The genomic improvement of meat quality traits in pigs

by

Kristin Lee

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Meat and carcass quality traits are of increasing interest to the swine industry due to their influence on customer purchasing and repurchasing decisions. However, meat quality traits must be measured post-mortem, meaning that these traits cannot be measured on the potential breeding candidates themselves, instead they must be measured on their siblings. For this reason, selection of meat quality traits using traditional selection methods is difficult, expensive, and lowly accurate, which is preventing the practical use of many of these traits in breeding programs today. Alternatively, genomic selection (GS) can be implemented for the improvement of meat quality traits. Using GS, breeding values are estimated using genomic relationships or genomic effects, providing a higher selection accuracy and an increased rate of genetic gain compared to traditional selection methods. Therefore, GS provides a significant opportunity to improve and predict meat and carcass quality. The main goal of this thesis was to improve our current understanding of the genetic and biological factors underlying meat and carcass quality traits to aid in the implementation of GS methods. In part 1 of this thesis, variance component estimates were used to calculate genetic parameters for meat quality traits in pigs. These results could be used to directly incorporate meat quality traits into selection procedures, including both traditional or GS methods. Secondly, the biological factors underlying meat quality traits were explored. In part 2, a genome wide association study (GWAS) was used to identify quantitative trait loci (QTL) and genes associated with drip loss (DL). Following this, in part 3, a single-SNP association analysis was used to determine the effects of two potential causative mutations on meat colour phenotypes. These two analyses were intended to contribute to an improved understanding of the genes and mutations underlying meat quality traits, which would not only improve the biological knowledge for meat quality traits, but also facilitate the future

implementation of alternative methods of GS that incorporate biological knowledge. Phenotypes were collected from either a purebred Duroc (n = 997), or commercial crossbred pig population (Duroc X Landrace/Large White, n = 1098). Meat quality traits included various colour measurements (Minolta L*, a*, and b*) from multiple muscle types, including the *longissimus thoracis et lumborum* (loin; LOINL, LOINA, and LOINB), loin fat (LOINFATL, LOINFATA, LOINFATB), ham *gluteus medius* (GLUTL, GLUTA, GLUTB), ham *quadriceps femoris* (QUADL, QUADA, QUADB), and ham *iliopsoas* (ILIOL, ILIOA, ILIOB), as well as drip loss (DL), and ultimate pH. Further, carcass traits included muscle depth (MD), fat depth (FD), loin eye area (LEA), and intramuscular fat (NSIF IMF). In part 1 of this thesis, meat colour traits showed heritabilities ranging between low to moderate (0.06±0.05 for QUADB to 0.44±0.09 for LOINA) and remaining meat quality traits showed moderate heritabilities, including DL (0.23±0.08) and pH (0.28±0.08). All carcass quality traits analyzed had moderate heritabilities, including MD (0.33±0.08), FD (0.39±0.08), LEA (0.39±0.09), and NSIF IMF (0.43±0.09). Moderate to high genetic correlations were observed between the same colour measurements from different muscle types (ranging between 0.50 to 0.96) and different colour measurements from the same muscle types (ranging between 0.56 to 0.92). Colour measurements also showed moderate to high genetic correlations with pH (ranging between -0.54 to -0.80) and DL (ranging between 0.38 to 0.69). The remaining meat quality traits showed a high negative correlation, between DL and pH (-0.65±0.16). Carcass quality traits showed moderate to high correlations, including LEA and MD (0.94±0.04), and FD and NSIF IMF (0.36±0.15). In addition, very few unfavorable correlations were observed between the meat and carcass quality traits. Overall, these results show that meat and carcass quality traits can be improved by genetic selection, to produce a high quality and lean pork product. In the remaining studies of this thesis, parts 2 and

3, the biological factors underlying meat quality traits were explored. However, both studies proved underpowered in their ability to identify genes and causative mutations that were significantly associated with meat quality traits. Nonetheless, the results from these studies provide a basis upon which future work can built.

Preface

This thesis is the original work of Kristin Lee. No part of this thesis has been previously published. This thesis is a part of a larger research project, "Identifying functional gene variants and non-additive effects to enhance the power of genomic selection of purebred pigs for crossbred performance", NSERC CRDPJ 485526, 01/06/2016. This project has received research ethics approval from the University of Alberta Animal Care and Use Committee. The animals used in this study were part of a commercial herd and raised following the Canadian Quality Assurance program and the Canadian Council on Animal Care (CCAC) guidelines. This project was considered as Category A, which involved tissues collected from the abattoir, with little to no experimental manipulation.

Acknowledgements

First and foremost, I want to express my thanks to Dr. Graham Plastow for presenting me with the opportunity to complete a MSc thesis. Thank-you for challenging me to generate my own thoughts and ideas but also always being available to provide expertise and guidance. You have helped me gain the skills necessary to be successful in my projects throughout my masters, and you have set me up to be successful in my future as a professional in the animal science industry.

Thanks to the members of Livestock Gentec for always being available to lend your knowledge and advice as well as for your kindness and friendship. A special thanks to Marzieh Heidaritabar for offering me her extensive knowledge on quantitative genetics and animal science. Thank-you for your discussion and feedback on my methods and results, and for helping me to come up with solutions for the reoccurring problem of non-convergence. Finally, thank-you for providing me with critiques and comments during my thesis writing process. Your guidance and encouragement have been integral to my success as a master's student.

Thanks to Dr. Heather Bruce for being a part of my committee, you have provided important feedback as well as invaluable expertise on the meat quality aspects of my thesis.

Thanks to the Natural Sciences and Engineering Research Council (NSERC) of Canada for providing the funding for this project. Thanks to Hypor Inc. as well for providing the animals and financial support required to complete this project. Thank-you to the members of the Hendrix and Hypor Inc. team that I was able to meet over the years, it was a pleasure to get to know and learn from everyone. Finally, thanks to Dr. Robert McKay of Genstat Consultants Inc.

for collecting the samples required for this project. Thank-you for your accommodation and advice during my visit at East 40 Packers, you made sample collection an exceptionally fun experience.

Finally, and most importantly, thanks to my family, I wouldn't be here if it wasn't for your unwavering support, positivity, and encouragement. Thank you for shaping me into the person that I am today.

Table of contents

Chapter 1. Literature review 1

1.1. Introduction 1

1.2. The conversion of muscle to meat 2

1.3. From traditional selection to genomic selection 9

1.4. Marker-assisted selection 11

1.5. Genomic selection 14

1.6. Statistical models 15

1.7. Accuracy of genomic selection 19

1.8. Alternative methods of genomic selection 20

1.9. Uncovering the biology of the trait 23

1.10. Benefits for genomic selection of meat quality traits in pigs 25

1.11. Challenges for genomic selection of meat quality traits in pigs 25

1.12. Conclusions and thesis outline 28

1.13. References 30

Chapter 2. Estimating genetic parameters for meat quality and carcass traits in purebred Duroc pigs 46

2.1. Introduction 46

2.2. Materials and methods 48

2.2.1. Data 48

2.2.2. Statistical analysis 50

2.3. Results and discussion 52

2.3.1. Phenotypic statistics 52

2.3.2. Heritability estimates 53
2.3.3. Correlation estimates 56
2.4. Conclusions 59
2.5. References 61

Chapter 3. A genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs 69
3.1. Introduction 69
3.2. Materials and methods 71
3.2.1. Data 71
3.2.2. Population stratification 72
3.2.3. Statistical analysis 73
3.2.4. Plots and correction for multiple testing 74
3.2.5. Quantitative trait loci detection and functional analysis 75
3.3. Results and discussion 76
3.3.1. Phenotype statistics 76
3.3.2. Principle component analysis 76
3.3.3. Genome-wide association study 77
3.3.4. Comparison with previous results 77
3.3.5. Comparison of statistical methods 79
3.3.6. Candidate genes 81
3.4. Conclusions 83
3.5. References 85

Chapter 4. Single-SNP association analysis for predicted causative mutations and meat colour phenotypes in purebred Duroc pigs 97

4.1. Introduction 97

4.2. Materials and methods 99

4.2.1. Bioinformatics 99

4.2.2. Data 100

4.2.3. Statistical analysis 101

4.3. Results and discussion 102

4.3.1. Phenotype statistics 102

4.3.2. Candidate genes 102

4.3.1. Bioinformatics 104

4.3.3. Single-SNP association analysis 105

4.4. Conclusions 107

4.5. References 109

Chapter 5. General discussion 120

5.1. Introduction 120

5.2. Increasing the statistical power of genome-wide association studies 122

5.3. Strategies for an effective post-GWAS analysis 126

5.4. Future perspectives for pig breeding 132

5.5 Conclusions 134

5.6. References 136

List of tables

Table 2.1. Descriptive statistics for meat and carcass quality phenotypes in purebred pigs: abbreviation, number of animals (N), minimum (Min.), mean, maximum (Max.), standard deviation (SD), and coefficient of variation (CV) 65

Table 2.2. Estimations for heritabilities (diagonal), as well as genetic (below diagonal), and phenotypic (above diagonal) correlations ± standard error (SE) for meat and carcass quality phenotypes in purebred pigs 66

Table 3.1. Quantitative trait loci (QTL) and the corresponding positional candidate genes identified for SNPs with the lowest P-values in a genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs 96

Table 4.1. Target, probe, and primer sequences for a quantitative PCR (qPCR) SNP genotyping assay for potential causative mutations located within the *MYOD1* (rs336462969) and *RTN4* (rs340803577) genes 115

Table 4.2. Descriptive statistics for pork colour phenotypes in purebred pigs: abbreviation, number of animals (N), minimum (Min.), mean, maximum (Max.), standard deviation (SD), and coefficient of variation (CV) 116

Table 4.3. Genotype and allele frequencies for potential causative mutations located within the *MYOD1* (rs336462969) and *RTN4* (rs340803577) genes in purebred pigs 118

Table 4.4. The least square means for genotype classes of potential causative mutations within the (A) *MYOD1* (rs336462969) and (B) *RTN4* (rs340803577) genes on meat colour traits in purebred pigs.....119

List of figures

Figure 3.1. Principle component analysis (PCA) of SNP genotypes from commercial crossbred pigs originating from a single population (Hypor Inc., Regina, SK, Canada).....94

Figure 3.2. Manhattan and (B) quantile-quantile (Q-Q) plots from a genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs 95

Figure 4.1. Multiple sequence alignments for regions within candidate genes *MYOD1* (rs336462969) and *RTN4* (rs340803577) containing potential causative mutations for meat colour traits in purebred pigs.....117

List of abbreviations

BF	Backfat depth
BLUP	Best linear unbiased prediction
CCAC	Canadian council of animal care
DFD	Dark, firm, and dry
DL	Drip loss
EBV	Estimated breeding value
eQTL	Expressed quantitative trait loci
FD	Fat depth
GBLUP	Genomic best linear unbiased prediction
GEBV	Genomic estimated breeding value
GFBLUP	Genomic feature best linear unbiased prediction
GLUTA	Ham *gluteus medius* Minolta a*
GLUTB	Ham *gluteus medius* Minolta b*
GLUTL	Ham *gluteus medius* Minolta L*
GRM	Genomic relationship matrix
GO	Gene ontology
GS	Genomic selection
GWAS	Genome-wide association study/studies
ILIOA	Ham *iliopsoas* Minolta a*
ILIOB	Ham *iliopsoas* Minolta b*
ILIOL	Ham *iliopsoas* Minolta L*
IMF	Intramuscular fat

IPA	Ingenuity pathway analysis
IBD	Identity-by-descent
IBS	Identity-by-state
KEGG	Kyoto encyclopedia of genes and genomes
LASSO	Least absolute shrinkage and selection operator
LD	Linkage disequilibrium
LEA	L*ongissimus thoracis et lumborum* (loin) eye area
LOINA	Loin Minolta a*
LOINB	Loin Minolta b*
LOINFATA	Loin fat Minolta a*
LOINFATB	Loin fat Minolta b*
LOINFATL	Loin fat Minolta L*
LOINL	Loin Minolta L*
LASSO	Least absolute shrinkage and selection operator
lncRNA	Long non-coding RNA
MAF	Minor allele frequency
MAS	Marker-assisted selection
MD	Muscle depth
miRNA	Micro-RNA
mRNA	Messenger RNA
MA-GBLUP	Marker-assisted genomic best linear unbiased prediction
NSIF IMF	National Swine Improvement Intramuscular Fat score
NRM	Numerator relationship matrix

NCBI	National Center for Biotechnology Information database
PCA	Principle component analysis
pH24	pH at 24 hours (ultimate pH)
PSE	Pale, soft, and exudative
PSS	Porcine stress syndrome
Q-Q	Quantile-quantile
QTL	Quantitative trait loci
QUADA	Ham *quadriceps femoris* Minolta a*
QUADB	Ham *quadriceps femoris* Minolta b*
QUADL	Ham *quadriceps femoris* Minolta L*
RRBLUP	Ridge regressions best linear unbiased prediction
SIFT	Sorting intolerant from tolerant
SNP	Single nucleotide polymorphism
SSC	*Sus scrofa* chromosome
SIFT	Sorting intolerant from tolerant
ssGBLUP	Single-step genomic best linear unbiased prediction
VEP	Variant effect predictor
WHC	Water-holding capacity
WGS	Whole-genome sequencing
wGBLUP	Weighted genomic best linear unbiased prediction

Chapter 1. Literature review

1.1. Introduction

Demand for animal protein is expected to double by 2050 due to increasing population size, urbanization, and income (FAO, 2019). However, this growth will be challenged by changing consumer attitudes and competition from plant-based and other alternative protein sources. Many countries, including Canada, the United States, China, and several European countries, suggest eating more plant proteins in their national food guides. Pork provides a lean and nutritious source of animal protein, but due to the increasing competition from alternative protein sources, such as plant-based products, pork producers, packers, and processors will all have to focus on meeting a high level of consumer expectation on appearance and eating quality. By providing a product to consumers that is both nutritious and consistently satisfies their quality preferences, consumers will be more willing to purchase and repurchase pork as their source of protein in the future. However, the preference for different meat quality characteristics (colour, leanness, intramuscular fat content, drip-loss, and flavor) can vary widely, especially across international markets (Dransfield et al., 2005; Ngapo et al., 2007). Exports make up 70% of Canadian pork production, much of which represent high value markets (Japan and China) (FCC, 2017), and in order to stay competitive in the future meat market, Canadian pork producers must be able to satisfy a wide variety of consumer preferences. For this reason, the swine industry has begun to focus on breeding and genetics as the means to improve and predict meat quality.

Traditional genetic selection methods are largely responsible for the highly efficient livestock present today, however, selection decisions for meat quality traits rely mainly on post-mortem

phenotypic measurements (expressed late in life), which cannot be measured on breeding candidates and must be measured on their relatives. This makes current selection methods difficult, and expensive for the improvement of meat quality traits, which is preventing the practical use of these traits in breeding programs today. Genomic selection (GS) methodologies allow for the selection of animals based on genomic relationships or genomic effects, which means animals can be selected earlier in life and with higher accuracy, without first requiring their phenotype to be measured on the breeding or selection candidate. Therefore, GS provides a substantial opportunity for the genetic improvement of meat quality traits.

1.2. The conversion of muscle to meat

Meat quality can refer to many aspects of the final pork product, including the wholesomeness, appearance, nutritional quality, and eating experience. When considering consumer purchasing intent, pork colour, firmness, and water loss (drip loss, exudation), are important indicators of meat quality (Buege & Griffin, 2015). In general, desirable pork is referred to as red, firm, and non-exudative (RFN) meat, which is red or bright pink in colour, firm in texture, and free of excess water. Large variation from this quality standard will result in an undesirable pork product referred to as: 1) pale, soft, and exudative (PSE), or 2) dark, firm, and dry (DFD). PSE meat is abnormally pale in colour, soft in texture, and will contain excessive water accumulation on the surface of the meat and in the product packaging. Alternatively, DFD pork is dark in colour, firm in appearance, and the surface of the pork appears dry. Dark meat is interpreted by consumers as being from old animals, or lacking freshness (Viljoen et al., 2002), and is also prone to spoilage, due to its high pH, overall translating to product and economic losses for the retailer (Newton & Gill, 1981).

After exsanguination, physical and chemical changes in the muscle will take place over time, which causes the conversion of muscle into meat. It is the rate and extent that these post-mortem changes take place that will influence the meat quality of the final product (RFN, PSE, or DFD). After exsanguination, the circulatory system can no longer function to transport oxygen and nutrients to the muscle or remove waste products from the muscle. For this reason, oxygen in the muscle will diminish, resulting in a shift in metabolism from aerobic to anaerobic (Huff-Lonergan & Page, 2006). Anaerobic glycolysis breaks down glucose into lactic acid to generate ATP for cellular activities. Since lactic acid cannot be removed from the muscle by the circulatory system, it will build up in the tissue, along with other by-products of anaerobic glycolysis (H^+, H_2O), which contributes to a pH decline in the muscle. Under normal conditions, the muscle pH will drop gradually, along with the muscle temperature as the carcass is chilled. The pH of the muscle will continue to drop until glycogen is used up or the glycolytic enzymes are no longer able to function. This will cause a change in pH from 7.4 in living tissue to an ideal pH of 5.6 to 5.9 in meat, in which case, the resulting pork will have desirable RFN quality (Huff-Lonergan & Page, 2006). However, if there are abnormalities in the post-mortem rate of metabolism or the concentrations of glycogen stores, then the final product will have undesirable qualities (PSE, or DFD).

PSE meat occurs when the rate of metabolism or the concentration of glycogen/glucose is high, which, after exsanguination, will cause pH to drop extensively (< 5.5) and very rapidly while the muscle temperature is still high (Wismer-Pederson & Briskey, 1961). The combination of low pH and high temperatures will cause the denaturation of muscle proteins (Wismer-Pederson & Briskey, 1961), which has a major impact on the quality of the final product. Muscle proteins are responsible for binding water and holding it within the muscle cell, and denaturation will

eliminate their ability to bind water (Huff-Lonergan & Lonergan, 2005). In addition, the remaining proteins that are not denatured will not bind water due to the low pH of the muscle. Proteins must possess a charge to bind with water, and as the pH of meat approaches 5.1, referred to as the isoelectric point, muscle proteins will have no net charge, and will not be able to interact with water (Huff-Lonergan & Lonergan, 2005). Overall, this low pH will contribute to large amounts of protein denaturation and unbound water in the muscle cell, therefore, compromising the integrity of the muscle, resulting in a product that appears soft. Further, upon the application of external pressures or cooking, water will escape from the muscle cell, resulting in excess water loss and exudative meat (Offer & Knight, 1988). In terms of meat colour, myoglobin (the muscle specific protein that gives meat its red colour) is of upmost importance (Mancini & Hunt, 2005), and the denaturation of myoglobin will reduce the colour intensity and redness of the meat (Pan & Myron, 1972). In addition to myoglobin, colour is also determined by the light scattering ability of meat (Offer & Knight, 1988). Increased light reflectance is caused by the unbound water within the muscle cell, along with the excess water accumulation on the surface of meat (Offer & Knight, 1988). Therefore, in PSE meat, light does not penetrate deep into the muscle tissue, and is reflected off the surface of the meat (Offer & Knight, 1988). This means the light that is directed on to the final pork product will be scattered in many different directions before it can be absorbed by the myoglobin pigment heme, further contributing to the appearance of pale pork.

DFD meat is also directly related to the pH decline post mortem, however DFD occurs when the rate of post-mortem metabolism is low or glycogen/glucose stores are depleted, resulting in a minimal drop in pH (> 6.0) (Hall et al., 1994). In this case, the high pH will have a major effect on the meat quality of the final product. The high pH permits interactions between muscle

proteins and water (Offer & Knight, 1988), resulting a large amount of water that is tightly bound within the muscle cell, which will make the muscle cells turgid, and the pork appear firm in texture and dry to the touch. In terms of colour, the high water holding capacity of meat will also contribute to the dark appearance of pork, as muscle cells with high levels of intracellular water will reflect less light (Offer & Knight, 1988). The high water-holding capacity of meat, along with the high concentration of intact myoglobin (due to reduced protein denaturation), means light can penetrate deep into the muscle tissue and be absorbed by myoglobin, resulting in pork that appears more red in colour (Offer & Knight, 1988). In addition, the oxidation status of myoglobin will also contribute to pork colour. Myoglobin has an iron (Fe^{2+}) containing heme group, which can reversibly bind oxygen. Upon processing and exposure to oxygen, myoglobin will change from a dark red/purple colour (deoxymyoglobin) to a desirable bright red colour (oxymyoglobin). However, when the muscle cells are swollen and tightly packed with water, and oxygen is restricted in its ability to penetrate the muscle and "bloom" the tissue, the product will appear dark (Hall et al., 1994).

Meat quality traits are complex (quantitative) in nature, and controlled by many factors that can be either environmental or genetic in origin, and for this reason, environmental management is integral to ensuring excellent meat quality. In terms of the environment, diet, pre-slaughter handling, and slaughter procedure each have significant impacts. Diet can alter pre-slaughter muscle glycogen reserves, which, as discussed previously, will affect the meat quality of the final product. Specifically, feed withdrawal 18 to 22 hours prior to slaughter has been shown to reduce the glycogen concentration in the *longissimus thoracis et lumborum* (loin), resulting in increased pH, darker meat, and reduced water loss (Eikelenboom et al., 1991; Guàrdia et al., 2004). Similarly, diets high in fat, and protein, but low in carbohydrates that are fed for three

weeks prior to slaughter will reduce the total amount of glycogen available at slaughter for conversion to lactic acid, providing similar results to feed withdrawal (Rosenvold et al., 2001a; Rosenvold et al., 2001b). In addition, supplementation with tryptophan (Guzik et al., 2006), magnesium (D'Souza et al., 2000), or creatine monohydrate (Young et al., 2005) have also shown to reduce the incidence of PSE pork and/or improve meat quality.

Pre-slaughter stress and fear can be experienced by the animal over the long-term, such as during on-farm handling, mixing, loading, transport, and unloading, as well as over the short-term, such as new lairage conditions, and handling at the abattoir. Both short and long-term stress can initiate a physiological stress response that can alter the meat quality of the final product. Stress will stimulate the release of stress hormones (cortisol and adrenaline) into the bloodstream, which will activate the metabolism and initiate glycogen breakdown to generate the energy for the "flight or fight" response. If pigs are exposed to short-term stress immediately prior to slaughter, then their metabolism will be activated (high glycolytic potential) and their body temperature will increase. After exsanguination, this will cause the rapid accumulation of lactic acid in the muscle while temperatures are high, resulting in PSE meat (Hambrecht et al., 2004; Dokmanović et al., 2014). Conversely, when pigs are exposed to long-term stress, glycogen reserves will be used up and depleted, and once an animal is exsanguinated, there will be limited accumulation of lactic acid in the muscle, resulting in an increased incidence of DFD pork (Murray & Jones, 1994; Martoccia et al., 1995). For these reasons, care should be taken with transport and handling practices to reduce stress prior to slaughter. For example, unfamiliar animals should not be mixed, to avoid the incidence of fighting to establish dominance and hierarchy. Proper footing is required during loading, distractions should be minimized, handheld panels should be used to guide animals into the trailer, and the use of electrical prods should be

minimized. With regards to transportation, trailers should be cleaned prior to loading to prevent slipping, and transport time, stocking density, and ventilation should all be considered. Finally, if stress is experienced during loading and transportation, then animals should be rested in the lairage for two to four hours prior slaughter, to allow time for animals to recover and glycogen stores to be restored (Grandin, 2003).

After exsanguination, the rate of chilling and the use of electrical stimulation will also affect meat quality. Rapid chilling of the carcass after exsanguination can slow the process of glycolysis, and prevent the conditions that contribute to PSE (high temperature, and rapid pH decline) (Kerth et al., 2001). However, cold shortening can occur if chilling occurs too rapidly. Normally, in the post mortem muscle, there is a gradual increase of calcium concentration due to leakage from the sarcoplasmic reticulum. However, upon rapid chilling or freezing, the integrity of the sarcoplasmic reticulum is compromised, causing a rapid influx of a large concentration of calcium into the muscle cell. Cold shortening occurs upon the rapid release of calcium into the muscle cell when ATP concentrations are still high. These conditions lead to severe muscle contraction and sarcomere shortening, resulting in reduced tenderness as well as an increased water loss (muscle contraction causes water to be squeezed from the muscle cell) (Honikel et al., 1983; Smulders et al., 1986). Therefore, rapid cooling combined with electrical stimulation can reduce the incidence of cold shortening. Electrical stimulation causes the muscle to contract and relax, which uses up ATP and hastens the completion of post-mortem glycolysis (the time to reach ultimate pH), then once muscle is chilled or frozen, calcium will be released, ATP will not be present, and cold shortening will not occur (Smulders et al., 1986).

It is important to mention other meat quality traits, such as intramuscular fat (IMF), tenderness, and flavor, as these are also important factors with regards to consumer eating experience, and

subsequent repurchasing decisions. Once the product is purchased, consumers will respond to the smell, taste, and mouthfeel during consumption, and will judge future purchasing decision based on the satisfaction of their past eating experiences (Jeremiah, 2006). Consumers often prefer a more tender and flavorful product, however, due to the perceived health implications of animal fat, consumers, especially in Canada and the United States, often prefer products with minimal visible fat (Jeremiah, 2006). In terms of biochemical pathways, factors that control fat deposition are important determinants of IMF (breed, muscle, and diet) (Wood et al., 2004), whereas tenderness is determined by the sarcomere length, collagen content, as well as post-mortem proteolysis (Huff-Lonergan et al., 1996; Wheeler et al., 2000). In addition, flavor is determined by the interaction and degradation of specific fats and low molecular weight compounds (amino acids, reducing sugars, vitamins and nucleotides) into intermediate and/or volatile compounds (Idolo Imafidon et al., 1994).What is more, IMF, tenderness, and flavor, each interact with each other to some extent. For example, IMF is necessary for a palatable meat product, as fat contains many of the compounds responsible for meat flavor, and different IMF compositions will give meat different flavor profiles (Cameron et al., 1990). Further, IMF is responsible for a proportion of the variability for meat tenderness, and high levels of IMF (> 8%) will improve the tenderness of a product by disrupting the connective tissues that are responsible for the toughness of meat (Nishimura et al., 1999). Overall, to consistently produce high quality pork product, there must be an understanding of the environmental factors that contribute to the variability in meat quality, but genetic factors must also be considered.

1.3. From traditional selection to genomic selection

Animals have been improved by selective breeding for hundreds of years. The method of selection has largely improved over the years, but the basic concepts of animal breeding have remained the same (Oldenbroek & van der Waaij, 2015). 1) Animals are selected based on a predefined breeding goal, which nowadays can be complex. Three breeding goals that are common in pig breeding due to their economic importance are: reproductive performance of the sow (birth weights, mothering ability, pigs weaned, slaughter pigs sold, rebreeding, conformation) or the boar (libido, semen quality and quantity), growth performance (daily gain, feed intake, gain to feed ratio, disease resistance), and slaughter performance (carcass yield, dressing percentage, fat and lean percent, meat quality). 2) Phenotypic measurements for these economically important traits are recorded along with pedigree records, which are used to select the best animals to produce the next generation (Oldenbroek & van der Waaij, 2015).

Over time, statistical and quantitative genetic tools were developed for the most accurate selection decisions. Traditional breeding methods use the statistical approach best linear unbiased prediction (BLUP), which uses observed phenotypic records of an individual or its relatives, and pedigree information (proportion of shared additive genetic relationships between animals) to estimate breeding value (EBV) of all animals within a population for a performance trait (Henderson, 1975). Further, breeding goals are often complex, and involve more than one trait, and for this reason, selection indexes are commonly calculated to ensure balanced and well-rounded breeding decisions. Selection indexes for individuals are calculated by multiplying EBVs by appropriate weighting factors (the proportion of emphasis placed on the trait depending on its economic importance), and summing each value to generate an index value. Then selection

index values can be used to rank animals based on their genetic potential, allowing for accurate breeding decisions (selection of best animals to produce the next generation) (Bourdon, 2014).

Traditional breeding methods have made considerable improvements in animal production traits over time, however, difficult to measure traits, such as meat and carcass quality traits, are not ideal candidates for traditional improvement. Meat quality traits are measured post-mortem, cannot be measured on the selection candidates themselves, and they must be inferred by measuring the phenotypes of their relatives. As a result, the success of traditional breeding methods relies on pedigree genetic relationships, which involves some uncertainty and inaccuracies due to Mendelian sampling (variability in breeding value between full-siblings due to the inheritance of a random sample of alleles from each parent) and incomplete pedigree (unknown relationships due to shared common ancestors that are not recorded in the pedigree) (Oldenbroek & van der Waaij, 2015). For this reason, genetic improvement of meat quality traits through this method is difficult and slow (Miar et al., 2014; Oldenbroek & van der Waaij, 2015).

Nowadays, the rise of high-throughput genotyping methodologies have led to the availability of thousands of single nucleotide polymorphism (SNP) markers and dense genotyping arrays, such as the 50K and 60K SNP panels (Ramos et al., 2009). Therefore, these technological advances along with improvements in computing power have enabled alternate approaches for genetic improvement of meat quality traits.

1.4. Marker-assisted selection

Firstly, the method termed marker assisted selection (MAS) was developed, which considers a few pre-identified markers to make selection decisions. Markers that are selected for use in MAS will either be: 1) causative mutations that directly code for the functional change, or 2) in very strong linkage disequilibrium (LD) with causative mutations (Dekkers, 2004). Subsequently, these SNP markers can be used in breeding programs to: 1) preselect animals prior to testing and breeding, or 2) they can be incorporated into the BLUP model as fixed or random effects for EBV estimation (Dekkers, 2004; Lopes et al., 2017). There are many examples of LD markers that have been identified for meat quality traits in pigs (Zhang et al., 2014) and could be used in MAS programs. In contrast, there are relatively few examples where the causative mutation is known. However, in the case that the causative mutation is known, the effect is usually large, and selection for the favorable allele has the potential to make significant improvements to meat quality traits in pigs. The following paragraphs will discuss these most notable genes and their alleles:

***Ryanodine receptor* (*RYR1*)**

The *ryanodine receptor* (*RYR1*) gene encodes a calcium release channel that localizes exclusively to the sarcoplasmic reticulum of skeletal muscle where it plays a role in the regulation of intracellular calcium concentration and muscle contraction (Fujii et al., 1991). The presence of a single *RYR1* allele (called *Hal*) is beneficial, as animals have been reported to have better feed conversion efficiency, higher carcass weight, higher muscling, and lower fat (Leach et al., 1996; Murray & Johnson, 1998). However, animals homozygous for *hal* become

susceptible to porcine stress syndrome (PSS), which is a single-gene disorder where stressful conditions, such as poor pre-slaughter handling and transportation, can cause uncontrolled muscle contraction and death in the animal, or an increased occurrence of pale, soft, and exudative (PSE) pork post-mortem (Fujii et al., 1991). A missense mutation located within *RYR1*, Hal-1843 (pArg615Cys), has been found directly responsible for the effects of *hal*, including PSS in numerous commercially important pig breeds (Pietrain, Yorkshire, Duroc, Landrace, and Hampshire). Correspondingly, Hal-1843 has also been shown to have a negative effect on the meat quality of both homozygous and heterozygous pigs, as these animals produce pork with lower pH 45 minutes after slaughter (pH_{45}), higher Minolta L* (lightness), higher Minolta b* (yellowness), and higher drip loss (DL) (Otto et al., 2007). The discovery of Hal-1843 and knowledge on its adverse relationship with meat quality has made a major impact on the swine industry, as it can now be used for genetic testing and removal of the allele, resulting in the elimination of a major contributor of PSS from the breeding population, and improvement of meat quality traits in pigs (Ciobanu et al., 2011).

***Protein kinase AMP-activated non-catalytic subunit gamma 3* (*PRKAG3*)**

Protein kinase AMP-activated non-catalytic subunit gamma 3 (*PRKAG3*) produces the protein product AMP/ATP-binding domain of the AMP-activated protein kinase (AMPK), which plays a role in the regulation of glycogen storage and energy homeostasis in skeletal muscle (Milan et al., 2000; Ciobanu et al., 2001). A dominant mutation in the *PRKAG3* gene, initially called RN^-, was mapped to a locus in the regulatory subunit of AMPK (pArg200Gln). This mutation was associated with an immense 70% increase in glycogen content in the skeletal muscle of pigs

post-mortem, adversely affecting the ultimate pH, DL, and cooking yield of the resulting pork product (Milan et al., 2000). Alternatively, some markers within the *PRKAG3* gene have been found to be associated with reduced glycogen content, and corresponding improvements in meat quality (pIle199Val, pThr30Asn, and pGly52Ser). Specifically, pIle199Val, which is located nearby the causative mutation for RN^-, causes the largest meat quality improvements, including higher ultimate pH, lower Minolta L* (lightness), higher Minolta b* (redness) (Ciobanu et al., 2001), and lower DL (Otto et al., 2007). The effects of pIle199Val, pThr30Asn, and pGly52Ser were smaller than those of RN^-, however, each allele was found in multiple commercially important pig breeds (Duroc, Landrace, Large White, and Berkshire), and provided significant effects on meat quality phenotypes, making them of use in MAS programs (Ciobanu et al., 2011).

***Calpastatin* (*CAST*)**

The *Calpastatin* (*CAST*) gene encodes a calcium activated protease inhibitor that acts on the muscle proteases, milli- and μ-calpain, and correspondingly regulates the rate and extent of protein degradation in skeletal muscle post-mortem (Ciobanu et al., 2004). For these reasons, *CAST* has been found to be an important determinant of meat tenderness. Multiple *CAST* alleles (pArg249Lys, and pSer638Arg) have been identified that influence the firmness and shear force measurement of raw pork, as well as the tenderness, juiciness, and chewiness of cooked pork (Ciobanu et al., 2004). In addition, SNPs have also been identified in the *CAST* regulatory regions (promoter and transcription factor binding sites) that are significantly associated with pork tenderness (Nonneman et al., 2011). Recently, *CAST* has also been found to significantly

influence additional meat quality traits, such as DL, pH, colour, and intramuscular fat (IMF) (Ropka-Molik et al., 2014). Similar to *PRKAG3*, these *CAST* alleles have proven to have significant effects on meat quality phenotypes in commercially important pig breeds (Duroc, Landrace, Large White, Berkshire, and Yorkshire) (Ciobanu et al., 2004; Nonneman et al., 2011; Ropka-Molik et al., 2014), which is beneficial for MAS programs that aim to improve meat quality traits in pigs.

1.5. Genomic selection

Each of the markers discussed above are associated with moderate to large effect sizes (explaining > 9% and > 25% of the genetic variance for moderate and large effect sizes, respectively) (Cohen, 1998), and have provided a significant opportunity to make considerable genetic improvement to meat quality traits. This is because these SNPs explain a significant proportion of the total genetic and phenotypic variation for a trait, which will translate to observable genetic improvement when these SNPs are selected individually. However, complex (polygenic) traits, such as meat and carcass quality, are at least in-part controlled by SNPs of small effects (Yang et al., 2011), explaining < 1% of the genetic variance (Cohen, 1998), which is problematic as these types of SNPs are very difficult to be identified by current association/fine mapping methods (genome-wide association studies, GWAS). Therefore, once the large effect markers reach fixation in the population, they will be difficult to replace to provide further genetic improvements. For these reasons, GS was proposed as an alternative to MAS, for complex traits, such as meat and carcass quality traits.

GS does not require the explicit identification of markers significantly associated with a trait and instead uses genome-wide dense marker panels with thousands or millions of SNPs spread across the entire genome that are assumed to be in LD with the unknown causative mutations for the trait (Meuwissen et al., 2001). Since explicit identification of causative mutations is not required, GS can consider the effects of many variants, including those with a small effect on the trait. Many small effect variants will sum to account for a large proportion of the total genetic variation for the trait, which can translate to potentially large genetic gain. There are two steps for GS: 1) genotyping and phenotyping a reference population for the traits to be genetically improved to identify regression parameters (the "training" step), and 2) statistical methods are applied to estimate a genomically-estimated breeding value (GEBV) to predict genetic potential of selection candidates that are only genotyped with no phenotypic records ("genomic prediction" step).

1.6. Statistical models

There are two types of GS statistical methods commonly studied today: 1) relationship-based methods (Nejati-Javaremi et al., 1997), and 2) SNP effect-based methods (Meuwissen et al., 2001; de los Campos et al., 2013). Relationship-based methods predict GEBVs by replacing the pedigree derived numerator relationship matrix (NRM, **A**) used in traditional BLUP methods with a genomic (or realized) relationship matrix (GRM, **G**), which estimates the covariance between individuals based on shared chromosomal segments (SNP marker genotypes) (VanRaden, 2008). This method, termed genomic BLUP (GBLUP) has been successful in improving breeding value prediction accuracy (Hayes et al., 2009), as the GRM is based on

identity-by-state (IBS) relationships between individuals. IBS is when individuals share chromosomal segments or SNP alleles that are similar in sequence (either due to a shared common ancestor or a mutation event in identical loci in genetically different lines), which accounts for genetic relationships that may not be possible from pedigree predictions (Makgahlela et al., 2013; Fernando et al., 2017). This includes instances such as sibling relationships (due to the uncertainty contributed by the Mendelian sampling term) or incomplete pedigree. Therefore, GBLUP is able to provide a more accurate estimate of relationships within a population compared to the pedigree derived NRM (Hayes et al., 2009).

GBLUP uses the following linear mixed model:

$$\boldsymbol{y} = \mathbf{1}\mu + \boldsymbol{Z}\boldsymbol{g} + \boldsymbol{e} \qquad [1]$$

Where **y** is a vector of phenotype values; ***1*** is a vector of ones; μ is the overall mean of the phenotype values; ***Z*** is a design matrix associating ***g*** with response variables; ***g*** is the vector of random additive genetic effects; and ***e*** is a vector of residual effects. Genetic and residual vectors (**g** and ***e***) are assumed to be normally distributed; $\mathbf{g} \sim \mathrm{N}\left(0, \mathbf{G}\sigma_{\mathrm{g}}^{2}\right)$, and $\mathbf{e} \sim \mathrm{N}(0, \mathbf{I}\sigma_{\mathrm{e}}^{2})$, respectively, where σ_{g}^{2} and σ_{e}^{2} are the additive genetic and residual variances, respectively; **G** is the realized GRM; and **I** is an identity matrix. Single-step GBLUP (ssGBLUP), which will be discussed below, uses a similar model, but where **u** is the vector of random additive genetic effects, which are assumed normally distributed; $\mathbf{u} \sim \mathrm{N}(0, \mathbf{H}\sigma_{\mathrm{u}}^{2})$; and **H** is the relationship matrix.

