ABSTRACT

Title of dissertation: GENETIC VARIATION IN NITROGEN AND PHOSPHORUS LEVELS IN BROILER EXCRETA: OPPORTUNITY FOR IMPROVING BOTH BIRDS AND THE ENVIRONMENT

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Doctor of Philosophy, 2010

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The increase in poultry meat consumption has resulted in intensified poultry farming operations with consequent concentration of excreta in major production areas. The nutrient content in the soil surrounding the poultry farms has increased as a result of the high content of nitrogen (**N**) and phosphorus (**P**) in the poultry excreta. The current study aimed to propose a strategy to reduce the N and P content in excreta through genetic selection of broilers for efficient nutrient utilization. The traits measured (on a dry matter basis) were the percentage of N in the excreta (**PNE**) and the percentage of P in the excreta (**PPE**). Individual 24-hr excreta samples were collected from 6 wk old birds. Excreta samples were collected from a commercial breeding farm at two different time periods from line A and line B birds respectively, and analyzed for PNE and PPE. Analysis of excreta samples collected during the first period (197 bird samples belonging to 15 sire families) and second period (278 birds belonging to 25 sire families) suggested a

heritability of 0.08, 0.16 for PNE and 0, 0.20 for PPE, respectively. Phenotypic and genetic correlations between the measured traits from the two lines were very low; however, phenotypic correlation analysis of PNE and PPE with other traits of commercial interest showed some favorable as well as neutral associations. Blood samples collected from the birds were used for an association study of the excreta traits with four candidate genes. The candidate genes were selected based on the results of previous research. Some of the SNPs from the candidate genes were found to have additive and dominance effect on the excreta and production traits and were usually favorably associated with mutations in higher frequency in the populations. The results suggest that genetic selection of birds for PNE and PPE could improve the environment and the market value of the birds.

GENETIC VARIATION IN NITROGEN AND PHOSPHORUS LEVELS IN BROILER EXCRETA: OPPORTUNITY FOR IMPROVING BOTH BIRDS AND THE ENVIRONMENT

by

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2010

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Dedication

I dedicate this dissertation to my loving family. Particularly to my understanding and patient wife, Ambili, who has put up with these many years of research. I must also thank my loving mother, and my equally loving in-laws who have great understanding and have given me their fullest support. Finally, I dedicate this work to my late father, Kochukrishnan Appukuttan, who supported my hopes and encouraged me greatly in the pursuit of excellence in science.

Acknowledgments

I owe my gratitude to all the people who have made this dissertation possible and because of whom my graduate experience has been one that I will cherish forever.

First and foremost I'd like to thank my advisor, Assistant Professor Frank Siewerdt for giving me an invaluable opportunity to work on challenging and extremely interesting projects over the past four years. He has always made himself available for help and advice and there has never been an occasion when I've knocked on his door and he hasn't given me time. It has been a pleasure to work with and learn from such an extraordinary individual.

I would also like to thank Professor Rick A. Kohn for allowing to use his laboratory to do my research work. Without his consent to use his laboratory, this dissertation would have been a distant dream. Thanks are due to Associate Professor C. Roselina Angel, Dr. George R. Wiggans and Dr. David L. Pollock for agreeing to serve on my thesis committee and for sparing their invaluable time reviewing the manuscript.

My colleagues at the laboratory have enriched my graduate life in many ways and deserve a special mention. José Adrián Carrillo, Taylor E. Callicrate, and Ashley D. Franklin were helpful in maintaining shared work culture in the laboratory without any complaints. I would like to give a special thanks to Dr. Telmo Oleas (Dr. Rick Kohn's laboratory) for helping me in analyzing the nitrogen and phosphorus content. He was extremely patient and spend a lot of his valuable time to help me.

I would also like to acknowledge help and support from Perdue Farm crew

during the sample collection period. Dr. Frank Siewerdt, Dr. David L. Pollock, Dr. Morteza Sadjadi, Dr. Alex Sandro Schierholt, and other Perdue crew helped me a lot in collecting excreta and blood samples from perdue broiler birds.

I would also like to think that I am very fortunate to attend some of the best lectures in Biometrics from Dr. Frank Siewerdt and Dr. Bahram Momen. I am also very thankful to all the faculty in Animal and Avian Science Department for all the help they have provided during the four years of study.

I owe my deepest thanks to my family - my mother, my late father, and my wife who have always stood by me and guided me through my career, and have pulled me through against impossible odds at times. Words cannot express the gratitude I owe them. I would also like to thank Dr. Kishor Kumar K. S. wholeheartedly for encouraging and instilling confidence in me to pursue a career in research.

My housemate at my place of residence have been a crucial factor in my finishing smoothly. I'd like to express my gratitude to Madhu Uppalapati for his friendship, support, and cooking.

I would like to acknowledge Perdue Farm, for all the projects discussed herein and for partially funding this work. I also like to thank J. P. Barin (São Paulo, Brazil) for assitance with the SNP analysis.

It is impossible to remember all, and I apologize to those I've inadvertently left out.

Lastly, thank you all and thank God!

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ATP	adenosine 5‘- triphosphate
BLUP	best linear unbiased prediction
BV	breeding value
BW	body weight
C	carbon
CAFO	concentrated animal feeding operations
CV	coefficient of variation
DM	dry matter
DNA	deoxyribonucleic acid
EBV	estimated breeding value
EPD	expected progeny differences
FAO	food and agricultural organization
FC	feed consumption
FCR	feed conversion ratio
GBV	genomic breeding value
GWS	genome-wide selection
HAP	high available phosphorus
$HPO_4{}^{2-}$	hydrogen phosphate ion
IBD	identity-by-descent
IP_6	inositol hexakisphosphate
LD	linkage disequilibrium
LE	linkage equilibrium
MAS	marker assisted selection
ME	metabolizable energy
MUN	milk urea nitrogen
N	nitrogen
N_2	nitrogen gas
NH_3	ammonia
NO	nitric oxide
NO_2	nitrogen dioxide
Nr	reactive nitrogen
O	oxygen
P	phosphorus
PBA	phytate phosphorus bioavailability
PNE	percentage of nitrogen in the excreta
PPE	percentage of phosphorus in the excreta
QTL	quantitative trait loci
RFC	residual feed consumption
RFLP	restriction fragment length polymorphism
S	sulfur
SE	standard error
SSLP	simple sequence length polymorphism
SNP	single nucleotide polymorphism
WG	weight gain

Chapter 1

Increase in Reactive Nitrogen: A myth or reality?

1.1 Introduction

Nitrogen (**N**) was considered to be discovered in the late 18th century through the works of several early chemists-Scheele (1742-1786, Sweden), Rutherford (1749-1819, Scotland), and Lavoisier (1743-1794, France) (Galloway and Cowling, 2002). Chemical elements like N, carbon (**C**), phosphorus (**P**), oxygen (**O**), and sulfur (**S**) are all essential for living organisms. With the exception of N, all other elements are readily available to sustain life forms (Galloway et al., 2003). The element N becomes part of molecules which build life as well as those that destroy life. Nitrogen is an integral part of nucleotides that determine the genetic composition all living organisms and the enzyme proteins that drive the metabolic machinery of every living cell. Nearly 80% of the total mass of Earth′s atmosphere is occupied by the triple-bonded nitrogen gas (N_2) (Galloway and Cowling, 2002). Even though the total amount of N in the atmosphere, soils, and waters of Earth is approximately 4×10^{21} grams (g)-greater than the mass of all the other four elements (Mackenzie, 1998), 99% of N is not available to more than 99% of living organisms (Galloway et al., 2003). Most organisms cannot utilize the huge reservoir of atmospheric N because of the inability to break the triple bond of N_2 molecule′s two atoms. For living organisms to grow and thrive, they need the element N in a reactive fixed

form that is bonded to C, H, or O. The reactive N (Nr) can be either in oxidative (nitrate, NO_3) or reductive (ammonia, NH_3) forms (Galloway and Cowling, 2002; Fields, 2004).

In nature, Nr is produced by certain unique microorganisms through the process of N_2 fixation. A few free-living bacteria, blue-green algae, and certain symbiotic bacteria are involved in producing the biologically active reduced forms of N such as ammonia, amines, and amino acids (Galloway and Cowling, 2002; Vitousek, 2002). Oxidative fixation of gaseous N_2 by natural processes such as lightning adds 3-10 teragrams (Tg) of Nr per year globally. The energy generated by lightning converts O and N into nitric oxide (NO), nitrogen dioxide (NO_2), and nitric acid (HNO_3). Nitric acid gets transported to the ground through rain, snow, or hail. Through this mode of transportation, nitric acid acts as an important source of Nr in areas with scarce N-fixing plants (Fields, 2004).

Biological N fixation was the dominant means by which new Nr was made available to living organisms in the pre-human world. The discovery of biological N fixation in the late 19th century coupled with the demand for food by increasing human populations enhanced the global search for natural deposits of Nr (Galloway and Cowling, 2002). In 1913, German scientists Fritz Haber and Carl Bosch developed the Haber-Bosch process for converting nonreactive atmospheric N_2 to ammonia (Fields, 2004). The Haber-Bosch process triggered the unlimited supply of Nr that could be used to grow both food and make explosives (Galloway and Cowling, 2002). Nitrogen fertilizer applications had increased their worldwide use to nearly 80 million tons (Mt) by the late 1980s (Smil, 2002). Currently, the Haber-Bosch

process is indirectly involved in the production of more than half of the food eaten by the human population (Smil, 2001).

1.2 Changing trends of Global Reactive Nitrogen (Nr) production: Past vs. Present

Even though the human population quadrupled from 1890 to 1990, world energy production has increased 50-fold and the volume of agricultural grain production has increased ten times. About 75% of the energy in the past was provided by biomass fuels and the rest by coal (Smil, 1994); use of fossil-fuel combustion was limited and it created only 0.6 Tg N/yr in 1890s through production of oxides of N (NO_x)(van Aardenne et al., 2001). Crop production was primarily carried out by utilizing recycled crop residue and manure before the Haber-Bosch revolution. Smil (1999) estimated a bulk share of Nr in the 1900s from cultivation (15 Tg N/yr) compared to guano mining (0.02 Tg N/yr), and nitrate deposits (0.13 Tg N/yr). In the 1890s, the total anthropogenic Nr creation rate for food production (around 15 Tg N/yr) was less than to the natural rate of Nr creation (300 Tg N/yr) by terrestrial ecosystems (100 Tg N/yr) and marine ecosystems (140 Tg N/yr). Lightning contributed fixing of additional 5 Tg N/yr (Galloway et al., 1995; Galloway and Cowling, 2002).

The world population has increased by 78% and Nr has increased by 120% since 1970 (Galloway et al., 2008). The most dramatic increase in Nr in the 1990s were attributable to human creation. In the 1990s, cultivation-induced Nr was estimated

to be 40 Tg N/yr (Galloway et al., 1995), while Haber-Bosch process generates 85 Tg N/yr (Smil, 2002) and fossil fuel NO_x emissions increased to 21 Tg N/yr (Levy et al., 1999; van Aardenne et al., 2001). Thus, in 1990, Nr created by anthropogenic activities was estimated to be around 140 Tg N/yr, which is 9-fold increase over 1890 in contrast to 3.5 fold increase in human population. Reactive nitrogen further increased to around 165 Tg N/yr in 2000 due to a 5 times increase in Nr from food production than the energy production (Galloway et al., 2003). Howarth et al. (2002) reports about half of the inorganic N ever used on the planet was applied in the past 15 years.

Despite the increase in human population, starvation and malnutrition have declined in many regions especially Asia (Townsend et al., 2003) which is made possible by conversion of natural grasslands and forests to croplands. This created a decrease in natural terrestrial N fixation from 100 Tg N/yr to 89 Tg N/yr in the 1990s (Galloway and Cowling, 2002).

A human population of 5.3 billion in the 1990s created about 110 Tg N/yr of Nr for food production, but needed only around 11 Tg (at 2 kg/person/yr of protein to survive). The excess 100 Tg N/yr that is produced will be distributed in the environment without entering the human body. The excess amount of Nr escapes into the environment in several steps of the N cycle. Based on the average estimation of Nr loss (Bleken and Bakken 1997; Mosier et al., 2000), efficiency of feeding humans by growing plant protein (14%) is higher when compared to growing animal protein (4%).

1.3 Reactive Nitrogen: Effects

Globally, Nr created by human action since 2000 (approximately 165 Tg N/yr) can either be accumulated (stored) within the ecosystem or denitrified. Currently, it is difficult to estimate the relative importance of these processes (Galloway and Cowling, 2002). Galloway et al. (2003) suggests that sustenance of the increasing large population of human beings increases the risk of wide dispersion and accumulation of Nr because Nr creation rates are greater than rates of denitrification to nonreactive N_2. In the United States, about two thirds of Nr inputs were denitrified or stored in soils and biota while one third was exported to riverine flux, food and feeds, as well as atmospheric advection to oceans in 1997. Annual denitrification from agroecosystems in the United States can produce as much as 6 Tg Nr/yr (Howarth et al., 2002) while studies done in Europe estimated denitrification from agroecosystems as 8 Tg Nr/yr (van Egmond et al., 2002). Generally, most of the Nr received in agroecosytems gets transferred to other systems while a smaller portion is denitrified to N_2. For watershed landscape units composed of agroecosystems, forests and urban areas, the average Nr input from atmospheric deposition, fertilizer use, food/feed import and N_2 fixation was 3420 kg Nr $km^{-2}yr^{-1}$, and of that the denitrified were around 48% (Galloway and Cowling, 2002). Even though a large proportion of Nr in aquatic ecosystems are denitrified, most anthropogenic Nr within agroecosystems is not converted to N_2. Aquatic ecosystems like groundwater are accumulating Nr at low rates although Nr loss occurs through denitrification and advection of nitrates to surface waters (Galloway et al., 2003). Wetlands are

highly efficient in denitrification but nearly half of the wetlands in the United States have been destroyed since 1780 (Gleick, 1993). Even though coastal ecosystems are more efficient than terrestrial ecosystems in denitrification, the period of time Nr spend in the coastal ecoystem can have obvious consequences like increase in anoxic or hypoxic water masses which are called dead zones (e.g. Gulf of Mexico, the Chesapeake Bay, Florida Bay, the Baltic Sea) (Diaz and Rosenberg, 1995). Since denitrification is not keeping pace with the creation of new Nr, it keeps on accumulating in environmental reservoirs like atmosphere, soils, and waters. The accumulation of Nr can cause undesirable effects on humans (Wolfe and Patz, 2002) and ecosystems (Rabalais, 2002).

Increased amount of Nr as NO_x can lead to production of tropospheric ozone (Chameides et al., 1994) and aerosols. Reactive airways disease (RAD), coughs, asthma, chronic respiratory diseases are induced and aggravated by ozone exposure (von Mutius, 2000; Townsend et al., 2003). Ammonia plays a role in the direct and indirect effects of aerosols on radiative forcing and on global climate change (Seinfeld and Pandis, 1998). Multiple studies have also shown a positive correlation between particulate matter air pollution and cardiovascular diseases, respiratory diseases, reduced lung function, and overall mortality (Pope et al., 2002).

Another major Nr with direct health effects is the presence of nitrate in drinking water. Even in the USA, the 10 ppm nitrate-N maximum standard for safe drinking water adopted by World Health Organization is exceeded in 10-20% of groundwater sources (Townsend et al., 2003). Swine and Poultry concentrated animal feeding operations (CAFO) play a major role in severe instance of groundwater

contamination (Mallin, 2000). Of the 33 TgN/yr of grain produced by crop agroecosystems fed to animals globally, only about 15% is consumed by humans. The remaining Nr is lost as manure and waste (Smil, 2001; Smil, 2002). Although, manure can be reused, Nr is lost through air emissions (NH_3, N_2O, NO and N_2) and leaching of nitrate to ground or surface waters (Galloway et al., 2003). Contamination of groundwater with nitrate associated with increased fertilizer use can cause diverse health effects including reproductive problems (Kramer et al., 1996), methemoglobinemia and cancer (Townsend et al., 2003). Weyer et al. (2001) reports increased risk of bladder and ovarian cancers associated with nitrate levels even below the 10 ppm standard in Iowa.

Addition of N to the environment due to human activity can also have indirect ecological and health implications. Increase in atmospheric Nr deposition can first increase and then decrease forest and grassland productivity. Decrease in biodiversity in many natural habitats and increased acidification of lakes and streams with addition of Nr are also highly probable (Aber et al., 1995; Vitousek et al., 1997). Galloway et al. (2003) suggests that Nr is responsible for eutrophication, hypoxia, loss of biodiversity, and habitat destruction in coastal ecosystems. Worldwide increase in harmful algal blooms has been linked to anthropogenic nutrient loading. Harmful algal blooms can cause neurological, amnesic, paralytic, and/or diarrheic shellfish poisoning, as well as toxins production by various cyanobacteria (Burkholder, 1998). Tropospheric ozone deposition can decrease productivity of crops, forests, and natural ecosystems (Galloway et al., 2003). Global climate change and stratospheric ozone depletion is also attributable to increased Nr which

have huge impacts on the health of humans and of several ecosystems (Cowling et al., 1998). Teng et al. (1998) reported a positive correlation between concentrations of inorganic N in surface water and larval abundance for malarial *Anopheles sp.* mosquitoes.

1.4 Nitrogen Cascade

Multiple linkages among the ecological and human health effects of Nr molecules as they move from one environmental system to another is termed as N cascade (Galloway, 1998). In Figure 1.2, energy production by fossil fuel combustion causes conversion of atmospheric N_2 (or fossil Nr) into NO_x. An atom of N mobilized as NO_x in the atmosphere can increase ozone concentrations thereby decreasing the atmospheric visibility and increase in concentrations of small particles with an increase in precipitation acidity. Upon deposition to the terrestrial ecosystem, the same N atom can increase soil acidity, decrease biodiversity, and either increase or decrease ecosystem productivity. If the N atom is discharged into aquatic ecosystem, it can increase surface water acidity and lead to coastal acidification. Nitrogen converted to N_2O and emitted to atmosphere has a residence time of 120 years which definitely increases the greenhouse warming and decreases stratospheric ozone. Similar cascade effects occur for Nr from food production. Atmospheric N_2 is converted to ammonia by Haber-Bosch process (Galloway et al., 2003). 75% of the Nr created globally by humans is added to agroecosystems to sustain food production. About 70% of this Nr comes from Haber- Bosch process and 30% from cultivation-induced

biological N fixation. About half of the Nr fertilizer applied to global agroecosystems gets incorporated into crops and used as human food and livestock feed (Smil, 1999). The other half gets transferred to atmosphere as ammonia, NO, N_2O, or N_2 or lost as nitrate to aquatic ecosystems. Microbial facilitated denitrification of agricultural soil N to NO and N_2O ranges from trace to nearly 10 kg N/ha/yr (Williams et al., 1992). Denitrification rates of NO and N atom transferred to these downstream systems becomes part of the cascade (Galloway et al., 2003).

The cascade of Nr from one system to another gets enhanced in conditions causing limited Nr accumulation or loss of N_2 through denitrification. Galloway et al. (2003) suggests two ways to decrease the total Nr: (1) decreasing the rate of Nr creation during food and energy production or (2) by converting Nr back to N_2 following Nr creation and use.

1.5 Future Strategies: Reduction of Nr creation rate and increasing Nr to N_2 conversion

Reductions of NO_x emissions in the fuel sector can be carried out boldly by either switching from fossil-inorganic Nr to hydrocarbon-based fuel or by eliminating NO_x and other Nr species from the combustion process (Bradley and Jones, 2002). It is possible to decrease Nr creation from fossil fuel combustion so that it becomes a minor disturbance to the global cycle (Galloway et al., 2003). In the northeastern United States, Nr deposition to the research sites in the study by Aber et al. (2003) would decrease by 50% if fossil fuel combustion was no longer a source of NO_x

(Ollinger et al., 1993).

Increasing the efficiency of Nr use in food production industry will definitely decrease the Nr creation rate (Galloway et al., 2003). The traditional agronomic practices of the 1890s extended to today′s 1500 Mha of cultivated land could feed only a maximum of 3.2 billion people or 2.4 billion people (40% of today′s total) if the average per capita yields in 1990 were considered. Today, about 40% of the survival of humanity depends on Haber-Bosch created Nr. It is possible to decrease Nr creation globally if the affluent countries reduce their N applications from current level (35% of global N fertilizers) by half (Smil, 2002). North America with only 5% of the world′s population creates about 20% of the world′s Nr (Galloway and Cowling, 2002). China and United States rank first and second respectively in the list of world′s largest producer and consumer of synthetic N fertilizers (Smil, 2002). Food and Agricultural Organization (FAO) projected N fertilizer use globally in the year 2030 as almost 40% above the 2000 level (Roy et al., 2002).

The benefit of Nr creation through Haber-Bosch process and cultivation induced biological N fixation is undercut by the huge amount of Nr leaks. Smil (1999) states that of about 170 TgN of Nr added to global crop agroecosystems in1995, only 12% ultimately reaches the human body. In other words, to produce 1 kg of edible N in plant foods and crop-derived animal foods, a supply of 8 kg of the element in the fields is needed and for producing 1 kg of edible N in meat, eggs, or dairy products, nearly 7 kg of feed N is needed (Smil, 2002). Thus, future goals for decreasing the Nr creation rate should include strategies for increasing the efficiency of N use in crop and animal agriculture (Cassman et al., 2002), increasing Nr recycling within

agroecosystems (Smil, 2002), increasing use of cultivation-induced biological N fixation (Roy et al., 2002), providing incentives to reduce over fertilization (Howarth et al., 2002b), redistribution of Nr from high Nr production areas to where there is a need for Nr for food production (Erisman et al., 2001), more efficient production of animal foods and gradual dietary transformations (Smil, 2002), and afforestation along with wetland restoration (Galloway et al., 2003). Grant et al. (2006) reported that the Danish Action Policy of afforestation and wetland restoration reduced a total of 1600 tonnes N emissions during the period 1998-2003. Some of the N-efficient practices adopted by US triggered an increase in the corn cropping average grain output from 42 kg of grain per kg of fertilizer N in 1980 to 57 kg/kg N in 2000 (Fixen and West, 2002). Similar type of efficient use of N fertilizers were reported for British winter wheat and Japanese rice (Smil, 2001).

Increasing the conversion of Nr to N_2 should be another way to reduce the impact of Nr on the ecosystem. Even though there is limitation in N_2 formation in most environmental reservoirs except wetland-stream-river-estuary-shelf continuum, there are suitable intervention points to reduce the detrimental effects of Nr accumulation. These include intervention of Nr losses from animal and human waste and Nr transfer from agroecosystems to surface waters (Galloway etal., 2003). Globally, 21 Tg N/yr of the total 100 Tg N/yr produced as Nr-containing waste by animals and humans is not reused and is easily collectible, thus increasing the potential for a denitrification intervention (Cassman et al., 2002). Second intervention will be to take advantage of the denitrification patterns of wetland and riparian areas giving emphasis on optimizing N_2 production relative to N_2O (Galloway et al., 2003).

1.6 Livestock Production and Nitrogen Efficiency

Trichopoulou et al. (1993) suggested that switching from traditional North American food consumption pattern to a Mediterranean diet will reduce the feed needed to produce meat protein by about 40%, which in effect reduces the overall Nr production. During the past generation, several European countries like Germany and France reported a drop in meat-eating habits of about 15% and 10% respectively. On the flip side, there are strong arguments to have meat proteins as an important part of human diet. Human adults and children cannot synthesize some amino acids (8 and 9 essential amino acids respectively) in the body and hence should be ingested from the food. An ideal protein should satisfy the dual requirement of containing all essential amino acids and also should be easily digestible. Egg, cow milk, meat, and fish proteins fit this requirement while plant proteins are deficient in at least one essential amino acid and are lower in digestibility (Smil, 2000). Net protein utilization is well above 80% for typical US mixed diets, but may be below 50% for pure vegetarian Asian diets (Huang and Lin, 1981). Overlooking these facts and suggesting a major change in diet habits will be putting the human health at risk. Thus, rather than reduction of animal based food products, it is important to find ways of efficient livestock production across the globe.

