A Genetic Investigation of Pork Fat and its Role in Meat Quality

By

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Abstract

Excellent pork meat quality for improved eating experience is becoming increasingly important to consumers and therefore the hog industry as a whole. One of the most interesting factors associated with eating experience is fat; fat plays a significant role in the acceptance (and rejection) of pork. Two depots of fat which significantly affect the industry are intramuscular fat (IMF) and backfat; IMF plays an important role in eating quality (tenderness, juiciness) and the backfat depth (BFD) ultimately determines the lean yield of the entire carcass, which is used for its valuation and producer payment. Therefore, the main objective of this study was to investigate the genetic underpinnings of IMF as well as its relationship to backfat, due to their economic relevance and importance for producers, consumers, and the whole industry.

Nine-hundred and ninety-seven *longissimus dorsi* and backfat samples from purebred Duroc gilts were analysed with near-infrared spectroscopy (NIR) in order to generate IMF and backfat composition phenotypes for further use in genetic studies. Resulting phenotypes were compared against reference methods to determine accuracy. The IMF phenotypes had a high, positive relationship between the reference method and predicted phenotypes (p <0.0001). Backfat composition phenotypes had a low degree of accuracy from the NIR algorithm when compared to the reference method values (p <0.0001). In order to better understand the relationship between IMF and backfat, variance components were estimated using IMF and BFD phenotypes measured by various collection methods (NIR, ultrasound, subjective, traditional). Crossbred animals were investigated in addition to the purebreds; phenotypes from a total of 916 crossbred (Duroc × [Large white × Landrace]) pigs were used and 997 purebred Duroc gilts were used. Heritability estimates (±SE) for IMF were moderate-high (0.38±0.09)

in the purebreds and moderate in the crossbreds (0.24±0.07); heritability estimates (±SE) for backfat were high in the purebreds (0.46 ± 0.09) and crossbreds (0.49 ±0.09). There were moderate-high positive genetic correlations between IMF and BFD for all measurement methods in the purebreds (0.36 ±0.14) and crossbreds (0.44 ± 0.63). This indicates that IMF and BFD are good indicator traits for one another; that the selection of one would also select for the other. There was a high positive coefficient of correlation (r) between NIRIMF and ultrasound IMF (UIMF) as well as BFD and ultrasound backfat thickness (UBFD) which indicates that the method of phenotype collection could be substituted for the other and maintain similar accuracy in further studies. Finally, three genome-wide association studies (GWAS') were conducted with IMF phenotypes from the purebred Durocs (UIMF, NIRIMF) and commercial crossbred pigs (UIMF) in order to investigate the genetic underpinnings of IMF. From this, five significant single nucleotide polymorphisms (SNPs) were identified in the purebred Durocs with association with IMF. No significant SNPs were identified in the crossbreds from GWAS; after application of an additional statistical method (LASSO) three SNPs with measurable effect size were identified. With further validation, the findings in these studies will help increase understanding of the genetic underpinnings of IMF and aide its independent manipulation from backfat. The findings from all studies can be utilised as reference for the hog industry to help develop novel genomic tools to identify animals with superior meat quality and improve Canadian pork.

Preface

This thesis is a part of a larger research project, which has received research ethics approval from the University of Alberta Research Ethics Board, project code NSERC CRDPJ 485526, July 1, 2016, titled “Identifying functional gene variants and non-additive effects to enhance the power of genomic selection of purebred pigs".

The research in this thesis focuses on the pork fat depots of intramuscular fat (IMF) and backfat, primarily the genetic architecture of IMF in order to ultimately improve this trait through breeding. This thesis consists of 7 chapters. Chapter 1 and 2 include a general introduction as well as a thorough review of related literature in this area.

Chapter 3 focuses on the generation of phenotypes for lean meat IMF content and backfat fatty acid composition from purebred Duroc samples through near-infrared spectroscopy (NIR). Samples were collected by Dr. Robert Mckay of Genstat consultants Inc (Brandon, MB) and shipped to the Agriculture and Agri-Food (AACF) in Lacombe. A portion of Chapter 3 was published in the Banff Pork seminar (2020) proceedings; including lean meat data analysis, predicted phenotypes and statistical analysis. I was responsible for the collection of NIR data and proximate analysis data as well as the abstract composition. Ivy Larsen with AACF Lacombe assisted with generation of prediction phenotypes through a SAS algorithm. Dr. Nuria Prieto was the primary author in the development of this algorithm; additional members contributing to this work include Drs. Graham Plastow (University of Alberta), Manuel Juárez (AAFC), Michael Dyck (University of Alberta) and Patrick Charagu (Hypor).

Chapter 4 of this thesis focuses on the generation of variance component and heritability estimations with IMF and BFD phenotypes obtained from a purebred Duroc and a commercial crossbred population. Data collection for the crossbred and purebred animals was

obtained from Hypor, save for the NIR phenotypes which were generated as described in Chapter 3. These estimates were subsequently compared to values in the literature. The data analysis was performed primarily by myself with assistance from Dr. Marzieh Heidaritabar.

Chapters 5 and 6 discuss the results of three genome-wide association studies (GWAS) performed with IMF phenotypes from the purebred Duroc and commercial crossbred pig populations. Data analysis was performed primarily by myself with assistance from Dr. Marzieh Heidaritabar.

Chapter 7 is a summary of all the findings in this thesis with emphasis on their impact to the Canadian Swine industry.

All chapters were written by myself with the assistance of my supervisor Dr. Graham Plastow, and reviewed by Dr. Michael Dyck, Dr. Manuel Juárez and Dr. Marzieh Heidaritabar

Dedication

For my parents, Brian and Heidi, who have always encouraged and believed in me, your love and support has meant everything; thank you for all you have done and continue to do for me.

Also, to my husband, Matt, for your never-ending support and love; and to my dogs, Lidsy and Fern, for their unconditional love and all of the joy they bring to my life.

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I would like to thank Dr. Manuel Juárez for being a valued member of my committee, for your encouragement, support and feedback throughout my project. Additionally, I would like to express my immense gratitude for the team at the Agriculture and Agri-Food Canada Lacombe Research and Development Centre, Ivy Larsen, Rhona Thacker, Haley Scott, Jeff Ranches & Mike Hamilton; your assistance and guidance during sample and statistical analysis was invaluable. I would also like to thank Dr. Robert McKay of Genstat Consultants Inc. for not only the collection, trimming and shipment of samples from Brandon, MB to Lacombe, AB but for your guidance and generosity (in both time and bacon ends) during my time at East 40 packers for sample collection. I have never had a more fun carcass data collection day!

To Dr. Marzieh Heidaritabar, I want to express my deepest gratitude for your advice, coaching and support during my statistical analysis and writing; your consistent encouragement and patient help has been integral to my success.

My sincere thank you to each members of Livestock Gentec who have supported me and given valuable advice over the years. To my family who have supported me in getting to where I am; my brother Dr. Mitchell Liddell and sister in-law Dr. Megan Engel who have not only provided me guidance and support while navigating my academic pursuits but are a consistent pillar of love, support and inspiration to me. My sweet niece, Laurelin Hildegard

Engel-Liddell for her welcomed calls to update me on her days adventures and remind me of the most important things in this world; thank you.

Finally, all animals, purebreds and crossbreds, in the project were provided from Hypor, along with funding (both cash and in kind) which I gratefully acknowledge. Funding was also provided by the Natural Sciences and Engineering Research Council (NSERC) of Canada for two Collaborative Research and Development Grants. The first was a collaboration with Hypor and Genesus looking at meat quality of commercial pigs; Chapters 4 and 6 include data from these crossbred animals.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADG	average daily gain
AFI	average feed intake
ASD	analytical spectral device
BFD	backfat depth
BLUP	best linear unbiased prediction
CCAC	Canadian Council on Animal Care
CPI	Canada Pork International
CV	Coefficient of Variation
DWG	daily weight gain
EBV	estimate breeding value
FAME	fatty acid methyl esters
FDR	False discovery rate
GC	chromatography
GEBV	genomic enhanced breeding value
GRM	genomic (realized) matrix
GS	genomic selection
GWAS	genome wide association study
IBD	identical by descent
IMF	intramuscular fat
IR	infra-red
IV	iodine value
LASSO	least absolute selection and shrinkage operator
LD	linkage disequilibrium
MAF	minor allele frequency
MAS	Marker assisted selection
MUFA	monounsaturated
NCBI	National Center for Biotechnology Information Database
NIR	near-infrared reflectance
NIRIMF	near-infrared intramuscular fat
NSIF	National Swine Improvement Federation
PCA	Principle component analysis
PHu	ultimate pH
PLS	Partial least square
PRESS	predictive error sum of squares
PSF	peak shear force
PUFA	polyunsaturated
Q-Q	quantile-quantile
QC	quality attributes
QA	quality characteristics
QTL	quantitative trait loci
QTN	quantitative trait nucleotide
RMSEP	root-mean-square error prediction
SFA	saturated fatty acid
SNP	single nucleotide polymorphism
SNV	standard normal variate
SUBIMF	subjective marbling score (visual IMF)
UBFD	ultrasound backfat depth
UFA	unsaturated fatty acid
UIMF	ultrasound Intramuscular fat
USDA	United States Department of Agriculture
WHC	water holding capacity

Chapter 1.0. General introduction

1.1 Introduction

Today, pork is the world's most commonly eaten meat with approximately 40% of global meat consumption (United States Department of Agriculture (USDA), foreign agricultural service, OECD, 2017). Consumption increased by 2% in 2018 from 2017 and is expected to continue to increase; Canadian pork exports alone have increased by 1.26% from 2016 to 2019 (Canada Pork International (CPI), 2020). To meet the growing demand and maximise profit margins, the focus of swine breeding over the last four decades has been on production efficiency and related traits such as feed efficiency, reproductive success, high growth rate and reduced backfat thickness for increased lean yield. Decreased carcass fatness was desirable, primarily in the 1980's, due to the prevailing science at the time which equated dietary fat to negative health consequences. This leanness was quickly achieved, however, traits with positive genetic relationships to backfat thickness also decreased; this has led to an overall decrease in meat quality and consumer satisfaction. For this reason, there is a demand for better quality pork and the industry has more recently shifted focus to improving meat quality and related traits (Van Wijk *et al*, 2005; Papanagiotou *et al*, 2012).

Meat quality is integral to the value of meat products, in fact, a negative experience with a type of meat may dissuade consumers from trying it again. Additionally, many *pre* and *post-mortem* factors culminate in final product quality (Miller, 2002), but all ultimately relate to a sensory experience for the consumer. Often, what is considered a desirable characteristic differs between countries or regions. Such traits include visual assessment and taste preferences like colour and visual marbling, but also consumption related traits like tenderness

and juiciness. In general, the factors which are associated with palatability of fresh or processed meat products coupled with those related to economic losses from processing and distribution are summed to define fresh meat quality (Bray, 1966). Further, meat quality can be divided into quality attributes (QA) and quality characteristics (QC). QA include features which a consumer is primarily concerned with, those which influence initial purchase and repurchase of meat products. These include visual appeal, flavour, juiciness, and tenderness upon consumption, these are referred to as organoleptic attributes, as they engage the consumers senses of sight, smell, and taste (Bray, 1966; Glitsch, 2000; Becker, 2002). One of the most important attributes for a positive eating experience is tenderness (Fortin *et al*, 2005; Teye *et al,* 2006) which is significantly correlated with the amount of intramuscular fat (IMF) present in a piece of meat (Wood *et al,* 1999; Cannata *et al,* 2010; Lim *et al,* 2016). Though a consumer cannot visually evaluate IMF content, as it requires more scientific measurements to obtain an exact content percentage, they can assess the visible fat content in the lean, which is known as marbling. In addition to the importance of IMF content, the quality of backfat is economically important as it heavily influences the attributes and acceptance of further processed products. Any backfat which is trimmed from primal and commercial cuts goes on for further processing in a number of products (Kouba and Sellier, 2011), and in these further processed products, fat is one of the most variable ingredients utilised. As mentioned, the selection and breeding focus on leanness and maximum lean yield through reduction of backfat thickness has resulted in unintended decreases of correlated meat quality traits which has negatively impacted the overall acceptance of pork in a number of ways. Firstly, reduction of backfat thickness has simultaneously reduced IMF content, as they are positively related traits with a genetic correlation of approximately 0.37 (Grindflek *et al*, 2001; Hernández-Sánchez *et al*, 2013) and secondly, as backfat thickness decreases the proportion of polyunsaturated fatty

acids (PUFA) in the fat increases, which devalues the fat. This devaluation is primarily due to increased propensity for rancidity due to oxidation, and poor, soft texture (Teye *et al*, 2006; Seman *et al,* 2013). Interestingly, total carcass adipose tissue deposition and IMF have a low-positive genetic correlation (r = 0.11) (Huff-Lonergan *et al,* 2002; Hernández-Sánchez *et al*, 2013), however, both traits have moderate to high heritability (h^2), averaging 0.69 (Rosenvold and Anderson, 2003) and 0.5, respectively (Sellier*,*1998; Rosenvold and Anderson, 2003; Zhao *et al,* 2009; Casellas *et al,* 2010; Lim *et al,* 2016). These values suggest that total carcass fatness and IMF content are independent traits which indicates the potential to breed for a commercial hog with desirable IMF content, but low total carcass fatness in order to maintain lean yield.

Breeding for desirable traits, including meat quality, litter size or milk production, is the ultimate goal of selective breeding; this can be done within a single breed (purebred) or through careful crossing of breeds (crossbred). Crossbreeding is used extensively in the Canadian hog industry. This is done for many reasons, including hybrid vigor (heterosis), breed complementarity and favourable breed combinations (Falconer and Mackay, 1996; Yadav *et al*, 2018). A number of the benefits of crossbreeding, primarily on litter size, carcass and meat quality from using purebred sires and crossbred sows was discussed by Fahmy *et al* (1987). Various sire and dam crosses were assessed, and this work formed the basis of the typical Canadian commercial meat pig. Nowadays, this has centered on a terminal three-way cross of a Duroc boar to an F1 progeny sow of Landrace × Large White (Miar *et al*, 2014); the three-breed cross with a terminal sire has many advantages over a two-breed terminal cross, as it produces maximum hybrid vigor in the offspring (Yadav *et al*, 2018). In general, breeding programs select a boar primarily for meat quality traits (Schwab *et al*, 2006), feed efficiency, growth and carcass size (Hypor, 2018). The Duroc breed, specifically, is known to have higher

IMF content than other potential sire breeds such as the Pietrain, but also higher growth and feed efficiency than fatter breeds which contain higher IMF, such as the Berkshire. Growth and feed efficiency are important to ensure profitability of the final carcass (Fahmy *et al*, 1987; Alfonso *et al*, 2010; Choi *et al*, 2014). The sow is chosen for mothering ability, litter size and longevity in the breeding herd (Blomberg, 2010; Camerlink, 2018). In Canada, commercial sows are typically an F1 cross of Large Whites and Landrace. One of the benefits of crossbreeding is that it helps to capitalise on differing trait expression of each breed, including any within-line selection which has taken place to improve the purebreds. The traditional breeding methods which utilise statistical models to select superior animals for breeding tend to offer slow improvement for meat quality traits; this is due in part to the measurement requirements of records for meat quality traits such as IMF content, as they are traits expressed late in life and can only be definitively assessed *post-mortem* (Miar *et al*, 2014), and cannot be measured directly on selection candidates. The within-line selection of purebreds is done primarily to improve the performance of crossbred progeny under field conditions (Tusell *et al,* 2016). This is beneficial for traits which cannot be improved simultaneously in two breeds, potentially due to opposing genetic correlations or results which oppose one another, such as meat quality traits and mothering traits; many meat quality traits do not benefit from heterosis as they are intended to be passed directly from the sire to the progeny. Meat quality traits have a wide range of reported heritabilities from low-high (Miar *et* al, 2014) and capitalising on heterosis is more useful for traits with low heritability. The study of purebreds has been shown to be able to accurately predict the phenotypes of crossbreds given that the genetic correlation of the crossbred and purebred animals for those traits is high (Bichard, 1971). Meat quality traits have been shown to have a high genetic correlation between crossbred and purebred animals (> 0.9) (Tusell *et al*, 2016). Additionally, the genetic improvement achieved in the

purebred herds directly affects the rate of genetic improvement in the commercial crossbred animals; a high rate of genetic progress in the purebreds will lead to a better result in the commercial crossbreds.

