic values for right ventricular weight and total ventricular weight. The genetic correlation between body weight and RATIO changed from 0.50 under normal conditions to -0.27 under cold conditions and the genetic correlation between body weight and haematocrit values changed from 0.55 to -0.23 (Pakdel et al., 2005b).

The results from Pakdel et al. (2005b) suggest that the genetic parameters for indicator traits are very sensitive to temperature conditions, and therefore, to the incidence of the disease. A low genetic correlation for body weight measured in cold and normal environments were estimated, which indicates that growth under normal and cold conditions are genetically different traits. Pakdel et al. (2005b) concluded that this genetic correlation indicates an interaction between environment and genotype: broilers with high genetic values for body weight under normal conditions do not necessarily have high genetic values for body weight under cold conditions. The observed genotype by environment interaction might be due to ascites. This is in agreement with Wideman (2001), who concluded that any increase in body weight for broilers that are clinically healthy can be related to increases in total ventricular weight as well as cardiac output and stroke volume.

Alternative Indicator Traits

Several studies have considered blood gas parameters as indirect criteria in selection for reduced incidence of ascites in broilers (Julian and Mirsalimi, 1992; Scheele et al., 2003; Wideman et al., 2003; Navarro et al., 2006; van As et al., 2010). It has been shown that broilers with right ventricular failure have significantly lower blood oxygen saturation (sO_2) compared to broilers with a normal heart (Julian and Mirsalimi, 1992). Further, an elevated RATIO is related to a higher partial pressure of carbon dioxide in venous blood (pvCO2), a lower partial pressure of oxygen in arterial blood, and increased bicarbonate (HCO_3) concentrations in arterial blood compared as compared to chickens with a normal RATIO (Wideman et al., 2003). Druyan et al. (2007) estimated a moderate heritability for blood oxygen saturation, indicating that blood oxygen saturation might be used as indicator for ascites susceptibility, although with limited efficacy. Based on a smaller study with 200 broilers, they concluded that blood $pvCO_2$ and pH in both male and female broilers seem to be critical factors in ascites pathophysiology and can be used as phenotypic traits to predict ascites susceptibility. Recent findings show that high $pvCO_2$ values together with low pH values (males) or high pH values (females) in the venous blood of young broilers can predict ascites (van As et al., 2010).

Quantitative Trait Loci for Ascites Related Traits

The purpose of QTL mapping studies is to identify genetic markers that are closely linked to the causal mutation. QTL mapping studies in chicken have identified chromosomal regions that contribute to variation in several economically important traits (Abasht et al., 2006). The associations between markers and traits

can be used in selection and can increase selection response, especially for traits that are difficult to measure, such as resistance to disease (Hocking, 2010). Genomic information also has been suggested as an effective means to select for reduced susceptibility to ascites in broilers (Pakdel et al., 2005a). Rabie et al. (2005) identified QTL affecting ascites indicator traits on chromosome 2, 4 and 6 and suggestive linkage was found on chromosome 5, 8, 10, 27 and 28. The detection of several QTL, each with relatively small effect, suggests a complex genetic background.

1.2 Aim and Outline of this Thesis

The aims of this thesis are to (i) identify and estimate genetic parameters for ascites indicator traits using commonly used and alternative statistical methods and (ii) perform a genome wide association study used to identify candidate genes involved in ascites.

Chapter 2 and chapter 3 present the estimation of genetic parameters. Several physiological studies have suggested the use of blood gas parameters as indicators of ascites. However, few studies have estimated genetic parameters for blood gas parameters. The objectives of chapter 2 were (i) to estimate the heritability for RATIO, body weight and blood gas parameters, and (ii) to estimate the genetic and phenotypic correlations between RATIO, body weight at two weeks, body weight at five weeks and blood gas parameters measured during week three and week four. It has been suggested that male broilers are more likely to develop ascites, because they tend to grow faster than female broilers. However, no studies have estimated genetic parameters for ascites indicator traits separately for male and females broilers. The aims of chapter 3 were (i) to estimate the heritability for RATIO and BW in male and female broilers, (ii) to estimate the genetic correlation between RATIO and BW separately for male and female broilers, and (iii) to estimate genetic correlations between BW for ascitic and non-ascitic broilers.

Traditionally the statistical genetic analyses of ascites data are performed using linear models. However, explicitly modeling healthy and diseased birds and the susceptibility to ascites, i.e. the actual trait of interest might be more appropriate. Chapter 4 presents the analysis of RATIO using a liability normal mixture model (LNM). The aim of chapter 4 was to apply the LNM model to the ascites-indictor trait RATIO and to use this model estimate heritablities.

The number of genetic markers that is available for broilers has increased dramatically over the last years and the cost for genotyping a single chicken has declined. This offers opportunities for detection and fine mapping of QTL related to ascites Chapter 5 present the results of a genome wide association study for RATIO. The aim of chapter 5 was to detect and characterize chromosomal regions affecting RATIO.

Chapter 6 is the general discussion where the four following issues are discussed: (i) genetic background of ascites in broilers, (ii) the use of Liability Normal Mixture Models in selection for disease resistance, (iii) genome wide association studies used for identify ascites, and (iv) comparison between species.

References

Abasht, B., J. C. M. Dekkers, and S. J. Lamont. 2006. Review of Quantitative Trait Loci Identified in the Chicken. Poultry Science 85(12):2079-2096.

Arthur, J. A., and G. A. A. Albers. 2003. Industrial Perspective on Problems and Issues Associated with Poultry Breeding. Pages 1-12 in Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S. E. Aggrey, ed. CABI Publishing, Wallingford, UK.

Baghbanzadeh, A., and E. Decuypere. 2008. Ascites syndrome in broilers: physiological and nutritional perspectives. Avian Pathology 37(2):117-126.

Bahadoran, S., and M. Hassanzadeh. 2010. Effect of early cold exposure on the endocrine responses of broiler chickens and the incidence of ascites syndrome. International Journal of Veterinary Research 4(1):11-16, 67.

Balog, J. M. 2003. Ascites syndrome (Pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian and Poultry Biology Reviews 14(3):99-125.

Cueva, S., H. Sillau, A. Valenzue, and H. Ploog. 1974. High-Altitude Induced Pulmonary-Hypertension and Right Heart-Failure in Broiler Chickens. Research in Veterinary Science 16(3):370-374.

Decuypere, E., J. Buyse, and N. Buys. 2000. Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. Worlds Poultry Science Journal 56(4):367-377.

Decuypere, E., M. Hassanzadeh, N. Buys, and J. Buyse. 2005. Further insights into the susceptibility of broilers to ascites. The Veterinary Journal 169(3):319-320.

Druyan, S., A. Shlosberg, and A. Cahaner. 2007. Evaluation of growth rate, body weight, heart rate, and blood parameters as potential indicators for

selection against susceptibility to the ascites syndrome in young broilers. Poult Sci 86(4):621-629.

Executive Guide to World Poultry Trends. 2010. A Statistical reference for poultry executives. in Watt Publishing Company, Mt. Morris, USA.

FAO stat. 2010. FAO statistical yearbook 2010. in http://www.fao.org.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82(10):1500-1508.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994. Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poult Sci 73(12):1785-1794.

Hocking, P. M. 2010. Developments in poultry genetic research 1960 – 2009. British Poultry Science 51(3 supp 1):44-51.

Julian, R. J. 1993. Ascites in Poultry. Avian Pathology 22(3):419-454.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult Sci 77(12):1773-1780.

Julian, R. J. 2000. Physiological, management and environmental triggers of the ascites syndrome: a review. Avian Pathology 29(6):519-527.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry - A review. Veterinary Journal 169(3):350-369.

Julian, R. J., and S. M. Mirsalimi. 1992. Blood-Oxygen Concentration of Fast-Growing and Slow-Growing Broiler-Chickens, and Chickens with Ascites from Right Ventricular Failure. Avian Diseases 36(3):730-732.

Lubritz, D. L., J. L. Smith, and B. N. Mcpherson. 1995. Heritability of Ascites and the Ratio of Right to Total Ventricle Weight in Broiler Breeder Male Lines. Poult Sci 74(7):1237-1241.

Maxwell, M. H., and G. W. Robertson. 1998. UK survey of broiler ascites and sudden death syndromes in 1993. British Poultry Science 39(2):203-215.

McGovern, R. H., J. J. R. Feddes, F. E. Robinson, and J. A. Hanson. 1999. Growth performance, carcass characteristics, and the incidence of ascites in broilers in response to feed restriction and litter oiling. Poult Sci 78(4):522-528.

Moghadam, H. K., I. McMillan, J. R. Chambers, and R. J. Julian. 2001. Estimation of genetic parameters for ascites syndrome in broiler chickens. Poult Sci 80(7):844-848.

Moynagh, J. 2000. EU Regulation And Consumer Demand For Animal Welfare . AgBioForum 3(107):114.

Navarro, P., P. M. Visscher, D. Chatziplis, A. N. M. Koerhuis, and C. S. Haley. 2006. Genetic parameters for blood oxygen saturation, body weight and breast

conformation in 4 meat-type chicken lines. British Poultry Science 47(6):659-670.

Olkowski, A. A., S. Nain, C. Wojnarowicz, B. Laarveld, J. Alcorn, and B. B. Ling. 2007. Comparative study of myocardial high energy phosphate substrate content in slow and fast growing chicken and in chickens with heart failure and ascites. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 148(1):230-238.

Pakdel, A., P. Bijma, B. J. Ducro, and H. Bovenhuis. 2005a. Selection strategies for body weight and reduced ascites susceptibility in broilers. Poult Sci 84(4):528-535.

Pakdel, A., J. A. A. Van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites-related traits in broilers. Poult Sci 81(9):1273-1279.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005b. Genetic parameters of ascites-related traits in broilers: effect of cold and normal temperature conditions. British Poultry Science 46(1):35-42.

Rabie, T. S. K. M., R. P. M. A. Crooijmans, H. Bovenhuis, A. L. J. Vereijken, T. Veenendaal, J. J. van der Poel, J. A. M. van Arendonk, A. Pakdel, and M. A. M. Groenen. 2005. Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop pulmonary hypertension syndrome. Animal Genetics 36(6):468-476.

Scheele, C. W., J. D. van der Klis, C. Kwakernaak, N. Buys, and E. Decuypere. 2003. Haematological characteristics predicting susceptibility for ascites. 1. High carbon dioxide tensions in juvenile chickens. British Poultry Science 44(3):476-483.

Scheele, C. W., J. D. van der Klis, C. Kwakernaak, R. A. Dekker, J. H. Van Middelkoop, J. Buyse, and E. Decuypere. 2005. Ascites and venous carbon dioxide tensions in juvenile chickens of highly selected genotypes and native strains. Worlds Poultry Science Journal 61(1):113-129.

Schmidt, C. J., M. E. Persia, E. Feierstein, B. Kingham, and W. W. Saylor. 2009. Comparison of a modern broiler line and a heritage line unselected since the 1950s. Poultry Science 88(12):2610-2619.

van As, P., M. G. Elferink, A. M. Closter, A. Vereijken, H. Bovenhuis, R. P. M. A. Crooijmans, E. Decuypere, and M. A. M. Groenen. 2010. The use of blood gas parameters to predict ascites susceptibility in juvenile broilers. Poult Sci 89(8):1684-1691.

van Kaam, J. B. C. H., J. A. M. van Arendonk, M. A. M. Groenen, H. Bovenhuis, A. L. J. Vereijken, R. P. M. A. Crooijmans, J. J. van der Poel, and A. Veenendaal.

1998. Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. Livestock Production Science 54(2):133-150.

Wideman, R. F. 2001. Pathophysiology of heart/lung disorders: pulmonary hypertension syndrome in broiler chickens. World's Poultry Science Journal 57:289-307.

Wideman, R. F., D. M. Hooge, and K. R. Cummings. 2003. Dietary sodium bicarbonate, cool temperatures, and feed withdrawal: Impact on arterial and venous blood-gas values in broilers. Poult Sci 82(4):560-570.

Zerehdaran, S., E. A. van Grevehof, E. H. van der Waaij, and H. Bovenhuis. 2006. A bivariate mixture model analysis of body weight and ascites traits in broilers. Poult Sci 85(1):32-38.

2

Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers

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Abstract

Ascites, also called pulmonary hypertension syndrome, is a metabolic disorder in chickens that have an insufficient pulmonary vascular capacity. The tendency of broilers to develop ascites is heritable, and successful selection against this susceptibility would benefit from good and easy-to-measure indicator traits. Blood gas parameters have been suggested as indicator traits for ascites susceptibility. Therefore, the aim of the present study was to estimate the heritability of blood gas parameters and the genetic and phenotypic correlations between blood gas parameters, heart ratio (postmortem indicator for ascites), and BW at 2 different ages. For this purpose, blood gas parameters, including the partial pressure of carbon dioxide in venous blood ($pvCO_2$), the partial pressure of oxygen in venous blood (pvO_2), and blood oxygen saturation, were measured at an average age of 22 d in nearly 3,000 broilers. To challenge the resistance of the birds to ascites, they were kept under cold conditions. Heritability for heart ratio was 0.43, and the heritability estimates were low: 0.02 for $pvCO_2$, 0.03 for pvO_2, and 0.07 for blood oxygen saturation. The estimated heritability for pH was 0.15, for bicarbonate was 0.19, and for total carbon dioxide content was 0.19. The genetic correlations between heart ratio and total carbon dioxide content (0.31 ± 0.15) and between heart ratio and bicarbonate (0.31 ± 0.15) were moderate and positive. For pvO_2, the genetic correlation with heart ratio was stronger and negative (−0.62 ± 0.21); however, this correlation could not be estimated accurately because of the low heritability of pvO2. For $pvCO_2$, the genetic correlation with the heart ratio was close to zero (−0.04 ± 0.45). Phenotypic correlations between traits were, in general, similar to the genetic correlations. Heritabilities for blood gas parameters and the genetic correlations between blood gas parameters and the heart ratio estimated in the present study do not support the suggestion that blood gas parameters measured during wk 3 or 4 are useful traits to select against the susceptibility for ascites.

Key words: broiler, ascites, blood gas parameter, heritability, correlation

2.1 Introduction

Ascites, also called pulmonary hypertension syndrome, is a metabolic disorder in chickens. The disorder is associated with an insufficient pulmonary vascular capacity and results in right ventricular failure (Julian et al., 1987; Julian, 1998; Balog et al., 2000). In most cases, ascites is caused by a disproportion between the oxygen requirement and the cardiovascular ability to supply oxygen (Julian and Mirsalimi, 1992; Scheele et al., 1992; Decuypere et al., 2000). Oxygen shortage puts pressure on the pulmonary vascular system and can lead to oxygen deficiency in the tissues, which will increase pulmonary arterial pressure. The high blood pressure and high work load of the heart lead to fluid accumulation in the abdominal cavity and eventually death (Shlosberg et al., 1992; Decuypere et al., 2000; Havenstein et al., 2003). Mortality caused by ascites ranges from 5 to 8% in populations worldwide and can be as great as 20 to 30% in heavier broiler flocks (Balog, 2003; Pavlidis et al., 2007).

The increase in the occurrence of ascites has been linked to genetic selection for increased growth rate, greater meat yield, and lower feed conversion ratio (Decuypere et al., 2000; Balog, 2003). It has been shown that fast-growing broilers are more susceptible to ascites than slow-growing broilers (Julian, 1993). Ascites in broiler flocks can be reduced by management measures, such as avoiding low temperatures, maintaining good air quality and high oxygen concentrations, and restricting feeding to restrict growth (Decuypere et al., 2000; Julian, 2000; Balog, 2003). A variety of physiological studies have evaluated specific traits asindicators for ascites susceptibility. Two of the most common clinical signs associated with ascites are right ventricular hypertrophy and fluid accumulation in the abdominal cavity (Decuypere et al., 2000; Moghadam et al., 2001; Balog et al., 2003; Pakdel et al., 2005a; Zerehdaran et al., 2006). The ratio of right to total ventricular weight (RATIO), which measures right ventricular hypertrophy, has been suggested as a good indicator for ascites (Julian, 1993; McGovern et al., 1999; Pakdel et al., 2005c).

Studies have shown genetic variation within lines (Wideman and French, 1999; Wideman et al., 1999; Deeb et al., 2002; Pakdel et al., 2002) and between lines (Lubritz et al., 1995; Buys et al., 1999a,b; Wideman and French, 2000; De Greef et al., 2001; Druyan et al., 2007, 2008) for susceptibility to ascites. However, current indicator traits, such as RATIO and fluid accumulation in the abdominal cavity, can only be measured postmortem. Therefore, selection against ascites susceptibility by using these indicators is complicated, and information for selection relies heavily

on information from relatives (McMillan and Quinton, 2002; Pakdel et al., 2005a). Thus, there is a need for alternative indicator traits that can be measured on living birds; blood gas parameters might be a good alternative. It has been shown that broilers with right ventricular failure have significantly lower blood oxygen saturation (sO_2) compared with broilers with a normal heart (Julian and Mirsalimi, 1992). Wideman et al. (2003) found that chickens with an elevated RATIO had a greater partial pressure of carbon dioxide in venous blood ($pvCO_2$), a lower partial pressure of oxygen in arterial blood, and greater bicarbonate (HCO_3) concentrations in arterial blood compared with chickens with a normal RATIO. Furthermore, by comparing 2 different broiler lines, Scheele et al. (2003) observed a relationship between ascites susceptibility and high $pvCO_2$ at d 11 in juvenile chickens and suggested that ascites could be eliminated by selecting for low $pvCO_2$. Navarro et al. (2006) demonstrated that sO_2 is heritable and suggested that ascites susceptibility could be decreased by selecting for increased sO_2 values. Druyan et al. (2007) reported a moderate heritability for sO_2 and indicated that sO_2 might serve as an indicator in selection against ascites susceptibility, although with limited efficacy.

In addition to the studies by Navarro et al. (2006) and Druyan et al. (2007), to our knowledge, no other studies have reported heritability estimates for blood gas parameters. Furthermore, to our knowledge, only Druyan et al. (2007) reported genetic correlations between blood gas parameters and other ascites indicator traits such as RATIO.

The objective of the present study was to estimate heritability, heart ratio, and genetic and phenotypic correlations between blood gas parameters measured during wk 3 and 4 and BW at 2 different ages in broilers.

2.2 Materials and Methods

Experimental Population and Phenotyping

Animal Material

The experiment was carried out by licensed and authorized personnel under approval of Hendrix Genetics. The experimental population consisted of 5,987 broilers. The chickens were from generations 7 and 8 of an advanced intercross line, which was a cross between 2 genetically different dam lines originating from the White Plymouth Rock breed. The data consisted of 2,413 males, 2,452 females, and 1,122 chickens of unknown gender. Birds from generations 3 of this population have been used in previous studies on ascites and meat quality traits (van Kaam et

al., 1998; Pakdel et al., 2002). The chickens in the experiment were kept under a cold temperature regimen to induce ascites. The temperature was 30°C at the time of hatching and was gradually reduced to 10°C at 22 d of age. The temperature remained at 10°C until the end of the experiment when the chickens were 5 wk of age. The chickens were group housed with 20 birds/m2, they had ad libitum access to a commercial broiler feed containing 12,970 KJ/kg, and they were exposed to 23 h of light per day during the entire experiment. Except for the temperature schedule applied, the chickens were kept under conditions that closely resemble commercial practice.

Venous blood samples were taken when the chickens were, on average, 22 d old (ranging from 19 to 27 d old). The blood gas parameters measured (GEM Premier 3000, Instrumentation Laboratories, Lexington, MA) were blood pH, $pvCO_2$, and partial pressure of oxygen in venous blood (pvO_2). Bicarbonate and total carbon dioxide content (TCO_2) were calculated from the pH and $pvCO_2$ by the following equations:

$$\text{Log } HCO_3 = \text{pH} + \log pvCO_2 - 7.608, \text{ and}$$
$$TCO_2 = HCO_3 + 0.03\ pvCO_2.$$

Blood sO_2 is an indicator of the percentage of hemoglobin saturated with oxygen at the time of the measurement; pvO_2, pH, and HCO_3 were used to calculate sO_2 with the following equation:

$$sO_2 = 100\frac{X^3 + 150X}{X^3 + 150X + 23400},$$

where $X = pvCO_2 \times 10^{[0.48\ (pH-7.4)-0.0013(HCO3-25)]}$.

The weight of the heart ventricles was determined at 5 wk of age. The RATIO was the weight of the right ventricle as a percentage of the total ventricle weight (TV). The chickens were weighed at 2 wk (BW_2) and 5 wk of age (BW_5). No postmortem dissection was performed on the animals that died before the end of the experiment; therefore, the cause of death was unknown. Animals that died before the end of the experiment were assigned a total mortality (MORT-TOT) score of 1 and birds that survived got a score of 0.

Statistical Analysis

Genetic parameter estimates were obtained by using ASREML software (Gilmour et al., 2006). To determine the importance of maternal effects, a model without a maternal effect and a model with a maternal environmental effect were used. The following model without a maternal environmental effect was used:

$$y_{ijkl} = \mu + sex_i + IHD_j + date_k + a_l + e_{ijkl}\,, \qquad [1]$$

where y_{ijkl} is the dependent variable of chicken ijkl of sex_i, which is the fixed effect of sex (i = female, male, or unknown); IHD_j is the fixed effect of individual hatching day (j = 1, 2, . . ., 34 d at hatching); $date_k$ is the fixed effect for date of blood gas measurement (k = 1, 2, . . ., 37); a_l is the random direct genetic effect of individual l with $a \sim N(0,A_a^2)$ and e_{ijkl} is the random residual effect with $e \sim N(0,I_a^2)$. The effect $date_k$ was used only in the model for the blood gas parameters.

The second model with a maternal environmental effect was

$$y_{ijklm} = \mu + sex_i + IHD_j + date_k + a_l + d_m + e_{ijklm}\,. \qquad [2]$$

This model is identical to the first model, except for the random maternal environmental effect of dam m (d_m) with $d \sim N(0,I_a^2)$. The fraction of the variation attributable to maternal environmental effects (m^2) was calculated as

$$m^2 = \frac{\sigma_d^2}{\sigma_a^2 + \sigma_d^2 + \sigma_e^2}$$

To test the significance of the maternal environmental effect, a likelihood ratio test with 1 df was used:

$$X_1^2 = 2\log_e L(F) - 2\log_e L(R),$$

where L(F) is the likelihood of the full model (model [2]), and L(R) is the likelihood of the reduced model (model [1]). Univariate analysis was used to estimate heritabilities and maternal environmental effects. Bivariate analysis was used to estimate genetic and phenotypic correlations between the traits.

Some of the animals died before the end of the experiment and had an observation for only BW_2. The animals that died might have been the ones that were most susceptible to ascites, and this selection might have had an impact on the estimated genetic parameters. Selection related to BW_2 can be accounted for by performing a multivariate analysis including BW_2 (Ouweltjes et al., 1988). Therefore, we also estimated heritabilities by using a bivariate analysis with BW_2 as a permanent trait. The effect of selection on genetic correlations was studied by performing a trivariate analysis with BW_2 as a permanent trait.

2.3 Results

Data Description

Means, SD, and CV of the traits measured under cold stress conditions are presented in Table 2.1. Of the 5,987 chickens retained for measurement of BW_2, 5,222 also had measurements for BW_5, 5,155 had measurements for RATIO, and 2,956 chickens were used for measuring blood gas parameters. Mortality recordings were missing for 210 chickens because of the loss of wing bands or because the trait was not recorded. The average venous blood pH was 7.38, the average sO_2 was 84%, the average $pvCO_2$ was 45.4 mmHg, and the average HCO_3 concentration was 26.88 mmol/L (Table 2.1). The average BW of broilers under cold stress conditions was 360 g at 2 wk and 1,146 g at 5 wk, and the average RATIO was 25%. The MORT-TOT was 10%. Coefficients of variation were moderate to high for most of the traits (e.g., 14.6% for $pvCO_2$, 20% for pvO_2, 29.4% for BW_2, 18.9% for BW_5, and 21.2% for RATIO). However, the CV for pH was very low (0.7%).

Genetic Analyses

Phenotypic variance, heritability, and maternal environmental effects for the ascites-related traits obtained from the univariate models are given in Table 2.2. The heritability for RATIO was 0.43. For some of the blood gas parameters, the heritabilities were close to zero: 0.02 for $pvCO_2$, 0.03 for pvO_2, and 0.07 for sO_2. However, for pH, HCO_3, and TCO_2, moderate heritabilities were found: 0.15, 0.19, and 0.19, respectively. The estimated heritabilities for the 2 BW measurements were 0.15 for BW_2 and 0.17 for BW_5. The traits BW_2, BW_5, TV, $pvCO_2$, and MORT-TOT were significantly affected by maternal environmental effects. The fraction of the total variation explained by maternal environmental effects was 0.05 for $pvCO_2$, 0.12 for BW_2, and 0.07 for BW_5.

Table 2.1 The mean, SD, and CV for BW, heart ratio, blood gas parameters, and mortality of the broiler chickens

Variable	Units	Abbreviation	Number	Mean	SD	CV[1] (%)
BW at 2 wk	G	BW_2	5,987	360	106	29.4
BW at 5 wk	G	BW_5	5,222	1,146	217	18.9
Heart ratio	%	RATIO	5,155	25.01	5.31	21.2
Right ventricular weight	G	RV	5,155	1.29	0.38	29.5
Total ventricular weight	G	TV	5,155	5.15	0.95	18.4
Blood pH	pH	pH	2,956	7.38	0.05	0.7
Partial pressure of carbon dioxide in venous blood	mmHg	$pvCO_2$	2,956	45.4	6.62	14.6
Partial pressure of oxygen in venous blood	mmHg	pvO_2	2,956	52.46	10.5	20
Blood bicarbonate concentration in venous blood	mmol/L	HCO_3	2,955	26.88	3.33	12.4
Total carbon dioxide in venous blood	mmol/L	TCO_2	2,956	28.28	3.44	12.2
Oxygen saturation in venous blood	%	sO2	2,952	83.98	7.02	8.4
Total mortality before slaugther	Died (0/1)	MORT-TOT	5,777	0.1	0.3	—

[1]CV (%) is the CV calculated by taking the SD to the mean.

No significant evidence for the presence of maternal environmental effects was found for the traits RATIO, pH, pvO_2, HCO_3, TCO_2, and sO_2. Using a model without a maternal effect gave a heritability estimate of 0.51 for BW_2, of 0.37 for BW_5, and of 0.15 for $pvCO_2$ (Table 2.2). Bivariate analysis with BW_2 as a permanent trait resulted in slightly greater heritability estimates; they were at maximum 0.03 greater than the heritabilities estimated by using a univariate model (results not shown). The heritability estimates for MORT-TOT were also analyzed by using a binary model (results not shown), and the results increased compared with heritabilities estimated from the linear model. These results were in agreement with heritabilities estimated by transforming the heritabilities from the linear model to the underlying scale (Lynch and Walsh, 1998)

Table 2.2. Phenotypic variance, heritability (h^2), and maternal effect (m^2) for model [1] and model [2]1

		Model [1]	Model [2]		Significance of log-likelihood test[2]
Trait	Phenotypic variance	h^2 (SE)	h^2 (SE)	m^2 (SE)	
BW_2	4,606	0.51 (0.06)	0.15 (0.09)	0.12 (0.04)	0
BW_5	34,280	0.37 (0.05)	0.17 (0.08)	0.07 (0.03)	0.029
RATIO	25.5	0.43 (0.06)			NS
RV	0.106	0.42 (0.06)			NS
TV	0.613	0.37 (0.05)	0.17 (0.07)	0.06 (0.03)	0.003
pH	0.002	0.15 (0.04)			NS
$pvCO_2$	26.19	0.15 (0.04)	0.02 (0.02)	0.05 (0.01)	0.006
pvO_2	93.25	0.03 (0.01)			NS
HCO_3	5.36	0.19 (0.05)			NS
TCO_2	5.8	0.19 (0.05)			NS
sO_2	36.3	0.07 (0.02)			NS
MORT-TOT	0.087	0.05 (0.02)	0.01 (0.02)	0.02 (0.01)	0.019

[1]BW_2 = BW at 2 wk; BW_5 = BW at 5 wk; RATIO = ratio of right to total ventricular weight; RV = right ventricular weight; TV = total ventricular weight; $pvCO_2$ = partial pressure of carbon dioxide in venous blood; pvO_2 = partial pressure of oxygen in venous blood; HCO_3 = bicarbonate; TCO_2 = total carbon dioxide in venous blood; sO_2 = oxygen saturation in venous blood; MORT-TOT = total mortality.