Comparisons of protein costs of animal foods (Figure 1.3) show that dairy foods can be produced most efficiently in terms of feed protein to food-protein conversion efficiency (Smil, 2000). If pork production converts feed protein to lean meat only half as efficiently as broilers do, then beef production can be termed as

the least efficient way of supplying dietary protein through animal feeding in certain regions where the food options are limited. During the late 1990s, average amount of concentrate feed to produce one kg of live-weight for all beef cattle was 5.5 times higher than broilers. Even though beef and pork are less-efficient, they make up 2/3 of the average supply of US animal protein. It is estimated that if the protein-rich US diet were composed of equal shares of dairy products, eggs, chicken, pork, and aquacultured fish, it would have used less than half of its concentrate feed and consequently half of N fertilizer (Smil, 2002). This clearly indicates the need for switching from beef and pork to chicken and dairy products for betterment in N efficiency.

1.7 Poultry Production in the United States: Reactive Nitrogen Concerns

In the 1940s, the average American ate less than 20 lbs of poultry (boneless weight) per year. By 1995, the per capita consumption of poultry by Americans increased to 63 lb (Figure 1.4). Most of this increase in consumption was from broiler meat (Lasley et al., 1988). Chicken consumption rose steadily from 1960 and it reached 86 lbs per person in 2006 while per capita beef consumption remained virtually unchanged and pork consumption declined. In addition, a combination of factors (genetics, nutrition, management changes) resulted in producing a more efficient broiler chicken in 2001, that required one-third the time and over a threefold decrease in feed consumption when compared to a 1957 broiler (Havenstein et al.,

2003). In the U.S., between 1960 and 2006, poultry prices rose by 2.7% per year while prices for other food rose by 4.2% per year according to Consumer Price Index (CPI). Thus, retail poultry became cheaper compared with other foods, encouraging a shift to poultry consumption (MacDonald, 2008).

The increase in per capita poultry consumption triggered the growth of poultry farms in U.S. In 2007, the total U.S. broiler production was 36.1 billion lbs and is expected to increase by 2% in 2008 (Haley, 2008). Until the middle part of the 20^{th} century, chickens were raised on farms and in backyards, but today, poultry production is concentrated in farms in the southeast, midwest, mid-Atlantic, and west coast of United States. In the late 1990s, almost all of the country′s poultry production was occurring in confined feeding operations of more than 100,000 birds each (Gardner, 1998). The changes in cost and profitability have led to interregional shifts and concentration of poultry production (Lasley et al., 1985). The direct effect of intensification is that much of the crop production is no longer fed to animals near the point of harvest; rather, crops are transported to animal feedlots while little animal waste is returned to the original fields where grains are harvested. Manure is applied at excessive rates to fields close to the feedlot operations or placed in lagoons. This trend is due to the direct result of availability of inexpensive inorganic N fertilizers, and so farmers were freed from recycling animal wastes for fertilizer (Howarth et al., 2002).

Intensive poultry farms emit large concentrations of NH_3, which gets deposited close to the source and increases oxidized NO and N_2O emissions (Skiba et al., 2006). Theobald et al. (2004) reported that livestock farms are the single largest source of

NH_3, thus intensification creates significant local hotspots of elevated atmospheric NH_3 concentrations. In the eastern United States, due to the intensification of poultry and other livestock farms, about 60% of anthropogenic NH_3 is contributed mainly by animal waste (Strader et al., 2001). The efficiency of N use for production of human-digestible protein from feed grains and forages on poultry farms is about 40% to 50% (Galloway et al., 2003). Increasing the efficiency of N use in poultry farms will certainly decrease the emission of environmental pollutants like NH_3 and other Nr. Other ways of decreasing the NH_3 loss include lowering the pH of the litter by applying aluminium sulfate (Moore et al., 1995). Thus, by adopting adequate measures for reducing NH_3 emissions in poultry farms and through increasing the efficiency of N use in poultry production, the growing concerns of N pollution from the poultry industry can be mitigated.

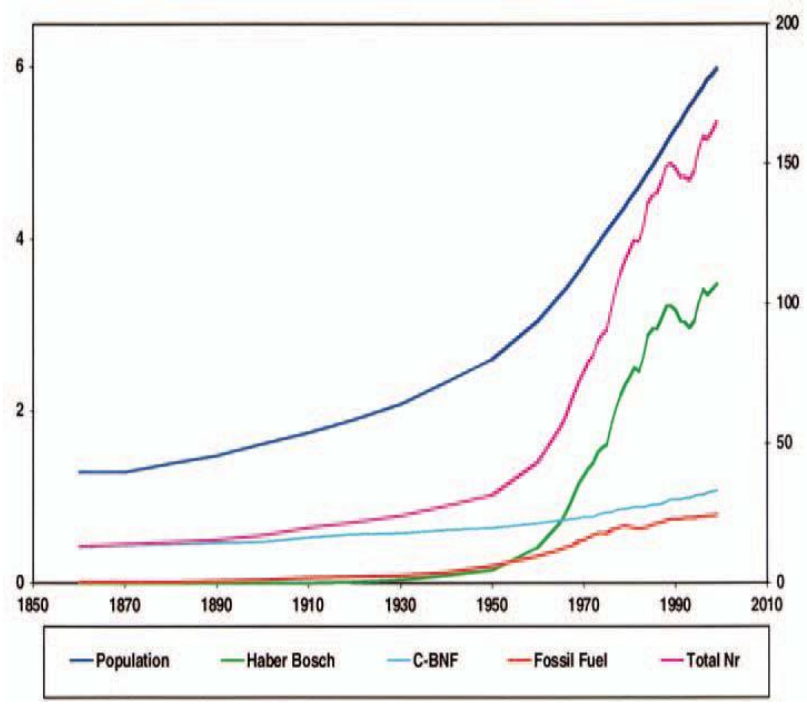

Figure 1.1: Global population trends from 1860 to 2000 (billions, left axis) and reactive nitrogen (Nr) creation (Tg N/yr, right axis) (Galloway et al., 2003).

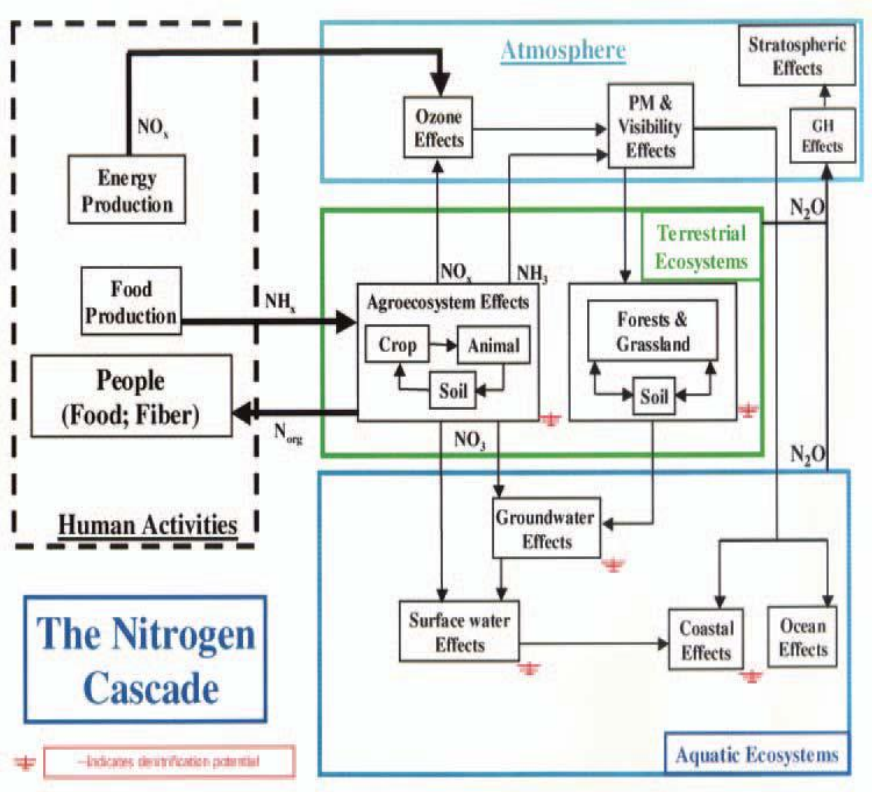

Figure 1.2: Illustration of the nitrogen (N) cascade showing sequential effects that a single atom of N can have in various reservoirs after it has been converted from a nonreactive to reactive form (Galloway et al., 2003).

	Milk	Carp	Eggs	Chicken	Pork	Beef
Feed conversion (kg of feed/kg^{-1} of live weight)	0.7	1.5	3.8	2.3	5.9	12.7
Feed conversion (kg of feed/kg^{-1} of edible weight)	0.7	2.3	4.2	4.2	10.7	31.7
Protein content (% of edible weight)	3.5	18	13	20	14	15
Protein conversion efficiency (%)	40	30	30	25	13	5

Figure 1.3: Protein contents of major animal foods and feed conversion efficiencies of their production (Smil, 2002).

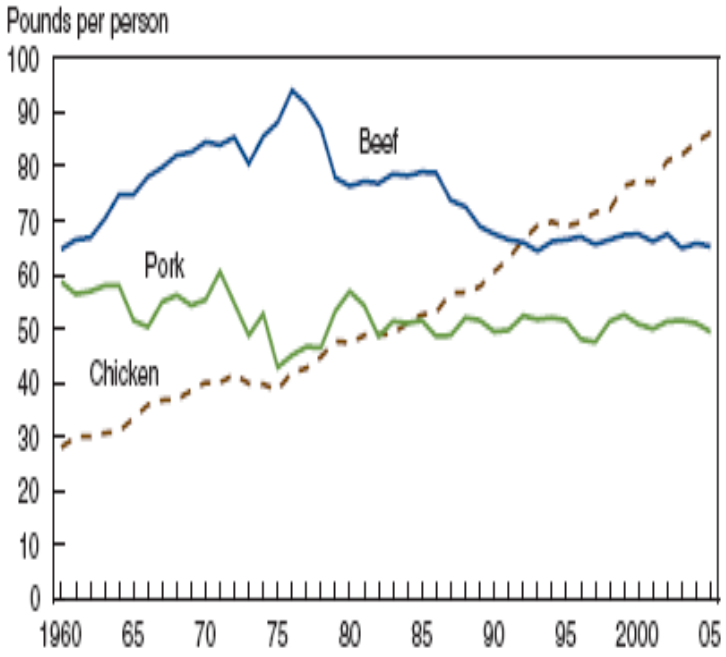

Figure 1.4: Trends in per capita consumption, 1960-2005 (MacDonald, 2008)

Chapter 2

The Specter of Phosphorus Pollution

2.1 Introduction

Phosphorus (P) is the eleventh most abundant element in the Earth′s crust (Macia, 2005) and it is number 15 in the periodic table (Gleason, 2007). The amount of P estimated on land (4.3×10^{20} g) and on the ocean floor (3.9×10^{20} g) is similar (Macia et al., 1997). The element P was discovered in the 17th century (1669) by German alchemist Henning Brand. He found a white material which glowed in the dark while distilling his urine to find some kind of "life essence" (Djodjic, 2001). This glow was explained plausibly by van Zee and Khan (1974) as a reaction of oxygen and surface P forming light emitting short-lived molecules HPO and P_2O_2. Phosphorus is highly reactive, thus it is never found in nature as a free element; it exists in physical form as either white or red solid. Elemental P does not have much usage; P compounds are widely used in baking powders (phosphoric acid), pesticides (phosphine), soaps and detergents (phosphorus trichloride). Polyphosphates and metaphosphates are used as water softeners in detergents (Djodjic, 2001).

2.2 Biological Role of Phosphorus

Many fundamental biochemical functions in living systems are performed by P compounds (Macia, 2005). Phosphorus plays a major role in the metabolism of carbohydrates, amino acids, and lipids (Zhang et al., 2003). The esters of phosphoric acid, including sugar phosphates and nucleotides, play leading role in biochemical processes like glycolysis and nucleic acid metabolism (Macia, 2005). Phosphorus compounds (phosphate groups) are also important in bonding the nucleotides together in DNA and RNA of living organisms.

Inside the living cell, complex processes like signal transduction systems (MAP kinase system, Ras system etc.) and cell cycle depend on phosphorylation of proteins. Phosphorylation/dephosphorylation of cellular compounds is a mechanism for regulating enzyme activity as well as transport and storage of cell compounds (Brown, 1999). Intracellular energy transfer is carried out by P-containing compound adenosine 5′- triphosphate (ATP); it is produced as energy source during the processes of photosynthesis and cellular respiration (Knowles, 1980). Some other cyclic nucleotide derivatives play significant role in biochemical activities of diverse hormones, in synaptic transmission of nervous system, and also in immune response. Also, crucial roles ranging from active carrier transport through cellular and mitochondrial membranes to bone metabolism are carried out by P-containing ions like HPO_4^{2-}.

2.3 Eutrophication

The United Nations (1998) estimates an increase in world population by an additional 2 billion by 2030. Therefore, finding ways to satisfy humanity′s water demands, while protecting the life-support functions of fresh water systems, will be one of the critical and difficult challenges of the 21st century. The global hydrological cycle annually makes available several times more fresh water than is needed to sustain the current world population of six billion people, but a large part of it is not accessible to human use due to quick runoffs such as floods (Postel, 2000).

The major challenge for societies in accomplishing cleaner water resources and sustainable agricultural systems will be to limit runoffs and leaching of nutrients supplied for plant growth while maintaining modest mineral nutrients in lakes, rivers, and estuaries for reasonable level of productivity (Correll, 1998). Mobilization of nutrients like P through land clearing, production and application of fertilizer, discharge of human waste, animal production, and combustion of fossil fuels can disturb the normal nutrient content in the aquatic ecosystem. Gilluly (1970) describes the complex process of eutrophication as the gradual change in productivity of lakes from oligotrophic to marshes and finally to solid land as a result of the supply and accumulation of silts and nutrients from streams. Historically, many water bodies have progressed from oligotrophic or low productivity to mesotrophic productive conditions, to hypertrophic or over-enriched eutrophic conditions (Correll, 1998). Eutrophication accounts for 50% of the impaired lake area and 60% of the impaired river reaches in the United States (U.S. EPA, 1996). Eutrophic conditions

can result in algal or cyanobacterial growth, anoxia, fish kills, and thus leading to a reduction in biodiversity (Likens, 1972; Jaworksi, 1981). Sometimes, cyanobacteria and other noxious microorganisms produce toxins which are dangerous to humans and livestock (Martin and Cooke, 1994).

Eutrophication and its effects are so important that at least 2000 articles regarding various aspects of eutrophication were published before 1972. Although eutrophication is less important in rivers, but larger and slow-flowing ones used for water supply are always at risk. In the 1970s, the most common cause of artificial eutrophication was sewage while agricultural wastes were less commonly responsible (Lund, 1972). After 1970s, tighter controls of point sources of nutrients (e.g. municipal wastewater treatment plants) decreased the nutrient loading from these sources into the aquatic environment while increase in agricultural activities shifted the focus on nonpoint sources of nutrients like agricultural runoff (Moore and Miller, 1994). Jaworski et al. (1992) suggest that runoffs from animal farms are generally linked to downstream eutrophication in freshwater systems such as rivers.

2.4 Role of Phosphorus in Eutrophication

While eutrophication is a complex phenomenon that varies from lake to lake in its character, rates and causes, most of the eutrophic waters in United States have P or N as its limiting nutrients, with P apparently considered as the major cause of eutrophication (Gilluly, 1970; Lund, 1972). Usually the lakes in North America have P as limiting nutrient for eutrophication, but nitrates are also attributed a key

role in eutrophication of lakes such as Lake Tahoe (Gilluly, 1970). Although P and N supplies contribute to freshwater eutrophication (OECD, 1982), P is attributed as the primary cause for eutrophication of lakes (Schindler, 1977).

During the period 1960-1970, some bioassay experiments were conducted in small bottles and it gave the evidence that C might play a major significant role in eutrophication (Lange, 1967; Lange, 1970). But the small bottle experiments excluded processes that occur in natural water bodies such as turbulence of water and interaction with overlying atmosphere. Also, the proportion of alkalinity supplied by hydroxyl ions affects the rate at which C invades the lake ecosystems (Schindler and Fee, 1973). These facts led Schindler (1977) to categorically conclude that control of C no longer deserves consideration as a method for managing eutrophication of natural waters. In most of the temperate estuaries and coastal ecosystems, N is primarily responsible for eutrophication (Howarth, 1988), but the global attention has been focused on P primarily because of the difficulty in controlling exchange of other essential nutrients like N and C between the atmosphere and water. Besides the atmospheric exchange, N is also fixed by some blue-green algae (Sharpley, 1999).

Carpenter et al. (1988) reported that P inputs into fresh water can accelerate eutrophication while there are reported growths of blue-green alga when P was added to small lake basins (Schindler, 1974). Maloney et al. (1972) conducted a series of bioassays in lake waters from the Great Lakes region of the U.S.; it showed that *Selenastrum capricornutum* cell number responded to addition of P rather than N. Experimental studies of mangrove forests on islands in Belize have concluded that mangroves are more severely P limited (Feller, 1995) whereas estuaries at their

upstream ends change from P limiting in the spring to N limiting in summer and fall (Lee et al., 1996). Based on these facts, P can be arguably considered as the most important limiting element for eutrophication.

Phosphorus can be discharged into receiving waters from both point and nonpoint sources. Nowadays, the intensification of land use created more supply of P from nonpoint sources while more efficient sewage treatment technologies reduced the point sources of P (Correll, 1999). According to Correll (1996), high-intensity pastures are serious nutrient sources because of high rate of application of exogenous inorganic and organic nutrients along with direct inputs of animal waste. When these nutrients reach a body of water through runoffs, they cause nutrient loading and subsequent eutrophication.

2.5 Poultry Production and Phosphorus Pollution in the United States

The biggest environmental challenges faced by the poultry industry today are atmospheric NH_3 emissions and nonpoint source P runoff from litter fertilized fields. Before the 1980s, farmers were unaware of the issues of P because N-based management had been practiced and advocated by the farm advisors. Now, farmers are becoming aware of the environmental problems caused by P (Sharpley, 1999). The rapid growth and intensification of crop and poultry production in many areas such as Southeastern States of U.S. has created local and regional imbalances in P inputs and outputs (MacDonald, 2008). Sharpley (1999) reports that only 30% of the fertilizer and feed P input to farming systems is incorporated into produced

agricultural crops and animal products. The annual on-farm P surplus in North Carolina for a typical modern broiler farm (6,380 kg) is almost 5-6 times large than for a dairy farm (1,141 kg) under traditional diet and management practices (Tarkalson and Mikkelsen, 2003). The high concentration of poultry litter can in effect cause nutrient pollution to air and water as well as risks from contagious spread of poultry diseases (MacDonald, 2008).

Phosphorus is the second most abundant mineral in the animal body and approximately 80% of P is found in the bones and teeth (Viveros et al., 2002). Poultry diets contain primarily ingredients of plant origin like cereal grains, cereal by-products, and oil seed meals (Zhang et al., 2003). About 60 to 82% of the total P in seeds of cereals, grain, legumes, and oil-bearing plants are constituted by phytate-P (Ravindran et al., 1994). Even though P is an essential element required by poultry for its normal growth and development (Zhang et al., 2003), chickens and other monogastric animals are lacking or limited in phytase activity in the digestive tract which is required to breakdown the phytate-bound P in plant feedstuffs (Raboy, 1990; Pallauf et al., 1994). The inadequacy of utilization of phytate-P in poultry along with the spatial separation of grain and animal production results in substantial losses of P through excreta and creates pollution threat when manure is applied to land (Ravindran et al., 1995; Sharpley, 1999). Previously, poultry litter application rates contributed to the increase in P load in soil; it was based on meeting the crop N requirements while overlooking the P content in the litter (Field et al., 1985). However, this strategy was changed in the mid-1990's; litter application rates based on mineral P fertilizer recommendations were introduced.

But, Robinson and Sharpley (1996) reported that manure application rates should be based on soil tests rather than just on mineral fertilizer trials.

In addition to the threat of pollution, P in the phytate form can also induce negative effects in physiological functions of birds. Singh and Krikorian (1982) reported that phytates in plant feedstuffs are able to bind to endogenous proteases such as trypsin and chymotrypsin in the gastrointestinal tract resulting in reduction in enzyme activity and subsequently decrease in digestibility of protein and amino acids. Also, fully phosphorylated myo-inositol (IP6) or phytic acid is a potent chelator of many mineral ions such as Ca, Mg, Zn, and Cu; thus it decreases the availability of these minerals for absorption (Morris, 1986; Selle et al., 2000).

2.6 Strategies for Reduction of Phosphorus Pollution from the Poultry Industry

Utilization of phytate-P in chickens has been the issue of research for decades. Nelson (1976) suggests that the ability of chickens to utilize phytate-P depends on hydrolysis of phytate to inositol and orthophosphoric acid. There were diverse reports suggesting the hydrolysis of phytate by phytase. While McCance and Widdowson (1944) reported some feed ingredients (e.g. wheat) have increased phytase activity compared with others (e.g. maize and seed meals), there were also reports of active bacterial phytase in the digestive tract (Warden and Schaible, 1962). Kiiskinen et al. (1994) showed that broilers were able to utilize phytate-P when animal feed was supplemented with microbial phytase. Exogenous phytase addition can

reduce the dietary nonphytate P formulations without affecting bird performance or bone mineralization and subsequently reduce the concentration of total P and water soluble P by 30 and 50% respectively in the litter of 6 wk-old male broilers (Applegate et al., 2003). Angel et al. (2005) reports that proper management of the addition of exogenous phytase and dietary P can reduce the mass of total P and water soluble P excretion. In addition to reduction in P excretion, significantly increased weight gain, percentage egg production, egg weights, feed consumption, and tibia weights were reported in laying hens when diets were supplemented with phytase (Van der Klis et al., 1996). The capability in broilers for using a portion of phytate-P is also reported (Van der Klis and Versteegh, 1996).

Ertl et al. (1998) developed a mutant variety of corn called lpa1-1, which has a 65% reduction in phytate-P and a molar-equivalent increase in inorganic P. Feeding of the genetically altered corn to broiler chicks caused fecal P reduction in the range of 9 to 40%. The lap1-1 allele of the corn gene was later bred into a hybrid called high available P (HAP) corn by a Dupont Company called Pioneer. The hybrid corn (i.e. HAP) was shown to reduce the total P requirement in broilers by 11% (Huff et al., 1998). There are also reports that the bioavailability of P is increased by about 2-3 times in low phytate content corn when compared with the conventional corn (Douglas et al., 2000).

In addition to the above methods, field management practices also help in reducing P pollution. Chemical additions such as alum, quick lime, slaked lime, ferrous chloride, ferric chloride etc. in the poultry litter can reduce the water soluble P levels from $>$ 2,000 mg P/kg litter to $<$ 1 mg P/kg litter (Moore and Miller, 1994).

Since dissolved inorganic P is directly available to algae, it is suggested that management practices aimed at decreasing the P runoff should consider the bioavailable-P load (Sonzogni et al., 1982). Therefore, treating litter with some of the chemicals prior to field application will reduce the amount of soluble P runoff from the pastures (Moore and Miller, 1994). It is recommended to spread alum-treated poultry litter at 7.3 t.ha^{-1} in field applications (Guo and Song, 2009). Mueller et al. (1984) reported that the concentration of algal-available P was increased for a no-till site relative to other tillage treatment sites. However, there is also evidence that no-tillage system reduced soil erosion by almost 50-fold (Triplett et al., 1978). So, a combination of management practices will be more effective in reducing the outflow of P from agricultural land.

Chapter 3

Genetic Reduction of Excreta Components in Broilers

3.1 Introduction

In the United States, poultry management practices such as production of broilers in concentrated animal feeding operations contribute to accumulation of nutrients, primarily nitrogen (**N**) and phosphorus (**P**), in places close to poultry farms (Skiba et al., 2006). In these areas, the excreta output is far greater than the capacity of sequestration by the soil. Sims and Coale (2002) reported that about 70% of N and 82% of P coming into the Chesapeake Bay are contributed solely by the poultry industry. Excess deposits of N and P can invade the surrounding water bodies and stimulate increased algal growth on the surface of water. The direct consequence can be a decrease in fish and shellfish populations.