Alongside the shift in breeding goals to improve meat quality, studies focusing on the genetic underpinnings of economically relevant traits have also taken place, primarily over the last two decades (Sellier, 1998; Rosenvold and Anderson, 2003; Van Wijk *et al*, 2005). Many of these studies have focused on the genetics, breed and nutritional implications of porcine adipogenesis, lipogenesis and deposition of IMF (Dalrymple and Guo 2017) with a common goal of the identification of causal mutations, candidate regions or candidate genes which explain some of the variance observed within the trait in order to better understand the role of genetics in trait expression. From these studies, there have been a number of potential candidate genes and regions identified including *LEPR* gene which regulates feed intake and energy metabolism through leptin (Ovilo *et al*, 2010), *DGAT2* gene, which is significantly associated with backfat thickness (Renaville *et al*, 2014; Zhang *et al*, 2014) and *MC4R* gene which plays an important role in regulation of food intake and body weight in mammals (Kim *et al*, 2000; Fan *et al*, 2009; Wang *et al*, 2013; Rothschild *et al,* 2014; Silva *et al*, 2019). However, despite the ramped-up focus in this area and widespread identification of potential candidate genes, there is little consistency in this identification between studies (Dalrymple and Guo, 2017). Meat quality traits are often influenced by more than a single gene (Davoli and Braglia, 2007), with their final expression coming from many genes with small influence (polygenic traits). The inconsistencies in identification of candidate genes between studies may be due to this polygenic nature, which can complicate their study, as the effect size for each marker may be small and not always be detected by certain statistical approaches (low power). Detection depends heavily on the methods of the study as well as the sample size. In general,

meat quality traits including IMF have low to moderate h^2 and are recognised as quantitative traits, meaning the phenotype is measurable and depends on the environment *pre* and *post-mortem* in addition to the cumulative action of many genes (Rosenvold and Anderson, 2003). Alternatively, carcass composition traits (backfat thickness) are generally moderate to highly heritable (Sellier, 1998; Ciobanu *et al*, 2011; Miar *et al*, 2014). The expression of these traits varies not only among individuals of the same breed, but also between breeds, resulting in a range of measurable phenotypes. The noted heritabilities along with phenotypic variation make both meat quality and carcass composition traits suitable for genetic improvement, as traits with moderate to high heritability typically see large genetic responses from the application of quantitative methods (Ciobanu *et al*, 2011). Heritability is only one aspect needed to understand the expression and response of meat quality traits to selection. Heritability captures the proportion of genetic variance due to additive genetic values (narrow-sense heritability, h^2) or the phenotypic variation due to genetic values including dominance and epistatic effects (broad-sense heritability, H^2) (Wray and Visscher, 2008). Calculated heritabilities are most accurate for the population from which they were calculated from (Hermesch *et al*, 2000) and as such should be applied to different populations with caution. Additionally, as mentioned, many meat quality traits including IMF are polygenic. Certain genomic technologies such as genome-wide association studies (GWAS) can be very useful for obtaining a better understanding of a studied trait, which is useful when investigating complex traits, particularly those which are polygenic.

Today, identification of large numbers of single nucleotide polymorphisms (SNPs), generation of sequence data and genome assemblies for many species, including pigs, have been made available. This has enabled the study of the genetic architecture of traits though investigation of the genetic association between markers and phenotypes. From this, a number

of quantitative trait loci (QTL) have been identified (Georges *et al,* 1995; Dekkers, 2004). Marker assisted selection (MAS) is able to exploit these identified QTL for selection purposes and has been utilised with success for traits controlled by a single gene (Cesar *et al*, 2017). However, a limitation of MAS is the consistent need to discover new SNPs as each SNP is associated with different traits. In order to overcome this, a new method of selection, genomic selection (GS), was proposed (Meuwissen *et al*, 2001). GS works with these large numbers of identified SNPs over the entire genome, rather than a few select identified QTL, in order to apply them into breeding programs (Van Der Steen *et al*, 2005; Goddard and Hayes, 2007). This provides an excellent opportunity for meat quality traits in particular as a genomic enhanced breeding value (GEBV), which are used to assess the value of an animal for potential breeding animals, can be predicted for an individual without a phenotypic record (Goddard and Hayes, 2007; De Los Campos *et al,* 2013). The GEBVs can be calculated through the estimation of SNP effects or the genomic relationships between the genotyped animal (selection candidate) and the reference (phenotyped and genotyped) population (Meuwissen *et al*, 2001); rather than requiring *post-mortem* analysis, the trait may be predicted in a breeding animal from their genotype. GS assumes that by using so many SNPs that all quantitative trait loci (QTL) will be in linkage disequilibrium[1] (LD) with at least one genotyped SNP. It is important to note however, that effective research and the application of GS programs relies on the integration of both phenotypic data collection and pedigree information with the genomic efforts (Meuwissen *et al*, 2001; Van Der Steen *et al*, 2005).

[1] Linkage disequilibrium is the non-random association of alleles at different loci within a population. When the frequency of association of their different alleles is different from expected (higher or lower), if the loci associated randomly and were independent, these loci are said to be in linkage disequilibrium (Slatkin, 2008).

Identification of QTL or SNPs which have associations with a certain trait, can be achieved through quantitative methods such as a GWAS and can subsequently be considered when potential breeding animals are selected. Many statistical methods have been used for GWAS' including single-SNP association analysis (Yang *et al*, 2017; Akanno *et al,* 2018). In pigs, the use of high-density SNP panels such as the Illumina PorcineSNP60 BeadChip (Ramos *et al*, 2009) and other higher density panels, such as 660K (Axiom Porcine 660K, https://www.thermofisher.com/order/catalog/product/550588#/550588), have been invaluable for the elucidation of candidate genes which explain variation observed in meat quality traits (Van Son *et al,* 2017). As such, appropriate phenotype collection to coincide with genotype records obtained from SNP chips is imperative. Precise and accurate phenotypes are necessary for the study of quantitative genetics, high-resolution linkage mapping, GWAS' and for training GS models (Cobb *et al*, 2013). If a phenotype is inaccurate genetic gain will not be obtained.

Increased understanding of the genetic architecture of a desired trait can be helpful for improved accuracy of certain quantitative selection methods (GS). The within-line selection of purebreds (nucleus herds) results in a modest amount of inbreeding in order to fix desirable alleles (Leroy *et al*, 2018); this can ensure that these are passed onto their crossbred progeny. Additionally, it has been shown that using crossbred information (pedigree or more favourably, genomic information) in combination with purebred information will improve the accuracy of selection (Wei and Van der Steen, 1991; Xiang *et al*, 2017; Sewell *et al*, 2018, Sevillano *et al*, 2019). For crossbred animals, since their genome is inherited from the different parental breeds, the breed from which breed a SNP-allele was inherited may influence different effects. These different allele effects arise for multiple reasons: 1) depending on which parental breed the QTL was inherited from it may be in LD with different SNPs (Lopes, 2016), 2) different

quantitative trait nucleotide (QTN)[2] could be underlying a QTL in different parental breeds, (Wientjes *et al*, 2015), and finally 3) the parental breeds may experience different epistatic[3] interactions (Mackay, 2014). As such, identification of markers in crossbred animals with high IMF content is helpful for further improving accuracy of GS in the purebred animals. Additionally, pigs have been shown to be an excellent candidate species for GS based on the many QTLs affecting meat quality which have already been detected. This can increase the rate of genetic improvement within all tiers of the Canadian hog breeding system.

1.2 Objectives

The overall objective of the study was to investigate fat, primarily IMF and backfat, and the genetic architecture of IMF and to relate these to fat's role in pork meat quality. The eventual goal, though out of the scope of this thesis, is the implementation of any newly identified SNPs into breeding programs to improve the crossbred expression of IMF, and thus improving meat quality traits. Positively (genetically) correlated traits generally increase and decrease together, proportional to their correlation and which trait is under direct selection. Genomics, particularly the incorporation of trait-specific markers can be useful by increasing the accuracy of GEBVs in a GS program for the trait under investigation and lessening the degree to which related traits are affected. For IMF and BFD, this is of particular importance, as the goal is to increase IMF without re-fattening pigs. This was achieved through assessment of IMF content, backfat fat composition (quality) estimates, examination of IMF and related traits in both purebred Durocs and commercial crossbred pigs and finally, implementation of a GWAS for IMF content in order to identify relevant SNPs and regions. The studies were completed

[2] A quantitative trait nucleotide (QTN) is an identified causative nucleotide within a quantitative trait loci (QTL) (Miles and Wayne, 2008).
[3] Epistasis is basically the interaction between genes at different loci (Cordell, 2002).

through discovery-based methods, which allowed us to freely investigate the traits of interest. The specific objectives and hypothesis for each study are as follows:

1. Phenotypic data generation for purebred Duroc IMF content of the loin as well as for backfat quality using near-infrared spectroscopy. Reference methods of proximate analysis (IMF) and gas chromatography (backfat) were used to validate the predicted phenotypes. The goal is to build on the body of literature validating near-infrared reflectance (NIR) as a method of reliable phenotype production and to utilise these phenotypes in further genetic studies. Based on the assessment of the literature of NIR as a reliable method for phenotype prediction including swine subcutaneous fat and meat composition, we expected to find a moderate-high positive relationship, indicative of accuracy, between the predicted phenotypes from NIR and those which were measured via reference methods (actual values). Our null hypothesis is that no relationship exists.
2. Investigation of the relationships between IMF content, measured by various methods, and backfat depth, measured by various methods, in a commercial crossbred and purebred Duroc population. In addition to estimating the variance components for each single trait (heritabilities for backfat depth (BFD), ultrasound backfat depth (UBFD), near-infrared IMF (NIRIMF), ultrasound IMF (UIMF), subjective marbling score (SUBJ) were calculated), we conducted a bivariate analysis to compute genetic and phenotypic correlation between the aforementioned phenotypes. All results were compared to the literature; based on the well documented relationship between IMF and BFD found in the literature we expected to identify moderate-high positive correlations between these traits, as well as moderate – high heritabilities for these traits. Our null

hypothesis is that there is no relationship or a negative relationship between the traits and that estimated heritabilities are outside the range of those previously reported.

3. Identification of SNPs and subsequent candidate genes related to IMF (UIMF and NIRIMF estimates of IMF) content in first a purebred Duroc population followed by a commercial crossbred pig population. This was done using a GWAS; the results of the GWAS were then compared to the current body of literature and to one another to investigate the genetic underpinnings of IMF in commercial crossbred and purebred pigs. We hypothesised that for all GWAS' that we will identify a SNP which has a large effect size, explaining a sizable amount of the phenotypic variance seen in IMF; in the event there are no single SNPs with large effect size we expect to identify SNPs with a smaller effect size which still pass the threshold of significance. We have no null hypothesis for the GWAS'.

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Chapter 2.0. Literature review

2.1 Canadian hog industry

Canada is home to a thriving agricultural and agri-food sector and pork is Canada's fourth largest agricultural commodity, following canola, dairy and beef cattle (Brisson, 2015). Canada is the world's 7th largest pork producer, hosting 2% of global production (Agriculture Canada, 2017) and exports continue to increase. In 2011, close to 60% of all domestic pork produced was exported (Maignel *et al,* 2011) and Canada Pork International (CPI) (2018) reports that in 2017, over 1.2 million tonnes of pork, pork by-products and value-added products were globally exported. This resulted in over $4 billion dollars of revenue, increased from $3.2 billion dollars in exports in 2016 (Aurora, 2017), and just over a billion dollars in 1998 (CPI, 2018). Canadian pork is primarily exported to the USA and Japan. The USA imports more tonnes of product; however, Japan spends more money on what is chosen to import. In 2019, the USA spent $1.251 billion dollars on just under 320 000 tonnes of Canadian pork and pork products, and Japan spent $1.403 billion on just under 262 000 tonnes of Canadian pork and pork products (CPI, 2019). The primary difference is import choices; Japan imports significantly less than the USA does in all categories (fresh/frozen/chilled fat, offal, and processed), but the products which it does import are of a higher economic value. Canada's entire agricultural and agri-food industry sector was reported as 6.7% of Canada's annual GDP in 2016, (Agriculture and Agri-food Canada, 2017), totalling $111.9 billion dollars. Pork exports alone contributed $3.5 billion dollars to Canada's GDP in 2012 (Canadian Pork Council, 2012).

Traditionally, Canadian pigs were grown on small-medium scale farms in conjunction with other livestock animals or crops, predominantly for subsistence (Brisson, 2015). Today,

however, farms are large, often specialised in a single animal product, and fewer in number. This change has made small-scale operations less economically viable and encourages their large-scale growth (Machum, 2005). Over the last 45 years, the meat packing industry has undergone a massive shift, coinciding with the changes in farm size (Macdonald, 2003). Significant consolidation of small plants and increased vertical integration allowed large firms to capitalise on economies of scale (Macdonald, 2003). For the pork industry specifically, this consolidation has allowed packers to procure large contracts with producers and space the arrival of specified numbers of hogs to maximise efficiency (Harper, 2009). Desired weight is specified in the contract and can be within a narrow window, therefore uniformity of animals significantly affects the producer's profit. Carcass homogenization and achievement of desired carcass traits are affected by many factors, including animal nutrition, environment, and genetics. Incentive payments according to the contract and carcass grading and purchase matrices, known as the payment grid, are incentives for producers to follow these guidelines and incorporate the genetics into their breeding herd. During the 1980's, a majority of pigs were sold on a live weight basis (Hayenga *et al*, 1985), just 10 to 12% were sold on a carcass basis. Some producers felt that more emphasis should be placed on carcass quality (Hayenga *et al*, 1985), which would help improve the consistency and quality of pork, drive up its demand, value and maximize payment received for an excellent carcass. Emphasis on carcass leanness has also been as a result of health-conscious consumers pressuring the industry for leaner meat, and to combat the poor perception of pork during the 1980's as a low quality, 'blue collar', protein source (Dougherty, 1987). During this time, the prevailing research also equated animal fats, particularly saturated fatty acids, and high fat intake with negative health consequences (Kritchevsky, 1998; La Berge, 2008). As a result, premiums for particular traits, primarily increased lean muscle and decreased backfat, were built into the purchase matrices by the

packers for producers to follow (Hayenga *et al,* 1985; Martin, 2008). The shift from fat pigs and fatty pork to lean pigs and lean pork was achieved rapidly, in just two decades of dedicated selection producers had made huge progress (Schwab *et al,* 2006). Selection and breeding focusing solely on leanness and reduction of backfat resulted in unintended decreases of correlated meat quality traits and negatively affected meat quality. The efficient production of these lean pigs was also a major goal. Feed comprises 60 to 70% of the cost of growing pigs (Renaudeau *et al,* 2012; Martinsen *et al*, 2015), and the energy costs of protein deposition compared to fat deposition ranges from 9.00 to 11.25 kcal/g and 12.73 to 12.96 kcal/g, respectively (Cleveland *et al*, 1983). Therefore, reduced feed intake whilst increasing average daily gain were some of the other traits selected for in order to grow a more efficient lean pig (Cleveland *et al*, 1983). Additionally, some drug therapies, specifically ractopamine, were also investigated for their role in growth efficiency. Ractopamine use significantly improves growth performance, lean growth, and carcass composition (Williams *et al*, 1994); although ractopamine is not banned in Canada, it is banned in many countries which Canada exports to. As such, all federally inspected abattoirs, which produce 97% of all Canadian pork, require hogs to be ractopamine free and producers comply to the Canadian ractopamine-free certification program (Canadian Pork Council, 2020).