Due to the costs associated with genotyping, only potential selection candidates in a breeding population may be genotyped, leaving many animals with phenotypes but no genotypes. For this

reason, ssGBLUP has been presented as a more practical method of GS compared to both traditional BLUP and GBLUP as it is able to utilize all the available data for a population in a single method (Legarra et al., 2009; Misztal et al., 2009; Christensen & Lund, 2010). ssGBLUP takes advantage of genotypes, phenotypes, and pedigree data in a one-step method for GEBV estimation by combining the NRM and the GRM into a single matrix (**H**) for all animals (Legarra et al., 2009; Misztal et al., 2009; Christensen & Lund, 2010). This technique has proven to predict breeding value more accurately compared to traditional selection methods, and is also able to increases the training population size for GBLUP, which results in improved prediction accuracies for traits with low heritability. In addition, ssGBLUP is also faster and simpler than more complicated models such as multi-step GBLUP. Successful implementation in this model has been shown in multiple livestock species, including pigs (Christensen et al., 2012; Fangmann et al., 2017).

Alternatively, for SNP effect-based methods, the effect of each SNP on the trait is estimated on the reference population. SNP effects are each estimated by fitting all SNP effects simultaneously as random variables drawn from a prior distribution. Then breeding animals are genotyped and the effect of each SNP is summed to generate a GEBV. In some studies, SNP effect-based methods have been shown to predict breeding value with higher accuracy compared to GBLUP, but the results depend on whether the true distribution of SNP effects for the trait matches the prior assumptions of the statistical method (Zhang et al., 2018).

The SNP-effect based method of GS uses the following model:

$$\boldsymbol{y} = \mathbf{1}\mu + \sum_i \boldsymbol{W}\boldsymbol{q}_i + \boldsymbol{e} \qquad [2]$$

Where **y** is a vector of phenotype values; ***1*** is a vector of ones; μ is the overall mean of the phenotype values; ***W*** is a design matrix relating genotypes coded 0, 1, 2 to observations; $\boldsymbol{q}_i$ is the effect of the i^{th} SNP; and ***e*** is a vector of residual effects. The genetic variance of SNP effects and residual variance equals: $\mathbf{WW'}\sigma_q^2$, and $\mathbf{I}\sigma_e^2$, respectively, where σ_q^2 and σ_e^2 are the additive genetic and residual variances, respectively; and **I** is an identity matrix.

Statistical methods for SNP effect-based methods include ridge regression BLUP (RRBLUP) (Whittaker et al., 2000), and various Bayesian methods. The most common Bayesian methods include, BayesA, BayesB (Meuwissen et al., 2001), BayesC (Habier et al., 2011), BayesR (Erbe et al., 2012), BayesRC (MacLeod et al., 2016), and Bayesian LASSO (Tibshirani, 1996), and each differs by their underlying assumptions of SNP effect distributions. BLUP methods (traditional BLUP, GBLUP, and RRBLUP) each follow an infinitesimal model. In this case, SNPs are assumed to be drawn from a normal distribution, where each has an equal and small effect on the trait, and no SNPs are expected to have a large effect on the trait (Meuwissen et al., 2001). Alternatively, the Bayesian methods assume a finite loci model, where few SNPs have moderate to large effect on the trait. BayesA and BayesB both assume a t-distribution, additionally, though, BayesA assumes there are many SNPs with small effects, and BayesB assumes a mixed distribution where a small, pre-defined proportion of SNPs (say 5%) have a non-zero effect but the rest of the SNPs have zero effect (Meuwissen et al., 2001). Bayes-C assumes that a proportion $(1 - \pi)$ of the SNPs has zero effects and that the remaining SNP effects are normally distributed (π) (Habier et al., 2011). BayesRC is unique and will be discussed further below, but this method is a modified version of BayesR. BayesR assumes SNPs come from a mixture of normal distributions, including one distribution that assumes SNPs have zero effect and the remaining three distributions that assume SNPs have increasing effects (Erbe et al.,

2012). Alternatively, BayesRC provides an advantage over BayesR in that it incorporates prior biological data. To do this, SNPs are allocated into one of four "classes" based on their predicted effect on the trait, then variance for each class is estimated individually (MacLeod et al., 2016). Finally, Bayesian LASSO assumes a double exponential distribution of SNP effects, where in addition to a few SNPs with moderate to large effect on the trait, many SNPs also have close to zero effect (Tibshirani, 1996).

1.7. Accuracy of genomic selection

Breeding value prediction accuracy is defined as the correlation between true and estimated breeding value (how reliable the phenotype of the breeding animal can be predicted). Since the true breeding value is unknown, accuracy is calculated as the correlation between breeding value (A) and phenotype (true breeding value (P) and environmental influences (E)):

$$r_{AP} = \frac{\sigma_{AP}}{\sqrt{\sigma_A^2 \times \sigma_P^2}} = \frac{\sigma_{AP}}{\sigma_A \times \sigma_P} = \frac{\sigma_A^2}{\sigma_A \times \sigma_P} = h \quad [3]$$

Where r_{AP} is breeding value prediction accuracy; $\sigma_{AP}$is the covariance between breeding value and phenotype; σ_A^2 is the additive genetic variance for the trait; σ_P^2 is the phenotypic variance for the trait; and h is the square root of heritability for the trait. The higher the accuracy of the prediction method, the more reliably the method will predict breeding value, and the higher potential genetic gain (Mrode & Thompson, 2005).

It is important to note that many factors will influence prediction accuracy, including the heritability of the trait, the effective population size, the size of the reference population

(Daetwyler et al., 2010), the relationship between individuals in training and validation populations (Clark et al., 2012), the mode of inheritance (additive or dominance) (Zeng et al., 2013), SNP panel density, LD between the QTL and SNPs (Meuwissen & Goddard, 2010), and the choice of statistical model (Goddard et al., 2010).

1.8. Alternative methods of genomic selection

Knowledge on the biological factors underlying meat quality traits could provide a significant opportunity to increase the breeding value prediction accuracy of GS. Nowadays, BLUP methods (traditional BLUP and ssGBLUP) are the most commonly practiced methods for estimating breeding value. However, these methods assume an infinitesimal model for SNP effects, which ignores any genetic or biological information known for the trait. SNPs associated with a trait may in fact be distributed non-uniformly throughout the genome, clustered in genes that are biologically relevant for the trait, and have varying effect sizes (from where the SNP has no biological function in the trait, to where the SNP displays a large effect on the trait) (Allen et al., 2010; Maurano et al., 2012). Therefore, knowledge on the biology of a trait is valuable as it can be incorporated into the statistical model used for GS to provide a more informed and customized procedure.

Multiple strategies to incorporate this biological knowledge into GS procedures have been proposed. One method, marker-assisted GBLUP (MA-GBLUP), derived from MAS, uses GWAS to identify markers with large effect on the trait, then incorporates them into the prediction model as fixed effects (Lopes et al., 2017). In pigs, incorporating the single SNP that explained the largest percentage of phenotypic variance for the trait into either BLUP or GBLUP

procedures was found to increase prediction accuracy between 0.021 to 0.124, and 0.003 to 0.043, respectively. What is more, incorporating all SNPs that explained greater than 1% of the phenotypic variance increased prediction accuracy even further (Lopes et al., 2017). The benefit of MA-GBLUP is that it requires little additional computational demands compared to BLUP or GBLUP, as it requires the incorporation of only a few additional fixed effects into the model, which makes this method easily implemented in practice.

Alternatively, GS methods can exploit biological information by using it to assign genetic variance to genomic regions enriched in biologically relevant factors. This can be done by differentially weighing of the GRM depending on the location and the effect of the genomic features, which takes advantage of the fact that genomic effects are distributed unevenly throughout the genome, and clustered in biologically relevant loci (Edwards et al., 2016). Therefore, the assumption of the model is no longer that each SNP shares an equal variance, but that the biologically relevant loci can account for a larger proportion of variance for the trait. One method, weighted GBLUP (wGBLUP), uses estimates of SNP effects to weigh the GRM for use in GBLUP. SNP effects have been estimated using Bayesian procedures, such as BayesB (Zhang et al., 2010), and Bayesian LASSO (Legarra et al., 2011), as well as GWAS results (de los Campos et al., 2013; Fragomeni et al., 2017). When Bayesian methods were used to weigh the GRM, wGBLUP predication accuracy was equivalent to that of the corresponding Bayesian model (Zhang et al., 2010; Legarra et al., 2011). Alternatively, the use of GWAS results was found to increase accuracy of GEBV estimations based on simulation studies (Fragomeni et al., 2017), and for some traits in real data studies in cattle, including various reproductive traits (Brøndum et al., 2015). Therefore, wGBLUP provides an ideal method of GS, especially when the genetic architecture of the trait is controlled by genes of large effect, as GEBV prediction

accuracy can be increased compared to GBLUP but without the additional computational burden imposed when using Bayesian procedures (Zhang et al., 2010; Legarra et al., 2011).

Another method, genomic feature BLUP (GFBLUP) uses prior biological information referred to as a "genomic features" to weight the GRM. Genomic features can be in the form of genes, chromosomes, biological pathways, gene ontologies, sequence annotation, transcriptomics data, or QTL regions, and genomic features that are relevant for the specific trait will be allocated a heavier weight (Edwards et al., 2016). An increase in 49 to 89%, and 3 to 164% in prediction accuracy for GFBLUP compared to GBLUP has been observed in real data studies in Drosophila and dairy cattle, respectively (Edwards et al., 2016; Fang et al., 2017). A similar Bayesian method exists, termed BayesRC, where SNPs are prioritized based on known biological data, then they are allocated into one of four different classes, which are expected to have a different probability of containing causative mutations for the trait, and thus explain a larger proportion of variance for the trait (MacLeod et al., 2016). Of course prediction accuracy for GFBLUP and BayesRC is improved when a larger proportion of the total genetic variance for the trait is accounted for, such as by the prioritization of causal variants, and accuracy is reduced by the incorrect prioritization of non-causal genetic variants (Edwards et al., 2016; Fang et al., 2017). These alternative GS methods highlight the potential of biological knowledge as a strategy to improve prediction accuracy. However, aside from these examples, relatively few genes have been found associated with meat and carcass quality traits, meaning a large proportion of the total genetic variance for the trait remains unaccounted for. Therefore, meat and carcass quality traits are good candidates for genomic analyses, such as genome-wide association studies (GWAS), to further our understanding on the genetic and biological factors underlying the trait.

1.9. Uncovering the biology of the trait

Association analysis is a powerful method to identify the genomic regions that are influencing a trait, with the ultimate goal to better understand the biology of complex phenotypes. Initial association analyses detected QTL using sparse microsatellite markers, however, these studies were limited by large confidence intervals (Hirschhorn & Daly, 2005). In some cases, these QTL could encompass entire chromosomes, and contain thousands of potential candidate genes and variants, which often required additional experiments for these to be fine mapped and identified (Dekkers, 2004). Today, high-density SNP genotyping data, along with a fully annotated pig genome (Pruitt et al., 2014; Zerbino et al., 2018), is now available for use in genome-wide association studies (GWAS). These tools have increased power and precision to test thousands of SNP markers across the entire genome for a significant association with a phenotype of interest, and to identify candidate genes nearby to significant SNPs with potentially biologically relevant roles (Yang et al., 2011). GWAS have been immensely successful in identifying quantitative trait loci (QTL) associated with observed variation in economically important traits in livestock. Tens of thousands of QTL have been reported on pig QTLDB for hundreds of different performance, disease, and carcass traits (Hu et al., 2005).

Despite the potential of GWAS methods, statistical difficulties present a major challenge, as they can prevent the identification of true, novel QTL. These include stringent P-values due to multiple testing, spurious associations due to population stratification, as well as the difficulty in detecting small effect, non-additive (dominance and epistasis), chromosomal (insertions, deletions, and translocations), and rare variants with low MAF (Manolio et al., 2009). Further, even if these statistical difficulties are overcome and statistically significant associations are identified, further challenges can arise due to long-range LD in livestock genomes. For this

reason, QTL, although much smaller than the confidence intervals of earlier studies, can still span several megabases in length, and contain multiple biologically relevant candidate genes for the trait. This makes pinpointing the exact gene associated with the trait difficult, limiting their use in alternative GS procedures.

Nonetheless, today GWAS can still benefit livestock breeding and genetics, especially in studies where sample sizes are large. GWAS could provide a significant opportunity to progress the current understanding of the genetic and biological factors underlying meat and carcass quality traits. As more QTL associated with economically important traits are identified, targeted studies can determine candidate genes and mutations within the genes and as well as their features. With regards to meat quality, the results of GWAS studies will not only increase our basic understanding of the post-mortem muscle biochemistry that determines meat and carcass quality, but as mentioned previously, can also be considered in GS procedure for the practical purpose of selecting breeding animals with superior meat quality.

Instead of relying on increasing sample sizes of GWAS to explore the biology of the trait, alternative methods are also being explored, which include but are not limited to, RNA expression (Wickramasinghe et al., 2014), metabolomics (Goldansaz et al., 2017), proteomics (D'Alessandro & Zolla, 2013), and epigenetics (DNA methylation and histone modification) (Gomez et al., 2013; Doherty & Couldrey, 2014), each provide a unique opportunity to improve the genetic and biological understanding of economically important traits in livestock.

1.10. Benefits for genomic selection of meat quality traits in pigs

Both relationship and SNP effect-based methods of GS provide a significant opportunity to select pigs with superior meat quality. GS can estimate breeding value of breeding candidates (selection candidates) accurately, by accounting for many variants of various effect sizes (depending on the model used), without having to collect phenotypes on the breeding candidate itself or on a large number of its close relatives. This allows for: 1) animals can be selected at an earlier age, and 2) with a higher prediction accuracy using GS compared to traditional selection methods, which taken together will greatly increase the rate of genetic gain for the trait (Meuwissen et al., 2001). However, since pigs already have a relatively short generation interval, the major power of GS in pig breeding will be realized through improving the accuracy of breeding values estimation. The current estimates for accuracy of GS on meat quality traits is low, for example, in a purebred Duroc population, GEBV accuracy ranged between 0.12 to 0.38 and 0.16 to 0.38, for carcass and meat quality traits, respectively, which in comparison to the accuracy of traditional selection estimates, is an increase between 6 to 33% and 7 to 38% for carcass and meat quality traits, respectively (Miar, 2015). Small increases in prediction accuracy can yield large genetic improvements, but further improvements to the accuracy of GEBV prediction will increase the rate of genetic gain for meat quality as well as the feasibility of including the trait in breeding programs.

1.11. Challenges for genomic selection of meat quality traits in pigs

GS presents a significant opportunity to improve the potential genetic gain for meat quality traits in pigs, however, multiple technical challenges still remain. In pig breeding, genetic selection is

implemented in purebred pigs, but the final product is a crossbred animal. Pig breeding uses a pyramidal structure composed of three tiers, which emphasizes crossbreeding throughout. 1) Genetic improvement is made at the top of the pig breeding pyramid, where animals are selected based on their own performance in a high-health, purebred nucleus herd. 2) Purebred dam lines are crossed (called an F1 cross) at multiplier farms to produce large numbers of breeding animals, which can be sold to commercial producers. Dam lines are often selected for their reproductive abilities, but these traits commonly have low heritability and are difficult and slow to improve. Therefore, the cross between purebred dam lines takes advantage of the phenomenon known as heterosis, where the performance of the crossbred offspring is better than that of the purebred parents. 3) The final stage of pig production occurs at the commercial level, where the crossbred dam lines are combined with purebred terminal sire lines. This final cross (called a terminal or three-way cross) produces animals with excellent growth, meat, and carcass quality that are destined for the market. Simultaneous improvement of both reproduction as well as meat quality, carcass quality, and growth is difficult and slow due to the adverse genetic relationships between these traits. Therefore, independent improvement of these traits in the dam and sire lines allows for the generation of dams that are highly productive, as well as an efficient and high-quality commercial product (a process called breed complementarity). The emphasis on crossbreeding throughout the pig production pyramid provides many benefits, which were mentioned, including the phenomenon known as heterosis (also known as outbreeding enhancement or hybrid vigor), and breed complementarity (Oldenbroek & van der Waaij, 2015), however, it also provides unique challenges for the implementation of GS.

GS relies on LD between SNPs and nearby causative mutations, and for this reason, recombination will occur between SNPs and causative mutations, reducing the accuracy of

GEBV estimation over generations (Meuwissen et al., 2001). If the markers used in GS were the causative mutations that were directly causing the phenotypic variance, then estimation of SNP effects would only need to be performed once in the reference population, and GEBVs for all the following generations could be predicted using these estimates of SNP effects (Meuwissen & Goddard, 2010). However, the majority of causative mutations remain unknown, and SNP effects need to be re-estimated over generations (Meuwissen et al., 2001). Additionally, for an accurate estimate of GEBV for meat quality, which is a low heritability trait, many animals will need to be included in the reference population (Daetwyler et al., 2010), which further emphasizes the need for a constant measurement of phenotypes and genotypes for re-estimation of SNP effects. In theory, SNP effects should be re-estimated every generation, due to a substantial drop in GEBV accuracy that is observed after the first generation (Wolc et al., 2011; Pszczola & Calus, 2016). In practice, this is easily achieved for most traits as phenotypes and genotypes of breeding animals are recorded continuously (Calus, 2010), but this becomes difficult and expensive for meat quality traits that must be measured post-mortem, since purebred breeding animals are not produced for the purpose of slaughter. Alternatively, continuous measurement of phenotypes could be done in a crossbred population. GS of purebred animals based on crossbred performance has been found to predict breeding value of purebred animals with either slightly lower (Toosi et al., 2010; Miar, 2014), or higher accuracy compared to selection based on purebred data. The resulting selection accuracy will depend on the model used. Current GS methods would assume that SNP effects are the same across breeds, but in fact, effects will vary across breeds, due to non-additive effects (dominance, and epistasis), and breed specific effects (Esfandyari et al., 2015). Additionally, implementing GS using crossbred data

will come with additional financial costs, as crossbred animals are not usually identified, genotyped, and their performance recorded (Esfandyari et al., 2015).

Since dominance and epistatic effects are likely the genetic basis of heterosis (Falconer, 1960), using a GS model that incorporates both additive and non-additive effects would be crucial for ensuring a large genetic gain (Esfandyari et al., 2015). Additionally, accounting for breed specific effects will also be important for accurate selection of purebreds based on crossbred performance. Breed specific effects occur when the same allele has a different effect depending on the specific breed, and are intensified when the relatedness between original purebreds is low. This can be due to incomplete LD between SNPs and causative mutations, where recombination between the SNP and causative mutation has occurred in one breed, but not the other (Esfandyari et al., 2015). Whole genome sequencing (WGS) data could solve the problem of incomplete LD, as theoretically it should include all possible variants, including causative mutations (Meuwissen & Goddard, 2010). Alternatively, the use of a genomic model that splits the additive genetic SNP effects that are estimated from crossbreds performance into breed specific components would allow for purebred animals to be selected based on their unique breed specific effects (Ibanez-Escriche et al., 2009).

1.12. Conclusions and thesis outline

Today, GS provides a significant opportunity to improve meat quality traits, which must be measured post-mortem, and are difficult, expensive, and slow to improve by traditional selection methods. The most commonly practiced method of GS is ssGBLUP, however, this method does not consider the biology of the trait, and instead assumes SNP effects are distributed uniformly

across the genome each with equal and small effects on the trait. Alternate methods of GS, such as wGBLUP, GFBLUP, or BayesRC that incorporate known biological information into their procedure have the potential to estimate breeding value with higher accuracy, which would improve the feasibility of including meat quality traits into breeding programs. However, considerable advances must first be made in understanding trait biology before these alternative methods are possible. Therefore, the main goal of this thesis was to improve our current understanding of the genetic and biological factors underlying meat and carcass quality traits to aid in the implementation of GS methods. Therefore, the purpose of this thesis was: 1) to estimate variance components and calculate genetic parameters for meat and carcass quality in pigs as an important first step in incorporating these traits into breeding programs. Then to use: 2) GWAS, and 3) a single-SNP association analysis of potential causative mutations, to improve the current understanding of the biological factors underlying meat quality traits.

1.13. References

Allen, H., Estrada, K., Lettre, G., Berndt, S., Weedon, M., Rivadeneira, F., Willer, C., Jackson, A., Vedantam, S., & Raychaudhuri, S. et al. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature, 467(7317), 832–838. https://doi.org/10.1038/nature09410

Bee, G., Biolley, C., Guex, G., Herzog, W., Lonergan, S. M., & Huff-Lonergan, E. (2006). Effects of available dietary carbohydrate and preslaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. Journal of Animal Science, 84(1), 191–203. https://doi.org/10.2527/2006.841191x

Bourdon, R. M. (2014). Understanding Animal Breeding (2nd ed.). Upper Saddle River, NJ: Prentice Hall: Pearson New International.

Brøndum, R. F., Su, G., Janss, L., Sahana, G., Guldbrandtsen, B., Boichard, D., & Lund, M. S. (2015). Quantitative trait loci markers derived from whole genome sequence data increases the reliability of genomic prediction. Journal of Dairy Science, 98(6), 4107–4116. https://doi.org/10.3168/jds.2014-9005

Buege, D., & Griffin, D. (2015). Variation in Pork Lean Quality Descriptors of Pork Lean Quality. Pork Information Gateway. Retrieved from http://porkgateway.org/wp-content/uploads/2015/07/variation-in-lean-pork-quality1.pdf

Calus, M. P. L. (2010). Genomic breeding value prediction: Methods and procedures. Animal, 4(2), 157–164. https://doi.org/10.1017/S1751731109991352

Cameron, N. D., Warriss, P. D., Porter, S. J., & Enser, M. B. (1990). Comparison of Duroc and British landrace pigs for meat and eating quality. Meat Science, 27(3), 227–247. https://doi.org/10.1016/0309-1740(90)90053-9

Christensen, O., & Lund, M. (2010). Genomic relationship matrix when some animals are not genotyped. Genetics Selection Evolution, 42(2), 1–8. https://doi.org/10.1186/1297-9686-42-2

Christensen, O., Madsen, P., Nielsen, B., Ostersen, T., & Su, G. (2012). Single-step methods for genomic evaluation in pigs. Animal, 6(10), 1565–1571. https://doi.org/10.1017/S1751731112000742

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics, 159(3), 1151–1162.

Ciobanu, D., Bastiaansen, J. W. M., Lonergan, S. M., Thomsen, H., Dekkers, J. C. M., Plastow, G. S., & Rothschild, M. F. (2004). New alleles in calpastatin gene are associated with meat quality traits in pigs. Journal of Animal Science, 82(10), 2829–2839. https://doi.org/10.2527/2004.82102829x

Ciobanu, D., Lonergan, S., & Huff-Lonergan, E. (2011). Genetics of Meat Quality and Carcass Traits. In The Genetics of the Pig, 2nd Edition (355–378).

Cohen, J. (1998). Statistical power analysis for the behavioral sciences (2nd edition). Hillsdale, N. J.: Erlbaum Associated.

D'Alessandro, A., & Zolla, L. (2013). Meat science: From proteomics to integrated omics towards system biology. Journal of Proteomics, 78, 558–577. https://doi.org/10.1016/j.jprot.2012.10.023

D'Souza, D. N., Warner, R. D., Leury, B. J., & Dunshea, F. R. (2000). The influence of dietary magnesium supplement type, and supplementation dose and duration, on pork quality and

the incidence of PSE pork. Australian Journal of Agricultural Research, 51(2), 185–189. https://doi.org/10.1071/AR99090

Daetwyler, H. D., Pong-Wong, R., Villanueva, B., & Woolliams, J. A. (2010). The impact of genetic architecture on genome-wide evaluation methods. Genetics, 185(3), 1021–1031. https://doi.org/10.1534/genetics.110.116855

Dokmanović, M., Velarde, A., Tomović, V., Glamočlija, N., Marković, R., Janjić, J., & Baltić, M. Z. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. Meat Science, 98(2), 220–226. https://doi.org/10.1016/j.meatsci.2014.06.003

de los Campos, G., Hickey, J. M., Pong-Wong, R., Daetwyler, H. D., & Calus, M. P. L. (2013). Whole-genome regression and prediction methods applied to plant and animal breeding. Genetics, 193(2), 327–345. https://doi.org/10.1534/genetics.112.143313

de los Campos, G., Vazquez, A. I., Fernando, R., Klimentidis, Y. C., & Sorensen, D. (2013). Prediction of Complex Human Traits Using the Genomic Best Linear Unbiased Predictor. PLoS Genetics, 9(7). https://doi.org/10.1371/journal.pgen.1003608

Dekkers, J. C. M. (2004). Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. Journal of Animal Science, 82(E-Suppl.) https://doi.org/10.2527/2004.8213_supplE313x

Doherty, R., & Couldrey, C. (2014). Exploring genome wide bisulfite sequencing for DNA methylation analysis in livestock: A technical assessment. Frontiers in Genetics, 5(126), 1–8. https://doi.org/10.3389/fgene.2014.00126

Dransfield, E., Ngapo, T. M., Nielsen, N. A., Bredahl, L., Sjödén, P. O., Magnusson, M., Campo, M. M., & Nute, G. R. (2005). Consumer choice and suggested price for pork as

influenced by its appearance, taste and information concerning country of origin and organic pig production. Meat Science, 69(1), 61–70. https://doi.org/10.1016/j.meatsci.2004.06.006

Edwards, S., Sørensen, I. F., Sarup, P., Mackay, T. F. C., & Sørensen, P. (2016). Genomic prediction for quantitative traits is improved by mapping variants to gene ontology categories in Drosophila melanogaster. Genetics, 203(4), 1871–1883. https://doi.org/10.1534/genetics.116.187161

Eikelenboom, G., Bolink, A. H., & Sybesma, W. (1991). Effects of feed withdrawal before delivery on pork quality and carcass yield. Meat Science, 29(1), 25–30. https://doi.org/10.1016/0309-1740(91)90020-Q

Erbe, M., Hayes, B., Matukumalli, L., Goswami, S., Bowman, P., Reich, C., Mason, B., & Goddard, M. (2012). Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. Journal of Dairy Science, 95(7), 4114–4129. https://doi.org/10.3168/jds.2011-5019

Esfandyari, H., Sørensen, A. C., & Bijma, P. (2015). Maximizing crossbred performance through purebred genomic selection. Genetics Selection Evolution, 47(1), 1–16. https://doi.org/10.1186/s12711-015-0099-3

Falconer, D. S. (1960). Introduction to Quantitative Genetics. New York, NY: Ronald Press.

Fang, L., Sahana, G., Ma, P., Su, G., Yu, Y., Zhang, S., Lund, M. S., & Sørensen, P. (2017). Exploring the genetic architecture and improving genomic prediction accuracy for mastitis and milk production traits in dairy cattle by mapping variants to hepatic transcriptomic regions responsive to intra-mammary infection. Genetics Selection Evolution, 49(1), 1–18. https://doi.org/10.1186/s12711-017-0319-0

Fangmann, A., Sharifi, R. A., Heinkel, J., Danowski, K., Schrade, H., Erbe, M., & Simianer, H. (2017). Empirical comparison between different methods for genomic prediction of number of piglets born alive in moderate sized breeding populations. Journal of Animal Science, 95(4), 1434–1443. https://doi.org/10.2527/jas2016.0991

FAO. (2019). Meat & Meat Products. Food and Agriculture Organization of the United Nations, Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/home.html

FCC. (2017). Economic Snapshot of Canada's Hog and Pork Sectors. Farm Credit Canada, Retrieved from https://www.fcc-fac.ca/content/dam/fcc/knowledge/ag-economist/2017-economic-snapshot-hog-pork-e.pdf

Fernando, R. L., Cheng, H., Sun, X., & Garrick, D. J. (2017). A comparison of identity-by-descent and identity-by-state matrices that are used for genetic evaluation and estimation of variance components. Journal of Animal Breeding and Genetics, 134(3), 213–223. https://doi.org/10.1111/jbg.12275

Fragomeni, B. O., Lourenco, D. A. L., Masuda, Y., Legarra, A., & Misztal, I. (2017). Incorporation of causative quantitative trait nucleotides in single-step GBLUP. Genetics Selection Evolution, 49(1), 1–11. https://doi.org/10.1186/s12711-017-0335-0

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., & Maclennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253(5018), 448–451. https://doi.org/10.1126/science. 1862346

Goddard, M., Hayes, B., & Meuwissen, T. (2010). Genomic selection in livestock populations. Genetics Research, 92(5–6), 413–421. https://doi.org/10.1017/S0016672310000613

Goldansaz, S. A., Guo, A. C., Sajed, T., Steele, M. A., Plastow, G. S., & Wishart, D. S. (2017). Livestock metabolomics and the livestock metabolome: A systematic review. PLoS ONE, 12(5), 1–26. https://doi.org/10.1371/journal.pone.0177675

Gomez, D., Shankman, L., A, N., & Owens, G. K. (2013). Detection of histone modifications at specific gene loci in single cells in histological sections. Nature Methods, 10(2), 171–177. https://doi.org/10.1038/nmeth.2332

Grandin, T. (2003). The welfare of pigs during transport and slaughter. Retrieved from https://www.grandin.com/references/pig.welfare.during.transport.slaughter.html

Guàrdia, M. D., Estany, J., Balasch, S., Oliver, M. A., Gispert, M., & Diestre, A. (2004). Risk assessment of PSE condition due to pre-slaughter conditions and RYR1 gene in pigs. Meat Science, 67(3), 471–478. https://doi.org/10.1016/j.meatsci.2003.11.020

Guzik, A. C., Matthews, J. O., Kerr, B. J., Bidner, T. D., & Southern, L. L. (2006). Dietary tryptophan effects on plasma and salivary cortisol and meat quality in pigs. Journal of Animal Science, 84(8), 2251–2259. https://doi.org/10.2527/jas.2005-292

Habier, D., Fernando, R. L., Kizilkaya, K., & Garrick, D. J. (2011). Extension of the bayesian alphabet for genomic selection. BMC Bioinformatics, 12(186). https://doi.org/10.1186/1471-2105-12-186

Hall, J., Latschar, E. E., & Mackintosh, D. L. (1994). Characteristics of dark cutting beef. Survey and preliminary investigation. Agriculture Experimental Station Technical Bulletin, 58(4).

Hambrecht, E., Eissen, J. J., Nooijen, R. I. J., Ducro, B. J., Smits, C. H. M., Den Hartog, L. A., & Verstegen, M. W. A. (2004). Preslaughter stress and muscle energy largely determine

pork quality at two commercial processing plants. Journal of Animal Science, 82(5), 1401–1409. https://doi.org/10.2527/2004.8251401x

Henderson, C. R. (1975). Best Linear Unbiased Estimation and Prediction under a Selection Model. Biometrics, 31(2), 423–447.

Hirschhorn, J. N., & Daly, M. J. (2005). Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics, 6(2), 95–108. https://doi.org/10.1038/nrg1521

Hu, Z. L., Dracheva, S., Jang, W., Maglott, D., Bastiaansen, J., Rothschild, M. F., & Reecy, J. M. (2005). A QTL resource and comparison tool for pigs: PigQTLDB. Mammalian Genome, 16(10), 792–800. https://doi.org/10.1007/s00335-005-0060-9

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of Specific Muscle Structural Proteins by μ-Calpain at Low pH and Temperature is Similar to Degradation in Postmortem Bovine Muscle. Journal of Animal Science, 74(5), 993–1008. https://doi.org/10.2527/1996.745993x

Huff-Lonergan, E., & Page, J. (2006). The Role of Carcass Chilling in the Development of Pork Quality. National Pork Board, American Meat Science Association Fact Sheet. Retrieved from http://porkgateway.org/resource/the-role-of-carcass-chilling-in-the-development-of-pork-quality/

Honikel, K. O., Roncalés, P., & Hamm, R. (1983). The influence of temperature on shortening and rigor onset in beef muscle. Meat Science, 8(3), 221–241. https://doi.org/10.1016/0309-1740(83)90046-3

Ibanez-Escriche, N., Fernando, R. L., Toosi, A., & Dekkers, J. C. M. (2009). Genomic selection of purebreds for crossbred performance. Genetics Selection Evolution, 41(12), 1–10. https://doi.org/10.1186/1297-9686-41-12

Idolo Imafidon, G, & Spanier, A. M. (1994). Unraveling the secret of meat flavor. Trends in Food Science and Technology, 5(10), 315–321. https://doi.org/10.1016/0924-2244(94)90182-1

Jeremiah, L. E. (2006). Marbling and Pork Tenderness. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/07/marbling-and-prok-tenderness1.pdf

Kerth, C. R., Carr, M. A., Ramsey, C. B., Brooks, J. C., Johnson, R. C., Cannon, J. E., & Miller, M. F. (2001). Vitamin-mineral supplementation and accelerated chilling effects on quality of pork from pigs that are monomutant or noncarriers of the halothane gene. Journal of Animal Science, 79(9), 2346–2355. https://doi.org/10.2527/2001.7992346x

Khan, M. I., Jo, C., & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors-A systematic review. Meat Science, 110, 278–284. https://doi.org/10.1016/j.meatsci.2015.08.002

Leach, L. M., Ellis, M., Sutton, D. S., McKeith, F. K., & Wilson, E. R. (1996). The Growth Performance, Carcass Characteristics, and Meat Quality of Halothane Carrier and Negative Pigs. Journal of Animal Science, 74(5), 934–943. https://doi.org/10.2527/1996.745934x

Legarra, A., Aguilar, I., & Misztal, I. (2009). A relationship matrix including full pedigree and genomic information. Journal of Dairy Science, 92(9), 4656–4663. https://doi.org/10.3168/jds.2009-2061

Legarra, A., Robert-Granié, C., Croiseau, P., Guillaume, F., & Fritz, S. (2011). Improved Lasso for genomic selection. Genetics Research, 93(1), 77–87. https://doi.org/10.1017/S0016672310000534

Lopes, M. S., Bovenhuis, H., van Son, M., Nordbø, Ø., Grindflek, E. H., Knol, E. F., & Bastiaansen, J. W. M. (2017). Using markers with large effect in genetic and genomic predictions. Journal of Animal Science, 95(1), 59. https://doi.org/10.2527/jas2016.0754

MacLeod, I., Bowman, P., Vander Jagt, C., Haile-Mariam, M., Kemper, K., Chamberlain, A., Schrooten, C., Hayes, B., & Goddard, M. (2016). Exploiting biological priors and sequence variants enhances QTL discovery and genomic prediction of complex traits. BMC Genomics, 17(1), 1–21. https://doi.org/10.1186/s12864-016-2443-6

Makgahlela, M. L., Strandén, I., Nielsen, U. S., Sillanpää, M. J., & Mäntysaari, E. A. (2013). The estimation of genomic relationships using breedwise allele frequencies among animals in multibreed populations. Journal of Dairy Science, 96(8), 5364–5375. https://doi.org/10.3168/jds.2012-6523

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. Meat Science, 71(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., McCarthy, M. I., Ramos, E. M., Cardon, L. R., Chakravarti, A., Cho, J. H., Guttmacher, A. E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C. N., Slatkin, M., Valle, D., Whittemore, A. S., Boehnke, M., Clark, A. G., Eichler, E. E., Gibson, G., Haines, J. L., MacKay, T. F.C., McCarroll, S. A., & Visscher, P. M. (2009). Finding the missing heritability of complex diseases. Nature, 461(7265), 747–753. https://doi.org/10.1038/nature08494

Martoccia, L., Brambilla, G., Macrì, A., Moccia, G., & Cosentino, E. (1995). The effect of transport on some metabolic parameters and meat quality in pigs. Meat Science, 40(2), 271–277. https://doi.org/10.1016/0309-1740(94)00015-Y

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., Reynolds, A. P., Sandstrom, R., Qu, H., Brody, J., Shafer, A., Neri, F., Lee, K., Kutyavin, T., Stehling-Sun, S., Johnson, A. K., Canfield, T. K., Giste, E., Diegel, M., Bates, D., Hansen, R. S., Neph, S., Sabo, P. J., Heimfeld, S., Raubitschek, A., Ziegler, S., Cotsapas, C., Sotoodehnia, N., Glass, I., Sunyaev, S. R., Kaul, R., & Stamatoyannopoulos, J. A. (2012). Systematic localization of common disease-associated variation in regulatory DNA. Science, 337(6099), 1190–1195. https://doi.org/10.1126/science.1222794

Meuwissen, T., & Goddard, M. (2010). Accurate prediction of genetic values for complex traits by whole-genome resequencing. Genetics, 185(2), 623–631. https://doi.org/10.1534/genetics.110.116590

Meuwissen, T., Hayes, B., & Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics, 157, 1819–1829. https://doi.org/11290733

Miar, Y. (2015). Genomic selection for pork quality and carcass traits in both cross- and pure-bred populations (Dissertation). University of Alberta. https://doi.org/10.7939/R3M32NH2S

Miar, Y., Plastow, G., Moore, S., Manafiazar, G., Charagu, P., Kemp, R., Van Haandel, B., Huisman, A., Zhang, C., Mckay, R., Bruce, H., & Wang, Z. (2014). Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livestock Production Science, 26(2), 119–135. https://doi.org/10.2527/jas2014-7685

Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robix, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronee, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., Chardon, P., & Andersson, L. (2000). A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. Science, 288(5469), 1248–1251. https://doi.org/10.1126/science.288.5469.1248

Misztal, I., Legarra, A., & Aguilar, I. (2009). Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. Journal of Dairy Science, 92(9), 4648–4655. https://doi.org/10.3168/jds.2009-2064

Mrode, R. A., & Thompson, R. (2005). Linear Models for the Prediction of Breeding Values. In CABI Publishing (Second Edition). Cambridge, MA.