Excess deposition of poultry litter in cropping systems can result in nitrate contamination of groundwater (Bitzer and Sims, 1988); high levels of nitrate in ground water were documented to cause methemoglobinemia, cancer, and respiratory illness in humans (Kelleher et al., 2002). In addition to polluting the water bodies, N in the poultry excreta can enter the atmosphere by the volatilization of ammonia (Marshall et al., 1998). According to Galloway et al. (2003), increased N concentration in the atmosphere can directly and indirectly impact the human and ecosystem health on a regional and global basis.

Nutrient excess in poultry excreta originates either from incomplete digestion or inefficient absorption of dietary nutrients. This is important in the U.S. as there has been an increase in broiler production. In 2008, the annual broiler production was 36.9 billion pounds (2008 figures, USDA, 2009). Therefore, efficient strategies must be adopted to counterbalance the nutrient buildup in the environment.

Traditionally, poultry excreta nutrient management has been dealt with by the implementation of two major strategies, dietary modification to improve the utilization of N (Applegate et al., 2008) or P (Huff et al., 1998) or industrial disposal of excreta through composting, anaerobic digestion, or direct combustion (Kelleher et al., 2002). A promising alternative to the above methods is to use genetic selection as a tool for increasing nutrient utilization by the birds.

Differences in efficiency of feed utilization will result in variation in composition of excreta among individual birds fed the same diet. The observed variation in excreta composition can be partly attributed to genetic makeup of each individual. However, there are relatively few animal studies carried out on the genetic basis for nutrient utilization.

Wiener (1979) provided evidence for genetic differences in plasma copper utilization in sheep. In another study, genetic differences in nutrient excretion were established between maternal and paternal lines of pigs (Crocker and Robison, 2002). In broilers, large individual differences in phytate-P utilization were reported by Carlos and Edwards (1997) while another study (Zhang et al., 2003) in Athens-Canadian randomly bred chickens reported an estimated heritability of 0.09 for phytate-P bioavailability (**PBA**). These studies suggest the feasibility of increasing

the efficiency in N and P utilization by genetic selection.

The current research proposes that poultry excreta nutrients can be efficiently reduced by selection using the nutrient content in the excreta as a selection goal. I hypothesized that the genotypes of broiler chickens can be altered in a way that results in birds that are more efficient users of nutrients. Verifying the existence of genetic variation in excreta composition is the first step in the selection process.

The long-term goal of this research is to develop a detailed understanding of the genetics of excreta composition in broiler chickens. The specific goals for this study include: 1) evaluate genetic variability of excreta components in broiler chickens through association of excreta nutrient composition with specific genetic line or individuals within a line, 2) determine biological relationships between excreta components and traits of commercial importance.

3.2 Materials and Methods

3.2.1 Experimental Birds and Layout

The study was conducted in a commercial broiler farm facility. A total of 475 three-wk-old pedigreed male broilers belonging to lines A (197 birds) and B (278 birds) were used for evaluation in 2 separate sampling periods in September 2007 and February 2008, respectively. Birds from lines A and B belonged to 15 and 25 sire families, respectively. Further instances of coancestry between the birds were established by acknowledging paternal half-sibs among the sires. Line A is employed as a terminal broiler sire while line B is used to generate a commercial hybrid female.

Birds were raised on floor pens through 18 d of age and then moved to individual cages (46 cm length, 36 cm width, and 33 cm height). After an adaptation period of 3 d the feed conversion trial started, lasting 21 d. Each cage had a feed bin and a nipple drinker. During the experimental period, feed and water were available for ad libitum consumption. The cages were arranged in four rows in the chicken house; each row was considered as a block to account for the time of processing and for slight differences in ambient temperature within the facility.

3.2.2 Sampling

3.2.2.1 Bird and Feeder Weights

Individual bird weights and feed weights were obtained on d 0 and 21 of the trial. Feed disappearance and BW gain were calculated. Feed conversion ratio (**FCR**) was expressed as the ratio of feed consumption by BW gain during the experimental period. The birds were given a single standard grower commercial corn and soy based diet containing 21.75% CP and 3080 kcal ME/kg of diet. The diet met or exceeded the National Research Council (1994) recommended nutrients for broilers for growth. The research protocol for this trial was approved by the University of Maryland Animal Care and Use Committee.

3.2.2.2 Excreta Samples - Collection and Processing

Individual 24 h excreta samples were collected at the end of the trial. Collection of excreta was carried out by placing metal trays lined with parchment paper

underneath the cages on d 20. After 24 h, the trays were removed; collection paper was folded, stored in sealable plastic bags, placed on ice for transportation, and frozen until processed. Samples were thawed, the paper removed, and samples homogenized before being oven-dried (55°C for 72 h). The dried excreta samples were ground to pass through a 0.25-mm screen and stored in plastic containers for further analysis.

3.2.3 Excreta Composition Traits: Feasibility and Chemical Analysis

The nutrients (N and P) expressed as a percentage of excreta DM i.e. percentage of N in the excreta (**PNE**) and percentage of P in the excreta (**PPE**) were the traits measured in the study. During the sample collection period, several practical difficulties were encountered such as variable loss of N from the excreta into the atmosphere, difficulty in keeping a strict 24 h collection period from individual cages, daily variation in the amount of excreta produced by a bird, and the possible contamination of excreta with feed and feathers. The traits PNE and PPE were defined in this trial after evaluation of all the factors mentioned above.

Samples were analyzed in duplicate and the average value was used for calculations. Analysis for DM was conducted by oven drying approximately 0.25 g of excreta sample at 100°C for 24 h while ash content was determined by placing the dried sample in a crucible and ashing overnight at 575°C. The ash left in the crucible was boiled with 20 mL of 4 N HCl and 0.5 mL of 70% HNO_3 for estimation of PPE.

The PPE of excreta samples were determined by a modified version of Fiske

and Subbarrow (1925) method, procedure No. 670 (Sigma Diagnostics, St. Louis, MO). The samples prepared were reacted with ammonium molybdate under acidic condition to form phosphomolybdate. The Fiske and Subbarrow reducer, i.e. a mixture of sodium bisulfite, sodium sulfite, and 1-amino-2-naphthol-4-sulfonic acid (Sigma No. 661-11), was used to reduce the phosphomolybdate to form phosphomolybdenum blue complex. Phosphate concentration was measured at 655 nm.

Analysis for PNE was carried out on a small aliquot (approximately 0.1 g) of clean, forced-draft oven-dried (55°C for 72 h), and ground (< 2 mm) sample. The PNE content in excreta samples was determined by a combustion method using an FP 2000 nitrogen analyzer (Leco, Corp, St. Joseph, MN) as described by Adeola and Bajjalieh (1997).

3.2.4 Statistical Analysis

Residual feed consumption (**RFC**) was estimated by linear regression of feed consumption (**FC**) on weight gain (**WG**); RFC was expressed as the deviation between the actual FC by the bird and its predicted FC based on the linear regression equation. Estimates of the (co)variance components and genetic parameters were obtained using GLM procedure of the SAS software (SAS Institute, Cary, NC) and by restricted maximum likelihood using the MTDFREML software package (Boldman et al., 1995; Riley et al., 2002).

Sire families were ranked by fitting an animal model:

$y = X\beta + Z\alpha + e$

where y is the data, β are the fixed effects of blocks (rows of cages), α are the additive genetic values, and e are the random, independently distributed errors; X and Z are the design and incidence matrices linking the fixed and random effects to the data, respectively.

Genetic relationships between individuals are accounted for by including the additive genetic relationship matrix in the animal model equations (Henderson, 1984).

$$\left[\begin{pmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}\frac{{\sigma_e}^2}{{\sigma_\alpha}^2} \end{pmatrix}\right]\left[\begin{pmatrix} \beta \\ \alpha \end{pmatrix}\right] = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

where A^{-1} is the inverse of additive genetic relationship matrix, ${\sigma_e}^2$ is the variance of the error, and ${\sigma_\alpha}^2$ is the variance of additive genetic values.

Trait heritabilities were initially calculated based on the SAS GLM procedure (SAS Institute, Cary, NC) of partitioning the total phenotypic variance of each trait into variance between sire families ($\hat{\sigma}^2_{SF}$) and the residual variance ($\hat{\sigma}^2_e$). Heritabilities were computed from the estimated variance components: $\hat{h}^2_{SF} = \frac{4\hat{\sigma}^2_{SF}}{\hat{\sigma}^2_{SF}+\hat{\sigma}^2_e}$ (Falconer and Mackay, 1996), under the assumption that the individuals of the same sire family are half sibs.

The estimates of heritability were also obtained by single-trait analysis by restricted maximum likelihood with MTDFREML software (Boldman et al., 1995). This same program was used to estimate phenotypic and genetic correlations between the traits were in a two-trait analyses, done in pairwise fashion . The analyses were conducted by using pedigree information for two generations of ancestors (line A) and one generation of ancestors (line B) in the relationship matrix. The es-

timated variances for the corresponding trait and random values from single-trait analysis were used as priors in two-trait analysis; the values of these priors were not allowed to change during the estimation of covariances. The process was repeated until -2log likelihood remained unchanged in the first 6 decimal positions. The single-trait and two-trait analyses were both done at the level of convergence criterion (10^{-6}). The standard errors of the genetic and phenotypic correlation were estimated in the two-trait analysis using the method of creating a dummy factor for each trait to properly account for the missing observations (Kachman and Van Vleck (2007). Phenotypic correlations were also estimated by using the CORR procedure of SAS.

3.3 Results

3.3.1 Descriptive Statistics

The means, SE, CV, and range of the values for lines A and B are presented in Table 3.1. For line A, PNE values ranged from 3.03% to 10.85%, with a mean of 5.26% and SE of 0.07% while the range for PPE was between 1.38% and 2.59% with a mean and SE of 1.98% and 0.02% respectively. The range of PNE in A (7.82%) and B lines (4.42%) indicates adequate phenotypic variability for the trait (Figure 3.1 and Figure 3.2). The CV for most traits were high with the exception of FCR in line B. Line A has a much higher selection emphasis on FCR than line B and this was reflected in the present point estimates for FCR (1.82 and 1.92, respectively).

3.3.2 Genetic Parameters

Genetic and environmental variance components are presented in Table 3.2. The heritability estimates from single-trait analysis for PNE and PPE are shown in Table 3.3. Heritability estimate for PNE from line A was found to be lower than the calculated value obtained using the variance estimates from GLM procedure (0.15 ± 0.182). The single-trait analysis heritability estimate from line A was approximately zero for PPE; however the calculated value of heritability estimate from GLM procedure was negative (-0.059 ± 0.11). Standard errors for heritabilities were calculated based on the methods described by Becker (1984). The difference in results are due to a very low genetic variance (approximately 0) for PPE in line A, creating a negative G matrix consisting of additive genetic variances on the diagonal and covariances elsewhere. Line B had a higher heritability estimate for PPE with respect to line A. The estimates of phenotypic correlation were consistently very low in lines A and B. There was a high negative genetic correlation between the traits in line A but the same correlation was moderately positive in line B. The inconsistency in those results may originate from the very low genetic variance for PPE in line A.

3.3.3 Correlations with Other Traits

In line A, PNE was negatively correlated ($P < 0.01$) with WG and FC while it was positively correlated ($P < 0.05$) with FCR. However, WG, FC and FCR were not significantly correlated with PPE in line A. In this same line, a positive correlation ($P < 0.01$) between WG and FC was observed whereas WG was negatively correlated

($P < 0.01$) with FCR; but FC and FCR were not found to be correlated. In line B, the correlations of PNE with WG and FCR were significant ($P < 0.01$); however, there was no correlation between FC and PNE. The correlations of PPE with WG, FC, and FCR in line B did not showed any significance. Correlations between WG and FC were positive ($P < 0.01$) while WG showed a negative correlation ($P < 0.01$) with FCR in line B. Similar to line A, the correlation between FC and FCR was not significant in line B. In lines A and B, the correlations of RFC with FC and FCR were highly significant ($P < 0.01$); however there were no correlations of RFC with WG and PPE. RFC and PNE in line B had a significant ($P < 0.05$) low positive correlation.

3.4 Discussion

In this article I approach a genetic solution to the environmental pollution generated from poultry farms. Selection experiments for improved feed conversion (Leenstra and Pit, 1988; Buyse et al., 1998), or increase in BW (Dunnington and Siegel, 1996; Nestor et al., 1996), and estimation of genetic parameters for fat, carcass, and body composition traits (Zerehdaran et al., 2004; Gaya et al., 2006) in broilers have been documented previously. However, selection experiments in broilers to reduce environmental output of N and P (Zhang et al., 2003) are scarce even though environmental concerns associated with animal agriculture are increasing every year. For example, pollution from the chicken industry, especially in the state of MD, is showing an increasing trend every year. So the state is considering diverse

approaches to handle the 294 million kg of chicken manure produced every year; but it is also a sensitive issue to handle since the MD economy is greatly benefited by the chicken industry alone (Urbina, 2008).

In the current study, the heritability estimates for PNE in line B were comparable with a study conducted by Mitchell et al. (2005) in Holstein cows; but these authors defined milk urea nitrogen (**MUN**) as the trait and obtained heritability estimates of 0.15 and 0.22 for wet chemistry MUN and infrared MUN, respectively. In chickens, there were no reports found on selection experiments using N in the excreta as part of the selection goals. The values in this research are believed to be the first ones to be reported.

The PPE estimate of heritability in line A was approximately equal to zero. The heritability estimate reported by Zhang et al. (2003) for PBA in Athens-Canadian randomly bred chickens was also low (0.09). The heritability estimate for PPE in line B was higher than those reported by Zhang et al. (2003). Variation in the heritability estimate for PPE between the current study and those reported by Zhang et al. (2003) might be related to the maintenance requirement of the experimental birds. Age was found to be one of the factors that influence the rate of protein synthesis and degradation in farm animals (Rimbach and Liebert, 1999). Therefore, broilers at 6 wk of age may have a different maintenance requirement when compared to 4 wk birds used by Zhang et al. (2003). Body size can also affect maintenance requirements. Based on the compilation of several records from nonpasserine birds, Lasiewski and Dawson (1967) reported a relationship between basal metabolism and body weight in layers. Also, maintenance requirement is

greater than the basal metabolism. Birds in different ages may have difference in growth rate and size; it can, therefore, impact the maintenance requirement. According to McCarthy and Siegel (1983), the maintenance requirement in meat-type birds has a substantial effect in masking the genetic variation of feed efficiency for growth.

The genetic correlation between the excreta traits was very high and negative in line A whereas it was moderately positive for line B. However, phenotypic correlations between the traits were very low and negative for both the lines. The different values between genetic correlation and phenotypic correlation can be due to pleiotropic effect of genes acting differently in both lines. It may also be due to difference in environmental conditions like diet, housing etc. for the two lines. The feed conversion trial for line A was conducted in the August-September months whereas line B had a winter sampling period. The high genetic correlation results obtained for line A may also be attributed to software limitations when dealing with a very low genetic variance for one trait.

The phenotypic correlations of PNE with WG (negative) and FCR (positive) in lines A and B indicates that efficient N utilizing birds might also have other beneficial marketable traits. The non-significant correlations of PPE with WG, FC, and FCR also suggest that selection towards improvement in P utilization may not have any adverse effect on broiler growth traits. However, there are reports (Edwards, 1983; Zhang et al., 2003) which suggests that improvement in PBA negatively affects growth rate.

According to Zhang et al. (2003), the genetic correlations between PBA and

growth traits (e.g. BW gain, FC) were moderately negative, but there was a low positive phenotypic correlation between PBA and those traits. Zhang et al. (2003) also reported a non-significant genetic correlation between PBA and FCR, thereby suggesting that selection for improvement in P utilization does not alter the current FCR chosen by the industry. A possible reason for the difference in correlation of P with other traits between the current and previous studies may be related to the difference in trait definition. The PPE trait used in the current study can be affected by a wide range of different genes as compared to PBA used by Zhang et al. (2003). The age difference between the birds used in the current study and those used by Zhang et al. (2003) can be another factor. The deposition of P in bones for growth and its excretion can be completely different in the birds used in the current study since the birds were 6 wk old.

The data in this study consistently demonstrate that even though excreta traits have low heritability, improvement in the utilization of N and P through an appropriate selection index will result in improvement, albeit slow, of major economic traits. Nutritionally, the study does have some limitations, particularly in the inability to differentiate whether the improvement in nutrient utilization is due to a difference in absorption of other components in excreta or from the absorption of N and P. It could be corrected if the birds were fed with diets containing N and P in higher and lower levels. Alternative strategies like selection on genomic breeding values (**GBV**) or integrating excreta component information to other economic traits could achieve progress through correlated response to selection. According to Muir (2007), selection using GBV is particularly efficient for low heritability traits.

Fine mapping of QTL for the whole genome or association studies based on candidate gene approach may be utilized in the future to incorporate genomic information into the breeding scheme.

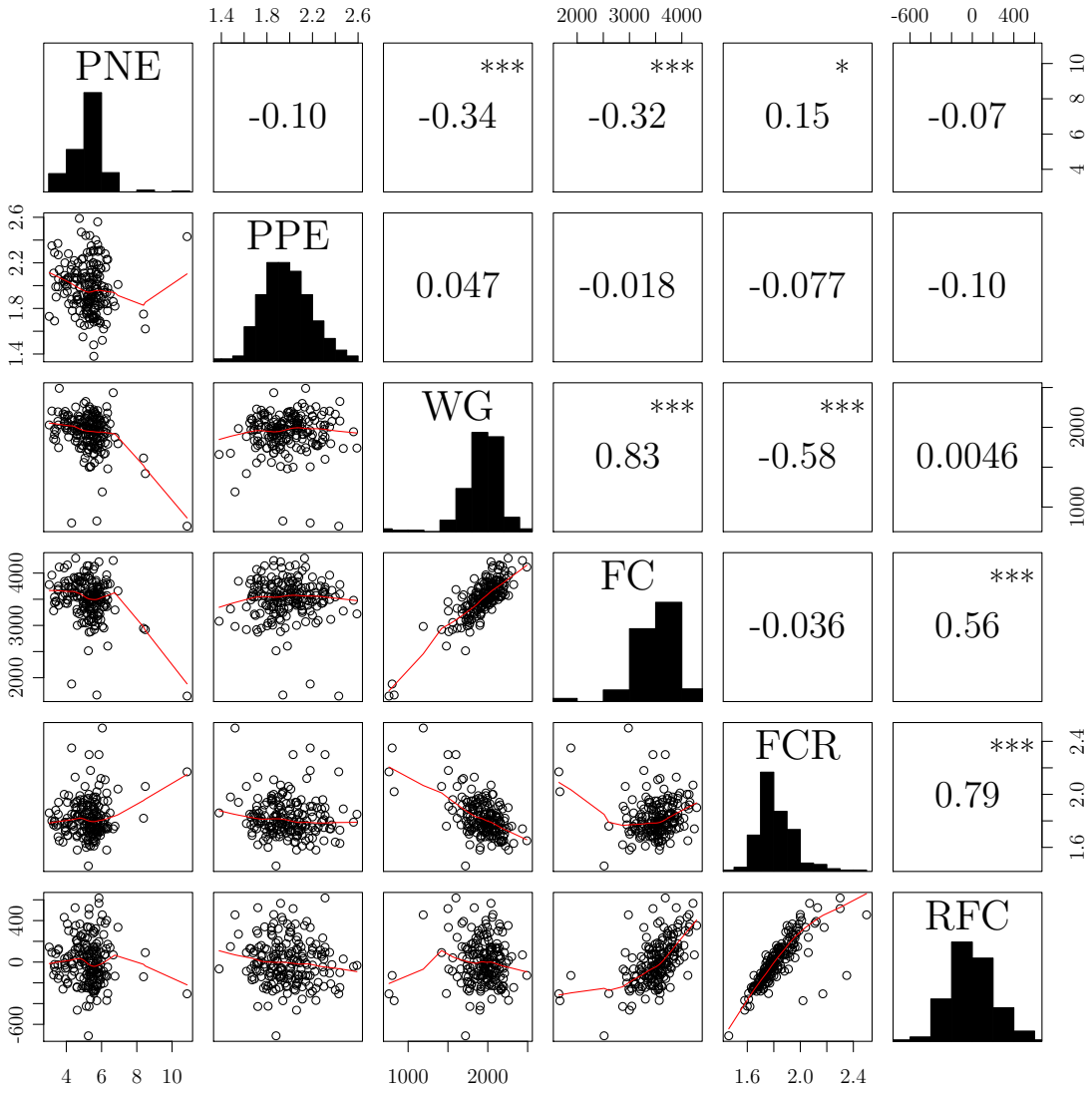

Figure 3.1: Scatter plot matrix for line A. The figure shows scatter plot matrix of the variables (PNE: Percentage of nitrogen in the excreta, PPE: Percentage of phosphorus in the excreta, WG: Weight gain during the 3 wk feed conversion trial, FC: Feed consumption during the 3 wk feed conversion trial, FCR: Feed conversion ratio, and RFC: Residual feed consumption) in the lower diagonal, histograms along the diagonal, and correlation coefficients with significance in the upper diagonal. Significance indicated by $*$.

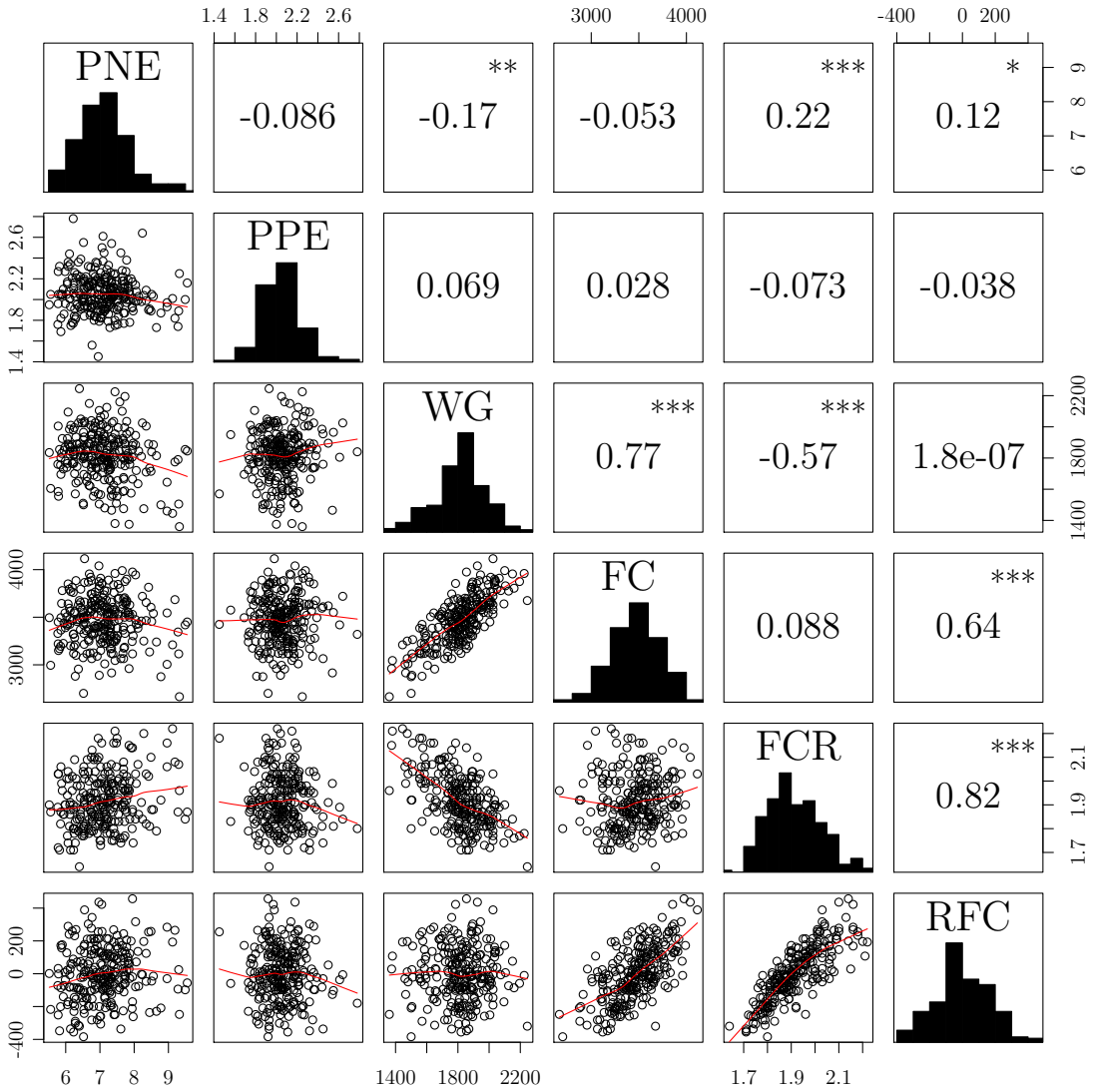

Figure 3.2: Scatter plot matrix for line B. The fig. shows scatter plot matrix of the variables (PNE: Percentage of nitrogen in the excreta, PPE: Percentage of phosphorus in the excreta, WG: Weight gain during the 3 wk feed conversion trial, FC: Feed consumption during the 3 wk feed conversion trial, FCR: Feed conversion ratio, and RFC: Residual feed consumption) in the lower diagonal, histograms along the diagonal, and correlation coefficients with significance in the upper diagonal. Significance indicated by $*$.