Though the lean meat goal had been achieved, decreases in certain meat quality characteristics, including intramuscular fat (IMF), and subsequent organoleptic qualities assessed by consumers, such as reduced tenderness and reduced juiciness created a new set of issues for the pork sector.

2.2 Pork fat and meat quality

Overview

Meat quality is a broad topic which encompasses intrinsic and extrinsic factors relating to consumption satisfaction of the final product; many *pre* and *post-mortem* factors also significantly affect consumption quality. This section will first focus on the general definition of pork meat quality and factors which influence this. Subsequently, this will narrow into an investigation of fat; fat quality, deposition, genetic influence of deposition, noteworthy depots (IMF), and finally, consumer preferences.

Pork meat quality

Overall quality of pork is a combination of technological quality, carcass traits, and consumer acceptability of the product (Lee *et al,* 2012). There are a large number of traits to assess when investigating meat quality, all of which ultimately relate to a sensory experience. Some of these include water holding capacity (WHC), peak shear force (PSF), colour of both muscle and fat, ultimate muscle pH, visual appeal, tenderness, juiciness and flavour (Zhang *et al*, 2015). These are all assessed *post-mortem* and can be affected by slaughter practices and carcass handling techniques. Additionally, *pre-mortem* factors can significantly affect the assessed traits; these include environment, diet, transportation to slaughter facilities, age, breed (genetics) and method of slaughter. Ultimately, the most important factors depend on the preference of those evaluating the product (Purslow, 2017) which can vary between individuals, regions and countries.

As a society progresses and changes, so does the colloquial definition of meat quality; if meat becomes readily available, safe and free of disease, consumers then look more closely at intrinsic qualities, such as preferred flavour and texture (Purslow, 2017). More narrowly, meat quality is divided into quality attributes (QA) or quality characteristics (QC), the former being the set of criteria used by consumers for qualitative inspection, and the latter being those measurable through scientific tests and processes (Becker, 2002). QA include the visual appeal,

flavour, juiciness, and tenderness upon consumption, and engage the consumers' senses of sight, smell, and taste; therefore, they are referred to as organoleptic attributes. These characteristics come into play upon purchasing and repurchase of meat products and are primarily based on consumer preference (Glitsch, 2000). QC are objective and quantitatively measured and thus have values calculated for evaluation (Becker, 2002). Some traits may be evaluated in both sets of standards, such as IMF and marbling (visual IMF in the lean). QC IMF can be estimated by precise instruments such as near-infrared (NIR) on meat samples, ultrasound in live animals, or through the use wet chemistry for an exact value. When evaluated under QA, it is called marbling; many standards exist for the subjective evaluation of visual marbling including the National Swine Improvement Federation Standards (NSIF), the National Pork Producers' Council standards (NPPC), and the Canadian loin marbling standards.

Upon carcass processing, a number of measurements may be completed to assess specific meat quality traits. Firstly, IMF (visual marbling), meat colour and fat colour may be subjectively assessed using reference standards provided by Canada Pork International for export quality (Canada Pork International, 2015). Additional measurements taken at the packer include ultimate pH (PHU), and carcass temperature; these measurements would not be done on every carcass, but rather on a representative percentage. Backfat thickness is measured on each carcass as it is this measurement which ultimately determines the valuation of the carcass and final payment for the producer. The equation used for carcass valuation is shown in Figure 2.1. In general, the thinner the backfat, the greater the lean yield calculated and greater payment.

Figure 2.1

A: Henessey grading probe = $67.2327 - 0.7877 (FAT) + 0.1086 (LEAN) + 0.0087 (FAT)^2 - 0.0004 (LEAN)^2 - 0.0002 (FAT \times LEAN)$

B: Canadian Lean Yield = **[**weight of side ribs + commercial belly +the muscles of the half carcass – (the weight of the jowl muscles + the muscles between the neck bones + the muscles around the picnic ribs)**]** *100 / **[**weight of the dissected tissues (skin, fat, lean, bone) of the carcass including the feet, tail and jowl**]**

Figure 2.1, which shows two equations used for the calculation of carcass lean yield.
A: Algorithm used by a well-known grading probe for calculation of estimated lean yield. Adapted from National Pork Carcass & meat quality evaluation standards handbook, 1997
B: Hand equation for Canadian lean yield calculation. Known in shorthand as LY2000. Adapted from Marcoux *et al,* 2007

The role of fat in pork, for satisfactory consumption of meat and processed products as well as for carcass economic valuation will be discussed in depth throughout this section, and the high value these depots have will become clear.

What is fat?

Triglycerides are the primary storage lipid in animals, they are deposited when energy supplied is in excess of biological needs and are the main constituent of fat depots. They are made of esters of fatty acid chains attached to a glycerol molecule. Fatty acids can be synthesised *de novo* or absorbed from dietary sources, and glycerol is derived from dietary glucose (Dunshea and D'Souza, 2003). Due to the innervation of blood vessels and capillaries throughout porcine adipose tissue (Seman, 2008), dietary glucose is easily supplied. One triglyceride is made of three esterified fatty acid chains which are attached to the glycerol backbone. Each fatty acid chain is either saturated (SFA), polyunsaturated (PUFA), or monounsaturated (MUFA). Unsaturation is a measure of the carbon-carbon double bonds in the hydrocarbon chain of the fatty acid, as carbon-carbon double bonds increase, carbon-hydrogen single bonds decrease (Ockerman, 1996). An unsaturated fatty acid contains less than the maximum amount of hydrogen due to the presence of the double bonds between the carbons in

the tail. MUFAs have one double bond, PUFAs have two or more double bonds and SFAs have none. The presence of double bonds, their location in the fatty acid and finally, the number of bonds influences the fatty acid's properties. Subsequently, the primary type of fat deposited will influence the properties associated with that fat depot.

Fat quality

Subcutaneous fat, further categorised into backfat, belly fat, intermuscular and IMF are the principle depots of adipose tissue in pigs with regards to carcass and meat quality. Quality, quantity, texture, colour and thickness of pork fat is economically relevant as it heavily influences meat quality parameters, including firmness, colour, peak shear force, organoleptic parameters for consumers, visual assessment prior to purchase, the attributes and acceptance of further processed products, and ultimately the value of an entire carcass (thickness) (Teye *et al,* 2005). Generally, fat is described by and ranked into one of two broad categories, 'good' or 'poor', based on colour and consistency/texture (Sørensen *et al*, 2012). Good fat is firm and white and comprised primarily of SFAs. Poor fat is oily, wet, off coloured, and contains a high concentration of UFAs and a high water: lipid ratio which contribute to an undesirable, soft texture (Kouba and Sellier, 2011). In the abattoir, carcass cut up follows an efficient and defined procedure; first, the carcass is split into halves (lengthwise) and is then further divided into four 'primal' cuts of the shoulder, leg, loin and belly (Marcoux *et al,* 2007; CFIA, 2012). These primal cuts are then marketed to grocery stores and butchers where they are further cut into sub-primal or retail cuts for consumer purchase and consumption, though some packers may do this step as well (Alberta Agriculture and Forestry, 2018). Texture-wise, hard fat is preferred from a packer perspective as the cohesive nature between the muscle and the fat keep large muscles of the primal cuts together prior to further cutting (Sørensen *et al,* 2012). Soft fat decreases cutting efficiency and causes uneven slicing due to sticky knives. The majority of

backfat and subcutaneous fat is trimmed from retail cuts prior to selling, and this fat goes for use in other value-added products (Kouba and Sellier, 2011). In these further processed products, fat is one of the most variable ingredients used; is it suggested, by the National Pork Producer's Council, that a good quality pork fat must contain less than 15% PUFAs. Overall, the fatty acid composition of subcutaneous fat plays an integral role in the texture, flavour and processing efficiency of value-added products. Fat comprised of SFAs is preferred for further processed products, as it provides positive eating attributes and a more consistent product for sausage producers (Baer *et al,* 2013). PUFAs are associated with negative properties, such as fat capping in hot dogs and oil/fat seepage (Baer *et al,* 2013). As discussed, PUFAs are also less oxidatively stable than SFA and have an increased propensity for lipid peroxidation which can lead to reduced shelf life and rapid development of rancidity, resulting in off-flavours noted during consumption (Cameron *et al*, 2000; Cannata *et al*, 2010). Animal nutrition significantly affects the final composition of fat. Feeding an antioxidant such as vitamin E at a supra-nutritional level to the pigs prior to slaughter can reduce this risk (Turner *et al,* 2014*)*, though this is not widely implemented.

As a result of breeding programs with the focus of efficient, lean growth, we have inadvertently attained low quality fat due to the relative increase in PUFA concentration in all adipose tissue which is associated with high-lean carcasses (Wood *et al,* 2008) and total carcass fat reduction. As backfat thickness decreases, the proportion of PUFA increases, devaluing the fat quality. It is well documented in the literature that decreased fat deposition results in an increase of unsaturation in the fat; lower deposition associated with leaner pigs results in higher PUFA and MUFA proportions (Correa *et al,* 2008). The level of unsaturation in swine adipose tissue follows a negative gradient from the outside to the inside of the body (Kouba and Sellier, 2011; Soladoye *et al,* 2017). This significantly affects subcutaneous fat

(backfat), which is generally deposited in two layers (Seman *et al,* 2008). The first layer deposited is the outermost layer, and it is more heavily concentrated in PUFA's than the second (innermost) layer, which contains higher levels of SFAs. This is attributed to less *de novo* synthesis of SFA and MUFA and a higher incorporation of dietary fatty acids in pigs during the first stages of lipid deposition, the early stages of growth (Wood *et al*, 2008). Interestingly, since backfat at the dorsal portion of the loin is highly accessible for ultrasound analyses and on-floor measurement it has been the chief site of assessment during the pursuit of leaner carcasses (Kouba and Sellier, 2011).

Measures of pork fat quality which are evaluated on the packing line include subjective colour, and texture (Seman, 2008). Any measures done in the abattoir must be quick, inexpensive and require minimal to no equipment, as the abattoir is a fast-paced environment where evaluators do not often have more than 15 seconds with each carcass (Sørensen *et al*, 2012). Such assessments are prone to error, lack repeatability and rely primarily on qualitative analyses; cumulatively, these tests are known as the subjective fat quality scores (Seman *et al,* 2013). A commonly used, more scientific method of fat quality analysis is Iodine Value (IV); IV is reported as the grams of iodine that 100 grams of a fatty acid takes ups in a halogenation reaction. Iodine monochloride is introduced to the fatty acid, and at the site of any C=C double bonds, an iodine molecule is incorporated to create a di-halogenated bond. One carbon is bonded to an iodine and one to a chloride, of which the total quantity can later be measured. Saturated fatty acids take up no iodine, and do not undergo the halogenation reaction (Vlab.Amrita, 2011), therefore their IV is zero. IVs greater than 70 indicate the fatty acids are primarily unsaturated and likely an oil, a value less than 70 indicates increased saturation of the fat. Generally, iodine value is calculated after the fatty acid profile of fat is determined via gas chromatography (GC) (Seman *et al,* 2013). However, as discussed, rapid methods are more

practical for kill-floor evaluations. The NitFom™ (Frontmatec, Denmark) is a hand-held device which uses NIR and is capable of rapid IV calculation from scanning carcass subcutaneous fat. The accuracy, when compared to GC, is very high (Christensen *et al*, 2019). Classification of carcasses based on their IV, soft or firm, can allow processors to sort carcasses for appropriate cutting recipes and to provide feedback to producers on their feeding programs (Christensen *et al*, 2019). Though a producer is not paid for improvement of fat quality, optimization of fat firmness will positively affect the hog industry by improving overall product quality via slicing efficiency and extend shelf life due to reduced PUFAs. For the estimation of individual fatty acids, categories of SFAs, UFAs and adipose tissue composition in meat or subcutaneous fat, methods have been developed using NIR technology (Prieto *et al*, 2009). This technology has been shown to provide highly accurate results at full production speed. NIR technology works by passing infra-red (IR) light through a sample via a probe. Certain molecular bonds absorb the IR light maximally at different wavelengths; the NIR instrument analyzes the proportion of light that is reflected by the sample material. This returns a spectrum which can then be analyzed against standards to determine the composition of that sample (Davies, 2005).

Fat deposition in pigs

Adipose tissue or fat tissue is differentiated from the mesenchyme cells during fetal development, and these cells then differentiate into loose connective tissue which are filled with lipid as the pig grows (Seman, 2008), these are known as adipocytes. Immature adipocytes contain barely any fat content, and that which is present contains high proportions of water and connective tissue, causing it to be grey and soft (Wood, 1984). As the animal matures, more energy is diverted into fat deposition and therefore these adipocytes fill with lipids, primarily triglycerides. This displaces water and lowers the relative percentage of

connective tissue in the fat depot, which alters the texture and increases quality of the fat (Seman, 2008). This tissue is also the site of a significant amount of *de novo* fatty acid synthesis. In fact, approximately 75% of fat deposits in pigs are from *de novo* synthesis in the adipose tissue. The remainder are absorbed and incorporated into tissues, unchanged, from dietary sources (O'Hea and Leveille, 1969; Lawrence and Fowler, 2002). As such, diet and feed intake play large roles in not only the cost of raising pigs, but also in deposition of fat. Additionally, sex is an important factor for fat deposition, as gilts tend to be fatter than boars (Farnworth and Kramer, 1987; Kouba and Sellier, 2011), and barrows fatter than gilts (Dunshea and D'Sousza, 2003).

Subcutaneous fat is subdivided into backfat and belly (omental) fat, along with inter and IMF which are the other primary adipose depositions. The allometric growth, the measurement of unequal development rates of different organs or parts of an animal, in regard to fat depots, is of interest for fat depot development (Collins Dictionary of Biology, 2005). The allometric growth of kidney fat is approximately 1.24 times, significantly higher than both subcutaneous fat and intermuscular fat at 1.01 and 0.87, respectively (Wood, 1984). IMF is deposited in the late/final stages of growth, making it a 'late' maturing fat depot. Feed energy supplied for heavy, fast growing animals to support protein deposition can be allocated to IMF deposition upon reaching their mature weight (Wood *et al,* 1999). Generally, fat is deposited when energy from the diet is supplied in excess to daily metabolic requirements. The accretion of fat tissue costs approximately four times more energy than the accretion of an equal amount of lean tissue (De Lange, 1998). Lean growth and deposition of muscle is very efficient in today's meat pigs. Too much fat deposition may result in producers receiving less money for a carcass based on the above described grid system, but also costs significant money in feed.