[2]Log-likelihood results indicate the significant difference between model [1] and model [2]. A univariate model was used for the estimations. NS = not significant.

The estimates for genetic correlations (above the diagonal) and the phenotypic correlations (below the diagonal) of the blood gas parameters RATIO, BW, and MORT-TOT are presented in Table 2.3. The greatest genetic correlation between RATIO as a postmortem indicator for ascites and blood gas parameters was found

for pvO_2 (−0.62 ± 0.21). However, genetic correlations between pvO_2 and other traits have high SE, mainly because of the low heritability for pvO_2. The genetic correlations between RATIO and the blood gas parameters TCO_2 (0.31 ± 0.15) and HCO_3 (0.31 ± 0.15) were positive and moderate. For $pvCO_2$ and RATIO, the genetic correlation was close to zero (−0.04 ± 0.45). The genetic correlation between BW_2 and RATIO was 0.19, whereas the genetic correlation between BW_5 and RATIO was −0.18. The genetic correlation between BW_2 and BW_5 was high (0.88). Phenotypic correlations between traits were, in general, similar to the genetic correlations. A trivariate model with BW_2 as a permanent trait had hardly any effect on the estimated genetic correlation between the traits: genetic correlations between RATIO and the blood gas parameters increased from 0.01 to 0.02 when using a trivariate model instead of a bivariate model (results not shown).

Table 2.3. The genetic correlations (above the diagonal) and the phenotypic correlations (below the diagonal)[1]

Trait	BW_2 (SE)	BW_5 (SE)	RATIO (SE)	RV (SE)	TV (SE)	pH (SE)	$pvCO_2$ (SE)	pvO_2 (SE)	HCO_3 (SE)	TCO_2 (SE)	sO2 (SE)	MORT-TOT (SE)
BW_2		0.88 (0.10)	0.19 (0.23)	0.52 (0.16)	0.49 (0.26)	0.79 (0.35)	NC	−0.24 (0.43)	0.59 (0.20)	0.58 (0.18)	0.4 (0.29)	−0.63 (0.63)
BW_5	0.75 (0.01)		−0.18 (0.16)	0.23 (0.17)	0.3 (0.27)	0.67 (0.23)	−0.90 (0.89)	0.13 (0.31)	0.45 (0.19)	0.53 (0.18)	0.6 (0.21)	NC
RATIO	−0.02 (0.03)	−0.15 (0.03)		0.81 (0.04)	−0.02 (0.16)	0.06 (0.17)	−0.04 (0.45)	−0.62 (0.21)	0.31 (0.15)	0.31 (0.15)	−0.12 (0.20)	NC
RV	0.36 (0.03)	0.35 (0.03)	0.76 (0.01)		0.62 (0.10)	0.13 (0.17)	0.00 (0.42)	−0.41 (0.24)	0.41 (0.14)	0.42 (0.14)	0.06 (0.20)	NC
TV	0.56 (0.03)	0.71 (0.02)	−0.05 (0.03)	0.6 (0.02)	0.27 (0.22)		−0.60 (0.90)	0.1 (0.29)	0.33 (0.20)	0.32 (0.20)	0.25 (0.23)	NC −0.29 (0.42)
pH	0.12 (0.03)	0.1 (0.03)	−0.04 (0.03)	−0.02 (0.03)	0.04 (0.03)	NC	−0.08 (0.26)	0.4 (0.30)	0.12 (0.19)	0.47 (0.17)		
$pvCO_2$	NC	−0.01 (0.03)	0.12 (0.03)	0.13 (0.03)	0.03 (0.03)	NC		−0.48 (0.45)	0.69 (0.16)	0.71 (0.15)	−0.82 (0.31)	−0.14 (0.67)
pvO_2	0.01 (0.02)	0.00 (0.02)	−0.06 (0.02)	−0.04 (0.02)	−0.01 (0.02)	0.05 (0.02)	−0.14 (0.02)		−0.59 (0.21)	0.77 (0.13)	−0.58 (0.22)	NC
HCO_3	0.1 (0.03)	0.04 (0.03)	0.15 (0.03)	0.15 (0.03)	0.09 (0.03)	0.21 (0.02)	0.58 (0.02)	−0.14 (0.02)		NC	−0.11 (0.22)	0.30 (0.32)
TCO_2	0.14 (0.03)	0.10 (0.03)	0.15 (0.03)	0.18 (0.03)	0.1 (0.03)	0.15 (0.02)	0.62 (0.01)	−0.14 (0.02)	NC		−0.15 (0.22)	0.49 (0.36)
sO_2	0.08 (0.02)	0.07 (0.02)	−0.08 (0.02)	−0.03 (0.02)	0.0004 (0.02)	0.32 (0.02)	−0.34 (0.02)	0.77 (0.01)	−0.07 (0.02)	−0.09 (0.02)		−0.70 (0.46)
MORT-TOT	−0.19 (0.02)	NC	NC	NC	NC	−0.07 (0.02)	0.04 (0.02)	NC	0.002 (0.02)	0.01 (0.02)	−0.06 (0.02)	

[1] BW_2 = BW at 2 wk; BW_5 = BW at 5 wk; RATIO = ratio of right to total ventricular weight; RV = right ventricular weight; TV = total ventricular weight; $pvCO_2$ = partial pressure of carbon dioxide in venous blood; pvO_2 = partial pressure of oxygen in venous blood; HCO_3 = bicarbonate; TCO_2 = total carbon dioxide in venous blood; sO_2 = oxygen saturation in venous blood; MORT-TOT = total mortality; NC = nonconverged.

2.4 Discussion

The objective of the present study was to estimate the heritability and genetic and phenotypic correlations between blood gas parameters measured at an average age of 22 d, BW at 2 different ages, and heart ratio in broilers. Body weight and RATIO were measured on 5,987 birds, and the blood gas parameters were measured on a subset of 2,956 birds. The study was performed under cold stress conditions to stimulate the metabolic rate resulting in an increased requirement for oxygen, which is known to increase the incidence of ascites in chickens (Decuypere et al., 2000). To evaluate whether specific blood gas parameters could be used in selecting against ascites susceptibility, we studied the heritability and genetic correlations with RATIO.

Severity of the Challenge

Previous studies have indicated that correlations between BW and ascites traits are dependent on the frequency of ascitic birds in the population, and therefore on the severity of the challenge (De Greef et al., 2001; Zerehdaran et al., 2006). In the current study, the average MORT-TOT was 10%. In comparison with the MORT-TOT of 16% found by Pakdel et al. (2002) under cold conditions, the mortality in the present study was not very high. However, mortality was much greater than the 4 to 5% mortality found in chickens reared under normal commercial conditions (Pakdel et al., 2002). In the study by Pakdel et al. (2002), an average RATIO of 28% was found, which is greater than the average value of 25% that was obtained in the current study. Julian et al. (1987) suggested that a RATIO of greater than 25% indicates susceptibility to ascites. This threshold would imply that, in our experiment, 45% of the birds showed signs of ascites. The BW_5 in the present study was also lower than under commercial conditions. This suppressed growth rate was likely due to the cold stress conditions under which the birds were kept.

In the current study, the average $pvCO_2$ was 45.4 mmHg. Scheele et al. (2003) found an average $pvCO_2$ at 3 wk of age of 53.8 mmHg in a high-risk broiler line and an average $pvCO_2$ of 43.9 mmHg in a low-risk line. Interestingly, however, the pvO_2 was lower (44.6 mmHg for the high-risk line and 46.9 mmHg for the low-risk line) than in the current study (52.46 mmHg). However, it should be noted that Scheele et al. (2003) used only male broilers, whereas in the current study, the average measurements were based on results from both males and females. In addition, the previous study compared ascites susceptibility between 2 genetically different stocks (high- and low-risk lines), whereas the current study investigated ascites susceptibility within one crossed line.

It can be concluded that birds in the current study were kept under circumstances that caused a mild increase in ascites. Because estimates of genetic parameters depend on the severity of the challenge (De Greef et al., 2001; Zerehdaran et al., 2006), the estimates presented in this study should be interpreted in this context.

Correlations between BW and RATIO

Pakdel et al. (2005c) found a negative genetic correlation between BW_5 and RATIO (−0.27). This is consistent with the negative genetic correlation (−0.18) found between RATIO and BW_5 in the present study. A positive genetic correlation (0.19) was observed between RATIO and BW_2. These results suggest that susceptible chickens tend to have a greater BW early in life (BW_2) and a lower BW later in life (BW_5). These results are in agreement with the general finding that correlations between traits are dependent on the frequency of ascitic birds in the population.

Maternal Effects

A maternal environmental effect may influence the phenotype of the individual, which, in case these effects play a role, should be accounted for in the statistical analysis (Clément et al., 2001). In the current study, noticeable changes were found in the heritabilities for TV, $pvCO_2$, and MORT-TOT when the maternal environmental effect was included in the model. De Smit et al. (2008) showed that ascites resistance is related to several physiological variables at the embryonic stage, which suggests that maternal effects might play a role in susceptibility. Several studies have reported a maternal effect for BW (Koerhuis and Thompson, 1997; van Kaam et al., 1998; Pakdel et al., 2002). Pakdel et al. (2002) found a significant maternal effect for RATIO, which could not be confirmed in the present study. Navarro et al. (2006) found little evidence for maternal (environmental) effects on sO_2; of the 4 broiler lines investigated, one of them exhibited significant evidence for the presence of maternal effects. In that line, maternal environmental effects explained approximately 2% of the total variance. In the present study, we did not find significant maternal effects on sO_2; however, a significant maternal effect was found for $pvCO_2$, which explains 5% of the phenotypic variation.

Blood Gas Parameters as Indicator Traits for Ascites Susceptibility

In the present study, we evaluated the suitability of blood gas parameters as indicator traits for ascites based on heritabilities and correlations with RATIO. For some of the blood gas parameters, heritabilities were close to zero ($pvCO_2$, pvO_2, and sO_2), whereas for others, they were moderate (pH, HCO_3, and TCO_2). The heritability estimate for sO_2 was in agreement with results by Navarro et al. (2006);

however, in that study, sO_2 was measured on 6-wk-old chickens that were not cold stressed. This might have affected the heritability estimates. Druyan et al. (2007) reported a considerably greater heritability estimate (0.49 ± 0.23) for sO_2 in chickens that were 7 d old. However, this estimate is not significantly different from the heritability estimate reported in the present study. The low heritabilities indicate that accurate estimates of breeding values for these traits cannot be obtained based on single observations. Accuracies might be improved by using repeated observations, but this will depend on the repeatability of the traits. Repeatability could not be estimated based on the present data; therefore, this is still an option that can be explored. In addition to high heritability, a suitable indicator trait should also have a high genetic correlation with ascites susceptibility. This was evaluated by studying correlations with RATIO. Several authors have suggested that RATIO is a good indicator trait for ascites susceptibility (Lubritz et al., 1995; Pakdel et al., 2002, 2005b). Julian et al. (1987) recommended the use of RATIO as an objective method for assessing right ventricular failure, and therefore of diagnosing ascites. However, others have questioned whether RATIO is a good indicator trait for birds kept under normal conditions (i.e., conditions that do not stimulate ascites; Pavlidis et al., 2007). In the present study, birds were kept under cold stress conditions.

Genetic correlations between RATIO and both HCO_3 and TCO_2 were moderate, and correlations between RATIO and pH or $pvCO_2$ were close to zero. The correlation between RATIO and pvO_2 was −0.62; however, the estimated heritability for pvO_2 was very low, resulting in very high SE for the genetic correlations with this trait. Therefore, results from the present study suggest that blood gas parameters are not useful as indicators for ascites susceptibility when measured at an average age of 22 d.

Experimental results from juvenile chickens (Korte et al., 1999; Scheele et al., 2003) showed that at the age of 11 d, a high $pvCO_2$ is associated with a greater incidence of ascites at the age of 5 to 7 wk. Scheele et al. (2005) stated that genetic selection for low $pvCO_2$ values at 11 d of age will be an effective method of reducing the occurrence of the ascites syndrome. However, results from the present study do not confirm this. The first explanation for the discrepancy between results from the present study and results by Scheele et al. (2003, 2005) may be the different ages of the chickens at which the blood gas parameters were measured. The pulmonary pressure index values are known to change rapidly over the first 2 wk of the life of a chicken. Particularly during the period of juvenile growth, the metabolic rate is

high and these conditions impose greater metabolic demands. The increased metabolism requires high O_2 intake and, at the same time, high maintenance requirements. These factors lead to the maximal potential delivery capacity of oxygen in the respiratory and cardiovascular systems, which are then exceeded and trigger the events that lead to ascites (Decuypere et al., 2000). In the current study, the blood gas parameters were measured when the chickens were, on average, 22 d old. However, in the study by Scheele et al. (2005), the differences in $pvCO_2$ values between the lines remained consistent until the end of the experiment, but did increase as the chickens became older. The pvO_2 values decreased as the chickens aged, and the differences between the mean pvO_2 values in the 2 lines became greater (Scheele et al., 2003). It should be mentioned that the severity of the challenge differed between the study by Scheele et al. (2003, 2005) and the current study. In the current study, the temperature was gradually reduced to 10°C at 22 d of age, whereas Scheele et al. (2003, 2005) gradually reduced the temperature to 15°C at 16 d of age. Although this did not result in a greater mortality, it is possible that even ascites-resistant broilers experienced problems with breathing because of the low temperature. This might have had an effect on the blood gas parameter values. The second explanation could be the lines that were used. Scheele et al. (2003) compared ascites susceptibility between 2 genetically different stocks (high- and low-risk lines), and several different lines were compared in the other study (Scheele et al., 2005), whereas in the current study, ascites susceptibility was investigated within one crossed line. A third explanation could be the fact that only males were used by Scheele et al. (2003, 2005), whereas both males and females were used in the current study. The female growth rate is slower than the male growth rate; thus, ascites-susceptible females will have a lower $pvCO_2$, on average. Therefore, differences in $pvCO_2$ between healthy and affected chickens will be smaller. Although previous studies (Scheele et al., 2003, 2005) reported the use of $pvCO_2$ as an indicator trait for ascites susceptibility in male broilers, the results from the current study do not support this. It might be concluded that the severity of the challenge, the genetic lines used, the sex of the chickens, and the time of measurement are critical factors.

Conclusion

The estimated heritabilities for the blood gas parameters $pvCO_2$ and pvO_2 were almost zero. This indicates that selection based on single measurements of these blood gas parameters is not feasible. The heritabilities of HCO_3 and TCO_2 showed enough variation in the population to be used for selection. However, the low genetic correlation between RATIO and these 2 blood gas parameters suggests that

they are not useful as indicators for ascites susceptibility. Therefore, the current data suggest that blood gas parameters measured at an average age of 22 d will not be very effective when used for selecting against susceptibility.

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References

De Smit, L., V. Bruggeman, M. Debonne, J. K. Tona, B. Kamers, N. Everaert, A. Witters, O. Onagbesan, L. Arckens, J. De Baerdemaeker, and E. Decuypere. 2008. The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. Poult. Sci. 87:551–560.

Decuypere, E., J. Buyse, and N. Buys. 2000. Ascites in broiler chickens: Exogenous and endogenous structural and functional causal factors. World's Poult. Sci. J. 56:367–377.

Deeb, N., A. Shlosberg, and A. Cahaner. 2002. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 4. Association between responses to heat stress and to coldinduced ascites. Poult. Sci. 81:1454–1462.

Druyan, S., Y. Hadad, and A. Cahaner. 2008. Growth rate of ascites-resistant versus ascites-susceptible broilers in commercial and experimental lines. Poult. Sci. 87:904–911. doi:10.3382/ ps.2008-00003.

Druyan, S., A. Shlosberg, and A. Cahaner. 2007. Evaluation of growth rate, body weight, heart rate, and blood parameters as potential indicators for selection against susceptibility to the ascites syndrome in young broilers. Poult. Sci. 86:621–629.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. ASReml User Guide. New South Wales Department of Primary Industries, Orange, Australia.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Julian, R. J. 1993. Ascites in poultry. Avian Pathol. 22:419–454.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773–1780.

Julian, R. J. 2000. Physiological, management, and environmental triggers of the ascites syndrome: A review. Avian Pathol. 29:519–527.

Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. Avian Dis. 31:130–135.

Julian, R. J., and S. M. Mirsalimi. 1992. Blood oxygen concentration of fast-growing and slow-growing broiler chickens, and chickens with ascites from right ventricular failure. Avian Dis. 36:730–732.

Koerhuis, A. N. M., and R. Thompson. 1997. Models to estimate maternal effects for juvenile body weight in broiler chickens. Genet. Sel. Evol. 29:229–249.

Korte, S. M., A. Sgoifo, W. Ruesink, C. Kwakernaak, S. van Voorst, C. W. Scheele, and H. J. Blokhuis. 1999. High carbon dioxide tension (PCO_2) and the incidence of cardiac arrhythmias in rapidly growing broiler chickens. Vet. Rec. 145:40–43.

Lubritz, D. L., J. L. Smith, and B. N. McPherson. 1995. Heritability of ascites and the ratio of right to total ventricle weight in broiler breeder male lines. Poult. Sci. 74:1237–1241.

Lynch, M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. 5th rev. ed. Sinauer Associates Inc., Sunderland, MA.

McGovern, R. H., J. J. Feddes, F. E. Robinson, and J. A. Hanson. 1999. Growth performance, carcass characteristics, and the incidence of ascites in broilers in response to feed restriction and litter oiling. Poult. Sci. 78:522–528.

McMillan, I., and V. M. Quinton. 2002. Selection strategies for limiting the increase in ascites while increasing growth in broilers. Poult. Sci. 81:737–744.

Moghadam, H. K., I. McMillan, J. R. Chambers, and R. J. Julian. 2001. Estimation of genetic parameters for ascites syndrome in broiler chickens. Poult. Sci. 80:844–848.

Navarro, P., P. M. Visscher, D. Chatziplis, A. N. M. Koerhuis, and C. S. Haley. 2006. Genetic parameters for blood oxygen saturation, body weight and breast conformation in 4 meat-type chicken lines. Br. Poult. Sci. 47:659–670.

Ouweltjes, W., L. R. Schaeffer, and B. W. Kennedy. 1988. Sensitivity of methods of variance component estimation to culling type of selection. J. Dairy Sci. 71:773–779.

Pakdel, A., P. Bijma, B. J. Ducro, and H. Bovenhuis. 2005a. Selection strategies for body weight and reduced ascites susceptibility in broilers. Poult. Sci. 84:528–535.

Pakdel, A., J. A. Van Arendonk, A. L. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites-related traits in broilers. Poult. Sci. 81:1273–1279.

Pakdel, A., J. A. M. Van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005b. Genetic parameters of ascites-related traits in broilers: Correlations with feed efficiency and carcass traits. Br. Poult. Sci. 46:43–53.

Pakdel, A., J. A. M. Van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005c. Genetic parameters of ascites-related traits in broilers: Effect of cold and normal temperature conditions. Br. Poult. Sci. 46:35–42.

Pavlidis, H. O., J. M. Balog, L. K. Stamps, J. D. Hughes Jr., W. E. Huff, and N. B. Anthony. 2007. Divergent selection for ascites incidence in chickens. Poult. Sci. 86:2517–2529.

Scheele, C. W., E. Decuypere, P. F. G. Vereijken, and F. J. G. Schreurs. 1992. Ascites in broilers. 2. Disturbances in the hormonal regulation of metabolic rate and fat metabolism. Poult. Sci. 71:1971–1984.

Scheele, C. W., J. D. Van Der Klis, C. Kwakernaak, N. Buys, and E. Decuypere. 2003. Haematological characteristics predicting susceptibility for ascites. 1. High carbon dioxide tensions in juvenile chickens. Br. Poult. Sci. 44:476–483.

Scheele, C. W., J. D. Van der Klis, C. Kwakernaak, R. A. Dekker, J. H. Van Middelkoop, J. Buyse, and E. Decuypere. 2005. Ascites and venous carbon dioxide tensions in juvenile chickens of highly selected genotypes and native strains. World's Poult. Sci. J. 61:113–129.

Shlosberg, A., I. Zadikov, U. Bendheim, V. Handji, and E. Berman. 1992. The effects of poor ventilation, low temperatures, type of feed and sex of bird on the development of ascites in broilers. physiopathological factors. Avian Pathol. 21:369–382.

van Kaam, J. B. C. H. M., J. A. M. van Arendonk, M. A. M. Groenen, H. Bovenhuis, A. L. J. Vereijken, R. P. M. A. Crooijmans, J. J. van der Poel, and A. Veenendaal. 1998. Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. Livest. Prod. Sci. 54:133–150.

Wideman, R. F. Jr., and H. French. 1999. Broiler breeder survivors of chronic unilateral pulmonary artery occlusion produce progeny resistant to pulmonary hypertension syndrome (ascites) induced by cool temperatures. Poult. Sci. 78:404–411.

Wideman, R. F. Jr., and H. French. 2000. Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. Poult. Sci. 79:396–401.

Wideman, R. F. Jr., D. M. Hooge, and K. R. Cummings. 2003. Dietary sodium bicarbonate, cool temperatures, and feed withdrawal: Impact on arterial and venous blood-gas values in broilers. Poult. Sci. 82:560–570.

Wideman, R. F. Jr., P. Maynard, and W. G. Bottje. 1999. Venous blood pressure in broilers during acute inhalation of five percent carbon dioxide or unilateral pulmonary artery occlusion. Poult. Sci. 78:1443–1451.

Zerehdaran, S., E. M. van Grevehof, E. H. van der Waaij, and H. Bovenhuis. 2006. A bivariate mixture model analysis of body weight and ascites traits in broilers. Poult. Sci. 85:32–38.

3

Genetic correlation between heart ratio and body weight as a function of ascites frequency in broilers split up into sex and health status

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Abstract

Ascites or pulmonary hypertension syndrome is a metabolic disorder in broilers. Male broilers have a higher BW and are therefore expected to be more prone to developing ascites than females. As genetic parameters might be affected by the ascites incidence, genetic parameters might differ between male and female broilers. The aims of this study were to estimate the heritability for the ratio of right ventricular weight to total ventricular weight (RATIO) and BW in male and female broilers, the genetic correlation between RATIO and BW separately for male and female broilers, and the genetic correlations between BW for ascitic and nonascitic broilers. Data were available from 7,856 broilers (3,819 males and 4,037 females). The broilers in the experiment were kept under a cold temperature regimen and increased CO_2 levels. In this study, we showed that the incidence of ascites is higher in male than in female broilers. Heritability estimates for BW at 7 wk of age were higher for male (0.22) than for female (0.17) broilers, and for RATIO heritability, estimates were higher for female (0.44) than for male (0.32) broilers. The genetic correlations between RATIO and BW measured at different ages changed from slightly positive at 2 wk of age to moderately negative at 7 wk. The change in genetic correlation was more extreme for male (from 0.01 to −0.62) than for female (from 0.13 to −0.24) broilers. The difference in ascites incidence between male and female broilers is the most likely reason for the difference in genetic correlations. The genetic correlation between BW traits measured in broilers with fluid in the abdomen and without fluid in the abdomen decreased from 0.91 at 2 wk to 0.69 at 7 wk. We conclude that under circumstances with ascites, data from male and female broilers should be analyzed separately.

Key words: broiler, ascites susceptibility, sex, heritability, genetic correlation

3.1 Introduction

Ascites or pulmonary hypertension syndrome is a metabolic disorder found in broilers that is associated with fluid accumulation in the abdominal cavity as a consequence of right ventricular failure (Julian et al.1987; Julian and Mirsalimi, 1992). Pulmonary hypertension syndrome is caused by increased pressure in the pulmonary arteries when the heart struggles to pump more blood through the lungs to meet increased oxygen demands. The overload of the blood volume increases the pressure on the right ventricle, which causes dilatation and hypertrophy of the right ventricular wall, valvular insufficiency, right ventricular failure, and ascites (Julian et al., 1987; Peacock et al., 1990; Julian andMirsalimi, 1992). The development of ascites leads to increased mortality (Julian, 1998; Druyan et al., 2007), reduced BW (Closter et al., 2009), and reduced meat quality, such as reddish color of the breast fillet (Pakdel et al., 2005b). Mortality due to ascites in commercial broiler flocks has been found to range from around 5% (Maxwell and Robertson, 1998) to up to 30% in some flocks (Balog, 2003).

Broiler breeding programs have, over the last decades, primarily been focusing on growth rate, feed efficiency, and meat yield and secondarily on health traits and fertility (Arthur and Albers, 2003; Balog, 2003; Havenstein et al., 2003). Health traits like ascites susceptibility have been included in the breeding goal to balance the economic losses due to mortality, decreased meat production caused by ascites, and to increase the welfare of the chickens (Maxwell and Robertson, 1998; Arthur and Albers, 2003). Genetic analyses have demonstrated that ascites is heritable, where heritabilities range from 0.21 to 0.45 for the ascites indicator trait; that is, ratio of right ventricular weight to total ventricular weight (RATIO; Lubritz et al., 1995; Pakdel et al., 2002), and 0.36 to 0.44 for fluid in the abdomen (Lubritz et al., 1995). The relative high heritabilities for ascites indicate that genetic factors play an important role in ascites susceptibility of broilers. Both simulation as well as experimental studies have shown that genetic correlations between RATIO and BW are a function of the ascites incidence (de Greef et al., 2001; Pakdel et al., 2005b; Zerehdaran et al., 2006; Closter et al., 2009): the genetic correlations become more negative when the incidence of ascites increases (de Greef et al., 2001; Pakdel et al., 2005b). The effect on the correlation between BW and RATIO from the incidence of ascites in a chicken population can be explained by the chickens suffering from ascites also showing reduced growth (Zerehdaran et al., 2006). Disease incidences have also been found to affect genetic correlations between production traits and disease in sheep (Bishop and Stear, 1999) and in rainbow trout (Kause et al., 2005).

Current statistical methods for estimating genetic parameters for ascites indicator traits do not account for differences between healthy and diseased chickens (Zerehdaran et al., 2006). However, traits in ascites-affected and non-ascites-affected broilers actually might be genetically different traits. Furthermore, most studies consider traits measured on male and female broilers to be genetically identical. Because male broilers have a higher BW, and therefore a higher oxygen demand, males are expected to be more prone to developing ascites than females (Decuypere et al., 2000). Differences in the incidence of ascites between male and female broilers might affect the estimated genetic parameters. The aims of this study were 1) to estimate the heritability for RATIO and BW in male and female broilers, 2) to estimate the genetic correlation between RATIO and BW separately for male and female broilers, and 3) to estimate genetic correlations between BW for ascitic and nonascitic broilers.