Table 3.1: Descriptive statistics for excreta traits and other economically important traits

Line	Trait[a]	Mean±SE	CV(%)	Min	Max	N
A	PNE	5.26±0.07	17.89	3.03	10.85	197
	PPE	1.98±0.02	10.87	1.38	2.59	195
	WG(g)	1936±17	12.37	760	2490	191
	WO(g)	2672±21	10.89	1340	3365	191
	FC(g)	3512±28	10.88	1650	4285	191
	FCR	1.82±0.01	7.86	1.46	2.5	191
B	PNE	7.12±0.05	11.07	5.13	9.55	276
	PPE	2.05±0.01	9.45	0.98	2.78	278
	WG(g)	1814±10	8.78	1360	2245	256
	WO(g)	2950±12	6.4	2390	3405	256
	FC(g)	3468±16	7.34	2665	4115	256
	FCR	1.92±0.01	5.79	1.64	2.22	256

[a]PNE: Percentage of nitrogen in the excreta (on a DM basis); PPE: Percentage of phosphorus in the excreta (on a DM basis); WG: Weight gained by the bird during the feed conversion trial (3 wk old - 6 wk old); WO: Weight of the bird at the end of feed conversion trial; FC: Feed consumed by the bird during the 3 wk of the feed conversion trial; FCR: Feed conversion ratio.

Table 3.2: Estimates of Variance Components for Excreta Traits from MTDFREML (shown outside the brackets), and SAS GLM (shown inside the brackets) analysis

Line	Trait[a]	Genetic Variance	Environmental Variance
A	PNE	0.073 (0.134)	0.804 (0.844)
	PPE	6.63×10^{-8} (0)[b]	0.047 (0.047)
B	PNE	0.093 (0.094)	0.500 (0.570)
	PPE	0.008 (0.008)	0.029 (0.036)

[a]PNE: Percentage of nitrogen in the excreta (on a DM basis); PPE: Percentage of phosphorus in the excreta (on a DM basis).

[b]Calculated genetic variance from GLM procedure was found to be -0.003

Table 3.3: Estimates of heritabilities (on diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations for excreta traits

Line	Trait[a,b]	PNE	PPE
A	PNE	0.08 (0.165)	-0.92[c] (NA)
	PPE	-0.12 (0.07)	0.14×10^{-5} (0.148)
B	PNE	0.16 (0.148)	0.44 (0.636)
	PPE	-0.09 (0.06)	0.20 (0.158)

[a]PNE: Percentage of nitrogen in the excreta; PPE: Percentage of phosphorus in the excreta.

[b]Standard errors for the estimates were shown in brackets (Computed based on method outlined by Kachman and Van Vleck (2007))

[c]High negative genetic correlation between the traits can be due to very low genetic variance of PPE

Chapter 4

Selection using genotypic information

4.1 Introduction

The productivity of agricultural plants and animals increased during the last 50 years. In fact, the rate of change in animal and plant productivity has been increasingly rapid in the last five to six decades through breeding and selection (Gjedrem, 1985; Alston et al., 2009). This fact was corroborated in broilers by Havenstein et al. (2003) who conducted a comparison study for growth and feed conversion between 1957 and 2001 broilers; the study concluded that in order to produce an 1815 g broiler, a 2001 broiler required less than one-third the time (32 d vs. 101 d) with over a threefold decrease in feed conversion rate (1.47 vs. 4.42) when compared to a 1957 broiler. Most of the increase in productivity can be attributed to genetic selection. According to two studies (Sherwood, 1977; Havenstein et al., 1994) performed on broilers, between 85 and 90% of the increase in BW in a modern broiler can be attributed to genetic selection strategies practiced by commercial breeders. Interestingly, in the previous decade, Pollock (1997) predicted that within the next 20 years, if the genetic improvement continued at the same rate then broilers at market weight will be aged 10 d less. Similar improvements in productivity for other animals and plants were also made possible by genetic selection.

According to Dekkers and Hospital (2002), increase in productivity through

genetic selection, in reality, was based on phenotypic characteristics rather than on the genetic makeup of the livestock species. Also, traditionally, breeding values were estimated from information available on phenotypes rather than based on the genes causing the observed phenotypes (Meuwissen et al., 2001). It was truly a reflection of the genetic progress through phenotypic selection. However, even during the 1980s, the estimated genetic progress was lower than the theoretical predicted values. For e.g. in Holstein dairy cattle, the estimated genetic progress was found to lie between 0.25 and 0.50 of the theoretical optimum per year (Everett and Henderson, 1972; Hintz et al., 1978).

Phenotypic selection based on genetic estimates calculated from phenotypic values was largely carried out in most of the economically important traits in livestock and plants; these traits are controlled by a large number of genes and are often termed as quantitative traits (e.g. growth, milk production, carcass yield). The genes controlling the quantitative traits can vary in number from hundreds (Mather and Jinks, 1971) to a few loci (Thoday and Thompson, 1976). However, defining the exact number of loci controlling quantitative traits is still controversial. Some of the debates regarding that matter started as early as 1921 by Castle (Wright, 1968).

Based on the number of genes affecting the quantitative traits, different statistical approaches have been proposed. In an infinitesimal model, quantitative traits are assumed to be controlled by an infinite number of unlinked and additive loci, each having infinitesimally small effect (Fisher, 1918). This model forms the basis for breeding value (**BV**) prediction theory (Henderson, 1984). However, the in-

finitesimal model had the assumption that all loci equally affect all the conceivable quantitative traits, so it makes less sense genetically (Mackay, 2001). Also, evidence from a study conducted with Drosophila melanogaster suggests that the actual number of loci controlling quantitative traits is much smaller than that assumed in the infinitesimal model (Thompson, 1975).

Another model, proposed by Robertson (1967), assumes that allelic effects have exponential distribution; it follows that very few loci have considerably large effects that cause most of the trait variation whereas a larger proportion of the loci have smaller effects. This model was also known as the finite loci model because a great proportion of the variation in quantitative traits is primarily attributed to a finite number of loci. The few genes having large effects on the phenotype are called major genes. Major genes are supposed to be located at quantitative trait loci (**QTL**) whereas the remaining unknown genes having smaller effects on the phenotype are termed as polygenes (Shrimpton and Robertson, 1988). The results of many studies in D. melanogaster for sensory bristle number (Breese and Mather, 1957; Shrimpton and Robertson, 1988; Nuzhdin et al., 1999), wing shape (Weber et al., 1999) support Roberston's model. The search for the QTL with moderate to large effect, and use of the QTL information for increased accuracy of genetic selection was driving research in the last two decades. The effects of the QTL reported in mapping experiments in both pigs and dairy cattle are shown in Figure 4.1 (Hayes, 2007).

For quantitative traits, the total genetic variation is determined by variation in both the major genes and polygenes. Accurately estimating the BVs for both these

components will provide a better understanding of the merit of expected progeny differences (**EPD**). The EPD values help comparing or ranking individual animals based on their relative superiority to the target population. So, accurate estimation of BV is a critical step to ensure maximum genetic progress. According to Muir and Stick (1998), improvement in the phenotypic performance of a population needs to focus on the total genetic value of the trait rather than just few major genes that were already known; they also showed that selection strategies that aim to quickly fix the major genes achieve less response to selection than traditional selection based on phenotypes. The simple sum of the best estimates of BVs of the major genes and polygenes gives the best estimate of an animal's total BV. This genetic selection model was used in a short term vs. long term simulation study (Gibson, 1994) and compared the results with phenotypic selection that did not use major gene information. The results of the study demonstrates that genotypic selection response was greater in the short term, but phenotypic selection achieved greater response in the longer term. The reason for lower long term genotypic selection response is that polygenes may get lost in the initial generations as the major gene gets fixed, compromising genetic gains in the polygenes in advanced generations. However, advances in analyses of quantitative traits using information from molecular genetics increased the power for identification of major genes located at the QTL. The information from the major genes will increase the accuracy of estimation of BV and must improve the phenotypic value of the trait if used judiciously.

Estimation of BV using traditional selection methods like sib selection and BLUP depends on phenotypic information of the individual and its relatives. Since

prediction of an individual's BV using phenotype has limited accuracy, selection based on traditional methods have less accuracy for genetic gains (Dekkers and Hospital, 2002). The expected increase in mean performance of a population per generation through genetic selection also depends on intensity of selection, and the existing genetic variation in the population.

4.2 Genetic Markers: Approach to uncover Quantitative Trait Loci

Sunnucks (2000) described genetic markers as heritable characters with multiple states at each character but with no function and no impact on animal performance. Based on the DNA sequence, molecular markers are classified into three types: restriction fragment length polymorphisms (RFLP), simple sequence length polymorphisms (SSLP or microsatellites), and single nucleotide polymorphisms (SNP) (Brown, 1999). Currently, SNP-based DNA chips are preferred as the total number of SNP is estimated in millions which makes it cost effective (Meuwissen et al., 2001). The definition of genetic marker by Sunnucks (2000) may not be applicable if there is a mutation of a SNP inside a gene; in this case the mutation can cause functional differences depending upon whether the mutation falls under the category of silent, missense or nonsense mutation.

Genetic markers may also be broadly classified as direct or indirect markers depending on their association with the QTL (van der Werf, 2000). Direct markers are located within a major gene, so it may not be affected by recombination but these are hard to find for quantitative traits; also the causality of mutation by direct

markers is difficult to prove (Dekkers and Hospital, 2002). Indirect markers are assumed to be non-functional markers that are linked to the QTL and are affected by recombination (Figure 4.2). Indirect markers are identified using either direct search by candidate gene approach in unstructured populations, or genome scans in specialized populations based on linkage mapping with anonymous DNA markers (Andersson, 2001).

Candidate gene approach assumes that a gene involved in the physiology of a trait could harbor a mutation causing variation in that trait. However, Dekkers and Hospital (2002) had the view that a candidate gene marker may not itself be the functional variant instead of the gene, then it will be statistically difficult to prove DNA differences causing the phenotypic differences; in that case, a statistical significance could not differentiate between the true functional variance in a gene and the candidate gene. Usually, candidate genes are sequenced in different animals and the association between variations in DNA sequence with variation in phenotypic trait is tested for significance. The advantage of using the candidate gene approach is that the marker is quite often tightly linked to the QTL, so this will reduce the recombination rate and the subsequent dilution of the QTL effect. Therefore, candidate gene approach is quite powerful and can detect very small effects provided that it represents a true causative gene, but there are also some practical disadvantages. Since, most of the quantitative traits are determined by a large number of genes, using candidate gene approach requires sequencing a large number of genes in several animals and so, many association studies should be carried out. This may lead to improper adjustment to significance threshold and spurious results can occur. Zhao

et al. (2003) compared the different tests of candidate gene for QTL analysis and concluded that F-drop test as the most attractive but the results should be interpreted with caution as it will show associations for both close and distant candidate gene loci. The high cost of genotyping also creates a disadvantage when large numbers of candidate genes are present. There is also a chance of having the causative mutation lie in a gene which is not regarded a priori as an obvious candidate for the concerned trait (Andersson, 2001).

Compared to candidate gene approach, genome scans or the QTL mapping approach identifies part of the chromosome that affects the trait. Usually, the length of this region is 10-20 cM, so it may contain an unknown number of QTL (Dekkers and Hospital, 2002). The basic assumption of QTL mapping is that the actual genes affecting the quantitative trait are not known. Neutral DNA markers are used to find association between allele variations at the marker with variation in quantitative traits. This will help to determine whether the DNA marker is linked to the QTL or not. But mapping QTL using linkage requires a large number of progeny per family to reduce the confidence intervals (expressed in cM) for QTL location on the chromosome according to the formula, $CI = \frac{3000}{kN\delta}$, where N is the number of individuals genotyped, δ is the allele substitution effect, and k is the number of informative parents per individual (Hayes, 2007).

In genome scans, the possibility of false-positive error rate can be also high. The reasons are: 1) multiple tests done to identify multiple markers in linkage to a QTL, and 2) identification of individual-by-marker combination that represent heterozygous QTL (Fernando et al., 2004). Southey and Fernando (1998) proposed

that reducing the proportion of false positive will ensure control of the false positive error rate. There are other potential problems like the markers being used not being physically close to the QTL. In such cases, the marker-QTL relationship may not persist across the population (Hayes, 2007).

4.3 Marker Assisted Selection

Animals can be selected based on genetic marker information alone. In such cases, the effects of the genes not covered by the marker or phenotypic information are ignored. Technically, selection based on information at genetic markers can be termed as marker assisted selection (**MAS**), but MAS usually combines information from both phenotypes and the genetic markers.

Hayes (2007) classified MAS into three categories: LE-MAS (DNA in linkage equilibrium with a QTL), LD-MAS (DNA in linkage disequilibrium or non-random association with QTL), and Gene-MAS (based on selection on actual mutation causing the QTL effect). These three strategies of MAS can be distinguished based on molecular score in selection, in combination with phenotype, or estimated BV (**EBV**) derived from phenotypic information. Among the three types of MAS, Gene-MAS and LD-MAS are preferred among the livestock breeding industry especially dairy cattle industry due to consistency in association between genotypes and phenotypes. When compared to conventional selection, MAS provides opportunity for improvement in response for low heritable traits and traits which are difficult to measure (Dekkers, 2004). Likewise, MAS is also helpful when the traits are limited

to one sex (van der Werf, 2000). The simulation results for a sex-limited trait measured in dairy cows suggested an increase in genetic gain by 38% and 21% after one and five generations of MAS respectively; however, increase in genetic gain permanently by MAS is only possible when there is a continuous input of newly identified QTL (Meuwissen and Goddard, 1996). This can happen when the selection was practised on a single trait. But, selection in the livestock industry will be generally for multiple traits. When multiple traits are selected, in a few generations, there is a probability for loss in linkage between genes involved with the traits due to recombination.

4.4 Genome-wide Selection

Application of genomic methods in breeding let the breeders to determine the shared genes within the animal population (VanRaden et al., 2008). In genome-wide selection (**GWS**), the total breeding value is predicted based on a large number of marker haplotypes across the entire genome (Meuwissen et al., 2001); it is superior to LE-MAS, LD-MAS, and Gene-MAS in capturing an increased proportion of additive genetic variance. Also, genomic selection fits all QTLs simultaneously, thus, it removes the effect of QTL in brackets adjacent to a true QTL, and gives smaller confidence intervals. In GWS, the whole genome is divided into chromosome segments defined by adjacent markers, and then all the segments are traced (Hayes, 2007). Genome-wide selection assumes that all markers are in LD with the QTL, which will not be seen in real populations. With the use of dense marker maps,

some of the markers get closer to the QTL to cause LD between the marker and the QTL. Meuwissen et al. (2001) combined those markers which are closer together as haplotypes and estimated the effect of quantitative trait of small chromosome segments defined by the haplotypes of marker alleles.

Genome-wide selection is implemented as a two-step process: 1) Estimation of effects of chromosome segments in reference population ("training" - Muir, 2007), and 2) prediction of genomic EBV (**GEBV**) for animals not in the reference population. Prediction of GEBVs for animals with genotypic information but no phenotype is carried out by summing the effect of chromosome segments across the genome:

$$GEBV = \sum_{1}^{n} X_i \hat{g}_i$$

where n is the number of chromosome segments, X_i is a design matrix allocating animals to the haplotype effects at segment i, and $\hat{g}_i$ is the vector of effects of haplotypes within chromosome segment i. Selection using GWS can be carried out using single markers (one effect per segment), marker haplotypes (multiple effects per segment), or identity-by-descent (IBD) approach. Estimation of single marker or haplotype effects in GWS can be done using least squares method, ridge regression and BLUP, or Bayesian methods (Hayes, 2007). Meuwissen et al. (2001) compared the accuracy of using these estimation methods in a simulation study based on an additive model and found that BayesB provided a higher correlation between the predicted and true breeding value or accuracy of selection (0.848 ± 0.012). BayesB method used a prior which had a high density (π) at

$$\sigma^2_{g_i} = 0$$

since in reality, most of the loci are not associated with any appreciable values of genetic variance.

4.5 Breeding Value Accuracy Using Genome-Wide Selection

The potential of GWS can be ascertained by comparing the accuracy of EBV using GWS and those obtained by BLUP. For example an animal with no records and only parental information has an EBV which is equal to, $\hat{A}_i = (0.5) \times \hat{A}_s + (0.5) \times \hat{A}_D$, where $\hat{A}_s$ and $\hat{A}_D$ are the EBV of the sire and dam, respectively. Therefore, the accuracy of EBV is $\rho_{A\hat{A}} = \sigma_{A\hat{A}}[\sigma_A \sigma_{\hat{A}}]^{-1}$, where $\sigma_{A\hat{A}}$ is the covariance between true breeding values and EBVs, and $\sigma_{\hat{A}}$ is the EBV standard deviation. If the parent EBVs have an accuracy of 1, $\sigma_{A\hat{A}} = (0.5){\sigma_A}^2 = (0.5){\sigma_{\hat{A}}}^2$, and $\rho_{A\hat{A}} = (0.5){\sigma_A}^2[(0.5){\sigma_A}^2{\sigma_A}^2]^{-\frac{1}{2}} = 0.71$, which is the upper bound of accuracy when BLUP is used. In the case of GWS using Bayesian methods, the accuracy increases to 0.85 (Meuwissen et al., 2001). According to Woolliams et al. (2002), the increase in accuracy using GWS must come from the accuracy of the Mendelian sampling estimate. The accuracy of the Mendelian sampling estimate can be ascertained if the between family variance (${\sigma_B}^2$) can be assumed to be ${\sigma_B}^2 = (0.5){\sigma_A}^2$. Therefore, the proportion of Mendelian sampling variance explained by GWS EBV is ${\rho_{M\hat{M}}}^2{\sigma_M}^2 = {\rho_{A\hat{A}}}^2{\sigma_A}^2 - 0.5({\sigma_A}^2)$, where ${\rho_{A\hat{A}}}^2$ is the proportion of ${\sigma_A}^2$ explained by EBV. If $\rho_{A\hat{A}} = 0.85$ in GWS, then $\rho_{M\hat{M}}$ =0.67. This increase in accuracy of Mendelian sampling terms ($\rho_{M\hat{M}}$) of GWP is large when compared to a value of zero for BLUP (Daetwyler et al., 2007).

4.6 Linkage Disequilibrium, Accuracy and Cost of Testing: Genome-Wide Selection

According to Nachman (2002), LD will be more expected in genomic regions with low recombination. However, LD is complicated by a number of other factors such as directional selection, recurrent mutations, and demographic processes like admixture or population growth. Selection can affect the extent of LD in two ways: 1) hitchhiking effect of a flanking haplotype along with a favored variant can increase the frequency of the adaptive haplotype rapidly and can lead to the fixation of the linked variants (Wang et al., 2002), or 2) epistatic selection for combinations of alleles at two or more loci on the same chromosome (Cannon, 1963). Beneficial mutations can also generate LD in asexual populations as a result of the increase in frequency of the modifier by genetic hitchhiking (Johnson, 1999). But, in sexual populations, the effect of beneficial mutations on evolution of mutation rates is negligible (Leigh, 1973). Among the other factors, rapid population growth decreases LD by reducing genetic drift. However, extreme inbreeding could produce high levels of LD without substantial reduction in variation; it was confirmed in a population structure study conducted in *Arabidopsis thaliana* (Nordborg et al., 2002).

Meuwissen et al. (2001) mentioned that improving accuracy in predicting BV requires LD between marker and QTL. But, realistically, due to the above mentioned factors, LD may be incomplete between marker and QTL thus the marker haplotypes may not be able to explain all the variance at the QTL. A simulation study by Meuwissen et al. (2001) used N_e =100, and a marker spacing of 2cM to achieve

the maximal accuracy of predicting genetic values of the offspring of recorded animals. Since LD is a function of $N_e c$ (where c is the recombination rate), at greater marker spacing or effective population size, LD decreases. In the simulation, maximal accuracy of prediction of true BV occurred when $N_e c < 2$. In most commercial livestock populations, the effective population size will be greater than 100. In such cases, either more informative markers should be used to allow a higher $N_e c$ value or a larger number of animals will be required in the reference population since only about 1-2% of the phenotypic variance will be usually accounted by even the largest QTL effects on some traits.

An assumption in the simulation conducted by Meuwissen et al. (2001) was that each centimorgan distance contains a QTL. But in reality, the QTLs affecting a trait might be found in clusters. There are also concerns about sampling errors while estimating the haplotype effects. The sampling error increases with an increase in ratio of the environmental variance and the number of genotyped and recorded animal. In traits with low heritability, therefore, sampling error increases. Data on more animals or records per animal will be necessary to achieve the same accuracy. This implies that the computational and financial cost of estimation will be higher for low heritability traits.

In GWS, the number of effects will be very large compared to the number of records. For example, in the simulation study by Meuwissen et al. (2001), the average number of haplotypes per 1 cM segment was around 50. Since segments of 100 cM each, on 10 chromosomes, were simulated, the total number of haplotypes to be estimated becomes around 50,000. The phenotypic records simulated were

about 2200. Thus, the effects could only be estimated using BLUP, or BayesB methods. Since the genome size in livestock species will be much larger than the simulated study, the effects to be estimated will also increase considerably which may ultimately reduce the accuracy of individual effects in GEBV; in GWS, even if the individual effects are not accurately estimated, their sum may be quite accurate.

Even though, GWS offers a lot of advantages, the practical utilization of GWS is still being questioned. Konig et al. (2009) compared a simulated BLUP based breeding program and genomic selection in dairy cattle to measure the economic merits of these programs. The results showed that genomic selection can be economically successful if progeny testing can be completely eliminated; however, Muir (2007) suggest that accuracy and persistence of accuracy on selection based on GEBV will be better with the use of both within and across generation phenotypic and genotypic data.

In poultry industry, selection by GWS may be economically ineffective presently. The cheapest dense marker bracket (DNA chip) available in the market currently costs around US$150 (H. Cheng, personal communication). Genotyping individual birds using this chip will generate a sizable cost; recovering the investment in broiler, e.g., is challenging given the relatively short reproductive life span of the breeders and the common practice of replacement of all breeders at each generation cycle, which typically happens between 44 and 56 weeks, depending on the selection goals. Size and generation interval will be also key factors in the implementation of GWS on a broader scale in birds. A short generation interval and the small size for birds will makes each individual less worthy in monetary terms for GWS. Based

on the facts mentioned above, the economic returns in the future may not cover the expenses incurred. Therefore, other options such as candidate gene approach or selective genotyping may be more realistic and viable.

4.7 Alternatives to Genome-Wide Selection: Candidate Gene approach and Selective Genotyping

The advantages and disadvantage of using candidate genes in selection were described above. An alternative method for determining associations of QTL and marker is selective genotyping. It was proposed by Darvasi and Soller (1992). Here, genotyping is carried out only in individuals from the high and low phenotypic tails of the population. This method will decrease the number of individuals that need to be genotyped. For quantitative traits, using individuals with high and low phenotypes in the population can increase the power of the analysis at the expense of an increase in the number of individuals being phenotyped (Lander and Botstein, 1989). However, this approach is mostly appropriate with single trait selection. When multiple traits are used, selection of extremes of each trait may select a large proportion of the population; therefore, no reduction in genotyping can occur (Darvasi, 1997). Thus, utilizing economically viable options is more feasible when resources are limited.