Growing heavy, lean pigs is more economically efficient than heavy fat pigs due to the energetic costs of deposition between lean and fat (De Lange, 1998), though meat quality and palatability decrease from lack of lipid (Frank *et al,* 2016). Late maturing breeds, such as Duroc or Yorkshire, tend to have less fat deposited at the time of slaughter than early maturing breeds, and what fat is deposited has a higher concentration of water due to the immaturity of adipocytes (Wood, 1984). The Duroc breed (modern type[4]), however, is also associated with higher IMF than other breeds (Schwab *et al,* 2006). Breed effects in fat deposition substantially influence total carcass fatness, as well as deposition to the various depots (Kouba and Sellier, 2011). Fat deposition in pigs follows a defined pattern, meaning the same depots will be deposited, however, fat partitioning sees significantly more genetic variation between breeds and populations (Kempster, 1980). This deposition of fat, its ultimate quantity and relationship to various factors including genotype is discussed in a study by Wood *et al.* (2004) where the effects of diet, breed and protein deposition were investigated in relation to fat deposition and subsequent eating quality of the meat. Four breeds were investigated: two modern, Duroc and Large White, and two traditional[5] (Tamworth and Berkshire). Carcasses of the traditional breeds were significantly fatter and had less lean deposition, indicating the genetics relating to fat deposition significantly affected the carcass composition of these animals. Different breeds grow at different rates, and inherently have different predispositions for fattening (Farnworth and Kramer, 1987).

Genetic influence on fat deposition

[4] Modern type breeds, also called conventional, improved or commodity, are breeds which have undergone selection in order to meet the demands of industry and consumers. Modern breeds are 'fast growing' types, their carcasses contain more lean protein deposited and less fat (than traditional breeds) when given the same diet (Wood *et al*, 2004).

[5] Traditional breeds, also called unimproved or heritage, are breeds which have not undergone significant selection to meet consumer and industry. They tend to be fatter and slower growing (Stachowiak *et al*, 2016).

In addition to dietary influences, sex, and other environmental factors, genetics play a large role in the deposition of fat. Although pork meat quality is predominantly polygenic, there are a few major genes specific to pork quality, namely the Halothane (*HAL*) gene (Fujii *et al,* 1991; Hamilton *et al,* 2000; Brewer *et al,* 2002; Rosenvold and Andersen, 2003; Yang *et al,* 2017) and the Rendement Napole (RN^-) gene (Le Roy *et al*, 1990; Hamilton *et al,* 2000; Brewer *et al*, 2002; Rosenvold and Andersen, 2003; Yang *et al,* 2017). Major genes have large, single gene effects on meat quality. They are considered major genes if the mean value for a homozygous individual compared to individuals not carrying the gene is equal to or greater than one phenotypic standard deviation for the affected trait (Sellier and Monin, 1994; Rosenvold and Andersen, 2003). These genes, along with their identified causative mutations and subsequent removal from breeding stock will be discussed in detail in section 2.4.

Differential breed expression (phenotypic variation) can significantly affect meat quality. The pyramidal structure of the hog industry capitalises on differential breed expression of selected traits and also take advantage of the benefits of crossbreeding in order to improve the quality of the final product. Since 2001, there have been over ninety studies done focusing on the genetics, breed and nutritional implications of porcine adipogenesis, lipogenesis and deposition of IMF with little consistency in specific genes implicated in these processes (Dalrymple and Guo 2017). From investigation of previous literature, a number of genes have been identified with association to IMF development in pigs. These are presented in Table S2.1 in Supplementary information 2.0. The majority of identified candidate genes which exert influence on fat deposition are involved in the various adipogenesis pathways as well as lipid metabolism (Kouba and Sellier, 2011; Xing *et al,* 2015). Four primary genes identified by Dalrymple and Guo (2017), which are supported by identification in five or more of the over

ninety studies, are *acetyl-CoA carboxylase* (*ACACA*), *fatty acid synthase* (*FASN*), *stearoyl-coA desaturase* (*SCD*), and *fatty acid binding protein* 4 (*FABP4* or *A-FABP*). *ACACA* encodes the protein acetyl-CoA carboxylase alpha, which catalyses the rate limiting step for *de novo* fatty acid synthesis (Xing *et al,* 2015). *FASN* is a lipogenic gene which encodes the fatty acid synthase protein (FAS), this protein catalyses the synthesis of SFAs (Jensen-Urstad and Semenkovich, 2012). *SCD* codes for an enzyme which catalyses the conversion of SFA stearic acid into MUFA oleic through addition of a double bond (Renaville *et al*, 2014). *FABP4* or *A-FABP* codes for the fatty acid binding protein used for lipid transport inside fat cells and is involved in regulation of IMF deposition (Ensembl 95, 2018 - https://uswest.ensembl.org/index.html). Xing *et al.* (2015) showed that full Songliao black pig siblings with opposite backfat phenotypes had differing upregulation of various genes. In individuals with high backfat, *ACACA*, *SCD,* and *FASN* were highly expressed, which are associated with fatty acid synthesis. There have been studies which suggest the possibility of a major gene relating to IMF content in pigs, specifically originating from the Meishan breed (Janss *et al,* 1997; de Koning *et al*, 1999). This gene, *MI,* has a recessive allele which increases IMF content. The same authors, though, subsequently elucidated a number of QTL with reduced individual effect (Janss *et al,* 1997). Similarly, in the Duroc population, the supposed major gene (*MI)* was actually found to correspond to two QTL after further analysis (Sanchez *et al,* 2007).

An important piece to understand in regard to genetic influence on fat are the genetic and phenotypic correlations of related traits, as well as their individual heritabilities. A genetic correlation (r_g) is the proportion of variance which two traits share due to genetic causes (Falconer and Mackay, 1996). Calculation of r_g can be done with the following formula;

$$r_g = \frac{cov(g_1 g_2)}{\sqrt{V_{g1} V_{g2}}} \quad (1)$$

where $cov(g_1, g_2)$ is the given genetic covariance between two traits, V_{g1} is the genetic variance of trait 1 and V_{g2} is the genetic variance of trait 2 (Searle, 1961; Falconer and Mackay, 1996). Correlations of 0 indicate that the genetic effects on one trait are independent of one another, however, a value of 1 implies that all genetic influences on the two traits are equal. Often, the genetic correlation between two traits mirrors the phenotypic correlation observed between them (Cheverud, 1988). Phenotypic correlation between two traits is affected by the individual trait's heritability as well as their genetic correlation and is the correlation between records of two traits on the same animal (Searle, 1961). A phenotypic correlation can be calculated using the following formula:

$$r_p = r_g \sqrt{h_1^2 h_2^2} / \sqrt{(1 - h_1^2)(1 - h_2^2)} \quad (2)$$

where h_1^2 and h_2^2 are the heritabilities of the two traits, and r_g is their genetic correlation (Searle, 1961). Understanding of a trait's heritability and genetic correlations are important for selection purposes. Traits can be independently highly heritable, but not be genetically correlated, similarly, they can be completely genetically correlated, but with low individual heritabilities. Many traits which comprise pork quality generally have low to moderate heritability (0.15 to 0.3) (Rosenvold and Andersen, 2003). An exception to this is IMF, which is moderately to highly heritable (h^2 = 0.47 to 0.53) (Hernández-Sánchez *et al,* 2013; Lim *et al,* 2016). Total carcass adipose tissue deposition and IMF have a low-positive genetic correlation (r^2 = 0.11) (Wood, 1990) which is much lower than the genetic correlation of IMF and backfat thickness, which ranges from 0.37 to 0.64 ($\pm$0.05) (Solanes *et al*, 2009; Hernández-Sánchez *et*

al, 2013). It is important to be mindful of correlated traits during selection, as they must be factored into the expected outcome (Miar *et al,* 2014). Simply selecting for animals with increased IMF will also increase backfat thickness, which is not the goal; an increased understanding of the genetic architecture of each trait is important for achieving a marbled carcass with decreased/maintained fat depth. This is a key point of focus for this thesis, as improving IMF in the crossbred is the ultimate goal but increasing backfat thickness (re-fattening pigs) is contradictive to industry goals and will harm overall profitability. It is evident that utilising genetic tools for the independent manipulation of these traits will be necessary.

Intramuscular fat (IMF)

The amount of IMF deposited can affect what the fatty acid composition of the depot will be; as discussed in the previous section, decreased fat deposition, associated with leaner pigs, results in higher PUFA and MUFA proportions in the fat (Correa *et al,* 2008; Webb and O'Neill, 2008). Additionally, regardless of total carcass fat deposition, remnant backfat and intermuscular fat is trimmed from retail cuts prior to consumption, (Kouba and Sellier, 2011; Listrat *et al*, 2016) which leaves IMF as a primary fat depot for consumption. For optimal eating quality, ideal IMF content as determined by sensory panels is considered to be > 3%, however the typical commercial hog in Canada averages just 1.5% in the *longissimus dorsi* (loin) muscle (Meadus *et al*, 2018). Low IMF is associated with a dry, flavourless and tough product. Unfortunately, overcooking often adds to this dryness. Pork is also commonly associated with *Trichinella spiralis*, a parasite which results in the disease trichinellosis in humans who eat undercooked pork products. *Trichinella spiralis* can infect pigs which are swill fed (uncooked garbage/kitchen scraps), those which are exposed to pig carcasses, raised outdoors with potential exposure to wild hogs or through vectors such as rats (Murrell *et al*, 2006). Today, however, the risk is much lower due to modern farming techniques (CFIA,

2013). Lingering food safety fears over *Trichinella spiralis* contributes to the overcooking pork, adding to the results of a dry, tough, and unappealing product, but is unnecessary based on these guidelines (USDA, 2020).

As discussed, marbling, or IMF, is one of the most important meat quality traits as it significantly affects the eating quality of meat as it is positively correlated with many eating quality traits, including juiciness, flavour and tenderness (Wood *et al,* 1999, Fernández *et al*, 1999b; Cannata *et al,* 2010; Lim *et al,* 2016). Tenderness, widely considered the most important attribute for eating quality (Fortin *et al*, 2004; Teye *et al,* 2006), is significantly correlated with the amount of IMF or marbling present. IMF is deposited in between individual muscle fibers and between bundles of muscle fibers and this is believed to contribute to opening of the muscle structure, diluting fibrous protein with fat, and therefore decreasing the shear force associated with the cut (Wood *et al,* 1999). Juiciness is increased in pork with increased IMF, as during cooking the fat helps to retain moisture, as well as increased flavour due to the Maillard reaction[6] (Teye *et al*, 2006). Total IMF content depends upon many factors, including genetics, breed expression, nutrition, and environment. Ultimately, it is the number and size of intramuscular adipocytes which determine the final IMF amount (Won *et al*, 2018), along with the genetic background of the animal. As discussed, in section 2.1, breeding focus, primarily in the 1980's, was placed on increased efficiency of lean growth and reduced backfat thickness for decreased carcass fatness due to the dominant health science at the time which equated fat (animal fat, particularly) with negative health consequences (Kritchevsky, 1998; La Berge, 2008). Unfortunately, the relatively high genetic correlation for backfat and IMF

[6] The Maillard reaction, also known as non-enzymatic browning, is the chemical reaction which occurs between amino acids and reducing sugars in the presence of heat (minimum 140°C) that results the browning of food while forming new aromas and flavors (Feiner, 2006).

indicates that selection for reduced backfat thickness would inadvertently also select for decreased IMF. When discussing IMF, we primarily investigate the loin (*longissimus dorsi)*, which is the most routinely consumed cut and it is also typically the leanest cut; this has been achieved through the reduction of visible fat (marbling), compounded by breeding goals to achieve the same (Webb and O'Neill, 2008). This reduction of marbling in pork was initially well received, so much so that the National Pork Board commissioned the 'other white meat' ad campaign, launched in 1987 to combat any lingering stigma of pork being regarded as a low quality, fatty meat. However, complaints of blandness in pork became abundant, as compared to the flavourful counterpart, which was historically available, this new lean meat lacked taste (Wood *et al,* 1999). A majority of consumers choose lean loins over more marbled loins based on visual inspection, however, during a taste panel with the same consumers, the taste, juiciness and tenderness associated with loins having increased IMF was unanimously preferred (Font-i-Furnols *et al*, 2012). This phenomenon is consistent in other markets, as well. The organoleptic experience associated with pork consumption is strongly correlated to fat quantity and quality. Such consumption-related attributes, including texture and flavour in meats are all associated with fat, thereby influencing its acceptability and palatability for consumers (Drewnowski, 1992). It is evident that a balance between leanness for initial purchase, and fatness for repurchase, of pork products must be achieved (Lim *et al*, 2016). It is therefore abundantly clear the importance that IMF plays in meat quality.

Consumer consumption preferences

Consumer perception of excellent quality pork relies on how important specific traits are to any given purchaser (Meuwissen *et al*, 2007); due to the importance of acceptance to the industry, high importance is placed on meat quality attributes which relate to eating experience. One of the most interesting factors associated with eating experience is fat; fat plays a

significant role in the acceptance (and rejection) of pork. As discussed, the visual inspection of pork prior to purchase is an important part of consumer acceptance and many consumers use marbling as a visual measure, and lean meat with minimal marbling is often preferred at that point (Font-i-Furnols *et al,* 2012). This holds true for a majority of North American consumers, although different cultures and countries have different preferences. Pigs from the standard Canadian meat pig cross have significant variation in their IMF content, but currently, it is often < 1% - 1.5% (Wood *et al,* 1990; Meadus *et al*, 2018). Export markets vary in their IMF preferences; USA consumers prefer 2.5 - 3%, whereas 1% is the preference in the United Kingdom (Listrat *et al,* 2016). Japan is of particular importance for Canada as they are a major customer for high value Canadian pork exports (Canada Pork International, 2019). In general, Asian consumers highly value flavour and juiciness over leanness, and therefore emphasis is placed on cuts with moderate to abundant IMF during purchasing (Frank *et al*, 2016). Marbling in Canadian pork is graded on a 6-point scale from Canada Pork International (CPI, 2015). Pork with a score of 3 or above is recommended as premium retail meat, which is then often exported to countries with this preference. Traditional breeds such as the Berkshire (Kurobota), are highly valued in Japan as their meat contains significantly more marbling than lean meat pigs, a trait highly valued in Asian culture (Frank *et al*, 2016). However, Berkshire pigs grow much slower and are more expensive to feed than commercial crossbred [Duroc x (Landrace x Large white)] pigs in Canada. Companies which supply genetics to the greater Canadian pork industry often have sires of different breeds and lines of a single breed which have been selected for traits valued by all consumer markets, such as IMF content, balanced with growth, in order to improve the profitability of a producer's herd (Hypor, 2018).

Consumers purchasing raw pork often use three to four meat characteristics to make a choice (Ngapo, 2017). Aside from marbling, colour of meat is generally one of the first

characteristics noted and preference for pork colour is diverse and can vary significantly within even a single country (Ngapo, 2017). High marbling content can distort the colour of a pork loin chop as it increases reflectance, recorded objectively as lightness (L*), and can cause it to appear paler to an untrained consumer eye. In extreme cases, this can result in pork being mistaken for PSE. Striking a balance for enough IMF to satisfy consumption preferences, but not excessive to devalue the visual appeal is important. Albertan consumers have been shown to prefer a dark red pork colour with little fat cover (Ngpao, 2017). Aside from colour, drip loss is another visual characteristic assessed by consumers to make their meat choice. Drip loss is the water lost from meat during storage, such as in the wrapped trays on retail shelves (Warner, 2014). Packages with excessive moisture content are visually unappealing and can indicate the product is inferior, as drip loss is considered an important trait for palatability and overall acceptance of meat (Warner, 2014; Dalrymple and Guo, 2017). Overall, consumer satisfaction with pork is the ultimate goal when setting breeding goals to achieve excellent meat quality and Canadian hog system utilises a specific breed cross in order to optimise meat quality for the consumer.