3.2 Materials and Method

Experimental Population

The broilers used in the current study were from a dam line originating from the White Plymouth Rock breed. The experimental population descended from 91 sires and 804 dams. One sire was mated with 2 to 28 dams, and one dam was mated with 1 to 3 sires. The number of offspring per mating ranged from 1 to 22. The experimental population consisted of 7,856 broilers of which 3,823 were males and 4,034 were females.

Experimental Conditions

The broilers in the experiment were kept under a cold temperature regimen and increased CO_2 levels to challenge the susceptibility to ascites. The temperature was 30°C at the time of hatching and was gradually reduced to 12°C when the broilers were 11 d of age. The temperature remained at 12°C until the end of the experiment when the broilers were 7 wk of age. At the same time, ventilation in the stables was reduced to increase the CO_2 level to approximately 1,500 ppm. The broilers were group-housed with 20 chickens/m2. They had ad libitum access to a commercial broiler feed containing 12,970 kJ/kg, and they were exposed to 23 h of light per day during the entire experiment. Except for the applied temperature schedule and increased CO_2 level, the broilers were kept under conditions that closely resemble commercial practice. The males were slaughtered at 45 d of age, and the females were slaughtered at 46 d of age. The experiment was carried out

by licensed and authorized personnel under the supervision of Hendrix Genetics BV.

Phenotypes

The broilers were weighed at 2 (BW_2), 5 (BW_5), and 7 wk (BW_7) of age. The BW_7 was measured on the day of slaughter. Animals that died before the end of the experiment were assigned a score of one for total mortality and the other chickens received a score of zero. Postmortem examination was performed on broilers that were slaughtered as well as on the broilers that died before the end of the experiment. Each broiler was examined for the presence of fluid in the abdomen as an indicator of ascites. The trait was scored as zero in the case that no fluid accumulation was observed in the abdomen and scored as one in the case that fluid had accumulated in the abdomen. Broilers that died before the end of the experiment and that showed fluid accumulation in the abdomen were considered to have died due to ascites. Hearts were collected from all broilers; that is, broilers that died before the end of the experiment as well as broilers that were slaughtered at the end of the experiment. The hearts were used to determine RATIO.

Statistical Analysis

Genetic parameter estimates were obtained using the ASREML software (Gilmour et al., 2006). The following animal model with a maternal environmental effect was used:

$$y_{ijkl} = \mu + sex_i + batch \times stable_j + a_k + d_l + e_{ijkl} \quad [1]$$

where y_{ijkl} is the dependent variable of broiler i_{jkl}; sex_i is the fixed effect of sex (i = male or female, the effect was only in the model when both sexes were analyzed simultaneously); batch × $stable_j$ is the effect of the interaction between batch and stable (j = 1, 2,…, 10), batch consisted of 5 trials and there were 2 stables; a_k is the random genetic effect of individual k with $a \sim N(0, A\sigma_a^2)$; d_l is the random maternal environmental effect of dam l with $d \sim N(0, I\sigma_d^2)$; and e_{ijkl} is the random residual effect with $e \sim N(0, I\sigma_e^2)$. For the trait RATIO, the fixed effect of the person who cut the heart ($Person_m$ = 1, 2,…, 13) was added to the model [1]. The heritability (h^2) was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_d^2 + \sigma_e^2} \qquad [2]$$

where σ_a^2 is the additive genetic variance, σ_d^2 is the maternal environmental variance, and σ_e^2 is the residual variance. Heritabilities are estimated using a univariate model.

To test the significance of the maternal environmental effect, a likelihood ratio test with one degree of freedom was used:

$$\chi_1^2 = 2\log_e L(F) - 2\log_e L(R) \qquad [3]$$

where L(F) is the likelihood of the full model [1] and L(R) is the likelihood of the reduced model; that is, without a maternal environmental effect.

The fraction of the variance due to maternal environmental effects (M^2) was calculated as:

$$M^2 = \frac{\sigma_d^2}{\sigma_a^2 + \sigma_d^2 + \sigma_e^2} \qquad [4]$$

Some of the animals died before the end of the experiment and had an observation only for BW_2. The animals that died might have been the ones that were most susceptible to ascites, and this selection might have an effect on the estimated genetic parameters. Selection related to BW_2 can be accounted for by performing a multivariate analysis including BW_2 (Ouweltjes et al., 1988). Therefore, we also estimated heritabilities by using a bivariate analysis with BW_2 as a permanent trait.

Correlations between RATIO and BW_2 were from a bivariate model. The possible effect of selection on genetic correlations between BW_5 and RATIO and between BW_7 and RATIO was accounted for by performing a trivariate analysis with BW_2 as a permanent trait.

The traits BW_2, BW_5, BW_7, and RATIO were also analyzed separately for males and females. Bivariate analyses were performed to estimate genetic correlations between the same trait in male and in female broilers.

Body weights (BW_2, BW_5, and BW_7) of ascitic and nonascitic broilers might be genetically different traits. Therefore, we distinguished between broilers with fluid in the abdomen (ascitic) and without fluid in the abdominal (nonascitic). Heritability and maternal environmental effects were estimated separately for both groups. Further, genetic and phenotypic correlations were estimated between BW_2, BW_5, and BW_7 in ascitic and nonascitic chickens using a bivariate analysis.

3.3 Results

Data Description

Means and SD for BW_2, BW_5, BW_7, and RATIO for all broilers (males and females) and for male and female broilers separately are presented in Table 3.1. The average weight of the broilers was 248 g at 2 wk, 1,296 g at 5 wk, and 2,075 g at 7 wk of age. The CV for BW was similar at different ages and around 14%. The BW_2 was similar for females (250 g) and for males (246 g). The BW_5 was 126 g higher for males than for females, and BW_7 was 181 g higher for males than for males. The average RATIO for all broilers was 28.7%, where RATIO was 3.4% higher for males than for females. Table 3.2 shows the mean and SD of traits for male and female chickens with or without fluid accumulated in the abdomen.

Both RATIO and mortality were higher for chickens with fluid accumulated in the abdomen, both for males and females. Further, in males and in females, BW_5 and BW_7 were higher for chickens without fluid in the abdomen. The BW_2 was lower for male chickens without fluid in the abdomen as compared with male chickens with fluid in the abdomen. For the females, BW_2 was very similar for both groups. Not all of the chickens had observations for fluid in the abdomen. Information about fluid in the abdomen was missing for 105 males and 85 females that had observations for BW_2, for 77 males and 58 females that had observations for BW_5, for 12 males and 36 females that had observations for BW_7 and for 33 males and 41 females that had observations for RATIO.

Table 3.1. The number of observations (N), mean and the standard deviation (S.D.) for BWs (BW) at three different ages, heart ratio and mortality for the entire population of broilers and for male and female broilers separately.

			All			Males			Females		
Variable	Units	Abbreviation	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
BW at 2 weeks	Gram	BW_2	7,803	248	38	3,791	246	40	4,012	250	35
BW at 5 weeks	Gram	BW_5	7,496	1,296	174	3,596	1,362	169	3,900	1,236	156
BW at 7 weeks	Gram	BW_7	7,121	2,075	295	3,351	2,171	310	3,770	1,990	253
Heart ratio	%	RATIO	7,687	28.7	7.6	3,723	30.5	7.8	3,964	27.1	7.1
Mortality	0/1	MORT-TOT	7,856	0.093	0.29	3,819	0.122	0.33	4,037	0.066	0.25

Table 3.2. The number of observations (N) , mean and the standard deviation (S.D.) for BWs (BW) at three different ages, heart ratio and mortality for male broilers and female broilers and for chickens without fluid accumulated in the abdomen (AS = 0) or with fluid accumulated in the abdomen (AS = 1).

	Males						Females					
Variable	AS = 0			AS = 1			AS = 02			AS = 1		
	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	M	SD
BW at 2 weeks	3,203	244	39	483	262	43	3,660	250	35	267	255	41
BW at 5 weeks	3,080	1,367	166	439	1,313	170	3,596	1, 40	152	246	1,190	189
BW at 7 weeks	3,030	2,207	285	309	1,828	321	3,559	2,006	238	175	1,667	337
Heart ratio	3,209	28.8	6.5	418	41.5	6.6	3,655	26.0	5.9	268	40.7	7.1
Mortality	3,230	0.06	0.24	484	0.36	0.48	3,681	0.03	0.18	270	0.34	0.48

Out of the 7,856 chickens, 3,353 males and 3,772 females survived until the end of the experiment, and 466 males and 265 females died before the day of slaughter (Table 3.3). It should be noted that 6 of the chickens that survived missed observations for BW_7 (Table 3.1). A total of 754 chickens had fluid accumulated in the abdomen, 484 males and 270 females. Using fluid accumulated in the abdomen as a direct indicator for ascites, it was concluded that 9.5% of the chickens developed ascites, 12.2% of the males and 6.6% of the females. In total, 265 chickens died (3.4%) before slaughter and also showed fluid accumulation in the abdomen. Of the 466 males with fluid in the abdomen, 172 died before the day of slaughter; that is, 4.5% of the total number of males died due to ascites. For the females, 93 chickens that died before slaughter showed fluid accumulated in the abdomen; that is, 2.3% of the total number of females died due to ascites (Figure 3.1). In the total population, 323 chickens (4.1%) died before slaughter and did not show signs of ascites (fluid in the abdomen) and 200 males (5.2%) and 123 females (3%) died without signs of ascites. For 94 males and 49 females, a score for accumulation of fluid in the abdomen was not available. Also, 34 males (one with fluid accumulated in the abdomen) and 18 females (one with fluid accumulated in the abdomen) died during the first 2 wk of the experiment.

Table 3.3. The number of observations for survived chickens, premature death chickens and chickens with fluid accumulated in abdomen.

	All	Male	Female
Total number of chickens	7856	3819	4037
Chickens that survived till day of slaughter	7125	3353	3772
Chickens that died before day of slaughter	731	466	265
Chickens with fluid accumulated in the abdomen	754	484	270
Survived chickens with fluid accumulated in the abdomen	489	312	177
Premature died chickens with fluid accumulated in the abdomen	265	172	93
Premature died chickens without fluid accumulated in the abdomen	323	200	123

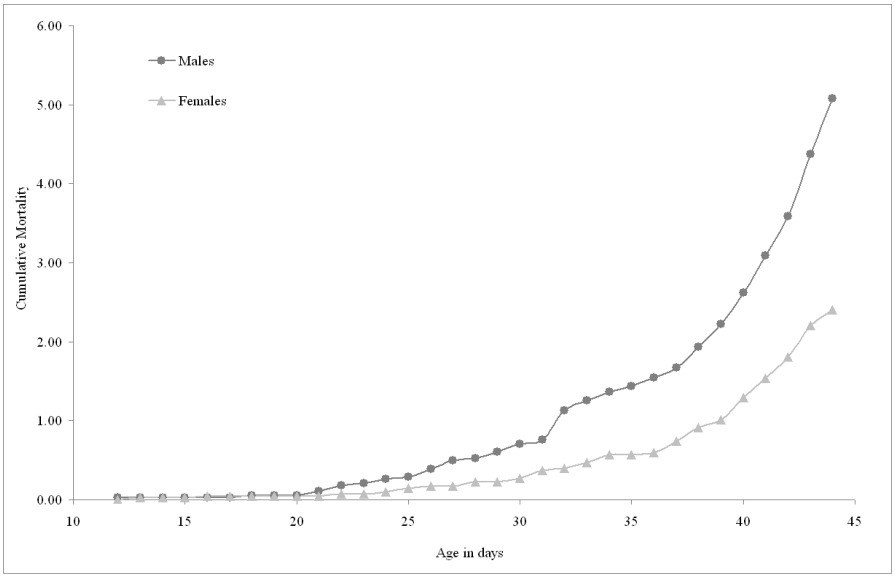

Figure 3.1 Cumulative mortality due to ascites, i.e. broilers with fluid in the abdomen at day of death, separately for males and females. The cumulative mortality is given as a percentage of the total number of male and female broilers.

Genetic Analyses

Heritability and Maternal Effects.

The likelihood ratio test indicated that BW at 2, 5, and 7 wk of age were significantly ($P < 0.05$) affected by maternal effects. For RATIO, the maternal effect was close to being significant ($P = 0.06$) and, therefore, we decided to include maternal environmental effect in the model. The heritability for RATIO decreased from 0.42 to 0.35 when the maternal environmental effect was in the model.

The heritability and the fraction of the total variance explained by maternal environmental effects for BW decreased with age. This was observed for both female and male broilers (Table 3.4). Heritability estimates for BW_2 and BW_5 were similar for female and male broilers. The heritability for BW_7 tended to be higher for male broilers (0.22) than for female broilers (0.17). Maternal environmental effects for BW at the 3 ages were similar for male and female broilers and decreased from 0.07 for BW_2 to about 0.04 at BW_7. The largest difference in heritability between male and female broilers was found for RATIO: 0.32 in male broilers and 0.44 in female broilers. The fraction of the total variance explained by maternal environmental effects for RATIO was 0.02 for female broilers and 0.01 for male broilers (Table 3.4).

Correlation between RATIO and BW.

The estimates for genetic and phenotypic correlations between RATIO and BW at 2, 5, and 7 wk of age are presented in Table 3.5. The results show that the genetic correlations between BW and RATIO change as the broilers get older. The genetic correlation between BW and RATIO estimated based on all data changed from 0.11 at 2 wk to −0.43 at 7 wk. When analyzing the genetic correlations separately for male and female chickens, the same trend was observed; however, the negative correlation between BW_7 and RATIO was much stronger in males than in females. The genetic correlation for males changed from 0.01 at 2 wk to −0.62 at 7 wk, and the genetic correlation for females changed from 0.13 at 2 wk to −0.24 at 7 wk. Phenotypic correlations between RATIO and BW showed the same trend as the genetic correlations.

Genetic Correlations between BW in Nonascitic and Ascitic Broilers.

The genetic and phenotypic correlations were also estimated between the same trait (BW_2, BW_5, or BW_7) measured in nonascitic (no fluid in abdomen) and in ascitic broilers (fluid in abdomen). For BW_2, 6,863 chickens were categorized as nonascitic and had a mean BW of 244 g for males and 250 g for females. There were 750 chickens that were ascitic and the males had a mean BW of 262 g and the females had a mean BW of 255 g. This indicates that chickens that developed ascites at a later age are heavier at 2 wk of age. For the nonascitic chickens, heritability for BW_2 was 0.37 (SE = 0.06) and the maternal effect was 0.05 (SE = 0.01). For the ascitic chickens, the heritability for BW_2 was 0.36 (SE = 0.11) and the maternal effect was 0.10 (SE = 0.05). At 5 wk of age, 6,676 chickens were categorized as nonascitic, and males had a mean BW of 1,367 g and females had a mean BW of 1,240 g. There were 685 chickens with ascites and the males had a mean BW of 1,313 g and the females had a mean BW of 1,190 g.

Table 3.4. Phenotypic variance, heritability (h^2) and maternal effect (M^2) for the entire population of broilers, male broilers and female broilers.

	Phenotypic variance[1)]			h^2 [2)]			M^2 [3)]		
Variable	All	Males	Females	All	Males	Females	All	Males	Females
BW_2	959	917	957	0.33	0.30	0.30	0.06	0.07	0.07
BW_5	25,910	28,440	23,310	0.22	0.23	0.22	0.05	0.05	0.06
BW_7	72,420	88,140	59,810	0.18	0.22	0.17	0.04	0.04	0.05
RATIO	49.3	53.7	45.2	0.35	0.32	0.44	0.02	0.02	0.01

Table 3.5. The genetic and the phenotypic correlations between RATIO and BW for entire population of broilers, for the males and the females. BW_5 and BW_7 have been estimated using a trivariate model including BW_2. Standard errors are in brackets.

	Genetic correlation			Phenotypic correlation		
Variable	All	Male	Female	All	Male	Female
BW_2	0.11 (0.12)	0.01 (0.14)	0.13 (0.14)	0.04 (0.02)	0.04 (0.02)	0.004 (0.03)
BW_5	-0.09 (0.13)	-0.24 (0.14)	0.05 (0.15)	-0.06 (0.02)	-0.04 (0.02)	-0.07 (0.02)
BW_7	-0.43 (0.11)	-0.62 (0.11)	-0.24 (0.15)	-0.25 (0.02)	-0.27 (0.02)	-0.22 (0.02)

The heritability for BW_5 in the nonascitic chickens was 0.24 (SE = 0.04) and the maternal effect was 0.05 (SE = 0.01). The heritability for BW_5 in the ascitic chickens was 0.23 (SE = 0.11) and the maternal effect was 0.05 (SE = 0.05). For BW_7, 6,589 chickens were categorized as nonascitic, and the males had a mean BW of 2,207 g and the females had a mean BW of 2,006 g. There were 484 ascitic birds, and the males had a mean BW of 1,828 g and the females had a mean BW of 1,667 g. The heritability for BW_7 in the nonascitic chickens was 0.19 (SE = 0.04) and the maternal effect was 0.04 (SE = 0.01). The heritability for BW_7 in the ascitic chickens was 0.16 (SE = 0.12) and the maternal effect was 0.12 (SE = 0.07). The genetic correlation between the same trait in the 2 groups (ascitic and nonascitic) went from 0.91 (SE = 0.09) for BW_2 to 0.81 (SE = 0.17) for BW5 and 0.69 (SE = 0.27) for BW_7. The phenotypic correlation between the 2 groups (ascitic and nonascitic) went from 0.41 (SE = 0.05) for BW_2 to 0.25 (SE = 0.05) for BW_5 and 0.14 (SE = 0.05) for BW_7.

3.4 Discussion

The present study included 7,856 broilers from a dam broiler line kept under ascites-inducing conditions: cold temperature and increased CO_2 level. We showed that of the chickens that died during the experiment, almost twice as many males as females showed signs of ascites (4.5 vs. 2.3%). Previous studies showed that genetic parameters are a function of the ascites incidence (de Greef et al., 2001; Pakdel et al., 2005a; Zerehdaran et al., 2006; Closter et al., 2009) and, therefore, we estimated genetic parameters separately for male and female broilers. We showed that heritability estimates for BW_7 are higher for male than for female broilers and for RATIO heritability estimates, are higher for female than for male broilers. The genetic correlations between RATIO and BW measured at different ages changed from slightly positive at 2 wk to moderately negative at 7 wk of age. The change in genetic correlation was more extreme for male than for female chickens. The genetic correlation between BW traits measured in chickens with fluid in the abdomen and without fluid in the abdomen decreased from 0.91 at 2 wk to 0.69 at 7 wk of age.

Literature

Total mortality in the present study was 9.3%, which is comparable with previous studies under ascites-inducing conditions (de Greef et al., 2001; Closter et al. 2009) but lower than mortalities reported by Pakdel et al. (2002). Mortality due to ascites mostly starts after d 30 and is highest between wk 6 and 7. The cumulative mortality rate for both males and females was lower than what Druyan et al. (2009) found for resistance and susceptible lines; however, these broilers were also reared

under experimental high-challenge ascites inducing conditions, which is more extreme than the ascites-inducing condition used in the current study. The average RATIO in the present study was 28.7, which is higher than values reported in other studies (de Greef et al., 2001; Pakdel et al., 2002; Closter et al., 2009). The average ratio found in the current study is in between values found by Druyan et al. (2009) for an ascites-susceptible line (36.1) and an ascites-resistance line (23.5). Heritability estimates based on combined male and female data for BW measurements and RATIO are in agreement with values reported by Pakdel et al. (2002) and Closter et al. (2009) but lower than estimates reported by de Greef et al. (2001), which can be explained by adjusting for maternal effects in the present study.

Male versus Female Broilers

In the present study, male broilers were more severely affected by ascites than female broilers; 4.2% of the males and 2.3% of the females died due to ascites. Further, RATIO, which is commonly used as an indicator for ascites susceptibility (Lubritz et al., 1995; Pakdel et al., 2002; Pakdel et al., 2005b), was noticeably higher for males (30.5%) than for females (27.1%). Wideman et al. (2010) did not find any difference between males and females for RATIO. However, the broilers from the Wideman et al. (2010) experiment were reared under standard commercial conditions, where the chickens from the present study were reared under cold conditions and increased CO_2 levels. Pakdel et al. (2005c) showed that ascites-related traits are influenced by the rearing conditions of the chicken. Higher total mortalities and RATIO for males as compared with females has also been reported in other studies (Burton et al., 1968; Wideman and French, 2000; Pakdel et al., 2002; Druyan et al., 2007). It has been suggested that the modern broiler, which has been selected for rapid growth and feed efficiency, has problems dealing with the high oxygen demand associated with rapid growth, resulting in chickens that are more susceptible to ascites syndrome. Therefore, the higher growth rate of male broilers is expected to result in an increased oxygen requirement, which might be the reason for increased susceptibility of males to ascites (Peacock et al., 1990; Balog, 2003).

Under normal production circumstances, heritability estimates for growth traits in poultry tend to be slightly higher in males than in females (Koerhuis and Thompson, 1997; Van Kaam et al., 1999; Mignon-Grasteau et al., 1999; Mulder et al., 2009; Wolc et al., 2009). However, in the present study, heritabilities for BW_7 were higher for males (0.22) than for females (0.17). On the contrary, heritabilities

for RATIO were higher in females than in males. Because broilers in the present study were kept under ascites-inducing conditions, the estimated heritabilities might be affected by ascites. This explanation is not in agreement with the estimated heritabilities in ascitic and nonascitic broilers; the heritability for BW_7 was higher (0.19) in nonascitic chickens than in ascitic chickens (0.16). However, standard error of especially the estimated heritability in ascitic chickens was large, and the difference between the heritabilities could not be considered to be significant. For BW_7, both the additive genetic and residual variances are higher in males than in females. However, for RATIO, the additive genetic variance is very similar in males and in females, but the higher residual variance in males resulted in a lower heritability for RATIO in males. Several studies have estimated genetic correlations between BW traits in males and females (Koerhuis and Thompson, 1997; Mignon-Grasteau et al., 1999; Van Kaam et al., 1999). Genetic correlations in general are high (>0.92) but, in some cases, significantly different from one (Koerhuis and Thompson, 1997; Mignon-Grasteau et al., 1999). Mignon-Grasteau et al. (1999) showed that females have a lower initial growth rate but a higher maturation rate than males. This suggests that from a genetic point of view there are small but significant differences in growth between males and females. These differences might be the cause for the remarkable difference in ascites susceptibility between sexes. It has been suggested that reduced early growth reduces the incidence of metabolic diseases later in life (Baghbanzadeh and Decuypere, 2008). Therefore, the lower initial growth rate in females, as reported by Mignon-Grasteau et al. (1999), might explain the lower incidence of ascites. Phenotypic and genetic correlations between BW_2 and RATIO were positive but very weak both in males and in females, which only weakly support a relationship between early growth and ascites. However, the BW_2 of nonascitic chickens (247 g) was lower than the BW_2 of chickens that developed ascites later in life (260 g) and the findings by Druyan et al. (2008), where mean BW on d 19 were approximately 5% lower in the broilers from the ascites-resistant line than in those from the ascites-susceptible line. Scheele et al. (2005) found that a combination of fast growth with a low feed conversion ratio showed by far the highest incidence of ascites. However, a very fast-growing broiler cross was much less sensitive to ascites than a slower-growing sire line (Scheele et al., 2005). Furthermore, Druyan et al. (2008) found that most broilers that remained healthy under the ascites-inducing conditions exhibited the same growth rate and BW during the first weeks as those that later developed ascites. Druyan et al. (2007) hypothesized that the tendency to develop ascites is genetically associated with rapid growth rate, because the threshold level of rapid growth to develop ascites is higher in ascites-

susceptible chickens. Based on these results and the relatively high incidence of ascites in a slow-growing line, Druyan et al. (2008) concluded that there is very little direct genetic association between ascites and genetic differences in potential growth rate and suggested that ascites-resistant broilers can be selected for higher growth rate and remain healthy. The relationship between growth and ascites might explain part of the differences in ascites susceptibility between male and female broilers, however, the relationship is not strong and therefore alterative explanations should not be excluded. For example, the higher thickness of the right ventricular wall at hatching in females as compared with males might play a role (Thaxton, 2002).

Change of Correlation due to Ascites Incidence

The genetic correlation between RATIO and BW changed from positive and weak to moderate and negative for both males and females. The change in genetic correlation between BW and RATIO over the experimental period was particularly noticeable for the males and less severe for the females. The change in correlation follows the same pattern as mortality due to ascites, as described in Figure 3.1. Previous studies have indicated that the genetic correlation between BW and RATIO is dependent on the frequency of ascitic chickens in the population (de Greef et al., 2001; Pakdel et al., 2005a,b; Zerehdaran et al., 2006; Closter et al., 2009).

The change in genetic parameters as a consequence of the increase in incidence of a disease has also been observed in other species. Bishop and Stear (1999) showed that the correlation between productivity and resistance to nematode infection in sheep changes as the level of infection or larval challenge changes. Kause et al. (2005) found that the genetic correlation between BW and skeletal deformations changed from positive to negative when trout got older and more fish showed skeletal deformations. Further, several studies indicated that somatic cell scores in healthy and infected cattle are genetically different traits (Boettcher et al., 2007; Madsen et al., 2008). When more energy is needed to overcome problems due to infections or metabolic disorders, less energy is available for production. Therefore, estimated correlations between productivity and disease traits are a function of the severity of the challenge and these correlations are likely to be lower than, or even in the opposite direction from, correlations estimated under normal conditions (Van der Waaij et al., 2000).

Most methods currently in use for estimating genetic parameters do not account for differences between ascitic and nonascitic chickens. Zerehdaran et al. (2006)

used a bivariate mixture model to study the relationships between BW and RATIO. Phenotypic correlations between BW and RATIO were 0.10 for the healthy chickens and −0.39 for the ascitic chickens. Based on simulation, Zerehdaran et al. (2006) showed that when ignoring the difference between healthy chickens and ascitic chickens, the estimated phenotypic correlation will be a function of the frequency of ascitic chickens in the population. Phenotypic correlations between BW and RATIO found in the present study are in line with correlations reported by Zerehdaran et al. (2006).

Healthy and Diseased Chickens

In the present study, BW in ascites-affected (showing fluid in abdomin) and nonascitic chickens (not showing fluid in abdomin) were analyzed using a bivariate model. de Greef et al. (2001) estimated heritabilities for BW and RATIO in the total data set as well as on a subset of nonascitic chickens. Heritability for BW in the nonascitic chickens was similar to that in the total data set in the study by de Greef et al. (2001). Heritability for RATIO was lower in the nonascitic chickens; however, the interpretation of that estimate is not straightforward, as RATIO was used to assign chickens to the nonascitic group. To our knowledge, this is the first study directly estimating the genetic correlation between BW in ascitic and nonascitic chickens. The genetic correlation decreased over time from 0.91 for BW_2 to 0.81 for BW_5 and 0.69 for BW_7. These results suggest that BW_5 and BW_7 may be considered genetically different traits in ascites-affected and nonascitic broilers.