4.8 Quantitative Trait Loci Mapping in Poultry

The use of genomic markers in selection programs is a recent approach being gradually adopted by the poultry industry. The main advantage of incorporating genomic information in a poultry selection program is that it will increase the genetic improvement of low heritability traits like hatchability and fertility. According to Pollock (1999), a 1% increase in hatchability, eviscerated carcass weight, or improvement in feed conversion ratio will provide considerable increase in income to a farmer that participates in vertical broiler production systems. Accuracy is essential for the improvement of low heritability traits. Genomic selection provides accuracy; however, cost effectiveness is currently one of its major drawbacks.

During the past decade, QTL mapping studies in chickens have identified chromosomal regions causing variation in economically important traits (Abasht et al., 2006). However, the goal of identifying genetic markers close to the QTL (LD markers) or direct markers was difficult due to polygenic inheritance, epistasis, incomplete penetrance, variable expressivity, and pleiotropy of QTL (Lander and Schork, 1994). Even though there is difficulty involved in QTL mapping, many studies have quite successfully detected QTL in chicken for economically important traits such as growth, carcass, and meat quality traits by using crossbred experimental populations (Abasht et al., 2006). Uemoto et al. (2009) detected four significant and five suggestive QTL on chromosomes 1, 2, 3, and 10 for growth traits, and three significant and eight suggestive QTL on chromosomes 1, 3, 5, and 6 for carcass traits. The QTL effects ranged from 3.7 to 9.3% (growth traits) and 4.0 to 10.5% (carcass

traits) of the total phenotypic variation. However the majority of the growth traits (e.g. breast muscle, abdominal fat percentage, muscle color etc.), have heritabilities ranging between 0.4 and 0.8 (Le Bihan-Duval et al., 1999; Zerehdaran et al., 2004). Le Bihan-Duval et al. (1999) used birds that originated from Quality lines whereas Zerehdaran et al. (2004) used outcross broiler lines originating from White Plymouth Rock.

For traits that have low heritability, Hillel (1997) suggests use of linkage analysis between marker loci and BV of sires. Feather pecking is one example of a trait with very low to insignificant heritability (Rodenburg et al., 2003). By use of genome-wide analysis, Buitenhuis et al. (2003) detected three different QTLs for gentle feather pecking in a paternal half-sib model.

In conclusion, it is possible to improve quantitative traits in poultry by application of MAS. However, application of MAS to complex traits requires an in-depth understanding of the trait itself. Effectiveness of using mapping technology in poultry also relies upon the use of cost-effective technology. Until then, there is a possibility for a lag period in wide spread implementation of MAS in poultry breeding.

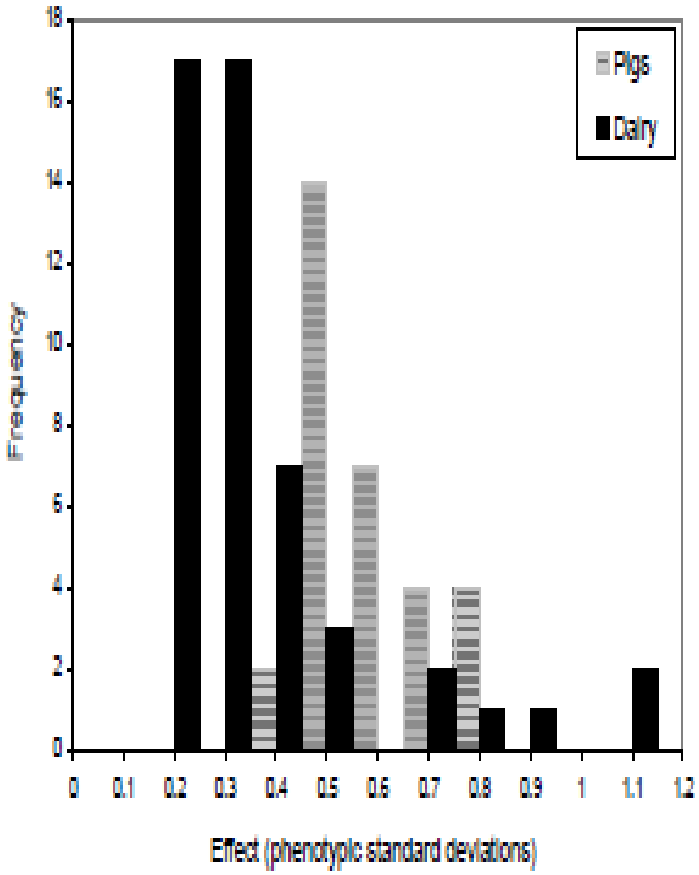

Figure 4.1: Distribution of additive (QTL) effects from pig experiments, scaled by the standard deviation of the relevant trait, and distribution of gene substitution (QTL) effects from dairy experiments scaled by the standard deviation of the relevant trait (Hayes, 2007).

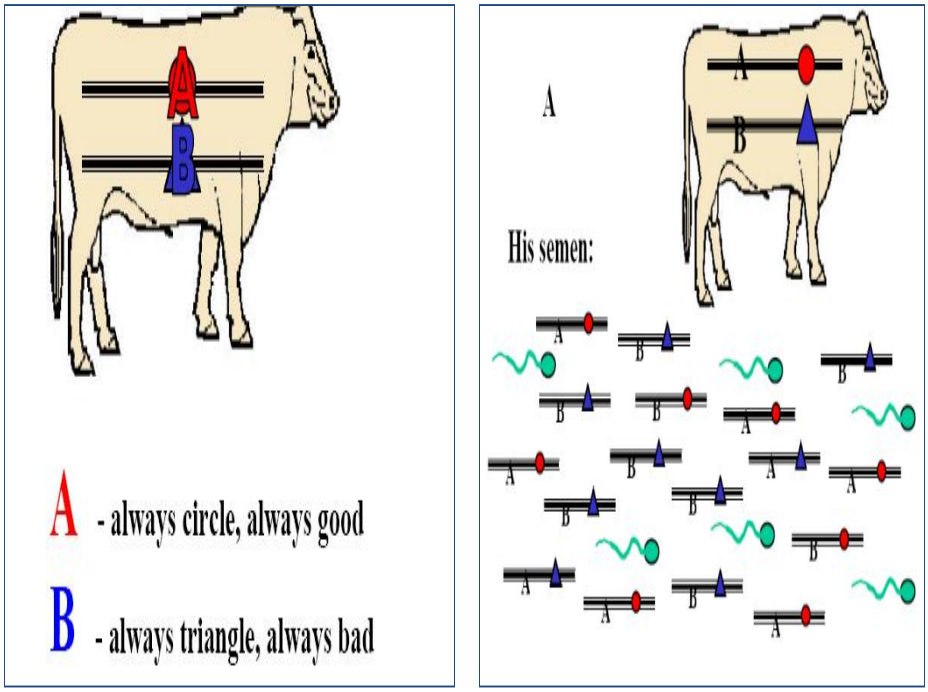

Figure 4.2: a. Direct genetic marker. b. Indirect genetic marker with recombination. Marker variants A and B linked to major gene variants 'o' and 'Δ' (van der Werf, 2000)

Chapter 5

An Association Study of Single Nucleotide Polymorphism with Excreta Traits in Broiler Chickens

5.1 Introduction

In the past 45 years, selection for rapid growth rate and increased body mass in broiler chickens has dramatically increased the yield in breast, wings, thighs, and whole body carcasses (Havenstein et al., 2003). In the United States, the total number and the revenue broilers generated increased by 1% and 7%, respectively, to reach 50.4 billion pounds and $23.1 billion in 2008, compared to a base year of 2007 (USDA, 2009). Poultry production practices like concentrated animal feeding operations (**CAFO**) also have a role in the increased production. Increase in poultry production from the CAFO released excess nutrients, primarily nitrogen (**N**) and phosphorus (**P**), in to the surrounding areas. High concentration of these nutrients can damage water supplies and can create a hostile environment to aquatic species (Shore and Pruden, 2009). Some human diseases like methemoglobinemia, cancer, and respiratory illness have been associated with nitrate contamination of groundwater (Kelleher et al., 2002). The reduction of nutrient content in poultry excreta is a great opportunity for the industry to improve production efficiency while exercising responsible environmental stewardship.

A previous study (Muchow, 2006), conducted at University of Maryland, studied lines of fat and lean chickens. Five differentially expressed candidate genes from the pituitary glands were identified: lysophosphatidic acid receptor-1 (**LPAR1**), extracellular superoxide dismutase (**SOD3**), aldo-keto reductase family 1, member B10 (**AKR1B10**), glypican 3 (**GPC3**), and syndecan 1 (**SDC1**). Large-scale sequencing of genomic data in fat and lean chickens by the same authors also identified 11 single nucleotide polymorphisms (**SNP**s) in the promoter regions of the candidate genes. Promoter regions may have a key role in altering transcriptional machinery binding sites, therefore, SNPs identified on these regions will be important in the regulation of the metabolism in chickens. Differences in metabolism can result in changes in the nutrient content of their excreta.

Pages et al. (2001) reported that LPAR1 gene has a role in regulating adipocyte differentiation. Further investigation (Muchow, 2006) in F2 chickens with extreme body fat revealed a T to C transversion, and it introduced a globin transcription factor 1 (**GATA1**) binding site. The last author suggested a possibility of resistance to differentiation in preadipocytes with the introduction of GATA1 factor binding site upstream of LPAR1 gene by the SNP. The GATA1 factor binding site may increase the expression of LPAR1 gene and it is suggested to be one of the factors regulating body composition in chickens. According to Pages et al. (2000), lysophosphatidic acid (**LPA**) gene is a potent bioactive phospholipid, involved in cell proliferation and differentiation. Pleiotropic trophic activity of LPA could be important in the nutrient regulation of proliferating cells.

Cellular metabolism can be drastically changed in aerobic organisms by the

formation of reactive oxygen species (**ROS**); it can lead to increased vascular oxidative stress (Jung et al., 2003). Pharmacological inhibition of SODs can attenuate endothelium-dependent relaxation (Mügge et al., 1991). According to Fattman et al. (2003), low levels of ROS such as nitric oxide (**NO**) are necessary for the regulation of vascular tone. Superoxide dismutase is one of the antioxidant enzymes with a possible role in mediating NO-induced signaling events.

The AKR1B10 gene belongs to the super family of aldo/keto reductase (**AKR**) genes. This family includes more than 40 enzymes and proteins. Chicken AKR has a unique feature of not having any activity with glucose (Crosas et al., 2001). In humans, AKR1B10 has been reported as a candidate gene for the development of diabetic retinopathy with Type 2 diabetes (Petrovič et al., 2004). Overexpression of AKR1B10 in the lung can cause squamous cell carcinoma (Fukumoto et al., 2005). It is also associated with human liver cancers (Cao et al., 1998).

Glypicans belong to the heparan sulfate proteoglycan family. They are involved in cell signaling pathways and tissue growth (Filmus, 2001). According to Weksberg and Squire (1997), GPC3 negatively regulates insulin-like growth factor II in an overgrowth condition (Beckwith Wiedemann syndrome) in humans. GPC3 is known to inhibit cell proliferation and can down-regulate certain types of tumors (Duenas Gonzales et al., 1998).

Syndecans represent another cell-surface proteoglycan (David, 1993). In mammals, there are four members of syndecan family and they differ in intracellular interaction and phosphorylation. SDC1 protein is generally involved in regulating cell signaling, cell binding, cell proliferation etc. (Kolset and Salmivirta, 1999).

Genes of interest controlling a particular trait can be identified using candidate gene approach (Rothschild and Soller, 1997). The present study analyzes the associative effect of candidate genes such as SOD3, AKR1B10, GPC3, and SDC1 on nutrient (N and P) output in the excreta. Previously, there was no documented evidence regarding the effect of these genes on the nutrient excretion. However, the results from a study conducted in fat and lean broiler chickens by Liu (2009) indicated four SNPs (SDC1 SNP1, GPC3 SNP1, AKR1B10 SNP1, and SOD3 SNP2) to be significantly associated with fat weight, fat yield, thigh yield, and breast yield.

The phenotypic correlations between the excreta trait, percentage of N in the excreta (**PNE**), and other marketable traits e.g. weight gain (**WG**), feed consumption (**FC**), and feed conversion ratio (**FCR**), from chapter 3 indicate that efficient nutrient utilization in broilers will have a favorable effect on the production traits. Therefore, the hypothesis for the present study is that the genes associated with production traits from a previous study (Liu, 2009) must also have an influence on the nutrient output from the bird. The goals for the current study were to determine: 1) the association of select SNP markers with excreta traits, 2) the correlation of results from chapter 3 with the SNP analysis, 3) whether marker assisted selection (**MAS**) can be implemented for selecting broilers with efficient nutrient utilization.

5.2 Materials and Methods

5.2.1 Experimental Birds and Layout

The birds used in this study have been previously described in chapter 3. A total of 475 three-wk-old pedigreed male broilers belonging to lines A (197 birds from 15 sire families) and B (278 birds from 25 sire families) were used for the association study. Males from line A have been traditionally used as a terminal sire for production of broilers; females of line B are mated to males from another line and their female progeny are used as commercial hybrid broiler breeders.

Birds were raised on floor pens until 18 d of age and then moved to individual cages for a 3 wk feed conversion trial. During the experimental period, ad libitum feed and water were provided.

5.2.2 Experimental Diet and Sampling

A single standard grower commercial corn/soy diet containing 21.75% CP and 3080 kcal ME/kg of diet was given to the birds. The diet met or exceeded the National Research Council (1994) recommended nutrients for broilers for growth. The research protocol for this trial was approved by the University of Maryland Animal Care and Use Committee (R-07-23).

Individual bird weights and feed weights were collected at the start (3-wk old) and end of the trial (6-wk old). Excreta samples were obtained for each bird over a 24 h collection period at the end of the trial. Further details on the processing of the excreta samples were presented in chapter 3. Once the measurement of feed dis-

appearance and WG were made, their ratio (FCR) was calculated to determine the efficiency of feed utilization. Also, residual feed consumption (**RFC**) was estimated by the linear regression of FC over WG.

Blood samples (4 mL) were collected from the wing vein of all birds at the end of the trial at 6-wk old. The blood samples were collected as a source of DNA to enable association analysis with pre-determined candidate genes. The samples were placed on ice for transportation, and frozen until processed.

5.2.3 SNP Genotyping

The protocol for extraction of DNA and the genotypic methodology closely followed those described in detail by Liu (2009). Briefly, after thawing, extraction of genomic DNA followed the modified phenol-chroloform methodology; the saran wrap method was used to quantify DNA (Sambrook and Russel, 2001). The nine chosen SNP were determined for each sample using ABI TaqMan® assays. The primers used are presented in Table A1 (Appendix). DNA fragments were first amplified by polymerase chain reaction using Taqman Universal PCR Master Mix. An aliquot of 2.5 μL of genomic DNA was used for each sample. Initial thermal cycles were 2 min at 50°C and 10 min at 90°C, followed by 50 cycles alternating 95°C for 15 sec and 60°C for 1 min. Fluorescent signals were read by the ABI Prism Sequence 7900HT using real-time PCR capabilities.

5.2.4 Statistical Analysis

Hardy-Weinberg equilibrium (**HWE**) was tested on each SNP separately on lines A and B to check the underlying allele and genotypic selection processes in the population. Deviations from the HWE indicate disturbing influence (e.g. non-random mating, mutation, selection, limited population size, or unequal progeny in sire families) on the equilibrium. The expected genotype frequencies were calculated according to the methods described in Falconer and Mackay (1996). Deviations from expected genotype frequencies were tested (Pearson goodness-of-fit chi-square test) using genetics library from R statistical software.

A linear model for each SNP was used to first estimate the associations between the SNPs and the traits in broiler chickens using the GLM procedure of SAS (SAS Institute, Cary, NC). The traits used for finding the association include excreta traits (PNE and percentage of P in the excreta (**PPE**)) and production traits (WG, FC, FCR, and RFC). The model used for the preliminary point analysis in lines A and B was:

$$y_{ij} = \mu + SNP_i + e_{ij}$$

where y_{ij} is a phenotypic observation on the ij^{th} individual, μ is the overall population mean, SNP_i is the fixed genetic effect associated with the i^{th} SNP genotype, and e_{ij} is the residual error for the ij^{th} individual.

Additive and dominance effects of each SNP were estimated from the GLM procedure using estimate statements. An additive effect was estimated as one-half of the difference between the two homozygotes, whereas the dominance effects were

calculated as the difference between the heterozygote and the mean of the two homozygotes.

Linear and quadratic regressions on each SNP were carried out separately using the REG procedure of SAS. Based on the point analysis ANOVA, linear and quadratic regression results on each SNP, the significant SNPs were included for a multiple regression analysis (backward elimination method) in lines A and B. The quadratic effect was included in the model to account for the SNPs with significant dominant effect. Regression analysis was carried out on the SNP genotypes represented as the number of 1 allele (0, 1, 2).

5.3 Results and Discussion

Candidate gene approach in genetic studies involves selection of genes with a known or inferred biological function. Those genes are selected based on the presumption that they may be linked to the observed phenotype. In the present study, selecting candidate genes for the excreta traits (PNE, PPE) in broiler chickens was not trivial given the inherently complex biology involved with the traits and also due to the limited genetic studies in this field of research. Our strategy aimed to address these problems by identifying the genes which have experimental evidence for regulation of metabolism. Identification of SNPs in lean and fat broiler chickens from previous research (Muchow, 2006; Liu, 2009) provided evidence that some of the SNPs were associated with the fat and lean traits (e.g. fat yield, carcass yield) in broilers. The traits defined in the present study have either direct or indirect

correlation with the metabolism of the bird. An association between the excreta traits and those SNPs seemed realistic enough to pursue under the above stated hypothesis.

The overall descriptive statistics of the variables are shown in Table 5.1. In this study, only 443 samples were able to be genotyped and that explains the slight difference in descriptive statistics from those reported in Table 3.1. Allele frequency, genotypic frequency, and χ^2-test results for HWE in broiler lines A, B, and the difference in frequencies in the two lines (lines A and B combined) for the nine SNPs are shown in Tables 5.2, 5.3, and 5.4 respectively. All the SNPs per sire family in line A were in HWE ($\alpha = 0.05$). In line B, SOD3 SNP3 and AKR1B10 SNP1 were found to deviate from HWE; the AKR1B10 SNP1 showed very low frequency for the TT genotype, implying that selection has occured against the TT homozygous genotype in line B. Lines A and B differ in the allele frequencies of most of the SNPs per sire family except SOD3 SNP2.

The summary of the association analysis between the SNPs and the excreta traits in lines A and B are shown in Figures 5.1 and 5.2 respectively. Significant associations were observed in line A between SOD3 SNP1 and PNE. In line B, AKR1B10 SNP1 and AKR1B10 SNP2 were associated with PNE and PPE, respectively. Liu (2009) reported associations between the AKR1B10 gene and fat yield. It is possible that AKR1B10 gene could be involved in multiple metabolism regulation pathways. When fat deposition is affected, it can affect the glucose metabolism, and ATP production. This can indirectly lead to variations in protein metabolism and growth. Therefore, the possibility of the AKR1B10 gene affecting N and P

metabolism cannot be ruled out. The SOD3 gene was reported to be involved with regulation of NO; therefore, it is possible to have an indirect association between SOD3 SNP1 and PNE.

The SNP genotype associations with excreta traits are shown in Figures 5.3, 5.4, and 5.5. In line A, birds with the CT genotype of SOD3 SNP1 were found to have a lower PNE than birds with TT genotype. Also, the frequency of TT genotype was very low. It implies that favorable (lower) values of PNE can be forecasted in broilers if the selection is against TT genotype, or if selection is done specifically against the homozygote genotypes of SOD3 SNP1. Similarly, for SDC1 SNP1, the lower genotypic effect of CC ensures that selection against GG may provide a beneficial effect for PNE in line A. SDC1 protein is known for cell proliferation (Kolset and Salmivirta, 1999). Therefore, SDC1 genes may have a role in nutrient metabolism for cellular growth. The effect of AA genotype in reducing PNE was found to be highly significant when compared to TT of AKR1B10 SNP1 in line B. The frequency of TT genotype was surprisingly low (1) from a sample of 247. The interesting question here is "Did correlated response to selection created a decreased PNE in line B?". However, line A analysis did not showed a decrease in TT genotypic frequency. Therefore, AKR1B10 gene might have a line specific effect in reducing PNE. The AKR1B10 SNP2 genotype GA showed an effect in lowering PPE when compared to the homozygous GG. It indicates AA genotype is favorable for the trait PPE.

The SNP associations with broiler production traits are shown in Figures 5.6, and 5.7. The line A results indicate associations of SOD3 SNP1 with WG and FC, AKR1B10 SNP1 with FC and RFC, GPC3 SNP1 with RFC, and GPC3 SNP2 with

FC and RFC. According to Duenas Gonzales et al. (1998), GPC3 gene can inhibit cell proliferation and it reduces tumor growth by down-regulation. So, GPC3 gene may regulate both nutrient metabolism and cellular growth. In line B, associations of AKR1B10 SNP1 with WG, FC, and FCR were found. These results indicate a commonality in effect of the genes (SOD3 and AKR1B10) between excreta and production traits.

The genotypic effect of the SNPs on the production traits are shown in Figures 5.8, and 5.9. In line A, the SOD3 SNP1 genotype CT showed a higher effect on WG and FC when compared to TT. The results for WG and PNE in line A indicate that SOD3 gene had a beneficial effect on both production and excreta traits. The AKR1B10 SNP1 genotype TT in line A had a lower FC when compared to the heterozygote. However, it was not found to be associated with either of the excreta traits in line A. In line B, the AKR1B10 SNP1 genotype TT was found to have a lower effect on WG when compared with AA. This result indicates AKR1B10 SNP1 genotype AA had a beneficial effect on both PNE and WG. Selection for AA genotype in line B, therefore, will be highly beneficial. Also, in line B, the AA genotype of AKR1B10 SNP1 had a higher effect on FC, which may suggest that the gene favors an efficient nutrient utilization with increased intake of feed. However, the AKR1B10 SNP1 genotype AA had a lower FCR when compared to the TT genotype in line B. In this particular case, we have to take into consideration the very low frequency of TT genotype. The low frequency may be due to unfavorable pleiotropic effects or to phase linkage with some allele in another locus that has been selected against ("hitchhiking"?). The small number of TT genotypes in line B

may have limited the statistical power to state its effect categorically. An effect of GPC3 SNP2 on RFC was also noticed in line A. The genotype CC had a lower effect on RFC when compared to TT. A divergent selection study (Bordas and Minvielle, 1999) for decreased RFC in layer chicken had previously shown an increase in body weight (BW) and BW gain in both males and females in their initial periods of growth. This indicates that selection on CC genotype of GPC3 SNP2 in line A would be beneficial to improve the marketability of the bird. The apparently non-significant effect of this gene on the excreta trait also suggests that selection on this GPC3 gene will improve the production trait without increasing nutrient content in the excreta.

The additive and dominance effect of the genotypes for each of the SNPs on the excreta traits and production traits in lines A and B are shown inTables 5.7, 5.8, 5.9, and 5.10. In line A, the additive and dominance effect of SOD3 SNP1, and dominance effect of AKR1B10 SNP1 were found to be significant for PNE whereas only the additive effect of SOD3 SNP3 was significant for PPE in the same line. Line B showed additive and dominance effect of AKR1B10 SNP1, and additive effect of GPC3 SNP1 to be significant for PNE. The effects on PPE in line B were showed by an additive on SOD3 SNP3, and dominance on SDC1 SNP1.

Both additive and dominant effect of SOD3 SNP1, and additive effect of SDC1 SNP1 for WG were significant in line A. Line B also had the same effect of SOD3 SNP1, additive effect on SOD3 SNP2, additive and dominance effect on AKR1B10 SNP1, additive effect of AKR1B10 SNP2, and dominance effect on SDC1 SNP3 for WG. This corroborates the association of SOD3 SNP1 and AKR1B10 SNP1

with WG and PNE. The additive effect on SOD3 SNP1, dominance effect on both AKR1B10 SNP1 and GPC3 SNP2, and additive and dominance effects on SDC1 SNP1 for FC were significant in line A. Line B also showed an additive effect on SOD3 SNP1, and additive effect on SDC1 SNP1 for FC. This indicates that both SOD3 and SDC1 genes have a common effect on the WG and contribute to additive genetic variation.