2.3 Crossbreeding

The Canadian hog industry is a pyramidal structure composed of three tiers; nucleus farms are at the top of the pyramid, and this is where genetic improvement is achieved (Figure S2.1, Supplementary information 2.0). Here, animals are selected based on their own performance in a purebred herd. Nucleus farms are also very high-health farms, which is very important to maximise the results of genetic improvement. By reducing the stress of disease and other potential environmental stressors, full genetic potential can be expressed and measured. The middle tier are the multiplier herds, where purebred dam lines are crossed (F1

animals) in order to produce large numbers of breeding animals. These crosses can be purchased by commercial farms as replacement gilts. The bottom tier is the commercial farms, which mates purebred sire semen with F1 dams to produce a terminal, F2 commercial cross which is destined for market. This structure is known as terminal crossbreeding.

Terminal crossbreeding offers many benefits for final product quality, and also daily performance of the animals, such as feed efficiency which improves overall profitability. The crossbred nature of market pigs exploits hybrid vigor, breed complementarity, beneficial breed combinations and differential breed expression of desired traits (Falconer and Mackay, 1996; Oldenbroek and Van Der Waaij, 2015; Yadav *et al*, 2018). In a technical bulletin from the Government of Canada (Fahmy *et al*, 1987), multiple combinations of purebred sires and crossbred dam were investigated for the desirability of progeny's carcass and meat quality, among other traits. This work founded the typical Canadian commercial meat pig; a three-way cross of a Duroc boar to an F1 progeny sow of Landrace × Large White (Miar *et al*, 2014). The Duroc breed, specifically, is known to have higher IMF content than other potential sire breeds such as the Pietrain, but also higher growth and feed efficiency than fatter breeds which contain higher IMF, such as the Berkshire; growth and feed efficiency are important to ensure profitability of the final carcass (Fahmy *et al*, 1987; Alfonso *et al*, 2010; Choi *et al*, 2014). The sow is chosen primarily for mothering ability, litter size and longevity in the breeding herd (Blomberg, 2010; Camerlink, 2018). The three-breed cross with a terminal sire has many advantages and produces maximum hybrid vigor of any crossbreeding system (Yadav *et al*, 2018). This maximum hybrid vigor is realised in both in the F1 sow and the commercial progeny; since replacement gilts are generally purchased from multiplier herds rather than produced on farm, sires can be chosen with no attention to maternal traits (Yadav *et al*, 2018). Hybrid vigor, also known as heterosis or outbreeding enhancement is the unique phenomenon

in which crossbred animals outperform the average performance of their purebred parents (Falconer and Mackay, 1996) and is one of the main reasons crossbreeding is applied. Heterosis can be calculated as the crossbred average for the trait minus the purebred average, divided by the purebred average (Spangler, 2013); heterosis can results from epistasis, complete- or over-dominance effects, which occurs when a heterozygous individual (Bb) with 1 copy of a particular allele will produce a phenotype that is equivalent or more beneficial than that of the homozygous individual (BB) (Falconer and Mackay, 1996; Oldenbroek and Van Der Waaij, 2015). This is particularly beneficial for traits with low heritability, such as PHU (h^2 = 0.11 - 0.20; Cameron *et al*, 1990; Hermesch *et al,* 2000; Van Wijk *et al*, 2005) which may be otherwise difficult to improve with selective breeding (Oldenbroek and Van der Waaij, 2015). In fact, traits with a high heritability have lower estimates for heterosis than those with lower heritability. Additionally, heterosis can mask deleterious alleles, as crossbreds tend to have more heterozygosity and alleles with negative effects are frequently recessive (Oldenbroek and Van der Waaij, 2015). Heterosis is not the exclusive goal from crossbreeding, and in fact for some traits, particularly those with high heritability and are additive in nature, such as many meat quality traits selected for in the purebred lines (IMF), breed complimentarity is more beneficial to the progeny.

Breed complementarity is another benefit of crossbreeding; which results from mating parents of different breeds which are specialised in different traits, as seen from the typical Canadian commercial cross. Breed complementarity is particularly beneficial for traits which may be negatively correlated but are both relevant for production, and as such cannot be easily improved in a single breed. Examples include loin muscle area (cm^2) with number of piglets weaned (-0.015 $\pm$ 0.008), and lean growth rate (kg/d) with number of piglets born alive (-0.082 $\pm$ 0.0033) (Chen *et al*, 2003).

Though there are significant benefits to crossbreeding, some limitations must be considered. In some cases, crossbreeding two genetically distant individuals or lines can result in outbreeding depression, a reduction of fitness in the progeny (Frankham *et al*, 2011). Additionally, traits with moderate-high heritability generally do not benefit from heterosis as significantly as those with low heritability do; this indicates that heterosis is not the primary mechanism through which these traits are improved in the progeny of a 3-way terminal cross. The genetic correlation between the performance of purebred and crossbred animals must be considered. If this is significantly less than one, the phenotype of the purebred parent may not be a good predictor of the crossbred's phenotype. In previous studies, the genetic correlation for purebred and crossbred performance for meat quality traits were found to be high and positive (ranging from 0.69 to 0.99) (Tusell *et al*, 2016), but some have noted lower correlations (0.36) (Götz and Peschke, 1993). Depression of genetic correlations can be due to various factors, such as genotype by environment (Schou *et al*, 2019); this is common in the Canadian system due to the environments in which the purebreds and crossbreds are raised, causing this genotype by environment interaction (Oldenbroek and Van Der Waiij, 2015). The within-line selection of purebreds is done primarily to improve the performance of crossbred descendants under field conditions (Tusell *et al,* 2016). The high-health herds seen at the nucleus level do not face the same disease challenges that a crossbred animal at the commercial level may see, and traits such as growth may be affected due to lowered immune status and onset of disease. An example of this is the genetic correlation between daily weight gain (DWG) and osteochondrosis lesions (0.31) (Kadarmideen *et al*, 2004) indicating that animals with more lesions will put on less weight daily and grow more slowly. Additionally, the use of three-tiered breeding programs such as this can lead to genetic improvement lag, which is the time taken for genetic improvements achieved in the upper tier to reach the tier below

(Bichard, 1971). Genetic lag is ultimately determined by the summation of the following factors: the rate of genetic progress within the nucleus herd, the number of steps in the breeding system, the genetic superiority of the nucleus boars and of the gilts used in multiplier herds and finally, the generation interval at each level of the pyramid (See, 1995). In the Canadian breeding scheme, the estimated improvement lag is approximately 3.5 years (Bichard, 1977; See, 1995). Genetic improvement achieved in the multiplier and commercial levels directly depends on the rate of the genetic improvement achieved in the nucleus, a high rate of genetic progress at the nucleus layer will lead to a higher rate of progress on subsequent levels (Bichard, 1971; See, 1995); increased rate of genetic progress in the nucleus helps to reduce the size of genetic lag, as any improvement seen in the nucleus is additive. The length of time that animals are used in the lower tiers (litters /sow, number of services per boar), and the relative genetic superiority of boars and gilts in the nucleus herd which are transferred to the lower tiers also affects the size of genetic lag (Bichard, 1971; See, 1995). Improved evaluation accuracy and subsequent selection in the nucleus herd and use of semen from these superior selected boars in the commercial level (artificial insemination, AI) both help to reduce genetic lag seen (Bichard, 1971).

2.4 Breeding approach: traditional to genomic

Traditional

Very basically, animal breeding is using selective mating of superior animals in order to increase the likelihood that these traits are expressed in the progeny and to increase the frequency of the best genes for the desired trait in the population (Falconer and Mackay, 1996). The statistical approach of best linear unbiased prediction (BLUP) is a traditional breeding method which uses observed phenotypic records of an individual or its relatives, and the

proportion of shared additive genetic relationships between animals (pedigree information) in order to estimate breeding value (EBV) of animals for each studied performance trait. Animals are ranked on their genetic potential through these EBVs, which allows for breeding decisions to be made which optimise the performance of the next generation (Henderson, 1975). This is the basis of genetic improvement programs, where the progeny of the individuals chosen for breeding are expected to have desirable expression of certain traits and is known as 'selective breeding' or a 'selective breeding program' (Hill, 2001). Such methods have been able to yield substantial improvements for animal production traits; however, carcass composition and meat quality traits are not as suitable for improvement by this method. This is because these traits must be measured definitively *post-mortem* and therefore cannot be measured on selection candidates, but rather, on relatives. It is therefore understood that genetic improvement of meat quality traits via traditional methods is both difficult and slow (Miar *et al*, 2014; Oldenbroek and Van Der Waiij, 2014). The accuracy of breeding value prediction is the correlation between true and estimated breeding value. This is estimated by regressing the estimated breeding value (A) onto the phenotype (P), which is the product of the true breeding value (A) and environmental influences (E) (Mrode, 2005). It is done with the following formula:

$$b_{AP} = \frac{cov(A,P)}{var(P)} = \frac{cov(A,A+E)}{\sigma_P^2} = \frac{\sigma_A^2}{\sigma_P^2} = h^2 \qquad (3)$$

where b_{AP} is breeding value prediction accuracy. The genetic variance for the trait is σ_A^2, σ_P^2 is the phenotypic variance for the trait and h^2 is the heritability of the trait. This calculation allows comparison of different selection method accuracies; the higher the accuracy, the better the method for prediction of breeding values, and therefore higher potential genetic gain (Mrode, 2005).

Marker-assisted selection (MAS)

Recently, advancements in computing power and technology has facilitated alternate approaches for breeding programs. Together, these have made genomic methods more common approaches for selective breeding programs, however, perhaps most importantly is the availability of many single nucleotide polymorphisms (SNPs) across the genome of many species, discovered by genome sequencing (Goddard and Hayes, 2007). Once a SNP is found to be associated with a certain trait, then the genotype at the SNP locus can then be considered when potential breeding animals are selected. This type of selection is known as marker-assisted selection (MAS); MAS utilises a small number identified markers for selection purposes (Dekkers, 2004). This method has been used for the improvement of some significant meat quality traits in pigs through the manipulation of two primary genes, namely the protein kinase AMP-activated non-catalytic subunit gamma 3 (*PRKAG3*) gene (originally called "Rendement Napole" (RN^-)) (Le Roy *et al*, 1990; Hamilton *et al,* 2000; Brewer *et al*, 2002; Rosenvold and Andersen, 2003; Yang *et al,* 2017), and the Halothane gene *(HAL*) (Hamilton *et al,* 2000; Brewer *et al,* 2002; Rosenvold and Andersen, 2003; Yang *et al,* 2017).

A mutation in the *PRKAG3* gene, which typically encodes for a muscle specific isoform of the regulatory gamma subunit of adenosine monophosphate-activated protein kinase (Rosenvold and Andersen, 2003) causes significant and prolonged pH decline *post-mortem* (Figure 2.3), leading to what has been termed "acid meat". This is attributed to higher than average glycolytic potential in the muscle which prolongs the pH drop and degrades glycogen *post-mortem* (Gao *et al*, 2007). The high glycolytic potential *post-mortem* and elevated residual glycogen also results in excessive drip loss due to poor WHC, which results in low cooking and processing yields (Salas and Mingala, 2017). Multiple markers in the *PRKAG3* genes have been found to be significantly associated with economically important meat quality traits. The first dominant allele identified in *PRKAG3* results in a substantial reduction of technological

yield (5 to 6%). This yield reduction is directly related to the high glycogen content in animals with this mutation (Fernández and Tornberg, 1991; Milan *et al,* 2000), which can be up to 70% greater than that of an unaffected animal (Lundström *et al*, 1996; Garíepy *et al*, 1999).

The second significant gene in pork quality which MAS was used is *HAL*. Pigs which are homozygous (nn) for the recessive *HAL* n (Hal-1843) allele have porcine stress syndrome (PSS) (Sather and Murray, 1989; Fujii *et al,* 1991). PSS is triggered when nn animals are subjected to pre-slaughter stress, even minorly, or exposed to the anesthetic gas halothane (Rosenvold and Andersen, 2003). Malignant hyperthermia susceptibility is a hallmark of this condition, and results in higher than average carcass temperatures, low pH early *post-mortem* and subsequent excessive protein degradation (Figure 2.2) (Gao *et al*, 2007) leading to pale, soft and exudative (PSE) meat. The mutation which causes this disorder, the Hal-1843 allele, was identified within the ryanodine receptor (*RYRI*) (SSC6) (Fujii *et al*, 1991), which is the calcium release channel in the sarcoplasmic reticulum; the biochemical alteration occurs in the calcium release channel (CRC1) of the sarcoplasmic reticulum of the muscle. The widespread testing for this identified mutation (HAL-1843) has allowed for the identification of normal (NN), carrier (Nn) and susceptible (nn) animals (Lazzaroni *et al*, 2007); this has helped to remove the deleterious allele from breeding stock (Dekkers, 2004). Interestingly, halothane positive animals (Nn and nn) have higher carcass yield and lean percentage than average. As such, some purebred lines maintain heterozygous animals in order to benefit from this (Lazzaroni *et al*, 2007). Overall, however these positive attributes are outweighed by the negative effects on meat quality (Gao *et al*, 2007) and the almost total removal of this deleterious allele has significantly improved pork meat quality (Sather and Murray, 1989; Fujii *et al*, 1991; Dekkers, 2004).

Figure 2.2

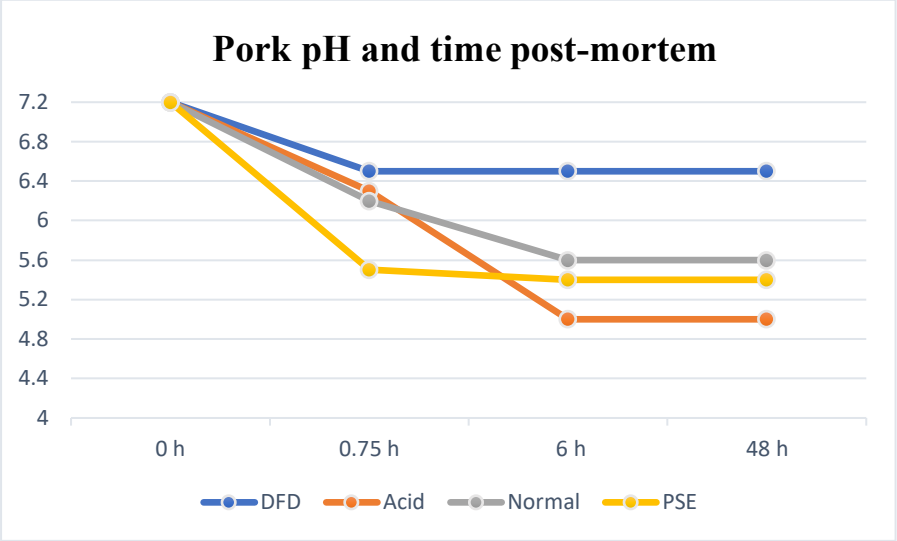

Figure 2.2 in which time *post-mortem*, in hours is shown with corresponding relationship with pH. Where dark, firm and dry is shown as DFD, pale, soft and exudative is shown as PSE. Figure and description adapted from OMAFRA factsheet: (Du, 2016)

The genes (alleles) in the examples above are known as 'major', meaning that they have large, single gene effects on meat quality. This provided excellent opportunity to make significant advances for pork quality through MAS, however, meat quality traits are predominantly polygenic, meaning the final phenotype is a result from many genes with small effects (Rosenvold and Anderson, 2003). This is problematic, as SNPs with low minor allele frequency (MAF) and low effect sizes are not easily detected and MAS can be limited by this issue (Dekkers, 2004; Van Der Steen *et al*, 2005). Early association analyses, which used sparse microsatellite markers to detect QTL, were also limited due to large confidence intervals which were often many mega-bases in length, and any detected QTL may contain thousands of variants and candidate genes. Additional experiments in fine mapping were required in order to identify these genes and variants (Dekkers, 2004). The availability of 'next' or 'second' generation sequencing technologies has enabled the efficient generation of large amounts of

sequence data and genome assemblies for many species, including pigs, at a much lower price than was previously possible, which has further enabled significant marker discovery. This identification of thousands of SNP variants made possible by next generation sequencing has also been able to alleviate these issues.