Murray, A. C., & Johnson, C. P. (1998). Impact of the halothane gene on muscle quality and pre-slaughter deaths in Western Canadian pigs. Canadian Journal of Animal Science, 78(4), 543–548. https://doi.org/10.4141/A97-122

Murray, A. C., & Jones, S. D. M. (1994). The effect of mixing, feed restriction and genotype with respect to stress susceptibility on pork carcass and meat quality. Canadian Journal of Animal Science, 74(4), 587–594. https://doi.org/10.4141/cjas94-086

Nejati-Javaremi, A., Smith, C., & Gibson, J. P. (1997). Effect of Total Allelic Relationship on Accuracy of Evaluation and Response to Selection. Journal of Animal Science, 75(7), 1738–1745. https://doi.org/10.2527/1997.7571738x

Newton, K. G., & Gill, C. O. (1981). The microbiology of DFD fresh meats: A review. Meat Science, 5(3), 223–232. https://doi.org/10.1016/0309-1740(81)90005-X

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2007). International preferences for pork appearance: I. Consumer choices. Food Quality and Preference, 18(1), 26–36. https://doi.org/10.1016/j.foodqual.2005.07.001

Nishimura, T., Hattori, A., & Takahashi, K. (1999). Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. Journal of Animal Science, 77(1), 93–104. https://doi.org/10.2527/1999.77193x

Nonneman, D., Lindholm-Perry, A. K., Shackelford, S. D., King, D. A., Wheeler, T. L., Rohrer, G. A., Bierman, C. D., Schneider, J. F., Miller, R. K., Zerby, H., & Moeller, S. J. (2011). Predictive markers in calpastatin for tenderness in commercial pig populations. Journal of Animal Science, 89(9), 2663–2672. https://doi.org/10.2527/jas.2010-3556

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 2. Drip losses. Developments in Meat Science, 4, 173–243.

Oldenbroek, K., & van der Waaij, L. (2015). Textbook Animal Breeding and Genetics for BSc Students. Retrieved from https://wiki.groenkennisnet.nl/display/TAB/Textbook+Animal+Breeding+and+Genetics

Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S., & Kalm, E. (2007). Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Science, 75(2), 185–195. https://doi.org/10.1016/j.meatsci.2006.03.022

Pan, B. S., & Myron, S. (1972). The effect of pH on bovine oxymyoglobin structure and stability. *Journal of Food Science*, *37*, 29–32.

Pruitt, K., Brown, G., Hiatt, S., Thibaud-Nissen, F., Astashyn, A., Ermolaeva, O., Farrell, C. M., Hart, J., Landrum, M. J., McGarvey, K. M., Murphy, M. R., O'Leary, N. A., Pujar, S., Rajput, B., Rangwala, S. H., Riddick, L. D., Shkeda, A., Sun, H., Tamez, P., Tully, R. E., Wallin, C., Webb, D., Weber, J., Wu, W., Dicuccio, M., Kitts, P., Maglott, D. R., Murphy, T. D., & Ostell, J. (2014). RefSeq: an update on mammalian reference sequences. Nucleic Acids Research, 42(1), 553–559. https://doi.org/10.1093/nar/gkt1114

Pszczola, M., & Calus, M. P. L. (2016). Updating the reference population to achieve constant genomic prediction reliability across generations. Animal, 10(6), 1018–1024. https://doi.org/10.1017/S1751731115002785

Ramos, A. M., Crooijmans, R. P. M. A., Affara, N. A., Amaral, A. J., Archibald, A. L., Beever, J. E., Bendixen, C., Churcher, C., Clark, R., Dehais, P., Hansen, M. S., Hedegaard, J., Hu, Z. L., Kerstens, H. H., Law, A. S., Megens, H. J., Milan, D., Nonneman, D., Rohrer, G. A., Rothschild, M. F., Smith, T. P. L., Schnabel, R. D., Van Tassell, C. P., Taylor, J. F., Wiedmann, R. T., Schook, L. B., & Groenen, M. A. M. (2009). Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PLoS ONE, 4(8). https://doi.org/10.1371/journal.pone.0006524

Ropka-Molik, K., Bereta, A., Tyra, M., Rózycki, M., Piórkowska, K., Szyndler-Nedza, M., & Szmatoła, T. (2014). Association of calpastatin gene polymorphisms and meat quality traits in pig. Meat Science, 97(2), 143–150. https://doi.org/10.1016/j.meatsci.2014.01.021

Rosenvold, K., Lærke, H. N., Jensen, S. K., Karlsson, A. H., Lundstrom, K., & Andersen, H. J. (2001a). Strategic finishing feeding as a tool in the control of pork quality. Meat Science, 59, 387–406. https://doi.org/10.1016/S0309-1740(01)00092-4

Rosenvold, K., Petersen, J. S., Lærke, H. N., Jensen, S. K., Therkildsen, M., Karlsson, A. H., Møller, H. S., & Andersen, H. J. (2001b). Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. Journal of Animal Science, 79(2), 382–391. https://doi.org/10.2527/2001.792382x

Smulders, F. J. M., Eikelenboom, G., & Logtestijn, J. G. Van. (1986). The Effect of Electrical Stimulation on the Quality of Three Bovine Muscles. Meat Science, 16, 91–101.

Sterten, H., Frøystein, T., Oksbjerg, N., Rehnberg, A. C., Ekker, A. S., & Kjos, N. P. (2009). Effects of fasting prior to slaughter on technological and sensory properties of the loin muscle (M. longissimus dorsi) of pigs. Meat Science, 83(3), 351–357. https://doi.org/10.1016/j.meatsci.2009.06.002

Tibshirani, R. (1996). Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society., 58(1), 267–288.

Toosi, A., Fernando, R. L., & Dekkers, J. C. M. (2010). Genomic selection in admixed and crossbred populations. Journal of Animal Science, 88(1), 32–46. https://doi.org/10.2527/jas.2009-1975

Viljoen, H. F., De Kock, H. L., & Webb, E. C. (2002). Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. Meat Science, 61(2), 181–185. https://doi.org/10.1016/S0309-1740(01)00183-8

Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. Meat Science, 86(1), 171–183. https://doi.org/10.1016/j.meatsci.2010.04.042

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. *Journal of*

Animal Science, *78*(4), 958–965. https://doi.org/10.2527/2000.784958x

Whittaker, J. C., Thompson, R., & Denham, M. C. (2000). Marker-assisted selection using ridge regression. Genetical Research, 75(2), 249–252. https://doi.org/10.1017/S0016672399004462

Wickramasinghe, S., Cánovas, A., Rincón, G., & Medrano, J. F. (2014). RNA-Sequencing: A tool to explore new frontiers in animal genetics. Livestock Science, 166(1), 206–216. https://doi.org/10.1016/j.livsci.2014.06.015

Wismer-Pederson, J., & Briskey, E. J. (1961). Rate of Anaerobic Glycolysis versus Structure in Pork Muscle. Nature, 189, 318–320. https://doi.org/10.1038/189318a0

Wolc, A., Arango, J., Settar, P., Fulton, J. E., O'Sullivan, N. P., Preisinger, R., Habier, D., Fernando, R., Garrick, D. J., & Dekkers, J. C. M. (2011). Persistence of accuracy of genomic estimated breeding values over generations in layer chickens. Genetics Selection Evolution, 43(1), 1–8. https://doi.org/10.1186/1297-9686-43-23

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. Meat Science, 78(4), 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019

Yang, J., Benyamin, B., McEvoy, B., Gordon, S., Henders, A., Dale, N., Madden, P., Heath, A., Martin, N., Montgomery, G., Goddard, M., & Visscher, P. (2011). Common SNPs explain a large proportion of heritability for human height. Nature Genetics, 42(7), 565–569. https://doi.org/10.1038/ng.608

Young, J. F., Bertram, H. C., Rosenvold, K., Lindahl, G., & Oksbjerg, N. (2005). Dietary creatine monohydrate affects quality attributes of Duroc but not Landrace pork. Meat Science, 70(4), 717–725. https://doi.org/10.1016/j.meatsci.2005.03.008

Zerbino, D. R., Achuthan, P., Akanni, W., Amode, M. R., Barrell, D., Bhai, J., et al. (2018). Ensembl 2018. Nucleic Acids Research, 46(D1), D754–D761. https://doi.org/10.1093/nar/gkx1098

Zhang, C., Kemp, R., Stothard, P., Wang, Z., Boddicker, N., Krivushin, K., Dekkers, J. C. M., Plastow, G. (2018). Genomic evaluation of feed efficiency component traits in Duroc pigs using 80K, 650K and whole-genome sequence variants. Genetics Selection Evolution, 50(1), 1–13. https://doi.org/10.1186/s12711-018-0387-9

Zhang, C., Wang, Z., Bruce, H. L., Janz, J., Goddard, E., Moore, S., and Plastow, G. S. (2014) Associations between single nucleotide polymorphisms in 33 candidate genes and meat quality traits in commercial pigs. Animal Genetics, 45(4), 508–516. https://doi.org/10.1111/age.12155

Zhang, Z., Liu, J., Ding, X., Bijma, P., de Koning, D. J., & Zhang, Q. (2010). Best linear unbiased prediction of genomic breeding values using a trait-specific marker-derived relationship matrix. PLoS ONE, 5(9), 1–8. https://doi.org/10.1371/journal.pone.0012648

Chapter 2. Estimating genetic parameters for meat quality and carcass traits in purebred Duroc pigs

2.1. Introduction

Carcass traits such as backfat depth (BF), loin muscle depth (MD), and carcass weight are of high economic importance due to their influence on carcass value. Larger carcasses with a higher percentage of lean meat are regarded as more valuable according to the Canadian and American pricing standards (Alberta Pork, 2017). Carcass traits may be easily inferred from the live animal using ultrasound (as an indicator trait) or they can be measured for a low cost directly on the carcass using a ruler or a grading probe. Due to their economic importance and ease of measurement, these carcass traits have been subject to intense selection and rapid genetic improvement. This has resulted in immense changes to pork carcass composition as well as a large increase in the value of Canadian pork. In contrast, meat quality traits have not commonly been included as a breeding goal in traditional breeding programs, as they must be measured post-mortem, and they cannot be predicted on potential breeding candidates with high accuracy (Miar, 2015). Nowadays, consumer awareness of meat quality is increasing and these traits are becoming of higher economic importance due to their influence on customer purchasing and repurchasing decisions. Therefore, breeding programs should emphasize meat quality in addition to carcass traits, to produce a high-quality lean pork, and further improve the value and the demand for Canadian pork products.

Genomic selection (GS) provides a practical solution for the genetic improvement of meat quality traits. An important first step for introducing any trait into a breeding program is the estimation of genetic parameters for the trait. In general, meat quality traits show low to

moderate heritability (between 0.10 to 0.39), and carcass traits show moderate to high heritability (between 0.22 to 0.63) depending on the specific population and breed (Ciobanu et al., 2011; Miar et al., 2014). The Duroc breed is a common terminal sire line, especially in Canada, as they are known to be highly productive (with regards to growth rate and feed efficiency) and provide meat with highly desirable quality characteristics. Duroc meat is dark red in colour, has intramuscular fat (IMF) throughout, lower drip loss (DL) , and higher pH (Tannas & Tannas, 2014). However, few studies have reported heritability and correlation estimates for meat quality traits in the Duroc breed, and those that do have mainly focused on carcass or fat traits (Suzuki et al., 2005; Hernández-Sánchez et al., 2013). Duroc animals represent an ideal breed for which to make genetic improvements for meat quality traits, therefore, the purpose of this study was to estimate genetic variance components for meat quality traits as well as to calculate the heritabilities and the genetic and phenotypic correlations between meat and carcass traits in a purebred Duroc pig population. It is hypothesized that meat quality traits are controlled at least in part by genetics ($h^2 > 0$), and that relationships among meat quality traits, as well as between meat and carcass traits, are at least in part due to shared genetic determinants between the traits ($r_g > 0$). The results of this study could be directly incorporated into selection procedures, or they can be used when selecting traits to include in the breeding goal.

2.2. Materials and methods

2.2.1. Data

Animals

A total of 997 purebred female Duroc pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used in this study. These animals were raised in a nucleus pig breeding herd following the Canadian Council on Animal Care (CCAC) guidelines and by protocol approved by the University of Alberta Animal Care and Use Committee. This study was characterized as a Category A animal experiment, which involved no experimental manipulation and tissues collected from the abattoir (CCAC, 2020).

The pigs used in this study were raised with *ad libitum* access to food and water. Pigs were harvested every third week on Friday from January of 2018 to March of 2019 (14 months) at a provincial slaughterhouse (East 40 Packers, Brandon, MB, Canada). Animals were shipped to the slaughter house in batches of 30-35 animals, held overnight at the slaughterhouse with *ad libitum* access to water and restricted access to food, then slaughtered the following morning. At the time of slaughter, pigs weighed an average of 121 kg and were on average 168 days of age. Meat quality measurements were recorded from the carcass within a 24-hour period after slaughter.

Phenotypes

Meat and carcass quality measurements were taken on both the ham and *longissimus thoracis et lumborum* (loin) muscles. The loin was harvested from the third to fourth last rib, which

corresponds to the Canadian grading site. Meat colour measurements were collected using a Minolta CR 310 colorimeter set at C illuminant (Minolta, Osaka, Japan). The colorimeter measures Minolta L*, a*, and b* values, which represent the lightness (L* = 0 is black, L* = 100 is white), redness (a* = +60 is red, a* = -60 is green), and yellowness (b* = +60 is yellow, b* = -60 is blue) of the muscle tissue, respectively. Loin Minolta L*, a*, and b* (LOINL, LOINA, and LOINB) were measured from four sites on the fresh cut and anterior surface of the boneless center cut loin muscle. The final value was taken as an average Minolta measurement of the four sites on the loin. Fat Minolta L*, a*, and b* (LOINFATL, LOINFATA, and LOINFATB) measurements were taken from the subcutaneous fat tissue above the loin muscle. Minolta L*, a*, and b* were also taken from three sites of the ham: the fresh cut surface on the inside of the ham *gluteus medius* (GLUTL, GLUTA, and GLUTB), the ham *quadriceps femoris* (QUADL, QUADA, and QUADB), and the ham *iliopsoas* (ILIOL, ILIOA, and ILIOB) muscles. The ultimate pH measurement (pH24) was taken 24 hours after exsanguination on the loin muscle at two of the Minolta colour score locations. The final value was taken as the average measurement of the two sites. DL was measured from a 3 cm defatted and deboned loin which were weighed, placed on a stainless-steel grid, and stored for 48 hours at 4°C. After incubation, loins were blotted dry and weighed again. DL (%) was calculated using the following formula: $\text{DL (\%)} = \frac{\text{starting weight} - \text{final weight}}{\text{starting weight}} * 100$. Loin eye area (LEA), fat depth (FD), and loin muscle depth (MD) were each measured on the loin muscle. LEA was determined using a 1 cm square grid and by manually counting the number of 1 cm squares that fit inside the loin face. FD and MD were both measured in mm using a ruler that was placed 5.5 cm off the midline, perpendicular to the skin. Subjective marbling score (NSIF IMF) was determined using the National Swine Improvement Federation (NSIF) marbling charts, which score the marbling on the face of the

loin muscle with a value between 1 to 6 (0 = devoid, 1 = practically devoid, 2 = trace, 3 = slight, 4 = small, 5 = moderate, and 6 = abundant) (NSIF, 1997).

Pedigree

The pedigree file used was composed of a total of 269,921 animals, with 100% of the animals in this study having both parents known. The pedigree included 2,322 sires and 16,744 dams over 25 generations.

2.2.2. Statistical analysis

The significance of fixed effects and covariates was determined using Wald F. statistics in ASReml software, and factors with P-value < 0.05 were included in subsequent analysis (Gilmour, 2015). A pairwise bivariate analysis was performed in ASReml software to estimate the variance components of meat and carcass traits using the following model (Gilmour, 2015):

$$\begin{bmatrix} \boldsymbol{y}_1 \\ \boldsymbol{y}_2 \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_1 & 0 \\ 0 & \boldsymbol{X}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{b}_1 \\ \boldsymbol{b}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_1 & 0 \\ 0 & \boldsymbol{Z}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{e}_1 \\ \boldsymbol{e}_2 \end{bmatrix} \quad [1]$$

Where $\boldsymbol{y}_1$ and $\boldsymbol{y}_2$ are vectors of phenotypic records for traits 1 and 2, respectively; $\boldsymbol{X}_1$ and $\boldsymbol{X}_2$ are design matrices that relate fixed effects to the observations; $\boldsymbol{b}_1$ and $\boldsymbol{b}_2$ are the vectors of fixed effects including slaughter batch for all traits and slaughter age for MD; $\boldsymbol{Z}_1$ and $\boldsymbol{Z}_2$ are design matrices associating $\boldsymbol{a}_1$ and $\boldsymbol{a}_2$ with phenotypic records (response variables); $\boldsymbol{a}_1$ and $\boldsymbol{a}_2$ are vectors of random additive genetic effects (animal effects); and $\boldsymbol{e}_1$ and $\boldsymbol{e}_2$ are vectors of residual effects.

The additive genetic effects and the residual effects are both assumed to be normally distributed; $\boldsymbol{a} \sim N(0, \boldsymbol{A}\sigma_a^2)$, $\boldsymbol{e} \sim N(0,\ \boldsymbol{I}\sigma_e^2)$, respectively, where σ_a^2 and σ_e^2 are the additive genetic and residual variances, respectively, $\boldsymbol{A}$ is the additive relationship matrix constructed using pedigree data, and $\boldsymbol{I}$ is an identity matrix.

Random effects were assumed to be independent. The (co)variance matrix of random variables is as follows:

$$Var\begin{pmatrix} a_1 \\ a_2 \\ e_1 \\ e_2 \end{pmatrix} = \begin{pmatrix} \boldsymbol{A}\sigma_{a1}^2 & \boldsymbol{A}\sigma_{a1a2} & 0 & 0 \\ \boldsymbol{A}\sigma_{a2a1} & \boldsymbol{A}\sigma_{a2}^2 & 0 & 0 \\ 0 & 0 & \boldsymbol{I}\sigma_{e1}^2 & \boldsymbol{I}\sigma_{e1e2} \\ 0 & 0 & \boldsymbol{I}\sigma_{e2e1} & \boldsymbol{I}\sigma_{e2}^2 \end{pmatrix} \quad [2]$$

Where $\boldsymbol{A}$ and $\boldsymbol{I}$ are defined above; σ_{a1}^2, σ_{a2}^2, σ_{e1}^2, and σ_{e2}^2 are direct additive genetic variance, and residual variances for traits 1 and 2, respectively; and $\sigma_{a1a2}/\sigma_{a2a1}$, and $\sigma_{e1e2}/\sigma_{e2e1}$ are the genetic and residual covariances between traits 1 and 2, respectively.

(Co)variance components estimated from bivariate analysis were used to estimate heritabilities (h^2) for each trait: $h^2 = \frac{\sigma_a^2}{\sigma_p^2}$, where σ_a^2 is the direct additive genetic variance of a trait; and σ_p^2 is the phenotypic variance of the trait: $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$. Maternal additive genetic effects for the meat and carcass quality traits analyzed were assumed negligible as indicated by previous studies (Miar et al., 2014). (Co)variance components were also used to estimate genetic (r_g) and phenotypic (r_p) correlations between meat and carcass traits: $r_g = \frac{\sigma_{a1a2}}{\sqrt{(\sigma_{a1}^2 \times \sigma_{a2}^2)}}$ (parameters defined above), and $r_p = \frac{\sigma_{p1p2}}{\sqrt{(\sigma_{p1}^2 \times \sigma_{p2}^2)}}$. Where σ_{p1p2} is the phenotypic covariance between traits: $\sigma_{p1p2} = \sigma_{a1a2} + \sigma_{e1e2}$; and σ_{p1}^2, and σ_{p2}^2 are the phenotypic variance of traits 1 and 2, respectively.

Significance for heritability and correlation estimates were declared using a 95% confidence interval. Confidence intervals were calculated using the following formula: $Estimate \pm 1.96 \times SE$, where $estimate$ can be either correlation or heritability estimates. A 95% confidence interval consists of all the values that are between $estimate$ - 1.96 standard errors and $estimate$ + 1.96 standard errors and the probability that the population parameter value lies within this confidence interval is:

$$P(\text{estimate} - 1.96\ \text{SE} \leq \text{parameter} \leq \text{estimate} + 1.96\ \text{SE}) = 0.95.$$

All confidence intervals that do not contain a 0 value were declared significantly different from 0 with P-value < 0.05.

2.3. Results and discussion

2.3.1. Phenotypic statistics

A total of 21 traits were analyzed in this study, and out of these, 17 were meat quality traits and 4 were carcass traits. The carcass traits used are traits commonly used to represent carcass leanness (LEA, FD, MD, and NSIF IMF). The descriptive statistics for each trait were calculated, and abbreviations, number of records (N), minimum (Min.), mean, maximum (Max.), standard deviation (SD), and coefficient of variation (CV), are recorded in Table 2.1. Minolta L*, a*, and b* measurements were found to average between 43.9 (ILIOL, SD = 3.11) to 53.3 (QUADL, SD = 3.67), 2.5 (QUADA, SD = 1.49) to 16.0 (ILIOA, SD = 2.16), and 8.34 (QUADB, SD = 1.41) to 11.3 (ILIOB, SD = 1.48), respectively, depending on the specific muscle type. Ideally, Minolta L* measurements should be within the preferred range of 38 to 50, to prevent the

observance of pale, soft, exudate (PSE), and dark, firm, dry (DFD) meat quality (Towers, 2016). The average Minolta L* measurements for each muscle type were within this range, with the exception of ham *quadriceps femoris*, which was found to have the lightest (Minolta L* = 53.3) and also palest (Minolta a* = 8.34) meat colour measurements. Both ultimate pH (5.87), and DL (1.07%) had average values within optimal ranges, of 6.1 to 5.7, and < 6%, respectively (Towers, 2016). FD, MD, and NSIF IMF were each found to average 10.3 mm, 75.3 mm, and 1.46, respectively.

2.3.2. Heritability estimates

Heritability estimates and their standard errors (SE) are reported on the diagonal in Table 2.2. Heritability estimates for colour traits vary largely depending on the carcass cut and the colour measurement. Fat colour measurements each showed low heritability estimates, LOINFATL (0.09±0.05), LOINFATA (0.11±0.05), and LOINFATB (0.18±0.06). In general, meat colour measurements for Minolta L* and b* showed low to moderate heritability, LOINL (0.11±0.06), LOINB (0.23±0.08), GLUTL (0.27±0.08), GLUTB (0.30±0.08), QUADL (0.07±0.05), QUADB (0.06±0.05), ILIOL (0.12±0.06), and ILIOB (0.09±0.06), and meat colour measurements for Minolta a* showed moderate heritabilities, LOINA (0.44±0.09), GLUTA (0.42±0.09), QUADA (0.31±0.08), and ILIOA (0.25±0.08). In this study, estimates of heritability for LOINL (0.11±0.06) were below the range of literature values (0.16 to 0.31), and LOINA (0.44±0.09) estimates were slightly higher (0.21 to 0.38) (de Vries et al., 1994; Larzul et al., 1997; Suzuki et al., 2005; Van Wijk et al., 2005; Miar et al., 2014). Minolta colour measurements for loin fat and ham components are novel traits in the literature, therefore limiting the ability to compare results,

but heritabilities for ham Minolta L*, a*, and b* have been reported previously by Miar et al. (2014) for a commercial crossbred population, and were also calculated for the purebred Duroc population used in this study (Heidaritabar, unpublished results, personal communication). In comparison, heritability estimates for GLUTL, GLUTA, QUADA, QUADB were relatively similar for the crossbred population, but notably, estimates for QUADL, ILIOL and ILIOB were found to be higher in the crossbred population compared to the purebred population (Miar et al., 2014). In addition, the animals used in this study were used in a second study, which estimated genetic parameters using a relationship matrix derived using imputed whole-genome sequence (WGS) data. In the related study, heritability estimates for most meat quality traits were higher when WGS data was used (by 0.01 to 0.09 units), but notably, estimates for GLUTA, GLUTB, and ILIOA, were found to be slightly higher in this study compared to when WGS data was used (by 0.01 to 0.06 units) (Heidaritabar, unpublished results, personal communication). Other meat quality measurements showed moderate heritability, 0.28±0.08 and 0.23±0.08 for pH and DL, respectively, which were at the high end of the literature ranges (0.07 to 0.39 and 0.01 to 0.31 for pH and DL, respectively) (de Vries et al., 1994; Larzul et al., 1997; Suzuki et al., 2005; Van Wijk et al., 2005; Ciobanu et al., 2011; Miar et al., 2014). Overall these results show that the Duroc breed has relatively high heritabilities for meat quality traits compared to other breeds, suggesting that this breed could provide a significant opportunity for improving meat quality traits in pigs, especially for loin and ham Minolta a*, DL, and pH.

In this study, heritability estimates for carcass composition traits were moderate to high, MD (0.33±0.08), LEA (0.39±0.09), FD (0.39±0.08), and NSIF IMF (0.43±0.09), but these estimates were on the low end of literature ranges with the exception of NSIF IMF, MD (0.31 to 0.52), LEA (0.36 to 0.47), FD (0.31 to 0.72), and NSIF IMF (0.23 to 0.44).

Differences between heritability estimates in this study compared to other literature values can be due to differences between the breeds and populations and their environments. Environmental differences are of particular importance when comparing purebred populations with crossbred populations, as purebreds housed in nucleus farms will live under different conditions compared to crossbred animals that are raised in commercial conditions. Therefore, different environmental conditions will interact differently with the same genetic factors, and as a result the same allele can have a different effect on the phenotype depending on the conditions the animal is raised under (Wientjes & Calus, 2017). In addition, differences in the genetic background of different populations will also affect heritability estimates. For example, populations may differ in allele frequencies due to different selection criteria and selection intensity, or populations may contain different alleles with different effect sizes due to new mutations or variants coming into the population over generations. Heritability estimates can also be affected by differences in the method of variance component estimation or the statistical model used for the analysis (fixed effects, random effects, or relationship matrices) (Mathevon et al., 1998; Wilson, 2008). The relationship matrix is of relevance when comparing the results of this study, which used a pedigree derived relationship matrix, to those using WGS data. Heritability is estimated from the resemblance between relatives by comparing their genetic relationship with their phenotypic correlation (Visscher et al., 2006). When pedigree data is used, expected relationship estimates across the entire genome are considered, but when genetic markers are used to estimate relationships, the proportion of genomic markers shared between animals is considered. For example, full sibs are expected to share 50% of alleles by identity-by-descent (IBD), however due to Mendelian sampling, these siblings may actually share between 40% to 60% of their genome, and if siblings that share 60% proportion of their genome are more phenotypically

similar than the siblings that share 40% of their genome, then the trait will be more strongly influenced by genetics (higher heritability) (Wray & Visscher, 2008). Therefore, in the case that pedigree data is used, phenotypic variance may be incorrectly attributed to the environment or residual variance, reducing the estimated heritability for the trait. Further, when WGS data is used to estimate relationships, the entirety of the additive genetic variance is expected to be accounted for, as WGS data is assumed to include all of the causative mutations responsible for the additive genetic variation of the trait (Meuwissen & Goddard, 2010). Therefore, genetic parameters estimated using WGS data should provide the most accurate estimates of relationships between animals, and the heritabilities estimated using WGS data should be higher compared to those estimated using lower density genotypes or pedigree data.

2.3.3. Correlation estimates

Correlations among meat quality traits

Genetic (below diagonal) and phenotypic (above diagonal) correlations between meat and carcass traits (±SE) are reported in Table 2.2. In some cases, phenotypic correlations can be substituted for genetic correlations when the latter are not precisely estimated (precise estimates require large samples sizes). However, in this study, many genetic correlations were statistically significant, and therefore phenotypic correlations will not be discussed here.

Meat colour measurements were moderately to highly correlated with each other, notably LOINL with GLUTL (0.75±0.24), QUADL (0.83±0.36), and ILIOL (0.96±0.23), LOINA with GLUTA (0.62±0.11), QUADA (0.65±0.14), and ILIOA (0.50±0.16), and LOINB with GLUTB

(0.76±0.14), QUADB (0.94±0.24), and ILIOB (0.57±0.26). Further, Minolta L*, a*, and b* measurements in the remaining muscle types displayed similar trends (Table 2.2). Relatively large and significant relationships were also observed between colour traits in Miar et al. (2014), LOINL with GLUTL (0.45±0.15), QUADL (0.66±0.14), and ILIOL (0.39±0.14), LOINA with GLUTA (0.55±0.11), QUADA (0.53±0.13), and ILIOA (0.43±0.16), and LOINB with GLUTB (0.39±0.21), QUADB (0.71±0.19), and ILIOB (0.48±0.17). These results show that selection for Minolta L*, a*, or b* colour in one muscle type, will cause corresponding changes to the same colour measurement in other muscle types. Therefore, if meat colour preferences are the same across different muscle types, then measurement of only one of the correlated traits is required for improvement across traits in the breeding program. Previous studies have reported a strong, significant correlation between LOINL and LOINB (0.51 to 0.60) (Van Wijk et al., 2005; Miar et al., 2014), for which a similar relationship was also observed in this study (0.55±0.21). Additionally, significant correlations were observed in this study between GLUTL and GLUTB (0.90±0.06), and ILIOL and ILIOB (0.59±0.24), as well as other studies (0.56±0.14 and 0.92±0.01, respectively) (Miar et al., 2014) showing that paler pork products are additionally more yellow in colour. Loin fat colour measurements did not show significant correlations with many meat quality traits, LOINFATA with LOINFATB (0.87±0.11), and LOINFATL with ILIOL (0.74±0.35) showed the only significant correlations. As mentioned above, these are novel traits that have not been found to be recorded in the literature for comparisons.

Some Minolta colour traits were found to have moderate to strong negative correlations with pH and positive correlations with DL, notably pH with LOINL (-0.78±0.15), LOINA (-0.54±0.15), and LOINB (-0.80±0.12), and DL with LOINL (0.67±0.24), LOINA (0.38±0.18,), and LOINB (0.69±0.17). Similar trends were observed for GLUT and QUAD L*, a*, and b* colour traits

with pH and DL in this study and by Miar et al. (2014). This relationship is well supported in the literature and in the pig production industry due to the incidence of pale, soft, and exudative (PSE) as well as dark, firm, and dry (DFD) pork traits. Paler pork tends to come from muscles with predominately white, fast twitch muscle fibers, which are mainly sustained by anaerobic glycolysis (Choe et al., 2007). Muscles with a high proportion of these fiber types have rapid and extensive pH decline post-mortem, resulting in excessive protein denaturation and water release, and PSE pork. Similarly, dark pork tends to come from muscles with predominately red, slow twitch muscle fibers, which are sustained by aerobic respiration (Choe et al., 2007). Muscles with a high proportion of these fiber types have slow pH decline post-mortem, resulting in water retention, and DFD pork (Seideman et al., 1984; Mancini & Hunt, 2005). Additionally, a strong significant correlation between pH and DL (-0.65±0.16) was observed in this study. This relationship is well supported by literature estimates of correlations (ranging between -0.13 to -0.99 depending on the population, technique used for trait measurement, and the time of measurement) (Van Wijk et al., 2005; Schwab et al., 2006; Ciobanu et al., 2011; Miar et al., 2014), as well as by the biological knowledge mentioned above (Seideman et al., 1984; Mancini & Hunt, 2005). Therefore, pH can potentially be used as an indicator trait for improvement of DL.

Correlations among carcass traits

The two measures of carcass muscle, LEA and MD, were highly and significantly correlated (0.94±0.04), but neither showed a significant correlation with FD. Marbling score (NSIF IMF) was low to moderately correlated with FD (0.36±0.15), but also did not show a significant

correlation with MD or LEA. These values fit within literature ranges, but MD/LEA and FD have commonly been found to have a negative relationship, as values have been shown to range between 0.00 to -0.39 (Van Wijk et al., 2005; Schwab et al., 2006; Ciobanu et al., 2011; Miar et al., 2014).

Correlations among meat quality and carcass traits

Few significant correlations were observed between the meat quality and carcass traits analyzed. However, two moderate and unfavorable correlations were identified between LEA with GLUTL (0.38±0.18) and ILIOA (-0.43±0.19), suggesting selection for increased muscle could have led to paler meat in these muscle types. This is supported by biological data, as rapid and extensive muscle growth is associated with an increased proportion of fast twitch muscle fibers, which contain less myoglobin and thus appear paler in colour (Choe et al., 2008; Choi & Kim, 2009). However, these results are in contrast to those of Miar (2014), which found a low positive correlation between LEA and GLUTL (0.12±0.03), and no significant correlation was found between LEA and ILIOA. Due to the larger SE shown in this study, the correlations among meat quality and carcass traits from Miar (2014) may be closer to the true values.

2.4. Conclusions

Carcass quality measurement and selection has been essential in improving the leanness of pork products. However, most meat quality traits have not commonly been included in selection programs due to the difficulty and expense of repeated measurement, therefore GS provides a

significant opportunity to incorporate these traits into breeding programs. The results of this study supported the original hypothesis, that meat quality traits are controlled in part by genetics ($h^2 > 0$), and that relationships among meat quality and carcass traits are in part due to shared genetic determinants between the traits ($r_g > 0$). Specifically, low to moderate heritabilities for meat quality traits were observed in this study, indicating rapid and significant improvements to these traits is possible. In addition, strong genetic correlations between colour traits and different muscle types, as well as pH and DL suggest that the improvement of one trait will cause similar changes in the correlated trait. Specifically, due to the difficulty and expense of measuring DL phenotypes (requires carcass destruction), improvement of DL instead can be accomplished by the indirect selection for pH. Few negative correlations between carcass and meat quality traits analyzed suggest that both can be improved simultaneously to produce a high-quality lean product. Duroc animals are known for their excellent meat quality traits, and this study has shown that the breed also provides an ideal genetic base for which to make improvements to meat quality in Canadian pigs. Overall, these results provide information on meat and carcass traits that can be useful to breeders for the identification of valuable indicator trait(s) for meat quality and for selecting the best parents for genetic improvement of these traits.

2.5. References

Alberta Pork. (2017). Alberta Pork Pricing and Marketing Information. Alberta Pork. Retrieved from https://www.albertapork.com/our-producer-services/pricing/canadian-pricing/

CCAC. (2020). Guide to the Care and Use of Experimental Animals, Volume 1, 2nd Edition. Canadian Council on Animal Care.

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., & Kim, B. C. (2008). The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Science, 80(2), 355–362. https://doi.org/10.1016/j.meatsci.2007.12.019

Choi, Y. M., & Kim, B. C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livestock Science, 122(2–3), 105–118. https://doi.org/10.1016/j.livsci.2008.08.015

Ciobanu, D., Lonergan, S., & Huff-Lonergan, E. (2011). Genetics of Meat Quality and Carcass Traits. In The Genetics of the Pig, 2nd Edition, 355–378.

de Vries, A. G., van der Wal, P. G., Long, T., Eikelenboom, G., & Merks, J. W. M. (1994). Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Production Science, 40(3), 277–289. https://doi.org/10.1016/0301-6226(94)90095-7

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., Thompson, R. (2015). ASReml User Guide Release 4.1, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-4.1-Functional-Specification.pdf

Hernández-Sánchez, J., Amills, M., Pena, R. N., Mercadé, A., Manunza, A., & Quintanilla, R. (2013). Genomic architecture of heritability and genetic correlations for intramuscular and back fat contents in Duroc pigs. Journal of Animal Science, 91(2), 623–632. https://doi.org/10.2527/jas.2012-5270

Kominakis, A. P. (2003). Phenotypic correlations as substitutes to genetic correlations in dairy sheep and goats. Journal of Animal Breeding and Genetics, 120(4), 269–281. https://doi.org/10.1046/j.1439-0388.2003.00397.x

Larzul, C., Lefaucheur, L., Ecolan, P., Gogué, J., Talmant, A., Sellier, P., Le Roy, P., Monin, G. (1997). Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. Journal of Animal Science, 75(12), 3126–3137. https://doi.org/10.2527/1997.75123126x

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. Meat Science, 71(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

Mathevon, M., Buhr, M. M., & Dekkers, J. C. M. (1998). Environmental, Management, and Genetic Factors Affecting Semen Production in Holstein Bulls. Journal of Dairy Science, 81(12), 3321–3330. https://doi.org/10.3168/jds.S0022-0302(98)75898-9

Meuwissen, T., & Goddard, M. (2010). Accurate prediction of genetic values for complex traits by whole-genome resequencing. Genetics, 185(2), 623–631. https://doi.org/10.1534/genetics.110.116590

Miar, Y. (2015). Genomic selection for pork quality and carcass traits in both cross- and pure-bred populations (Dissertation). University of Alberta. https://doi.org/10.7939/R3M32NH2S

Miar, Y., Plastow, G., Moore, S., Manafiazar, G., Charagu, P., Kemp, R., Van Haandel, B., Huisman, A., Zhang, C., Mckay, R., Bruce, H., & Wang, Z. (2014). Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livestock Production Science, 26(2), 119–135. https://doi.org/10.2527/jas2014-7685

NSIF. (1997). Guidelines for uniform swine improvement programs. National Swine Improvement Federation. Retrieved from http://www.nsif.com

Schwab, C. R., Baas, T. J., Stalder, K. J., & Mabry, J. W. (2006). Effect of long-term selection for increased leanness on meat and eating quality traits in Duroc swine. Journal of Animal Science, 84(6), 1577–1583. https://doi.org/10.2527/2006.8461577x

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors Associated with Fresh Meat Color: a Review. Journal of Food Quality, 6(3), 211–237. https://doi.org/10.1111/j.1745-4557.1984.tb00826.x

Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M., & Nishida, A. (2005). Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. Journal of Animal Science, 83(9), 2058–2065. https://doi.org/10.2527/2005.8392058x

Tannas, L., & Tannas, V. (2014). Better Meat Quality with Duroc Genetics. The Pig Site. Retrieved from https://thepigsite.com/news/2014/03/better-meat-quality-with-duroc-genetics-1

Towers, L. (2016). Pork Meat Quality: Understanding Industry Measurements and Guidelines. The Pig Site. Retrieved from https://thepigsite.com/articles/pork-meat-quality-understanding-industry-measurements-and-guidelines

Van Wijk, H. J., Arts, D. J. G., Matthews, J. O., Webster, M., Ducro, B. J., & Knol, E. F. (2005). Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. Journal of Animal Science, 83(2), 324–333. https://doi.org/10.2527/2005.832324x

Visscher, P. M., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Zhu, G., Cornes, B. K., Montgomery, G. W., & Martin, N. G. (2006). Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. PLoS Genetics, 2(3), 0316–0325. https://doi.org/10.1371/journal.pgen.0020041

Wientjes, Y. C. J., & Calus, M. P. L. (2017). The purebred-crossbred correlation in pigs: A review of theory, estimates, and implications. Journal of Animal Science, 95(8), 3467. https://doi.org/10.2527/jas2017.1669

Wilson, A. J. (2008). Why h2 does not always equal VA/VP? Journal of Evolutionary Biology, 21(3), 647–650. https://doi.org/10.1111/j.1420-9101.2008.01500.x

Wray, N., & Visscher, P. (2008). Estimating Trait Heritability. Nature Education, 1(1), 29. Retrieved from https://www.nature.com/scitable/topicpage/estimating-trait-heritability-46889/

Tables and figures

Table 2.1. Descriptive statistics for meat and carcass quality phenotypes in purebred pigs: abbreviation, number of animals (N), minimum (Min.), mean, maximum (Max.), standard deviation (SD), and coefficient of variation (CV).