Conclusion

In this study, we showed that the incidence of ascites is higher in male than in female broilers. This is the most likely reason for the difference in heritabilities and genetic correlations observed between males and females. Therefore, under circumstances with ascites, data from male and female broilers should be analyzed separately. Recently, mixture models have been used to perform genetic analyses of somatic cell count in dairy cattle (Odegård et al., 2003; Odegård et al., 2005; Madsen et al., 2008). Application of similar methods for the analysis of ascites in broilers seems of interest to further disentangle relations between traits for ascitic and nonascitic chickens

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References

Arthur, J. A., and G. A. A. Albers. 2003. Industrial perspective on problems and issues associated with poultry breeding. Pages 1–12 in Poultry Genetics, Breeding, and Biotechnology. W. M. Muir and S. E. Aggrey, ed. CABI Publ., Wallingford, UK.

Baghbanzadeh, A., and E. Decuypere. 2008. Ascites syndrome in broilers: Physiological and nutritional perspectives. Avian Pathol. 37:117–126.

Balog, J. M. 2003. Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian Poult. Biol. Rev. 14:99–125.

Bishop, S. C., and M. J. Stear. 1999. Genetic and epidemiological relationships between productivity and disease resistance: Gastrointestinal parasite infection in growing lambs. Anim. Sci.69:515–524.

Boettcher, P. J., D. Caraviello, and D. Gianola. 2007. Genetic analysis of somatic cell scores in us holsteins with a Bayesian mixture model. J. Dairy Sci. 90:435–443.

Burton, R. R., E. L. Besch, and A. H. Smith. 1968. Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. Am. J. Physiol. 214:1438–1442.

Closter, A. M., P. van As, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2009. Genetic and phenotypic relationships between blood gas parameters and ascites related traits in broilers. Poult. Sci. 88:483–490.

de Greef, K. H., L. L. G. Janss, A. L. J. Vereijken, R. Pit, and C. L. M. Gerritsen. 2001. Disease-induced variability of genetic correlations: Ascites in broilers as a case study. J. Anim. Sci. 79:1723–1733.

Decuypere, E., J. Buyse, and N. Buys. 2000. Ascites in broiler chickens: Exogenous and endogenous structural and functional causal factors. World's Poult. Sci. J. 56:367–377.

Druyan, S., A. Ben-David, and A. Cahaner. 2007. Development of ascites-resistant and ascites-susceptible broiler lines. Poult. Sci. 86:811–822.

Druyan, S., Y. Hadad, and A. Cahaner. 2008. Growth rate of ascites resistant versus ascites-susceptible broilers in commercial and experimental lines. Poult. Sci. 87:904–911.

Druyan, S., D. Shinder, A. Shlosberg, A. Cahaner, and S. Yahav. 2009. Physiological parameters in broiler lines divergently selected for the incidence of ascites. Poult. Sci. 88:1984–1990.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. ASReml User Guide Release 2.0. VSN International Ltd., Hemel Hempstead, UK.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult. Sci. 77:1773–1780. Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. Avian Dis. 31:130–135.

Julian, R. J., and S. M. Mirsalimi. 1992. Blood-oxygen concentration of fast-growing and slow-growing broiler chickens and chickens with ascites from right ventricular failure. Avian Dis. 36:730–732.

Kause, A., O. Ritola, T. Paananen, H. Wahlroos, and E. A. Mäntysaari. 2005. Genetic trends in growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding programme for rainbow trout (Oncorhynchus mykiss). Aquaculture 247:177–187.

Koerhuis, A. N. M., and R. Thompson. 1997. Models to estimate maternal effects for juvenile body weight in broiler chickens. Genet. Sel. Evol. 29:225–249.

Lubritz, D. L., J. L. Smith, and B. N. Mcpherson. 1995. Heritability of ascites and the ratio of right to total ventricle weight in broiler breeder male lines. Poult. Sci. 74:1237–1241.

Madsen, P., M. M. Shariati, and J. Ødegård. 2008. Genetic analysis of somatic cell score in Danish Holsteins using a liability-normal mixture model. J. Dairy Sci. 91:4355–4364.

Maxwell, M. H., and G. W. Robertson. 1998. UK survey of broiler ascites and sudden death syndromes in 1993. Br. Poult. Sci. 39:203–215.

Mignon-Grasteau, S., C. Beaumont, E. Le Bihan-Duval, J. P. Poivey, H. de Rochambeau, and F. H. Ricard. 1999. Genetic parameters of growth curve parameters in male and female chickens. Br. Poult. Sci. 40:44–51.

Mulder, H. A., W. G. Hill, A. Vereijken, and R. F. Veerkamp. 2009. Estimation of genetic variation in residual variance in female and male broiler chickens. Animal 3:1673–1680.

Ødegård, J., J. Jensen, P. Madsen, D. Gianola, G. Klemetsdal, and B. Heringstad. 2003. Detection of mastitis in dairy cattle by use of mixture models for repeated somatic cell scores: A Bayesian approach via Gibbs sampling. J. Dairy Sci. 86:3694–3703.

Ødegård, J., P. Madsen, D. Gianola, G. Klemetsdal, J. Jensen, B. Heringstad, and I. R. Korsgaard. 2005. A Bayesian threshold normal mixture model for analysis of a continuous mastitis-related trait. J. Dairy Sci. 88:2652–2659.

Ouweltjes, W., L. R. Schaeffer, and B. W. Kennedy. 1988. Sensitivity of methods of variance component estimation to culling type of selection. J. Dairy Sci. 71:773–779. Pakdel, A., P. Bijma, B. J. Ducro, and H. Bovenhuis. 2005a. Selection strategies for body weight and reduced ascites susceptibility in broilers. Poult. Sci. 84:528–535.

Pakdel, A., J. A. A. Van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites related traits in broilers. Poult. Sci. 81:1273–1279.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005b. Genetic parameters of ascites-related traits in broilers: Correlations with feed efficiency and carcass traits. Br. Poult. Sci. 46:43–53.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005c. Genetic parameters of ascites-related traits in broilers: Effect of cold and normal temperature conditions. Br. Poult. Sci. 46:35–42.

Peacock, A. J., C. Pickett, K. Morris, and J. T. Reeves. 1990. Spontaneous hypoxaemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. Comp. Biochem. Physiol. A Comp. Physiol. 97:537–541.

Scheele, C. W., J. D. van der Klis, C. Kwakernaak, R. A. Dekker, J. H. Van Middelkoop, J. Buyse, and E. Decuypere. 2005. Ascites and venous carbon dioxide tensions in juvenile chickens of highly selected genotypes and native strains. World's Poult. Sci. J. 61:113–129.

Thaxton, J. P. 2002. Heart growth in broilers. Br. Poult. Sci. 43:24–27.

van der Waaij, E. H., P. Bijma, S. C. Bishop, and J. A. van Arendonk. 2000. Modeling selection for production traits under constant infection pressure. J. Anim. Sci. 78:2809–2820.

Van Kaam, J. B. C. H., M. A. M. Groenen, H. Bovenhuis, A. Veenendaal, A. L. J. Vereijken, and J. A. M. Van Arendonk. 1999. Whole genome scan in chickens for quantitative trait loci affecting carcass traits. Poult. Sci. 78:1091–1099.

Wideman, R. F., M. L. Eanes, K. R. Hamal, and N. B. Anthony. 2010. Pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome: Age and sex comparisons. Poult. Sci. 89:1815–1824.

Wideman, R. F., and H. French. 2000. Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. Poult. Sci. 79:396–401.

Wolc, A., I. M. S. White, S. Avendano, and W. G. Hill. 2009. Genetic variability in residual variation of body weight and conformation scores in broiler chickens. Poult. Sci. 88:1156–1161.

Zerehdaran, S., E. A. van Grevehof, E. H. van der Waaij, and H. Bovenhuis. 2006. A bivariate mixture model analysis of body weight and ascites traits in broilers. Poult. Sci. 85:32–38.

4

Genetic Analysis of Ascites in Broilers using a Liability-Normal Mixture Model

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Abstract

Observations on the occurrence of ascites as well as for the ascites indicator trait ratio of right ventricle to the total ventricular weight (RATIO) for 7,613 broilers chickens were analyzed with a two- component liability-normal mixture model. This model is used to analyze the genetic background of the ascites syndrome and genetic parameters, liability and individual disease risk were estimated. The heritability for liability was 0.54, demonstrating that susceptibility to develop ascites is highly heritable. Of all chickens in the study 24 % were estimated to be affected by the ascites syndrome. Heritability for RATIO of diseased chickens was 0.31 and for the healthy chickens the heritability was 0.32. The genetic correlation between RATIO of healthy and diseased chickens was 0.75, indicating that RATIO is a different trait in healthy and diseased chickens. The genetic correlation between RATIO and liability was positive for healthy chickens (0.27) whereas the genetic correlation between RATIO and liability for diseased chickens is negative (-0.32). This negative correlation indicates that chickens with high RATIO are less susceptible to the disease, whereas the positive correlation between liability and RATIO for the healthy chickens indicates that chickens with high RATIO are more susceptible to the disease. Analyses using the LNM model indicate that RATIO is not the same trait in healthy and diseased chickens and that almost a quarter of the chickens in the analyzed population are affected by ascites.

Key words: Broiler, ascites, liability normal mixture model, heart ratio

4.1 Introduction

Genetic improvement for health traits has received increasing attention in breeding programs for production animals (Groen et al., 1997). Health status is typically recorded as healthy vs. subclinical and clinically diseased or as diseased vs. healthy. However, direct selection to reduce the incidence of specific diseases is often complicated by difficulties in recording health status of individual animals. Alternatively, disease-indicator traits can be used as indirect selection criteria to lower the frequency of disease. Examples of indicator trait are ratio of right ventricle to the total ventricular weight (RATIO) for ascites in broiler, however, a trait that is difficult and time consuming to measure (Pakdel et al., 2005a). Another often used indicator trait is milk somatic cell count (somatic cell score, SCS) and used routinely to determine mastitis status in dairy cattle (Young et al., 1960). A complication when using indicator traits is that depending upon the health status the trait may have distinct, but possibly overlapping distributions (Detilleux and Leroy, 2000; Ødegård et al., 2003).

The distribution of the disease-indicator trait may be modeled as a mixture of distributions from healthy and diseased animals when disease status itself is not observable. Modeling of disease-indicator traits using mixture distributions was introduced by Detilleux and Leroy (2000) and Ødegård et al. (2003). Detilleux and Leroy (2000) proposed a finite mixture model approach with genetic effects and for the analysis of SCS, as a selection criterion to improve mastitis resistance in dairy cattle in absence of direct information regarding mastitis status. They used the EM-algorithm for inference by maximum likelihood. The model proposed by Ødegård et al. (2003) assumes that the distribution of the random effects in the model were independent of the health status. Ødegård et al. (2005) refined their original two-component normal mixture by using a hierarchical model, the Liability Normal Mixture model (LNM). After adjusting for systematic environmental effects the LNM model allows estimating probabilities for individual animals of being diseased. Health status is modeled in the LNM model as an unobserved binary variable, assumed to be fully determined by an also unobserved underlying liability. The LNM model is based on an observed disease-indicator trait and predicts genetic effects for both the disease-indicator trait and the unobserved liability based on observed disease-indicator trait (Ødegård et al., 2005). The LNM model has been used to analyze somatic cell count data from dairy cattle (Boettcher et al., 2007; Jamrozik and Schaeffer, 2010; Madsen et al., 2008), but not for ascites in broilers.

Ascites, also known as pulmonary hypertension syndrome, is a metabolic disorder found in broilers. Ascites is associated with fluid accumulation in the abdomen as a consequence of a reduced right ventricular function (Julian et al., 1987; Julian and Mirsalimi, 1992). The characteristic symptoms of ascites are enlarged heart, especially the right ventricle, liver abnormalities and accumulation of fluid in the abdomen (Julian et al., 1987; Julian and Mirsalimi, 1992; Peacock et al., 1990). However, not all chickens that show enlarged right ventricular develop accumulation of fluid in the abdomen. These chickens might be considered to have subclinical ascites. The development of ascites leads to reduced growth (Closter et al., 2009; Pakdel et al., 2005b), increased disapproval at slaughter and increased mortality (Druyan et al., 2007; Julian, 1998). Mortality caused by ascites ranges from 5 to 8% in populations worldwide and can be as great as 20 to 30% in heavier broiler flocks (Balog, 2003; Maxwell and Robertson, 1998).The estimated heritability of the ascites indicator trait RATIO is between 0.21 and 0.45 (de Greef et al., 2001; Lubritz et al., 1995; Pakdel et al., 2002) suggesting a genetic component in the development of ascites. Zerehdaran et al. (2006) fitted a two-component mixture distribution to RATIO and provided evidence that the two distributions relate to healthy and diseased chickens. Using a multivariate mixture model Zerehdaran et al. (2006) showed that the incidence of the disease will affect the phenotypic correlation between RATIO and body weight. Closter et al. (2012) analyzed body weights at two, five and seven weeks of age separately for ascites diseased and healthy broilers classified according to the presence of fluid in the abdomen and found that body weight and the relation between RATIO and BW may have a distinct genetic bases in ascites diseased and healthy broilers. Instead of classifying diseased and healthy broilers based on the presence or absence of fluid in the abdomen, using a LNM provides a way to model a continuous transition between healthy and diseased birds. The LNM model allows estimating probabilities for individual chicken of being ascitic after adjusting for systematic environmental effects. Further, instead of analyzing the trait RATIO as is done a traditional animal model, the LNM model explicitly models the susceptibility or the underlying liability to ascites, i.e. the actual trait of interest.

The aim of the current study is to apply the LNM model to the ascites-indictor trait RATIO and to estimate genetic parameters. Specifically, two hypotheses were investigated: first, that a broiler population reared under ascites inducing conditions can be modeled by a two component mixture distribution of RATIO, where each observation is sampled from one of the components depending on diseases status, and second, that based on the modeling of the phenotypic

distributions the underlying liability for the disease can be identified, genetic parameters can be estimated and for each individual a probability of being diseased can be inferred.

4.2 Materials and Methods

Experimental Population

Data for this study was obtained from a broiler dam line originating from the White Plymouth Rock breed. The chickens descended from 91 sires and 800 dams. Each sire was mated to between 2 and 28 dams, and a dam was mated to between 1 and 3 sires. The number of offspring per mating ranged from 1 to 202. The total number of offspring with information on RATIO was 7,613 (Table 4.1).

Table 4.1. Statistics summary of the pedigree

Number of broilers	7,613
Number of observations on each chicken	1
Number of sires	91
Number of dams	800
Number of animals in pedigree	11,863
Number of generations in pedigree	25

Experimental Conditions

The broilers were kept under a cold temperature regime. The temperature was 30°C at the time of hatching and was gradually reduced reaching 12°C when the broilers were 11 days of age. The temperature remained at 12°C until the end of the experiment when the broilers were seven weeks of age. Ventilation in the stables was reduced to increase the CO_2 level to approximately 1500 ppm. The animals were exposed to low temperature and increased CO_2 levels in order to induce ascites. The broilers were group housed with 20 chicken/m2. They had ad libitum access to a commercial broiler feed containing 12,970 KJ/kg. They were exposed to 23h of light per day during the entire experiment. Except for the applied temperature schedule and increased CO_2 level, the broilers were kept under conditions closely resembling commercial practice. The experiment was divided up into five periods with approximate 1,600 chickens per period. All the chicken from each period were kept in one stable from day one till day seven. At day seven, the chicken were sexed and divided into two separate stables depending on sex. Males were slaughtered at 45 days of age and females at 46

days of age. The experiment was carried out by licensed and authorized personnel under supervision of Hendrix Genetics BV.

Phenotypes

Post-mortem examination was performed on the chickens dying during the experiment and on the chicken slaughtered at the end of the experiment. Chickens that showed signs of ascites syndrome (fluid in the abdomen) were assigned a health status $ABD_i = 1$, i.e. ascites, and chickens that showed no signs of ascites syndrome (no fluid in the abdomen) were assigned an $ABD_i = 0$, i.e. no ascites (van As et al., 2010).

Hearts were collected from all broilers. The right ventricular (including the valve) was cut from the left ventricle and septum. The right ventricular was weighed, the left ventricle and septum were added, and the total ventricular was weighed. The weight of the right ventricle as a percentage of the total ventricle weight is referred to as RATIO (Julian, 1987). Descriptive statistics of the observed data are in Tables 4.1 and 4.2.

Table 4.2. Number of chickens, mean, median and the standard deviation (S.D.) for RATIO (the weight of the right ventricle as a percentage of the total ventricle weight), ABD_i (presence of fluid in the abdomen as an indicator of ascites assigned a health status). $ABD_i = 0$ refers to no fluid in the abdomen and $ABD_i = 1$ refers to present of fluid in the abdomen

	RATIO	RATIO ($ABD_i = 0$)	RATIO ($ABD_i = 1$)	ABD_i
Number of chickens	7,613	6,864	749	7,613
Mean	29	27	41	0.10
Median	27	26	42	0
SD	7.6	6.3	6.8	0.30
Skewness	0.83	0.70	-0.65	2.70
Range	0.09 – 0.60	0.09 – 0.54	0.19 – 0.60	0 – 1

Statistical Model

The observed trait RATIO for each of the chickens can be viewed as a variable drawn from a mixture of two normal distributions, depending on the health status (ABD_i) of the chicken, e.g. with or without fluid in the abdomen. Therefore, RATIO was assumed to follow a mixture of two distributions. For the LNM model, observations on RATIO were assigned to one of the two components, depending upon their ABD_i. The mixture model allows for differences in location (mean) and

dispersion parameters (variances) according to ABD_i. The choice of mixture component is determined by an unobservable (*n x 1*) vector health status, *HS* = [HS_i] of binary values. Health status is determined by an underlying unobservable liability.

The observations of RATIO were assigned to the vector y. The distribution and genetic parameters for y and liability were estimated based on the additive genetic effect in addition to a random residual. Conditionally on z, the following liability-normal mixture model (Ødegård et al., 2005) was used in the genetic analyses, given the vector of indicator variables HS_i (health status):

$$\begin{bmatrix} y \\ \lambda \end{bmatrix} = \begin{bmatrix} X_y\beta_0 + M_{HS}1\beta_1 + Z_a a_0 + M_{HS}Z_a a_1 + e_y \\ X_\lambda \beta_\lambda + Z_a a_\lambda + e_\lambda \end{bmatrix} \quad [1]$$

where y is the vector of length *n* of observation of RATIO, λ is a vector of length n of unobservable liabilities for ascites, β_0 is a vector of length 20 of the fixed effects including effects of sex (2 levels), period (5 levels), age of death (1 regression coefficient) and person cutting the heart (12 levels); β_1 are the (scalar) fixed effect of a chicken being classified as diseased (i.e. $HS_i = 1$). β_1 are the increase in the fixed effects for the diseased chickens as a deviation from the β_0. Observations are related to fixed effects through the incidence matrix X_y corresponding to β_0; M_{HS} is an (*n x n*) diagonal matrix with diagonal elements $M_{ii} = HS_i$, β_λ is a vector of length 21 containing the same fixed effects as β_0 augmented by the effect of having observed fluid in abdomen and therefore ascites (ABD_i); X_λ is the incidence matrix corresponding to β_λ (including ABD_i); a_0 is a vector of length q_a of random genetic effects for healthy chicken (base level), a1 is a vector of length q_a of additional random additive genetic effects for the chicken with HS_i=1 (reaction) to RATIO; a_λ is a vector of length q_a of random additive genetic effects for all the chickens on liability wherein q_a is the number individuals for which breeding values are predicted; Z_a is an incidence matrix corresponding to random additive genetic effects and the vector containing observed fluid in abdomen (ABD_i) for all observations. The model samples y_i either from the distribution of healthy chickens ($HS_i = 0$) or diseased chickens ($HS_i = 1$).

Assumptions for random effects:

$$a = \begin{bmatrix} a_0 \\ a_1 \\ a_\lambda \end{bmatrix} \sim N(0, G \otimes A) \qquad [2]$$

where *A* is the additive relationship matrix. G is the genetic covariance matrix for components of *a* corresponding to a particular individual:

$$G = \begin{pmatrix} \sigma_0^2 & \sigma_{0,1} & \sigma_{0\lambda} \\ \sigma_{1,0} & \sigma_1^2 & \sigma_{1\lambda} \\ \sigma_{\lambda,0} & \sigma_{\lambda,1} & \sigma_\lambda^2 \end{pmatrix} \qquad [3]$$

e_R and e_λ are vectors of length n of random residuals distributed as:

$$e_y \sim N\left(\bar{0}, (1 - M_{HS})\sigma_{e_0}^2 + M_{HS}\sigma_{e_1}^2\right) \qquad [4]$$

And

$$e_y \sim N(\bar{0}, I) \qquad [5]$$

In each Gibbs cycle the records were assigned to the intercept or healthy chickens (a_0), the reaction of being diseased (a_1) and the liability (a_λ) scale. The genetic (co)variances for RATIO for healthy and diseased animals and the liability scale are obtained as:

$$G^* = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} G \begin{pmatrix} 1 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \qquad [6]$$

The parameter $\sigma_{e_0}^2$ is the residual variance when $HS_i = 0$ and $\sigma_{e_1}^2$ is the residual variance when $HS_i = 1$.

The Gibbs sampling procedure as implemented in the DMU package was used (Madsen and Jensen, 2010). Prior distributions for the co-variance components of the LNM model were scaled inverted Wishart distributions, Bernoulli for the health status (HS_i), a beta distribution for the mixing proportions and uniform prior distributions were assumed for the fixed effects. In total 1,000,000 cycles were generated. The first 10,000 were discarded as burn-in. Posterior means and standard deviations were calculated based on every 10th Gibbs cycle.
Only one observation was available per animal. In this situation analysis of a binary trait (like HS_i) using an animal threshold model may not work (Ødegård et al., 2010). This problem was circumvented by sampling the genetic (co)variance components based on parental animals only using the algorithm described of Ødegård et al. (2010). Posterior means of parameters are indicated by hats. Heritability of RATIO in the healthy chickens is estimated as

$$\hat{h}_0^2 = \frac{\hat{\sigma}_0^2}{\hat{\sigma}_0^2 + \hat{\sigma}_{e_0}^2} \qquad [7]$$

Correspondingly the heritability among diseased chickens is estimated as

$$\hat{h}_1^2 = \frac{G_{22}^*}{G_{22}^* + \hat{\sigma}_{e_0}^2} + \frac{\hat{\sigma}_0^2 + 2\hat{\sigma}_{01} + \hat{\sigma}_1^2}{\hat{\sigma}_0^2 + 2\hat{\sigma}_{01} + \hat{\sigma}_1^2 + \hat{\sigma}_{e_0}^2} \qquad [8]$$

where σ_{01} is the genetic covariance between the random residual of RATIO among healthy chickens and the increase observed among diseased animals, and G_{22}^* is the 2,2 element of G^*.

Significance of the difference between two correlations
The z-value was calculated using the the Fisher r-to-z transformation in order to assess the significance of the difference between two correlation coefficients.

4.3 Results

Summary statistics for the traits measured are presented in Tables 4.1 and 4.2. Descriptive statistics for RATIO are shown for the whole population and for the population split up by presence or absence of fluid in the abdomen (ABD_i). The distribution of RATIO is shown in Figure 4.1. It has positive skew which is consistent with the phenotypic distribution being a mixture of multiple normal distributions.

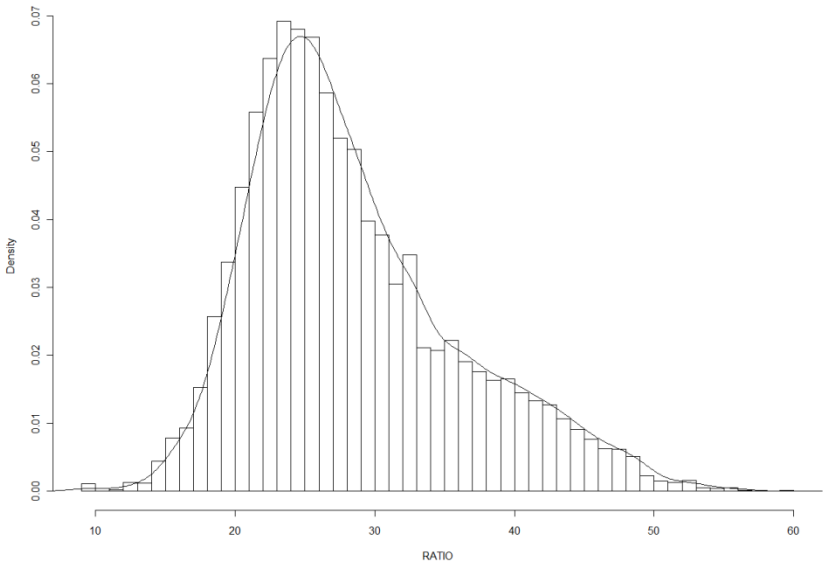

Figure 4.1 The distribution of unadjusted phenotypic observations for RATIO (right ventricular weight as percentage of total ventricular weight) of 7,613 birds kept under cold temperature conditions and high CO_2 levels.

The data shows a significant difference in RATIO between chickens without fluid in the abdomen (ABD_i = 0) and chickens with fluid in the abdomen (ABD_i = 1). Based on the presence or absence of fluid in abdomen 10% for the chickens were categorized as being affected by ascites. The average RATIO for chicken with ABD_i = 1 (41.2) was higher than for the chicken with ABD_i =0 (27.3). However, separating the observations based on ABD_i (0 or 1) does not yield normally distributed data. The skewness of the ABD_i = 0 and ABD_i = 1 groups deviated from zero (Table 4.2). This may be due to the imperfect diagnosis of chickens based on the presence or absence of fluid in abdomen.

Performance of the Gibbs sampler

Trace plots (not shown) were inspected and the sampler was found to be mixing. Convergence of the Gibbs sampler was checked by visual inspection of trace plots and the method of batching (Schmeiser, 1982). A burn-in period of 10,000 rounds was sufficient for the Gibbs chains to converge. The batching method was also used to estimate the effective posterior sample size. The post Gibbs analysis was conducted for all dispersion parameters, the mixing proportion as well as derived

parameters such as heritabilities and correlations. Table 4.3 shows the effective samples size for each of the variables and was determined based on the Gibbs cycles. The effective sample sizes varied form 129 for the residual variance of diseased chickens to 1319 for the residual variance of healthy chickens (Table 4.3). Effective sample size of variance for a1 was smaller than effective sample sizes of the other parameters.

Table 4.3. Effective sample size and posterior means for dispersion parameters from the liability-normal mixture models (posterior standard deviations in parentheses), which were directly estimated from the LNM model. a_0 is the intercept, a_1 is the reaction of being diseased and a_λ is the liability. Posterior means of the genetic (co)variance components for a_0 and a_1 were multiplied with 10^4

	a_0	a_1	a_λ
Effective sample size			
Genetic (co)variance			
a_0	757		
a_1	329	233	
a_λ	803	498	144
Residual variance	1319	129	NA
Posterior means			
Genetic (co)variance			
a_0	5.79 (0.82)		
a_1	0.21 (1.01)	5.06 (2.21)	
a_λ	0.68 (0.35)	-1.95 (0.75)	1.22 (0.40)

The posterior means for the disease frequency (i.e. mixing proportion), the genetic (co)variances, residual variances and the genetic correlations are presented in Table 4.4. The average for the posterior probability of disease ($P(HS_i=1)$) was 24%, i.e. about one quarter of the chickens was classified as diseased. RATIO in diseased chickens is 12.6 (standard deviation of 1.3) higher than RATIO of healthy chickens (Results not in table).