Genome-wide association study (GWAS)

Genome-wide association studies (GWAS') are a commonly used technique to identify QTL which are significantly associated with a trait of interest, providing insights to the genetic architecture behind a trait (Korte and Farlow, 2013). A GWAS utilises the principle of LD at the population level, relying on the LD between genotyped SNPs and ungenotyped causal variants (Visscher *et al*, 2012). In order to capture this, we utilise SNP marker panels of varying densities. The development of commercially available high-density SNP panels, such as the Illumina Porcine 60k BeadChip (Ramos *et al*, 2009), used for genotyping large number of animals, has been instrumental to the dissection of economically important traits through high-throughput analyses (Ramos *et al*, 2009). Previous studies have suggested various methods for selecting markers to use on SNP chips, including random, uniform or based on the principles of LD (Shashkova *et al*, 2019). Understanding that the strength of the statistical associations between alleles at different loci depends upon their allele frequencies (Visscher *et al*, 2012) has contributed to the recommendations for the selection of panel SNPs. SNPs are selected on the basis of their frequencies in order to increase the chance of detecting associations with nearby causative loci (Wray, 2005). As such, SNP chips are comprised primarily of common SNPs with MAF typically > 0.05 (Visscher *et al*, 2012), as rare variants (MAF < 0.01) will have low LD, with nearby variants, even if they are ultimately mapped to the same recombination interval (Visscher *et al*, 2012). Increasing the density of the SNPs on a chip would increase the likelihood that an ungenotyped causal variant will be in LD with a

genotyped SNP on the panel. Some chips are as low as 10K SNPs and can be up to 660K (Van Son *et al,* 2017; Zhang *et al*, 2018; Van Son *et al,* 2019), depending upon the availability of marker data for that species; the density of chip used for a given study may also depend on the funds available. Lower density chips are less expensive to use, and the use of imputation programs such as FImpute (Sargolzaei *et al*, 2014) or Beagle (Browning *et al*, 2018) can be used to infer high density genotypes from low density panels. Accuracy of 90% from imputation is sufficient in order to obtain genomic evaluations which are identical to those from using high-density panels (Wellmann *et al*, 2013). GWAS' have been instrumental in the identification of quantitative trait loci (QTL) which contribute to the observed variation in economically important traits in many livestock species, including pigs. QTL for various carcass and performance traits have been reported on Pig QTL database in the tens of thousands (Hu *et al*, 2005).

A number of limitations, however, exist when using GWAS', particularly in regard to the statistical approaches and assumptions inherent to the method. These include stringent *p*-values due to multiple testing, random associations due to population stratification, difficulty in detection of small, non-additive variants and rare variants with low MAF (Manolio *et al*, 2009). Fortunately, many of these potential limitations can be addressed, and we understand GWAS' are still widely used by animal scientists for investigation of various traits. Significant opportunity presents itself through GWAS' as these may allow progress into the understanding and identification of genes and biological pathways which contribute to the expression of investigated traits. Particularly, since knowledge regarding the genetic basis of many meat quality traits is limited due to their complex nature, GWAS' provides an excellent opportunity for these difficult to study traits. As we uncover more QTL and genes associated with our studied traits, further and more targeted work can be done to identify the causative mutations in

these genes and lead to a better understanding of the polymorphisms which contribute to the studied trait. Practically, identified variants which are significantly associated with relevant traits can be used in animal breeding programs in order to improve accuracy and aide in the selection for superior breeding stock. A GWAS is able to detect the underpinnings of genetic variation, however in order to move forward and act with these identified regions, an additional method, genomic selection, is required (Przeworski *et al*, 2005). With these identified markers incorporated into a GS model, the ability to independently manipulate IMF from other genetically correlated traits becomes feasible; this is the ultimate goal for meat quality, improving IMF whilst maintaining backfat levels so as to not decrease the lean yield of the carcass.

Genomic selection

Genomic selection (GS) has been proposed as a practical solution for improving difficult to measure traits. Instead of using clearly identified markers which are significantly associated with a trait, like traditional MAS, GS uses genome-wide marker panels which consists of thousands of SNPs and considers all of these effects (Meuwissen *et al,* 2001; Goddard and Hayes, 2007). Phenotypes are regressed on all available markers (Meuwissen *et* al, 2001), and it is assumed that all QTL will be in linkage disequilibrium (LD) with at least one marker. GS is a two-stage process in which the population set to undergo improvement must 1) be genotyped and phenotyped for the desired traits (training population), the effects of each QTL genotype are then estimated and finally all the QTL effects are summed in order 2) to obtain a genomic enhanced breeding value (GEBV) for potential breeding animals (selection candidates) (Goddard and Hayes, 2007). Prediction methods are used for estimation of GEBV to predict the genotypic value of selection candidates (validation population) which are not necessarily phenotyped.

The GEBV can be calculated based on either the estimation of SNP effects or the genomic relationships between the genotyped individuals in the population (Meuwissen *et al*, 2001). These methods are described in the following:

As discussed by De Los Campos *et al.* (2013), SNP-effect methods provide excellent opportunity for improvement of meat quality traits. There are many statistical methods which can be used for SNP-effect based GS, including ridge regression BLUP (rrBLUP) and Bayesian least absolute selection and shrinkage operator (LASSO). These methods differ primarily based on the assumptions made about the distribution of SNP effects and how the SNP effect sizes are handled (Tibshirani, 1996; Meuwissen *et al*, 2001; Arbet *et al*, 2017). It is important to note that the effect sizes of markers associated with a trait may not be normally distributed throughout the genome, as is assumed in the infinitesimal models used in BLUP methods. Finite-loci models are used in other Bayesian methods, in which a few SNPs are expected to be associated with the trait, and all SNPs have a different size of effect on the trait. The assumptions in a model are important to take into consideration as in Bayesian methods, the accuracy depends significantly on whether the genetic architecture of the trait fits with the assumptions of the model (Zhang *et al*, 2018). In general, Bayesian methods are beneficial because of their use of prior distributions and assumptions of SNP effect distribution. The primary methods of BayesA and BayesB were first introduced by Meuwissen *et al.* (2001) and later, BayesC and BayesD were introduced and discussed by Habier *et al.* (2011). There are additional approaches which have emerged recently, which are variations on the original methods.

BayesA assumes an inverse chi-square (χ^2) prior distribution for the markers which are also assumed to have different variances. BayesA considers all markers and requires significant computing time and power due to the large numbers included in the model, and none of these

are assumed to be zero (Meuwissen *et al*, 2001). BayesB, alternatively, assumes many markers to have zero genetic variance and only a few markers to be non-zero; it also assumes an inverse χ^2 prior distribution for those markers assumed to be non-zero. Finally, BayesC estimates common variance from the marker data as a prior distribution (Kizilkaya *et al,* 2010) and BayesD, which also uses a marker distribution that is an estimated scale parameter of the scaled inverse χ^2 rather than a set prior distribution (Habier *et al*, 2011).

Two additional Bayes methods, BayesR and BayesRC, are also of interest to discuss, particularly for practical animal breeding. BayesR has been shown to be equal to or superior in accuracy for QTL mapping and genomic prediction when compared to linear mixed models (Macleod *et al*, 2016). BayesR assumes that each variant is equally likely to affect the trait and no prior biological knowledge is included in the model. Additional assumptions are that the real SNP effects are derived from a series of normal distributions; the first distribution has zero variance and subsequent distributions go up to 1% of genetic variance (Erbe *et al*, 2012). This prior assumes that not all markers are in LD with a QTL and therefore have zero effect, with others having small to moderate effects (Meuwissen *et al*, 2001; Erbe *et al,* 2012). Any available biological knowledge is applied after the analysis in order to confirm candidate genes or mutations (Macleod *et al*, 2016). However, the way that this biological knowledge is applied has been criticized as arbitrary and biased. The BayesRC method is based on BayesR with small modifications; BayesRC incorporates prior biological knowledge into its analysis (Macleod *et al*, 2016). This incorporation is done by categorizing variants into classes which are likely to be enriched in causal mutations, as it is understood that these mutations may cluster in genes which are biologically relevant to the trait (Lango Allen *et al*, 2010), and each variant class is believed to differ in the probability that they contain causal variants for trait expression. The biological information can be obtained from numerous places, such as already

known causal variants, lists of previously identified candidate genes, or known genes of importance for trait expression (Macleod *et al*, 2016). In BayesRC, the prior distribution used is uniform across all variant classes, which ensures that only the biological information included will influence the analysis (Macleod *et al*, 2016). These statistical methods can be used to predict GEBVs and help to select superior animals.

The second common group of statistical methods of GS are relationship-based methods; these methods use a genomic (realized) matrix (GRM) to predict GEBVs and can accurately predict relationships for individuals without their own phenotypic record. It does so by estimating the proportion of shared chromosomal segments between animals based on the similarities seen in SNP marker genotypes (Vanraden, 2007; Hayes and Goddard, 2010). A GRM replaces the pedigree – derived relationship matrix (A matrix) from traditional BLUP (Hayes *et al*, 2009). In a GRM between individuals, the realized proportion of the genome recognises those which are identical by descent (IBD[7]) between pairs of individuals (Hayes *et al*, 2009).The assumption of this approach is that only the additive genetic covariance between relatives is proportional to this realized IBD portion (Thompson, 2013). This method is termed a genomic BLUP (GBLUP) and is commonly used in livestock breeding programs and has proven successful in improving GEBV prediction accuracy (Hayes *et al*, 2009). Further, GBLUP was improved upon through implementation of a matrix which combines pedigree and genotype information in order to estimate the relationships between each pair of animals (genotyped and non-genotyped). This is known as single-step GBLUP (ssGBLUP) which uses

[7] A segment of DNA is deemed to be identical by descent (IBD) if it is identical between two or more individuals and is assumed to be inherited from a common ancestor without recombination. If all alleles on a maternal or paternal chromosome are identical, they are considered a match. Segments can appear to be IBD through new mutations, but this is known as identical by state (IBS) (Thompson, 2013).

all phenotypic, pedigree and genotypic information simultaneously, including phenotypic information for animals which are not genotyped (Teissier *et al*, 2018). The accuracy of prediction using genomic methods have been reported to be higher (0.07 - 0.31) than pedigree-only based BLUP (0.09) (Daetwyler *et al*, 2012; Teissier *et al*, 2018).

All BLUP methods mentioned (BLUP, GBLUP, ssGBLUP and rrBLUP) follow an infinitesimal model of SNP distribution, meaning that is assumed SNPs have a normal distribution and all contribute equally to the trait (Goddard *et al*, 2010); this variance per locus is calculated where the variance per locus = $\frac{\text{total genetic variance}}{\text{n}}$ and n is the total number of loci (Vanraden, 2007; Goddard *et al*, 2010).

Based on the discussed attributes, it is understood meat quality traits are good candidates for improvement via GBLUP and rrBLUP methods (Samorè and Fontanesi, 2016). Additionally, the generation interval along with the accuracy of breeding values also affect genetic progress (Falconer, 1989). In order to reduce the amount of genetic lag, GS allows to considerably improve the accuracy of breeding values and decrease the generation interval (Lillehammer *et al*, 2011; Baby *et al*, 2014). Thus, genotyped animals, who may not yet display the phenotype of interest (or perhaps it is not obviously visible) to be included in breeding much earlier.

Finally, the increased ability to predict crossbred performance based on purebred performance is a significant benefit to implementation of GS in pig breeding (Hidalgo *et al*, 2016). The structure of the Canadian hog relies on this, as improvement of pork quality at the commercial level is ultimately what drives the acceptance and demand for pork. The selection in purebred lines at the nucleus herd is ultimately to improve crossbred performance in the field conditions; and circumvent the challenges posed by collection of commercial phenotypes

(fast processing speed, high labour and equipment costs, high quality of data required) (Dekkers, 2007). A number of studies have shown that GS can be successfully applied in purebred populations over traditional methods, this is due to the ability to account for genetic differences in crossbred and purebred animals, environmental effects and non-additive gene effects (Daetwyler *et al*, 2007; Dekkers, 2007).

2.5 Conclusions

Pork quality is extremely important for consumer satisfaction and to maintain the domestic and international demand for Canadian pork. Backfat thickness ultimately determines lean yield, subsequent payment for a producer and is positively genetically correlated to IMF content. Through selection for a leaner pig to meet the demands of consumers and packers for increased lean yield and less fatty meat, pork has suffered a decrease in meat quality due to increased dryness and related traits from reduced IMF. It is evident that fat content is key in the acceptance of pork and pork products as intrinsic factors affecting pork quality, such as tenderness and flavour are vital in consumer satisfaction. Pork fat quality and quantity are integrated into many visual and flavour attributes assessed by consumers at the grocery store, and at home during consumption. The pyramidal breeding structure used in the Canadian hog industry is able to capitalise on breed expression of selected traits and also take advantage of the many benefits of crossbreeding in order to improve the quality of the final product. Genetic improvement occurs in the nucleus herds and a higher rate of progress here will translate to progress in the commercial level. Additionally, meat quality traits have been shown to have a high genetic correlation between crossbred and purebred animals and the study of purebreds for crossbred performance of these traits may be extremely beneficial to the improvement of meat quality for the market hog due to the discussed benefits of crossbreeding.

The collection of high-quality phenotypes to use in genetic selection programs can be a huge cost associated with these studies as they often contain thousands of animals. The collection can also be laborious, particularly for meat quality traits, however the complete neglect to collect phenotypes would be a mistake as both the confirmation of success and the goals of a GS program lay in these phenotypes. As such, effective research and the application of GS programs rely on the integration of both phenotypic data collection and pedigree information with the genomic efforts (Van Der Steen *et al*, 2005). Over generations accuracy of GEBVs will decrease and retraining of the GS models is required. Additionally, it is understood that phenotypic records in a population will help to improve accuracies of GEBVs and subsequently of the selection program. Because populations and environments are not static, routine reintegration of new phenotypic information into the GS program is important for continued accuracy. Therefore, ongoing phenotype collection is important for GS programs and, as such, the development of inexpensive and accurate methods for phenotype collection would be extremely beneficial. Additionally, collection of phenotypes from commercial animals can help gauge the success and rate of genetic improvement.

Despite the practicality of GS in livestock breeding programs, GWAS' are still an integral and practical method we can use to investigate the genetic underpinnings meat quality traits, particularly in research circumstances. A GWAS can be useful for obtaining a better understanding of a studied trait, which is useful when investigating complex traits, particularly those which are polygenic. Relying solely on GS and neglecting to investigate the underlying mechanisms of the trait could result in unwanted consequences in the resulting animals. Through the principle LD, we can understand and identify SNPs which may have a relationship to an ungenotyped causal variant. Perhaps most importantly, this increased understanding as incorporation of unique SNPs can allow us to manipulate IMF content independent of overall

carcass fatness (backfat) as increasing carcass fatness is not the goal. This can add to the growing body of work which investigates meat quality genetics and through studying relevant breeds to the Canadian hog system, any results can be practically applied to breeding programs by being added to custom SNP chips. Increased knowledge of functional gene variants will foster increased understanding and insights on the genomic architecture of economically relevant traits which impact pork quality. Through the integration of these identified functional gene variants into GS programs, we can more accurately estimate the overall genetic effects, additive and non-additive, of a trait which may be difficult or expensive to measure such as IMF. The generation of this new information can be additionally utilised to create novel genomic tools for the improvement of Canadian selection programs and breeding. Ultimately this can aide in the prediction of phenotypes in selection candidates themselves, as well as, perhaps most importantly, improve the accuracies of GEBVs and increase the rate of genetic improvement.