None of the markers were found to have an effect on FCR in line A, however, there was a dominance effect on SOD3 SNP1, and additive and dominance effect of AKR1B10 SNP1 for FCR in line B. In reference to RFC, only a dominance effect of AKR1B10 SNP1 in line A could be found to be significant.

Based on these results, a multiple regression analysis was performed to find the best combination of SNPs in each of the SNPs on the excreta traits in lines A and B. The multiple regression results can help in identifying marginal signifiance of SNPs; the information obtained from multiple regression analysis could be used in selection processes. The results are shown in Tables 5.5, and 5.6. It indicates that AKR1B10 SNP1 has an effect on PNE, and SOD3 genes are found to have effect on PPE in both lines.

These results have some commonality with those reported by Liu (2009). In that study, BW was found to be associated with SOD3 and SDC1 genes. The association of SOD3 SNP1 with BW was significant at 3^{rd}, 4^{th}, and 5^{th} wk of age. Also, breast yield was found to be significantly associated with SOD3 SNP1, and thigh yield to SDC1 SNP1. The correlation between production trait and excreta trait in the present study can be explained by the possibility that increase in body

mass conserves more nutrients in the body, and less in excreta. Therefore, it is likely that SOD3 will have an effect in regulation of the nutrient content in the excreta.

5.4 Conclusion

The results from chapter 3 and chapter 5 indicate that there is scope for improvement in broiler excreta nutrients if genetic selection is implemented judiciously. The high cost of genotyping, short generation interval, and small size of birds are some factors preventing the application of genome-wide selection in broiler selection experiments. The use of a high-density chicken SNP chip with large number of validated genetic markers coupled with more efficient and accurate statistical methods for determining breeding values provides hope for the implementation of GWS on chickens in the near future. Known associations between markers and genes will weaken in future generations because of recombination and, to a lesser extent, mutations. Large-scale adoption of GWS in broiler breeding programs will require data training every four or five generations in order to keep high accuracy in the prediction of GBV. There may not be enough economic benefit to justify using a dense SNP chip to genotype each broiler candidate so the candidate gene approach may be a viable option to incorporate genotypic information in selection decisions. The current research blends the traditional and modern approach in the most practical and economic way and provides evidence that environmental improvement through genetic selection could be fulfilled without any depreciation in production traits, but there is a possibility of reallocation of selection pressure. This research also corrobo-

rates the previous association of some genes with production traits. In addition, the results from this research can be useful for application in the poultry industry and for further investigation to find more genes associated with the quantitative excreta traits.

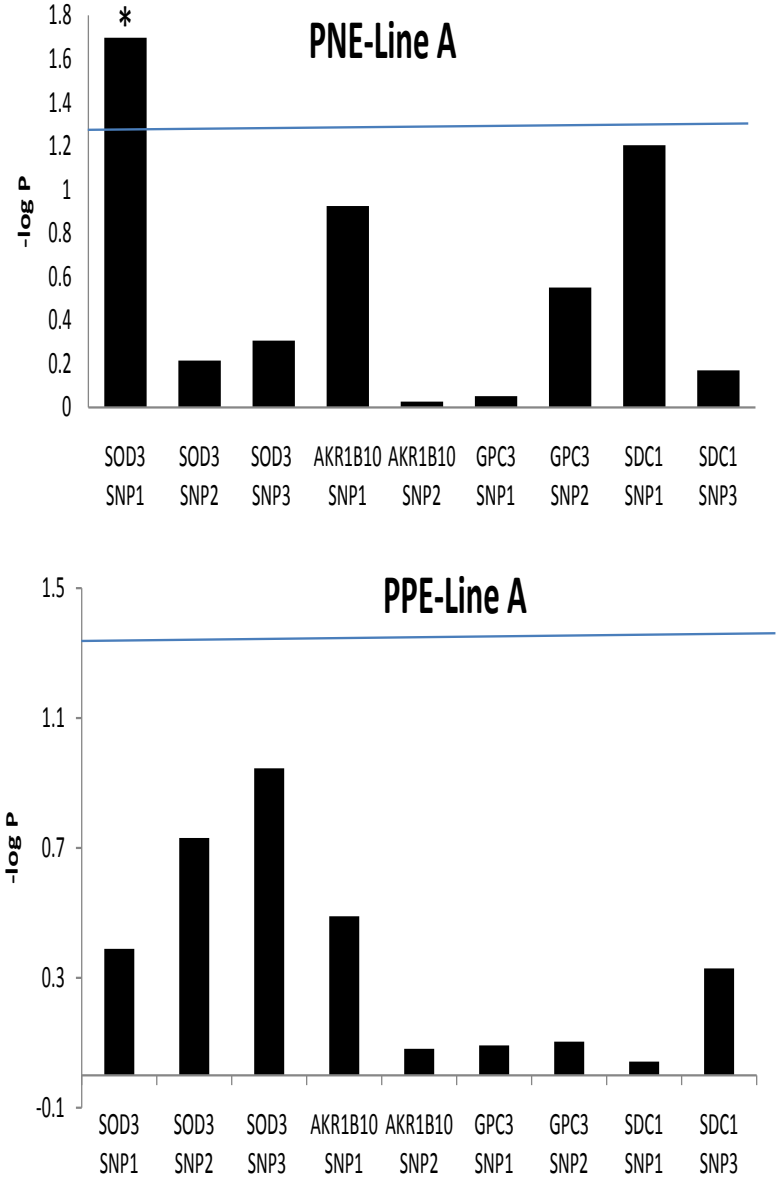

Figure 5.1: Line A. Association between SNPs and excreta traits: Percentage of N in the excreta (PNE), Percentage of P in the excreta (PPE).

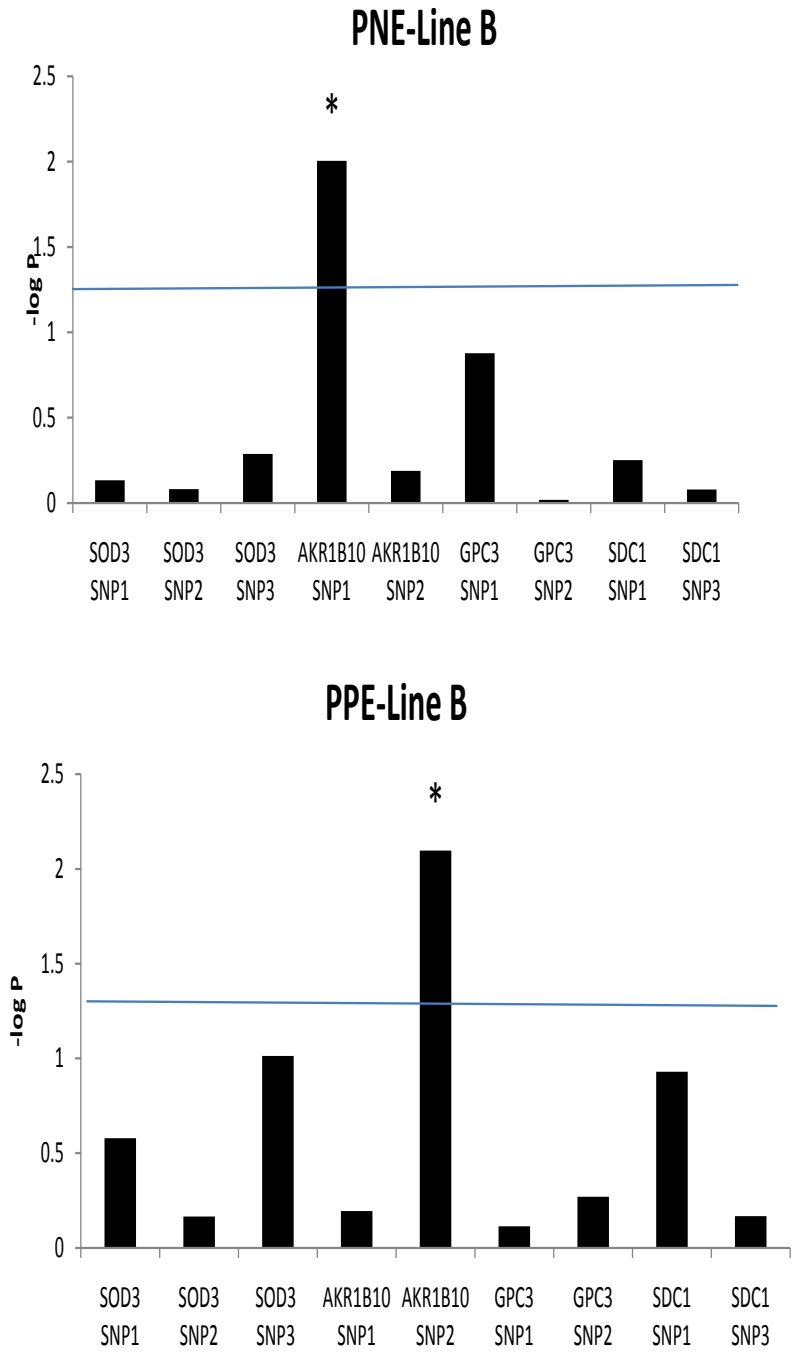

Figure 5.2: Line B. Association between SNPs and excreta traits: Percentage of N in the excreta (PNE), Percentage of P in the excreta (PPE).

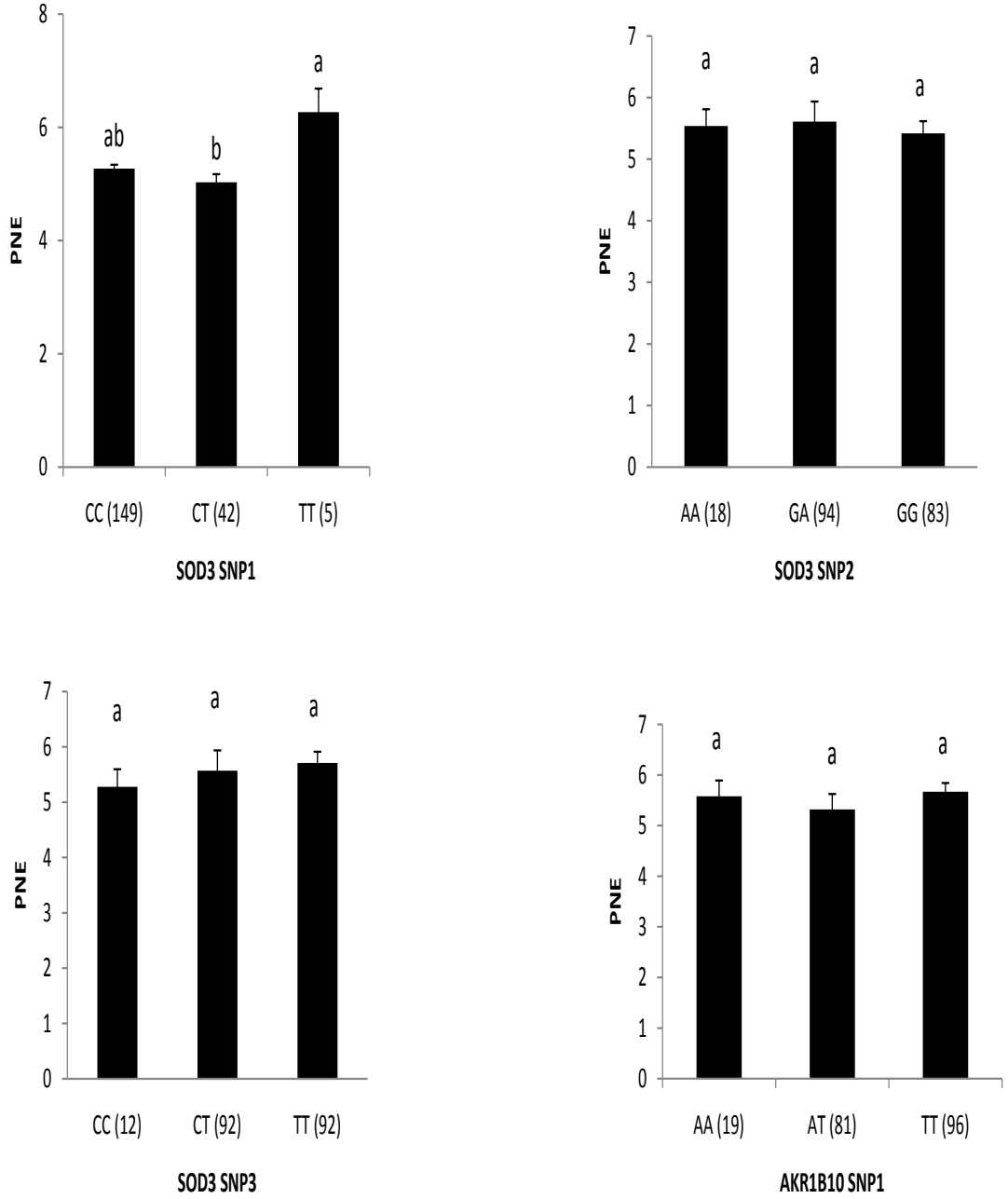

Figure 5.3: Line A. Association analysis between SNP genotypes and excreta traits: Percentage of N in the excreta (PNE)[1]. Tukey results are shown above each bar

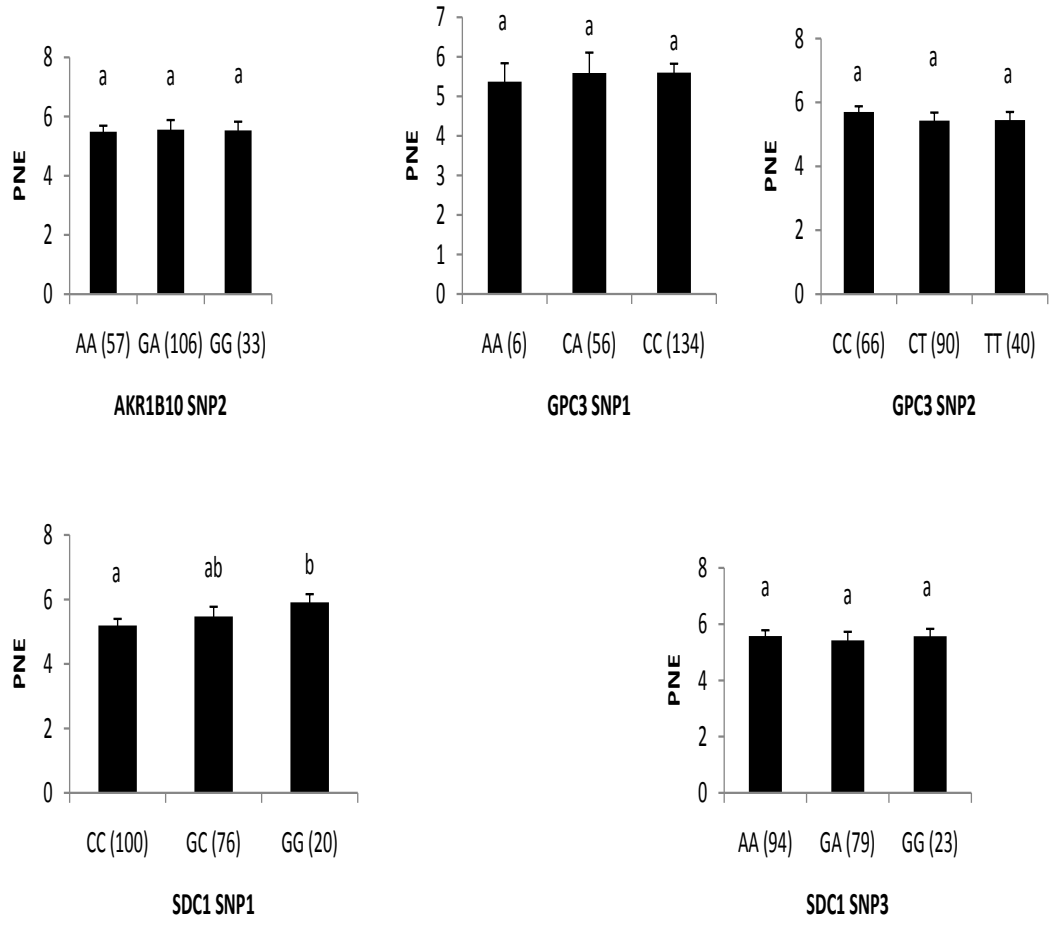

Figure 5.4: Line A. Association analysis between SNP genotypes and excreta traits: Percentage of N in the excreta (PNE)[2]. Tukey results are shown above each bar

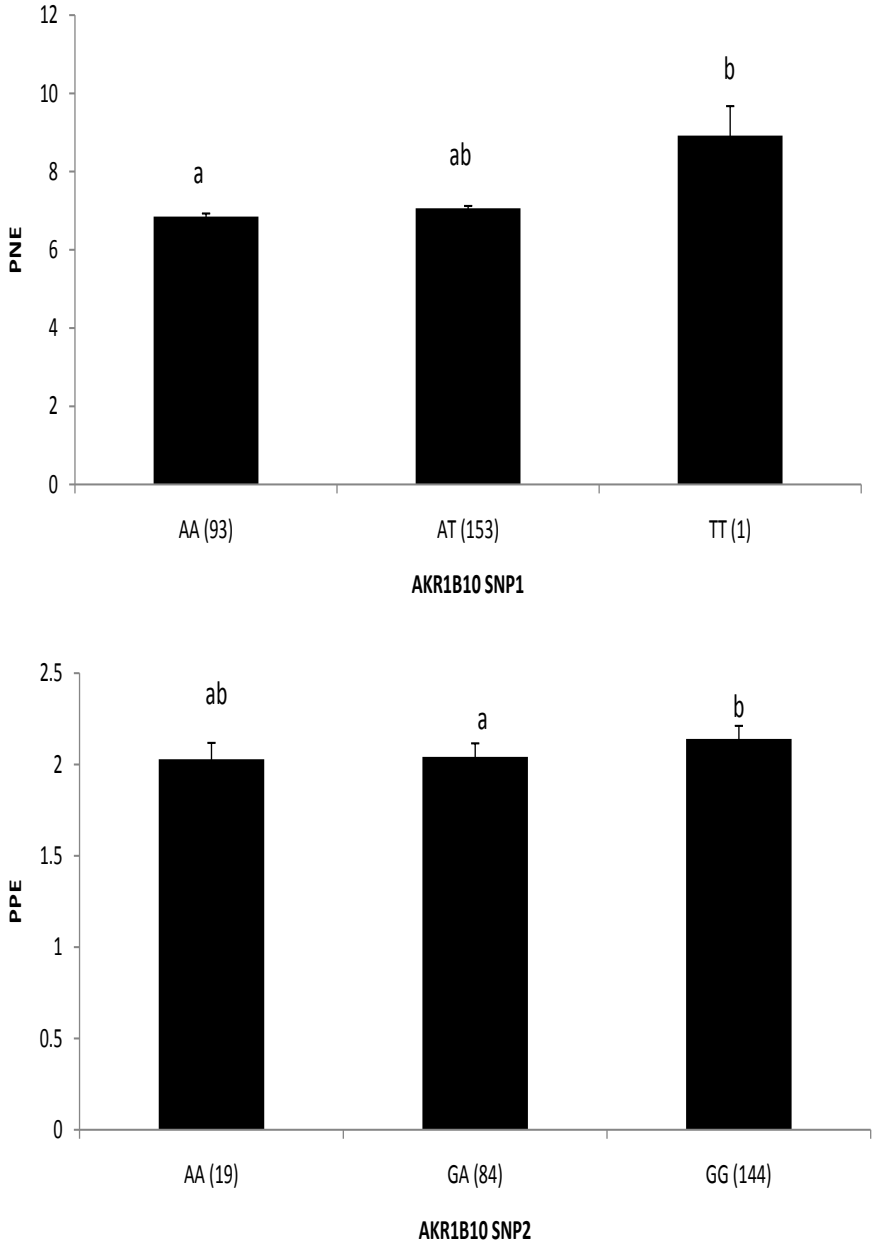

Figure 5.5: Line B. Association analysis between SNP genotypes and excreta traits: Percentage of N in the excreta (PNE), Percentage of P in the excreta (PPE). Tukey results are shown above each bar (Only charts with more than one Tukey groupings are shown)

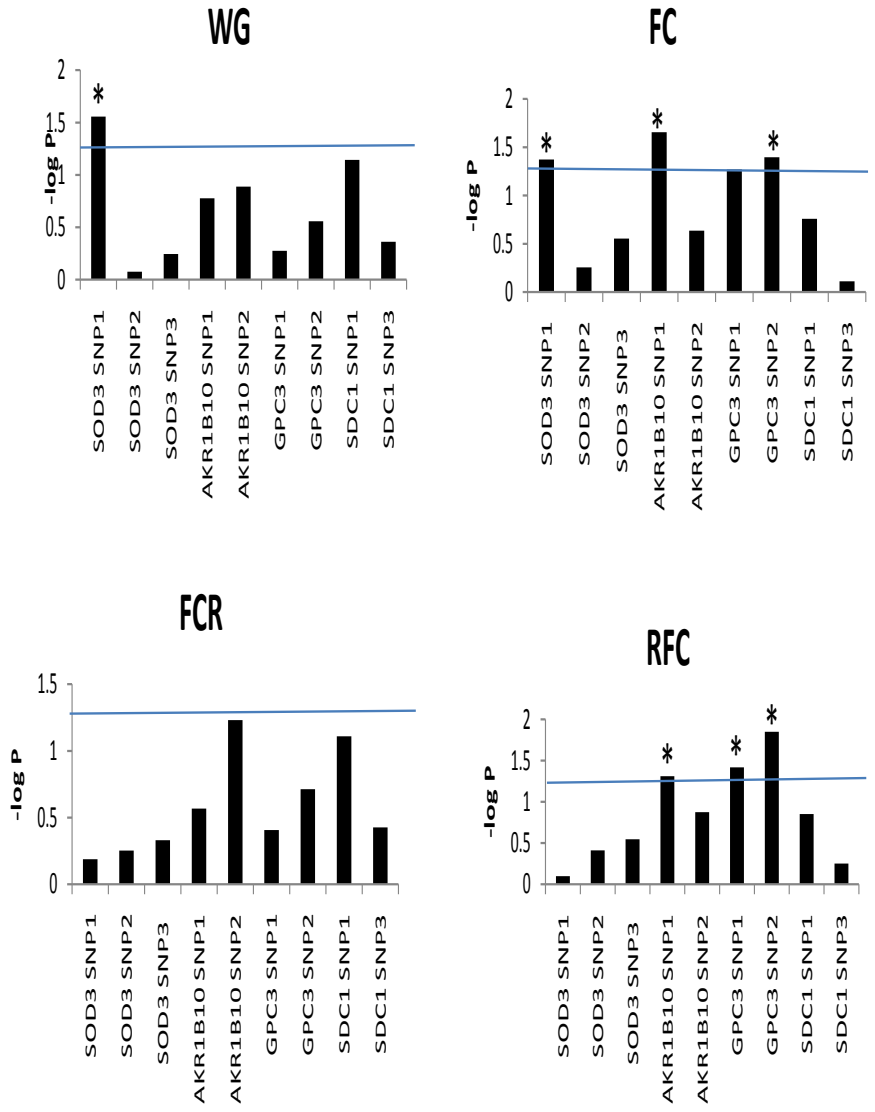

Figure 5.6: Line A. Association between SNPs and production traits: Weight gain (WG), Feed consumption (FC), Feed conversion ratio (FCR), Residual feed consumption (RFC).