Trait	Abbreviation	N	Min.	Mean	Max.	SD	CV (%)
Loin[1] Minolta L*	LOINL	997	41.40	48.10	55.70	2.52	5.24
Loin Minolta a*	LOINA	997	1.07	4.59	8.75	1.12	24.40
Loin Minolta b*	LOINB	997	5.95	9.37	13.90	1.22	13.00
Loin fat Minolta L*	LOINFATL	993	70.50	79.00	84.60	2.34	2.96
Loin fat Minolta a*	LOINFATA	993	-0.50	3.00	12.40	1.61	53.70
Loin fat Minolta b*	LOINFATB	993	5.90	10.90	17.20	1.76	16.20
Ham *gluteus medius* Minolta L*	GLUTL	997	39.30	47.40	57.20	2.66	5.61
Ham *gluteus medius* Minolta a*	GLUTA	997	1.20	5.51	9.60	1.26	22.90
Ham *gluteus medius* Minolta b*	GLUTB	997	5.40	8.91	12.70	1.11	12.50
Ham *quadriceps femoris* Minolta L*	QUADL	996	36.50	53.30	68.90	3.67	6.89
Ham *quadriceps femoris* Minolta a*	QUADA	996	-1.00	2.50	10.50	1.49	59.60
Ham *quadriceps femoris* Minolta b*	QUADB	996	4.70	8.34	14.50	1.41	16.90
Ham *iliopsoas* Minolta L*	ILIOL	996	34.70	43.90	55.60	3.11	7.08
Ham *iliopsoas* Minolta a*	ILIOA	996	8.80	16.00	23.00	2.16	13.50
Ham *iliopsoas* Minolta b*	ILIOB	996	7.10	11.30	15.80	1.48	13.10
Ultimate pH	ph24	996	5.52	5.84	6.59	0.16	2.74
Drip loss (%)	DL	997	0.27	1.07	5.20	0.49	45.80
Loin muscle area (cm^2)	LEA	996	26.50	57.10	74.50	5.75	10.10
Backfat depth (mm)	FD	997	4.00	10.30	19.00	2.45	23.80
Loin depth (mm)	MD	997	58.00	75.30	92.00	4.78	6.35
NSIF marbling score	NSIF IMF	997	0.00	1.46	3.00	0.59	40.50

[1]*Longissimus thoracis et lumborum*

Table 2.2. Estimates for heritabilities (diagonal), as well as genetic (below diagonal), and phenotypic (above diagonal) correlations ± standard error (SE) for meat and carcass quality phenotypes in purebred pigs.

Trait	LOINL	LOINA	LOINB	LOINFATL	LOINFATA	LOINFATB	GLUTL	GLUTA
LOINL	0.11±0.06	**0.33±0.03**	**0.74±0.02**	**0.11±0.03**	**-0.13±0.03**	**-0.10±0.03**	**0.32±0.03**	**0.09±0.03**
LOINA	-0.03±0.29	**0.44±0.09**	**0.78±0.01**	0.01±0.03	-0.02±0.03	-0.06±0.04	**0.11±0.04**	**0.39±0.03**
LOINB	**0.55±0.21**	**0.78±0.08**	**0.23±0.08**	0.03±0.03	**-0.08±0.03**	-0.06±0.03	**0.25±0.03**	**0.26±0.03**
LOINFATL	0.52±0.36	-0.08±0.29	0.26±0.33	0.09±0.05	**-0.62±0.02**	**-0.33±0.03**	**0.09±0.03**	-0.04±0.03
LOINFATA	0.01±0.39	0.04±0.26	0.02±0.31	-0.46±0.30	**0.11±0.05**	**0.73±0.02**	-0.02±0.03	0.01±0.03
LOINFATB	0.17±0.33	-0.09±0.22	0.01±0.26	-0.28±0.32	**0.87±0.11**	**0.18±0.06**	-0.04±0.03	-0.02±0.04
GLUTL	**0.75±0.24**	0.20±0.19	**0.59±0.21**	0.26±0.30	0.16±0.29	-0.18±0.24	**0.27±0.08**	0.04±0.04
GLUTA	0.11±0.27	**0.62±0.11**	**0.45±0.18**	-0.16±0.29	0.08±0.26	-0.01±0.22	0.22±0.19	**0.42±0.09**
GLUTB	**0.70±0.23**	**0.57±0.14**	**0.76±0.14**	0.32±0.30	0.16±0.28	-0.10±0.24	**0.90±0.06**	**0.63±0.12**
QUADL	**0.83±0.36**	-0.35±0.40	0.14±0.37	0.54±0.43	-0.35±0.41	-0.19±0.37	**0.74±0.30**	0.18±0.31
QUADA	-0.19±0.29	**0.65±0.14**	**0.43±0.20**	-0.12±0.31	0.33±0.27	0.10±0.23	0.16±0.21	**0.58±0.14**
QUADB	0.47±0.41	**0.78±0.23**	**0.94±0.24**	0.47±0.45	-0.04±0.44	-0.08±0.39	0.61±0.49	**0.68±0.23**
ILIOL	**0.96±0.23**	-0.27±0.26	0.26±0.28	**0.74±0.35**	-0.42±0.34	-0.37±0.28	**0.73±0.22**	-0.16±0.26
ILIOA	-0.13±0.32	**0.50±0.16**	0.20±0.24	0.53±0.35	-0.05±0.30	0.09±0.25	-0.01±0.23	0.35±0.19
ILIOB	**0.81±0.28**	0.29±0.25	**0.57±0.26**	**0.89±0.51**	-0.54±0.38	-0.28±0.34	**0.64±0.27**	-0.02±0.30
ph24	**-0.78±0.15**	**-0.54±0.15**	**-0.80±0.12**	0.02±0.33	-0.27±0.30	-0.30±0.24	**-0.60±0.17**	**-0.38±0.18**
DL	**0.67±0.24**	**0.38±0.18**	**0.69±0.17**	-0.03±0.35	0.08±0.31	-0.01±0.26	**0.68±0.17**	0.37±0.19
LEA	0.12±0.27	0.11±0.18	0.28±0.21	0.18±0.28	0.33±0.28	0.19±0.22	**0.38±0.18**	-0.09±0.17
FD	0.25±0.26	0.30±0.16	**0.46±0.18**	0.37±0.29	**-0.80±0.20**	**-0.46±0.20**	0.03±0.19	0.13±0.17
MD	0.21±0.28	0.13±0.18	0.25±0.22	**0.58±0.23**	-0.20±0.26	-0.06±0.23	0.36±0.19	-0.06±0.18
NSIF IMF	0.11±0.33	0.22±0.20	0.21±0.25	-0.05±0.35	-0.24±0.30	0.10±0.27	-0.18±0.24	0.01±0.22

Table 2.2. Continued

Trait	GLUTB	QUADL	QUADA	QUADB	ILIOL	ILIOA	ILIOB
LOINL	**0.29±0.03**	**0.29±0.03**	0.03±0.03	**0.22±0.03**	**0.31±0.03**	0.06±0.03	**0.28±0.03**
LOINA	**0.29±0.03**	**0.10±0.03**	**0.28±0.03**	**0.23±0.03**	**0.10±0.03**	**0.27±0.03**	**0.23±0.03**
LOINB	**0.37±0.03**	**0.21±0.03**	**0.18±0.03**	**0.28±0.03**	**0.23±0.03**	**0.16±0.03**	**0.28±0.03**
LOINFATL	0.01±0.03	0.04±0.03	-0.04±0.03	0.01±0.03	**0.09±0.03**	-0.05±0.03	0.05±0.03
LOINFATA	0.02±0.03	-0.02±0.03	0.02±0.03	-0.01±0.03	**-0.09±0.03**	0.02±0.03	-0.06±0.03
LOINFATB	0.02±0.03	-0.02±0.03	-0.03±0.03	0.01±0.03	**-0.11±0.03**	0.01±0.03	**-0.07±0.03**
GLUTL	**0.69±0.02**	**0.22±0.03**	0.04±0.04	**0.16±0.03**	**0.23±0.03**	-0.01±0.03	**0.17±0.03**
GLUTA	**0.58±0.02**	**0.14±0.03**	**0.32±0.03**	**0.26±0.03**	0.07±0.03	**0.24±0.03**	**0.19±0.03**
GLUTB	**0.30±0.08**	**0.20±0.03**	**0.19±0.03**	**0.27±0.03**	**0.21±0.03**	**0.13±0.03**	**0.26±0.03**
QUADL	0.64±0.34	0.07±0.05	-0.05±0.033	**0.55±0.02**	**0.21±0.03**	0.01±0.03	**0.16±0.03**
QUADA	0.33±0.19	**-0.99±0.40**	**0.31±0.08**	**0.61±0.02**	-0.04±0.03	**0.31±0.03**	**0.12±0.03**
QUADB	**0.92±0.27**	-0.48±0.80	**0.78±0.21**	0.06±0.05	**0.10±0.03**	**0.19±0.03**	**0.18±0.03**
ILIOL	**0.60±0.23**	**0.99±0.31**	**-0.46±0.23**	0.58±0.46	**0.12±0.06**	**0.12±0.03**	**0.73±0.02**
ILIOA	0.04±0.23	-0.44±0.40	**0.51±0.17**	0.27±0.35	**-0.74±0.25**	**0.25±0.08**	**0.60±0.02**
ILIOB	0.47±0.26	0.52±0.41	0.01±0.30	0.68±0.43	**0.59±0.24**	0.17±0.32	0.09±0.06
ph24	**-0.62±0.15**	-0.30±0.32	-0.27±0.20	-0.47±0.30	-0.09±0.29	-0.32±0.22	-0.47±0.25
DL	**0.53±0.19**	0.47±0.30	**0.47±0.19**	**0.92±0.26**	0.45±0.26	0.03±0.25	0.51±0.27
LEA	0.31±0.19	0.08±0.32	-0.24±0.18	0.07±0.34	0.27±0.24	**-0.43±0.19**	-0.20±0.29
FD	0.10±0.19	0.22±0.28	0.04±0.19	0.37±0.31	0.22±0.25	0.10±0.20	0.42±0.29
MD	0.35±0.19	-0.01±0.34	-0.15±0.20	0.09±0.35	0.39±0.25	-0.23±0.21	0.02±0.30
NSIF IMF	-0.05±0.24	0.48±0.41	-0.37±0.23	0.42±0.34	0.03±0.26	0.01±0.20	0.03±0.29

Table 2.2. Continued

Trait[1]	ph24	DL	LEA	FD	MD	NSIF IMF
LOINL	**-0.54±0.02**	**0.35±0.03**	**0.15±0.03**	**0.12±0.03**	**0.18±0.03**	**0.08±0.03**
LOINA	**-0.36±0.03**	**0.26±0.03**	0.05±0.04	**0.14±0.04**	0.03±0.04	**0.11±0.04**
LOINB	**-0.49±0.03**	**0.41±0.03**	**0.13±0.03**	**0.17±0.03**	**0.14±0.03**	**0.12±0.04**
LOINFATL	**-0.07±0.03**	0.04±0.03	**0.08±0.03**	**0.09±0.03**	**0.13±0.03**	0.01±0.03
LOINFATA	0.03±0.03	-0.01±0.03	-0.05±0.03	**-0.26±0.03**	**-0.13±0.03**	**-0.14±0.03**
LOINFATB	0.04±0.03	0.01±0.03	-0.01±0.03	**-0.24±0.03**	**-0.08±0.03**	**-0.13±0.03**
GLUTL	**-0.30±0.03**	**0.20±0.03**	**0.11±0.04**	0.01±0.04	**0.08±0.04**	0.04±0.04
GLUTA	**-0.18±0.04**	**0.13±0.04**	0.01±0.04	0.03±0.04	0.01±0.04	0.03±0.04
GLUTB	**-0.26±0.03**	**0.19±0.03**	**0.11±0.04**	0.02±0.04	**0.11±0.04**	0.05±0.04
QUADL	**-0.26±0.03**	**0.21±0.03**	**0.10±0.03**	-0.03±0.03	**0.07±0.03**	0.02±0.03
QUADA	**-0.20±0.03**	**0.17±0.03**	-0.05±0.04	-0.01±0.04	-0.05±0.04	0.06±0.04
QUADB	**-0.27±0.03**	**0.22±0.03**	0.02±0.03	0.01±0.03	-0.01±0.03	0.06±0.03
ILIOL	**-0.26±0.03**	**0.21±0.03**	**0.13±0.03**	**0.09±0.03**	**0.08±0.03**	-0.01±0.03
ILIOA	**-0.14±0.03**	**0.12±0.03**	0.01±0.04	-0.03±0.04	-0.03±0.04	-0.01±0.04
ILIOB	**-0.30±0.03**	**0.22±0.03**	**0.11±0.03**	0.05±0.03	0.05±0.03	-0.03±0.03
ph24	**0.28±0.08**	**-0.38±0.03**	**-0.08±0.04**	-0.05±0.04	**-0.08±0.04**	-0.01±0.04
DL	**-0.65±0.16**	**0.23±0.08**	0.05±0.04	-0.01±0.04	0.01±0.04	-0.02±0.04
LEA	0.06±0.20	-0.09±0.21	**0.39±0.09**	0.05±0.04	**0.70±0.02**	-0.01±0.04
FD	-0.10±0.19	0.16±0.20	0.10±0.17	**0.39±0.08**	0.05±0.04	**0.41±0.03**
MD	-0.10±0.21	-0.09±0.22	**0.94±0.04**	0.30±0.17	**0.33±0.08**	**0.37±0.03**
NSIF IMF	-0.07±0.20	0.02±0.21	0.09±0.17	**0.36±0.15**	0.12±0.23	**0.43±0.09**

Note: Significant values (P-value < 0.05) are bolded.

[1] **LOINL** = *Longissimus thoracis et lumborum (*loin*)*Minolta L*; **LOINA** = Loin Minolta a*; **LOINB** = Loin Minolta b*; **LOINFATL** = Loin fat Minolta L*; **LOINFATA** = Loin fat Minolta a*; **LOINFATB** = Loin fat Minolta b*; **GLUTL**= Ham *gluteus medius* Minolta L*; **GLUTA** = Ham *gluteus medius* Minolta a*; **GLUTB** = Ham *gluteus medius* Minolta b*; **QUADL** = Ham *quadriceps femoris* Minolta L*; **QUADA** = Ham *quadriceps femoris* Minolta a*; **QUADB** = Ham *quadriceps femoris* Minolta b*; **ILIOL** = Ham *iliopsoas* Minolta L*; **ILIOA** = Ham *iliopsoas* Minolta a*; **ILIOB** = Ham *iliopsoas* Minolta b*; **ph24** = Ultimate pH; **DL** = Drip loss (%); **LEA** = Loin muscle area (cm^2); **FD** = Backfat depth (mm); **MD** = Loin depth (mm); **NSIF IMF** = NSIF marbling score.

The significant correlations are bolded (P < 0.05).

Chapter 3. A genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs

3.1. Introduction

Drip loss (DL) or purge, the loss of water and soluble proteins during postmortem storage, is an economically important trait for pork processors and retailers to meet a high level of quality standards domestically, and to further expand into high value export markets. Excessive DL has negative consequences on profitability, and nutritional content, as both product weight and muscle proteins are lost during the process (Offer & Knight, 1988; Savage, 1990). Further, consumers commonly prefer a pork product with no drip, as excessive drip gives the meat an undesirable watery appearance, diminishing the attractiveness of the product. DL also has unfavorable correlations with important cooking and sensory traits, i.e. cooking loss and tenderness/shear force, 0.14±0.03 and -0.10±0.03, respectively, which can affect eating experience and willingness of consumers to repurchase the product (Ngapo et al., 2007; Miar et al., 2014). Unacceptably high DL is most often observed in pale, soft, and exudative (PSE) pork products, corresponding to DL measurements greater than 6% (Towers, 2016) and causes estimated losses of $12 per carcass (CCSI, 2001). Therefore, reducing the amount of DL postmortem would have important economic benefits for the pork production chain in Canada.

Both environmental and genetic factors influence pork quality traits such as DL. Therefore, DL can be improved in part by environmental management. The environmental factors influencing DL are well established. Pre-slaughter transport and handling (Channon et al., 2000; Baltić et al., 2014), and the rate of carcass temperature decline post-slaughter (Holmer et al., 2008; Rybarczyk, et al, 2015) each play significant roles in determining the extent of product purge.

Even so, genetic factors also play an important role in determining meat quality traits. DL traits have low to moderate heritability (0.08 to 0.21) depending upon the specific breed or population (de Vries et al., 1994; Van Wijk et al., 2005; Miar et al., 2014). Specifically, the data used in this study is part of a previous study by Miar et al. (2014), which found DL to be moderately heritable (0.21±0.09), demonstrating that a proportion of its phenotypic variability can be explained by genetics (Miar et al., 2014). Further, alleles of the *ryanodine receptor 1* (*RYR1*) (Otto et al., 2007), *protein kinase AMP-activated non-catalytic subunit gamma 3* (*PRKAG3*) (Zhang et al., 2015), and *calpastatin* (*CAST*) (Ropka-Molik et al., 2014) genes have each been shown to effect DL phenotypes. Knowledge on the biological factors influencing meat quality phenotypes provides a significant opportunity to make considerable genetic gain to these traits, as in pig breeding programs, complex traits such as DL can be genetically improved by selection based on the known genetic factors, such as genes and causative mutations (Goddard et al., 2016). However, aside from these examples, relatively few genes have been found associated with DL, meaning a large proportion of the total genetic variance for the trait remains unaccounted for. Therefore, DL is a good candidate for genomic analyses, such as genome-wide association studies (GWAS).

GWAS has been a very successful technique used to identify many new quantitative trait loci (QTL) that are associated with complex traits. GWAS uses dense single nucleotide polymorphism (SNP) markers to identify associations between marker genotypes and phenotypes. Results from GWAS provide significant opportunities to learn about the genetics underlying these traits as it can be used to identify specific genes and important biological pathways influencing the trait, as well as reveal the genetic architecture underlying the trait (Korte & Farlow, 2013; Goddard et al., 2016). Therefore, the goal of this study was to identify

the QTL and the potential candidate genes underlying DL phenotypes observed in a Canadian crossbred commercial pig population. Since DL is known to be heritable and in part determined by genetic factors, it is hypothesized that at least one marker should be significantly associated with the trait. The results of this study could subsequently be used for practical purposes, for the implementation into genetic selection programs and to expand the biological and genetic knowledge of the trait.

3.2. Materials and methods

3.2.1. Data

Animals

This project was approved by the University of Alberta Animal Care and Use Committee. The animals used in this study were raised in a commercial herd following the Canadian Quality Assurance program and the Canadian Council on Animal Care (CCAC) guidelines (CCAC, 2020).

A total of 1098 commercial crossbred pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study. The pigs resulted from a three-way cross between a Duroc sire and a F1 hybrid dam (Landrace X Large White). This three-way cross represents a major proportion of the commercial lines in Canadian pig production. Feeding, raising, and slaughter protocol have been described in previous studies (Miar et al., 2014; Zhang et al., 2015; Yang et al., 2017).

Phenotypes

Protocol for measurement of DL phenotypes was described previously in this thesis (Chapter 2 Section 2.2.1.).

Genotypes

DNA was extracted from tissue samples (ear punch) using the manufacturer's protocol (Thermo Fisher Scientific Ltd., Ottawa, ON, Canada). Genotyping was performed by Delta Genomics (Edmonton, AB, Canada) using the Illumina Porcine SNP60 V2 genotyping beadchip (Illumina Inc., San Diego, CA, USA). Quality control for genotyping data excluded SNPs with the following features: minor allele frequency (MAF) < 0.01, genotype call rate < 0.95, departure from Hardy-Weinberg Equilibrium (test of genotyping accuracy) > 0.15 (Wiggans et al., 2009; Misztal et al., 2018), and duplicated or unmapped SNPs. Sex chromosomes (X and Y) were also excluded. Any missing genotypes were imputed using FImpute version 2.2 (Sargolzaei et al., 2014). After filtration of the total number of 61,565 SNPs, 40,438 SNPs and 951 animals had adequate genotype and phenotype records for subsequent analyses.

3.2.2. Population stratification

In GWAS, spurious associations have been attributed to a mixture of multiple subpopulations that have differing allele frequencies for SNPs and differing phenotypic values (Manolio et al.,

2009). Therefore, principal component analysis (PCA) was used to test the population substructure for stratification. Analysis was conducted using the *stats* package version 3.7.0 in R software (Ihaka & Gentleman, 1996).

3.2.3. Statistical analysis

The significance of fixed effects and covariates was determined using Wald F. statistics in ASReml software, and factors with P-value < 0.05 were incorporated into the statistical model (Gilmour, 2015). A single-SNP GWAS (single marker GWAS), which uses a genomic relationship matrix (GRM, **G**), was performed. The following statistical model was used in ASReml software (Gilmour, 2015):

$$\boldsymbol{y} = \mathbf{1}\mu + \boldsymbol{Xb} + \boldsymbol{Zg} + \boldsymbol{v\alpha} + \boldsymbol{e} \quad [1]$$

Where $\boldsymbol{y}$ is a vector of phenotype values; $\mathbf{1}$ is a vector of ones; μ is the overall mean of DL phenotypes; $\boldsymbol{X}$ is a design matrix that relates fixed effects to observations; $\boldsymbol{b}$ is the vector of fixed effects including pen during test (approximately 70 – 115 kg) and contemporary group (consisting of slaughter order and year of slaughter); $\boldsymbol{Z}$ is a design matrix associating $\boldsymbol{g}$ with response variables; $\boldsymbol{g}$ is the vector of random additive genetic effects that is assumed to be normally distributed; $\boldsymbol{v}$ is a vector is SNP genotypes coded 0, 1, 2; $\boldsymbol{\alpha}$ is a vector of additive SNP effects; and $\boldsymbol{e}$ is a vector of residual effects that is assumed to be normally distributed. The additive genetic effects and the residual effects are both assumed to be normally distributed; $\boldsymbol{g} \sim N\left(0, \boldsymbol{G}\sigma_g^2\right)$ that accounted for the (co)variances between individuals due to genomic relationships, $\boldsymbol{e} \sim N(0,\ \boldsymbol{I}\sigma_e^2)$, respectively, where σ_g^2 and σ_e^2 are the additive genetic

and residual variances, respectively, ***G*** is the realized genomic relationship, and ***I*** is an identity matrix. The ***G*** matrix was constructed using GCTA software (Yang et al., 2011). The formula for calculation of the **G** matrix is as follows: $A_{jk} = \frac{1}{N} \sum \frac{(X_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1 - p_i)}$, where A_{jk} is a genome wide relationship between individual j and k; N is the total number of SNPs; x_{ij} is the SNP genotype coded 0, 1, 2 of the j^{th} individual at the i^{th} SNP; x_{ik} is a SNP genotype coded 0, 1, 2 of the k^{th} individual at the i^{th} SNP; and p_i the allele frequency of the i^{th} SNP (Yang et al., 2011).

3.2.4. Plots and correction for multiple testing

To account for population structure, the GWAS P-values for each SNP was corrected for their corresponding genomic inflation factor (lambda, λ). Quantile-quantile (Q-Q) plot for each trait was used to assess the inflation of P-values by comparing the genome wide distribution of -log10 of the P-values with the expected median of the corresponding normal distribution. Genomic inflation factor (λ) was calculated by dividing median observed χ^2 by the median expected χ^2 with 1 degree of freedom assumed. The χ^2 test statistics were computed from the P-values. Manhattan plots were also constructed to display the -log10 (P-values) of SNPs with respect to their genomic position (Mbp). Both Q-Q and Manhattan plots were constructed using the R package *qqman* (Turner, 2014).

Bonferroni correction was used to avoid false positives due to multiple testing using the following formula (Weller et al., 1998): $\text{Bonferroni corrected P} - \text{value} = \frac{\propto}{N}$, where α is the type I error threshold; and N is the total number of SNPs. Thresholds of 10% and 5% type I error were used to declare suggestive and genome-wide significance, respectively.

3.2.5. Quantitative trait loci detection and functional analysis

The associations detected by GWAS are being caused by either the SNP with the lowest P-value or a nearby SNP that is linked with it, therefore, the region surrounding the SNP with the lowest P-value was searched for potential candidate genes. It is expected that the SNP with the lowest P-value and the surrounding SNPs may be in linkage disequilibrium (LD). Therefore, QTL were defined as a 1 Mbp window surrounding the SNP with the lowest P-value identified by GWAS (0.5 Mbp on each side of the SNP). A 0.5 Mbp region was chosen because in crossbred pig populations the average LD (r^2) for distances up to 0.5 Mbp is 0.15 (Badke et al., 2012; Grossi et al., 2017). Therefore, genetic loci located up to 0.5 Mbp from the SNPs with the lowest P-values were analyzed, as the linkage within this region is considered high despite some LD decay.

The potential QTL regions identified in this study were compared with previously identified QTL in the pigQTL database (Hu et al., 2005). QTL that overlapped with genomic regions previously found to be associated with DL, or highly correlated traits such as water holding capacity (WHC, the ability of meat to retain water upon the application of external pressures), and pH, could provide additional evidence for the associations with the lowest P-values identified in this study.

QTL regions were further examined for candidate genes using the National Center for Biotechnology Information database (NCBI) (http://www.ncbi.nlm.nih.gov) and Ensembl Genome Browser (http://www.ensembl.org). The functional information for candidate genes was inferred from the NCBI gene database (http://www.ncbi.nlm.nih.gov). GeneCards: The Human Gene Database (http://www.genecards.org) was also used to determine gene function as the

biological function of human genes is relatively well established and likely to translate to livestock. If no functional candidate gene was identified, the gene nearest to the SNP (up to 0.5 Mbp) with the lowest P-value was assessed.

3.3. Results and discussion

3.3.1. Phenotypic statistics

The descriptive statistics for the DL phenotype used in the current study were determined. DL was measured as the percentage of total volume of fresh loin muscle lost over a time period of 48-hours. A total of 1098 animals were characterized for phenotype in this study, and the following descriptive statistics were calculated: minimum of 0.06%, mean of 1.16%, maximum of 4.43%, standard deviation of 0.56, and coefficient of variation (CV) of 48.38%.

3.3.2. Principal component analysis

Figure 3.1 shows a plot of the first two principal components from the PCA. No subpopulations or outliers were observed for this analysis, implying little to no stratification in the population. These results were expected as all the animals originated from the same farm and breeding population. Therefore, no principal components were included in subsequent analysis.

3.3.3. Genome-wide association study

Figure 3.2 shows the Manhattan plot and the corresponding Q-Q plot for a GWAS on DL. 40,438 SNPs were tested for association with the trait, however, no SNPs showed significance at either 5% (P-value < 1.2×10^{-6}) or 10% (P-value < 2.5×10^{-6}) false positive rates, and the genomic inflation factor was largely deflated (0.76). Nonetheless, a 1 Mbp region surrounding five SNPs (0.5 Mbp each side) with the lowest P-value was studied further. Five such regions on SSC- 2, 3, 13, and 14 showed the SNPs with the lowest P-values (< 5×10-4), and explained between 0.97 to 2.12% of the total phenotypic variance for the trait (Table 3.1.). QTL regions were defined as a 0.5 Mbp on each side of the SNPs with the lowest P-values.

3.3.4. Comparison with previous results

Relatively few QTL have been recorded in the literature for DL, however, DL does show high negative genetic correlations with WHC and pH (> -0.9), indicating either potential pleiotropy or high LD between the genes controlling the different traits (Jennen et al., 2007; Miar et al., 2014), thus providing some additional sources for comparisons of results. Previous GWAS have identified QTL for DL on SSC-1, 4, 5, 6, 9, 13, and 15 (Ma et al., 2013; Nonneman et al., 2013; Liu et al., 2015; Casiró et al., 2017), WHC on SSC-8 (Sato et al., 2016), and pH on SSC-1, 2, 3, 4, 6, 7, 9, 10, 11, 13, 15, and 17 (Ma et al., 2013; Nonneman et al., 2013; Liu et al., 2015; Zhang et al., 2015; Sato et al., 2016; Casiró et al., 2017; Verardo et al., 2017; Heidaritabar, unpublished results, personal communication). Although there is chromosomal overlap between the QTL identified in the different studies, there was very little overlap between any of the QTL positions. The exception being the region containing *PRKAG3*, located on SSC-15, which was found to be

associated with DL and pH in multiple studies (Zhang et al., 2015; Casiró et al., 2017; Verardo et al., 2017; Heidaritabar, unpublished results, personal communication).

There was a single case of overlap that was found between one QTL identified in this study and a QTL that was found to be significantly associated with pH in the results of Heidaritabar (unpublished results, personal communication). In the indicated study, a single-SNP GWAS using whole-genome sequence (WGS) data was performed, which incorporated the data of the commercial crossbred population from this study, combined with data from a second population of purebred Duroc pigs. The results identified a QTL on SSC-2 that was significantly associated with pH (Heidaritabar, unpublished results, personal communication), and this region overlapped with one of the regions with the lowest P-values identified in this study (SSC-2, position 150.8 to 151.8) (Table 3.1). Nonetheless, it is also important to note that although the marker panel density and sample size were much greater in the study by Heidaritabar (unpublished results, personal communication), there were still no significant QTL identified for DL (discussed further below).

Overall, these results suggest that single-SNP GWAS, such as the one performed in this study, are greatly underpowered in their ability to detect significant associations for traits with small effect variants or variants that explain a small proportion of the total genetic variation, limiting the ability to learn more about the genetics of the trait. Increasing samples size has, in some cases, been found to linearly increase the number of significant SNPs discovered after a minimum sample size threshold has been reached (Visscher et al., 2012). Increasing SNP panel density has also successfully increased the number of significant SNPs detected by GWAS by increasing the number of markers in linkage disequilibrium (LD) with causal mutations as well as increasing the strength of their association (Daetwyler et al., 2014; Yan et al., 2017; Van Den

Berg et al., 2019). However, considering the results from the GWAS performed by Heidaritabar, (unpublished results, personal communication), the higher sample size and marker panel density were still not sufficient to identify genomic regions significantly associated with DL. This emphasizes that DL is likely to be a highly polygenic (quantitative) trait that is controlled by many genes with very small effects. Therefore, in subsequent studies, WGS data, combined with much larger sample sizes will be required for the discovery of associated QTL for DL. However, other statistical approaches should also be considered to improve the power of GWAS.

3.3.5. Comparison of statistical methods

A surprising result of this study is the lack of a significant association between DL and the region on SSC-15 that contains *PRKAG3*. The protein product of *PRKAG3* is the AMP/ATP-binding subunit of the AMP-activated protein kinase (AMPK), which plays a role in the regulation of glycogen storage, and mutations within *PRKAG3* have been shown to have a major effect on post-mortem lactate production in skeletal muscle (Milan et al., 2000; Ciobanu et al., 2001). Changes in lactate production affects the post-mortem pH decline, which can alter the product purge (Huff-Lonergan & Lonergan, 2005). Multiple GWAS and single-SNP association studies have detected associations between DL and *PRKAG3* alleles (Otto et al., 2007; Rohrer et al., 2012; Zhang et al., 2015; Casiró et al., 2017; Verardo et al., 2017), whereas other studies have not (Ma et al., 2013; Nonneman et al., 2013; Liu et al., 2015; Sato et al., 2016), including the related sturdy of Heidaritabar (unpublished results, personal communication) described above. This is expected as different populations can harbor population-specific QTL (Raymond et al., 2018). However, the data used in this study, combined with data from a second

commercial crossbred population (Genus Genetics), was part of a GWAS by Zhang et al. (2015), which found a significant association between a SNP near *PRKAG3* and DL. This SNP was shown to explain a relatively small proportion of the total genetic variation (0.73%) with a low additive effect size of -0.043±0.010 (Zhang et al., 2015). The low effect size of the SNP and the low proportion of total variation explained by the SNP suggests a high statistical power is needed for its detection by GWAS. Presumably, the overall population size in the study by Zhang et al. (2015), as well as the statistical method used, was sufficient (a higher statistical power) to detect genetic associations, which may not have been the case in this study.

Zhang et al. (2015) used a Bayesian statistical method for their analysis. Single-SNP GWAS and Bayesian methods differ in terms of the way that the significance of SNP effects was estimated (different assumptions for the two models). With single-SNP GWAS, many different statistical tests were performed where each SNP is fitted as a fixed effect to detect a marker effect. Since tens of thousands of tests are performed, a very stringent significance threshold is needed, and SNPs should explain a considerable amount of variation to pass the significance threshold. If a SNP explains a small proportion of variation, it is very unlikely that the SNP will reach the significance threshold using single-SNP GWAS, resulting in a high type II error rate (false negative) and low statistical power (Hayes, 2013). Alternatively, BayesB simultaneously fits each SNP as a random effect, meaning only a single statistical test is performed, eliminating the requirement for a stringent significance threshold, improving the power to detect smaller effect SNPs (Hayes, 2013). This difference in statistical power between the two GWAS methods could contribute to the different results observed in the two studies.

A second Bayesian method was performed in this study using the adaptive least absolute shrinkage and selection operator (LASSO). Previously, LASSO has shown to be a powerful

method for detecting QTL associated with complex traits, where LASSO is able to detect overlapping as well as unique QTL compared to other Bayesian methods (BayesB and BayesC) (Yang et al., 2015; Yang et al., 2017). Yang et al. (2017) found LASSO to be a very successful approach for detecting QTL for meat colour phenotypes compared to BayesB, as LASSO was able to detect 3 out of 6 overlapping QTL as well as an additional 17 QTL (Zhang et al., 2015; Yang et al., 2017). However, in this study, GWAS using LASSO did not detect any genomic regions significantly associated with DL (results not shown here). One reason for the lack of results for the LASSO method in this study may be the smaller sample size used compared to previous and successful GWAS that used Bayesian statistical methods (Zhang et al., 2015; Yang et al., 2017). However, the success of a Bayesian method will also depend on how closely the assumption of the statistical method for the prior distribution of SNP effects matches the underlying genetic architecture of the trait (Zhang et al., 2018). Different Bayesian methods assume a different prior distribution of SNP effects, where LASSO assumes a non-normal, double exponential distribution of SNP effects with many SNPs that are equal to zero. Therefore, the distribution assumed by LASSO seemingly does not closely resemble the underlying genetic architecture of DL.

3.3.6. Candidate genes

The positional candidate genes that were found nearest to the SNP with the lowest P-value in each QTL window were assessed for a potential biological function in determining DL (Table 3.1). Five positional candidate genes were identified, *EPS15L1*, *NR3C1*, *KLHL29*, *CHST2*, and *DRG1* on SSC- 2, 2, 3, 13 and 14, respectively, which were found to function in biological

processes such as endocytosis, transcriptional regulation, enzymatic reactions, and microtubule dynamics (Kuo et al., 2015; Schellhaus et al., 2017). *EPS15L1* and *NR3C1* are the most interesting genes as their protein functions could potentially be implicated in post-mortem muscle biochemistry. The gene product, EPS15L1 could influence DL through its interactions with the transferrin receptor (TFR), which plays an important role in cellular iron uptake and homeostasis. Iron is a crucial component of myoglobin, which plays an important role in aerobic metabolism and the rate of energy metabolism within the muscle (Oexle et al., 1999). Anaerobic metabolism is thought to be the dominate source of ATP/H^+ production and pH decline in the post-mortem muscle, however, aerobic respiration also plays a role (England et al., 2018). Therefore, variability in the rate of aerobic metabolism likely influences the DL observed for meat through its contribution to pH decline post-mortem. This is because, if aerobic metabolism is active after slaughter, the switch to anaerobic metabolism will de delayed, and there will be no lactic acid production during this time, overall resulting in a less extensive pH decline observed post-mortem.

Additionally, the role of the gene product NR3C1 in glucose homeostasis could provide evidence for its role in DL. NR3C1 is a glucocorticoid receptor (GR), which regulates glucose uptake, glycogenolysis, as well as glycogen synthesis (Kuo et al., 2015). During times of stress (such as during slaughter), GRs will alter glucose concentrations in the liver and skeletal muscle (Kuo et al., 2015). Variability in the amount of glycogen in the muscle post-mortem is an important indicator of lactic acid production and the ultimate pH of the meat (Huff-Lonergan & Lonergan, 2005). Interestingly, in previous studies this *NR3C1* was also found to be significantly associated with meat quality phenotypes. Reyer et al. (2014) found an association between two mutations within the *NR3C1* gene with both DL and pH in a commercial crossbred pig population (Pietrain

X Landrace/Large White). Similarly, Terenina et al. (2013) detected a significant association between a mutation in *NR3C1* and DL in Large White and Meishan pig breeds. The *NR3C1* gene was also found to overlap with a QTL found to be significantly associated with pH in a previous GWAS that used WGS (Heidaritabar, unpublished results, personal communication). In their study, *NR3C1* was not located nearest to the lead SNP in this QTL, but it was located within a 1 Mbp radius (0.5 Mbp on either side of the lead SNP), indicating that the lead SNP and the gene were likely to be in high LD (Heidaritabar, unpublished results, personal communication).

Both *EPS15L1* and *NR3C1* are implicated in DL through their potential influence on post-mortem pH decline and the ultimate pH of meat. Post-mortem variability in the cellular pH has a major effect on meat quality as it effects protein degradation, net protein charge, as well as the lateral shrinkage of the muscle fiber, each play an important role in determining the ability of the muscle cell to hold water, and the extent of DL observed for meat (Huff-Lonergan & Lonergan, 2005). These results provide some evidence for true associations on SSC-2 despite the lack of a significant association after correction for type I error rates of 5% and 10%.