Supplementary information 2.0

Table S2.1 Previously identified genes in literature with an association to intramuscular fat (IMF) in pigs.

Gene	Breed	Sample size	Chromosome	Method of identification	Function	Reference
H-FABP	1 and 2. (Landrace X Large white or duroc) X (landrace X large white) 3. Goland 4. Danbred	614	SSC6	Single locus associations using Proc Mixed in SAS 9.2	Fatty acid binding proteins involved in intracellular fatty acid transport	Renaville *et al,* 2014
A-FABP	Duroc	983	SSC4	Isolation of A-FABP containing phage clones, microsatellite analysis, chromosomal localisation and sequence analysis.	A-FABP, also called FABP4 is exclusively expressed in adipocytes and is one of the eight identified members of the fatty acid-binding protein (FABP) family.	Gerbens *et al*, 1998b.
LEPR	Commercial Canadian three-way cross	398	SSC6	Custom SNP panel for meat quality traits and multimarker analysis using GLM procedure in SAS 9.2	Candidate gene for meat quality, regulates feed intake and energy metabolism through leptin. High expression stimulates lipolysis of adipocytes	Zhang *et al*, 2014.
FASN	1 and 2. (Landrace X Large white or duroc) X (landrace X large white) 3. Goland 4. Danbred	614	SSC12	Single locus associations using Proc Mixed in SAS 9.2	Fatty acid synthase (FAS), encoded by the FASN gene, catalyses de novo synthesis of	Renaville *et al,* 2014

					saturated fatty acids,	
DGAT2	1 and 2. (Landrace X Large white or duroc) X (landrace X large white) 3. Goland 4. Danbred	614	SSC9	Single locus associations using Proc Mixed in SAS 9.2	Significantly associated with backfat thickness, L* fat colour. Plays a more dominant role in triglyceride biosynthesis in mammals	Renaville *et al,* 2014
IGF-2	Large White X Meishan	703	SSC2	Marker-assisted segregation analysis, DNA sequencing, genotyping, Northern blot analysis and real time PCR analysis for identification and categorization after phenotypic data collection.	Implicated in myogenesis and lean meat content. A mutation in a single based of IGF2 has been described as a QTN, causing a major QTL effect on muscle growth and fat deposition in pigs	Gao *et al*, 2007; Van Laere *et al,* 2003
MC4R	Duroc Synthetic boar X F1 (landrace X large white) sow Duroc X Shanzhu	205	SSC1	GWAS with results from 60k SNP panel and subsequent estimates of genomic regions underlying correlations and genomic breeding values estimated. SNP position found using *Sus Scrofa 10.2* xin FASTPHASE.	Expressed primarily in the nervous system, plays a large role in the regulation of food intake, energy balance and body weight in mammals.	Rothschild *et al*, 2014, Silva *et al*, 2019; Wang *et al,* 2013
ACACA	Duroc Songliao black	350 (sub samples 70).	SSC12	Functional categorisation of expression analysis with results from 70 RNA samples, as well as	ACACA encodes the protein acetyl-CoA carboxylase alpha, which catalyses the rate limiting	Cánovas *et al*, 2010 ; Xing *et al*, 2015.

				biological pathway analysis.	step for de novo fatty acid synthesis.	
SCD	1 and 2. (Landrace X Large white or duroc) X (landrace X large white) 3. Goland 4. Danbred	614	SSC14	Single locus associations using Proc Mixed in SAS 9.2	SCD codes for an enzyme which catalyses the conversion of SFA stearic acid into MUFA oleic through addition of a double bond acid	Renaville *et al,* 2014; Xing *et al*, 2015

Figure S2.1

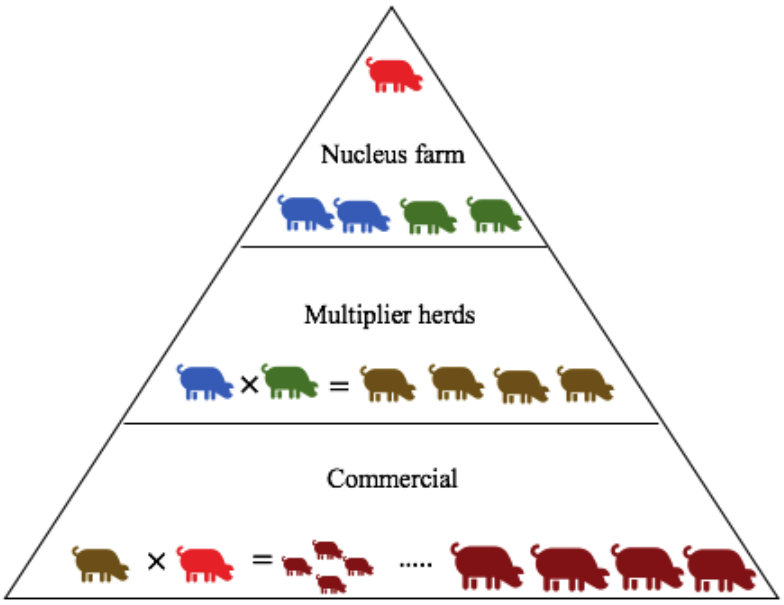

Figure S2.1 visual representation of the 3-tiered pyramidal structure of the Canadian hog system.

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Chapter 3.0 Phenotype prediction of loin intramuscular fat (IMF) and fatty acid content of backfat with near-infrared spectroscopy

3.1 Introduction

The use of accurate and consistent phenotypes for meat quality traits in genetic studies, such as GWAS', is extremely important. In fact, effective research programs and practical application of GS programs rely on the integration of both phenotypic data collection and pedigree information with the genomic efforts (Van Der Steen *et al*, 2005). For genetic studies, which typically include thousands of animals, the costs of collecting phenotypic data may represent a major expense. Ensuring quality is of particular importance for phenotypes which are not direct measurements but rather subjective approximations or mathematical predictions of a meat quality trait, such as visual marbling or ultrasound IMF, respectively. In the case of predicted values, validation is needed in order to ensure that choices subsequently made for which SNPs or QTLs to utilise in future breeding or genetic selection (GS) programs. Accuracy of phenotypes for GS programs can help to increase the genetic improvement of meat quality traits in the selection stock and thereby improve the overall quality of product seen in the crossbreds.

Two carcass and meat quality traits which are of particular importance to the industry are intramuscular fat (IMF) and backfat depth (BFD). Currently, there is focus on improvement of IMF independent from BFD. IMF is traditionally measured using wet lab techniques, such as soxhlet extraction. These methods are destructive and can be time consuming and expensive from a training, labour and equipment standpoint. Though the results from proximate analysis are very accurate and provide the most representative phenotype, it is not a methodology which can be utilised in situations which require fast results for many carcasses, such as in the

abattoir. In such situations, visual methods are widely preferred for fast evaluation of pork marbling, which is the term used to describe the appearance of visible IMF (Hocquette *et al,* 2010). Visual assessments are at a disadvantage when compared to wet chemistry techniques due to their subjective nature (Cheng *et al,* 2015). Poor precision, inaccuracies and inter-observer reliability when visually assessing carcasses has been noted in multiple studies (Strappini *et al*, 2011; Huertas *et al*, 2003). Backfat thickness and texture (firm or soft) are additional carcass traits measured at the abattoir. Backfat is then categorised into one of two broad categories, 'good' or 'poor' (Sørensen *et al*, 2012). In general, firm fat contains high concentrations of saturated fatty acids (SFA) and soft fat is primarily unsaturated fatty acids (UFA). The fatty acid composition of this fat depot plays an integral role in the texture, flavour and processing efficiency of meat products, as a majority of backfat is trimmed from the primals and commercial cuts and goes on for further processing (Kouba and Sellier, 2011). Fat comprised of SFAs is preferred for further processed products, as it contributes to product consistency and positive eating attributes (Baer *et al,* 2013); the importance of backfat composition is evident. Rapid and subjective measures, such as the thumb test[8] are not the most reliable or precise methods.

In light of all this, more scientific measures for estimation of fat quality and IMF content are needed. For backfat quality, the calculation of the iodine value (IV), a measure of fat unsaturation can be used. Values greater than 70 indicate the fatty acids are primarily unsaturated, while values lower than 70 indicate more saturation. Low IVs typically result in

[8] Thumb tests, also known as 'finger testing' or 'thumbing' is the manipulation of fat by hand (Seman *et al*, 2013). This method is similar to what butchers would perform when assessing product; fat can be allocated to one a category (hard, medium/hard, medium, medium/soft, soft) based upon this hand feel (Maw *et al*, 2001).

higher cutting yield, longer product shelf life and a firmer fat, whereas high IVs have lower cutting yield, shorter product shelf life and soft fat. Today, IV can be calculated after the fatty acid profile of fat is determined via gas chromatography (GC) (Seman *et al,* 2013), the reference method. On the kill-floor, however, GC is not performed due to time, labour and cost, and destruction of the product required. Rapid methods which utilise hand-held equipment, such as the NitFom^{TM} (Frontmatec, Denmark), are more practical for kill-floor evaluations. For the estimation of individual fatty acids, broad categories of SFAs, UFAs and adipose tissue composition in lean meat (IMF), methods have been developed using near-infrared (NIR) technology (Prieto *et al*, 2009). This technology has been shown to provide highly accurate results at full production speed (Sørensen *et al*, 2012; Gjerlaug-Enger *et al*, 2011; Ripoche and Guillard, 2001). NIR technology works by passing infra-red (IR) light through a sample via a probe, a brief scan of the meat or subcutaneous fat sample is all that is needed for estimation of composition. Certain molecular bonds absorb the IR light maximally at different wavelengths; the NIR instrument analyzes the proportion of light that is reflected by the sample material. Returned spectrum are analyzed against standards to determine the composition of that sample (Davies, 2005).

The speed and ease with which an evaluation method can be adopted by the industry is important. Processing lines in commercial abattoirs are fast-paced environments where graders do not often have more than 15 seconds with each carcass (Sørensen *et al*, 2012). In order for methodologies of phenotypic collection to become widely accepted and implemented they must be low cost and fast. For research purposes, the time, cost and complexity of phenotypic collection may be less of a barrier, but it is not best practice to utilise technologies which will never be feasibly implemented to industry. Therefore, accuracy must also be at the forefront of rapid collection technologies.

Overall, the generation of accurate, high quality phenotypes for many animals could provide multiple benefits, including increased understanding of the phenotypic variation seen in the commercial animals. The study described in this chapter aims to generate these phenotypes for IMF content and backfat quality, and through provision of these phenotypes for genetic efforts we can increase the accuracy of GS programs. We hypothesised, based on the strong, growing body of literature supporting NIR as an accurate phenotype generation method, that we would find a positive relationship between the predicted phenotypes and those measured by reference methods.

3.2 Materials and methods

Ethics statement

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 (Canadian Council on Animal Care (CCAC, 1993).

Animals

A total of 997 purebred Duroc female pigs (gilts) originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study. Feeding, raising and slaughter protocols, as well as meat quality measurements obtained have been described in previous studies (Miar *et al*, 2014; Zhang *et al*, 2015;Yang *et al*, 2017).

Samples

Lean meat samples were collected from the *longissimus dorsi* muscle between the second and third last ribs. All samples were obtained from the left side of the animal (facing the cranial end of the animal) The chop was weighed after all subcutaneous fat was trimmed;

this fat was independently bagged with the unique pig ID and used as the backfat sample in this study. All backfat samples were frozen in individual bags with their unique pig ID tag. All lean samples then underwent a 48-hour drip loss analysis prior to being frozen at -20°C and shipped to the Agriculture and Agri-Food Canada Lacombe Research and Development Centre (AAFC-Lacombe, Lacombe, AB). In total, 997 lean meat samples and 996 backfat samples were used in this study.

In the first shipment of fat and lean samples from Manitoba, a large number of loin samples near the top of the cooler thawed during the journey. There was noticeable drip loss and leakage seen in the sample bags which was not able to be accounted for during later moisture corrections as all samples were immediately placed into the freezer and the unique sample identifications were not recorded. It was not possible to separate these samples from the larger group for data analysis.

Sample preparation

In preparation for NIR scanning at AAFC-Lacombe, the frozen lean meat sample weights were recorded. Samples were removed from the -10°C freezer 24 hours before scanning was to begin and placed in a 4°C cooler to thaw. Thawed samples were blotted with paper towels to remove excess moisture and reweighed. The unique pig identification number was recorded at the time of each weight, and this tag remained with the sample throughout the preparation process. Samples were cut into quarters and then ground using a Blixer® 3 Series D 3 ½ Quart Robot coupe for a minimum of ten turns of the manual handle. Ground samples were placed on a black plastic layer on the laboratory benchtop with their unique ID tag on top.

In preparation for NIR scanning, the frozen backfat samples were counted out and placed back into the freezer. Samples were removed from the -10°C freezer 24 hours before scanning was to take place and placed in a 4°C cooler to thaw. When scanning, five samples at

a time were taken from the 4°C cooler and placed on a black plastic layer with their unique identification tag. Pre-scan, all samples were measured with calipers from the skin to the thickest portion.

Near infrared spectroscopy

All spectra were collected using an analytical spectral device (ASD) – portable standard-Res spectrophotometer model LabSpec®4 (Malvern Panalytical, 2019, Boulder, CO). The probe used was determined based on what type of sample was set to be scanned during that sample analysis period. If scanning fat, the ASD Proreflectance probe (Model C950230) was selected, while, for the lean samples, an ASD high intensity fiber optic contact probe (Model A122300) was used. After attachment of the appropriate probe, the machine was allowed to equilibrate for a minimum of 15 minutes. After equilibration, calibration of the software and NIR was conducted. All absorbance values were reported as the logarithm of the reciprocal of reflectance, log (1/R).

Lean meat protocol

Each sample was scanned four times using the ASD high intensity fiber optic contact probe (Model A122300) moving the probe to a new location upon completion of each scan. Each scan took 50 readings (~5 seconds) over visible and NIR range (350-2500 nm) in reflectance mode and spectra was averaged by the equipment software. Once scanning was completed, samples were repackaged into whirlpack™ bags with unique ID tags, and the probe was wiped clean with a Kimwipes® Low-Lint Wiper and 70% ethanol. Approximately 5% of samples were chosen at random for proximate analyses as a validation for the phenotypes of IMF content predicted from the spectra. Prior to the next sample, a new ID corresponding to the unique pig ID was entered into the software to track the samples.

Backfat protocol

The ASD LabSpec 4 Hi-Res analytical spectrometer (Malvern Panalytical, 2019, Boulder, CO) was set up as above. Each sample was scanned once using the handheld ASD Proreflectance probe (Model C950230). Each scan (~10 s) took 250 readings over the Visible and NIR range (350-2500 nm) in reflectance mode, and spectra averaged by the equipment software. In between scanning each sample, the probe was wiped with Kimwipes® Low-Lint Wiper and 70% ethanol, and a new ID corresponding to the unique pig ID was entered into the software to track the samples. When scanning, if the sample was less than 7.5mm thick, it was folded lengthwise to ensure enough fat surface area for the 6.5 mm probe to be in full contact with the sample throughout the scan. Finally, approximately 5% of samples were set aside for further analysis by gas chromatography (GC) as a validation method for the NIR predicted phenotypes.