The estimated probability for each chicken of being classified as diseased or healthy was plotted against the observed values for RATIO, and a line representing the average probability to be diseased for each observed value of RATIO was added (Figure 4.2). Generally, chicken with high RATIO also had a high probability of being

classified as being diseased and chicken with low RATIO had a high probability of being classified as being healthy. On average, chickens with a RATIO above 25% had a probability of being diseased above 0.5. The estimated residual variance for RATIO was much higher for diseased chickens as compared to healthy chickens (Table 4.4 and Figure 4.3).

Table 4.4 Residual variance and mixing proportion from the liability-normal mixture models (posterior standard deviations in parentheses) for RATIO for healthy chickens, and RATIO of diseased chickens and the liability. Posterior means of the residual variance components for healthy and diseased were multiplied with 10^4. The parameters are calculated from Table 4.3.

	RATIO (healthy)	**RATIO (diseased)**	**Liability**
Residual variance	12.20 (0.65)	24.45 (2.41)	1.00 (–)
Mixing proportion	0.76 (–)	0.24 (0.01)	–

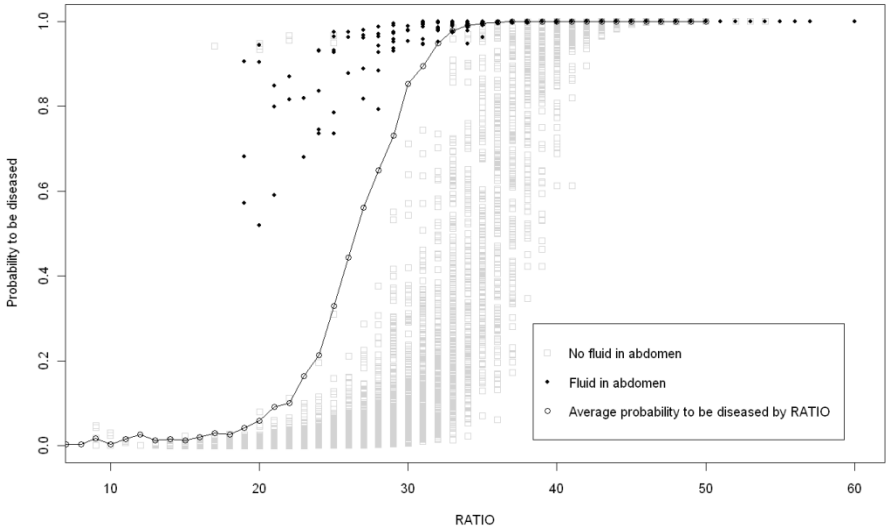

Figure 4.2 The unadjusted phenotypic values of RATIO plotted against probability to be diseased. The light grey squares are chickens with no fluid in abdomen and the black dots are chickens with fluid in abdomen. The dark grey circles with the straight line are the average probability to be diseased for each observed value of RATIO.

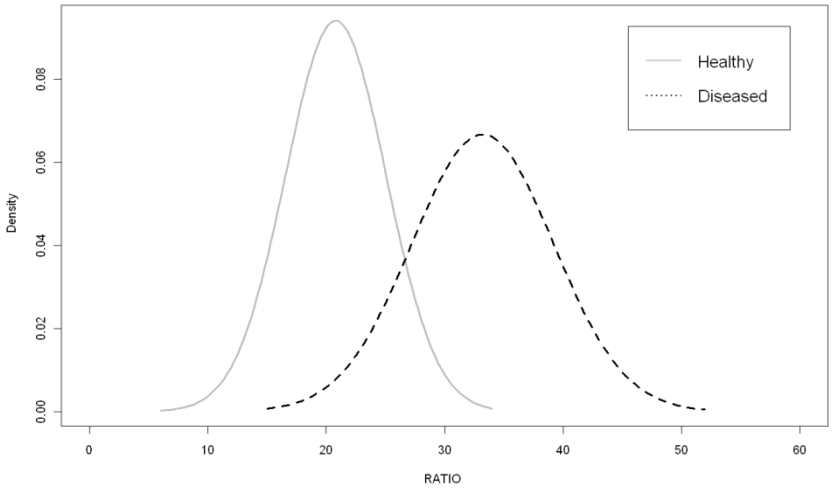

Figure 4.3 Fitted distributions for ratio of right ventricular weight to total ventricular weight (RATIO, in %) for the healthy (grey straight line) and diseased chickens (black dashed line) based on LNM model.

Genetic parameters

The genetic parameters (heritability and genetic correlation) are presented for RATIO, both for healthy and diseased chickens, and for liability (Table 4.5). Posterior means for heritabilities of RATIO for healthy chicken (0.32) and for diseased chickens (0.31) were not significantly different. The estimated heritability for liability was 0.54 and considerably higher than the heritability of RATIO. The genetic correlation between RATIO in diseased and healthy chickens was 0.75. Based on the Fisher r-to-z transformation this genetic correlation was significantly different from one. The estimated genetic correlation between liability and RATIO in the diseased chickens was weak and negative (-0.32), whereas the genetic correlation between liability and RATIO for the healthy was weak and positive (0.27). Based on the Fisher r-to-z transformation these to genetic correlations were significant different from each other. The negative correlation between liability and RATIO in the diseased chickens indicates that chickens with high RATIO are less susceptible to the disease, whereas the positive correlation between liability and RATIO for the healthy chickens indicates that chickens with high RATIO are more susceptible to the disease.

Table 4.5. Posterior means and standard deviations (in parentheses) for heritability and genetic correlation for the two mixture components) for RATIO for healthy chickens, and RATIO of diseased chickens and the liability. The parameters are calculated from Table 4.3 based on equation [8].

	RATIO (healthy)	RATIO (diseased)	Liability
Heritability	0.32 (0.04)	0.31 (0.07)	0.54 (0.09)
Genetic correlation			
RATIO (healthy)	1.00 (–)	–	–
RATIO (diseased)	0.75 (0.10)	1.00 (–)	–
Liability	0.27 (0.15)	-0.32 (0.17)	1.00 (–)

4.4 Discussion

Using the LMN allows a coherent modeling of uncertainty for classification, genetic architecture as well as differences in mean and variance between diseased and healthy chickens in a situation where health status in itself is unobservable or difficult to diagnose.

Previous analyzes of the current ascites data using a traditional animal model showed that splitting the data based on presence of fluid in the abdomen suggested that body weight may be considered a genetically different trait in ascites-affected and non-ascitic broilers (Closter et al. 2012). Classifying chickens as healthy or diseased based on the presence of fluid in the abdomen may be a crude way to assess the susceptibility to ascites as this is one of the final stages of the disease. Further, the trait RATIO of chickens with no fluid in the abdomen does not show a normal distribution which suggests that this still represents a mixture of healthy and diseased birds. Zerehdaran et al. (2006) analyzed broiler data using a bivariate mixture model where the chickens where reared either under normal, commercial temperature conditions or reared under cold temperature conditions. This analysis showed that there was a mixture of two distributions under cold temperature conditions, but just one distribution under normal temperature conditions. Analyses by Zerehdaran et al. (2006) were performed at the phenotypic level and did not consider genetic differences between birds. Results by Closter et al. (2012) suggest that RATIO and body weight can be considered as genetically different traits in ascites affected and non-ascitic broilers when classified according to the presence fluid in the abdomen. The LNM model takes into a count that a trait is a mixture of two normal distributions, allows for a different genetic background of both distributions and models the underlying liability.

Although the statistical evidence supporting the use of LNM model is strong, questions remain about the biological implications of applying a LNM model, and about the precise meaning of the genetic parameters for RATIO for the healthy chickens, RATIO for the diseased chickens and for liability resulting from a LNM model. However, the posterior means of predicted breeding values for liability to be diseased from the LNM model indicates that the LNM model gives a better description of the data and probably more correct selection criteria, compared to a standard linear model using an indicator trait like RATIO as selection criteria. Using a standard linear animal model, selection would be for lower RATIO, with the intention of reducing incidence of ascites (Ødegård et al., 2005).

Residual variance of diseased and healthy chickens

The LNM model provides estimates of the probability for each chicken being diseased given its own RATIO and the RATIO of relatives. The residual variances were considerably different between the healthy and diseased chickens: the estimated residual variance for diseased chickens was twice high as the residual variance for healthy chickens. Similar results were found by Madsen et al. (2008) when analyzing somatic cell count data using the LNM model. The reason that diseased animals have a higher unexplained variation might be that the part of the health condition has not been identified, but still is systematic.

Mixing Proportion

An essential part of the LNM model is the estimation of the unobserved liability and the mixing proportion. The estimated mixing proportion was 24 %. Therefore, the model classifies approximately a quarter of the population as being diseased. Based on fluid in the abdomen approximately 10% of the broilers reared under cold conditions are considered to be affected by ascites and therefore, diseased (Closter et al., 2009). The use of a liability based models suggests that the observed cases of fluid in the abdomen do not reveal the true disease frequency. The analysis based on the LNM model suggests that using fluid in the abdomen as ascites indicator misses out on 14% of the chickens which are affected, but do not (yet) show accumulation of fluid in the abdomen.

Since ascites is a term for the accumulation of fluid in the abdomen, and only 10% of the chickens were observed to have fluid accumulation in the abdomen, the term diseased is used to characterize the fraction of chickens with an abnormally high RATIO. RATIO is used in selection against ascites susceptibility, since RATIO indicate the onset of ascites (Balog et al., 2003; Burton et al., 1968; Cueva et al.,

1974), however, measurement of RATIO is very labour-intensive. However, there is no clear threshold for classifying chickens as healthy or diseased. Several studies suggest that a RATIO above 27% to 30% is indicative of right ventricular hypertrophy and ascites (Balog et al., 2003; Cueva et al., 1974; Wideman et al., 1998). Results from the current study indicate that chickens with a RATIO above 25% have on average a probability of 50% being diseased.

Heritability for Liability

The estimated heritability for liability was higher than the heritability for RATIO, either of healthy or diseased chickens. The heritability for liability was 0.54 suggesting that susceptibility to ascites is highly heritable. The heritability for RATIO for the diseased chicken was 0.31 and 0.32 for healthy chickens, but these two heritabilities were not significantly different. These heritabilities give an indication that the fraction of phenotype variability that can be attributed to genetic variation is similar in diseased and the healthy chickens.

The heritability for liability is higher than heritabilities that have been reported for RATIO using a linear animal model without (Lubritz et al., 1995) or with a maternal effect (Pakdel et al., 2005c; Closter et al., 2009). Closter et al. (2012) did not found significant evidence for the presence of a maternal environmental effect on RATIO in the current data. Based on the same data as used in the present study but using a linear animal model Closter et al. (2012) estimated heritability for RATIO of 0.35. This is slightly higher than the heritability's based on the LNM model of 0.32 for RATIO in healthy chickens and 0.31 for RATIO in diseased chickens. Heritability on the observed scale is always smaller than that on the liability scale, due the loss of information by the grouping into two categories (Dempster and Lerner, 1950).
Genetic correlation between RATIO conditional on health status

The estimated genetic correlation between RATIO for healthy chickens and the RATIO for diseased chickens was 0.75 (Table 4.5) and was significantly different from one, and therefore, the correlation indicates that RATIO in healthy and diseased chickens is not genetically the same trait. The high genetic correlation indicates that chickens that have a high RATIO when healthy are also more likely to have a high RATIO when having developed ascites. At the same time, it suggests that RATIO for healthy chickens and RATIO for the diseased chickens can be assumed as two different traits (Robertson, 1959).

The genetic correlation between RATIO for healthy chickens and liability was positive and 0.27 (Table 4.5), indicating that a high genetic value for RATIO in healthy chickens is associated with a higher susceptibility to ascites. The genetic correlation between RATIO for diseased chickens and liability, however, is negative and -0.32 (Table 4.5), which indicates that high genetic value for RATIO in diseased chickens is associated with a lower susceptibility to ascites. It should be noted that these estimated genetic correlations had high standard error and do not differ significantly from zero. However, the two correlation are significant different from each other. A possible explanation for the negative genetic correlation between RATIO and liability in diseased chickens could be that some chickens have the ability to adapt to the higher oxygen demands. Adaptation in these chickens might be by increasing the number of red blood cells which in a later stage result in a higher RATIO. These chickens have a higher RATIO but the adaptation might be sufficient to prevent them from being ascitic.

Conclusion

Genetic parameters, liability and individual disease risk were estimated. The heritability for liability was 0.54, demonstrating that ascites susceptibility is highly heritable. Of all chickens in the study 24 % were estimated to be affected by ascites.

Acknowledgments

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References

Balog, J. M. 2003. Ascites syndrome (Pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian Poultry Biol. Rev. 14: 99-125.

Boettcher, P. J., D. Caraviello, and D. Gianola. 2007. Genetic Analysis of Somatic Cell Scores in US Holsteins with a Bayesian Mixture Model. J Dairy Sci. 90:435-443.

Burton, R. R., E. L. Besch, and A. H. Smith. 1968. Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. AJP – Legacy. 214: 1438-1442.

Closter, A. M., P. van As, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2009. Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. Poult Sci. 88:483-490.

Closter, A. M., P. van As, M. G. Elferink, R. P. M. A. Crooijmanns, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2012. Genetic correlation between heart ratio and body weight as a function of ascites frequency in broilers split up into sex and health status. Poult Sci. 91:556-564.

Cueva, S., H. Sillau, A. Valenzuela and H. Ploog. 1974. High-Altitude Induced Pulmonary-Hypertension and Right Heart-Failure in Broiler Chickens. Res Vet Sci. 16: 370-374.

de Greef, K. H., L. L. G. Janss, A. L. J. Vereijken, R. Pit, and C. L. M. Gerritsen. 2001. Disease-induced variability of genetic correlations: Ascites in broilers as a case study. J. Anim Sci. 79:1723-1733.

Dempsteer, E. R. and I. M. Lerner. 1950. Heritability of threshold characters. Genetics 35: 212- 236.

Detilleux, J. and P. L. Leroy. 2000. Application of a Mixed Normal Mixture Model for the Estimation of Mastitis-Related Parameters. J Dairy Sci. 83:2341-2349.

Druyan, S., A. Ben-David, and A. Cahaner. 2007. Development of ascites-resistant and ascites-susceptible broiler lines. Poult Sci. 86:811-822.

Groen, A. F., T. Steine, J. J. Colleau, J. Pedersen, J. Pribyl, and N. Reinsch. 1997. Economic values in dairy cattle breeding, with special reference to functional traits. Report of an EAAP-working group. Livestock Production Science. 49: 1-21.

Jamrozik, J. and L. R. Schaeffer. 2010. Application of multiple-trait finite mixture model to test-day records of milk yield and somatic cell score of Canadian Holsteins. J Anim Breed Genet 127: 361-368.

Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The Relationship of Right Ventricular Hypertrophy, Right Ventricular Failure, and Ascites to Weight-Gain in Broiler and Roaster Chickens. Avian Dis. 31:130-135.

Julian, R. J. and S. M. Mirsalimi. 1992. Blood-Oxygen Concentration of Fast-Growing and Slow-Growing Broiler-Chickens, and Chickens with Ascites from Right Ventricular Failure. Avian Dis. 36:730-732.

Julian, R. J. 1998. Rapid growth problems: Ascites and skeletal deformities in broilers. Poult Sci. 77:1773-1780.

Lubritz, D. L., J. L. Smith, and B. N. Mcpherson. 1995. Heritability of Ascites and the Ratio of Right to Total Ventricle Weight in Broiler Breeder Male Lines. Poult Sci. 74:1237-1241.

Madsen, P. and J. Jensen. 2010. DMU: a user's guide. A package for analysing multivariate mixed models.

Madsen, P., M. M. Shariati, and J. Ødegård. 2008. Genetic Analysis of Somatic Cell Score in Danish Holsteins Using a Liability-Normal Mixture Model. J Dairy Sci. 91:4355-4364.

Maxwell, M. H. and G. W. Robertson. 1998. UK survey of broiler ascites and sudden death syndromes in 1993. Br Poult Sci. 39:203-215.

Ødegård, J., J. Jensen, P. Madsen, D. Gianola, G. Klemetsdal, and B. Heringstad. 2003. Detection of Mastitis in Dairy Cattle by Use of Mixture Models for Repeated Somatic Cell Scores: A Bayesian Approach via Gibbs Sampling. J Dairy Sci. 86: 3694-3703.

Ødegård, J., P. Madsen, D. Gianola, G. Klemetsdal, J. Jensen, B. Heringstad, and I. R. Korsgaard. 2005. A Bayesian Threshold-Normal Mixture Model for Analysis of a Continuous Mastitis-Related Trait. J Dairy Sci. 88: 2652-2659.

Ødegård, J., T.M. Meuwissen, B. Heringstad and P. Madsen. 2010. A simple algorithm to estimate genetic variance in an animal threshold model using Bayesian inference. Genet Sel Evol 42: 29.

Pakdel, A., J. A. A. Van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites-related traits in broilers. Poult Sci. 81:1273-1279.

Pakdel, A., P. Bijma, B. J. Ducro, and H. Bovenhuis. 2005a. Selection strategies for body weight and reduced ascites susceptibility in broilers. Poult Sci. 84:528-535.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005b. Genetic parameters of ascites-related traits in broilers: correlations with feed efficiency and carcase traits. Br Poult Sci. 46:43-53.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis 2005c. Genetic parameters of ascites-related traits in broilers: effect of cold and normal temperature conditions. Br Poult Sci 46: 35-42.

Peacock, A. J., C. Pickett, K. Morris, and J. T. Reeves. 1990. Spontaneous hypoxaemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. Comp Biochem Physiol A Comp Physiol. 97:537-541.

Robertson, A. 1959. The Sampling Variance of the Genetic Correlation Coefficient. Biometrics 15: 469-485.

Schmeiser, Bruce. 1982. Batch Size Effects in the Analysis of Simulation Output. Operations Research 30: 556-568.

van As, P. M.G. Elferink, A.M. Closter, A. Vereijken, H. Bovenhuis, R.P.M.A. Crooijmans, E. Decuypere, and M.A.M. Groenen. 2010. The use of blood gas parameters to predict ascites susceptibility in juvenile broilers. Poult Sci. 89: 1684-1691.

Wideman, R.F., T. Wing, Y.K. Kirby, M.F. Forman, N. Marson, C.D. Tackett and C.A. Ruiz-Feria. 1998. Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. Poult Sci. 77: 1565-1573.

Young, C. W., J. E. Legates, and J. G. Lecce. 1960. Genetic and Phenotypic Relationships between Clinical Mastitis, Laboratory Criteria, and Udder Height. J Dairy Sci. 43:54-62.

Zerehdaran, S., E. A. van Grevehof, E. H. van der Waaij, and H. Bovenhuis. 2006. A bivariate mixture model analysis of body weight and ascites traits in broilers. Poult Sci. 85:32-38.

5

Genome-wide association study for pulmonary hypertension syndrome in chickens

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Abstract

Pulmonary hypertension syndrome (PHS) in broilers, also known as ascites, corresponds to initial increase in blood pressure within the pulmonary circulation. The pathophysiological progression of ascites is associated with fluid accumulation in the abdominal cavity and leads to increased mortality. Ascites related traits like ratio of right ventricular weight to total ventricular weight (RATIO), exhibit high genetic variation referred to as high heritability. We performed a genome wide association study based on two generations of broilers. The parent generation consisted of 895 chickens genotyped for 17,790 SNPs, and the offspring generation consisted of 7,857 chickens phenotyped for ascites related traits and body weight (BW) under ascites inducing conditions. The GWAS was performed using a single SNP analysis. The parent generation with own genotype were used together with phenotypes information from progeny by calculating the Average Adjusted Progeny Trait Value (AAPTV). In total, 25 SNPs were calculated to be significant (FDR <0.30) associated with RATIO. Significant association was detected on chromosome 1, 2, 3, 7, 8, 10, 11 and 20. The most significant SNPs were found on chromosome 1, 8 and on 22. This study also identified 16 SNP that were significant associated both with RATIO and with fluid in abdomen. Out of these 16 SNPs nine SNPs were also found to be significant associated with BW. There were no unambiguous mode of genetic inherence for ascites indicator traits and BW at different ages.

Key words: Broiler, ascites, Genome-wide association study, heart ratio, body weight, fluid in abdomen

5.1 Introduction

The metabolic disorder, pulmonary hypertension syndrome ("PHS or ascites syndrome") is a problem in commercial broiler production. When growth is enhanced e.g. with high energy pelleted feed, at high altitude and under cold temperature conditions, the incidence of ascites can increase up to 25% (Maxwell and Robertson, 1998; Balog, 2003). However, mortality due to ascites is rarely higher than 5% (Druyan et al., 2007) and even 1% mortality due to ascites causes considerable economic losses (Maxwell and Robertson, 1998; Balog, 2003). Environmental conditions like low ambient temperature, increased CO_2 levels, or high altitude tend to increase the incidence of ascites due to increased oxygen requirement and subsequent increased hypoxia (Julian, 1993; Balog et al., 2003).
Physiological studies have shown that ascites is associated with an insufficient pulmonary vascular capacity and eventually results in right ventricular failure. The main contributor to ascites development is hypoxia that results from a disproportion between oxygen requirement and the cardiovascular ability to supply oxygen (Currie, 1999; Decuypere et al., 2005). Hypoxia increases the pressure on the pulmonary vascular system (Wideman et al., 2010), which leads to pulmonary hypertension and progresses to right ventricular hypertrophy (Julian et al., 1987). The increase of the right ventricle leads to right ventricular failure, liver congestion and eventually excretion of fluid into the abdominal cavity (McGovern et al., 1999). The fluid accumulates occurs most frequently in the two ventral hepatic, peritoneal, or pericardial spaces (Maxwell and Robertson, 1998).

Genetic studies of ascites indicator traits such as fluid in abdomen (ABD) and ratio of right ventricular weight to total ventricular weight (RATIO) have shown that susceptibility to ascites is partly due to genetic factors. Heritability for ascites indicator traits have been estimated to range from 0.11 to 0.45 (Lubritz et al., 1995; Moghadam et al., 2001; Pakdel et al., 2005b; Closter et al., 2009). The substantial genetic variation for ascites indicator traits indicates that selection can be used to reduce the incidence of ascites. Selection, however, is complicated as ascites or indicator traits for ascites can in most cases not be measured directly on selection candidates, e.g. RATIO can only be measured post-mortem. Therefore, identifying chromosomal regions associated with ascites and understanding the genetic basis of ascites indicator traits is of interest both from a physiological point of view as well as from a breeding point of view. Rabie et al. (2005) performed a linkage analysis to detect QTL involved in ascites, and found significant evidence on chromosomes 2, 4 and 6 and suggestive evidence on chromosomes 5, 8, 10, 27 and 28. The identification of large numbers of single-nucleotide polymorphism (SNP)

has made it possible to move from linkage studies to genome-wide association studies (GWAS). GWAS present a powerful approach to identify QTL associated with complex traits (e.g. McCarthy et al., 2008), such as ascites.

To unravel the genetic basis of ascites we performed a GWAS for the ascites indicator trait RATIO using approximately 18,000 SNPs. Further we analyzed the effect of the chromosomal regions associated with RATIO for its effects on body weight at different ages and on fluid accumulation in the abdomen.

5.2 Materials and Methods

Experimental Population.

The chickens used in the current study were from a purebred broiler dam line originating from the White Plymouth Rock breed that has been selected for breast meat percentage. The experimental population used consisted of two generations; parents and offspring. The parents were genotyped, while phenotypic observations were collected on the offspring. The parent generation consisted of 91 males and 804 females. Sires were mated to multiple dams ranging from two to 28, and dams were mated to one to three sires. The number of offspring per mating ranged from one to 22. The offspring consisted of 3,823 male and 4,034 female birds.

Experimental Conditions

The offspring were kept under a cold temperature regime. The temperature was 30°C at the time of hatching and was gradually reduced to 12°C when the broilers were eleven days of age. The temperature remained at 12°C until the end of the experiment when the broilers were seven weeks of age. Ventilation in the stables was reduced to increase the CO_2 level to approximately 1500 ppm. The animals were exposed to low temperature and increased CO_2 levels in order to induce ascites (Wideman et al. 1999). The broilers were group housed with 20 chicken/m2. They had ad libitum access to a commercial broiler feed containing 12,970 KJ/kg. They were exposed to 23h of light per day during the entire experiment. Except for the applied temperature schedule and increased CO_2 level, the broilers were kept under conditions that closely resemble commercial practice. The experiment was divided up into five periods with approximate 1600 chicken per period. All the chicken from each period were kept in one stable from day one till day seven. At day seven, the chicken were sexed and divided into two separate stables depending on sex. Males were slaughtered at 45 days of age and females at 46 days of age. The experiment was carried out by licensed and authorized personnel under supervision of Hendrix Genetics BV.

Genotypes

All parents were genotyped with an Illumina Infinium iSelect Beadchip containing 17,790 SNPs (for details see Elferink et al., 2010). Genotyping was performed using the standard protocol for Infinium iSelect Beadchips. Data were analysed with Beadstudio Genotyping v3.0.19.0, and quality control was performed according to the guidelines from the Infinium genotyping data analysis protocol (Illumina Inc, 2007). For the current study only markers located on autosomes were used. In total, 9,577 autosomal SNPs which are segregating in the current population were used in a single SNP association study, implying that 8,213 SNPs were not segregating in the population under study. Position of the SNPs was based on chicken genome build WASHUC2.

Phenotypic observations

Hearts were collected and post-mortem examinations were performed on all broilers that died during the experiment and on all broilers that were sacrificed at the end of the experiment. For each heart the ratio between the right ventricular weight and the total ventricular weight (RATIO) was determined. The broilers were weighed at two weeks (BW_2), five weeks (BW_5), and seven weeks (BW_7) of age. BW_7 was measured at the day of slaughter. Each broiler was examined for the presence of fluid in the abdomen (ABD). This trait was scored as zero in case no fluid accumulation was observed in the abdomen, and scored as one in case fluid had accumulated in the abdomen.

Statistical Analysis of phenotypic observations

The following animal model accounting for a maternal environmental effect was used:

$$y_{ijklm} = \mu + sex_i + batch \times stable_j + Person_k + a_l + d_m + e_{ijklm} \qquad [1]$$

where y_{ijklm} is the dependent variable of broiler ijklm; sex_i is the fixed effect of sex (i = 1, 2 male or female); batch × $stable_j$ is the effect of the interaction between batch and stable (j = 1, 2,…, 10), batch consisted of 5 trials and there were 2 stables; $Person_k$ is the fixed effect of the person who cut the heart (k = 1, 2,…, 13); a_l is the random genetic effect of individual l with $\mathrm{a} \sim \mathrm{N}(0, \mathrm{A}\sigma_{\mathrm{a}}^2)$; d_m is the random maternal environmental effect of dam m with $\mathrm{d} \sim \mathrm{N}(0, \mathrm{I}\sigma_{\mathrm{d}}^2)$; and e_{ijklm} is the

random residual effect with $e \sim N(0, I\sigma_e^2)$. Genetic parameter estimates were obtained using the ASREML software (Gilmour et al., 2006).