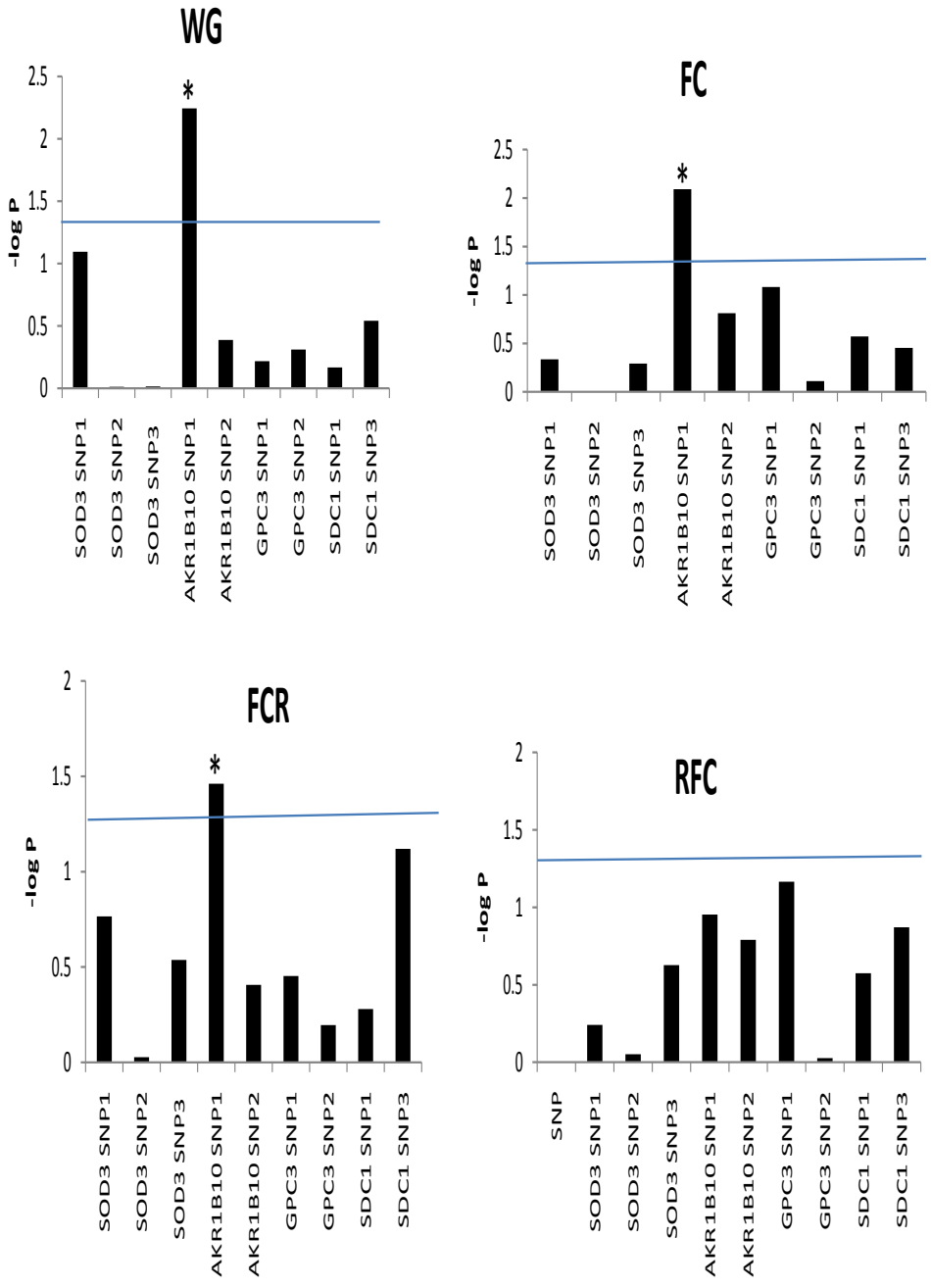

Figure 5.7: Line B. Association between SNPs and production traits: Weight gain (WG), Feed consumption (FC), Feed conversion ratio (FCR), Residual feed consumption (RFC).

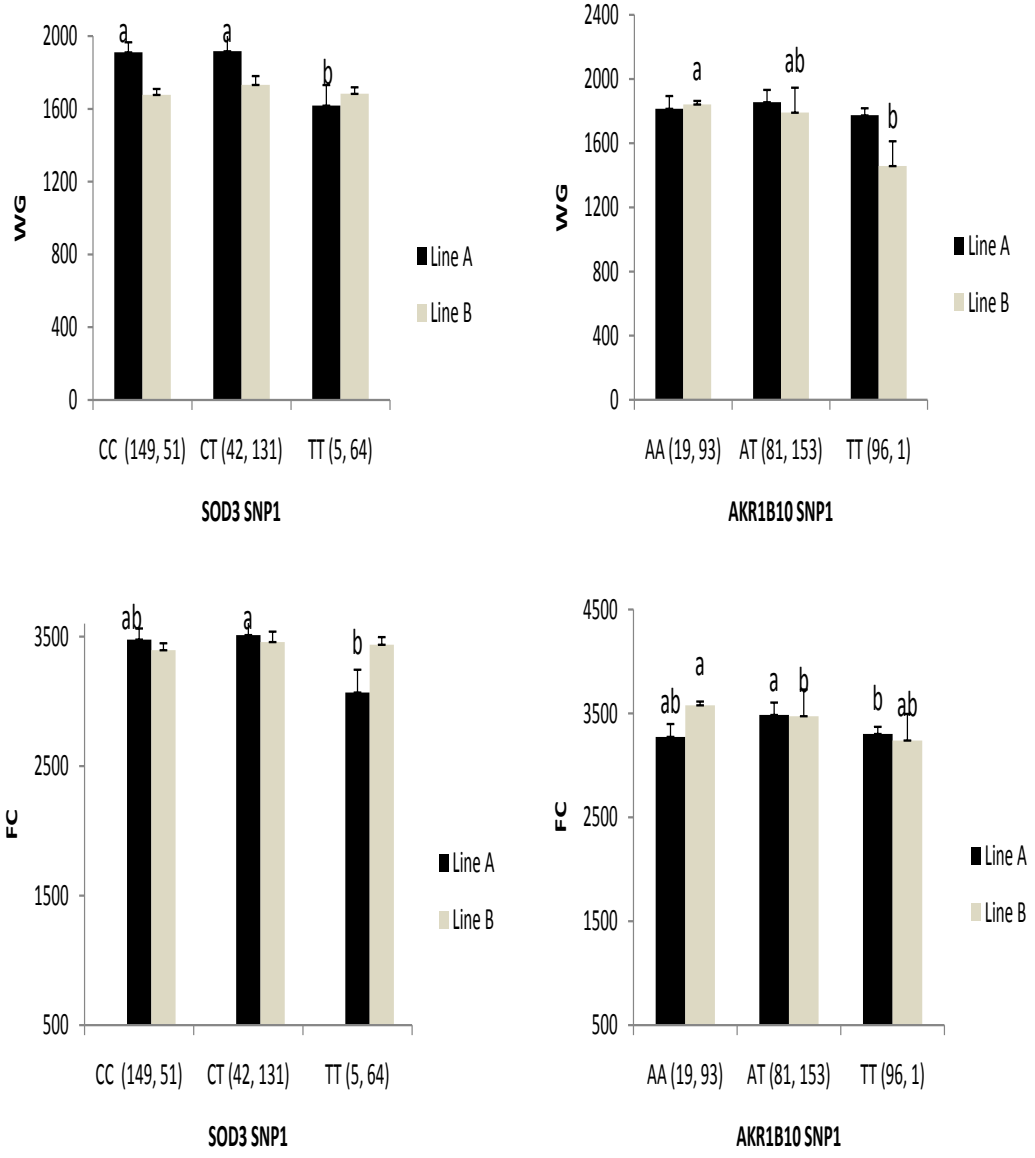

Figure 5.8: Association analysis between SNP genotypes and production traits: Weight gain (WG), Feed consumption (FC). Tukey results are shown above each bar (Only charts with more than one Tukey groupings are shown)

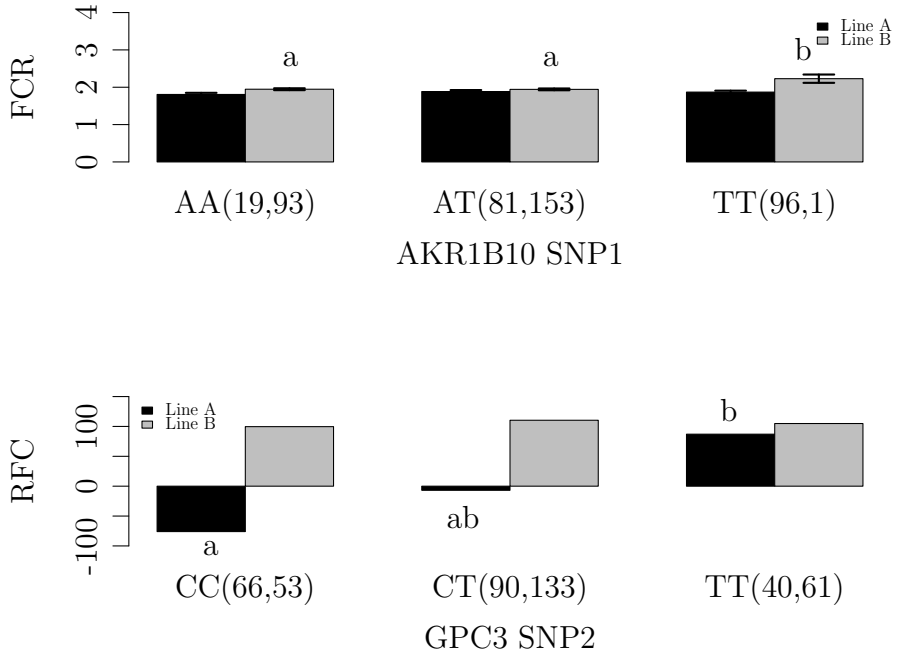

Figure 5.9: Association analysis between SNP genotypes and production traits: Feed conversion ratio (FCR), Residual feed consumption (RFC). Tukey results are shown above each bar (Only charts with more than one Tukey groupings are shown)

Table 5.1: Descriptive statistics for excreta traits and other economically important traits

Line	Trait[a]	Mean±SE	CV(%)	Min	Max	N
A	PNE	5.26±0.07	17.92	3.03	10.85	196
	PPE	1.98±0.02	10.87	1.38	2.58	194
	WG(g)	1936±17	12.37	760	2490	191
	FC(g)	3512±28	10.88	1650	4285	191
	FCR	1.82±0.01	7.86	1.46	2.50	191
B	PNE	7.08±0.05	10.82	5.52	9.55	247
	PPE	2.05±0.01	8.93	1.45	2.78	247
	WG(g)	1820±9.68	8.32	1360	2225	245
	FC(g)	3478±16	7.23	2665	4115	245
	FCR	1.92±0.01	5.62	1.71	2.22	245

[a]PNE: Percentage of nitrogen in the excreta (on a DM basis); PPE: Percentage of phosphorus in the excreta (on a DM basis); WG: Weight gained by the bird during the feed conversion trial (3 wk old - 6 wk old); FC: Feed consumed by the bird during the 3 wk of the feed conversion trial; FCR: Feed conversion ratio.

Table 5.2: Observed and expected numbers and percentages of SNP genotypes and allele frequencies in the Line A

	Genotype			Allele Frequency	
SOD3 SNP1	CC	CT	TT	C	T
Observed	149 (76%)	42 (21%)	5 (3%)	0.87	0.13
Expected	147.5 (75.2%)	45.1 (23%)	3.45 (1.7%)		
χ^2 test	p value=0.3466				
SOD3 SNP2	GG	GA	AA	G	A
Observed	83 (43%)	94 (48%)	18 (9%)	0.67	0.33
Expected	87.02(44%)	87.02 (44%)	21.77 (11%)		
χ^2 test	p value=0.2639				
SOD3 SNP3	TT	TC	CC	T	C
Observed	92 (47%)	92 (47%)	12 (6%)	0.70	0.30
Expected	96.04 (49.5%)	82.3(41%)	15.68 (8%)		
χ^2 test	p value=0.08				
AKR1B10 SNP1	TT	TA	AA	T	A
Observed	96 (49%)	81 (41%)	19 (10%)	0.70	0.30
Expected	95.1 (48.5%)	82.3 (42%)	17.64 (9%)		
χ^2 test	p value=0.7372				
AKR1B10 SNP2	GG	GA	AA	G	A
Observed	33 (17%)	106 (54%)	57 (29%)	0.44	0.56
Expected	37.8 (19.3%)	96.4 (49.2%)	61.5 (31.5%)		
χ^2 test	p value=0.1939				
GPC3 SNP1	CC	CA	AA	C	A
Observed	134 (68%)	56 (29%)	6 (3%)	0.83	0.17
Expected	133.8 (68.3%)	56.05 (28.6%)	5.88 (3%)		
χ^2 test	p value=1				
GPC3 SNP2	TT	TC	CC	T	C
Observed	40 (20%)	90 (46%)	66 (34%)	0.43	0.57
Expected	36.84 (18.8%)	96.04 (49%)	62.85 (32.07%)		
χ^2 test	p value=0.3834				
SDC1 SNP1	CC	CG	GG	C	G
Observed	100 (51%)	76 (39%)	20 (10%)	0.70	0.30
Expected	97.15 (49.57%)	81.53 (41.6%)	15.68 (8%)		
χ^2 test	p value=0.3903				
SDC1 SNP3	GG	GA	AA	G	A
Observed	23 (12%)	79 (40%)	94 (48%)	0.32	0.68
Expected	19.91 (10.1%)	85.06 (43.4%)	90.92 (46.39%)		
χ^2 test	p value=0.3248				

Table 5.3: Observed and expected numbers and percentages of SNP genotypes and allele frequencies in the Line B

	Genotype			Allele Frequency	
SOD3 SNP1	CC	CT	TT	C	T
Observed	51 (21%)	131 (53%)	64 (26%)	0.47	0.53
Expected	55.1 (22.4%)	122.5 (49.8%)	68.14 (27.7%)		
χ^2 test	p value=0.3091				
SOD3 SNP2	GG	GA	AA	G	A
Observed	103 (42%)	120 (49%)	24 (10%)	0.66	0.34
Expected	107.4 (43.5%)	110.65 (44.8%)	28.4 (11.56%)		
χ^2 test	p value=0.2561				
SOD3 SNP3	TT	TC	CC	T	C
Observed	159 (64%)	70 (28%)	18 (7%)	0.79	0.21
Expected	152.2 (61.6%)	82.9 (33.6%)	11.4 (4.6%)		
χ^2 test	p value=0.014*				
AKR1B10 SNP1	TT	TA	AA	T	A
Observed	1 (0%)	153 (62%)	93 (38%)	0.31	0.69
Expected	24.2 (9.8%)	106.2 (43%)	116.1 (47.1%)		
χ^2 test	p value < 0.01**				
AKR1B10 SNP2	GG	GA	AA	G	A
Observed	144 (58%)	84 (34%)	19 (8%)	0.75	0.25
Expected	140 (56.7%)	91.4 (37%)	14.8 (6%)		
χ^2 test	p value=0.1738				
GPC3 SNP1	CC	CA	AA	C	A
Observed	131 (53%)	104 (42%)	12 (5%)	0.74	0.26
Expected	135.4 (54.8%)	94.79 (38.4%)	16.54 (6.7%)		
χ^2 test	p value=0.1834				
GPC3 SNP2	TT	TC	CC	T	C
Observed	61 (25%)	133 (54%)	53 (21%)	0.52	0.48
Expected	65.7 (26.6%)	123 (49.8%)	57.8 (23.4%)		
χ^2 test	p value=0.2525				
SDC1 SNP1	CC	CG	GG	C	G
Observed	164 (66%)	75 (30%)	8 (3%)	0.82	0.18
Expected	164.3 (66.5%)	74.1 (30%)	8.4 (3.4%)		
χ^2 test	p value=1				
SDC1 SNP3	GG	GA	AA	G	A
Observed	168 (68%)	70 (28%)	9 (4%)	0.82	0.18
Expected	166.7 (67.5%)	72.1 (29.2%)	7.83 (3.2%)		
χ^2 test	p value=0.6625				

Table 5.4: Allele frequency differences between Line A and Line B

Line	SNP	Allele Frequency	
	SOD3 SNP1	C	T
A		0.87	0.13
B		0.47	0.53
χ^2 test:	p value < 0.01*		
	SOD3 SNP2	G	A
A		0.67	0.33
B		0.66	0.34
χ^2 test:	p value=0.8331		
	SOD3 SNP3	T	C
A		0.70	0.30
B		0.79	0.21
χ^2 test:	p value=0.005*		
	AKR1B10 SNP1	T	A
A		0.70	0.30
B		0.31	0.69
χ^2 test:	p value < 0.01*		
	AKR1B10 SNP2	G	A
A		0.44	0.56
B		0.75	0.25
χ^2 test:	p value < 0.01*		
	GPC3 SNP1	C	A
A		0.83	0.17
B		0.74	0.26
χ^2 test:	p value=0.0023*		
	GPC3 SNP2	T	C
A		0.43	0.57
B		0.52	0.48
χ^2 test:	p value < 0.014*		
	SDC1 SNP1	C	G
A		0.70	0.30
B		0.82	0.18
χ^2 test:	p value < 0.01*		
	SDC1 SNP3	G	A
A		0.32	0.68
B		0.82	0.18
χ^2 test:	p value < 0.01*		

Table 5.5: Line A Multiple Regression analysis (Backward Elimination-Final step) using SNPs (α=0.10)

Source	Dependent variable[a]	p value
SOD3 SNP1	PNE	0.0272
Quad: SOD3 SNP1	PNE	0.0050
AKR1B10 SNP1	PNE	0.08
Quad: AKR1B10 SNP1	PNE	0.0125
SDC1 SNP1	PNE	0.0497
SOD3SNP2	PPE	0.0302

[a]PNE: Percentage of N in the excreta, PPE: Percentage of P in the excreta

Table 5.6: Line B Multiple Regression analysis (Backward Elimination-Final step) using SNPs (α=0.10)

Source	Dependent variable[a]	p value
Quad: AKR1B10 SNP1	PNE	0.0052
GPC3 SNP1	PNE	0.0087
SOD3 SNP3	PPE	0.0236
Quad: SOD3 SNP3	PPE	0.0463
AKR1B10 SNP2	PPE	0.0421
SDC1 SNP1	PPE	0.0248
Quad: SDC1 SNP1	PPE	0.0310

[a]PNE: Percentage of N in the excreta, PPE: Percentage of P in the excreta

Table 5.7a: Line A, SNP point analysis results. PNE: Percentage of nitrogen in the excreta. PPE: Percentage of phosphorus in the excreta. p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
1	SOD3 SNP1	CC	A	PNE	5.225	0.0759	0.5664		0.0078**
2	SOD3 SNP1	CT	A	PNE	5.125	0.1431		-0.6967	0.0068**
3	SOD3 SNP1	TT	A	PNE	6.388	0.4147			
4	SOD3 SNP1	CC	A	PPE	1.980	0.0178	0.0008		0.9865
5	SOD3 SNP1	CT	A	PPE	1.962	0.0334		-0.0195	0.7430
6	SOD3 SNP1	TT	A	PPE	1.982	0.0967			
7	SOD3 SNP2	AA	A	PNE	5.259	0.217	-0.0371		0.7579
8	SOD3 SNP2	GA	A	PNE	5.317	0.097		0.0954	0.5383
9	SOD3 SNP2	GG	A	PNE	5.185	0.104			
10	SOD3 SNP2	AA	A	PPE	2.052	0.0490	-0.0544		0.0465*
11	SOD3 SNP2	GA	A	PPE	1.991	0.0223		-0.0071	0.8392
12	SOD3 SNP2	GG	A	PPE	1.943	0.0234			
13	SOD3 SNP3	CC	A	PNE	5.002	0.2724	0.1598		0.2711
14	SOD3 SNP3	CT	A	PNE	5.224	0.0984		0.0624	0.7218
15	SOD3 SNP3	TT	A	PNE	5.321	0.0983			
16	SOD3 SNP3	CC	A	PPE	1.848	0.0616	0.0748		0.0237*
17	SOD3 SNP3	CT	A	PPE	1.972	0.0222		0.0488	0.2191
18	SOD3 SNP3	TT	A	PPE	1.998	0.0225			
19	AKR1B10 SNP1	AA	A	PNE	5.336	0.2128	0.0464		0.6906
20	AKR1B10 SNP1	AT	A	PNE	5.033	0.1031		-0.3495	0.0258*
21	AKR1B10 SNP1	TT	A	PNE	5.429	0.0947			
22	AKR1B10 SNP1	AA	A	PPE	2.002	0.0509	-0.0176		0.5260
23	AKR1B10 SNP1	AT	A	PPE	1.982	0.0240		-0.003	0.9354
24	AKR1B10 SNP1	TT	A	PPE	1.9669	0.0222			
25	AKR1B10 SNP2	AA	A	PNE	5.1825	0.1246	-0.0456		0.6582
26	AKR1B10 SNP2	GA	A	PNE	5.3468	0.0914		0.2101	0.1288
27	AKR1B10 SNP2	GG	A	PNE	5.0912	0.1639			
28	AKR1B10 SNP2	AA	A	PPE	1.9687	0.0285	-0.0083		0.7247
29	AKR1B10 SNP2	GA	A	PPE	1.9882	0.0212		0.0277	0.3829

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Table 5.7b: Contd. Line A, SNP point analysis results. PNE: Percentage of nitrogen in the excreta. PPE: Percentage of phosphorus in the excreta. p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
30	AKR1B10 SNP2	GG	A	PPE	1.9521	0.0376			
31	GPC3 SNP1	AA	A	PNE	4.9717	0.3852	0.1676		0.3954
32	GPC3 SNP1	CA	A	PNE	5.1645	0.1261		0.0251	0.9145
33	GPC3 SNP1	CC	A	PNE	5.3070	0.0815			
34	GPC3 SNP1	AA	A	PPE	1.8516	0.0876	0.0691		0.1244
35	GPC3 SNP1	CA	A	PPE	1.9577	0.0286		0.0368	0.4889
36	GPC3 SNP1	CC	A	PPE	1.9899	0.0187			
37	GPC3 SNP2	CC	A	PNE	5.4605	0.1152	-0.1501		0.1110
38	GPC3 SNP2	CT	A	PNE	5.1486	0.0986		-0.1617	0.2362
39	GPC3 SNP2	TT	A	PNE	5.1603	0.1479			
40	GPC3 SNP2	CC	A	PPE	1.9754	0.0268	0.0059		0.7880
41	GPC3 SNP2	CT	A	PPE	1.9723	0.0228		-0.0089	0.7774
42	GPC3 SNP2	TT	A	PPE	1.9872	0.0346			
43	SDC1 SNP1	CC	A	PNE	5.2414	0.0939	0.1833		0.1126
44	SDC1 SNP1	GC	A	PNE	5.1826	0.1077		-0.2421	0.1261
45	SDC1 SNP1	GG	A	PNE	5.608	0.2099			
46	SDC1 SNP1	CC	A	PPE	1.9915	0.0217	-0.0313		0.2383
47	SDC1 SNP1	GC	A	PPE	1.9692	0.0247		0.0089	0.8050
48	SDC1 SNP1	GG	A	PPE	1.9290	0.0482			
49	SDC1 SNP3	AA	A	PNE	5.2219	0.0975	0.0679		0.5376
50	SDC1 SNP3	GA	A	PNE	5.2669	0.1064		-0.0229	0.8812
51	SDC1 SNP3	GG	A	PNE	5.3578	0.1973			
52	SDC1 SNP3	AA	A	PPE	1.9620	0.0222	0.0331		0.1875
53	SDC1 SNP3	GA	A	PPE	1.9783	0.0245		-0.0168	0.6317
54	SDC1 SNP3	GG	A	PPE	2.0282	0.0448			