3.4. Conclusions

In this study, a single-SNP GWAS was performed to identify genomic regions responsible for variability of DL phenotypes in commercial crossbred pigs. It was hypothesized that at least one SNP on the marker panel would be significantly associated with DL phenotypes. However, the results of this study did not support this hypothesis. It was concluded that the GWAS method used in this study was underpowered to detect QTL with significant effects as no associations were identified at either genome-wide significance or suggestive thresholds. However, based on

post-GWAS analysis of the top five QTL with the lowest P-values, two could potentially be implicated in DL through their biological functions. Genomic regions on SSC-2 were found to harbor the genes *EPS15L1* and *NR3C1*, which have functions linked to aerobic metabolism, and glucose homeostasis. These biological pathways could both potentially influence the extent of post-mortem pH decline and correspondingly the DL phenotypes of the meat. However, further studies with larger sample sizes and/or marker panel densities, as well as alternative statistical methods with different prior assumptions, should be explored to improve the power of GWAS and to confirm these claims. Additional research could lead to insights on the genetic architecture and biology underlying DL and could be used to improve meat quality of pigs through marker assisted selection (MAS) or genomic selection (GS) methods that incorporate prior biological information.

3.5. References

Badke, Y. M., Bates, R. O., Ernst, C. W., Schwab, C., & Steibel, J. P. (2012). Estimation of linkage disequilibrium in four US pig breeds. BMC Genomics, 13(1), 1–10. https://doi.org/10.1186/1471-2164-13-24

Baltić, M. Ž., Tomović, V., Marković, R., Velarde, A., Dokmanović, M., Janjić, J., & Glamočlija, N. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. Meat Science, 98(2), 220–226. https://doi.org/10.1016/j.meatsci.2014.06.003

CCSI. (2001). RN Gene Testing in Canadian AI Boars. Canadian Center for Swine Improvement. Retrieved from http://www.ccsi.ca/newsletters/RNgene.PDF

Casiró, S., Velez-Irizarry, D., Ernst, C. W., Raney, N. E., Bates, R. O., Charles, M. G., & Steibel, J. P. (2017). Genome-Wide association study in an F2 Duroc x Pietrain resource population for economically important meat quality and carcass traits. Journal of Animal Science, 95(2), 545–558. https://doi.org/10.2527/jas2016.1003

CCAC. (2020). Guide to the Care and Use of Experimental Animals, Volume 1, 2nd Edition. Canadian Council on Animal Care.

Channon, H. A., Payne, A. M., & Warner, R. D. (2000). Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. Meat Science, 56(3), 291–299. https://doi.org/10.1016/S0309-1740(00)00056-5

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics, 159(3), 1151–1162.

Daetwyler, H., Capitan, A., Pausch, H., Stothard, P., Van Binsbergen, R., Brøndum, R., Liao, X., Djari, A., Rodriguez, S., Grohs, C., Esquerré, D., Bouchez, O., Rossignol, M., Klopp, C., Rocha, D., Fritz, S., Eggen, A., Bowman, P., Coote, D., Chamberlain, A., Anderson, C., Vantassell, C., Hulsegge, I., Goddard, M., Guldbrandtsen, B., Lund, M., Veerkamp, R., Boichard, D., Fries, R., & Hayes, B. (2014). Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nature Genetics, 46(8), 858–865. https://doi.org/10.1038/ng.3034

de Vries, A. G., van der Wal, P. G., Long, T., Eikelenboom, G., & Merks, J. W. M. (1994). Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Production Science, 40(3), 277–289. https://doi.org/10.1016/0301-6226(94)90095-7

England, E. M., Matarneh, S. K., Mitacek, R. M., Abraham, A., Ramanathan, R., Wicks, J. C., Shi, H., Scheffler, T. L., Oliver, E. M., Helm, E. T., & Gerrard, D. E. (2018). Presence of oxygen and mitochondria in skeletal muscle early postmortem. Meat Science, 139, 97–106. https://doi.org/10.1016/j.meatsci.2017.12.008

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., Thompson, R. (2015). ASReml User Guide Release 4.1, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-4.1-Functional-Specification.pdf

Goddard, M., Kemper, K., MacLeod, I., Chamberlain, A., & Hayes, B. (2016). Genetics of complex traits: Prediction of phenotype, identification of causal polymorphisms and genetic architecture. Proceedings of the Royal Society B: Biological Sciences, 283(1835). https://doi.org/10.1098/rspb.2016.0569

Grossi, D. A., Jafarikia, M., Brito, L. F., Buzanskas, M. E., Sargolzaei, M., & Schenkel, F. S. (2017). Genetic diversity, extent of linkage disequilibrium and persistence of gametic phase in Canadian pigs. BMC Genetics, 18(1), 1–13. https://doi.org/10.1186/s12863-017-0473-y

Hayes, B. (2013). Overview of Statistical Methods for Genome-Wide Association Studies (GWAS). In Genome-Wide Association Studies and Genomic Prediction, 149–169. https://doi.org/10.1007/978-1-62703-447-0_10

Holmer, S. F., McKeith, F. K., & Killefer, J. (2008). The influence of early post-mortem enhancement and accelerated chilling on pork quality. Meat Science, 79(2), 211–216. https://doi.org/10.1016/j.meatsci.2007.09.004

Hu, Z. L., Dracheva, S., Jang, W., Maglott, D., Bastiaansen, J., Rothschild, M. F., & Reecy, J. M. (2005). A QTL resource and comparison tool for pigs: PigQTLDB. Mammalian Genome, 16(10), 792–800. https://doi.org/10.1007/s00335-005-0060-9

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Ihaka, R., & Gentleman, R. (1996). R: A Language for Data Analysis and Graphics. Journal of Computational Graphical Statistics, 5(3), 299–314.

Jennen, D. G. J., Brings, A. D., Liu, G., Jüngst, H., Tholen, E., Jonas, E., Tesfaye, D., Schellander, K., & Phatsara, C. (2007). Genetic aspects concerning drip loss and water-holding capacity of porcine meat. Journal of Animal Breeding and Genetics, 124(s1), 2–11. https://doi.org/10.1111/j.1439-0388.2007.00681.x

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: A review. Plant Methods, 9(1), 1-9. https://doi.org/10.1186/1746-4811-9-29

Kuo, T., McQueen, A., TC, C., & Wang, J. (2015). Regulation of Glucose Homeostasis by Glucocorticoids. Advances in Experimental Medicine and Biology, 872, 99-126. https://doi.org/10.1007/978-1-4939-2895-8_5

Liu, X., Xiong, X., Yang, J., Zhou, L., Yang, B., Ai, H., Ma, H., Xie, X., Huang, Y., Fang, S., Xiao, S., Ren, J., Ma, J., & Huang, L. (2015). Genome-wide association analyses for meat quality traits in Chinese Erhualian pigs and a Western Duroc x (Landrace × Yorkshire) commercial population. Genetics Selection Evolution, 47(1), 1–11. https://doi.org/10.1186/s12711-015-0120-x

Ma, J., Yang, J., Zhou, L., Zhang, Z., Ma, H., Xie, X., Zhang, F., Xiong, X., Cui, L., Yang, H., Liu, X., Duan, Y., Xiao, S., Ai, H., Ren, J., & Huang, L. (2013). Genome-Wide Association Study of Meat Quality Traits in a White Duroc × Erhualian F2 Intercross and Chinese Sutai Pigs. PLoS ONE, 8(5). https://doi.org/10.1371/journal.pone.0064047

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., McCarthy, Mark I., Ramos, E. M., Cardon, L. R., Chakravarti, A., Cho, J. H., Guttmacher, A. E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C. N., Slatkin, M., Valle, D., Whittemore, A. S., Boehnke, M., Clark, A. G., Eichler, E. E., Gibson, G., Haines, J. L., MacKay, T. F.C., McCarroll, S. A., & Visscher, P. M. (2009). Finding the missing heritability of complex diseases. Nature, 461(7265), 747–753. https://doi.org/10.1038/nature08494

Miar, Y., Plastow, G., Moore, S., Manafiazar, G., Charagu, P., Kemp, R., Van Haandel, B., Huisman, A., Zhang, C., Mckay, R., Bruce, H., & Wang, Z. (2014). Genetic and

phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livestock Production Science, 26(2), 119–135. https://doi.org/10.2527/jas2014-7685

Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robix, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronee, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., Chardon, P., & Andersson, L. (2000). A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. Science, 288(5469), 1248–1251. https://doi.org/10.1126/science.288.5469.1248

Misztal, I., S. Tsuruta, D. A. L. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2018. Manual for BLUPF90 family programs. University of Georgia. http://nce.ads.uga.edu/wiki/doku.php?id=documentation

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2007). International preferences for pork appearance: I. Consumer choices. Food Quality and Preference, 18(1), 26–36. https://doi.org/10.1016/j.foodqual.2005.07.001

Nonneman, D., Shackelford, S. D., King, D. A., Wheeler, T. L., Wiedmann, R. T., Snelling, W. M., & Rohrer, G. A. (2013). Genome-wide association of meat quality traits and tenderness in swine. Journal of Animal Science, 91(9), 4043–4050. https://doi.org/10.2527/jas2013-6255

Oexle, H., Gnaiger, E., & Weiss, G. (1999). Iron-dependent changes in cellular energy metabolism: Influence on citric acid cycle and oxidative phosphorylation. Biochimica et Biophysica Acta - Bioenergetics, 1413(3), 99–107. https://doi.org/10.1016/S0005-2728(99)00088-2

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 2. Drip losses. Developments in Meat Science, 4, 173–243.

Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S., & Kalm, E. (2007). Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Science, 75(2), 185–195. https://doi.org/10.1016/j.meatsci.2006.03.022

Raymond, B., Bouwman, A. C., Schrooten, C., Houwing-Duistermaat, J., & Veerkamp, R. F. (2018). Utility of whole-genome sequence data for across-breed genomic prediction. Genetics Selection Evolution, 50(1), 1–12. https://doi.org/10.1186/s12711-018-0396-8

Reyer, H., Ponsuksili, S., Wimmers, K., & Murani, E. (2014). Association of N-terminal domain polymorphisms of the porcine glucocorticoid receptor with carcass composition and meat quality traits. Animal Genetics, 45(1), 125–129. https://doi.org/10.1111/age.12083

Rohrer, G. A., Nonneman, D., Miller, R. K., Zerby, H., & Moeller, S. J. (2012). Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs. Meat Science, 92(4), 511–518. https://doi.org/10.1016/j.meatsci.2012.05.020

Ropka-Molik, K., Bereta, A., Tyra, M., Rózycki, M., Piórkowska, K., Szyndler-Nedza, M., & Szmatoła, T. (2014). Association of calpastatin gene polymorphisms and meat quality traits in pig. Meat Science, 97(2), 143–150. https://doi.org/10.1016/j.meatsci.2014.01.021

Rybarczyk, A., Karamucki, T., Pietruszka, A., Rybak, K., & Matysiak, B. (2015). The effects of blast chilling on pork quality. Meat Science, 101, 78–82. https://doi.org/10.1016/j.meatsci.2014.11.006

Sargolzaei, M., Chesnais, J. P., & Schenkel, F. S. (2014). A new approach for efficient genotype imputation using information from relatives. BMC Genomics, 15(1). https://doi.org/10.1186/1471-2164-15-478

Sato, S., Uemoto, Y., Kikuchi, T., Egawa, S., Kohira, K., Saito, T., Sakuma, H., Miyashita, S., Arata, S., Kojima, T., & Suzuki, K. (2016). SNP- and haplotype-based genome-wide association studies for growth, carcass, and meat quality traits in a Duroc multigenerational population. BMC Genetics, 17(1). https://doi.org/10.1186/s12863-016-0368-3

Savage, A. W. J., Warriss, P. D., & Jolley, P. D. (1990). The amount and composition of the proteins in drip from stored pig meat. Meat Science, 27(4), 289–303. https://doi.org/10.1016/0309-1740(90)90067-G

Schellhaus, A. K., Moreno-Andrés, D., Chugh, M., Yokoyama, H., Moschopoulou, A., De, S., Bono, F., Hipp, K., Schäffer, E., & Antonin, W. (2017). Developmentally Regulated GTP binding protein 1 (DRG1) controls microtubule dynamics. Scientific Reports, 7(1), 1–16. https://doi.org/10.1038/s41598-017-10088-5

Terenina, E., Babigumira, B. M., Le Mignon, G., Bazovkina, D., Rousseau, S., Salin, F., Bendixen, C., & Mormede, P. (2013). Association study of molecular polymorphisms in candidate genes related to stress responses with production and meat quality traits in pigs. Domestic Animal Endocrinology, 44(2), 81–97. https://doi.org/10.1016/j.domaniend.2012.09.004

Towers, L. (2016). Pork Meat Quality: Understanding Industry Measurements and Guidelines. The Pig Site. Retrieved from https://thepigsite.com/articles/pork-meat-quality-understanding-industry-measurements-and-guidelines

Turner, S. (2014). qqman: a R package for visualizing GWAS results using Q-Q and manhattan plots. BiorXiv. https://doi.org/https ://doi.org/10.1101/005165

Van Den Berg, S., Vandenplas, J., Van Eeuwijk, F. A., Bouwman, A. C., Lopes, M. S., & Veerkamp, R. F. (2019). Imputation to whole-genome sequence using multiple pig populations and its use in genome-wide association studies. Genetics Selection Evolution, 51(1), 1–13. https://doi.org/10.1186/s12711-019-0445-y

Van Wijk, H. J., Arts, D. J. G., Matthews, J. O., Webster, M., Ducro, B. J., & Knol, E. F. (2005). Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. Journal of Animal Science, 83(2), 324–333. https://doi.org/10.2527/2005.832324x

Verardo, L. L., Sevón-Aimonen, M. L., Serenius, T., Hietakangas, V., & Uimari, P. (2017). Whole-genome association analysis of pork meat pH revealed three significant regions and several potential genes in Finnish Yorkshire pigs. BMC Genetics, 18(1), 1–15. https://doi.org/10.1186/s12863-017-0482-x

Visscher, P. M., Brown, M. A., McCarthy, M. I., & Yang, J. (2012). Five years of GWAS discovery. American Journal of Human Genetics, 90(1), 7–24. https://doi.org/10.1016/j.ajhg.2011.11.029

Weller, J. I., Song, J. Z., Heyen, D. W., Lewin, H. A., & Ron, M. (1998). A New Approach to the Problem of Multiple Comparisons in the Genetic Dissection of Complex Traits. Genetics, 150(4), 1699–1706.

Wiggans, G. R., Sonstegard, T. S., Vanraden, P. M., Matukumalli, L. K., Schnabel, R. D., Taylor, J. F., Schenkel, F. S., & Van Tassell, C. P. (2009). Selection of single-nucleotide polymorphisms and quality of genotypes used in genomic evaluation of dairy cattle in the United States and Canada. Journal of Dairy Science, 92(7), 3431–3436.

Yan, G., Qiao, R., Zhang, F., Xin, W., Xiao, S., Huang, T., Zhang, Z., & Huang, L. (2017). Imputation-Based Whole-Genome Sequence Association Study Rediscovered the Missing QTL for Lumbar Number in Sutai Pigs. Scientific Reports, 7(1), 1–10. https://doi.org/10.1038/s41598-017-00729-0

Yang, J., Lee, S. H., Goddard, M., & Visscher, P. (2011). GCTA: A tool for genome-wide complex trait analysis. American Journal of Human Genetics, 88(1), 76–82. https://doi.org/10.1016/j.ajhg.2010.11.011

Yang, T., Plastow, G., & Wang, Z. (2015). Variable selection in whole-genome association studies using LASSO-based techniques. Plant and Animal Genome Conference XXIII. San Diego, CA, USA.

Yang, T., Wang, Z., Miar, Y., Bruce, H., Zhang, C., Plastow, G. (2017). A genome-wide association study of meat colour in commercial crossbred pigs. Journal of Animal Science, 97(4), 721–733. https://doi.org/10.1139/cjas-2016-0248

Zhang, C., Kemp, R., Stothard, P., Wang, Z., Boddicker, N., Krivushin, K., Dekkers, J. C. M., & Plastow, G. (2018). Genomic evaluation of feed efficiency component traits in Duroc pigs using 80K, 650K and whole-genome sequence variants. Genetics Selection Evolution, 50(1), 1–13. https://doi.org/10.1186/s12711-018-0387-9

Zhang, C., Wang, Z., Bruce, H., Kemp, R., Charagu, P., Miar, Y., Yang, T., Plastow, G. (2015). Genome-wide association studies (GWAS) identify a QTL close to PRKAG3 affecting meat pH and colour in crossbred commercial pigs. BMC Genetics, 16(1), 1–12. https://doi.org/10.1186/s12863-015-0192-1

Tables and figures

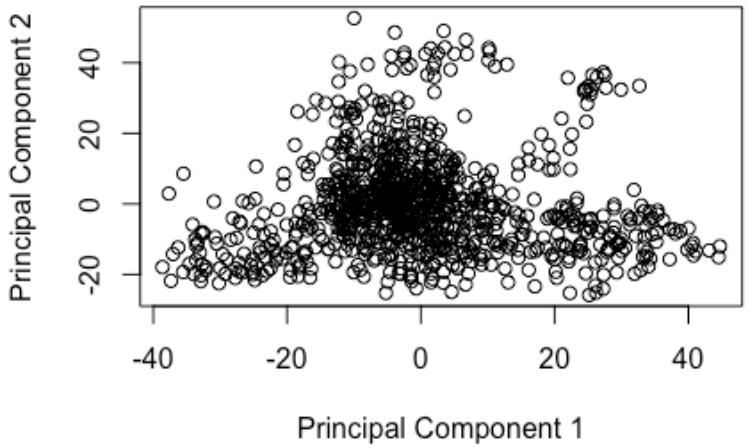
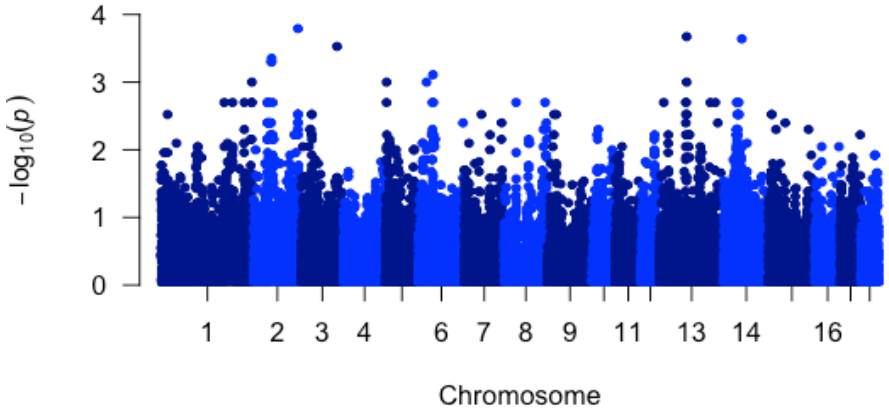


Figure 3.1. Principal component analysis (PCA) of SNP genotypes from commercial crossbred pigs originating from a single population (Hypor Inc., Regina, SK, Canada). Principle components 1 (1.7% total variation) and 2 (1.3% variation) were plotted against one another to visualize potential subpopulations.

(A)



(B)

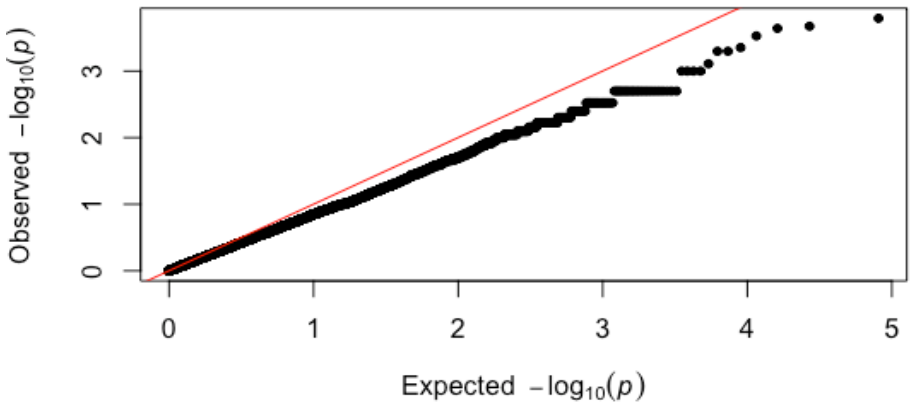


Figure 3.2. (A) Manhattan and (B) quantile-quantile (Q-Q) plots from a genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs. (A) The Manhattan plot shows the chromosome of the SNP marker along the x-axis and the -log10(P-values) representing the significance of the association along the y-axis. Bonferroni correction was used to control for multiple testing, with P-values of 0.05 (-log10 = 5.91), and 0.10 (-log10 = 5.61). (B) The Q-Q plot shows the expected null distribution of -log10 (p-values) (solid red line) compared to the actual distribution (dotted black line). The genomic inflation factor (λ) was calculated as 0.76.

Table 3.1. Quantitative trait loci (QTL) and the corresponding positional candidate genes identified for the SNPs with the lowest P-values in a genome-wide association study (GWAS) for drip loss (DL) in commercial crossbred pigs.

Chr[1]	SNP ID[2]	MAF[3]	Phenotypic Variance (%)[4]	QTL Position (Mbp)[5]	# QTL[6]	Positional Candidate Gene[7]	Gene Function[8]	Distance (Mbp)[9]
2	rs81293087	0.47	1.36	60.3-61.3	0	*EPS15L1*	Receptor-mediated endocytosis	0.0206
2	rs81261395	0.23	1.79	150.8-151.8	3	*NR3C1*	Glucocort-icoid receptor	0.189
3	rs81475257	0.01	1.37	121.6-122.6	0	*KLHL29*	Cul3-RING ubiquitin ligase	0.0002
13	rs81256965	0.36	2.12	91.2-92.2	0	*CHST2*	Carbohyd-rate sulfotrans-ferase	0.173
14	rs80883284	0.21	0.97	50.7-51.7	0	*DRG1*	GTPase	0.0089

[1]The chromosome the SNP with the lowest P-value is located.
[2]The SNP ID of the SNP with the lowest P-value.
[3]The minor allele frequency (MAF) of the SNP with the lowest P-value.
[4]The phenotypic variance explained by the SNP with the lowest P-value.
[5]The 1 Mbp QTL window surrounding the SNP with the lowest P-value.
[6]The number of previously identified QTL overlapping the QTL identified in this study.
[7]The ID of the candidate gene located nearest to the SNP with the lowest P-value.
[8]The known function of the candidate gene.
[9]The distance of the gene from the SNP with the lowest P-value.
Note: Chromosomal positions are according to the pig genome assembly Sscrofa10.2.

Chapter 4. Single-SNP association analysis of potential causative mutations in *MYOD1* and *RTN4* for meat colour in purebred Duroc pigs

4.1. Introduction

Meat colour is the first and most important visual factor influencing consumer perception at the point of purchase (Seideman et al., 1984; Ngapo et al., 2007; Ngapo, 2017). Pork meat is expected to be red or bright pink in colour, which indicates to the customer the freshness and wholesomeness of the product (Seideman et al., 1984). Interestingly, both light and dark coloured pork are equally preferred, and meat colour preferences change depending on the consumer segment (Ngapo et al., 2007; Ngapo, 2017). For example, Canadian consumers prefer light coloured pork, whereas darker coloured pork is chosen in Taiwan, Japan, China, and Korea (Ngapo et al., 2007). However, overall, any meat products that are excessively light or dark in colour or with inconsistencies and discolourations must be sold as a processed, trimmed, or discounted product leading to substantial economic losses (Seideman et al., 1984). Therefore, pork colour is an economically important trait, and being able to predict and produce a consistent product depending on colour preferences would be valuable to satisfy a wide variety of consumer demands.

Fresh meat colour is controlled by multiple different biological and environmental factors. Important environmental factors include pre-slaughter handling (Channon et al., 2000; Matthews et al., 2001), stunning method (Channon et al., 2000; Velarde et al., 2001), diet (Hamilton et al., 2002), and available pen space (Matthews et al., 2001; Gentry et al., 2002). Further, the genotype

of an animal will have an effect on pork colour through its influence on muscle biology and interactions with the environment. Mutations within the *ryanodine receptor 1* (*RYR1*) (Fujii et al., 1991; Otto et al., 2007) and *protein kinase AMP-activated non-catalytic subunit gamma 3* (*PRKAG3*) genes exhibit large effects on pork colour (Milan et al., 2000; Ciobanu et al., 2001). Causative mutations such as the ones identified in *RYR1* and *PRKAG3* are useful as they can be incorporated into marker (MAS) or genomic (GS) selection procedures. Selection methods that rely on linkage disequilibrium (LD) between SNPs on the marker panel and nearby causative mutations can be problematic as LD can decay over generations, and across populations, due to recombination. As a result, the marker panel will not be able to explain all of the genetic variance contributing to a trait, and accuracy of GEBV prediction will be low (Meuwissen et al., 2001). Further, if the causative mutation has a low minor allele frequency (MAF), then it is less likely to be in high LD with the common variants on a marker panel, which further exacerbates the risk of LD decay (Van Den Berg et al., 2016). Therefore, if more causative mutations were to be identified and directly incorporated into selection procedures, then this risk of recombination will no longer be apparent, which is anticipated to improve breeding value prediction accuracy and genetic gain as well as prevent the deterioration of accuracy over generations (Meuwissen & Goddard, 2010; MacLeod et al., 2014).

Novel QTL harboring potential candidate genes with biological roles relevant to meat colour traits have been identified by genome-wide association studies (GWAS) (Zhang et al., 2015; Yang et al., 2017). Specifically, the myoblast determination protein 1 (*MYOD1*) and reticulon 4 (*RTN4*) have both been found to be associated with pork colour traits in Canadian pig populations (Yang et al., 2017). Both genes have been biologically implicated in muscle cell differentiation during development and muscle fiber type specification in adult tissues, and are

likely influencing post-mortem meat colour phenotypes through their effect on muscle fiber type in the adult muscle (Hughes et al., 1997; Tang et al., 2007; Chen et al., 2010; Lee et al., 2012). SNPs within these genes could be influencing meat colour phenotypes and thus could be used as markers for the improvement of meat quality traits in pigs. However, GWAS are often not followed up with a search for potential causative mutations directly underlying the observed effects, limiting the practical use of candidate genes such as *MYOD1* and *RTN4* in selection programs. Therefore, the goal of this study was to identify causative mutations located within the potential candidate genes, *MYOD1* and *RTN4*. It is hypothesized that SNPs within the coding region of these genes, that are predicted to be missense and deleterious, could be influencing meat colour phenotypes by altering protein structure and function. The results of this study could indicate useful markers, which could be incorporated into MAS or GS for the genetic improvement of meat colour traits in pigs.

4.2. Materials and methods

4.2.1. Bioinformatics

The *MYOD1* and *RTN4* genes are located within quantitative trait loci (QTL) that are significantly associated with meat colour phenotypes in a GWAS (Yang et al., 2017), and were chosen for use in this study based on their known biological functions and potential effects on meat colour phenotypes. The Ensembl genome database (https://uswest.ensembl.org/index.html) was used to identify all the SNPs located within the *MYOD1* and *RTN4* genes in pigs, as well as their predicted consequences (Zerbino et al., 2018). The Ensembl genome database uses the

variant effect predictor (VEP) (https://uswest.ensembl.org/info/docs/tools/vep/index.html) tool as well as the sorting intolerant from tolerant (SIFT) program to predict the consequence of the amino acid change caused by each SNP (Hunt et al., 2018; Zerbino et al., 2018). VEP determines how the gene, transcript, and protein sequences are affected by a SNP, synonymous, non-synonymous, stop gain/loss, missense, and frameshift. (Hunt et al., 2018), and SIFT uses sequence homology and sequence conservation to assign the SNP a value between 0 and 1 that indicates its effect on the protein function, deleterious (0 to 0.05) or tolerated (0.06 to 1) (Hunt et al., 2018). Further, the location of the SNP within the protein, such as in an important protein domain (affecting protein folding, stability, or protein-protein interactions) and/or a highly conserved region of the protein, could provide further evidence of its functional importance. The location of the SNP within the protein was determined using the universal protein resource (UniProt) (Apweiler, 2009) and Clustal Omega Sequence Alignment tool (Sievers et al., 2011). Non-synonymous and deleterious SNPs that were located within the gene coding regions (functional domains and conserved protein sequences) were chosen for use in this study.

4.2.2. Data

Animals

A total of 437 female purebred Duroc pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used in this study. The ethical statement, raising protocol, phenotypes, and pedigree information were all described previously in this thesis (Chapter 2 Section 2.2.1.)

Genotypes

Genotyping was performed using Thermo Fisher TaqMan genotyping assays (Thermo Fisher Scientific Ltd., Ottawa, ON, Canada). DNA was extracted from tissue samples using Thermo Fisher protocol (Thermo Fisher Scientific Ltd., Ottawa, ON, Canada). Animals were genotyped for two SNPs, rs336462969 and rs340803577, which reside in the *MYOD1* and *RTN4* gene coding regions, respectively. Probes and primers were designed using Thermo Fisher Custom TaqMan assay design tool, which identifies DNA within the pre-defined target sequence that are optimized for probe and primer binding (minimizing non-specific binding). The target sequence, along with primer and probe sequences are shown in Table 4.1. The PCR reaction mixture was prepared following the Thermo Fisher standard protocol for a 96-well Fast 10 μL reaction. A standard thermal cycling setting for the PCR reaction was also used: Taq-polymerase enzyme activation (10 minutes hold), followed by 40 cycles of denaturation (95 ºC for 15 seconds), and annealing/extension (60 ºC for 1 minute).

4.2.3. Statistical analysis

The significance of fixed effects and covariates was determined using Wald F. statistics in ASReml software, and factors with P-value < 0.05 were included in the following analysis (Gilmour, 2015). A single-SNP association analysis was performed using the following statistical model in ASReml software (Gilmour, 2015):

$$\boldsymbol{y} = \mathbf{1}\mu + \boldsymbol{Xb} + \boldsymbol{S\alpha} + \boldsymbol{Za} + \boldsymbol{e} \quad [1]$$

Where $\boldsymbol{y}$ is a vector of phenotypic values; $\mathbf{1}$ is a vector of ones; μ is the overall mean for meat colour phenotypes; $\boldsymbol{X}$ is a design matrix that relates fixed effects to the observations; $\boldsymbol{b}$ is the vector of fixed effects including slaughter date; $\boldsymbol{S}$ is a vector of SNP genotypes coded 0, 1, 2; $\boldsymbol{\alpha}$ is the additive SNP effect; $\boldsymbol{Z}$ is a design matrix associating $\boldsymbol{a}$ with response variables; $\boldsymbol{a}$ is the vector of random additive genetic effects that is assumed to be normally distributed; and $\boldsymbol{e}$ is a vector of residual effects. The additive genetic effects and the residual effects are both assumed to be normally distributed; $\boldsymbol{a} \sim N(0,\ \boldsymbol{A}\sigma_a^2)$, $\boldsymbol{e} \sim N(0,\ \boldsymbol{I}\sigma_e^2)$, respectively, where σ_a^2 and σ_e^2 are the additive genetic and residual variances, respectively, $\boldsymbol{A}$ is the additive relationship matrix constructed using pedigree data, and $\boldsymbol{I}$ is an identity matrix.

4.3. Results and discussion

4.3.1. Phenotypic statistics

The descriptive statistics for pork color phenotypes used in this study were calculated. Abbreviations, number of records (N), minimum, mean, maximum, standard deviation (SD), and coefficient of variation (CV) are recorded in Table 4.2.

4.3.2. Candidate genes

MYOD1 is part of the MyoD family of transcription factors that regulates the process of myogenesis. *MYOD1* is critical in specifying the identity of myoblasts during muscle cell differentiation in animal development (Blau, 1988), but has also been found to play a role in

specifying muscle fiber type in adult muscles (Hughes et al., 1997). The latter has been confirmed in pigs as two SNPs in the *MYOD1* gene have shown a significant association with muscle fiber type and pork lightness (Lee et al., 2012).

RTN4 is a member of the reticulon (RTN) family of proteins, which almost exclusively localize to the endoplasmic reticulon where they often play a role in calcium signaling and homeostasis (Jozsef et al., 2014). Less is known about the *RTN4* gene and its exact function in skeletal muscle, but the gene has shown differential expression in muscle fiber types and the protein has been found to interact with MyoD family member MYF5 (Tang et al., 2007; Chen et al., 2010). Further, direct associations between expression levels of the *RTN4* gene and meat quality phenotypes (pH) have been identified in pigs, which altogether provides evidence for a role in muscle fiber type specification as well as skeletal muscle metabolism in adult muscle tissues (Te Pas et al., 2013).

Fiber type specification has a direct influence on the colour of meat post-mortem. Slow twitch muscle fibers have predominantly aerobic metabolism and require high concentrations of myoglobin (the muscle specific protein that gives meat its red colour) and oxygen to sustain, therefore muscles with a high proportion of slow twitch muscle fibers appear red in colour. Fast twitch muscle fibers are sustained by anaerobic metabolism and alternatively require glycogen to generate energy, resulting in a muscle that appears paler (Seideman et al., 1984; Mancini & Hunt, 2005). Further, muscles with a high proportion of fast twitch muscle fibers also have a high glycolytic potential prior to slaughter, which causes a rapid drop in pH post-mortem, resulting in an increase in protein denaturation, and light reflecting off the surface of the pork, resulting in pale pork (Choe et al., 2008).

4.3.3. Bioinformatics

A total of 12 SNPs were identified within the *MYOD1* gene coding region, with 7 synonymous (SNPs that do not change the amino acid sequence of a protein), and 5 missense (SNPs that change the amino acid sequence of a protein). The SIFT scores for the missense variants ranged from 0.05 to 0.6. The SNP that was chosen for analysis (rs336462969) had the only deleterious SIFT score of 0.05, which was assigned as the SNP caused a non-synonymous amino acid change from arginine (R) to proline (P). The change from a bulky and polar amino acid to one that is non-polar and compact indicates a potentially major change to the protein structure, which could result in impaired or altered protein function. Additionally, Figure 4.1 (A) shows a multiple sequence alignment of a portion of the myogenic basic domain that contains rs336462969 across mammals, *Sus scrufa* (pig), *Bos taurus* (cow), *Ovis aries* (sheep), *Mus musculus* (mouse), and *Homo sapiens* (humans), with the arginine (R) amino acid showing complete conservation of across every species analyzed. The MyoD family of proteins have relatively well conserved structure. Each family member contains a highly conserved helix-loop-helix DNA binding domain, as well as a myogenic basic domain, which shows sequence conservation to a lesser extent (Olson, 1990). The sequence conservation of arginine (R) could indicate its structural importance, for which non-synonymous and deleterious changes could potentially indicate impaired or altered protein function.

A total of 43 SNPs were located within the *RTN4* gene coding region, with 22 synonymous, 20 missense, and 1 caused a start-loss (SNPs that change the transcript start codon). The SIFT scores for these missense variants ranged from 0 to 0.41. The SNP chosen for analysis

(rs340803577) had a SIFT score of 0 and was prioritized as the top SNP for potentially affecting protein function, which was assigned as the SNP caused a non-synonymous amino acid change from proline (P) to threonine (T). The change from non-polar and compact amino acid to one that is polar and bulky could indicate important functional changes to the protein. Additionally, Figure 4.1 shows a multiple sequence alignment of a portion of the N-terminal cytoplasmic domain of the *RTN4* gene containing rs340803577, with the SNP showing high conservations of the proline (P) amino acid across mammalian species with the exception of humans. However, humans showed a proline (P) to leucine (L) amino acid change, but similar amino acid properties were maintained despite the change in protein sequence(small, hydrophobic, and non-polar). The *RTN* gene family contain a C-terminus reticulon homology domain (RHD), which is a functionally important cytoplasmic domain flanked by two transmembrane domains. RHD is highly conserved across many different species and across RTN paralogs (Yang & Strittmatter, 2007). On the other hand, the N-terminal cytoplasmic domain of the RTN genes, outside of RHD, displays low sequence similarity across species and between paralogs within the same species (Yang & Strittmatter, 2007). However, as shown in this study (Figure. 4.1), this domain does show some sequence conservation across mammals. Similar to previous results, this sequence conservation of the proline (P) amino acid across mammals could indicate structural and functional importance of the SNP.

4.3.4. Single-SNP association analysis

Table 4.4 shows the least squares mean values and standard errors (SE) of each genotype class for each pork colour trait. Each genotype class was tested for an association with the trait,

however, none showed a significant effect on meat colour phenotypes (P-value < 0.05) (Table 4.4). A previous study by Yang et al. (2017) identified a significant association between *longissimus thoracis et lumborum* (loin) Minolta a* (LOINA) and a QTL harboring the *MYOD1* gene, and between *quadriceps femoris* Minolta L* (QUADL), *quadriceps femoris* Minolta b* QUADB, and *iliopsoas* Minolta L* (ILIOL) and a QTL harboring *RTN4*. However, in this study, the P-value for the association between a mutation within *MYOD1* (rs336462969) and LOINA was low, with animals homozygous for the minor allele (GG) showing slightly higher LOINA values (redness), but this association was declared non-significant (P-value = 0.132). This could be due to either the lack of an association, or the low number of samples used in this study. The MAF of rs340803577 was somewhat low (0.16), which means a larger sample could be needed to detect a significant result. Conversely, the P-values for the association between a mutation within *RTN4* (rs340803577) and QUADL, QUADB, and ILIOL were all high, with P-values equal to 0.997, 0.396, and 0.488, respectively. These results are likely indicating the SNP is not a potential causative mutation for pork colour in the population.

In this study, the SNPs were chosen based primarily on their predictive effects (missense, deleterious, with a SIFT score < 0.05), however, the position of the SNP within the protein may have been more important than the SNP effects themselves. The rs336462969 and rs340803577 were located within the protein coding region that were evolutionarily conserved in mammals, but were located outside of the major important protein functional domains, indicating that they may not have had a large effect on the protein structure or function. Therefore, restricting the search for a causative mutation within these evolutionarily conserved and functionally important regions, as well as reducing the stringent SIFT score requirement in this study to include non-deleterious SNPs, could have been a more successful approach for predicting causative

mutations, as even minor changes in highly conserved regions could translate to major changes in protein structure and function (Thusberg & Vihinen, 2009). Further, more attention is now being directed towards investigating non-coding and regulatory region of the genome (such as transcription factor binding sites, enhancer regions, and histone or other DNA modification sites), as changes in these regions of the genome could result in the gene being expressed in the wrong place or at the wrong time, or it could reduce, eliminate, or increase the expression of a protein, potentially contributing to variability of phenotypes (Vockley et al., 2017). Interestingly, a previous study by Te Pas et al. (2013) found a significant association between expression levels of *RTN4* and meat pH. Therefore, the causative mutation for *RTN4* may not be located within its coding region, and may instead be found in its regulatory region.