Whole-Genome Association for RATIO

Genotypes were available on the parents and phenotypes were available on the offspring. Therefore, for the parents average adjusted progeny trait values were calculated. First adjusted trait values were calculated by correcting the phenotypic observations for fixed and maternal environmental effect in the model (for details see van Kaam et al. 1998). The adjusted trait values for each progeny were calculated as

$$ATV = y - \hat{\mu} - X\hat{b} - \hat{d} \qquad [2]$$

where ATV are the adjusted trait values; y is a vector (unadjusted) trait values; $\hat{\mu}$ is the estimated overall mean; X is the design matrix for the fixed effects; $\hat{b}$ is a vector with estimated fixed effects; $\hat{d}$ is the estimated maternal environmental effect. Estimates were obtained from model [1]; ATVs were adjusted for the estimated breeding value (EBV) of the mate and averaged over the progeny of a parent. For each offspring two adjusted trait values were calculated; one adjusted for the EBV of the sire and one adjusted for the EBV of the dam. ATV was then combined to one average adjusted progeny trait values (AAPTV) for each parent (van Kaam et al., 1998).

The genome-wide association study for RATIO was performed using a mixed model with the AAPTV as dependent variable. The single SNP analysis was performed using the ASREML software (Gilmour et al., 2006) and the analyses were performed using following statistical model:

$$y_{ij}^{*} = \mu + SNP_i + Animal_j + e_{ij} \qquad [3]$$

Where y_{ij}^{*} is the average adjusted progeny trait value (AAPTV) of the chicken ij; μ is the overall sample mean; SNP_i is the fixed effect of the SNP; $Animal_j$ is the random genetic effect of individual and e_{ij} is the random residual effect. A weighted analysis

was performed using the ASREML software (Gilmour et al., 2006). The weight for record y_{ij}^{*} depended on the number of progeny that contributed to the average.

Significance Thresholds

Significance thresholds were obtained by calculating the false discovery rate (FDR) using the QVALUE software package (Storey and Tibshirani, 2003) as implemented in R (R Development Core Team, 2011). The FDR was calculated based on the p-values obtained from model [3]. SNPs with a FDR<0.3 are reported.

Single SNP analysis for body weight and fluid accumulation in abdomen

In order to characterize the effect of the SNPs with a FDR<0.3 for RATIO, these SNPs were also analyzed for their effects on BW_2, BW_5, BW_7, and ABD. Similar as for RATIO the AAPTV for these traits was calculated using estimates from model [1] and using [2]. For BW_2, BW_5, BW_7, and ABD the effect of Person was not included in model [1]. The SNP analyses were performed using model [3]. SNPs with a $p < 0.05$ were considered significant.

5.3 Results

Descriptive statistics

Means and standard deviations for the traits RATIO, ABD, BW_2, BW_5 and BW_7 for the offspring generation are presented in Table 5.1.

Table 5.1. Number of observations from offspring generation (N), mean and the standard deviation (S.D.) for heart ratio, fluid in abdominal and body weight (BW) for the entire population of broilers

Variable	Units	Abbreviation	N	Mean	S.D.
Heart Ratio	%	RATIO	7,687	28.7	7.6
Fluid in abdominal	0/1	ABD	7,665	0.10	0.30
BW at 2 weeks	Gram	BW_2	7,803	248	37.8
BW at 5 weeks	Gram	BW_5	7,496	1,296	174
BW at 7 weeks	Gram	BW_7	7,121	2,075	295

During the experiment body weight of the broiler increased from 248 g at two weeks to 2,075 g at seven weeks and ten percent of the broilers had fluid

accumulated in the abdomen. Not all the chickens had observations for RATIO and fluid in the abdomen. RATIO was missing for 116 broilers and a score for ABD was missing for 138 broilers. Due to premature death, 307 broilers did have no observation for body weight at five weeks and 682 broilers had no body weight recorded at seven weeks.

Whole-Genome Association for RATIO

The whole genome association study for RATIO resulted in 25 SNPs, with an FDR between 0.19 and 0.30 ($-\log10_{P\text{-values}}$ between 3.00 and 4.50). Figure 5.1 shows the Manhattan plot of the $-\log10_{p\text{-values}}$ for the trait RATIO. Regions associated with RATIO were found on chromosomes 1, 2, 3, 7, 8, 10, 11 and 22 (Table 5.2).

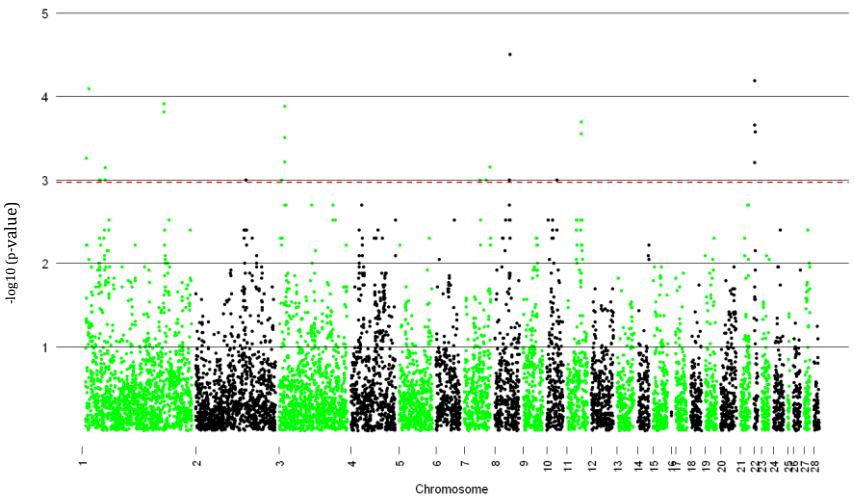

Figure 5.1 $-\log10_{P\text{-values}}$ for association of SNPs with RATIO. The position is represented along the x-axis and chromosome numbers are given. The solid black lines indicate grid lines and the dashed lines reflect cutoff points for false discovery rate <0.30.

Effects on body weight and fluid accumulation in abdomen

SNPs with a significant effect on RATIO were analyzed for their effects on ABD, BW_2, BW_5 and BW_7. Out of the 25 SNP significantly (FDR<0.30) associated with RATIO a total of seven SNPs also had a significant effect ($p < 0.05$) on ABD (Table 5.2). The genotypes of the SNPs are either associated with high RATIO and high ABD or associated with low RATIO and low ABD. Additional seven SNPs also had significant effect for RATIO, ABD and at least one of the BW measurement ($p < 0.05$, Table 5.2). The highest $-\log10_{P\text{-values}}$ were found for ABD on chromosome 1 for the two SNPs rs14899763 ($-\log10_{P\text{-value}} = 4.36$) and rs13952858 ($-\log10_{P\text{-value}} = 5.84$).

The genotypes of the SNP rs14899763 and SNP rs13952858 (both on chromosome 1) influence all traits (RATIO, ABD, BW_2, BW_5 and BW_7) in same direction, which can be specified with reduced RATIO and ABD together with increased BW or with increased RATIO and ABD together with decreased BW. These two SNPs are within one region and almost fully in LD with each other. Similar, the genotypes for SNP rs16183608 (chromosome 22) showed to influenced the traits RATIO, ABD, BW_2 and BW_5, in either a favourable direction with reduced RATIO, low ABD and high BW or in unfavourable direction with increased RATIO, high ABD and low BW. For SNPs, rs13841399 and rs13747646 on chromosome 1 and rs14965732 on chromosome 11, the genotypes associated with a higher RATIO and ABD were associated with lower values for BW_2 and BW_5. SNP rs13599609 (chromosome 7) was associated with higher RATIO and lower values for ABD and BW_7.

Table 5.2 SNPs with a significant (FDR<030) effect on RATIO and the significance ($-\log_{10}$ (p-value)) of these SNPs on body weight at 2 (BW_2), 5 (BW_5) and 7 (BW_7) weeks and fluid in abdomen (ABD). RATIO, BW_2, BW_5, BW_7 and ABD

SNP ID	Position (in bp)	Region [1]		RATIO[2]	FDR -RATIO	BW_2[2]	BW_5[2]	BW_7[2]	ABD[2]
Chromosome 1									
rs14789557	2,124,737	777,036	3,459,640	3.26	0.30	NS			1.39
rs16079719	7,880,127	7,136,958	8,623,298	4.10	0.19				1.51
rs13841399	27,984,447	27,566,929	29,087,815	3.00	0.30	4.97	2.05	NS	2.10
rs13747646	28,523,800			3.00	0.30	2.70	NS		1.12
rs14811108	37,855,143	37,378,125	38,721,903	3.00	0.30	NS			NS
rs15236245	38,231,869			3.15	0.30				NS
rs14899763	148,951,482	148,523,109	149,533,408	3.91	0.19	1.66	1.89	2.52	4.36
rs13952858	149,173,568			3.81	0.19	1.54	1.74	2.70	5.84
Chromosome 2									
rs14218633	92,844,354	92,247,793	93,302,291	3.00	0.30	NS			NS
Chromosome 3									
rs15257935	3,581,788	3,427,137	3,742,795	3.00	0.30				1.14
rs16225894	7,578,368			3.88	0.19	NS			1.28
rs15272751	7,646,095	7,353,248	8,010,947	3.51	0.21				NS
rs14317011	7,804,975			3.22	0.30				NS

Table 5.2 continued

SNP ID	Position (in bp)	Region [1]		RATIO[2]	FDR -RATIO	BW_2[2]	BW_5[2]	BW_7[2]	ABD[2]
Chromosome 7									
rs14617579	25,555,091	24,880,599	26,234,348	3.00	0.30	NS			1.27
rs13599609	33,065,420	32,622,689	33,431,198	3.00	0.30	NS		1.40	2.70
rs16615527	37,568,882	36,352,314	38,754,684	3.15	0.30	NS			NS
Chromosome 8									
rs15920819	19,019,020	18,317,667	20,023,086	3.00	0.30	NS			NS
rs15921649	19,584,684			4.50	0.19				1.89
Chromosome 10									
rs15580567	13,992,235	13,753,162	14,231,310	3.00	0.30	NS			2.52
Chromosome 11									
rs14965732	15,491,746	14,962,343	16,074,125	3.55	0.21	1.27	1.15	NS	1.35
rs14965814	15,744,073			3.69	0.21	NS			
Chromosome 22									
rs15187369	531,581			3.66	0.21	NS			
rs16183544	557,109	263,725	1,050,313	3.21	0.30				
rs16183608	786,137			4.19	0.19	2.10	2.22	NS	1.82
rs15187555	906,582			3.58	0.21	NS			

1) The region of interest was determined as +/- 1 cM from the position of the SNP, unless genes were partially located at one of the ends. In that case, the window size was increased in order to include the entire gene. Overlapping regions are merged. FDR = false discovery rate. NS = Not significant.

2) –log10 (p-value)

5.4 Discussion

The genetic factors involved in ascites susceptibility in broilers are not known and, therefore, a genome wide association study (GWAS) study was performed. The present study is the first GWAS for ascites in a broiler population. The GWAS is based on 895 genotyped individuals and over 7857 individuals with phenotypes. The post-mortem trait RATIO was used as an indicator for ascites and a number of regions were associated with RATIO. In total 25 SNPs were associated (FDR < 0.3) with RATIO and the SNPs were located on chromosomes 1, 2, 3, 7, 8, 10, 11 and 22. Although we detected a number of chromosomal regions associated with ascites, no regions with major effects were detected. Therefore it seems that ascites is affected by a large number of genes, each with a small effect.

For SNPs significantly associated with RATIO (FDR<0.3), we also estimated effects on the traits ABD and BW at 2, 5 and 7 weeks of age. Several SNPs were found to be associated with phenotypic variation in more than one trait. Out of the 25 SNPs associated with RATIO, 14 SNPs also have an effect on ABD and seven of these SNPs were also found to be association with BW

Genetic architecture of ascites

The detection of multiple chromosomal regions, each with a relatively small effect shows that ascites in the current population is a polygenic trait rather than a trait that is influenced by a single or a limited number of major genes as were suggested by some authors (Navarro et al., 2006; Druyan et al., 2007). This is in agreement with Rabie et al. (2005), whom, based on results from another broiler line, also concluded that ascites is influenced by multiple genes each with a small effect. Furthermore, Wideman and French (2000) stated that a number of genes seem to be partly responsible for ascites susceptibility in commercial broilers. The studies suggesting that either a polygenic or a monogenic basis for ascites is based on different broiler populations. Therefore, it cannot be excluded that different alleles are segregating in other populations.

SNPs associated with RATIO

Some of the chromosomal regions detected in the present study were located close to regions identified in a linkage study using a cross between two dam broilers lines by Rabie et al. (2005). Rabie et al. (2005) detected significant evidence for QTL on chromosome 2 for the ascites related traits right and total ventricular weight as percentage for body weight. The trait RATIO reached suggestive association on chromosome 2 and on chromosome 8 was there detected suggestive association

for traits right ventricular weight as percentage for body weight and for BW_5 (Rabie el at., 2005). For the region on chromosome 8, Rabie et al. (2005) identified association to total ventricular weight as percentage of body weight and BW_5, however in the present study was it only RATIO that was associated with rs15920819. The SNP on chromosome 10 is close to the region identified by Rabie et al. (2005) affecting mortality due to ascites and BW_5. Comparing results of Rabie et al. (2005) with the ones of the present study, we see that in the case of ascites indicator traits and traits are associated with the same regions. The current study confirms some of the chromosomal regions that have been identified by Rabie et al. (2005); however, both studies also identified a number of unique QTL regions.

Significant SNPs for RATIO affecting body weight and fluid in abdominal

The present study focused mainly on the identification and the location of SNPs associated with RATIO. Information regarding the effect of SNPs on other traits might help to understand how a potential candidate gene could influence both RATIO and BW traits. Therefore, the secondary focus of the study was to characterize these significant regions in terms of their effects on traits as ABD and BW at two, five and seven weeks of age.

Ascites eventually results in fluid accumulation in the abdominal cavity as a consequence of right ventricular failure (Julian et al., 1987; Julian and Mirsalimi, 1992). The genetic correlation between RATIO and ABD has been estimated to be 0.82 (Pakdel et al., 2005b) and therefore, at least some of the SNPs with a significant effect on RATIO also are expected to show an effect on ABD. In total seven SNPs were found to be significantly associated with only the traits RATIO and ABD. The SNPs associated with ABD were found on chromosome 1, 7, 8, 10, 11 and 22. The genotypic effects of the SNPs showed that the allele that increased RATIO also is associated with higher values for ABD. Since ascites causes major heart valve problems leading to a build-up of fluid in the tissues, fluid in the abdomen can be considered as one of the last stages of ascites. Therefore, it seems that the SNPs that affect both RATIO and ABD, affects the development of ascites over a longer period during the life the effected chicken.

In a total of seven SNPs located on chromosome 1, 7, 11 and 22 were found to be significantly associated with RATIO, ABD and BW. Several of the significant SNPs were found to either influence increased RATIO, increased ABD or lower BW, or to influence decreased RATIO, decreased ABD or higher BW. These effects indicate

that the SNPs had an effect on both the development of ascites, on early growth and late growth. Some of the significant regions had also an effect on BW_2; suggesting that potential candidate genes might be involved in early growth. Furthermore, some of the SNP alleles which are associated with a higher RATIO and higher values for ABD also are associated with higher BW_2 and higher BW_5. This is line to some publications stating that increased oxygen demand of rapid growth and high metabolic rate need to be related to ascites in broiler (Julian, 2000). This could imply that the underlying mutation has an effect on early growth which later in life leads to the development of ascites. The effects on BW_5 and BW_7 are also affected when the chickens have developed ascites, since chickens with ascites often also have a lower body weight. A possibility could be that a chicken with high genetic potential for growth, but does not have a decent potential to provide oxygen to sustain that growth. Deeb et al. (2002) found that chickens that have a higher potential for growth rate under normal temperature conditions are more likely to suffer from ascites under cold conditions as compared to chickens with a lower potential for growth rate.

Estimated genetic correlations between the traits BW and RATIO are generally low (Pakdel et al., 2005b; Closter et al., 2009; Closter et al., 2012) with correlations close to zero. The genetic correlation between RATIO and BW was found to range from 0.19 for body weight at week two and -0.18 for body weight at week five (Closter et al., 2009). Where in in the current data the genetic correlation between RATIO and BW was found to range from 0.11 for body weight at week two, -0.09 for body weight at week five and -0.43 for body weight at week seven (Closter et al., 2012). This indicates that under cold conditions the genetic potential for growth shows a weak correlation with the genetic potential for RATIO. The weak correlation proposes that there are genes with a positive effect on both traits. These genes have a positive effect on one trait and a negative effect on the other trait as well as genes with an effect on only one of both traits, e.g. two SNPs on chromosome 1 and one on chromosome 11 had a higher value for effect for RATIO and ABD compared lower values for BW_2 and BW_5 or only for BW_2. These different trends for the SNP effect for the associated SNPs designate the complexity of the development of the ascites. Therefore, complexity of the development of the ascites suggests that there is a relation between BW, susceptibility to ascites and temperature.

Enhanced Understanding of ascites

As RATIO is a good indicator for susceptibility to ascites, obtaining insights knowledge to how chromosome regions are associated with RATIO will lead to more efficient selection for ascites resistance in broiler chickens. Several regions on the chromosomes were found to be significant associated with the ascites indicator trait RATIO. In the literature have there not been conclusive evidence if ascites is a monogenic or a polygenetic trait. The conclusion based on this study is that there are no single regions with a major effect for ascites. Therefore it can be concluded that ascites in the current population is affected by a large number of genes, each with a small effect.

This study also identified some of the SNPs which are significantly associated with RATIO and ABD. However, since only some of the SNPs was associated with both RATIO and ABD, and other SNPs only associated with RATIO, it seem that the development of ascites is altered by the different stages of the disease. The results of the present study indicate that part of analysed SNPs had a pleiotropy effect were ascites indicator traits and BW are affected by some SNP simultaneously. The pleiotropic loci are associated with multiple traits with opposite effects, e.g. higher (or lower) RATIO and ABD compared lower (or higher) with BW. A notable outcome of this study was that improved knowledge of the genomic background of ascites indicator trait compared with the results of another indicator trait (ABD). The current study resulted in the identification of a number of genomic regions that seem to play a role in the development of ascites in broilers, which have increased the understanding of genetic architecture of ascites.

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References

Balog, J. M. 2003. Ascites syndrome (Pulmonary hypertension syndrome) in broiler chickens: Are we seeing the light at the end of the tunnel? Avian and Poultry Biology Reviews 14(3):99-125.

Balog, J. M., B. D. Kidd, W. E. Huff, G. R. Huff, N. C. Rath, and N. B. Anthony. 2003. Effect of cold stress on broilers selected for resistance or susceptibility to ascites syndrome. Poult Sci 82(9):1383-1387.

Closter, A. M., P. van As, M. G. Elferink, R. P. M. A. Crooijmans, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2012. Genetic correlation between heart ratio and body weight as a function of ascites frequency in broilers split up into sex and health status. Poult Sci 91: 556–564

Closter, A. M., P. van As, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2009. Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. Poult Sci 88(3):483-490.

Currie, R. J. W. 1999. Ascites in poultry: Recent investigations. Avian Pathology 28(4):313-326.

Decuypere, E., M. Hassanzadeh, N. Buys, and J. Buyse. 2005. Further insights into the susceptibility of broilers to ascites. The Veterinary Journal 169(3):319-320.

Deeb, N., A. Shlosberg, and A. Cahaner. 2002. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 4. Association between responses to heat stress and to cold-induced ascites. Poult Sci 81(10):1454-1462.

Druyan, S., A. Ben-David, and A. Cahaner. 2007. Development of ascites-resistant and ascites-susceptible broiler lines. Poult Sci 86(5):811-822.

Elferink, M. G., P. van As, T. Veenendaal, R. P. M. A. Crooijmans, and M. A. M. Groenen. 2010. Regional differences in recombination hotspots between two chicken populations. BMC Genetics 11(1):11.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. ASReml User Guide Release 2.0. in VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Infinium genotyping data analysis protocol. http://www.illumina.com/Documents/products/technotes/technote_infinium_genotyping_data_analysis.pdf, Accessed September 2007.

Julian, R. J. 1993. Ascites in Poultry. Avian Pathology 22(3):419-454.

Julian, R.J., 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathology 29, 519-527.

Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The Relationship of Right Ventricular Hypertrophy, Right Ventricular Failure, and Ascites to Weight-Gain in Broiler and Roaster Chickens. Avian Diseases 31(1):130-135.

Julian, R. J., and S. M. Mirsalimi. 1992. Blood-Oxygen Concentration of Fast-Growing and Slow-Growing Broiler-Chickens, and Chickens with Ascites from Right Ventricular Failure. Avian Diseases 36(3):730-732.

Lubritz, D. L., J. L. Smith, and B. N. Mcpherson. 1995. Heritability of Ascites and the Ratio of Right to Total Ventricle Weight in Broiler Breeder Male Lines. Poult Sci 74(7):1237-1241.

Maxwell, M. H., and G. W. Robertson. 1998. UK survey of broiler ascites and sudden death syndromes in 1993. British Poultry Science 39(2):203-215.

McCarthy, M. I., G. R. Abecasis, L. R. Cardon, D. B. Goldstein, J. Little, J. P. A. Ioannidis, and J. N. Hirschhorn. 2008. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9(5):356-369.

McGovern, R. H., J. J. R. Feddes, F. E. Robinson, and J. A. Hanson. 1999. Growth performance, carcass characteristics, and the incidence of ascites in broilers in response to feed restriction and litter oiling. Poult Sci 78(4):522-528.

Moghadam, H. K., I. McMillan, J. R. Chambers, and R. J. Julian. 2001. Estimation of genetic parameters for ascites syndrome in broiler chickens. Poult Sci 80(7):844-848.

Navarro, P., P. M. Visscher, D. Chatziplis, A. N. M. Koerhuis, and C. S. Haley. 2006. Segregation analysis of blood oxygen saturation in broilers suggests a major gene influence on ascites. British Poultry Science 47(6):671-684.

Pakdel, A., P. Bijma, B. J. Ducro, and H. Bovenhuis. 2005a. Selection strategies for body weight and reduced ascites susceptibility in broilers. Poult Sci 84(4):528-535.

Pakdel, A., J. A. M. van Arendonk, A. L. J. Vereijken, and H. Bovenhuis. 2005b. Genetic parameters of ascites-related traits in broilers: effect of cold and normal temperature conditions. British Poultry Science 46(1):35-42.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rabie, T. S. K. M., R. P. M. A. Crooijmans, H. Bovenhuis, A. L. J. Vereijken, T. Veenendaal, J. J. van der Poel, J. A. M. van Arendonk, A. Pakdel, and M. A. M. Groenen. 2005. Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop pulmonary hypertension syndrome. Animal Genetics 36(6):468-476.

Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 100(16):9440-9445.

van Kaam, J. B. C. H., J. A. M. van Arendonk, M. A. M. Groenen, H. Bovenhuis, A. L. J. Vereijken, R. P. M. A. Crooijmans, J. J. van der Poel, and A. Veenendaal. 1998. Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. Livestock Production Science 54(2):133-150.

Wideman, R. F., M. L. Eanes, K. R. Hamal, and N. B. Anthony. 2010. Pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome: Age and sex comparisons. Poult Sci 89(9):1815-1824.

Wideman, R. F., and H. French. 2000. Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. Poult Sci 79(3):396-401.

Wideman, R. F., P. Maynard, and W. G. Bottje. 1999. Venous blood pressure in broilers during acute inhalation of five percent carbon dioxide or unilateral pulmonary artery occlusion. Poult Sci 78(10):1443-1451.

6

General discussion

6.1 Introduction

This thesis is part of a joint project between Hendrix Genetics BV, Cobb-Vantress, and Wageningen University (the Netherlands) on "The characterization of genes involved in pulmonary hypertension syndrome in chickens", which is financially supported by the Technology Foundation, the Netherlands (STW). Previous studies identified heritable variation in phenotypic characteristics related to ascites. In addition, some chromosomal regions associated with ascites have been identified. The quantitative genetic background of ascites is well established. The use of genomic information in selection can substantially increase response to selection especially for complex traits or diseases that cannot be measured on breeding animals.

In this general discussion, the first section deals with genetic aspects of general health of broilers and ascites in particular. The second section deals with the liability normal mixture (LNM) model versus the linear mixed model with special emphasis on estimated breeding values from the different models. Furthermore, the second section deliberates on whether selection of broilers should be based on estimated breeding values (EBV) from a linear mixed model (animal model) or EBV from a LNM model. The third section of this chapter compares results from genome wide association studies (GWAS) using different models: a model that accounts only for relations due to sires versus an animal model that accounts for all family relationships Furthermore, an alternative model based on phenotypic trait values of the individuals and genotype probabilities calculated based on the parental genotypes was also examined. The last section of the discussion focuses on lessons to be learned from ascites related phenomena in other species.

6.2 Genetic Background of Ascites in Broilers

The economic importance of genetically improving production and liveability differs. Further, heritabilities and genetic and phenotypic correlations differ between production and liveability traits. In order to achieve maximum genetic progress towards the stated breeding goal and increase the economic efficiency of production animals, the aim is to use simultaneous selection for both production and liveability traits and in this way improving the total breeding value of animals. Ascites is a metabolic disorder, which is linked to growth rate and causes mortality (Maxwell and Robertson, 1998). Ascites is associated with insufficient oxygen supply of the tissue of the growing broiler (Julian, 1993), and both genetic and environmental factors contribute to the development of ascites. Although there

has been increased emphasis on liveability traits, health problems of the modern broiler have not been fully resolved and, therefore, need further attention.

Current selection of animals is mainly based on Best Linear Unbiased prediction (BLUP) methods. Animals used for breeding are selected based on estimated breeding values (EBV) for production traits like growth rate and feed conversion. However, selection for production traits can result in undesirable responses for liveability traits, because of antagonistic relationships that might exist between production traits and liveability traits, also called genetic trade-offs. The antagonistic genetic relationship between liveability traits on the one hand and production traits on the other hand complicates selection of animals. Therefore, phenotypic information on both production and liveability traits are required in order to improve total efficiency of livestock production. Including a disease trait in a breeding program requires a good recording system for the disease itself or a related indicator trait. Indicator traits are often used in selection for disease resistance as is the case for ascites in broilers, where the ratio of right ventricular weight to total ventricular weight (RATIO) is used as indicator for ascites (de Greef et al., 2001). Another often used indicator trait is milk somatic cell counts (somatic cell score, SCS) which is used to determine mastitis status in dairy cattle (Young et al., 1960). In the case of ascites, the development of the disease typically occurs under specific (unfavourable) conditions, which implies that the testing environment needs to be chosen carefully.

6.3 The Use of Liability Mixture Models in Selection for Disease Resistance

Liveability traits receive increasing attention in breeding programs for production animals. Selection for liveability traits is commonly based on indicator traits which are often measured on a continuous scale. The indicator traits might represent a mixture of (at least) two distributions: healthy and diseased. Mixture models offer the opportunity to account for this mixture of distributions. In chapter 4 it was assumed that the distribution of RATIO can be regarded as a mixture of two components depending on the ascites status of a broiler. In the two-component normal mixture model used by Ødegård et al. (2003), the disease related trait is assumed to be normally distributed with heterogeneous residual variance. However, this model assumes identical prior probabilities of the health status for all observations. This assumption might not be correct and therefore, Ødegård et al. (2005) suggested an LNM model where health status is modeled as an

unobserved binary variable. This unobserved binary variable is assumed to be fully determined by the (unobserved) underlying liability. The LNM model predicts genetic effects for both the disease indicator trait and for the unobserved liability. Estimated breeding values for liability can be used in selection to improve disease resistance. Using the LNM model, genetic and residual variances tend to be larger in diseased animals as compared to healthy animals. Further, in some studies heritabilities for somatic cell score (SCS) in diseased animals were higher than in healthy animals (Madsen et al., 2008).