Table 5.8a: Line B, SNP point analysis results. PNE: Percentage of nitrogen in the excreta. PPE: Percentage of phosphorus in the excreta. p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
1	SOD3 SNP1	CC	B	PNE	7.1543	0.1068	-0.1306		0.0694
2	SOD3 SNP1	CT	B	PNE	7.1508	0.0667		0.1270	0.1953
3	SOD3 SNP1	TT	B	PNE	6.8931	0.0954			
4	SOD3 SNP1	CC	B	PPE	2.0414	0.0258	0.0002		0.9920
5	SOD3 SNP1	CT	B	PPE	2.0666	0.0161		0.0250	0.2907
6	SOD3 SNP1	TT	B	PPE	2.0417	0.0230			
7	SOD3 SNP2	AA	B	PNE	7.3037	0.1560	-0.1534		0.0778
8	SOD3 SNP2	GA	B	PNE	7.1151	0.0697		-0.0352	0.7517
9	SOD3 SNP2	GG	B	PNE	6.9968	0.0753			
10	SOD3 SNP2	AA	B	PPE	2.0533	0.0376	-0.0029		0.8904
11	SOD3 SNP2	GA	B	PPE	2.0620	0.0168		0.0115	0.6671
12	SOD3 SNP2	GG	B	PPE	2.0475	0.0181			
13	SOD3 SNP3	CC	B	PNE	6.9716	0.1812	0.0675		0.4809
14	SOD3 SNP3	CT	B	PNE	7.0618	0.0919		0.0226	0.8643
15	SOD3 SNP3	TT	B	PNE	7.1066	0.0609			
16	SOD3 SNP3	CC	B	PPE	2.1577	0.0429	-0.0549		0.0159*
17	SOD3 SNP3	CT	B	PPE	2.0453	0.0217		-0.0575	0.0682
18	SOD3 SNP3	TT	B	PPE	2.0478	0.0144			
19	AKR1B10 SNP1	AA	B	PNE	6.9704	0.0782	1.0747		0.005**
20	AKR1B10 SNP1	AT	B	PNE	7.1399	0.0609		-0.9052	0.0192*
21	AKR1B10 SNP1	TT	B	PNE	9.1200	0.7542			
22	AKR1B10 SNP1	AA	B	PPE	2.0555	0.0191	-0.0227		0.8063
23	AKR1B10 SNP1	AT	B	PPE	2.0552	0.0149		0.0225	0.8109
24	AKR1B10 SNP1	TT	B	PPE	2.010	0.1843			
25	AKR1B10 SNP2	AA	B	PNE	7.0821	0.1765	-0.0074		0.9372
26	AKR1B10 SNP2	GA	B	PNE	7.1134	0.0839		0.0387	0.7586
27	AKR1B10 SNP2	GG	B	PNE	7.0673	0.0641			
28	AKR1B10 SNP2	AA	B	PPE	2.0195	0.0417	0.0305		0.1706
29	AKR1B10 SNP2	GA	B	PPE	2.0198	0.0198		-0.0302	0.3112

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Table 5.8b: Contd. Line B, SNP point analysis results. PNE: Percentage of nitrogen in the excreta. PPE: Percentage of phosphorus in the excreta. p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
30	AKR1B10 SNP2	GG	B	PPE	2.0804	0.0152			
31	GPC3 SNP1	AA	B	PNE	6.5866	0.2189	0.2917		0.0114*
32	GPC3 SNP1	CA	B	PNE	7.0332	0.0744		0.1549	0.2574
33	GPC3 SNP1	CC	B	PNE	7.1701	0.0663			
34	GPC3 SNP1	AA	B	PPE	2.0808	0.0532	-0.0109		0.6927
35	GPC3 SNP1	CA	B	PPE	2.0475	0.0181		-0.0223	0.5007
36	GPC3 SNP1	CC	B	PPE	2.0588	0.0161			
37	GPC3 SNP2	CC	B	PNE	7.0907	0.1053	-0.0624		0.3866
38	GPC3 SNP2	CT	B	PNE	7.1357	0.0665		0.1074	0.2742
39	GPC3 SNP2	TT	B	PNE	6.9659	0.0981			
40	GPC3 SNP2	CC	B	PPE	2.0211	0.0252	0.02034		0.2390
41	GPC3 SNP2	CT	B	PPE	2.0656	0.0159		0.02417	0.3037
42	GPC3 SNP2	TT	B	PPE	2.0618	0.0234			
43	SDC1 SNP1	CC	B	PNE	7.15585	0.0595	-0.1761		0.2035
44	SDC1 SNP1	GC	B	PNE	6.9572	0.0881		-0.0226	0.8903
45	SDC1 SNP1	GG	B	PNE	6.8037	0.2696			
46	SDC1 SNP1	CC	B	PPE	2.0573	0.0143	0.0632		0.057
47	SDC1 SNP1	GC	B	PPE	2.0366	0.0211		-0.0838	0.0334*
48	SDC1 SNP1	GG	B	PPE	2.1837	0.0645			
49	SDC1 SNP3	AA	B	PNE	7.1800	0.2547	-0.0201		0.8777
50	SDC1 SNP3	GA	B	PNE	6.9384	0.0914		-0.2214	0.1663
51	SDC1 SNP3	GG	B	PNE	7.1397	0.0589			
52	SDC1 SNP3	AA	B	PPE	2.0522	0.0614	0.0005		0.9860
53	SDC1 SNP3	GA	B	PPE	2.0598	0.0220		0.0071	0.8542
54	SDC1 SNP3	GG	B	PPE	2.0533	0.0142			

Table 5.9a: Line A, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
1	SOD3 SNP1	CC	A	WG	1938	19.6	-129.4		0.0172*
2	SOD3 SNP1	CT	A	WG	1963	37.4		154.7	0.0193*
3	SOD3 SNP1	TT	A	WG	1679	105.9			
4	SOD3 SNP1	CC	A	FC	3526	31.5	-177.9		0.041*
5	SOD3 SNP1	CT	A	FC	3502	60.1		153.8	0.145
6	SOD3 SNP1	TT	A	FC	3170	169.9			
7	SOD3 SNP1	CC	A	FCR	1.832	0.012	0.0290		0.3715
8	SOD3 SNP1	CT	A	FCR	1.785	0.023		-0.075	0.0575
9	SOD3 SNP1	TT	A	FCR	1.890	0.064			
10	SOD3 SNP1	CC	A	RFC	12.42	17.7	-6.85		0.888
11	SOD3 SNP1	CT	A	RFC	-45.19	33.8		-50.77	0.393
12	SOD3 SNP1	TT	A	RFC	-1.27	95.7			
13	SOD3 SNP2	AA	A	WG	1949	58.2	-18.9		0.556
14	SOD3 SNP2	GA	A	WG	1955	24.9		24.9	0.539
15	SOD3 SNP2	GG	A	WG	1912	26.7			
16	SOD3 SNP2	AA	A	FC	3575	93.1	-35.7		0.4867
17	SOD3 SNP2	GA	A	FC	3507	39.8		-31.9	0.6231
18	SOD3 SNP2	GG	A	FC	3503	42.6			
19	SOD3 SNP2	AA	A	FCR	1.840	0.034	0.003		0.8609
20	SOD3 SNP2	GA	A	FCR	1.801	0.015		-0.043	0.0785
21	SOD3 SNP2	GG	A	FCR	1.847	0.016			
22	SOD3 SNP2	AA	A	RFC	48.89	51.71	-10.7		0.7063
23	SOD3 SNP2	GA	A	RFC	-29.67	22.11		-64.8	0.0735
24	SOD3 SNP2	GG	A	RFC	24.43	23.69			
25	SOD3 SNP3	CC	A	WG	1832	75.45	66.1		0.098
26	SOD3 SNP3	CT	A	WG	1921	24.87		22.52	0.6319
27	SOD3 SNP3	TT	A	WG	1964	25.29			
28	SOD3 SNP3	CC	A	FC	3400	120.61	79.38		0.2135
29	SOD3 SNP3	CT	A	FC	3478	39.76		-1.447	0.9846

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Table 5.9b: Contd. Line A, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
30	SOD3 SNP3	TT	A	FC	3559	40.43			
31	SOD3 SNP3	CC	A	FCR	1.887	0.0454	-0.034		0.1617
32	SOD3 SNP3	CT	A	FCR	1.821	0.0149		-0.033	0.2474
33	SOD3 SNP3	TT	A	FCR	1.819	0.0152			
34	SOD3 SNP3	CC	A	RFC	26.43	67.95	-8.1		0.8223
35	SOD3 SNP3	CT	A	RFC	-12.85	22.40		-31.2	0.4610
36	SOD3 SNP3	TT	A	RFC	10.31	22.78			
37	AKR1B10 SNP1	AA	A	WG	1914	54.89	-0.084		0.9978
38	AKR1B10 SNP1	AT	A	WG	1969	27.09		54.5	0.1803
39	AKR1B10 SNP1	TT	A	WG	1914	24.68			
40	AKR1B10 SNP1	AA	A	FC	3430	86.64	13.65		0.7742
41	AKR1B10 SNP1	AT	A	FC	3596	42.76		152.44	0.0181*
42	AKR1B10 SNP1	TT	A	FC	3458	38.95			
43	AKR1B10 SNP1	AA	A	FCR	1.793	0.033	0.0132		0.4657
44	AKR1B10 SNP1	AT	A	FCR	1.836	0.0163		0.029	0.2220
45	AKR1B10 SNP1	TT	A	FCR	1.819	0.0148			
46	AKR1B10 SNP1	AA	A	RFC	-52.00	48.68	13.76		0.6069
47	AKR1B10 SNP1	AT	A	RFC	42.17	24.03		80.43	0.0263*
48	AKR1B10 SNP1	TT	A	RFC	-24.49	21.88			
49	AKR1B10 SNP2	AA	A	WG	1956	32.31	8.18		0.7614
50	AKR1B10 SNP2	GA	A	WG	1916	23.39		-48.291	0.1772
51	AKR1B10 SNP2	GG	A	WG	1972	43.04			
52	AKR1B10 SNP2	AA	A	FC	3495	51.62	46.48		0.2810
53	AKR1B10 SNP2	GA	A	FC	3498	37.36		-44.05	0.4402
54	AKR1B10 SNP2	GG	A	FC	3588	68.76			
55	AKR1B10 SNP2	AA	A	FCR	1.7915	0.0192	0.0143		0.3737
56	AKR1B10 SNP2	GA	A	FCR	1.842	0.0139		0.0359	0.0913
57	AKR1B10 SNP2	GG	A	FCR	1.820	0.0256			
58	AKR1B10 SNP2	AA	A	RFC	-42.07	28.79	35.66		0.1386

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Table 5.9c: Contd. Line A, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
59	AKR1B10 SNP2	GA	A	RFC	13.39	20.84		19.79	0.5338
60	AKR1B10 SNP2	GG	A	RFC	29.26	38.35			
61	GPC3 SNP1	AA	A	WG	2010	97.76	-30.99		0.5360
62	GPC3 SNP1	CA	A	WG	1899	32.59		-79.19	0.1861
63	GPC3 SNP1	CC	A	WG	1948	20.92			
64	GPC3 SNP1	AA	A	FC	3704	155.1	-84.12		0.2901
65	GPC3 SNP1	CA	A	FC	3431	51.69		-189.2	0.0470
66	GPC3 SNP1	CC	A	FC	3535	33.18			
67	GPC3 SNP1	AA	A	FCR	1.842	0.0588	-0.007		0.7933
68	GPC3 SNP1	CA	A	FCR	1.816	0.0196		-0.017	0.6308
69	GPC3 SNP1	CC	A	FCR	1.826	0.0126			
70	GPC3 SNP1	AA	A	RFC	95.25	87.27	-43.13		0.3351
71	GPC3 SNP1	CA	A	RFC	-32.39	29.09		-84.52	0.1143
72	GPC3 SNP1	CC	A	RFC	8.99	18.67			
73	GPC3 SNP2	CC	A	WG	1907	29.95	10.39		0.6699
74	GPC3 SNP2	CT	A	WG	1961	25.54		43.15	0.2228
75	GPC3 SNP2	TT	A	WG	1928	38.36			
76	GPC3 SNP2	CC	A	FC	3403	47.01	65.87		0.0862
77	GPC3 SNP2	CT	A	FC	3580	40.08		111	0.0464*
78	GPC3 SNP2	TT	A	FC	3535	60.22			
79	GPC3 SNP2	CC	A	FCR	1.800	0.0179	0.0185		0.2048
80	GPC3 SNP2	CT	A	FCR	1.835	0.0153		0.0162	0.4429
81	GPC3 SNP2	TT	A	FCR	1.837	0.0229			
82	GPC3 SNP2	CC	A	RFC	-70.17	26.17	52.13		0.0151*
83	GPC3 SNP2	CT	A	RFC	35.91	22.32		53.95	0.0817
84	GPC3 SNP2	TT	A	RFC	34.10	33.52			
85	SDC1 SNP1	CC	A	WG	1973	23.59	-98.38		0.0007**
86	SDC1 SNP1	GC	A	WG	1931	27.34		55.66	0.1615
87	SDC1 SNP1	GG	A	WG	1777	52.22			

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Table 5.9d: Contd. Line A, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
88	SDC1 SNP1	CC	A	FC	3546	37.84	-141.6		0.0024**
89	SDC1 SNP1	GC	A	FC	3533	43.84		128.9	0.0439*
90	SDC1 SNP1	GG	A	FC	3263	83.76			
91	SDC1 SNP1	CC	A	FCR	1.804	0.0145	0.0313		0.0756
92	SDC1 SNP1	GC	A	FCR	1.838	0.0167		0.0019	0.9366
93	SDC1 SNP1	GG	A	FCR	1.867	0.0318			
94	SDC1 SNP1	CC	A	RFC	-14.28	21.61	-11.58		0.6595
95	SDC1 SNP1	GC	A	RFC	29.42	25.04		55.28	0.1292
96	SDC1 SNP1	GG	A	RFC	-37.44	47.83			
97	SDC1 SNP3	AA	A	WG	1917	24.95	-7.54		0.7946
98	SDC1 SNP3	GA	A	WG	1968	27.09		58.39	0.1424
99	SDC1 SNP3	GG	A	WG	1902	52.22			
100	SDC1 SNP3	AA	A	FC	3521	40.03	-29.47		0.5263
101	SDC1 SNP3	GA	A	FC	3514	43.47		21.91	0.7309
102	SDC1 SNP3	GG	A	FC	3462	83.78			
103	SDC1 SNP3	AA	A	FCR	1.850	0.0147	-0.0134		0.4335
104	SDC1 SNP3	GA	A	FCR	1.793	0.0160		-0.044	0.0611
105	SDC1 SNP3	GG	A	FCR	1.823	0.031			
106	SDC1 SNP3	AA	A	RFC	34.83	22.14	-19.49		0.4486
107	SDC1 SNP3	GA	A	RFC	-39.96	24.04		-55.29	0.1177
108	SDC1 SNP3	GG	A	RFC	-4.16	46.34			

Table 5.10a: Line B, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
1	SOD3 SNP1	CC	B	WG	1759	20.89	38.96		0.0060**
2	SOD3 SNP1	CT	B	WG	1837	13.09		38.39	0.0466*
3	SOD3 SNP1	TT	B	WG	1837	18.79			
4	SOD3 SNP1	CC	B	FC	3416	35.00	53.79		0.0232*
5	SOD3 SNP1	CT	B	FC	3482	21.92		12.19	0.7052
6	SOD3 SNP1	TT	B	FC	3524	31.49			
7	SOD3 SNP1	CC	B	FCR	1.946	0.0149	-0.0117		0.2412
8	SOD3 SNP1	CT	B	FCR	1.901	0.009		-0.0334	0.0152*
9	SOD3 SNP1	TT	B	FCR	1.923	0.0134			
10	SOD3 SNP1	CC	B	RFC	15.16	22.85	6.16		0.6887
11	SOD3 SNP1	CT	B	RFC	-13.44	14.31		-34.76	0.0992
12	SOD3 SNP1	TT	B	RFC	27.48	20.56			
13	SOD3 SNP2	AA	B	WG	1765	30.77	37.03		0.0313*
14	SOD3 SNP2	GA	B	WG	1816	13.82		13.56	0.5380
15	SOD3 SNP2	GG	B	WG	1839	14.92			
16	SOD3 SNP2	AA	B	FC	3446	51.38	27.34		0.3392
17	SOD3 SNP2	GA	B	FC	3465	23.07		-8.72	0.8125
18	SOD3 SNP2	GG	B	FC	3501	24.92			
19	SOD3 SNP2	AA	B	FCR	1.955	0.0219	-0.0233		0.0572
20	SOD3 SNP2	GA	B	FCR	1.913	0.0098		-0.0178	0.2551
21	SOD3 SNP2	GG	B	FCR	1.908	0.0106			
22	SOD3 SNP2	AA	B	RFC	37.96	33.53	-17.93		0.3368
23	SOD3 SNP2	GA	B	RFC	-5.27	15.06		-25.29	0.2921
24	SOD3 SNP2	GG	B	RFC	2.09	16.26			
25	SOD3 SNP3	CC	B	WG	1858	35.62	-25.15		0.1821
26	SOD3 SNP3	CT	B	WG	1841	18.19		8.55	0.7441
27	SOD3 SNP3	TT	B	WG	1807	12.02			
28	SOD3 SNP3	CC	B	FC	3526	58.89	-37.59		0.2276
29	SOD3 SNP3	CT	B	FC	3527	30.08		38.16	0.3785

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Table 5.10b: Contd. Line B, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
30	SOD3 SNP3	TT	B	FC	3451	19.88			
31	SOD3 SNP3	CC	B	FCR	1.902	0.0254	0.006		0.6421
32	SOD3 SNP3	CT	B	FCR	1.921	0.0130		0.0132	0.4799
33	SOD3 SNP3	TT	B	FCR	1.914	0.009			
34	SOD3 SNP3	CC	B	RFC	4.980	38.65	-6.84		0.7376
35	SOD3 SNP3	CT	B	RFC	25.84	19.74		27.71	0.3300
36	SOD3 SNP3	TT	B	RFC	-8.70	13.05			
37	AKR1B10 SNP1	AA	B	WG	1849	15.43	-202.1		0.0074**
38	AKR1B10 SNP1	AT	B	WG	1806	12.11		158.5	0.0375*
39	AKR1B10 SNP1	TT	B	WG	1445	148.81			
40	AKR1B10 SNP1	AA	B	FC	3539	25.65	-164.7		0.1868
41	AKR1B10 SNP1	AT	B	FC	3442	20.13		67.47	0.5928
42	AKR1B10 SNP1	TT	B	FC	3210	247.4			
43	AKR1B10 SNP1	AA	B	FCR	1.918	0.0110	0.151		0.0052**
44	AKR1B10 SNP1	AT	B	FCR	1.911	0.009		-0.158	0.0039**
45	AKR1B10 SNP1	TT	B	FCR	2.220	0.106			
46	AKR1B10 SNP1	AA	B	RFC	28.45	16.89	82.38		0.3153
47	AKR1B10 SNP1	AT	B	RFC	-15.51	13.25		-126.3	0.1289
48	AKR1B10 SNP1	TT	B	RFC	193.2	162.8			
49	AKR1B10 SNP2	AA	B	WG	1745	34.43	46.00		0.0127*
50	AKR1B10 SNP2	GA	B	WG	1809	16.47		18.02	0.4653
51	AKR1B10 SNP2	GG	B	WG	1837	12.55			
52	AKR1B10 SNP2	AA	B	FC	3378	57.54	55.56		0.0708
53	AKR1B10 SNP2	GA	B	FC	3481	27.53		47.10	0.2538
54	AKR1B10 SNP2	GG	B	FC	3489	20.97			
55	AKR1B10 SNP2	AA	B	FCR	1.940	0.0246	-0.0178		0.1741
56	AKR1B10 SNP2	GA	B	FCR	1.928	0.0118		0.006	0.7211
57	AKR1B10 SNP2	GG	B	FCR	1.904	0.009			
58	AKR1B10 SNP2	AA	B	RFC	-5.441	37.68	-0.678		0.9730

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Table 5.10c: Contd. Line B, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
59	AKR1B10 SNP2	GA	B	RFC	18.95	18.03		25.07	0.3535
60	AKR1B10 SNP2	GG	B	RFC	-6.797	13.74			
61	GPC3 SNP1	AA	B	WG	1831	43.91	-7.970		0.7286
62	GPC3 SNP1	CA	B	WG	1827	14.98		3.885	0.8874
63	GPC3 SNP1	CC	B	WG	1815	13.34			
64	GPC3 SNP1	AA	B	FC	3425	72.79	29.56		0.4379
65	GPC3 SNP1	CA	B	FC	3476	24.85		21.04	0.6437
66	GPC3 SNP1	CC	B	FC	3485	22.12			
67	GPC3 SNP1	AA	B	FCR	1.873	0.031	0.0259		0.1114
68	GPC3 SNP1	CA	B	FCR	1.909	0.011		0.0103	0.5941
69	GPC3 SNP1	CC	B	FCR	1.924	0.009			
70	GPC3 SNP1	AA	B	RFC	-63.05	47.24	39.30		0.1127
71	GPC3 SNP1	CA	B	RFC	-7.46	16.13		16.29	0.5811
72	GPC3 SNP1	CC	B	RFC	15.56	14.35			
73	GPC3 SNP2	CC	B	WG	1808	20.82	18.94		0.1860
74	GPC3 SNP2	CT	B	WG	1814	13.19		-12.88	0.5082
75	GPC3 SNP2	TT	B	WG	1846	19.56			
76	GPC3 SNP2	CC	B	FC	3460	34.53	29.69		0.2114
77	GPC3 SNP2	CT	B	FC	3467	21.88		-22.46	0.4870
78	GPC3 SNP2	TT	B	FC	3519	34.45			
79	GPC3 SNP2	CC	B	FCR	1.917	0.0149	-0.0026		0.7993
80	GPC3 SNP2	CT	B	FCR	1.916	0.009		0.0009	0.9429
81	GPC3 SNP2	TT	B	FCR	1.912	0.0139			
82	GPC3 SNP2	CC	B	RFC	-1.0728	22.61	6.53		0.6742
83	GPC3 SNP2	CT	B	RFC	-1.249	14.33		-6.71	0.7511
84	GPC3 SNP2	TT	B	RFC	11.98	21.25			
85	SDC1 SNP1	CC	B	WG	1812	11.84	50.32		0.0859
86	SDC1 SNP1	GC	B	WG	1830	17.46		-32.14	0.3455
87	SDC1 SNP1	GG	B	WG	1913	57.14			

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Table 5.10d: Contd. Line B, SNP point analysis results. WG: Weight gain (g). FC: Feed consumption (g). FCR: Feed conversion ratio. RFC: Residual feed consumption (g). p-value: p-values for additive and dominance effect.

	SNP	Genotype	Line	Trait	Mean	S.E.	Additive	Dominance	p value
88	SDC1 SNP1	CC	B	FC	3458	19.52	109.6		0.0235*
89	SDC1 SNP1	GC	B	FC	3503	28.78		-64.09	0.2540
90	SDC1 SNP1	GG	B	FC	3677	94.22			
91	SDC1 SNP1	CC	B	FCR	1.913	0.0084	0.007		0.7329
92	SDC1 SNP1	GC	B	FCR	1.919	0.0125		-0.0008	0.9734
93	SDC1 SNP1	GG	B	FCR	1.927	0.0408			
94	SDC1 SNP1	CC	B	RFC	-7.856	12.82	48.13		0.1289
95	SDC1 SNP1	GC	B	RFC	15.46	18.90		-24.81	0.5010
96	SDC1 SNP1	GG	B	RFC	88.40	61.87			
97	SDC1 SNP3	AA	B	WG	1777	49.95	14.52		0.5715
98	SDC1 SNP3	GA	B	WG	1863	18.04		71.34	0.0237*
99	SDC1 SNP3	GG	B	WG	1806	11.59			
100	SDC1 SNP3	AA	B	FC	3478	83.31	-11.82		0.7825
101	SDC1 SNP3	GA	B	FC	3535	30.09		68.05	0.1944
102	SDC1 SNP3	GG	B	FC	3455	19.34			
103	SDC1 SNP3	AA	B	FCR	1.9667	0.0358	-0.0243		0.1882
104	SDC1 SNP3	GA	B	FCR	1.901	0.0129		-0.041	0.0697
105	SDC1 SNP3	GG	B	FCR	1.918	0.008			
106	SDC1 SNP3	AA	B	RFC	56.08	54.77	-29.58		0.2938
107	SDC1 SNP3	GA	B	RFC	7.338	19.78		-19.16	0.5777
108	SDC1 SNP3	GG	B	RFC	-3.075	12.71			

Appendix

Table A1: Primer sequence for genotypiing

SNP	Forward Primer Sequence	Reverse Primer Sequence
SOD3 SNP1	AGTGCGAGTGTGCACAGT	TGTTGCCCACATGCAGACT
AKR1B10 SNP1	GTGGGAAAGTGAAAGGAACATGTG	GTGTCTTCTAAATGCTTCCCACCAT
AKR1B10 SNP2	ACATCACTCTTGGAAGTGGTCAG	CTGTGCTTGTCACTATGGTATCTGA
GPC3 SNP1	AACTGGAGATGCCATTGGGAAT	CTAAGGAAGCATGGCCTGACA
SDC1 SNP1	GCATGACAGTGTTGGTTTTCTTTGT	CCCCGTATTCACACAGTCTTGT
SDC1 SNP3	GGTTGGGCCCTGGAATGG	TCTGAGAGCAGTGTCCAAATGC

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