4.4. Conclusions

A single-SNP association analysis was performed to identify potential causative mutations associated with meat colour traits in a Canadian pig population. *MYOD1* and *RTN4* genes were located within a QTL that was declared significantly associated with meat colour phenotypes in a previous GWAS (Yang et al., 2017). However, potential causative mutations within these candidate genes were not directly tested for an association with meat quality traits, limiting their use in selection programs. In this study, potential causative mutations were identified within *MYOD1* and *RTN4* based on the predicted effect of the SNPs as well as their location within the protein coding region. It was hypothesized that SNPs within the coding regions of these genes, with missense and deleterious effects, were responsible for the effects observed in the previous GWAS. However, no significant relationships were found between potential causative mutations

and meat colour phenotypes. These results suggest that the causative mutations responsible for the previously observed significant associations must reside in an alternative region within the QTL, which could either be within a different protein region in the candidate genes (regulatory region or evolutionarily conserved protein domains) or within a different gene entirely. Further studies are needed to identify causative mutations so that they can be included as informative markers for meat colour traits in MAS or GS programs.

4.5. References

Apweiler, R. (2009). The universal protein resource (UniProt) in 2010. Nucleic Acids Research, 38(s.1), D142–D148. https://doi.org/10.1093/nar/gkp846

Blau, H. M. (1988). Hierarchies of regulatory genes may specify mammalian development. Cell, 53(5), 673–674. https://doi.org/10.1016/0092-8674(88)90082-7

Channon, H. A., Payne, A. M., & Warner, R. D. (2000). Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. Meat Science, 56(3), 291–299. https://doi.org/10.1016/S0309-1740(00)00056-5

Chen, Y. C., Wu, B. K., Chu, C. Y., Cheng, C. H., Han, H. W., Chen, G. D., Lee, M. T., Hwang, P. P., Kawakami, K., Chang, C. C., & Huang, C. J. (2010). Identification and characterization of alternative promoters of zebrafish Rtn-4/Nogo genes in cultured cells and zebrafish embryos. Nucleic Acids Research, 38(14), 4635–4650. https://doi.org/10.1093/nar/gkq230

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., & Kim, B. C. (2008). The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Science, 80(2), 355–362. https://doi.org/10.1016/j.meatsci.2007.12.019

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics, 159(3), 1151–1162.

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., & Maclennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor

associated with malignant hyperthermia. Science, 253(5018), 448–451. https://doi.org/10.1126/science.1862346

Gentry, J. G., McGlone, J. J., Blanton, J. R., & Miller, M. F. (2002). Alternative housing systems for pigs: Influences on growth, composition, and pork quality. Journal of Animal Science, 80(7), 1781–1790. https://doi.org/10.2527/2002.8071781x

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., Thompson, R. (2015). ASReml User Guide Release 4.1, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-4.1-Functional-Specification.pdf

Hamilton, D. N., Ellis, M., McKeith, F. K., & Eggert, J. M. (2003). Effect of level, source, and time of feeding prior to slaughter of supplementary dietary magnesium on pork quality. Meat Science, 65(2), 853–857. https://doi.org/10.1016/S0309-1740(02)00291-7

Houben, J. H., Eikelenboom, G., & Hoving-Bolink, A. H. (1998). Effect of the dietary supplementation with vitamin E on colour stability and lipid oxidation in packaged, minced pork. Meat Science, 48(3–4), 265–273. https://doi.org/10.1016/S0309-1740(97)00103-4

Hughes, S. M., Koishi, K., Rudnicki, M., & Maggs, A. M. (1997). MyoD protein is differentially accumulated in fast and slow skeletal muscle fibers and required for normal fiber type balance in rodents. Mechanisms of Development, 61(1–2), 151–163. https://doi.org/10.1016/S0925-4773(96)00631-4

Hunt, S. E., McLaren, W., Gil, L., Thormann, A., Schuilenburg, H., Sheppard, D., Parton, A., Armean, I. M., Trevanion, S. J., Flicek, P., & Cunningham, F. (2018). Ensembl variation

resources. Database: The Journal of Biological Databases and Curation, 2018(8), 1–12. https://doi.org/10.1093/database/bay119

Jozsef, L., Tashiro, K., Kuo, A., Park, E., Skoura, A., Albinsson, S., Rivera-Molina, F., Harrison, K. D., Iwakiri, Y., Toomre, D., & Sessa, W. C. (2014). Reticulon 4 is necessary for endoplasmic reticulum tubulation, STIM1-Orai1 coupling, and store-operated calcium entry. Journal of Biological Chemistry, 289(13), 9380–9395. https://doi.org/10.1074/jbc.M114.548602

Lee, E. A., Kim, J. M., Lim, K. S., Ryu, Y. C., Jeon, W. M., & Hong, K. C. (2012). Effects of variation in porcine MYOD1 gene on muscle fiber characteristics, lean meat production, and meat quality traits. Meat Science, 92(1), 36–43. https://doi.org/10.1016/j.meatsci.2012.03.018

MacLeod, I., Hayes, B., & Goddard, M. (2014). The effects of demography and long-term selection on the accuracy of genomic prediction with sequence data. Genetics, 198(4), 1671–1684. https://doi.org/10.1534/genetics.114.168344

Matthews, J. O., Southern, L. L., Bidner, T. D., & Persica, M. A. (2001). Effects of betaine, pen space, and slaughter handling method on growth performance, carcass traits, and pork quality of finishing barrows. Journal of Animal Science, 79(4), 967–974. https://doi.org/10.2527/2001.794967x

Meuwissen, T., & Goddard, M. (2010). Accurate prediction of genetic values for complex traits by whole-genome resequencing. Genetics, 185(2), 623–631. https://doi.org/10.1534/genetics.110.116590

Meuwissen, T., Hayes, B., & Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics, 157, 1819–1829. https://doi.org/11290733

Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robix, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronee, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., Chardon, P., & Andersson, L. (2000). A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. Science, 288(5469), 1248–1251. https://doi.org/10.1126/science.288.5469.1248

Ngapo, T. M. (2017). Consumer preferences for pork chops in five Canadian provinces. Meat Science, 129, 102–110. https://doi.org/10.1016/j.meatsci.2017.02.022

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2007). International preferences for pork appearance: I. Consumer choices. Food Quality and Preference, 18(1), 26–36. https://doi.org/10.1016/j.foodqual.2005.07.001

Olson, E. N. (1990). MyoD family: a paradigm for development? Genes and Development, 4(9), 1454–1461. https://doi.org/10.1101/gad.4.9.1454

Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S., & Kalm, E. (2007). Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Science, 75(2), 185–195. https://doi.org/10.1016/j.meatsci.2006.03.022

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors Associated with Fresh Meat Color: A Review. Journal of Food Quality, 6(3), 211–237. https://doi.org/10.1111/j.1745-4557.1984.tb00826.x

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7(539). https://doi.org/10.1038/msb.2011.75

Tang, Z., Li, Y., Wan, P., Li, X., Zhao, S., Liu, B., Fan, B., Zhu, M., Yu, M., & Li, K. (2007). LongSAGE analysis of skeletal muscle at three prenatal stages in Tongcheng and Landrace pigs. Genome Biology, 8(6). https://doi.org/10.1186/gb-2007-8-6-r115

Te Pas, M. F. W., Kruijt, L., Pierzchala, M., Crump, R. E., Boeren, S., Keuning, E., Hoving-Bolink, R., Hortós, M., Gispert, M., Arnau, J., Diestre, A., & Mulder, H. A. (2013). Identification of proteomic biomarkers in M. Longissimus dorsi as potential predictors of pork quality. Meat Science, 95(3), 679–687. https://doi.org/10.1016/j.meatsci.2012.12.015

Thusberg, J., & Vihinen, M. (2009). Pathogenic or not? and if so, then how? Studying the effects of missense mutations using bioinformatics methods. Human Mutation, 30(5), 703–714. https://doi.org/10.1002/humu.20938

Van Den Berg, I., Boichard, D., Guldbrandtsen, B., & Lund, M. S. (2016). Using sequence variants in linkage disequilibrium with causative mutations to improve across-breed prediction in dairy cattle: A simulation study. G3: Genes, Genomes, Genetics, 6(8), 2553–2561. https://doi.org/10.1534/g3.116.027730

Velarde, A., Gispert, M., Faucitano, L., Alonso, P., Manteca, X., & Diestre, A. (2001). Effects of the stunning procedure and the halothane genotype on meat quality and incidence of hemorrhages in pigs. Meat Science, 58(3), 313–319. https://doi.org/10.1016/S0309-1740(01)00035-3

Vockley, C. M., Barrera, A., & Reddy, T. E. (2017). Decoding the role of regulatory element polymorphisms in complex disease. Current Opinion in Genetics and Development, 43, 38–45. https://doi.org/10.1016/j.gde.2016.10.007

Wiegand, B. R., Sparks, J. C., Beitz, D. C., Parrish, F. C., Horst, R. L., Trenkle, A. H., & Ewan, R. C. (2002). Short-term feeding of vitamin D3 improves color but does not change tenderness of pork-loin chops. Journal of Animal Science, 80(8), 2116-2121. https://doi.org/10.1093/ansci/80.8.2116

Yang, T., Wang, Z., Miar, Y., Bruce, H., Zhang, C., Plastow, G. (2017). A genome-wide association study of meat colour in commercial crossbred pigs. Journal of Animal Science, 97(4), 721–733. https://doi.org/10.1139/cjas-2016-0248

Yang, Y. S., & Strittmatter, S. M. (2007). The reticulons: A family of proteins with diverse functions. Genome Biology, 8(12), 1–10. https://doi.org/10.1186/gb-2007-8-12-234

Zerbino, D. R., Achuthan, P., Akanni, W., Amode, M. R., Barrell, D., Bhai, J., et al. (2018). Ensembl 2018. Nucleic Acids Research, 46(D1), D754–D761. https://doi.org/10.1093/nar/gkx1098

Zhang, C., Wang, Z., Bruce, H., Kemp, R., Charagu, P., Miar, Y., Yang, T., Plastow, G. (2015). Genome-wide association studies (GWAS) identify a QTL close to PRKAG3 affecting meat pH and colour in crossbred commercial pigs. BMC Genetics, 16(1), 1–12. https://doi.org/10.1186/s12863-015-0192-1

Tables and figures

Table 4.1. Target, probe, and primer sequences for a quantitative PCR (qPCR) SNP genotyping assay for potential causative mutations located within the *MYOD1* (rs336462969) and *RTN4* (rs340803577) genes. Primers and probes were designed using Thermo Fisher Custom TaqMan assay design tool and applied following the standard protocol for a 96-well Fast 10 uL reaction (Thermo Fisher Scientific Ltd., Ottawa, ON, Canada).

SNP ID (Gene)[1]	Target Sequence	Forward Primer	Reverse Primer	Probe 1	Probe 2
rs336462969 (*MYOD1*)	TCA[C/G]GAGC TCCCGGGGCC GGGTGCNCTG C	CGCGCGCACA TGCT	CCTAAAGCCC GAGGAACACT	CGGGAGCTCG TGAGGA	CGGGAGCTCC TGAGGA
rs340803577 (*RTN4*)	TGCCTGCCCTG GACTCNGGAG GNGG[G/A]CGC CTTCCCAGCCA TGCCCTTGTCC	GAAAAACTCA GTGCTTCACCA TCAC	GGTCAATGTTG AAACTTTGTCA GGTAATA	CTTTTCAGCCC AATTTA	CTTTTCAGACC AATTT

[1]Gene name in which the potential causative mutation is located.

Table 4.2. Descriptive statistics for pork colour phenotypes in purebred pigs: abbreviation, number of animals (N), minimum (Min.), mean, maximum (Max.), standard deviation (SD), and coefficient of variation (CV).

Trait	Abbreviation	N	Min.	Mean	Max.	SD	CV (%)
Loin[1] Minolta L*	LOINL	437	41.42	48.77	55.72	2.44	5.01
Loin Minolta a*	LOINA	437	1.07	4.61	8.75	1.18	25.57
Loin Minolta b*	LOINB	437	5.97	13.90	13.90	1.24	0.28
Ham *gluteus medius* Minolta L*	GLUTL	437	41.20	48.00	56.80	2.65	5.520
Ham *gluteus medius* Minolta a*	GLUTA	437	1.20	5.39	9.60	1.31	24.45
Ham *gluteus medius* Minolta b*	GLUTB	437	5.40	8.82	12.40	1.16	13.20
Ham *quadriceps femoris* Minolta L*	QUADL	437	42.10	53.15	68.40	3.41	6.42
Ham *quadriceps femoris* Minolta a*	QUADA	437	-1.00	2.40	13.40	1.56	65.00
Ham *quadriceps femoris* Minolta b*	QUADB	437	4.70	7.98	14.30	1.30	16.29
Ham *iliopsoas* Minolta L*	ILIOL	437	34.70	44.97	55.60	2.88	6.41
Ham *iliopsoas* Minolta a*	ILIOA	437	8.80	16.48	22.40	2.17	13.19
Ham *iliopsoas* Minolta b*	ILIOB	437	7.40	11.68	15.80	1.34	11.54

[1]*Longissimus thoracis et lumborum*

(A)

```
Sus_scrufa          EHSHFPAAAHPAPGAREDEHVRAPSGHHQAGR
Bos_taurus          EHSHFPAAAHPAPGAREDEHVRAPSGHHQAGR
Ovis_aries          EHSHFPAAAHPAPGAREDEHVRAPSGHHQAGR
Mus_musculus        EHAHFSTAVHPGPGAREDEHVRAPSGHHQAGR
Homo_sapien         EHSHFPAAVHPAPGAREDEHVRAPSGHHQAGR
```

(B)

```
Sus_scrofa          LSASPSLEGGKPYLESFQPNLDTTKDTLLPDKVSTLTQ
Bos_taurus          LSASPSPEGGKPYLESFQPSLGITKDTLAPDEVSALTQ
Ovis_aries          LSASPPPEGGKPYLESFQPSLGITKDTLAPDEVSALTQ
Mus_musculus        LSASP-QEVGKPYLESFQPNLHITKDAA-SNEIPTLTK
Homo_sapien         LSALP-PEGGKPYLESFKLSLDNTKDTLLPDEVSTLSK
```

Figure 4.1. Multiple sequence alignments for regions within candidate genes *MYOD1* (rs336462969) and *RTN4* (rs340803577) containing potential causative mutations for meat colour traits in purebred pigs. (A) Exon one of *MYOD1* contains the deleterious missense mutation R76P (highlighted). The protein sequences for *MYOD1* were obtained from the NCBI database, accession numbers NP_001002824, NP_001035568, NP_001009390, NP_034996, NP_002469. (B) Exon three of *RTN4* contains the deleterious missense mutation P754T (highlighted). The protein sequences for *RTN4* were obtained from the NCBI database, accession numbers XP_005662597, XP_005212628, XP_027821518, NP_918943, NP_065393.

Table 4.3. Genotype and allele frequencies for potential causative mutations located within the MYOD1 (rs336462969) and RTN4 (rs340803577) genes in purebred pigs.

Gene	SNP ID	Genotype / Allele	Genotype and allele frequency
MYOD1	rs336462969	CC	0.70 (307)[1]
		CG	0.26 (114)
		GG	0.04 (16)
		C	0.84
		G	0.16
RTN4	rs340803577	CC	0.51 (222)
		AA	0.42 (184)
		CA	0.07 (31)
		C	0.71
		A	0.29

[1]Number of animals in each genotype class.

Table 4.4. The least square means for genotype classes of potential causative mutations within the (A) *MYOD1* (rs336462969) and (B) *RTN4* (rs340803577) genes on meat colour traits in purebred pigs.

(A)

Geno-type	LOIN L	LOIN A	LOIN B	GLUT L	GLUT A	GLUT B	QUAD L	QUAD A	QUAD B	ILIOL	ILIOA	ILIOB
CC	48.69 ± 0.20	4.53 ± 0.20	9.26 ± 0.14	48.03 ± 0.43	5.32 ± 0.24	8.86 ± 0.15	53.05 ± 0.35	2.25 ± 0.30	8.01 ± 0.11	45.01 ± 0.46	16.63 ± 0.34	11.77 ± 0.13
CG	49.01 ± 0.26	4.77 ± 0.22	9.46 ± 0.16	47.83 ± 0.47	5.17 ± 0.25	8.73 ± 0.17	53.39 ± 0.43	2.25 ± 0.32	8.00 ± 0.14	44.63 ± 0.50	16.45 ± 0.30	11.66 ± 0.16
GG	48.69 ± 0.59	4.69 ± 0.35	9.18 ± 0.31	48.63 ± 0.78	5.23 ± 0.39	9.09 ± 0.29	52.99 ± 0.91	2.33 ± 0.50	8.09 ± 0.33	45.18 ± 0.87	17.03 ± 0.62	11.72 ± 0.35
P-values	0.345	**0.132**	0.323	0.972	0.401	0.755	0.538	0.930	0.923	0.848	0.273	0.518

(B)

Geno-type	LOIN L	LOIN A	LOIN B	GLUT L	GLUT A	GLUT B	QUAD L	QUAD A	QUAD B	ILIOL	ILIOA	ILIOB
CC	48.79 ± 0.21	4.53 ± 0.20	9.26 ± 0.14	48.16 ± 0.46	5.33 ± 0.23	8.91 ± 0.16	53.12 ± 0.36	2.134 ± 0.30	7.98 ± 0.11	44.87 ± 0.48	16.48 ± 0.32	11.74 ± 0.15
CA	48.82 ± 0.21	4.67 ± 0.20	9.26 ± 0.15	47.87 ± 0.46	5.19 ± 0.23	8.71 ± 0.16	53.22 ± 0.37	2.32 ± 0.30	8.01 ± 0.12	44.97 ± 0.48	16.56 ± 0.32	11.76 ± 0.15
AA	48.54 ± 0.42	4.71 ± 0.28	9.40 ± 0.23	47.64 ± 0.63	5.47 ± 0.31	8.93 ± 0.23	52.92 ± 0.66	2.70 ± 0.40	8.23 ± 0.23	44.85 ± 0.68	16.62 ± 0.48	11.68 ± 0.26
P-values	0.764	0.221	0.312	0.200	0.812	0.268	**0.997**	0.064	**0.396**	**0.488**	0.65	0.903

Note: Bolded P-values represent a previously identified significant associated between the colour traits and a QTL that harbored the potential causative mutations (Zhang et al., 2015).

Chapter 5. General discussion

5.1. Introduction

The economic importance of meat quality is increasing, and providing consumers with a product that is both lean and consistently satisfies their quality preferences today, will secure Canadian pork as a top protein choice in the future. Genomic selection (GS) provides a significant opportunity to select for pigs with superior meat quality, as this trait is difficult and expensive to measure. In general, GS follows a two-step procedure: firstly, animals in a reference population must be genotyped and phenotyped (training population), then if the genetic parameters for the trait in the specific population are known, the breeding value of selection candidates can be predicted using only its genotype information (Meuwissen et al., 2001). There are multiple GS methods, but the most common methods are, genomic best linear unbiased prediction (GBLUP), or a similar method single-step GBLUP (ssGBLUP). The underlying assumptions of these GBLUP methods are simple, each SNP contributes an equal and small effect on a complex trait. This assumption has been critical in the implementation of GS, as identification of the exact gene or causative mutation responsible for complex trait phenotype is not required. However, this ignores any genetic or biological information known for the trait, as in fact SNPs associated with a trait may be distributed non-uniformly throughout the genome, and clustered in genes that are biologically relevant for the trait (Allen et al., 2010; Maurano et al., 2012). Therefore, alternative methods, such as weighted GBLUP (wGBLUP) (Zhang et al., 2010; Brøndum et al., 2015), genomic feature BLUP (GFBLUP) (Edwards et al., 2016), or BayesRC (MacLeod et al., 2016), that consider the biology underlying traits have been proposed. Although, these methods remain

limited as much still remains unknown regarding the genes and variants determining traits as well as their exact function in livestock animals. Nowadays, high-throughput sequencing technologies allowing high-density SNP genotyping, along with advancements in the annotation of livestock genomes, have improved the ability to detect genes and variants underlying complex trait phenotypes as well as their associated biological pathways. For this reason, research has begun to focus on the genetic factors underlying economically important traits in livestock. Results from these studies contribute to the overall understanding of trait biology, and can subsequently be incorporated into GS procedures to improve breeding value prediction accuracy, and further accelerate genetic improvement, over generations, and across populations.

Genome-wide association studies (GWAS) have been a primary tool to determine the biology underlying complex traits. GWAS uses single nucleotide polymorphisms (SNPs), which are spread across the entire genomes of plant and animal species, and can be used to detect regions of the genome associated with traits of interest (quantitative trait loci, QTL). Numerous GWAS have been performed in pigs for meat and carcass quality traits, for which QTL have been found across all chromosomes, and include multiple genes that contribute large effects (Hu et al., 2005). Results from GWAS studies can provide insights into the genetic architecture underlying traits, such as the candidate genes or causative mutations responsible for the genetic variation of complex phenotypes. This will help to progress the current understanding the post-mortem muscle biochemistry, which overall will be important for the future implementation of alternative GS methods for improving the meat quality in pigs.

The purpose of this thesis was: firstly, to use variance component estimates to calculate genetic parameters for meat and carcass quality traits in purebred Canadian Duroc pigs. This provided useful estimates of heritability for meat and carcass quality traits, as well as insights into the

genetic relationships between traits, which can be used to incorporate these traits into current breeding procedures (traditional or GS) (chapter 2). Secondly, the biological factors underlying meat quality traits were explored using single-SNP association analysis, which included a GWAS to identify QTL and genes associated with drip loss (DL) in chapter 3, as well as a single-SNP association analysis of two potential causative mutations affecting meat colour in chapter 4. These two research chapters were intended to contribute to an improved understanding of the biology underlying meat quality traits, which would facilitate the use of alternative methods of GS that incorporate biological knowledge so that they can be implemented in the future. In response to the results of these chapters, the following will discuss: 1) increasing the statistical power of GWAS to detect QTL, 2) strategies for an effective post-GWAS analysis, 3) future perspectives for pig breeding.

5.2. Increasing the statistical power of genome-wide association studies

GWAS has been found to be a powerful tool to study trait biology, however, a major downside of this method, that was also experienced in this study, is that GWAS can often only account for a small proportion of the total genetic variation underlying complex traits. In chapter three, a single-SNP GWAS was performed with the intention to improve the biological understanding of DL, however this study was underpowered in its ability to detect QTL significantly associated with DL phenotypes. A Bayesian least absolute shrinkage and selection operator (LASSO) approach was also used in this study, as it has been previously shown to improve detection power of GWAS compared to other Bayesian methods (Yang et al., 2015, Yang et al., 2017), but this method was also unsuccessful. The positive and negative aspects of each statistical method

was discussed in detail in chapter three, but in short, Bayesian methods tend to perform best for traits controlled by fewer genes of moderate to large effect size, so it is likely that the genetic architecture of DL does not align with the underlying assumption of Bayesian LASSO. Therefore, additional research is needed to improve detection power of GWAS and reveal the biological basis of meat quality traits.

The underlying assumption of GWAS follows the "common disease-common variant" hypothesis, which assumes that common variants, are responsible for complex trait phenotypes (Botstein & Risch, 2003). However, common variants, individually or in combination, have only been able to account for a small proportion of the total genetic variation underlying complex traits, a phenomenon that has been termed the "missing heritability" problem (Yang, et al., 2011). Human height provides a classic example of the missing heritability problem, where thousands of variants have been found associated with human height, but these variants only translate to approximately 25% of the total variance for the trait, which proves that a few genes of moderate effect, and intermediate frequency are not responsible for complex traits phenotypes (Yengo et al., 2018). The most common hypothesis explaining this so-called "missing heritability" problem is that common variants with small effect sizes are underlying complex traits (Yang, et al., 2011). Yang et al. (2011) showed that by considering all SNPs simultaneously, common variants could explain 45% of the total phenotypic variance for human height ($h^2 = 0.85$), but due to lack of statistical power, small effect common variants are unable to pass the significance threshold required by single-SNP GWAS. Yang et al. (2011) also hypothesized that the remaining variance, which could not be accounted for in their study, is due to causative mutations that are not in complete (LD) with SNP markers on the genotyping panel. This is likely due to differences in minor allele frequencies (MAF), and causes a reduction in the

power to detect the variants underlying complex traits (Yang, et al., 2011). This is because markers on the genotyping panel will not always be associated with the nearby causative mutation, and thus SNP markers will not reliably be associated with the complex trait. This problem is especially exacerbated when causative mutations have low MAF (< 0.01), making their detection especially difficult. Therefore, ensuring adequate statistical power (the probability of correctly declaring a statistically significant association of a defined effect size with P-value ≤ α (significance level)) in subsequent GWAS, for complex traits such as DL, will ensure studies are able to detect at least a larger proportion of the variants responsible for the underlying genetic variation of complex traits. This can be done by either: 1) increasing sample size, or 2) increasing marker panel density.

The first method to improve power is to increase sample size. The power to detect common, moderate to large effect SNPs is high, even in situations where the sample size may be small, however, if the causative mutations for the trait have low effect size, then they will require additional power for detection (Spencer et al., 2009). For example, a simple power analysis was performed using a chi-squared statistical test in the *pwr* package version 1.3-0 of R software (Champely et al., 2020). Given the circumstances of the GWAS performed in this study (n = 1098, $\alpha = 1.2\times10^{-6}$), the power to detect a significant association from a moderate (0.2) to high (0.5) effect size variant is high, 0.97, and 1.0, respectively. On the other hand, the power to detect a low effect size variant (0.1) is exceptionally low, 0.06 (type II error rate = 0.94), and consequently, in this study, there was only a 6% chance that a significant association for low effect size variants would be correctly detected. Therefore, to detect a low effect size (0.1) variant with a standard power of 0.8, a much larger sample size than what was used in this study

will be required (n = 3,246). As a result, it can be concluded that DL is a complex trait controlled by many genes of small effect sizes.

Alternatively, the power of GWAS could be improved by increasing marker panel density. Today, standard genotyping chips contain 50/60K SNP markers, but higher density marker panels are available (660/770K), as well as whole genome sequence (WGS). Increasing marker panel density will either increase the strength of LD between SNPs and causative mutations, or in the case that WGS data is used, then this will reveal causative mutations that are not genotyped in the marker panels. WGS provides the most potential for improving statistical power, as it eliminates the need for markers that are in high LD with causative mutations, as causative mutations are expected to be included in WGS data. However, although the cost of WGS has gone down in recent years (WGS costs approximately $1000 per individual), the cost of sequencing thousands of animals for a GWAS would greatly outweigh the potential benefits. As a result, WGS incorporating imputation has been proposed as a low-cost alternative to improve the power of GWAS. To do this, a small subset of the population must be sequenced, and the remainder of the animals can be genotyped using a low-density marker panel, then the missing genotypes will be inferred (imputed) from the WGS data. This method has successfully improved QTL detection for multiple traits in pigs (Yan et al., 2018; Wu et al., 2019; Li et al., 2020).

At first glance, increasing the number of animals is the most straightforward and effective solution to improving GWAS power, however, with regards to meat and carcass quality traits, this may not be the most feasible and cost-effective solution. In pig breeding, selection generally occurs at the level of purebred animals, however the breeding animals, which genotype data is commonly recorded, are not sent to slaughter where their phenotypes could be recorded. On the

other hand, recording the phenotypes of crossbred animals, which are produced for the purpose of slaughter, may provide a feasible solution to increase the number of phenotyped individuals, however, these animals are not commonly genotyped, and doing so would generate a substantial increase in cost. Therefore, implementing WGS and imputation methods to increase marker panel density, so that a smaller population of either purebred or crossbred animals can be used, may provide the best solution to improving the power of GWAS for meat and carcass quality traits.

5.3. Strategies for an effective post-GWAS analysis

The purpose of GWAS is to identify QTL that are significantly associated with complex trait phenotypes to reveal the underlying biology. However, GWAS results do not explicitly specify the gene or causative mutation that is actually responsible for the significant association. Therefore, strategies that relate SNP markers to the appropriate candidate genes, causative mutations, or other genomic elements are the crucial last step to the success of GWAS.

The interpretation and fine mapping of GWAS results can be complicated by adjacent SNP markers can be in strong linkage disequilibrium (LD) (Amaral et al., 2008). As a result, QTL can span several megabases in length and contain dozens of potential candidate genes, or, in some cases, QTL may not contain any genes at all. Further, SNPs that are closest to the causative mutation will be indistinguishable from the neighboring SNP, limiting the use of GWAS for identifying the biological factors that are underlying the observed phenotype variation for the trait of interest. This shows that even if sample sizes and marker panel densities are increased, and more QTL are found to be significantly associated with complex traits, there is no guarantee

that genetic factors contributing to the trait will be identified. Therefore, strategies are necessary to improve post-GWAS analysis, such as: 1) further annotation of the pig genome, and 2) fine mapping and gene set enrichment.

Structural and functional annotation of the pig genome, describing the location of genes and features as well as their functions, are essential for biological insights from GWAS. However, annotation of livestock genomes is limited. Gene coding regions have been annotated to some extent, but the regulatory elements of livestock genomes remain mostly unknown. This is problematic as most known SNPs are located in the non-coding and regulatory regions of genomes (Auton et al., 2015). Further, in both humans and cattle, SNPs within regulatory regions have been found to represent a large proportion of SNPs significantly associated with economically important traits (Van Laere et al., 2003; Schaub et al., 2012; Koufariotis et al., 2014). Studies such as the Functional Annotation of Animal Genomes (FAANG, https://www.animalgenome.org/community/ FAANG/) aim to fill this gap in knowledge by generating genome-wide and tissue-specific datasets and functional maps in livestock species (similar projects have been completed in humans, the Encyclopedia of DNA Elements (ENCODE) project (https://www.encodeproject. org/) (Dunham et al., 2012; Andersson et al., 2015). Additionally, a project stemming from FAANG, called the regulatory GENomE of SWIne and CHicken (GENE-SWitCH), focuses specifically on the coding and regulatory elements of pigs and chicken genomes in tissues and timepoints that are relevant to sustainable meat production. Then this information can be used to develop new genomic prediction models, which are subsequently directly validated and integrated into commercial pig and chicken populations (https://www.geneswitch.eu/project. html). These FAANG projects will use tools such as RNA-sequencing (RNA-seq) to determine tissue specific gene expression as well as to identify novel

gene transcripts, gene isoforms, and non-coding RNA elements (miRNA, lncRNA). Tissue specific epigenetic maps will also be constructed using techniques such as, RNA-seq, bisulfite sequencing, chromatin immunoprecipitation followed by sequencing (ChIP-Seq), and DNAse hypersensitivity assays to identify sites of DNA-methylation, histone tail modifications, and open chromatin regions. ChIP-Seq can also be used to sequence and localize DNA-binding sites for transcription factors or other types of DNA-protein interactions (such as promoters, enhancers, silencers, and insulators) (Andersson et al., 2015). Once these genomic elements are known, this information can aid in post-GWAS analysis to connect genotype information to observed phenotype. SNPs overlapping regulatory elements could be altering regulatory function by changing chromatin conformation, disrupting binding sites, and preventing DNA-protein interactions, resulting in altered transcriptional activity, thus contributing to the phenotypic variation of complex traits. Alternatively, SNPs in regulatory elements could be influencing gene expression by affecting post-transcriptional processes such as the stability of the mRNA (Pai et al., 2015). Further, expression QTL (eQTL) analysis will also be performed to detect a significant association between SNPs and transcriptomics data (the sum of all messenger RNA (mRNA) molecules expressed within a particular tissue at a particular time). Results from these studies will further increase the value of regulatory information, as it will connect regulatory elements to their target genes, implicating their biological roles and pathways.

Fine mapping of GWAS results to identify causative genes and mutations is essential for the understanding of the biological mechanism underlying traits. In chapters three and four of this thesis, candidate genes were chosen based on known biological information on genes (Yang et al., 2017), then in chapter four, potential causative mutations were found in the Ensembl Genome Browser and selected based on their predicted effects (non-synonymous, missense variants) as

well as their location within the protein sequence (functional domains). These methods were somewhat successful, as in chapter three, two out of the five genomic regions that were investigated contained genes with biologically relevant roles in DL. Conversely, this method was unsuccessful in chapter four, as no potential causative mutations were found to be significantly associated with meat colour traits. More sophisticated methods to fine map causative genes and mutations that incorporate population specific variants as well as regulatory and functional data are available for use in livestock animals, and should be implemented in subsequent studies to identify potential candidate genes and causative mutations.

The most straightforward fine-mapping approaches look for overlap between population-specific SNP variants and functional elements (Broekema et al., 2020). WGS followed by imputation can be used to identify all of the variants segregating within a population, then this information can be overlapped with QTL to determine if these variants reside in functional elements (genes, promoters, enhancers, methylation sites, or other regulatory elements). Further, the filtering of WGS variants based on known functional effects will help to narrow down potential candidate genes and variants, as not all variants will cause a functional disruption of the gene or regulatory element (Broekema et al., 2020). For example, a non-synonymous SNP located within the exons of a gene, such as those used in chapter four of this study, are more likely to be causative variants than those located in the intron. Alternatively, in a GWAS by Velez-Irizarry et al. (2019), eQTL data was used to infer SNP functionality. In this case, RNA-seq analysis of the *longissimus thoracis et lumborum (*loin*)*muscle of Duroc X Pietran pigs was used to identify tissue specific eQTL. Subsequently, these eQTL were overlapped with QTL that were found to be significantly associated with growth and meat quality phenotypes. This technique greatly reduced the number of potential candidate genes to 16 genes for 21 QTL. Additionally, the

resulting candidate genes were found to be biologically relevant in post-mortem meat biochemistry, and included pathways such as calcium signaling, energy metabolism, and redox homeostasis, (Velez-Irizarry et al., 2019). One downside of using expression data that functional disruption of regulatory elements causing changes in gene expression does not guarantee causality. This is because, in some cases, gene expression may be controlled post-translationally, meaning changes in gene expression do not result in changes to downstream processes (Broekema et al., 2020). This highlights the importance of extra omics data to enable more accurate predictions of complex trait phenotypes. Other types of functional QTL data, that can be used similarly to eQTL data, include the quantity of, proteins (pQTL), metabolites (mQTL), DNA methylation patterns (meQTL), microbiota (miQTL), or cells (cell-count, ccQTL) (Broekema et al., 2020).

In addition to the use of functional elements, statistical fine-mapping approaches can greatly narrow down QTL regions and the list of potential causative genes or variants. Bayesian methods can be used to determine the probability that a SNP marker is causative by assessing LD structure and patterns of association, resulting in a set of SNPs that are isolated to much shorter genomic regions (Gallagher & Chen-Plotkin, 2018). Li et al. (2020) used this approach for a GWAS for litter size in pigs. Two QTL declared statistically significant in a GWAS were fine-mapped using a Bayesian factor modelling via Markov Chain Monte Carlo (BayesFM-MCMC) approach. This narrowed QTL regions of approximately one and two megabases down to 100 and 870 kilobases, respectively. Although the fine-mapped QTL contained too many SNPs for identification of causal mutations, 71 and 432 SNPs, respectively, four potential candidate genes with biologically relevant roles were identified using this method (Li et al., 2020). It is important to note that these fine mapping approaches need not assume a single gene or variant is

responsible for the variation contributed a single GWAS peak. In many cases, it is likely that multiple variants or even multiple genes are responsible for the GWAS signal. Therefore, a method termed conditional analysis can be performed to determine if a GWAS signal is due to a single locus in an area of high LD or multiple loci that are either weakly linked or unlinked, then this information can be considered during fine-mapping analysis (Gallagher & Chen-Plotkin, 2018).

A final step that can be used to extract biological information from GWAS results is known as gene set enrichment. This tool can be used to determine if a predefined set of genes (such as the candidate genes identified in GWAS) is enriched for a specific function or biological pathway than would be expected by chance (Kao et al., 2017). Online databases are available, such as Gene Ontology (GO) (Ashburner et al., 2000), the Kyoto Encyclopedia of Genes and Genomes pathway analysis (KEGG) (Ogata et al., 1999), and ingenuity pathway analysis (IPA) (Krämer et al., 2014), which allow you to test gene sets for an enrichment in functional pathways, networks, gene ontologies, or associated gene sets (Kao et al., 2017). Gene set enrichment has been shown to be a successful approach for the study of complex trait biology in both pigs (Falker-Gieske et al., 2019) and cattle (Cai et al., 2018). The only downside being that this method is biased in terms of available biological knowledge, and those genes with minimal information on their biological function or pathway will not prioritized as a top candidate gene even if it does play a role in the complex trait phenotype (Kao et al., 2017).

5.4. Future perspectives for pig breeding

This chapter has discussed common challenges associated with GWAS that were experienced in the research chapters of this thesis, as well as some methods to improve the detection of GWAS signals and their appropriate genes or variants. However, it is important to note that, although improving statistical power to identify QTL and advancing the knowledge of gene and regulatory annotation will greatly improve the ability to detect the genes and variants associated with complex traits in the future, as simply identifying a mass amount of candidate genes may not be sufficient for the useful application of biological information into alternative GS procedures to improve accuracy of selection. Gallagher et al. (2018) suggested that there should be an increased emphasis on the downstream functional activity of GWAS loci, as the identification of more and more GWAS signals with smaller and smaller effect sizes, could simply result in the identification of all the genes expressed in the "disease-relevant" or "complex trait-relevant" cell or tissue type, with no knowledge on the pathways or interactions that connect them. This hypothesis stems from an "omnigenic" perspective, which was proposed by Boyle et al. (2018), where every component that acts within a specific cell or tissue type, is expected to some extent influence complex trait phenotypes, with only a few core genes making a large and direct influence on the trait of interest (Boyle et al., 2018). Therefore, going forward, it may be best to consider the larger picture.

GWAS results should be integrated with other relevant and informative biological data, or they can be used to point downstream research towards other methods that could be used to explore the functional consequences of SNPs that are associated with complex traits. Systems genetic approaches, which have been reviewed previously (Suravajhala et al., 2016; Georges et al., 2019), attempt to explain how DNA variants relate to phenotype, using data from

transcriptomics, proteomics, metabolomics, epigenomics, and other regulatory information. Even more, gene function as well as the consequences of potential causative mutations can also be studied using molecular experimental approaches, such as genome-editing (CRISPR/Cas9) (Hsu et al., 2014).