Broilers can be divided in healthy and ascites diseased broilers. However, there are no clear criteria to distinguish between these two subpopulations. The use of mixture models gives the possibility to assign broilers to one of these two subpopulations via probabilities estimated from trait observations (Detilleux and Leroy, 2000). The LNM model predicts genetic effects for both RATIO and the unobserved liability to ascites. Estimated breeding values based from a LNM model for the underlying liability can be useful in selection to improve ascites resistance in broilers, i.e. assuming that the ascites status is completely specified by the underlying liability.

Estimated breeding values from the Liability Normal Mixture model

The LNM model was used for the genetic analysis of RATIO as described in chapter 4. This analysis also results in breeding values for the (unobserved) liability to ascites. EBVs for liability based on the LNM analysis might differ from EBVs for RATIO using the linear animal model, which was used in chapter 3. Figure 6.1 shows the relation between the EBVs obtained from both analyses. The EBVs are presented for broilers with phenotypic observation on RATIO, i.e. in total 8503 broilers.The correlation between the EBVs resulting from both models is 0.72 indicating that based on the EBVs from both models partly the same broilers will be selected. However, breeding values predicted with one model only explain 51.4% of the variation in EBVs of the other model, which emphasizes that the models do not result in identical EBV. This indicates that broilers are not ranked exactly the same and differences in selection response are expected. Figure 6.1 suggests that the differences between the two models are smaller for broilers with a low EBV for RATIO and a low EBV for liability, i.e. the broilers which are favoured.

It should be noted that clinical ascites is not necessarily the same trait as subclinical ascites. Therefore, using the LNM model, selection could be based on EBV for liability to ascites rather than by selecting for lower values for RATIO, as is the case

in the linear mixed model (animal model). The advantage of using the linear animal model is that it requires less computing time and is implemented in routine breeding value evaluation software.

Note that an important disadvantage of using RATIO as indicator trait is that RATIO is not an easy trait to measure: measuring RATIO is very labour intensive. Furthermore, RATIO cannot be measured on the selection candidates themselves as they have to be sacrificed and consequently cannot be used for reproduction. Therefore RATIO is not included in routine evaluation of broilers.

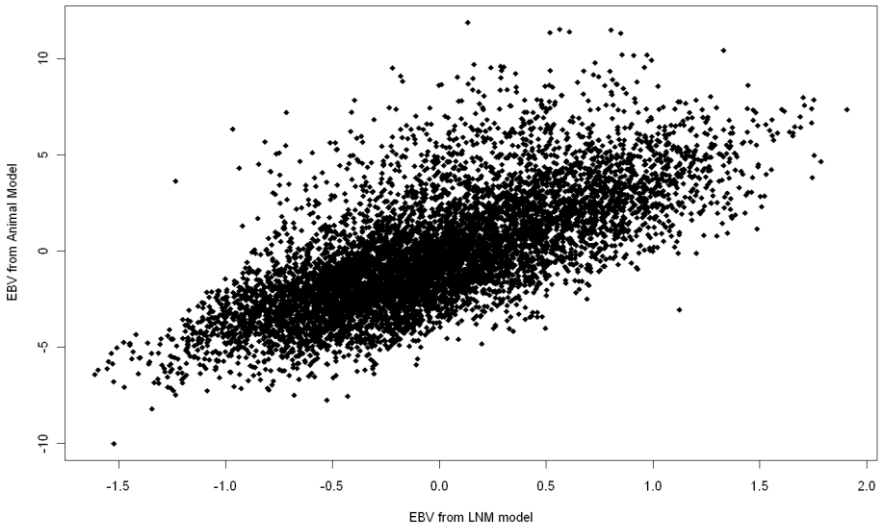

Figure 6.1 Scatter plots of estimated breeding values (EBV) for liability based on a normal mixture model and EBV for RATIO using an animal model. The EBVs are presented for approximately 8500 broilers with a phenotypic observation.

6.4 Genome Wide Association Studies for Ascites

A GWAS is aimed at studying common genetic variation across the entire genome and to identify genetic associations between specific regions with the observed trait. GWAS requires high-density SNP genotypes, which have recently come available. Since GWAS examines SNPs across the genome, it represents a promising way to study complex, common diseases in which many genes are expected to contribute to the risk of being diseased. A linkage study suggested that ascites is influenced by several genes each with a small effect (Rabie et al., 2005). The results

presented in chapter 5 supports that ascites has a polygenic origin. The findings from the current study are a further step towards understanding the genetic architecture of this complex disorder.

Statistical models for GWAS

Ignoring relationships between individuals in a GWAS can result in false positive associations (Goddard and Hayes, 2009). However, analyzing data using a mixed model which accounts for all relationships between individuals is computationally demanding, especially when the analysis has to be repeated for many SNPs. Alternatively, one could account for only part of the family relations. E.g. Bouwman et al. (2011) screened the bovine genome using a general linear model accounting for family relations due to sires only. In a second step Bouwman et al. (2011) verified signficant regions from the first step using an animal model accounting for all family relations. Bouwman et al (2011) showed that for a typical dairy cattle population accounting for relationships due to sires is a very good approximation: a correlation of 0.95 was found between the $-\log_{10}$ (P-values) of the general linear model (Bouwman et al, 2011) and the mixed model (Bouwman et al., 2012). This high correlation indicates that the general linear model correctly identified the chromosomal regions of interest and results from the general linear model are comparable with results from the mixed model (Bouwman et al. 2012). I compared results from both models based on the poultry data described in chapter 5.

A general linear model

The genome-wide association study was based on SNP genotypes of the parents and average adjusted progeny trait values for RATIO. This genome-wide association study was based on the following general linear model:

$$y_{ij}^{*} = \mu + sire_i + SNP_j + e_{ij}$$

where y* was the average adjusted progeny trait value for RATIO. Phenotypes were adjusted for systematic environmental effects. Sire was the fixed effect of sire; SNP was the fixed effect of the SNP genotype; and e was the random residual. A sire effect was included in the model to account for paternal half-sib relations only, i.e. relations between sires are not accounted for. A weighted analysis was performed in SAS. The weight for record y_{ij}^{*} depended on the number of progeny that contributed to the average.

This analysis resulted in several chromosomal regions with highly significant (P < 0.0001) effects on the ascites indicator trait RATIO (figure 6.2). In total 67 SNPs were found to have significant effects (p value smaller than 0.0001) and a further 154 SNPs had suggestive significant effects (p value smaller than 0.001). In the case of significant (or suggestive) evidence for the presence of a QTL, the region would have been reanalyzed using the animal model accounting for all relationships.

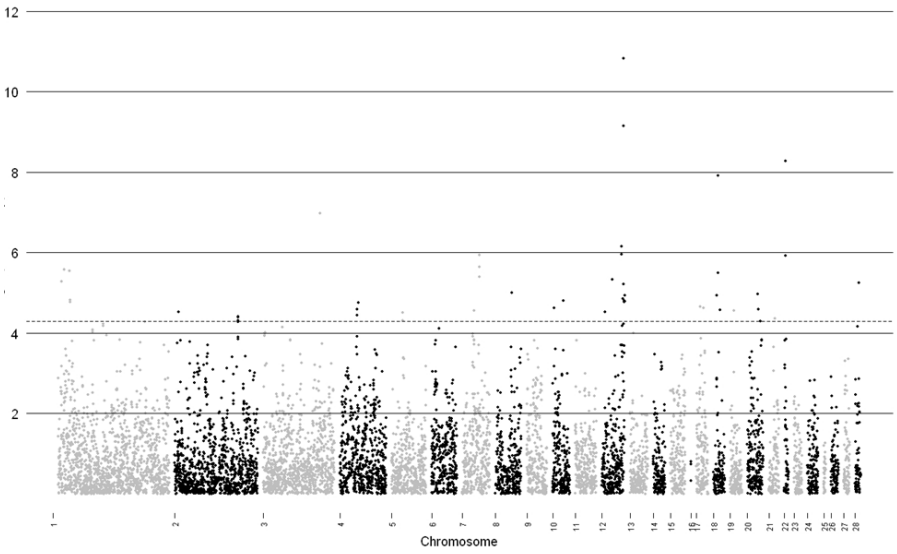

Figure 6.2 Manhattan plot for single SNP analysis using a general linear model accounting only for paternal half sib relations among animals. -$\log_{10}$P-values for association of SNPs with RATIO. The position is represented along the x-axis and chromosome numbers are given. The dashed line reflects cutoff for p = 0.0001.

The Animal Model

The second approach was also using SNP genotypes of the parents and average adjusted progeny trait values for RATIO but was extended by accounting for pedigree relationship between the broilers, i.e. the analysis described in chapter 5

$$y_{ij}^{*} = \mu + SNP_i + Animal_j + e_{ij}$$

Where y_{ij}^{*} is the average adjusted progeny trait value (AAPTV) of the chicken ij; μ is the overall sample mean; SNP_i is the fixed effect of the SNP; $Animal_j$ is the random

genetic effect of individual and the pedigree is used to account for relationships among animals. e_{ij} is the random residual effect. A weighted analysis was performed using the ASREML software (Gilmour et al., 2006). The weight for record y_{ij}^{*} depended on the number of progeny that contributed to the average.

Comparing the $-\log_{10}(p)$ values of the SNP analysed using the two models showed poor resemblance as quantified by the coefficient of determination of 0.22 (figure 6.3). Furthermore, the significance level decreased considerably when accounting for all relationships with the animal model instead of only accounting for relationships due to sires (figure 6.3 and 6.4). Figure 6.4 shows the Manhattan plot of the linear model (above the x-axis) and the animal model (below the x-axis). It is evident that there are some regions which are highly significant using the linear model that do not show up when using the animal model. Other regions are significant when using the animal model, but not when using the linear model.

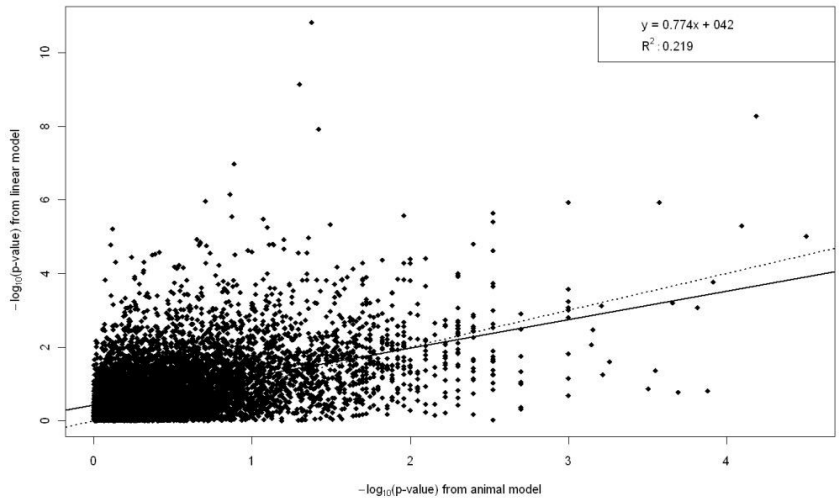

Figure 6.3 Scatter plot of $-\log_{10}$ (p-values) based on the single SNP analysis using a linear model accounting only for paternal half sib relations versus $-\log_{10}$ (p-values) based on the animal model accounting for the full pedigree. The broken line is the line y=x. and the other line is a fitted regression line.

The conclusion of this analysis is that results from both models are not consistent. The application of a linear model correcting for sire implies that only relations due to sires are accounted for and the main benefit of using the sire model is that the

analysis is simple and less computationally demanding. However, with the linear model, relationships due to dams are not accounted for. This resulted in an inflation of the test statistic and increased the number of "significant" SNP effects. These results differ from the situation in dairy cattle as described by Bouwman et al (2011). In dairy cattle accounting for relations due to sires results in a very good approximation of the results obtained using a model accounting for all relationships. The difference can be explained by the relevance of maternal family relationships in poultry versus dairy cattle: in poultry multiple offspring per female are common whereas in dairy cattle this would be an exception. Therefore, it was decided to perform the whole genome association study using a mixed animal model which accounts for all family-relationship based on the parent generation with own genotype, but average adjusted progeny trait values (chapter 5).

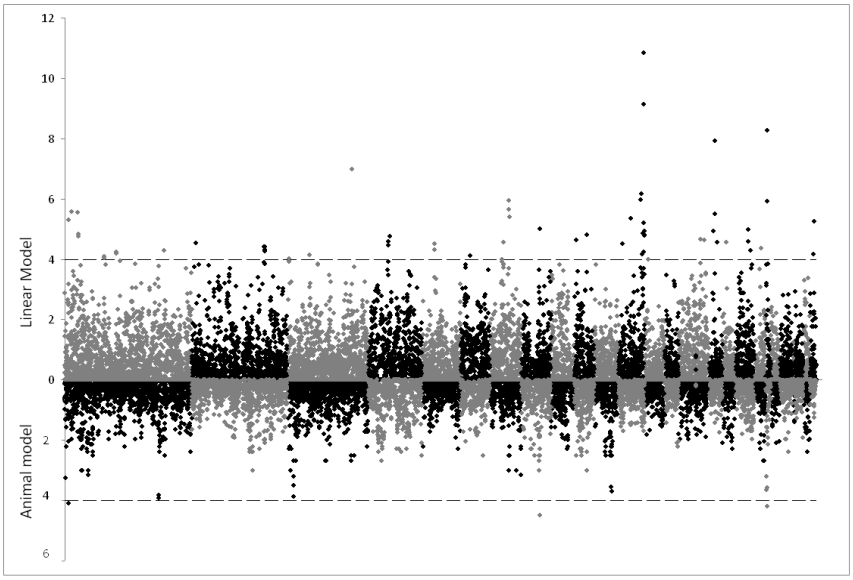

Figure 6.4 Two Manhattan plots for single SNP analysis using a general linear model accounting only for paternal half sib relations among animals (above the x-axis) and single SNP analysis using a animal model accounting for all relations among animals (below x-axis). $-\log_{10}$P-values for association of SNPs with RATIO. The position is represented along the x-axis. The dashed line reflects cutoff for p = 0.0001.

Alternative GWAS Model

In our study phenotypes were recorded on the offspring and genotypes were only available on the parents. To bring the genotypic information together with the phenotypic information we calculated average adjusted progeny trait values for

each parent which was the dependent variable in our analyses. In a sense the phenotypic observations were moved from the offspring to the parents. Alternatively, to bring the genotypic information together with the phenotypic information we could have moved the genotypes from the parents to the offspring. This analysis then can be performed based on phenotypic trait values of the individuals and genotype probabilities which can be calculated based on the parental genotypes. Each broiler in the offspring generation needed to be assigned a genotype probability based on the genotypes of its sire and its dam (table 6.1). The probabilities presented in Table 6.1 can be including in the analyses as co-variables. The first regression coefficient results in an estimate of the additive effect and second regression coefficients results in an estimate of the dominance effect.

Table 6.1. Genotype probabilities and co-variables depending upon the genotypes of the sire and the dam. The first regression coefficient (p(AA)-p(CC)) results in an estimate of the additive effect and second regression coefficient (P(AC)) results in an estimate of the dominance effect.

Mating Type	P(Offspring genotype)			Offspring mean	P(AA)-P(CC)	P(AC)
	AA	AC	CC			
AA * AA	1	0	0	A	1	0
AA * AC	½	½	0	½a+½d	½	½
AA * CC	0	1	0	D	0	1
AC * AC	¼	½	¼	½d	0	½
AC * CC	0	½	½	-½a+½d	-½	½
CC * CC	0	0	1	-a	-1	0

The following animal model could be used:

$$y_{ijklm} = \mu + sex_i + batch \cdot stable_j + \beta_1 \cdot \left[p(AA) - p(CC)\right]_{ijklm} + \beta_2 \cdot \left[p(AC)\right]_{ijklm} + a_l + d_m + e_{ijklm}$$

where y_{ijklm} is the dependent variable of broiler ijkl; sex_i is the fixed effect of sex (i = male or female); batch ·$stable_j$ is the effect of the interaction between batch and stable (j = 1, 2,…, 10), batch consisted of 5 trials and there were 2 stables; $\left[p(AA) - p(CC)\right]_{ijklm}$ and $\left[p(AC)\right]_{ijklm}$ are genotype probabilities for each individual ijklm; a_l is the random genetic effect of individual l; d_m is the random maternal environmental effect of dam l; and e_{ijklm} is the random residual effect. The maternal effect could be included in the model in case this significantly affects the phenotype (e.g. in the body weight analysis).

6.5 Comparison between Species

Ascites has been recognized in poultry for several decades, both at low and high altitudes (Julian, 2005), and the genetic susceptibility to this disorder has been of interest for broiler breeders for a long period of time. Originally ascites was only observed in broilers reared at high altitude. Diseases with symptoms similar to ascites in broilers are also observed in other species, e.g. pulmonary hypertension and acute altitude sickness in humans or brisket disease in cattle. Both animals and humans that are not adapted to high altitude will show an increased risk for developing pulmonary or cerebral oedema. Without adequate acclimatization extended periods at high altitude leads to chronic pulmonary hypertension and related complications (Julian, 1993; Ahola et al., 2006; Scheinfeldt and Tishkoff, 2010). Both physiological and genome wide association studies performed in different species provide information on pulmonary hypertension which can be used for an increased understanding of ascites in broilers.

Chronic mountain sickness in humans

Chronic mountain sickness (CMS) is a syndrome observed in humans, which can develop during extended time living at high altitudes. CMS develops when the respiratory frequency and ventilator capacity is unable to compensate for extreme hypoxia. As a consequence humans might develop severe pulmonary hypertension (Zubieta-Castillo, Sr. et al., 2006). While the development of acute mountain sickness is experienced shortly after moving to high altitude, chronic mountain sickness may develop after many years of living at high altitude. Physiological differences among groups of human populations living at high altitude in different continents can be caused by strong natural selection during longer exposures to hypoxia at high altitude or genetic differences in founder populations (Powell 2003).

Humans living at high altitude are generally characterized by smaller body size than humans living at low altitude. In a study by Tripathy and Gupta (2007) Tibetans living at high altitude outside Tibet and Tibetans living at low altitude outside Tibet were compared. It was found that the Tibetan living at low altitude outside Tibet were advanced in terms of height, weight, skinfold thickness at triceps and upper arm circumference compared to Tibetans at high altitude outside Tibet (Tripathy and Gupta, 2007). Further, this study indicates that genetic factors account for a large proportion of phenotypic variance in haemoglobin concentration in the two populations; the results do not identify a specific genetic factor underlying intrapopulation or interpopulation differences in response to altitude.

Comparative methods have been used to study populations living at low altitude with populations living at high altitude native from Andes, Himalayas, the Tabetan plateay and East Africa (Powell, 2003). The heritability for improved arterial oxygen saturation estimated for the Tibetan population suggests the presence of a major gene (Beall et al., 1998; Beall, 2006). A genome wide association study based on high- and low-altitude human populations identified candidate genes and QTL regions in the two long-resident high-altitude populations; the Andeans population and the Tibetans population (Bigham et al., 2010). Several chromosomal regions show evidence of association with unique adaptation traits. Some regions are unique to either Andeans or Tibetans, suggesting a lack of evolutionary convergence between these two highland populations indicating that the change to high altitude has triggered different types of adaptation. However, convergence of evolution between Andeans and Tibetans is suggested for other chromosomal regions: the signal detected for the Hypoxia-inducible factor (HIF) regulatory gene *EGLN1* was found in both populations. Furthermore, a second HIF regulatory gene, *EPAS1* and two HIF targeted genes, PRKAA1 and NOS2A, have been identified as candidate genes in Tibetans (*EPAS1*) or Andeans (*PRKAA1, NOS2A*) (Bigham et al., 2010) . *PRKAA1* and *NOS2A* play major roles in physiological processes essential to human reproductive success (Wilson et al., 2007). These studies indicate that HIF-regulatory genes play an important role in adaptation to high altitude in Andeans and Tibetans (Bigham et al., 2010). HIF genes are ubiquitously expressed heterodimeric transcription factors that mediate adaptive responses to hypoxia in all nucleated cells of metazoan organisms. Zhang et al. (2013) recently showed that HIF genes might be associated with the development of ascites in broiler.

Pulmonary hypertension in cattle

Brisket disease, also known as high mountain disease or pulmonary hypertension is similar to ascites in broilers and results in elevated pulmonary arterial pressures or pulmonary hypertension. Brisket disease is caused primarily by an oxygen shortage increasing the pressure on the heart. The pressure can continue to build up until fluids leaks out of the blood stream and collects in the chest cavity, the brisket, and other places. Eventually, the demand on the heart increases too much and the animal dies. Pulmonary hypertension occurs as a consequence of a right ventricular overload, right-sided heart failure develops in cattle and very similar to the ascites in broilers.

Brisket disease is a diseases found among cattle living at high altitudes (Ahola et al., 2006; Shirley et al., 2008). The mortality due to brisket disease can differ depending

on the origin of the cattle. Some cattle are able to tolerate living at high altitude for a longer period of time, while others die quickly. In cattle born and raised at high altitudes, the losses tend to be lower (0.5% to 5%) than lowland cattle which are moved to higher altitude later in live (30% to 40%). Brisket disease in cattle affects both sexes and has been found across all breeds including crossbreds and heritability of brisket disease have been estimated to ranging from 0.42 to 0.77.

The reduced amount of available oxygen at higher altitudes can affect the lungs of the cattle and cause artery walls to thicken similar to the broilers that are developing ascites. The decrease of blood pressure in the arteries and subsequent pulmonary hypertension, make it difficult for the right ventricular to pump blood into the lungs. The right ventricular muscle enlarges from the additional work needed to pump blood, resulting in the right ventricle to lose its ability to contract. As blood pressure increases and starts to flow back into the heart, it can expand the valves of the right ventricle. Some affected cattle develop oedema in the neck and brisket, as well as fluid accumulation in the jaws, or along the abdomen. Affected cattle may develop problems early in life, or shortly after being brought to high altitudes from lower altitudes. Often the sires which are superior in meat production and other desired traits are not tested at high altitudes. Therefore, these sires are not tested for brisket disease and may produce progeny that develop brisket disease. A suggested way to reduce cattle that are susceptible to develop brisket disease is to identify signalling pathways in cattle suffering from brisket disease and to identify chromosomal regions associated with brisket disease.

Gained knowledge about ascites from other species

Comparative physiology has been established as a useful method to understand how animals function under different environmental conditions and therefore can be used to identify potential adaptations to environmental oxygen levels. Comparing the studies of ascites related diseases in other species is of interest for the identification of genes and genetic variation that is related to these diseases. Although the causes and symptoms of ascites related diseases show variation among the different species, the information and experience gained for other species might help in understanding the genetic background of ascites in broilers and to identify potential candidate genes involved in ascites in broilers.

There is a general interest in the identification of genes subject to both positive and negative selection for adaption to the high altitude diseases in both human and

animals. Genetic variation that is of importance for positive selection in locally adapted populations are expected to show higher levels of population differentiation and in some cases extended regions of allelic association or linkage disequilibrium (Scheinfeldt and Tishkoff, 2010). The heritability for oxygen saturation estimated in human populations differs from the heritability for oxygen saturation estimated from our estimate in a broiler population (chapter 2). In our study the estimated heritability for oxygen saturation in venous blood was close to zero (0.07) (Closter et al., 2009).

Several physiological traits indicate how human populations in Asia, Africa and America have uniquely adapted to living at high altitude. Studies of these human populations have shown that these populations also from a genetic perspective adapted in a unique way to living at high altitude. This supports the hypothesis that this adaptation is not due to a single gene but rather a polygenic trait. On the one hand, this complicates the genetic analysis but in the case of production animals, it also suggests that selection for improved adaptation is possible.

References

Ahola, J. K., R. M. Enns, and T. Holt. 2006. Examination of potential methods to predict pulmonary arterial pressure score in yearling beef cattle. Journal of Animal Science 84:1259-1264.

Arthur, J. A. and G. A. A. Albers. 2003. Industrial Perspective on Problems and Issues Associated with Poultry Breeding. Pages 1-12 in Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S. E. Aggrey eds. CABI Publishing, Wallingford, UK.

Beall, C. M., G. M. Brittenham, K. P. Strohl, J. Blangero, S. Williams-Blangero, M. C. Goldstein, M. J. Decker, E. Vargas, M. Villena, R. Soria, A. M. Alarcon, and C. Gonzales. 1998. Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. American American Journal of Physical Anthropology: 106:385-400.

Beall, C. M. 2006. Andean, Tibetan, and Ethiopian patterns of adaptation to high-altitude hypoxia. Integrative and Comparative Biology 46:18-24.

Bigham, A., M. Bauchet, D. Pinto, X. Mao, J. M. Akey, R. Mei, S. W. Scherer, C. G. Julian, M. J. Wilson, D. López Herráez, T. Brutsaert, E. J. Parra, L. G. Moore, and M. D. Shriver. 2010. Identifying Signatures of Natural Selection in Tibetan and Andean Populations Using Dense Genome Scan Data. PLoS Genet 6:e1001116.

Bouwman, A., H. Bovenhuis, M. Visker, and J. van Arendonk. 2011. Genome-wide association of milk fatty acids in Dutch dairy cattle. BMC Genetics 12:43.

Bouwman, A., M. Visker, J. van Arendonk and H. Bovenhuis. 2012. Genomic regions associated with bovine milk fatty acids in both summer and winter milk samples. BMC Genetics 13: 93-106

Closter, A. M., P. van As, M. A. M. Groenen, A. L. J. Vereijken, J. A. M. van Arendonk, and H. Bovenhuis. 2009. Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. Poult Sci 88:483-490.

de Greef, K. H., L. L. G. Janss, A. L. J. Vereijken, R. Pit, and C. L. M. Gerritsen. 2001. Disease-induced variability of genetic correlations: Ascites in broilers as a case study. Journal of Animal Science 79:1723-1733.

Detilleux, J., and P. L. Leroy. 2000. Application of a mixed normal mixture model for the estimation of mastitis-related parameters. J. Dairy Sci. 83:2341–2349.

Goddard M.E. and Hayes B.J.. 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics. 10:381-391.

Julian, R.J. 1993. Ascites in Poultry. Avian Pathology 22:419-454.

Madsen, P., M. M. Shariati, and J. Ødegård. 2008. Genetic Analysis of Somatic Cell Score in Danish Holsteins Using a Liability-Normal Mixture Model. Journal of Dairy Science 91:4355-4364.

Maxwell, M. H. and G. W. Robertson. 1998. UK survey of broiler ascites and sudden death syndromes in 1993. British Poultry Science 39:203-215.

Newman J.H., T.N. Holt, L.K. Hedges, B. Womack, S.S. Memon, E.D. Willers, L. Wheeler and J.A. Phillips 3rd, and R. Hamid R. 2011. High-altitude pulmonary hypertension in cattle (brisket disease): Candidate genes and gene expression profiling of peripheral blood mononuclear cells. Pulmonary Circulation:1(4):462-9.

Ødegård, J., J. Jensen, P. Madsen, D. Gianola, G. Klemetsdal, and B. Heringstad. 2003. Detection of Mastitis in Dairy Cattle by Use of Mixture Models for Repeated Somatic Cell Scores: A Bayesian Approach via Gibbs Sampling. Journal of Dairy Science 86: 3694-3703.

Ødegård, J., P. Madsen, D. Gianola, G. Klemetsdal, J. Jensen, B. Heringstad, and I. R. Korsgaard. 2005. A Bayesian Threshold-Normal Mixture Model for Analysis of a Continuous Mastitis-Related Trait. Journal of Dairy Science 88: 2652-2659.

Pakdel, A., J. A. Van Arendonk, A. L. Vereijken, and H. Bovenhuis. 2002. Direct and maternal genetic effects for ascites-related traits in broilers. Poulty Science. 81:1273–1279.

Powell. 2003. Functional genomics and the comparative physiology of hypixia. Annual Reviews Physiology. 65: 203-230

Rabie, T. S. K. M., R. P. M. A. Crooijmans, H. Bovenhuis, A. L. J. Vereijken, T. Veenendaal, J. J. van der Poel, J. A. M. van Arendonk, A. Pakdel, and M. A. M. Groenen. 2005. Genetic mapping of quantitative trait loci affecting susceptibility in chicken to develop pulmonary hypertension syndrome. Animal Genetics 36:468-476.

Shirley, K. L., D. W. Beckman, and D. J. Garrick. 2008. Inheritance of pulmonary arterial pressure in Angus cattle and its correlation with growth. Journal of Animal Science: 86:815-819.

Scheinfeldt, L. B. and S. A. Tishkoff. 2010. Living the high life: high-altitude adaptation. Genome Biology 11.

Tripathy, V. and R. Gupta. 2007. Growth among Tibetans at high and low altitudes in India. American Journal of Human Biology 19:789-800.

van Kaam, J. B. C. H. M., J. A. M. van Arendonk, M. A. M. Groenen, H. Bovenhuis, A. L. J. Vereijken, R. P. M. A. Crooijmans, J. J.van der Poel, and A. Veenendaal. 1998. Whole genome scan for quantitative trait loci affecting body weight in chickens using a three generation design. Livestock Production Science. 54:133–150.

Wilson, M. J., M. Lopez, M. Vargas, C. Julian, W. Tellez, A. Rodriguez, A. Bigham, J. F. Armaza, S. Niermeyer, M. Shriver, E. Vargas, and L. G. Moore. 2007. Greater uterine artery blood flow during pregnancy in multigenerational (Andean) than shorter-term (European) high-altitude residents. American Journal 0of Physiology - Regulatory, Integrative and Comparative Physiology 293:R1313-R1324.

Young, C. W., J. E. Legates, and J. G. Lecce. 1960. Genetic and Phenotypic Relationships between Clinical Mastitis, Laboratory Criteria, and Udder Height. Journal of Dairy Science 43:54-62.

Zhang J., X. Feng, L. Zhao, W. Wang, M. Gao, B. Wu and J. Qiao. 2013 Expression of hypoxia-inducible factor alpha mRNA in hearts and lungs of broiler chickens with ascites syndrome induced by excess salt in drinking water. Poultry Science 92(8):2044-52. doi: 10.3382/ps.2013-03097.

Zubieta-Castillo, G., Sr., G. R. J. Zubieta-Calleja, and L. Zubieta-Calleja. 2006. Chronic mountain sickness: the reaction of physical disorders to chronic hypoxia. Journal of Physiology and Pharmacoly: 57: 431-442

Summary

Summary

The pulmonary hypertension syndrome (PHS) in broilers, also known as ascites, occurs in all species of poultry. Ascites is characterized by fluid accumulation in the abdominal cavity as a result of defects or abnormalities in the heart. Other internal organs like the liver and the kidneys can also show abnormalities. The occurrence of ascites differs around the world, but it is a recognized problem especially in broilers populations. Ascites results in a greater mortality and rejections at slaughter. The **aim** of this thesis was to estimate genetic parameters and identify QTL that are involved in the complex multi-factorial metabolic disorder, ascites, by a combination of genetic and genomics techniques. The main objectives of the project were (1) to identify and genetically characterise traits related to the development of ascites, and (2) to identify genomic regions associated with the development of ascites.

The general introduction (**chapter 1)** provides an overview of the developments in poultry production with particular focus on commercial broilers and how selection for health has been introduced in breeding programs for broilers. Furthermore, the physiological background of the development of ascites is reviewed as well as the current knowledge regarding the genetic background of ascites.

Blood gas parameters have been suggested as indicator traits for ascites susceptibility. Therefore, the aim of the study presented in Chapter 2 was to estimate the heritability of blood gas parameters and the genetic and phenotypic correlations between blood gas parameters, heart ratio (postmortem indicator for ascites), and body weight at 2 different ages. For this purpose, blood gas parameters, including the partial pressure of carbon dioxide in venous blood ($pvCO_2$), the partial pressure of oxygen in venous blood (pvO_2), and blood oxygen saturation, were measured at an average age of 22 days in nearly 3,000 broilers. To challenge the resistance of the birds to ascites, they were kept under cold conditions. Heritability for heart ratio was 0.43. The heritability estimates for blood gas parameters were low: 0.02 for $pvCO_2$, 0.03 for pvO_2, and 0.07 for blood oxygen saturation. The genetic correlations between heart ratio and total carbon dioxide content (0.31 S.E. = 0.15) and between heart ratio and bicarbonate (0.31 S.E. = 0.15) were moderate and positive. For pvO_2, the genetic correlation with heart ratio was stronger and negative (−0.62 S.E. = 0.21); however, this correlation could not be estimated accurately because of the low heritability of pvO2. For $pvCO_2$, the genetic correlation with the heart ratio was close to zero (−0.04 S.E. = 0.45). Phenotypic correlations between traits were, in general, similar to the genetic

correlations. The estimated heritabilities for blood gas parameters and the genetic correlations between blood gas parameters and the heart ratio do not support the suggestion that blood gas parameters measured during week 3 or 4 are useful traits in selecting against the susceptibility for ascites.

Male broilers have a higher body weight and are therefore expected to be more prone to developing ascites than females. As genetic parameters might be affected by the ascites incidence, genetic parameters might differ between male and female broilers. The aims of the study described in Chapter 3 were to estimate the heritability for the ratio of right ventricular weight to total ventricular weight (RATIO) and body weight in male and female broilers, the genetic correlation between RATIO and body weight separately for male and female broilers, and the genetic correlations between body weight for ascitic and non-ascitic broilers. Data were available from 7,856 broilers (3,819 males and 4,037 females). The broilers in the experiment were kept under a cold temperature regimen and increased CO_2 levels. In this study, we showed that the incidence of ascites is higher in male than in female broilers. Heritability estimates for body weight at 7 weeks of age were higher for male (0.22) than for female (0.17) broilers, and for RATIO heritability, estimates were higher for female (0.44) than for male (0.32) broilers. The genetic correlations between RATIO and body weight measured at different ages changed from slightly positive at 2 weeks of age to moderately negative at 7 weeks. The change in genetic correlation was more extreme for male (from 0.01 to −0.62) than for female (from 0.13 to −0.24) broilers. The difference in ascites incidence between male and female broilers is the most likely reason for the difference in genetic correlations. The genetic correlation between body weight traits measured in broilers with fluid in the abdomen and without fluid in the abdomen decreased from 0.91 at 2 weeks to 0.69 at 7 weeks. Based on these results it was concluded that under circumstances with ascites, data from male and female broilers should be analyzed separately.

Alternatively, it seems of interest to apply the liability normal mixture (LNM) method for the analysis of ascites in broilers. The LNM model has been used in other species to disentangle relations between traits for diseased and healthy animals when the assignment of individuals to the classes diseased or healthy is unknown. In **chapter 4** a liability normal mixture (LNM) model was fitted assuming separate (co)variance components for RATIO of healthy and diseased chickens. Observations on the occurrence of ascites as well as for the ascites indicator trait ratio of right ventricle to the total ventricular weight (RATIO) for 7,613 broilers

chickens were available. The heritability for liability was 0.54, demonstrating that susceptibility to develop ascites is highly heritable. Of all chickens in the study 24 % were estimated to be affected by the ascites syndrome. Heritability for RATIO of diseased chickens was 0.31 and for the healthy chickens the heritability was 0.32. The genetic correlation between RATIO of healthy and diseased chickens was 0.75, indicating that RATIO is a different trait in healthy and diseased chickens. The genetic correlation between RATIO and liability was positive for healthy chickens (0.27) whereas the genetic correlation between RATIO and liability for diseased chickens is negative (-0.32). This negative correlation indicates that chickens with a high RATIO are less susceptible to the disease, whereas the positive correlation between liability and RATIO for the healthy chickens indicates that chickens with high RATIO are more susceptible to the disease. The LNM model allowed for richer and more detailed inference about disease status and underlying structure than previous models did, since using the LNM model reveals hidden structure in data by properly modeling uncertainty of disease classification. This also opens up new opportunities for selection against ascites susceptibility.

Chapter 5 describes a genome wide association study using the parent generation, which was genotyped for SNPs, and the offspring generation, which was phenotyped for ascites related traits (chapter 3). The parent generation consisted of 895 chickens genotyped for 17,790 SNPs, and the offspring generation consisted of 7,857 chickens phenotyped for ascites related traits and body weight, recorded under ascites inducing conditions. The GWAS was performed using a single SNP analysis. The genotyped parent generation was combined with phenotypic information from their progeny by calculating the Average Adjusted Progeny Trait Values (AAPTV). In total, 25 SNPs were significantly (FDR <0.30) associated with RATIO. Significant associations were detected on chromosome 1, 2, 3, 7, 8, 10, 11 and 20. The most significant SNPs were found on chromosome 1, 8 and on 22. This study also identified 16 SNP that were significantly associated both with RATIO and with fluid in abdomen. Out of these 16 SNPs nine SNPs were also found to be significantly associated with body weight.

Finally, the general discussion in **chapter 6** discusses a number of topics: selection against ascites in broilers, the use of LNM model in selection for disease resistance, genome wide association studies for ascites and comparison between species. The first topic briefly discusses selection against ascites in broilers. The second topic discusses the use of LNM models in selection for disease resistance and focuses on comparing the estimated breeding values (EBV) for liability based on the LNM

analysis with EBVs for RATIO using the animal model. The third topic of the general discussion is on using alternative models for the genome wide association study. The fourth and last topic is on diseases in other species which are similar or have similar symptoms as ascites in broilers. Results from these studies are of interest for the identification of chromosomal regions or genes related ascites. Pulmonary hypertension and acute altitude sickness disease have been observed in humans and Brisket disease in cattle. While causes and symptoms of ascites related diseases differ among species, the information and experience gained in other species might help in understanding the genetic background and identifying potential candidate genes involved in ascites in broilers.

Samenvatting

Samenvatting

Het Pulmonale Hypertensie Syndroom (PHS), ook bekend als ascites, komt voor bij alle moderne vleeskuikenrassen. Ascites wordt gekenmerkt door de accumulatie van vocht in de buikholte als gevolg van afwijkingen aan het hart. Tegelijkertijd kan er ook sprake zijn van afwijkingen aan andere organen zoals de lever en de nieren. De frequentie waarin ascites voorkomt kan sterk verschillen maar in het algemeen wordt ascites gezien als een probleem dat leidt tot hogere mortaliteit en het afkeuren van kippen bij de slacht. Het doel van dit onderzoek was om genetische parameters te schatten en om QTL te detecteren die betrokken zijn bij de complexe metabole ziekte ascites door gebruik te maken van een combinatie van genetische en genomische technieken. De belangrijkste doelstellingen van het project waren (1) het identificeren en genetisch karakteriseren van kenmerken die verband houden met de ontwikkeling van ascites, (2) het identificeren van genomische gebieden die geassocieerd zijn met de ontwikkeling van ascites.

De algemene introductie in hoofdstuk 1 geeft een overzicht van de ontwikkelingen op het gebied van de pluimveeproduktie met speciale aandacht voor de selectie op gezondheid. Daarnaast geeft dit hoofdstuk een overzicht van de fysiologische veranderingen die optreden gedurende de ontwikkeling van ascites bij vleeskuikens en een samenvatting van de huidige kennis omtrent de genetische achtergrond van ascites.

Bloedgaswaarden worden wel genoemd als mogelijke indicatoren voor ascites. Het doel van het onderzoek beschreven in hoofdstuk 2 was daarom om de erfelijkheidsgraden van bloedgaswaarden te schatten en om genetische en fenotypische correlaties tussen bloedgaswaarden, RATIO (verhouding tussen het gewicht van de rechter hartkamer en het totale gewicht van beide hartkamers – een post-mortem indicator voor ascites) en lichaamsgewicht op twee leeftijden te schatten. Hiervoor zijn bloedgaswaarden gemeten aan bijna 3000 vleeskuikens op een leeftijd van gemiddeld 22 dagen. De volgende bloedgaswaarden zijn gemeten: de partiële koolstofdioxidespanning in het veneuze bloed (pvCO2), de partiële zuurstofspanning in het veneuze bloed (pvO2) en de zuurstofverzadiging van het bloed. Om de gevoeligheid van de vleeskuikens voor ascites te toetsen werden ze onder koude omstandigheden gehouden. De erfelijkheidsgraad voor RATIO was 0.43. De erfelijkheidsgraadschattingen voor de bloedgasparameters waren laag: 0.02 voor pvCO2, 0.03 voor pvO2, en 0.07 voor de bloedzuurstofverzadiging. De genetische correlaties tussen RATIO en totaal kooldioxide gehalte (0.31 S.E. = 0.15) en tussen RATIO en bicarbonaat gehalte (0.31 S.E.= 0.15) waren gematigd en positief. De genetische correlatie tussen pvO2 en RATIO was sterker en negatief

(−0.62 S.E. = 0.21). Deze correlatie kon echter niet nauwkeurig worden geschat vanwege de lage erfelijkheid van pvO2. De genetische correlatie tussen pvCO2 en RATIO was ongeveer 0 (−0.04 S.E. = 0.45). De fenotypische correlaties tussen kenmerken kwamen in het algemeen overeen met de genetische correlaties. De resultaten in dit hoofdstuk geven geen aanleiding om te veronderstellen dat bloedgasparameters gemeten op een leeftijd van 3 of 4 weken een grote bijdrage kunnen leveren aan de selectie tegen ascites.

Haantjes hebben een hoger lichaamsgewicht en zijn daarom naar verwachting gevoeliger voor ascites. Omdat de genetische parameters mogelijk afhankelijk zijn van de ascites incidentie kunnen de genetische parameters verschillen tussen haantjes en hennetjes. Het doel van het onderzoek beschreven in hoofdstuk 3 was daarom om: 1) de erfelijkheidsgraad voor RATIO en lichaamsgewicht afzonderlijk te schatten in haantjes en in hennetjes, 2) de genetische correlatie tussen RATIO en lichaamsgewicht afzonderlijk te schatten voor haantjes en hennetjes, 3) de genetische correlatie tussen lichaamsgewicht en ascites afzonderlijk te schatten in vleeskuikens met ascites en in gezonde vleeskuikens. Voor dit onderzoek waren gegevens beschikbaar van 7856 vleeskuikens (3819 haantjes en 4037 hennetjes). Om de gevoeligheid van de vleeskuikens voor ascites te toetsen werden ze gehuisvest bij een verlaagde temperatuur en bij een verhoogd CO2 niveau. Dit onderzoek laat zien dat de incidentie van ascites hoger is in haantjes dan in hennetjes. De erfelijkheidsgraden voor lichaamsgewicht op 7 weken waren hoger voor de haantjes (0.22) dan voor de hennetjes (0.17). Voor RATIO waren de erfelijkheidsgraadschattingen hoger voor de hennetjes (0.44) dan voor de haantjes (0.32). De genetische correlaties tussen RATIO en lichaamsgewicht gemeten op verschillende leeftijden veranderde van licht positief op een leeftijd van 2 weken naar gematigd negatief op een leeftijd van 7 weken. Deze verandering van de genetische correlatie was sterker in de haantjes (van 0.01 naar −0.62) dan in de hennetjes (van 0.13 naar −0.24). Het verschil in ascites incidentie tussen haantjes en hennetjes is de meest voor de hand liggende verklaring voor de verandering in genetische correlatie. De genetische correlatie tussen lichaamsgewicht in vleeskuikens met vocht in de buikholte (ascites) en die zonder vocht in de buikholte (gezond) nam af van 0.91 op 2 weken tot 0.69 op 7 weken. Wij concluderen dat gegevens van haantjes en hennetjes afzonderlijk moeten worden geanalyseerd in situaties waarin ascites een rol speelt.

Als alternatief kan de data worden geanalyseerd met een Gemengd-Ziektegevoeligheid-Model (GZM). Dit model kan rekening houden met verschillen

tussen gezonde en zieke vleeskuikens. In hoofdstuk 4 is een Gemengde-Ziektegevoeligheid-Model (GZM) gebruikt voor de analyse van RATIO. In dit model kunnen (co)variantie componenten verschillen tussen gezonde en zieke vleeskuikens. Dit model is gebruikt om genetische parameters te schatten voor RATIO in gezonde en zieke vleeskuikens maar ook om de gevoeligheid voor ascites te schatten. In de analyse is gebruik gemaakt van informatie over ascites (vocht in de buikholte) en RATIO gemeten aan 7613 vleeskuikens. De erfelijkheidsgraad voor ascitesgevoeligheid was 0.54 wat aangeeft dat de aanleg om ascites te ontwikkelen sterk genetisch is bepaald. Van alle vleeskuikens in deze studie heeft naar schatting 24% ascites. De erfelijkheid van RATIO in zieke kippen was 0.31 en in gezonde kippen was dit 0.32. De genetische correlatie tussen RATIO in gezonde en zieke kippen was 0.75 wat aangeeft dat RATIO in gezonde en zieke kippen genetisch gezien niet hetzelfde kenmerk is. De genetische correlatie tussen RATIO en ziektegevoeligheid was positief voor gezonde kippen (0.27) terwijl de genetische correlatie tussen RATIO en ziektegevoeligheid negatief was voor zieke kippen (-0.32). Deze negatieve correlatie geeft aan dat kippen met een hoge RATIO minder gevoelig zijn voor ascites. De positieve correlatie tussen ziektegevoeligheid en RATIO voor de gezonde kippen geeft aan dat kippen met een hoge RATIO gevoeliger zijn voor de ziekte. Het Gemengde-Ziektegevoeligheid-Model maakt een gedetailleerde analyse van de data mogelijk. Een dergelijke gedetailleerde analyse is niet mogelijk met het voorheen gebruikte lineair model. Het Gemengde-Ziektegevoeligheid-Model biedt daarom nieuwe mogelijkheden om te selecteren tegen ascitesgevoeligheid.

Hoofdstuk 5 beschrijft de resultaten van een genoomwijde-associatie studie. Voor deze studie zijn genotypes beschikbaar van de ouderdieren en ascites gerelateerde fenotypes van de nakomelingen. De 895 beschikbare ouderdieren zijn getypeerd voor 17790 SNPs en 7857 nakomelingen hebben fenotypes. De genetische associatie studie is voor elke SNP afzonderlijk uitgevoerd. In de analyses zijn de genotypes van de ouders gebruikt in combinatie met een Gecorrigeerd-Nakomelingen-Gemiddelde (GNG). In totaal waren 25 SNPs significant geassocieerd met RATIO (FDR<0.30). Significante associaties werden gevonden op chromosoom 1, 2, 3, 7, 8, 10, 11 en 20. De meest significante associaties werden gevonden op chromosoom 1, 8 en 22. In deze studie zijn 16 SNPs gevonden die significant geassocieerd waren met zowel RATIO als met het voorkomen van vocht in de buikholte. Van deze 16 SNPs waren er 9 die ook significant geassocieerd zijn met lichaamsgewicht.

Tenslotte worden in hoofdstuk 6 de volgende onderwerpen bediscussieerd: selectie tegen ascites, het gebruik van het Gemengde-Ziektegevoeligheid-Model in de selectie op ziekteresistentie, alternatieve statistische modellen voor de genoomwijde associatie studie en een overzicht van aan ascites gerelateerde kenmerken in verschillende diersoorten. Het eerste onderwerp bediscussieerd kort de selectie tegen ascites in vleeskuikens. Het tweede onderwerp richt zich op het gebruik van het Gemengde-Ziektegevoeligheid-Model in de selectie op ziektegevoeligheid. Hiertoe zijn de geschatte fokwaarden voor ziektegevoeligheid op basis van het Gemengde-Ziektegevoeligheid-Model vergeleken met de fokwaarden voor RATIO op basis van het diermodel. Het derde onderwerp richt zich op alternatieve statische modellen voor de genoomwijde associatiestudie. Het vierde en laatste onderwerp richt zich op ziektes bij andere diersoorten die soortgelijke symptomen vertonen als ascites bij vleeskuikens. Pulmonale Hypertensie en hoogteziekte komen voor bij de mens en Brisket ziekte komt voor bij rundvee. Terwijl oorzaak en symptomen van aan ascites verwante ziektes in andere diersoorten kunnen variëren, kan kennis omtrent de ziekte in ander soorten mogelijk bijdragen aan het begrip van de genetische achtergrond van ascites in vleeskuikens.

Curriculum Vitae

Ane Marie Closter was born in Lyngby Tårnbæk (Denmark) 6th of July 1978. In 1998 she obtained her high school diploma at the Amtsgymnasiet in Sønderborg. In 1998, Ane moved to Ireland and worked at Kildangan Stud, Co. Kildare before started her study on Equine Science University of Limerick, Ireland. An internship of eight months was performed at the Irish Equine Centre, Co. Kildare. She obtained her bachelor's degree in 2003. After obtaining her bachelor's degree an internship of two months was performed at the Del Mar Thoroughbred Club, California, USA. In 2003, Ane started her Master in Agricultural Science at the Royal Veterinary and Agricultural University, Copenhagen, Denmark. An internship of five months was performed at the Dept. of Pig Breeding at the Danish Pig Research Centre, Copenhagen. Ane obtained her degree in 2006 after completing his thesis at the Department of Molecular Biology and Genetics, Research Centre Foulum, Danish Institute of Agricultural Sciences. After obtaining her Master's degree Ane started her PhD project "The characterisation of genes involved in pulmonary hypertension syndrome in chicken" at the Animal Breeding and Genomics Centre, a project that was funded by the Dutch Technology Foundation (STW). This project resulted in this thesis. Currently, Ane is continuing her scientific career as an adviser Knowledge Centre for Agriculture, Cattle, Aarhus, Denmark. Ane is currently living in Randers, Denmark with Lars and they have two children, Cornelius (3 years old) and Benedikte (1 year old).

List of publications

Peered – reviewed articles

A.M. Closter, P. van As, M.A. Groenen, A.L. Vereijken, J.A. van Arendonk, and H. Bovenhuis, 2009. Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. Poult Sci. 88(3): 483-90.

P. Van As, M.G. Elferink, **A.M. Closter**, A.L. Vereijken, H. Bovenhuis, R.P. Crooijmans, E. Decuypere and M.A Groenen, 2010. The use of blood gas parameters to predict ascites susceptibility in juvenile broilers. Poult Sci. 89(8): 1684-91

A.M. Closter, B. Guldbrandtsen, M. Henryon, B. Nielsen and P.Berg, 2011. Consequences of elimination of the Rendement Napole allele from Danish Hampshire. J. Anim Breed Genet. 128(3): 192-200

A. M. Closter, M. G. Elferink, P. van As, R. P. M. A. Crooijmans, A. L. J. Vereijken, A. M. van Arendonk, M. A. M. Groenen and H. Bovenhuis, 2012. Genetic correlation between heart ratio and body weight as a function of ascites frequency in broilers split up into sex and health status. Poult Sci. 91: 556–564

Publications in review or preparation

A.M. Closter, P .Madsen, H. Bovenhuis, M.G. Elferink, M. A. M. Groenen and B. Guldbrandtsen. (2014, submitted to JAS). Genetic Analysis of Ascites in Broilers using Liability-Normal Mixture Model. Journal of animal science

Conference proceedings / abstract

A.M. Closter, B. Guldbrandtsen, M. Henryon, B. Nielsen and P.Berg, 2006, "The elimination of the allele Rendement Napole (RN) in the Danish Hampshire Pig has reduced genetic gain and decreased inbreeding". 57th European Federation for Animal Science (EAAP), Antalya, Turkey

A.M. Closter, P. Van As, M.G. Elferink, R.P. Crooijmans, A.L. Vereijken, G.A. Albars, M.A. Groenen and H. Bovenhuis, 2007, "Whole genome scan for the detection of genes affecting ascites in broilers". 5th European Poultry Genetic Symposium (EPGS), Brædstrup, Denmark

A.M. Closter, P. Van As, M.A. Groenen, A.L. Vereijken, J.A. Van Arendonk and H. Bovenhuis, 2008, "Genetic relationships between blood gas parameters and ascites in broilers". International Society of Aniaml Genetics (ISAG), Amsterdam, Netherlands

A.M. Closter, M.G. Elferink, P. Van As, A.L. Vereijken, J.A. Van Arendonk, R.P.M.A Crooijmans, M.A. Groenen, and H. Bovenhuis, 2009, "Genetic relationships between ascites and body weight at different ages in broilers". 60th European Federation for Animal Science (EAAP), Barcelona, Spain

A.M. Closter, M.G. Elferink, P. Van As, R.P. Crooijmans, M.A Groenen and H. Bovenhuis 2010. Genome-Wide Association Analysis Identifies Loci That Influence Ascites In broilers. 9th World Congress on Genetics Applied to Livestock Production (WCGalp) Liepzig, Germany 2010

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Training and Supervision Plan

The Basic Package (3 ECTS)	
Course on philosophy of science and/or ethics	2007
WIAS Introduction Course	2008

Scientific Exposure (12 ECTS) *Conferences, Seminars and workshops*	
European Poultry Genetic Symposium (*poster*)	2007
ISAG Conference (*poster*)	2008
EAAP annual meeting (*poster*)	2009
World congress on Genetics Applied to Livestock Production (*oral*)	2010
XI QTLMAS workshop	2007, 2009
Genetics of Milk Quality	2009
Genome-Wide Evaluation of Populations	2009
WIAS Science Day (oral 2011)	2007 – 2011

In-Depth Studies (19 ECTS)	
Modern Statistics for the Life Sciences	2007
Fortran 95 for Animal Breeders	2007
Understanding Genotype Environment Interactions	2007
Summer School in Statistical Genetics	2007
QTL Mapping, MAS, and Genomic Selection	2008
Epigenesis – Epigenetics	2008
Use of High-density SNP Genotyping for Genetic Improvement of Livestock	2009
Design of Animal Experiments	2009
Quantitative discussion group (Animal breeding and Genomics Centre)	2007-2010

Professional Skills Support Courses (3 ECTS)	
Dutch Language course 1st level,	2007
Course Techniques for Scientific Writing	2008
Teaching and supervising thesis students	2009

Research Skills Training (2 ECTS)	
Faculty of Agricultural Sciences , Aarhus University, Denmark	2010

Didactic Skills Training (10 ECTS)	
Lecturing Animal Breeding and Genetic, BSc course	2009
Supervision practical's Animal Breeding and Genetic, BSc course	2008, 2009
Supervising 2 MSc and 1 BSC students	2008, 2009

Total credits (ECTS): 49

Colophon

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