If more becomes known about the biologically relevant pathways and their components that are controlling meat quality phenotypes, then this information can be used to classify variants based on their potential predictive ability, and even those that haven't been identified in GWAS, can subsequently be used to inform GS procedure and improve breeding value prediction accuracy. If biological pathways and their constituents are known to influence a specific trait, then these regions can be assigned a higher weight or can be assumed to account for a higher proportion of SNP effects. Eventually, this biological knowledge may even be able to be used to identify a set of genes which could be used as targets for genome editing to induce variation into livestock populations and improve complex trait phenotypes (Georges et al., 2019).

Three major genome editing systems exist for livestock, including the Zinc-Finger Nucleases (ZNF) (Miller et al., 1985), Transcription Activator-Like Effector Nucleases (TALENs) (Göhre & Robatzek, 2008), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated 9 (Cas9) system (Horvath & Barrangou, 2010). These genome editing tools work by inducing double stranded breaks (DSB) into a target site in the DNA, which activates DNA repair pathways. One repair pathway is called non-homologous end joining (NHEJ), where the DSB is ligated together without the need for a repair template. This method is error prone and results in small insertions or deletions within the gene, often causing its loss of function (called a knock-out, KO). Alternatively, if a homologous DNA repair template is present, then DSBs are repaired by homology-directed repair (HDR), where precise

modifications are introduced according to the template sequence (Georges et al., 2019). Genome editing techniques have been successfully used in multiple types of livestock species, including pigs, cattle, sheep, and chicken, but some examples of the implementation in pigs include the *CD163* and myostatin (*MSTN*) genes. Modification of the *CD163* gene using CRISPR/Cas 9 resulted in pigs resistant to porcine reproductive respiratory syndrome virus (PRRSV) (Whitworth et al., 2016). Further, pigs with modified *myostatin* (*MSTN*) genes have been produced to improve meat and carcass yield (Hauschild et al., 2011; Cyranoski, 2015; Wang et al., 2015). Previously, in livestock, the main purpose of genome editing was to produce laboratory animals for the study of human diseases, but once more is known of the biology underlying complex and economically important traits in livestock, then this technology can be used to induce variation or novel changes that are not present in the breeding population (Georges et al., 2019). A method termed "promotion of alleles by genome editing" (PAGE) has been proposed to combine genome editing with GS, where the breeding animal would be edited by HDR for causative variants so they are homozygous for the favorable allele. This method is predicted to increase the rate of genetic gain by two to four times (Georges et al., 2019).

5.5. Conclusions

The results of this study can be used to help improve the meat quality of pigs in Canada. By incorporating novel meat and carcass quality traits into breeding program, and being able to select for these traits with a high accuracy, then rapid genetic gain can be accomplished. This could have a major effect on the pig industry, as by improving consumer appeal to purchase and repurchase pork as their source of protein in the future meat market, product demand and value

will improve, and the potential profit of each participant in the pork production chain will be improved. Although additional research, of sufficient power, will be required before some of the results of this thesis can be directly applied to breeding and selection decisions, they provide important background for which future research can be built upon.

5.6. References

Allen, H., Estrada, K., Lettre, G., Berndt, S., Weedon, M., Rivadeneira, F., Willer, C., Jackson, A., Vedantam, S., & Raychaudhuri, S. et al. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature, 467(7317), 832–838. https://doi.org/10.1038/nature09410

Amaral, A. J., Megens, H. J., Crooijmans, R. P. M. A., Heuven, H. C. M., & Groenen, M. A. M. (2008). Linkage disequilibrium decay and haplotype block structure in the pig. Genetics, 179(1), 569–579. https://doi.org/10.1534/genetics.107.084277

Andersson, L., Archibald, A. L., Bottema, C. D., Brauning, R., Burgess, S. C., Burt, D. W., et al. (2015). Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome Biology, 16(1), 4–9. https://doi.org/10.1186/s13059-015-0622-4

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene ontology: tool for the unification of biology. Nature Genetics, 25, 25–29. https://doi.org/10.1038/75556

Auton, A., Abecasis, G. R., Altshuler, D. M., Durbin, R. M., Bentley, D. R., Chakravarti, A., et al. (2015). A global reference for human genetic variation. Nature, 526(7571), 68–74. https://doi.org/10.1038/nature15393

Botstein, D., & Risch, N. (2003). Discovering genotypes underlying human phenotypes: Past successes for mendelian disease, future approaches for complex disease. Nature Genetics, 33, 228–237. https://doi.org/10.1038/ng1090

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2018). An expanded view of complex traits: from polygenic to omnigenic. Cell, 169(7), 1177–1186. https://doi.org/doi:10.1016/j.cell.2017.05.038

Broekema, R. V., Bakker, O. B., & Jonkers, I. H. (2020). A practical view of fine-mapping and gene prioritization in the post-genome-wide association era. Open Biology, 10(1). https://doi.org/10.1098/rsob.190221

Brøndum, R. F., Su, G., Janss, L., Sahana, G., Guldbrandtsen, B., Boichard, D., & Lund, M. S. (2015). Quantitative trait loci markers derived from whole genome sequence data increases the reliability of genomic prediction. Journal of Dairy Science, 98(6), 4107–4116. https://doi.org/10.3168/jds.2014-9005

Cai, Z., Guldbrandtsen, B., Lund, M. S., & Sahana, G. (2018). Prioritizing candidate genes post-GWAS using multiple sources of data for mastitis resistance in dairy cattle. BMC Genomics, 19(1), 1–11. https://doi.org/10.1186/s12864-018-5050-x

Champely, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., Ford, C., & Volcic, R. (2020). Basic Functions for Power Analysis. 1–22. https://cran.r-project.org/web/packages/pwr/pwr.pdf

Cyranoski, D. (2015). Super-muscly pigs created by small genetic tweak. Nature, 523, 13–14. https://doi:10.1038/523013a

Dunham, I., Kundaje, A., Aldred, S. F., Collins, P. J., Davis, C. A., Doyle, F., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. Nature, 489(7414), 57–74. https://doi.org/10.1038/nature11247

Edwards, S., Sørensen, I. F., Sarup, P., Mackay, T. F. C., & Sørensen, P. (2016). Genomic prediction for quantitative traits is improved by mapping variants to gene ontology

categories in Drosophila melanogaster. Genetics, 203(4), 1871–1883. https://doi.org/10.1534/genetics.116.187161

Falker-Gieske, C., Blaj, I., Preuß, S., Bennewitz, J., Thaller, G., & Tetens, J. (2019). GWAS for meat and carcass traits using imputed sequence level genotypes in pooled F2-designs in pigs. G3: Genes, Genomes, Genetics, 9(9), 2823–2834. https://doi.org/10.1534/g3.119.400452

Gallagher, M. D., & Chen-Plotkin, A. S. (2018). The Post-GWAS Era: From Association to Function. American Journal of Human Genetics, 102(5), 717–730. https://doi.org/10.1016/j.ajhg.2018.04.002

Georges, M., Charlier, C., & Hayes, B. (2019). Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 20(3), 135–156. https://doi.org/10.1038/s41576-018-0082-2

Göhre, V., & Robatzek, S. (2008). Breaking the Barriers: Microbial Effector Molecules Subvert Plant Immunity. Annual Review of Phytopathology, 46(1), 189–215. https://doi.org/10.1146/annurev.phyto.46.120407.110050

Hauschild, J., Petersen, B., Santiago, Y., Queisser, A. L., Carnwath, J. W., Lucas-Hahn, A., Zhang, L., Meng, X., Gregory, P. D., Schwinzer, R., Cost, G. J., & Niemann, H. (2011). Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. Proceedings of the National Academy of Sciences of the United States of America, 108(29), 12013–12017. https://doi.org/10.1073/pnas.1106422108

Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of Bacteria and Archaea. Science, 327(5962), 167–170. https://doi.org/10.1126/science.1179555

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

Hu, Z. L., Dracheva, S., Jang, W., Maglott, D., Bastiaansen, J., Rothschild, M. F., & Reecy, J. M. (2005). A QTL resource and comparison tool for pigs: PigQTLDB. Mammalian Genome, 16(10), 792–800. https://doi.org/10.1007/s00335-005-0060-9

Kao, P. Y. P., Leung, K. H., Chan, L. W. C., Yip, S. P., & Yap, M. K. H. (2017). Pathway analysis of complex diseases for GWAS, extending to consider rare variants, multi-omics and interactions. Biochimica et Biophysica Acta - General Subjects, 1861(2), 335–353. https://doi.org/10.1016/j.bbagen.2016.11.030

Koufariotis, L., Chen, Y. P. P., Bolormaa, S., & Hayes, B. J. (2014). Regulatory and coding genome regions are enriched for trait associated variants in dairy and beef cattle. BMC Genomics, 15(1). https://doi.org/10.1186/1471-2164-15-436

Krämer, A., Green, J., Pollard, J., & Tugendreich, S. (2014). Causal analysis approaches in ingenuity pathway analysis. Bioinformatics, 30(4), 523–530. https://doi.org/10.1093/bioinformatics/btt703

Li, Y., Li, B., Yang, M., Han, H., Chen, T., Wei, Q., Miao, Z., Yin, L., Wang, R., Shen, J., Li, X., Xu, X., Fang, M., & Zhao, S. (2020). Genome-Wide Association Study and Fine Mapping Reveals Candidate Genes for Birth Weight of Yorkshire and Landrace Pigs. Frontiers in Genetics, 11, 1–10. https://doi.org/10.3389/fgene.2020.00183

MacLeod, I., Bowman, P., Vander Jagt, C., Haile-Mariam, M., Kemper, K., Chamberlain, A., Schrooten, C., Hayes, B., & Goddard, M. (2016). Exploiting biological priors and

sequence variants enhances QTL discovery and genomic prediction of complex traits. BMC Genomics, 17(1), 1–21. https://doi.org/10.1186/s12864-016-2443-6

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., Reynolds, A. P., Sandstrom, R., Qu, H., Brody, J., Shafer, A., Neri, F., Lee, K., Kutyavin, T., Stehling-Sun, S., Johnson, A. K., Canfield, T. K., Giste, E., Diegel, M., Bates, D., Hansen, R. S., Neph, S., Sabo, P. J., Heimfeld, S., Raubitschek, A., Ziegler, S., Cotsapas, C., Sotoodehnia, N., Glass, I., Sunyaev, S. R., Kaul, R., & Stamatoyannopoulos, J. A. (2012). Systematic localization of common disease-associated variation in regulatory DNA. Science, 337(6099), 1190–1195. https://doi.org/10.1126/science.1222794

Meuwissen, T., Hayes, B., & Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics, 157, 1819–1829. https://doi.org/11290733

Miller, J., McLachlan, A. D., & Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. The EMBO Journal, 4(6), 1609–1614. https://doi.org/10.1002/j.1460-2075.1985.tb03825.x

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., & Kanehisa, M. (1999). KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 27(1), 29–34. https://doi.org/10.1093/nar/27.1.29

Pai, A. A., Pritchard, J. K., & Gilad, Y. (2015). The Genetic and Mechanistic Basis for Variation in Gene Regulation. PLoS Genetics, 11(1). https://doi.org/10.1371/journal.pgen.1004857

Schaub, M. A., Boyle, A. P., Kundaje, A., Batzoglou, S., & Snyder, M. (2012). Linking disease associations with regulatory information in the human genome. Genome Research, 22(9), 1748–1759. https://doi.org/10.1101/gr.136127.111

Spencer, C. C. A., Su, Z., Donnelly, P., & Marchini, J. (2009). Designing genome-wide association studies: Sample size, power, imputation, and the choice of genotyping chip. PLoS Genetics, 5(5). https://doi.org/10.1371/journal.pgen.1000477

Suravajhala, P., Kogelman, L. J. A., & Kadarmideen, H. N. (2016). Multi-omic data integration and analysis using systems genomics approaches: methods and applications in animal production, health and welfare. Genetics Selection Evolution, 48(1), 1–14. https://doi.org/10.1186/s12711-016-0217-x

Van Laere, A. S., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A. L., Haley, C. S., Buys, N., Tally, M., Andersson, G., Georges, M., & Andersson, L. (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature, 425(6960), 832–836. https://doi.org/10.1038/nature02064

Velez-Irizarry, D., Casiro, S., Daza, K. R., Bates, R. O., Raney, N. E., Steibel, J. P., & Ernst, C. W. (2019). Genetic control of longissimus dorsi muscle gene expression variation and joint analysis with phenotypic quantitative trait loci in pigs. BMC Genomics, 20(1), 1–19. https://doi.org/10.1186/s12864-018-5386-2

Wang, K., Ouyang, H., Xie, Z., Yao, C., Guo, N., Li, M., Jiao, H., & Pang, D. (2015). Efficient Generation of Myostatin Mutations in Pigs Using the CRISPR/Cas9 System. Scientific Reports, 5, 1–11. https://doi.org/10.1038/srep16623

Whitworth, K. M., Rowland, R. R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., Samuel, M. S., Lightner, J. E., McLaren, D. G., Mileham, A. J., Wells, K. D., & Prather, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology, 34(1), 20–22. https://doi.org/10.1038/nbt.3434

Wu, P., Wang, K., Yang, Q., Zhou, J., Chen, D., Liu, Y., Ma, J., Tang, Q., Jin, L., Xiao, W., Lou, P., Jiang, A., Jiang, Y., Zhu, L., Li, M., Li, X., & Tang, G. (2019). Whole-genome re-sequencing association study for direct genetic effects and social genetic effects of six growth traits in Large White pigs. Scientific Reports, 9(1), 1–12. https://doi.org/10.1038/s41598-019-45919-0

Yan, G., Guo, T., Xiao, S., Zhang, F., Xin, W., Huang, T., Xu, W., Li, Y., Zhang, Z., & Huang, L. (2018). Imputation-Based Whole-Genome Sequence Association Study Reveals Constant and Novel Loci for Hematological Traits in a Large-Scale Swine F2 Resource Population. Frontiers in Genetics, 9, 1–12. https://doi.org/10.3389/fgene.2018.00401

Yang, J., Benyamin, B., McEvoy, B., Gordon, S., Henders, A., Dale, N., Madden, P., Heath, A., Martin, N., Montgomery, G., Goddard, M., & Visscher, P. (2011). Common SNPs explain a large proportion of heritability for human height. Nature Genetics, 42(7), 565–569. https://doi.org/10.1038/ng.608

Yang, T., Plastow, G., & Wang, Z. (2015). Variable selection in whole-genome association studies using LASSO-based techniques. Plant and Animal Genome Conference XXIII. San Diego, CA, USA.

Yang, T., Wang, Z., Miar, Y., Bruce, H., Zhang, C., Plastow, G. (2017). A genome-wide association study of meat colour in commercial crossbred pigs. Journal of Animal Science, 97(4), 721–733. https://doi.org/10.1139/cjas-2016-0248

Yengo, L., Sidorenko, J., Kemper, K. E., Zheng, Z., Wood, A. R., Weedon, M. N., Frayling, T. M., Hirschhorn, J., Yang, J., & Visscher, P. M. (2018). Meta-analysis of genome-wide association studies for height and body mass index in ~700 000 individuals of European

ancestry. Human Molecular Genetics, 27(20), 3641–3649. https://doi.org/10.1093/hmg/ddy271

Zhang, Z., Liu, J., Ding, X., Bijma, P., de Koning, D. J., & Zhang, Q. (2010). Best linear unbiased prediction of genomic breeding values using a trait-specific marker-derived relationship matrix. PLoS ONE, 5(9), 1–8. https://doi.org/10.1371/journal.pone.0012648

References

Adeyemi, K. D., & Sazili, A. Q. (2014). Efficacy of Carcass Electrical Stimulation in Meat Quality Enhancement: A Review. Asian-Australasian Journal of Animal Sciences, 27(3), 447–456. https://doi.org/https://doi.org/10.5713/ajas.2013.13463

Alberta Pork. (2017). Alberta Pork Pricing and Marketing Information. Alberta Pork. Retrieved from https://www.albertapork.com/our-producer-services/pricing/canadian-pricing/

Allen, H., Estrada, K., Lettre, G., Berndt, S., Weedon, M., Rivadeneira, F., Willer, C., Jackson, A., Vedantam, S., & Raychaudhuri, S. et al. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature, 467(7317), 832–838. https://doi.org/10.1038/nature09410

Amaral, A. J., Megens, H. J., Crooijmans, R. P. M. A., Heuven, H. C. M., & Groenen, M. A. M. (2008). Linkage disequilibrium decay and haplotype block structure in the pig. Genetics, 179(1), 569–579. https://doi.org/10.1534/genetics.107.084277

Andersson, L., Archibald, A. L., Bottema, C. D., Brauning, R., Burgess, S. C., Burt, D. W., et al. (2015). Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome Biology, 16(1), 4–9. https://doi.org/10.1186/s13059-015-0622-4

Apple, J. (2015). Nutrition effects on pork quality in swine production. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/08/nutritiona-effects-on-pork-quality.pdf

Apweiler, R. (2009). The universal protein resource (UniProt) in 2010. Nucleic Acids Research, 38(s.1), D142–D148. https://doi.org/10.1093/nar/gkp846

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L.,

Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene ontology: tool for the unification of biology. Nature Genetics, 25, 25–29. https://doi.org/10.1038/75556

Auton, A., Abecasis, G. R., Altshuler, D. M., Durbin, R. M., Bentley, D. R., Chakravarti, A., et al. (2015). A global reference for human genetic variation. Nature, 526(7571), 68–74. https://doi.org/10.1038/nature15393

Badke, Y. M., Bates, R. O., Ernst, C. W., Schwab, C., & Steibel, J. P. (2012). Estimation of linkage disequilibrium in four US pig breeds. BMC Genomics, 13(1), 1–10. https://doi.org/10.1186/1471-2164-13-24

Ball, R. D. (2013). Designing a GWAS: Power, Sample Size, and Data Structure. https://doi.org/10.1007/978-1-62703-447-0_3

Baltić, M. Ž., Tomović, V., Marković, R., Velarde, A., Dokmanović, M., Janjić, J., & Glamočlija, N. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. Meat Science, 98(2), 220–226. https://doi.org/10.1016/j.meatsci.2014.06.003

Bee, G., Biolley, C., Guex, G., Herzog, W., Lonergan, S. M., & Huff-Lonergan, E. (2006). Effects of available dietary carbohydrate and preslaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. Journal of Animal Science, 84(1), 191–203. https://doi.org/10.2527/2006.841191x

Berg, E., & Eilert, S. (2006). Critical Points Affecting Fresh Pork Quality within the Packing Plant. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/07/ critical-points-affecting-fresh-pork-quality-within-the-packing-plant1.pdf

Blau, H. M. (1988). Hierarchies of regulatory genes may specify mammalian development. Cell, 53(5), 673–674. https://doi.org/10.1016/0092-8674(88)90082-7

Botstein, D., & Risch, N. (2003). Discovering genotypes underlying human phenotypes: Past successes for mendelian disease, future approaches for complex disease. Nature Genetics, 33, 228–237. https://doi.org/10.1038/ng1090

Bourdon, R. M. (2014). Understanding Animal Breeding (2nd ed.). Upper Saddle River, NJ: Prentice Hall: Pearson New International.

Bowker, B. C., Grant, A. L., Forrest, J. C., & Gerrard, D. E. (2000). Muscle metabolism and PSE pork. Journal of Animal Science, 79(E-Suppl_1), 1-8. https://doi.org/10.2527/jas.00.079es1001c

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2018). An expanded view of complex traits: from polygenic to omnigenic. Cell, 169(7), 1177–1186. https://doi.org/doi:10.1016/j.cell.2017.05.038

Broekema, R. V., Bakker, O. B., & Jonkers, I. H. (2020). A practical view of fine-mapping and gene prioritization in the post-genome-wide association era. Open Biology, 10(1). https://doi.org/10.1098/rsob.190221

Brøndum, R. F., Su, G., Janss, L., Sahana, G., Guldbrandtsen, B., Boichard, D., & Lund, M. S. (2015). Quantitative trait loci markers derived from whole genome sequence data increases the reliability of genomic prediction. Journal of Dairy Science, 98(6), 4107–4116. https://doi.org/10.3168/jds.2014-9005

Buege, D., & Griffin, D. (2015). Variation in Pork Lean Quality Descriptors of Pork Lean Quality. Pork Information Gateway. Retrieved from http://porkgateway.org/wp-content/uploads/2015/07/variation-in-lean-pork-quality1.pdf

Cai, Z., Guldbrandtsen, B., Lund, M. S., & Sahana, G. (2018). Prioritizing candidate genes post-GWAS using multiple sources of data for mastitis resistance in dairy cattle. BMC Genomics, 19(1), 1–11. https://doi.org/10.1186/s12864-018-5050-x

Calus, M. P. L. (2010). Genomic breeding value prediction: Methods and procedures. Animal, 4(2), 157–164. https://doi.org/10.1017/S1751731109991352

Cameron, N. D., Warriss, P. D., Porter, S. J., & Enser, M. B. (1990). Comparison of Duroc and British landrace pigs for meat a and eating quality. Meat Science, 27(3), 227–247. https://doi.org/10.1016/0309-1740(90)90053-9

CCSI. (2001). RN Gene Testing in Canadian AI Boars. Canadian Center for Swine Improvement. Retrieved from http://www.ccsi.ca/newsletters/RNgene.PDF

Casiró, S., Velez-Irizarry, D., Ernst, C. W., Raney, N. E., Bates, R. O., Charles, M. G., & Steibel, J. P. (2017). Genome-Wide association study in an F2 Duroc x Pietrain resource population for economically important meat quality and carcass traits. Journal of Animal Science, 95(2), 545–558. https://doi.org/10.2527/jas2016.1003

CCAC. (2020). Guide to the Care and Use of Experimental Animals, Volume 1, 2nd Edition. Canadian Council on Animal Care.

Champely, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., Ford, C., & Volcic, R. (2020). Basic Functions for Power Analysis. 1–22. https://cran.r-project.org/web/packages/pwr/pwr.pdf

Channon, H. A., Payne, A. M., & Warner, R. D. (2000). Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. Meat Science, 56(3), 291–299. https://doi.org/10.1016/S0309-1740(00)00056-5

Chen, Y. C., Wu, B. K., Chu, C. Y., Cheng, C. H., Han, H. W., Chen, G. D., Lee, M. T., Hwang, P. P., Kawakami, K., Chang, C. C., & Huang, C. J. (2010). Identification and characterization of alternative promoters of zebrafish Rtn-4/Nogo genes in cultured cells and zebrafish embryos. Nucleic Acids Research, 38(14), 4635–4650. https://doi.org/10.1093/nar/gkq230

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., & Kim, B. C. (2008). The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. Meat Science, 80(2), 355–362. https://doi.org/10.1016/j.meatsci.2007.12.019

Choi, Y. M., & Kim, B. C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livestock Science, 122(2–3), 105–118. https://doi.org/10.1016/j.livsci.2008.08.015

Christensen, O., & Lund, M. (2010). Genomic relationship matrix when some animals are not genotyped. Genetics Selection Evolution, 42(2), 1–8.

Christensen, O., Madsen, P., Nielsen, B., Ostersen, T., & Su, G. (2012). Single-step methods for genomic evaluation in pigs. Animal, 6(10), 1565–1571. https://doi.org/10.1017/S1751731112000742

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics, 159(3), 1151–1162.

Ciobanu, D., Bastiaansen, J. W. M., Lonergan, S. M., Thomsen, H., Dekkers, J. C. M., Plastow, G. S., & Rothschild, M. F. (2004). New alleles in calpastatin gene are associated with

meat quality traits in pigs. Journal of Animal Science, 82(10), 2829–2839. https://doi.org/10.2527/2004.82102829x

Ciobanu, D., Lonergan, S., & Huff-Lonergan, E. (2011). Genetics of Meat Quality and Carcass Traits. In The Genetics of the Pig, 2nd Edition (355–378).

Cohen, J. (1998). Statistical power analysis for the behavioral sciences (2nd edition). Hillsdale, N. J.: Erlbaum Associated.

Cyranoski, D. (2015). Super-muscly pigs created by small genetic tweak. Nature, 523, 13–14. https://doi:10.1038/523013a

D'Alessandro, A., & Zolla, L. (2013). Meat science: From proteomics to integrated omics towards system biology. Journal of Proteomics, 78, 558–577. https://doi.org/10.1016/j.jprot.2012.10.023

D'Souza, D. N., Warner, R. D., Leury, B. J., & Dunshea, F. R. (2000). The influence of dietary magnesium supplement type, and supplementation dose and duration, on pork quality and the incidence of PSE pork. Australian Journal of Agricultural Research, 51(2), 185–189. https://doi.org/10.1071/AR99090

Daetwyler, H., Capitan, A., Pausch, H., Stothard, P., Van Binsbergen, R., Brøndum, R., Liao, X., Djari, A., Rodriguez, S., Grohs, C., Esquerré, D., Bouchez, O., Rossignol, M., Klopp, C., Rocha, D., Fritz, S., Eggen, A., Bowman, P., Coote, D., Chamberlain, A., Anderson, C., Vantassell, C., Hulsegge, I., Goddard, M., Guldbrandtsen, B., Lund, M., Veerkamp, R., Boichard, D., Fries, R., & Hayes, B. (2014). Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nature Genetics, 46(8), 858–865. https://doi.org/10.1038/ng.3034

Daetwyler, H. D., Pong-Wong, R., Villanueva, B., & Woolliams, J. A. (2010). The impact of genetic architecture on genome-wide evaluation methods. Genetics, 185(3), 1021–1031. https://doi.org/10.1534/genetics.110.116855

de los Campos, G., Hickey, J. M., Pong-Wong, R., Daetwyler, H. D., & Calus, M. P. L. (2013). Whole-genome regression and prediction methods applied to plant and animal breeding. Genetics, 193(2), 327–345. https://doi.org/10.1534/genetics.112.143313

de los Campos, G., Vazquez, A. I., Fernando, R., Klimentidis, Y. C., & Sorensen, D. (2013). Prediction of Complex Human Traits Using the Genomic Best Linear Unbiased Predictor. PLoS Genetics, 9(7). https://doi.org/10.1371/journal.pgen.1003608

de Vries, A. G., van der Wal, P. G., Long, T., Eikelenboom, G., & Merks, J. W. M. (1994). Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Production Science, 40(3), 277–289. https://doi.org/10.1016/0301-6226(94)90095-7

Dekkers, J. C. M. (2004). Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. Journal of Animal Science, 82(E-Suppl), 313-328. https://doi.org/10.2527/2004.8213_supplE313x

Doherty, R., & Couldrey, C. (2014). Exploring genome wide bisulfite sequencing for DNA methylation analysis in livestock: A technical assessment. Frontiers in Genetics, 5(126), 1–8. https://doi.org/10.3389/fgene.2014.00126

Dokmanović, M., Velarde, A., Tomović, V., Glamočlija, N., Marković, R., Janjić, J., & Baltić, M. Z. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. Meat Science, 98(2), 220–226. https://doi.org/10.1016/j.meatsci.2014.06.003

Dransfield, E., Ngapo, T. M., Nielsen, N. A., Bredahl, L., Sjödén, P. O., Magnusson, M., Campo, M. M., & Nute, G. R. (2005). Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production. Meat Science, 69(1), 61–70. https://doi.org/10.1016/j.meatsci.2004.06.006

Dunham, I., Kundaje, A., Aldred, S. F., Collins, P. J., Davis, C. A., Doyle, F., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. Nature, 489(7414), 57–74. https://doi.org/10.1038/nature11247

Edwards, L. (2019). What are the impacts of stress on pork quality? The Pig Site. Retrieved from https://thepigsite.com/news/2010/11/how-stress-affects-pig-meat-quality-1

Edwards, S., Sørensen, I. F., Sarup, P., Mackay, T. F. C., & Sørensen, P. (2016). Genomic prediction for quantitative traits is improved by mapping variants to gene ontology categories in Drosophila melanogaster. Genetics, 203(4), 1871–1883. https://doi.org/10.1534/genetics.116.187161

Eikelenboom, G., Bolink, A. H., & Sybesma, W. (1991). Effects of feed withdrawal before delivery on pork quality and carcass yield. Meat Science, 29(1), 25–30. https://doi.org/10.1016/0309-1740(91)90020-Q

England, E. M., Matarneh, S. K., Mitacek, R. M., Abraham, A., Ramanathan, R., Wicks, J. C., Shi, H., Scheffler, T. L., Oliver, E. M., Helm, E. T., & Gerrard, D. E. (2018). Presence of oxygen and mitochondria in skeletal muscle early postmortem. Meat Science, 139, 97–106. https://doi.org/10.1016/j.meatsci.2017.12.008

Erbe, M., Hayes, B., Matukumalli, L., Goswami, S., Bowman, P., Reich, C., Mason, B., & Goddard, M. (2012). Improving accuracy of genomic predictions within and between

dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. Journal of Dairy Science, 95(7), 4114–4129. https://doi.org/10.3168/jds.2011-5019

Esfandyari, H., Sørensen, A. C., & Bijma, P. (2015). Maximizing crossbred performance through purebred genomic selection. Genetics Selection Evolution, 47(1), 1–16. https://doi.org/10.1186/s12711-015-0099-3

Falconer, D. S. (1960). Introduction to Quantitative Genetics. New York, NY: Ronald Press.

Falker-Gieske, C., Blaj, I., Preuß, S., Bennewitz, J., Thaller, G., & Tetens, J. (2019). GWAS for meat and carcass traits using imputed sequence level genotypes in pooled F2-designs in pigs. G3: Genes, Genomes, Genetics, 9(9), 2823–2834. https://doi.org/10.1534/g3.119.400452

Fang, L., Sahana, G., Ma, P., Su, G., Yu, Y., Zhang, S., Lund, M. S., & Sørensen, P. (2017). Exploring the genetic architecture and improving genomic prediction accuracy for mastitis and milk production traits in dairy cattle by mapping variants to hepatic transcriptomic regions responsive to intra-mammary infection. Genetics Selection Evolution, 49(1), 1–18. https://doi.org/10.1186/s12711-017-0319-0

Fangmann, A., Sharifi, R. A., Heinkel, J., Danowski, K., Schrade, H., Erbe, M., & Simianer, H. (2017). Empirical comparison between different methods for genomic prediction of number of piglets born alive in moderate sized breeding populations. Journal of Animal Science, 95(4), 1434–1443. https://doi.org/10.2527/jas2016.0991

FAO. (2019). Meat & Meat Products. Food and Agriculture Organization of the United Nations, Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/home.html

FCC. (2017). Economic Snapshot of Canada's Hog and Pork Sectors. Farm Credit Canada. Retrieved from https://www.fcc-fac.ca/content/dam/fcc/knowledge/ag-economist/2017-economic-snapshot-hog-pork-e.pdf

Feng, S., Wang, S., Chen, C. C., & Lan, L. (2011). GWAPower: A statistical power calculation software for genome-wide association studies with quantitative traits. BMC Genetics, 12(1), 12. https://doi.org/10.1186/1471-2156-12-12

Fernando, R. L., Cheng, H., Sun, X., & Garrick, D. J. (2017). A comparison of identity-by-descent and identity-by-state matrices that are used for genetic evaluation and estimation of variance components. Journal of Animal Breeding and Genetics, 134(3), 213–223. https://doi.org/10.1111/jbg.12275

Fragomeni, B. O., Lourenco, D. A. L., Masuda, Y., Legarra, A., & Misztal, I. (2017). Incorporation of causative quantitative trait nucleotides in single-step GBLUP. Genetics Selection Evolution, 49(1), 1–11. https://doi.org/10.1186/s12711-017-0335-0

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J., & Maclennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253(5018), 448–451. https://doi.org/10.1126/science. 1862346

Gallagher, M. D., & Chen-Plotkin, A. S. (2018). The Post-GWAS Era: From Association to Function. American Journal of Human Genetics, 102(5), 717–730. https://doi.org/10.1016/j.ajhg.2018.04.002

Gentry, J. G., McGlone, J. J., Blanton, J. R., & Miller, M. F. (2002). Alternative housing systems for pigs: Influences on growth, composition, and pork quality. Journal of Animal Science, 80(7), 1781–1790. https://doi.org/10.2527/2002.8071781x

Georges, M., Charlier, C., & Hayes, B. (2019). Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 20(3), 135–156. https://doi.org/10.1038/s41576-018-0082-2

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., Thompson, R. (2015). ASReml User Guide Release 4.1, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. https://asreml.kb.vsni.co.uk/wp-content/uploads/sites/3/2018/02/ASReml-4.1-Functional-Specification.pdf

Goddard, M., Hayes, B., & Meuwissen, T. (2010). Genomic selection in livestock populations. Genetics Research, 92(5–6), 413–421. https://doi.org/10.1017/S0016672310000613

Goddard, M., Kemper, K., MacLeod, I., Chamberlain, A., & Hayes, B. (2016). Genetics of complex traits: Prediction of phenotype, identification of causal polymorphisms and genetic architecture. Proceedings of the Royal Society B: Biological Sciences, 283(1835). https://doi.org/10.1098/rspb.2016.0569

Göhre, V., & Robatzek, S. (2008). Breaking the Barriers: Microbial Effector Molecules Subvert Plant Immunity. Annual Review of Phytopathology, 46(1), 189–215. https://doi.org/10.1146/annurev.phyto.46.120407.110050

Goldansaz, S. A., Guo, A. C., Sajed, T., Steele, M. A., Plastow, G. S., & Wishart, D. S. (2017). Livestock metabolomics and the livestock metabolome: A systematic review. PLoS ONE, 12(5), 1–26. https://doi.org/10.1371/journal.pone.0177675

Gomez, D., Shankman, L., A, N., & Owens, G. K. (2013). Detection of histone modifications at specific gene loci in single cells in histological sections. Nature Methods, 10(2), 171–177. https://doi.org/10.1038/nmeth.2332

Grandin, T. (2003). The welfare of pigs during transport and slaughter. Retrieved from https://www.grandin.com/references/pig.welfare.during.transport.slaughter.html

Grossi, D. A., Jafarikia, M., Brito, L. F., Buzanskas, M. E., Sargolzaei, M., & Schenkel, F. S. (2017). Genetic diversity, extent of linkage disequilibrium and persistence of gametic phase in Canadian pigs. BMC Genetics, 18(1), 1–13. https://doi.org/10.1186/s12863-017-0473-y

Guàrdia, M. D., Estany, J., Balasch, S., Oliver, M. A., Gispert, M., & Diestre, A. (2004). Risk assessment of PSE condition due to pre-slaughter conditions and RYR1 gene in pigs. Meat Science, 67(3), 471–478. https://doi.org/10.1016/j.meatsci.2003.11.020

Guzik, A. C., Matthews, J. O., Kerr, B. J., Bidner, T. D., & Southern, L. L. (2006). Dietary tryptophan effects on plasma and salivary cortisol and meat quality in pigs. Journal of Animal Science, 84(8), 2251–2259. https://doi.org/10.2527/jas.2005-292

Habier, D., Fernando, R. L., Kizilkaya, K., & Garrick, D. J. (2011). Extension of the bayesian alphabet for genomic selection. BMC Bioinformatics, 12(186). https://doi.org/10.1186/1471-2105-12-186

Hall, J., Latschar, E. E., & Mackintosh, D. L. (1994). Characteristics of dark cutting beef. Survey and preliminary investigation. Agriculture Experimental Station Technical Bulletin, 58(4).

Hambrecht, E., Eissen, J. J., Nooijen, R. I. J., Ducro, B. J., Smits, C. H. M., Den Hartog, L. A., & Verstegen, M. W. A. (2004). Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. Journal of Animal Science, 82(5), 1401–1409. https://doi.org/10.2527/2004.8251401x

Hamilton, D. N., Ellis, M., McKeith, F. K., & Eggert, J. M. (2003). Effect of level, source, and time of feeding prior to slaughter of supplementary dietary magnesium on pork quality. Meat Science, 65(2), 853–857. https://doi.org/10.1016/S0309-1740(02)00291-7

Hauschild, J., Petersen, B., Santiago, Y., Queisser, A. L., Carnwath, J. W., Lucas-Hahn, A., Zhang, L., Meng, X., Gregory, P. D., Schwinzer, R., Cost, G. J., & Niemann, H. (2011). Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. Proceedings of the National Academy of Sciences of the United States of America, 108(29), 12013–12017. https://doi.org/10.1073/pnas.1106422108

Hayes, B. (2013). Overview of Statistical Methods for Genome-Wide Association Studies (GWAS). In Genome-Wide Association Studies and Genomic Prediction (149–169). https://doi.org/10.1007/978-1-62703-447-0_10

Henderson, C. R. (1975). Best Linear Unbiased Estimation and Prediction under a Selection Model. Biometrics, 31(2), 423–447.

Hernández-Sánchez, J., Amills, M., Pena, R. N., Mercadé, A., Manunza, A., & Quintanilla, R. (2013). Genomic architecture of heritability and genetic correlations for intramuscular and back fat contents in Duroc pigs. Journal of Animal Science, 91(2), 623–632. https://doi.org/10.2527/jas.2012-5270

Hirschhorn, J. N., & Daly, M. J. (2005). Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics, 6(2), 95–108. https://doi.org/10.1038/nrg1521

Holmer, S. F., McKeith, F. K., & Killefer, J. (2008). The influence of early post-mortem enhancement and accelerated chilling on pork quality. Meat Science, 79(2), 211–216. https://doi.org/10.1016/j.meatsci.2007.09.004

Honikel, K. O., Roncalés, P., & Hamm, R. (1983). The influence of temperature on shortening and rigor onset in beef muscle. Meat Science, 8(3), 221–241. https://doi.org/10.1016/0309-1740(83)90046-3

Horvath, P., & Barrangou, R. (2010). CRISPR/Cas, the immune system of Bacteria and Archaea. Science, 327(5962), 167–170. https://doi.org/10.1126/science.1179555

Houben, J. H., Eikelenboom, G., & Hoving-Bolink, A. H. (1998). Effect of the dietary supplementation with vitamin E on colour stability and lipid oxidation in packaged, minced pork. Meat Science, 48(3–4), 265–273. https://doi.org/10.1016/S0309-1740(97)00103-4

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

Hu, Z. L., Dracheva, S., Jang, W., Maglott, D., Bastiaansen, J., Rothschild, M. F., & Reecy, J. M. (2005). A QTL resource and comparison tool for pigs: PigQTLDB. Mammalian Genome, 16(10), 792–800. https://doi.org/10.1007/s00335-005-0060-9

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Olson, D. G., & Robson, R. M. (1996). Proteolysis of Specific Muscle Structural Proteins by μ-Calpain at Low pH and Temperature is Similar to Degradation in Postmortem Bovine Muscle. Journal of Animal Science, 74(5), 993–1008. https://doi.org/10.2527/1996.745993x

Huff-Lonergan, E., & Page, J. (2006). The Role of Carcass Chilling in the Development of Pork Quality. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/07/role-of-carcass-chilling-in-the-development-of-pork-quality1.pdf

Hughes, S. M., Koishi, K., Rudnicki, M., & Maggs, A. M. (1997). MyoD protein is differentially accumulated in fast and slow skeletal muscle fibers and required for normal fiber type balance in rodents. Mechanisms of Development, 61(1–2), 151–163. https://doi.org/10.1016/S0925-4773(96)00631-4

Hunt, S. E., McLaren, W., Gil, L., Thormann, A., Schuilenburg, H., Sheppard, D., Parton, A., Armean, I. M., Trevanion, S. J., Flicek, P., & Cunningham, F. (2018). Ensembl variation resources. Database: The Journal of Biological Databases and Curation, 2018(8), 1–12. https://doi.org/10.1093/database/bay119

Ibanez-Escriche, N., Fernando, R. L., Toosi, A., & Dekkers, J. C. M. (2009). Genomic selection of purebreds for crossbred performance. Genetics Selection Evolution, 41(12), 1–10. https://doi.org/10.1186/1297-9686-41-12

Idolo Imafidon, G, & Spanier, A. M. (1994). Unraveling the secret of meat flavor. Trends in Food Science and Technology, 5(10), 315–321. https://doi.org/10.1016/0924-2244(94)90182-1

Ihaka, R., & Gentleman, R. (1996). R: A Language for Data Analysis and Graphics. Journal of Computational Graphical Statatistics, 5(3), 299–314.

Jennen, D. G. J., Brings, A. D., Liu, G., Jüngst, H., Tholen, E., Jonas, E., Tesfaye, D., Schellander, K., & Phatsara, C. (2007). Genetic aspects concerning drip loss and water-

holding capacity of porcine meat. Journal of Animal Breeding and Genetics, 124(s1), 2–11. https://doi.org/10.1111/j.1439-0388.2007.00681.x

Jeremiah, L. E. (2006). Marbling and Pork Tenderness. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/07/marbling-and-prok-tenderness1.pdf

Jozsef, L., Tashiro, K., Kuo, A., Park, E., Skoura, A., Albinsson, S., Rivera-Molina, F., Harrison, K. D., Iwakiri, Y., Toomre, D., & Sessa, W. C. (2014). Reticulon 4 is necessary for endoplasmic reticulum tubulation, STIM1-Orai1 coupling, and store-operated calcium entry. Journal of Biological Chemistry, 289(13), 9380–9395. https://doi.org/10.1074/jbc.M114.548602

Kao, P. Y. P., Leung, K. H., Chan, L. W. C., Yip, S. P., & Yap, M. K. H. (2017). Pathway analysis of complex diseases for GWAS, extending to consider rare variants, multi-omics and interactions. Biochimica et Biophysica Acta - General Subjects, 1861(2), 335–353. https://doi.org/10.1016/j.bbagen.2016.11.030

Kerth, C. R., Carr, M. A., Ramsey, C. B., Brooks, J. C., Johnson, R. C., Cannon, J. E., & Miller, M. F. (2001). Vitamin-mineral supplementation and accelerated chilling effects on quality of pork from pigs that are monomutant or noncarriers of the halothane gene. Journal of Animal Science, 79(9), 2346–2355. https://doi.org/10.2527/2001.7992346x

Khan, M. I., Jo, C., & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors-A systematic review. Meat Science, 110, 278–284. https://doi.org/10.1016/j.meatsci.2015.08.002

Klein, R. J. (2007). Power analysis for genome-wide association studies. BMC Genetics, 8, 1–8. https://doi.org/10.1186/1471-2156-8-58

Kominakis, A. P. (2003). Phenotypic correlations as substitutes to genetic correlations in dairy sheep and goats. Journal of Animal Breeding and Genetics, 120(4), 269–281. https://doi.org/10.1046/j.1439-0388.2003.00397.x

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: A review. Plant Methods, 9(1), 1–9. https://doi.org/10.1186/1746-4811-9-29

Koufariotis, L., Chen, Y. P. P., Bolormaa, S., & Hayes, B. J. (2014). Regulatory and coding genome regions are enriched for trait associated variants in dairy and beef cattle. BMC Genomics, 15(1). https://doi.org/10.1186/1471-2164-15-436

Krämer, A., Green, J., Pollard, J., & Tugendreich, S. (2014). Causal analysis approaches in ingenuity pathway analysis. Bioinformatics, 30(4), 523–530. https://doi.org/10.1093/bioinformatics/btt703

Kuo, T., McQueen, A., TC, C., & Wang, J. (2015). Regulation of Glucose Homeostasis by Glucocorticoids. Advances in Experimental Medicine and Biology, 872, 99–126. https://doi.org/https://doi.org/10.1007/978-1-4939-2895-8_5

Larzul, C., Lefaucheur, L., Ecolan, P., Gogué, J., Talmant, A., Sellier, P., Le Roy, P., Monin, G. (1997). Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. Journal of Animal Science, 75(12), 3126–3137. https://doi.org/10.2527/1997.75123126x

Leach, L. M., Ellis, M., Sutton, D. S., McKeith, F. K., & Wilson, E. R. (1996). The Growth Performance, Carcass Characteristics, and Meat Quality of Halothane Carrier and Negative Pigs. Journal of Animal Science, 74(5), 934–943. https://doi.org/10.2527/1996.745934x

Lee, E. A., Kim, J. M., Lim, K. S., Ryu, Y. C., Jeon, W. M., & Hong, K. C. (2012). Effects of variation in porcine MYOD1 gene on muscle fiber characteristics, lean meat production, and meat quality traits. Meat Science, 92(1), 36–43. https://doi.org/10.1016/j.meatsci.2012.03.018

Legarra, A., Aguilar, I., & Misztal, I. (2009). A relationship matrix including full pedigree and genomic information. Journal of Dairy Science, 92(9), 4656–4663. https://doi.org/10.3168/jds.2009-2061

Legarra, A., Robert-Granié, C., Croiseau, P., Guillaume, F., & Fritz, S. (2011). Improved Lasso for genomic selection. Genetics Research, 93(1), 77–87. https://doi.org/10.1017/S0016672310000534

Li, Y., Li, B., Yang, M., Han, H., Chen, T., Wei, Q., Miao, Z., Yin, L., Wang, R., Shen, J., Li, X., Xu, X., Fang, M., & Zhao, S. (2020). Genome-Wide Association Study and Fine Mapping Reveals Candidate Genes for Birth Weight of Yorkshire and Landrace Pigs. Frontiers in Genetics, 11, 1–10. https://doi.org/10.3389/fgene.2020.00183

Liu, X., Xiong, X., Yang, J., Zhou, L., Yang, B., Ai, H., Ma, H., Xie, X., Huang, Y., Fang, S., Xiao, S., Ren, J., Ma, J., & Huang, L. (2015). Genome-wide association analyses for meat quality traits in Chinese Erhualian pigs and a Western Duroc x (Landrace × Yorkshire) commercial population. Genetics Selection Evolution, 47(1), 1–11. https://doi.org/10.1186/s12711-015-0120-x

Lonergan, S. (2012). Pork quality: pH decline and pork quality. Pork Information Gateway. Retrieved from https://porkgateway.org/wp-content/uploads/2015/07/pork-quality-ph-and-pork-quality1.pdf

Lopes, M. S., Bovenhuis, H., van Son, M., Nordbø, Ø., Grindflek, E. H., Knol, E. F., & Bastiaansen, J. W. M. (2017). Using markers with large effect in genetic and genomic predictions. Journal of Animal Science, 95(1), 59. https://doi.org/10.2527/jas2016.0754

Lundström, K., Andersson, A., & Hansson, I. (1996). Effect of the RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. Meat Science, 42(2), 145–153. https://doi.org/10.1016/0309-1740(95)00034-8

Ma, J., Yang, J., Zhou, L., Zhang, Z., Ma, H., Xie, X., Zhang, F., Xiong, X., Cui, L., Yang, H., Liu, X., Duan, Y., Xiao, S., Ai, H., Ren, J., & Huang, L. (2013). Genome-Wide Association Study of Meat Quality Traits in a White Duroc × Erhualian F2 Intercross and Chinese Sutai Pigs. PLoS ONE, 8(5). https://doi.org/10.1371/journal.pone.0064047

MacLeod, I., Bowman, P., Vander Jagt, C., Haile-Mariam, M., Kemper, K., Chamberlain, A., Schrooten, C., Hayes, B., & Goddard, M. (2016). Exploiting biological priors and sequence variants enhances QTL discovery and genomic prediction of complex traits. BMC Genomics, 17(1), 1–21. https://doi.org/10.1186/s12864-016-2443-6

MacLeod, I., Hayes, B., & Goddard, M. (2014). The effects of demography and long-term selection on the accuracy of genomic prediction with sequence data. Genetics, 198(4), 1671–1684. https://doi.org/10.1534/genetics.114.168344

Makgahlela, M. L., Strandén, I., Nielsen, U. S., Sillanpää, M. J., & Mäntysaari, E. A. (2013). The estimation of genomic relationships using breedwise allele frequencies among animals in multibreed populations. Journal of Dairy Science, 96(8), 5364–5375. https://doi.org/10.3168/jds.2012-6523

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. Meat Science, 71(1), 100–121. https://doi.org/10.1016/j.meatsci.2005.03.003

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., McCarthy, M. I., Ramos, E. M., Cardon, L. R., Chakravarti, A., Cho, J. H., Guttmacher, A. E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C. N., Slatkin, M., Valle, D., Whittemore, A. S., Boehnke, M., Clark, A. G., Eichler, E. E., Gibson, G., Haines, J. L., MacKay, T. F.C., McCarroll, S. A., & Visscher, P. M. (2009). Finding the missing heritability of complex diseases. Nature, 461(7265), 747–753. https://doi.org/10.1038/nature08494

Martoccia, L., Brambilla, G., Macrì, A., Moccia, G., & Cosentino, E. (1995). The effect of transport on some metabolic parameters and meat quality in pigs. Meat Science, 40(2), 271–277. https://doi.org/10.1016/0309-1740(94)00015-Y

Mathevon, M., Buhr, M. M., & Dekkers, J. C. M. (1998). Environmental, Management, and Genetic Factors Affecting Semen Production in Holstein Bulls. Journal of Dairy Science, 81(12), 3321–3330. https://doi.org/10.3168/jds.S0022-0302(98)75898-9

Matthews, J. O., Southern, L. L., Bidner, T. D., & Persica, M. A. (2001). Effects of betaine, pen space, and slaughter handling method on growth performance, carcass traits, and pork quality of finishing barrows. Journal of Animal Science, 79(4), 967–974. https://doi.org/10.2527/2001.794967x

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., Reynolds, A. P., Sandstrom, R., Qu, H., Brody, J., Shafer, A., Neri, F., Lee, K., Kutyavin, T., Stehling-Sun, S., Johnson, A. K., Canfield, T. K., Giste, E., Diegel, M., Bates, D., Hansen, R. S., Neph, S., Sabo, P. J., Heimfeld, S., Raubitschek, A., Ziegler, S., Cotsapas, C., Sotoodehnia, N., Glass, I., Sunyaev, S. R., Kaul, R., & Stamatoyannopoulos, J. A. (2012).

Systematic localization of common disease-associated variation in regulatory DNA. Science, 337(6099), 1190–1195. https://doi.org/10.1126/science.1222794

Meuwissen, T., & Goddard, M. (2010). Accurate prediction of genetic values for complex traits by whole-genome resequencing. Genetics, 185(2), 623–631. https://doi.org/10.1534/genetics.110.116590

Meuwissen, T., Hayes, B., & Goddard, M. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics, 157, 1819–1829. https://doi.org/11290733

Miar, Y. (2015). Genomic selection for pork quality and carcass traits in both cross- and pure-bred populations (Dissertation). University of Alberta. https://doi.org/10.7939/R3M32NH2S

Miar, Y., Plastow, G., Moore, S., Manafiazar, G., Charagu, P., Kemp, R., Van Haandel, B., Huisman, A., Zhang, C., Mckay, R., Bruce, H., & Wang, Z. (2014). Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livestock Production Science, 26(2), 119–135. https://doi.org/10.2527/jas2014-7685

Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robix, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronee, H., Lundstrom, K., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., Chardon, P., & Andersson, L. (2000). A Mutation in PRKAG3 Associated with Excess Glycogen Content in Pig Skeletal Muscle. Science, 288(5469), 1248–1251. https://doi.org/10.1126/science.288.5469.1248

Miller, J., McLachlan, A. D., & Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. The EMBO Journal, 4(6), 1609–1614. https://doi.org/10.1002/j.1460-2075.1985.tb03825.x

Misztal, I., Legarra, A., & Aguilar, I. (2009). Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. Journal of Dairy Science, 92(9), 4648–4655. https://doi.org/10.3168/jds.2009-2064

Misztal, I., S. Tsuruta, D. A. L. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, Z. Vitezica. 2018. Manual for BLUPF90 family programs. University of Georgia. http://nce.ads.uga.edu/wiki/doku.php?id=documentation

Mrode, R. A., & Thompson, R. (2005). Linear Models for the Prediction of Breeding Values. In CABI Publishing (Second Edi). Cambridge, MA.

Murray, A. C., & Johnson, C. P. (1998). Impact of the halothane gene on muscle quality and pre-slaughter deaths in Western Canadian pigs. Canadian Journal of Animal Science, 78(4), 543–548. https://doi.org/10.4141/A97-122

Murray, A. C., & Jones, S. D. M. (1994). The effect of mixing, feed restriction and genotype with respect to stress susceptibility on pork carcass and meat quality. Canadian Journal of Animal Science, 74(4), 587–594. https://doi.org/10.4141/cjas94-086

NSIF. (1997). Guidelines for uniform swine improvement programs. National Swine Improvement Federation. Retrieved from http://www.nsif.com

Nejati-Javaremi, A., Smith, C., & Gibson, J. P. (1997). Effect of Total Allelic Relationship on Accuracy of Evaluation and Response to Selection. Journal of Animal Science, 75(7), 1738–1745. https://doi.org/10.2527/1997.7571738x

Newton, K. G., & Gill, C. O. (1981). The microbiology of DFD fresh meats: A review. Meat Science, 5(3), 223–232. https://doi.org/10.1016/0309-1740(81)90005-X

Ngapo, T. M. (2017). Consumer preferences for pork chops in five Canadian provinces. Meat Science, 129, 102–110. https://doi.org/10.1016/j.meatsci.2017.02.022

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2007). International preferences for pork appearance: I. Consumer choices. Food Quality and Preference, 18(1), 26–36. https://doi.org/10.1016/j.foodqual.2005.07.001

Nishimura, T., Hattori, A., & Takahashi, K. (1999). Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. Journal of Animal Science, 77(1), 93–104. https://doi.org/10.2527/1999.77193x

Nonneman, D., Lindholm-Perry, A. K., Shackelford, S. D., King, D. A., Wheeler, T. L., Rohrer, G. A., Bierman, C. D., Schneider, J. F., Miller, R. K., Zerby, H., & Moeller, S. J. (2011). Predictive markers in calpastatin for tenderness in commercial pig populations. Journal of Animal Science, 89(9), 2663–2672. https://doi.org/10.2527/jas.2010-3556

Nonneman, D., Shackelford, S. D., King, D. A., Wheeler, T. L., Wiedmann, R. T., Snelling, W. M., & Rohrer, G. A. (2013). Genome-wide association of meat quality traits and tenderness in swine. Journal of Animal Science, 91(9), 4043–4050. https://doi.org/10.2527/jas2013-6255

Oexle, H., Gnaiger, E., & Weiss, G. (1999). Iron-dependent changes in cellular energy metabolism: Influence on citric acid cycle and oxidative phosphorylation. Biochimica et Biophysica Acta - Bioenergetics, 1413(3), 99–107. https://doi.org/10.1016/S0005-2728(99)00088-2

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 2. Drip losses. Developments in Meat Science, 4, 173–243.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., & Kanehisa, M. (1999). KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 27(1), 29–34. https://doi.org/10.1093/nar/27.1.29

Oldenbroek, K., & van der Waaij, L. (2015). Textbook Animal Breeding and Genetics for BSc Students. Retrieved from https://wiki.groenkennisnet.nl/display/TAB/Textbook+Animal+Breeding+and+Genetics

Olson, E. N. (1990). MyoD family: a paradigm for development? Genes and Development, 4(9), 1454–1461. https://doi.org/10.1101/gad.4.9.1454

Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., Plastow, G. S., & Kalm, E. (2007). Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. Meat Science, 75(2), 185–195. https://doi.org/10.1016/j.meatsci.2006.03.022

Pai, A. A., Pritchard, J. K., & Gilad, Y. (2015). The Genetic and Mechanistic Basis for Variation in Gene Regulation. PLoS Genetics, 11(1). https://doi.org/10.1371/journal.pgen.1004857

Pan, B. S., & Myron, S. (1972). The effect of pH on bovine oxymyoglobin structure and stability. Journal of Food Science, 37, 29–32.

Pruitt, K., Brown, G., Hiatt, S., Thibaud-Nissen, F., Astashyn, A., Ermolaeva, O., Farrell, C. M., Hart, J., Landrum, M. J., McGarvey, K. M., Murphy, M. R., O'Leary, N. A., Pujar, S., Rajput, B., Rangwala, S. H., Riddick, L. D., Shkeda, A., Sun, H., Tamez, P., Tully, R. E., Wallin, C., Webb, D., Weber, J., Wu, W., Dicuccio, M., Kitts, P., Maglott, D. R., Murphy, T. D., & Ostell, J. (2014). RefSeq: an update on mammalian reference sequences. Nucleic Acids Research, 42(1), 553–559. https://doi.org/10.1093/nar/gkt1114

Pszczola, M., & Calus, M. P. L. (2016). Updating the reference population to achieve constant genomic prediction reliability across generations. Animal, 10(6), 1018–1024. https://doi.org/10.1017/S1751731115002785

Ramos, A. M., Crooijmans, R. P. M. A., Affara, N. A., Amaral, A. J., Archibald, A. L., Beever, J. E., Bendixen, C., Churcher, C., Clark, R., Dehais, P., Hansen, M. S., Hedegaard, J., Hu, Z. L., Kerstens, H. H., Law, A. S., Megens, H. J., Milan, D., Nonneman, D., Rohrer, G. A., Rothschild, M. F., Smith, T. P. L., Schnabel, R. D., Van Tassell, C. P., Taylor, J. F., Wiedmann, R. T., Schook, L. B., & Groenen, M. A. M. (2009). Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PLoS ONE, 4(8). https://doi.org/10.1371/journal.pone.0006524

Raymond, B., Bouwman, A. C., Schrooten, C., Houwing-Duistermaat, J., & Veerkamp, R. F. (2018). Utility of whole-genome sequence data for across-breed genomic prediction. Genetics Selection Evolution, 50(1), 1–12. https://doi.org/10.1186/s12711-018-0396-8

Reyer, H., Ponsuksili, S., Wimmers, K., & Murani, E. (2014). Association of N-terminal domain polymorphisms of the porcine glucocorticoid receptor with carcass composition and meat quality traits. Animal Genetics, 45(1), 125–129. https://doi.org/10.1111/age.12083

Rohrer, G. A., Nonneman, D., Miller, R. K., Zerby, H., & Moeller, S. J. (2012). Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs. Meat Science, 92(4), 511–518. https://doi.org/10.1016/j.meatsci.2012.05.020

Ropka-Molik, K., Bereta, A., Tyra, M., Rózycki, M., Piórkowska, K., Szyndler-Nedza, M., & Szmatoła, T. (2014). Association of calpastatin gene polymorphisms and meat quality traits in pig. Meat Science, 97(2), 143–150. https://doi.org/10.1016/j.meatsci.2014.01.021

Rosenvold, K., Lærke, H. N., Jensen, S. K., Karlsson, A. H., Lundstrom, K., & Andersen, H. J. (2001). Strategic finishing feeding as a tool in the control of pork quality. Meat Science, 59, 387–406.

Rosenvold, K., Petersen, J. S., Lærke, H. N., Jensen, S. K., Therkildsen, M., Karlsson, A. H., & Andersen, H. J. (2001). Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. Journal of Animal Science, 79(2), 382–391. https://doi.org/10.2527/2001.792382x

Rybarczyk, A., Karamucki, T., Pietruszka, A., Rybak, K., & Matysiak, B. (2015). The effects of blast chilling on pork quality. Meat Science, 101, 78–82. https://doi.org/10.1016/j.meatsci.2014.11.006

Sargolzaei, M., Chesnais, J. P., & Schenkel, F. S. (2014). A new approach for efficient genotype imputation using information from relatives. BMC Genomics, 15(1). https://doi.org/10.1186/1471-2164-15-478

Sather, A. P., & Murray, A. C. (1989). the Development of a Halothane-Sensitive Line of Pigs. Canadian Journal of Animal Science, 69(2), 323–331. https://doi.org/10.4141/cjas89-036

Sato, S., Uemoto, Y., Kikuchi, T., Egawa, S., Kohira, K., Saito, T., Sakuma, H., Miyashita, S., Arata, S., Kojima, T., & Suzuki, K. (2016). SNP- and haplotype-based genome-wide association studies for growth, carcass, and meat quality traits in a Duroc multigenerational population. BMC Genetics, 17(1). https://doi.org/10.1186/s12863-016-0368-3

Savage, A. W. J., Warriss, P. D., & Jolley, P. D. (1990). The amount and composition of the proteins in drip from stored pig meat. Meat Science, 27(4), 289–303. https://doi.org/10.1016/0309-1740(90)90067-G

Schaub, M. A., Boyle, A. P., Kundaje, A., Batzoglou, S., & Snyder, M. (2012). Linking disease associations with regulatory information in the human genome. Genome Research, 22(9), 1748–1759. https://doi.org/10.1101/gr.136127.111

Schellhaus, A. K., Moreno-Andrés, D., Chugh, M., Yokoyama, H., Moschopoulou, A., De, S., Bono, F., Hipp, K., Schäffer, E., & Antonin, W. (2017). Developmentally Regulated GTP binding protein 1 (DRG1) controls microtubule dynamics. Scientific Reports, 7(1), 1–16. https://doi.org/10.1038/s41598-017-10088-5

Schwab, C. R., Baas, T. J., Stalder, K. J., & Mabry, J. W. (2006). Effect of long-term selection for increased leanness on meat and eating quality traits in Duroc swine. Journal of Animal Science, 84(6), 1577–1583. https://doi.org/10.2527/2006.8461577x

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors Associated with Fresh Meat Color: a Review. Journal of Food Quality, 6(3), 211–237. https://doi.org/10.1111/j.1745-4557.1984.tb00826.x

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7(539). https://doi.org/10.1038/msb.2011.75

Smulders, F. J. M., Eikelenboom, G., & Logtestijn, J. G. Van. (1986). The Effect of Electrical Stimulation on the Quality, of Three Bovine Muscles. Meat Science, 16, 91–101.

Spencer, C. C. A., Su, Z., Donnelly, P., & Marchini, J. (2009). Designing genome-wide association studies: Sample size, power, imputation, and the choice of genotyping chip. PLoS Genetics, 5(5). https://doi.org/10.1371/journal.pgen.1000477

Sterten, H., Frøystein, T., Oksbjerg, N., Rehnberg, A. C., Ekker, A. S., & Kjos, N. P. (2009). Effects of fasting prior to slaughter on technological and sensory properties of the loin muscle (M. longissimus dorsi) of pigs. Meat Science, 83(3), 351–357. https://doi.org/10.1016/j.meatsci.2009.06.002

Suravajhala, P., Kogelman, L. J. A., & Kadarmideen, H. N. (2016). Multi-omic data integration and analysis using systems genomics approaches: Methods and applications in animal production, health and welfare. Genetics Selection Evolution, 48(1), 1–14. https://doi.org/10.1186/s12711-016-0217-x

Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M., & Nishida, A. (2005). Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. Journal of Animal Science, 83(9), 2058–2065. https://doi.org/10.2527/2005.8392058x

Tang, Z., Li, Y., Wan, P., Li, X., Zhao, S., Liu, B., Fan, B., Zhu, M., Yu, M., & Li, K. (2007). LongSAGE analysis of skeletal muscle at three prenatal stages in Tongcheng and Landrace pigs. Genome Biology, 8(6). https://doi.org/10.1186/gb-2007-8-6-r115

Tannas, L., & Tannas, V. (2014). Better Meat Quality with Duroc Genetics. The Pig Site. Retrieved from https://thepigsite.com/news/2014/03/better-meat-quality-with-duroc-genetics-1

Te Pas, M. F. W., Kruijt, L., Pierzchala, M., Crump, R. E., Boeren, S., Keuning, E., Hoving-Bolink, R., Hortós, M., Gispert, M., Arnau, J., Diestre, A., & Mulder, H. A. (2013).

Identification of proteomic biomarkers in M. Longissimus dorsi as potential predictors of pork quality. Meat Science, 95(3), 679–687. https://doi.org/10.1016/j.meatsci.2012.12.015

Terenina, E., Babigumira, B. M., Le Mignon, G., Bazovkina, D., Rousseau, S., Salin, F., Bendixen, C., & Mormede, P. (2013). Association study of molecular polymorphisms in candidate genes related to stress responses with production and meat quality traits in pigs. Domestic Animal Endocrinology, 44(2), 81–97. https://doi.org/10.1016/j.domaniend.2012.09.004

Thusberg, J., & Vihinen, M. (2009). Pathogenic or not? and if so, then how? Studying the effects of missense mutations using bioinformatics methods. Human Mutation, 30(5), 703–714. https://doi.org/10.1002/humu.20938

Tibshirani, R. (1996). Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society., 58(1), 267–288.

Toosi, A., Fernando, R. L., & Dekkers, J. C. M. (2010). Genomic selection in admixed and crossbred populations. Journal of Animal Science, 88(1), 32–46. https://doi.org/10.2527/jas.2009-1975

Towers, L. (2016). Pork Meat Quality: Understanding Industry Measurements and Guidelines. The Pig Site. Retrieved from https://thepigsite.com/articles/pork-meat-quality-understanding-industry-measurements-and-guidelines

Turner, S. (2014). qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. BiorXiv. https://doi.org/https ://doi.org/10.1101/005165

Van Den Berg, I., Boichard, D., Guldbrandtsen, B., & Lund, M. S. (2016). Using sequence variants in linkage disequilibrium with causative mutations to improve across-breed

prediction in dairy cattle: A simulation study. G3: Genes, Genomes, Genetics, 6(8), 2553–2561. https://doi.org/10.1534/g3.116.027730

Van Den Berg, S., Vandenplas, J., Van Eeuwijk, F. A., Bouwman, A. C., Lopes, M. S., & Veerkamp, R. F. (2019). Imputation to whole-genome sequence using multiple pig populations and its use in genome-wide association studies. Genetics Selection Evolution, 51(1), 1–13. https://doi.org/10.1186/s12711-019-0445-y

Van Laere, A. S., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A. L., Haley, C. S., Buys, N., Tally, M., Andersson, G., Georges, M., & Andersson, L. (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature, 425(6960), 832–836. https://doi.org/10.1038/nature02064

Van Wijk, H. J., Arts, D. J. G., Matthews, J. O., Webster, M., Ducro, B. J., & Knol, E. F. (2005). Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. Journal of Animal Science, 83(2), 324–333. https://doi.org/10.2527/2005.832324x

Velarde, A., Gispert, M., Faucitano, L., Alonso, P., Manteca, X., & Diestre, A. (2001). Effects of the stunning procedure and the halothane genotype on meat quality and incidence of hemorrhages in pigs. Meat Science, 58(3), 313–319. https://doi.org/10.1016/S0309-1740(01)00035-3

Velez-Irizarry, D., Casiro, S., Daza, K. R., Bates, R. O., Raney, N. E., Steibel, J. P., & Ernst, C. W. (2019). Genetic control of longissimus dorsi muscle gene expression variation and joint analysis with phenotypic quantitative trait loci in pigs. BMC Genomics, 20(1), 1–19. https://doi.org/10.1186/s12864-018-5386-2

Verardo, L. L., Sevón-Aimonen, M. L., Serenius, T., Hietakangas, V., & Uimari, P. (2017). Whole-genome association analysis of pork meat pH revealed three significant regions and several potential genes in Finnish Yorkshire pigs. BMC Genetics, 18(1), 1–15. https://doi.org/10.1186/s12863-017-0482-x

Viljoen, H. F., De Kock, H. L., & Webb, E. C. (2002). Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. Meat Science, 61(2), 181–185. https://doi.org/10.1016/S0309-1740(01)00183-8

Visscher, P. M., Brown, M. A., McCarthy, M. I., & Yang, J. (2012). Five years of GWAS discovery. American Journal of Human Genetics, 90(1), 7–24. https://doi.org/10.1016/j.ajhg.2011.11.029

Visscher, P. M., Medland, S. E., Ferreira, M. A. R., Morley, K. I., Zhu, G., Cornes, B. K., Montgomery, G. W., & Martin, N. G. (2006). Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. PLoS Genetics, 2(3), 0316–0325. https://doi.org/10.1371/journal.pgen.0020041

Vockley, C. M., Barrera, A., & Reddy, T. E. (2017). Decoding the role of regulatory element polymorphisms in complex disease. Current Opinion in Genetics and Development, 43, 38–45. https://doi.org/10.1016/j.gde.2016.10.007

Wang, K., Ouyang, H., Xie, Z., Yao, C., Guo, N., Li, M., Jiao, H., & Pang, D. (2015). Efficient Generation of Myostatin Mutations in Pigs Using the CRISPR/Cas9 System. Scientific Reports, 5, 1–11. https://doi.org/10.1038/srep16623

Wang, X., Yang, Z., & Xu, C. (2015). A comparison of genomic selection methods for breeding value prediction. Science Bulletin, 60(10), 925–935. https://doi.org/10.1007/s11434-015-0791-2

Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. Meat Science, 86(1), 171–183. https://doi.org/10.1016/j.meatsci.2010.04.042

Weller, J. I., Song, J. Z., Heyen, D. W., Lewin, H. A., & Ron, M. (1998). A New Approach to the Problem of Multiple Comparisons in the Genetic Dissection of Complex Traits. Genetics, 150(4), 1699–1706.

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78(4), 958–965. https://doi.org/10.2527/2000.784958x

Whittaker, J. C., Thompson, R., & Denham, M. C. (2000). Marker-assisted selection using ridge regression. Genetical Research, 75(2), 249–252. https://doi.org/10.1017/S0016672399004462

Whitworth, K. M., Rowland, R. R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., Samuel, M. S., Lightner, J. E., McLaren, D. G., Mileham, A. J., Wells, K. D., & Prather, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology, 34(1), 20–22. https://doi.org/10.1038/nbt.3434

Wickramasinghe, S., Cánovas, A., Rincón, G., & Medrano, J. F. (2014). RNA-Sequencing: A tool to explore new frontiers in animal genetics. Livestock Science, 166(1), 206–216. https://doi.org/10.1016/j.livsci.2014.06.015

Wiegand, B. R., Sparks, J. C., Beitz, D. C., Parrish, F. C., Horst, R. L., Trenkle, A. H., & Ewan, R. C. (2002). Short-term feeding of vitamin D3 improves color but does not change

tenderness of pork-loin chops. Journal of Animal Science, 80(8), 2116–2121. https://doi.org/https://doi.org/10.1093/ansci/80.8.2116

Wientjes, Y. C. J., & Calus, M. P. L. (2017). The purebred-crossbred correlation in pigs: A review of theory, estimates, and implications. Journal of Animal Science, 95(8), 3467. https://doi.org/10.2527/jas2017.1669

Wiggans, G. R., Sonstegard, T. S., Vanraden, P. M., Matukumalli, L. K., Schnabel, R. D., Taylor, J. F., Schenkel, F. S., & Van Tassell, C. P. (2009). Selection of single-nucleotide polymorphisms and quality of genotypes used in genomic evaluation of dairy cattle in the United States and Canada. Journal of Dairy Science, 92(7), 3431–3436.

Wilson, A. J. (2008). Why h2 does not always equal VA/VP? Journal of Evolutionary Biology, 21(3), 647–650. https://doi.org/10.1111/j.1420-9101.2008.01500.x

Wismer-Pederson, J., & Briskey, E. J. (1961). Rate of Anaerobic Glycolysis versus Structure in Pork Muscle. Nature, 189, 318–320. https://doi.org/10.1038/189318a0

Wolc, A., Arango, J., Settar, P., Fulton, J. E., O'Sullivan, N. P., Preisinger, R., Habier, D., Fernando, R., Garrick, D. J., & Dekkers, J. C. M. (2011). Persistence of accuracy of genomic estimated breeding values over generations in layer chickens. Genetics Selection Evolution, 43(1), 1–8. https://doi.org/10.1186/1297-9686-43-23

Wood, J. D., Nute, G. R., Richardson, R. I., Whittington, F. M., Southwood, O., Plastow, G., … Chang, K. C. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Science, 67(4), 651–667. https://doi.org/10.1016/j.meatsci.2004.01.007

Wray, N., & Visscher, P. (2008). Estimating Trait Heritability. Nature Education, 1(1), 29. Retrieved from https://www.nature.com/scitable/topicpage/estimating-trait-heritability-46889/

Wu, P., Wang, K., Yang, Q., Zhou, J., Chen, D., Liu, Y., Ma, J., Tang, Q., Jin, L., Xiao, W., Lou, P., Jiang, A., Jiang, Y., Zhu, L., Li, M., Li, X., & Tang, G. (2019). Whole-genome re-sequencing association study for direct genetic effects and social genetic effects of six growth traits in Large White pigs. Scientific Reports, 9(1), 1–12. https://doi.org/10.1038/s41598-019-45919-0

Yan, G., Guo, T., Xiao, S., Zhang, F., Xin, W., Huang, T., Xu, W., Li, Y., Zhang, Z., & Huang, L. (2018). Imputation-Based Whole-Genome Sequence Association Study Reveals Constant and Novel Loci for Hematological Traits in a Large-Scale Swine F2 Resource Population. Frontiers in Genetics, 9, 1–12. https://doi.org/10.3389/fgene.2018.00401

Yan, G., Qiao, R., Zhang, F., Xin, W., Xiao, S., Huang, T., Zhang, Z., & Huang, L. (2017). Imputation-Based Whole-Genome Sequence Association Study Rediscovered the Missing QTL for Lumbar Number in Sutai Pigs. Scientific Reports, 7(1), 1–10. https://doi.org/10.1038/s41598-017-00729-0

Yang, J., Benyamin, B., McEvoy, B., Gordon, S., Henders, A., Dale, N., Madden, P., Heath, A., Martin, N., Montgomery, G., Goddard, M., & Visscher, P. (2011). Common SNPs explain a large proportion of heritability for human height. Nature Genetics, 42(7), 565–569. https://doi.org/10.1038/ng.608

Yang, J., Lee, S. H., Goddard, M., & Visscher, P. (2011). GCTA: A tool for genome-wide complex trait analysis. American Journal of Human Genetics, 88(1), 76–82. https://doi.org/10.1016/j.ajhg.2010.11.011

Yang, T., Plastow, G., & Wang, Z. (2015). Variable selection in whole-genome association studies using LASSO-based techniques. Plant and Animal Genome Conference XXIII. San Diego, CA, USA.

Yang, T., Wang, Z., Miar, Y., Bruce, H., Zhang, C., Plastow, G.(2017). A genome-wide association study of meat colour in commercial crossbred pigs. Journal of Animal Science, 97(4), 721–733. https://doi.org/10.1139/cjas-2016-0248

Yang, Y. S., & Strittmatter, S. M. (2007). The reticulons: A family of proteins with diverse functions. Genome Biology, 8(12), 1–10. https://doi.org/10.1186/gb-2007-8-12-234

Yengo, L., Sidorenko, J., Kemper, K. E., Zheng, Z., Wood, A. R., Weedon, M. N., Frayling, T. M., Hirschhorn, J., Yang, J., & Visscher, P. M. (2018). Meta-analysis of genome-wide association studies for height and body mass index in ~700 000 individuals of European ancestry. Human Molecular Genetics, 27(20), 3641–3649. https://doi.org/10.1093/hmg/ddy271

Young, J. F., Bertram, H. C., Rosenvold, K., Lindahl, G., & Oksbjerg, N. (2005). Dietary creatine monohydrate affects quality attributes of Duroc but not Landrace pork. Meat Science, 70(4), 717–725. https://doi.org/10.1016/j.meatsci.2005.03.008

Zerbino, D. R., Achuthan, P., Akanni, W., Amode, M. R., Barrell, D., Bhai, J., et al. (2018). Ensembl 2018. Nucleic Acids Research, 46(D1), D754–D761. https://doi.org/10.1093/nar/gkx1098

Zhang, C., Kemp, R., Stothard, P., Wang, Z., Boddicker, N., Krivushin, K., Dekkers, J. C. M., Plastow, G. (2018). Genomic evaluation of feed efficiency component traits in Duroc pigs using 80K, 650K and whole-genome sequence variants. Genetics Selection Evolution, 50(1), 1–13. https://doi.org/10.1186/s12711-018-0387-9

Zhang, C., Wang, Z., Bruce, H., Kemp, R., Charagu, P., Miar, Y., Yang, T., Plastow, G. (2015). Genome-wide association studies (GWAS) identify a QTL close to PRKAG3 affecting meat pH and colour in crossbred commercial pigs. BMC Genetics, 16(1), 1–12. https://doi.org/10.1186/s12863-015-0192-1

Zhang, Z., Liu, J., Ding, X., Bijma, P., de Koning, D. J., & Zhang, Q. (2010). Best linear unbiased prediction of genomic breeding values using a trait-specific marker-derived relationship matrix. PLoS ONE, 5(9), 1–8. https://doi.org/10.1371/journal.pone.0012648

Zhang, C., Wang, Z., Bruce, H. L., Janz, J., Goddard, E., Moore, S., and Plastow, G. S. (2014) Associations between single nucleotide polymorphisms in 33 candidate genes and meat quality traits in commercial pigs. Animal Genetics, 45(4), 508–516. https://doi.org/10.1111/age.12155