Proximate analysis

Randomly selected lean samples, previously scanned with the spectrophotometer and repackaged into a whirlpack bag, were taken to a separate lab. Proximate analysis was run in accordance with the SOP from the meat and lipid laboratory at AAFC-Lacombe. Briefly, using AOAC approved methods (AOAC, 2016), one in twenty lean samples was analysed for total fat and moisture content using a CEM SMART system5 moisture analyser (Ontario, Canada) and a CEM Smart TracII fat analyzer (Ontario, Canada).

All samples were analysed in minimum of duplicate, and in cases where fat moisture values did not match within 2.5% of the standard error of the mean, triplicate or quadruplicate samples were done; sample repetitions were done until two results were within 2.5% of the standard error of the mean. Values were recorded and exported to a USB stick upon completion of each set of samples.

Gas chromatography

Fatty acid profile of the randomly selected backfat samples were analysed by gas chromatography (Varian 3800 GC, equipped with a 8100 autosampler, Varian, Walnut Creek, CA, USA) on a 30m capillary column (SP-2340, i.d. 25 μm, Supelco, Bellefonte, PA, USA) under conditions previously described by Dugan *et al* (2007), in accordance with the SOP from the meat and lipid laboratory at AAFC-Lacombe. Freeze dried adipose tissue was methylated directly using base catalyst to form fatty acid methyl esters (FAME) according to Yurawecz *et al* (1999). Briefly, 0.5 mL of internal standard (c10-17:1, 8mg/mL hexane) was added to 40 mg of freeze dried adipose tissue and methylated using 2 mL 0.5N sodium methoxide in methanol (Sigma-Aldrich Canada Co., 2149 Winston Park Dr., Oakville , Ontario, L6H 6J8) at 50 °C for 15 minutes. Hexane (1.5mL) and water (5mL) were then added, and the upper (hexane) layer containing FAME taken and diluted for GC analysis. The FAME of the upper level were identified by comparison to standards (GLC- 463 and GLC-603, Nu-Chek Prep, Inc. Elysian, MN, USA) and integrated using Varian Star Chromatography Workstation software v6.41 (Varian, Walnut Creek, CA, USA) (Turner *et al,* 2013., Dugan *et al,* 2007, Prieto *et al* 2017).

Data analysis

Spectral cleaning

All spectra were prepared for analysis using Camo's 'The Unscrambler®' software (Version 10.3, CAMO, Trondheim, Norway). This was done in two separate groups, lean meat and backfat, discussed respectively. First, the four scans for each lean meat sample were averaged to obtain a single value. Wavelengths between 400-1900nm were selected for analyses; 350:399 and 1901:2500 were removed due to excessive noise or chatter at beginning and end of scan. All spectral data underwent transformation from reflectance to absorbance. Absorbance data was then transformed to first and second Savitsky – Golay derivative, as well

as standard normal variate (SNV). SNV data then had the detrending correction applied, SNV-D. The first and second Savitsky-Golay derivative was then applied to the detrended data. This method of spectral pre-treatments for smoothing and cleaning was then applied to the spectra collected from the lean meat and backfat samples to ensure that the averaging of four scans per sample did not result in missing of outliers. This set of smoothed NIR data also underwent to following statistical analysis.

Statistical analysis

Once spectra had been cleaned and smoothed, we utilised SAS® statistical analysis software (Copyright 2019 SAS Institute Inc., Cary, NC, USA) for the prediction of phenotypes. We utilised 339 fresh lean meat samples and 357 backfat samples from a previous study (Prieto *et al,* 2017) to train the model as a calibration set of samples. The statistical protocol and algorithms used for both phenotype predictions of IMF from lean meat and fatty acid prediction from backfat is previously described in Prieto *et al* (2017). Briefly, partial least squares regression (PLSR) was utilised for the estimation of IMF content (%) in the lean meat as well as for the FA content, FA ratios and the IV for the backfat samples. In this, individual spectra were treated as independent variables. An analysis of variance (ANOVA) was performed to obtain the accuracy of prediction of the NIR phenotypes to the proximate analysis data. Overfitting due to too many PLS factors was avoided and the accuracy of prediction was evaluated using the adjusted R^2 (R^2_{adj}) coefficient of determination and the root mean square error prediction (RMSEP). In linear regression models such as the one used in this study, R^2_{adj} values are a commonly used statistical measure to see how well the model predicts the dependent variable. The R^2_{adj} value measures the proportion of the variance for the dependent variable which is explained by the independent variable in that model (Harel, 2009) which is

the spectra in this study. The range of the R^2_{adj} measurement is -1 to 1, with values closer to 1 indicating a strong, positive correlation and is calculated by the following formula;

$$R^2_{adj} = 1 - \left[\frac{(1-R^2)(n-1)}{n-k-1}\right] \quad (1)$$

Additionally, the RSMEP value is calculated by adding the squared prediction errors during cross-validation and is an efficient way to measure the uncertainty present in NIR predictions. Cross-validation, also known as bootstrapping, is used to check the validity of the multivariate model. Though this validity is also represented by the given F-value, this is an additional method to ensure goodness of fit of the model to the data (Ross, 2004). In this method, the data is split up into two sets, a training set and a test set. The training set is used to set up the model and then the test set is used for validation of the model. The RSMEP is calculated by the below formula.

$$RMSEP = \sqrt{\frac{PRESS}{n}} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{y} - y_i)^2} \quad (2)$$

Where PRESS is the predictive error sum of squares (PRESS) (Ross, 2004), n is the number of samples in the calibration set, $\hat{y}_i$ is the estimated responses which were gotten through cross-validation, and y_i is the number of measured responses (Prieto *et al,* 2017). This was used for validation of the lean meat model. Additionally, the coefficient of variation (CV) as used as an indication of the extent of variability in relation to the mean (Abdi, 2010). It is calculated using the following formula,

$$CV = \frac{SD}{\mu} \quad (3)$$

Where SD is the standard deviation and μ is the mean. This can also be expressed as a percentage by

$$CV = \frac{SD}{\mu} \times 100 \quad (4)$$

In this study, CV is expressed by the former. The range of acceptable CV depends upon the area of study; in general, a CV which is < 5% is thought of as very good (Campbell *et al*, 2010).

Required sample corrections

The lean meat samples underwent essentially two purge losses; the first drip loss prior to shipment to the AAFC-Lacombe facility and the second during thawing. In order to account for this, fat in the initial sample was calculated from the proximate analysis results by the following formula.

$$\text{Fat in initial sample} = 100 \times \left(\frac{\text{total fat in sample(\%)}}{\text{Initial chop weight(g)}} \right) \quad (5)$$

Where the total fat in the initial sample is calculated by the proximate analysis fat result multiplied by the final chop weight; final chop weight being the chop after all purge loss events.

Purge loss, total water weight and final moisture content were also calculated for all samples which underwent proximate analysis. These values were calculated in order to evaluate the accuracy of the phenotypes predicted from the spectral data. Based on the discrepancy seen between the predicted phenotypes and the calculated value for fat in the initial sample an adjustment factor was calculated. This was done by finding the average difference between the predicted fat phenotype and the actual fat in in the initial sample and subsequently applying this value to all the predicted NIR phenotypes.

The backfat samples did not lose significant moisture from the single freeze thaw during shipment and as such, no purge loss corrections were calculated for these samples.

3.3 Results and discussion

Lean meat

Proximate analysis

The average IMF and moisture content from proximate analysis were 2.20 $\pm$ 0.41 % and 73.22 $\pm$ 0.73% respectively. After correction for moisture, the fat in the initial, pre-drip loss sample, was calculated; this value averaged 2.05 $\pm$ 0.39%. Figure S3.1 in Supplementary information 3.0 contains these values, which represents the actual values for comparison and validation of the predicted phenotypes from NIR spectral data.

Phenotype prediction

The phenotypes of IMF predicted by the SAS algorithm (Prieto *et al,* 2017) for all 997 samples as well as all statistical values calculated are provided in the Supplemental Workbook Dataset A (Appendix A). The SAS algorithm was run on the cleaned, averaged spectral data for both the SNV-D trended first and second derivative; the R^2_{adj} values for the predicted phenotypes was 0.76 and 0.78 , respectively. The RMSEP value was 0.183 for the second derivative and 0.193 for the first derivative, both with *p*-values < 0.0001. Though both derivatives returned promising values, moving forward, the results for the second derivative were used as this was the most accurate mathematical pre-treatment, based on the higher R^2_{adj} value and the lower RMSEP value. The IMF predicted from NIR for all 997 samples averaged 3.33 $\pm$ 042 %, which is higher than the calculated moisture corrected proximate analysis value by 1.28%, which grossly overestimates the fat content in the sample. This discrepancy would significantly change the composition of the sample and the eating quality. Additionally, if these phenotypes were used in a selection-based program the results would be unexpected and poor. As such, the predicted phenotypes for lean meat would not be recommended for decision making in selection-based programs such as genetic selection or breeding programs. In order to continue exploring the data for use in further studies, an adjustment factor was calculated

(Materials and methods, section 3.2; Table S3.2, Supplemental information 3.0). This adjustment factor, -1.26, was applied to all the predicted NIR phenotypes; this application resulted in the predicted IMF content for all 997 samples to an average of 2.06% $\pm$ 0.42, just 0.01% higher than the calculated IMF from proximate analysis (Supplemental Workbook Dataset A, Appendix A). These corrected phenotypes are significantly more accurate, and as such, the further use these phenotypes would be appropriate. The level of IMF found in these samples, 2.06% is ideal for Canadian markets as minimum of 1.5% IMF is suggested to satisfy Canadian consumers (Listrat *et al,* 2016). Additionally, many consumers use marbling as a visual measure when choosing meat to purchase; preferences for marbling vary greatly, even within Canada (Ngpao *et al*, 2016), however consumers generally prefer lean meat with minimal marbling (Ngapo *et al,* 2016). As such, the level of IMF found in this study's population would provide a positive eating experience for these consumers through appropriate fat content, increasing tenderness and juiciness (Wood *et al,* 1999; Fortin *et al*, 2005; Teye *et al,* 2005, Cannata *et al,* 2010; Lim *et al,* 2016), but would not discourage consumers by appearing too fatty.

Limitations

The calculated coefficient of variation was 8.9; high CV indicates a greater level of diffusion around the mean and can indicate limitations to the study. Such limitations may include the use of averaged spectra for each sample instead of individual scans. This may have led to the use of outliers or imperfect scans, so in order to investigate this the individual scans were cleaned and checked. The individual scans did not show any outliers for fat or moisture, and so it was established that the averaged data was appropriate. Another potential limitation was the fact that the training samples were fresh lean meat, but the study samples were previously frozen. This may have led to some discrepancies due to moisture loss, and though

this was accounted for with moisture loss calculations and the calculated adjustment factor, it may still have affected the final predicted phenotypes. This is supported by the low R^2_{adj} value for the moisture relationship of 0.12. This indicates a very low positive relationship for the predicted moisture and the calculated moisture, as this was calculated on the non-adjusted phenotypes (p-value < 0.05). The values resulting from the adjustment factor application averaged exactly the same as the actual values calculated from proximate analysis values. The actual, predicted and adjusted values along with their means are represented in figure S3.2 in Supplementary information 3.0. The factor was only applied to those predicted values that had corresponding proximate analysis values, in order to assess the efficacy of the adjustment factor to obtain more accurate values. The resulting improvement in accuracy of the predicted phenotypes is promising, showing that the widespread application to all predicted phenotypes would offer extremely accurate predicted phenotypes for IMF content.

Backfat

Gas chromatography

The average SFA, PUFA, and mono-unsaturated fatty acid content (MUFA) were 32.17 $\pm$1.45%, 23.92 $\pm$ 2.77% and 42.75 $\pm$ 2.48%, respectively. Calculated IV was 76.53 $\pm$ 3.20 and ω -3 and 6 fatty acids were 1.96 $\pm$ 0.28 and 21.96 $\pm$ 2.6% respectively. All values calculated by gas chromatography can be found in Supplemental Workbook Dataset B (Appendix A).

Phenotype prediction

The SAS algorithm (Prieto *et al*, 2017) was run on the cleaned, averaged spectral data for both the SNV-D trended first and second Savitsky-Golay derivative. The mathematical pre-treatment which provided the lowest RMSEP for IV was the second derivative and as such will

be the values further presented. The average IV from predicted values was 90.81$\pm$4.88 compared to those calculated from gas chromatography results noted above. All iodine values are shown in table S3.3 in Supplementary information 3.0. The R^2_{adj} value was 0.4, indicating a weakly positive correlation, with a p-value < 0.001, RMSEP of 2.81 and low coefficient of variation at 3.24. These IV values are high for solid backfat samples, as typically values greater than 70 indicate that the unsaturation of the fats is so high that the sample may be an oil. None of the samples used in the study were excessively greasy or soft, though some were noted as quite thin. Additionally, there is a well-documented inverse relationship noted with proportion of 18:2 ω -6 PUFA, linoleic acid, in subcutaneous fat and the total amount of fat, as indicated by backfat thickness (Wood *et al*, 2008). The measured thicknesses can be found in Supplemental Workbook Dataset B (Appendix A). These high IVs may indicate a limitation of this technique as a reliable method of fat quality; though IV has been widely utilised as a quality measurement, it may not be able to tell the full story of fat quality. As an example, IV is often used, in part, to assess the hardness of pork bellies, which is of particular importance for bacon yield, one of the most economically important cuts of pork (Mandigo, 2000). It is interesting to note that different bellies can have the same IV value yet vary in their structural stability. This is because pork bellies can vary in thickness, which accounts for approximately 30% of their variation in firmness; just 14% was accounted for by their IV value (Soladoye *et al,* 2015). Due to this, it has been suggested that alternate methods of fat quality be looked at in addition to IV, in order to capture the full spectrum of fat quality.

The predicted PUFA is an interesting phenotype to investigate based on the recommendations of good quality fat from an abattoir perspective. White, hard fat is preferred, as during carcass cut up, the cohesive nature between the muscle and the fat keep large muscles

of the primal cuts together prior to further cutting (Sørensen et al, 2012). Soft fat decreases cutting efficiency and causes uneven slicing due to sticky knives. As discussed above, fat is often used as an ingredient in value added products, such as sausages. In these further processed products, fat is one of the most variable ingredients utilised. The National Pork Producer's Council suggests that a good quality pork fat must contain less than 15% PUFA and greater than 15% stearic acid (C18:0) in particular (NPPC, 2010; Soladoye *et al*, 2017) for preferential texture and palatability. As individual fatty acids were not predicted, this was evaluated using the gas chromatography result. These also offer us more reliable values to investigate with, as the predicted phenotypes all had low R^2_{adj} values, indicating weak correlations to the actual values obtained via GC. No samples had great than 12% stearic acid. The predicted PUFA values, however, were all above 15%, both predicted and measured from gas chromatography. As thickness decreases, the proportion of PUFA in the fat increases, devaluing the fat quality from the processing perspective. Table S3.4 in Supplementary information 3.0 contains all values for stearic acid (C18:0) and PUFA category as well as the origin of their values. These levels of PUFA, seen both in the GC results and predicted values, may indicate that although the samples were not suffering from excessive soft or oily texture that they may experience oxidative instability more rapidly than fat which is higher in SFA. Such fat may not be ideal for further processing in value added products due to this and the increase propensity for rancidity due to increased unsaturation.

In regard to all remaining predicted phenotypes; weak, positive relationships based on R^2_{adj}, varying RMSEP values and coefficient of variation. Despite the low R^2_{adj} values, the majority have correspondingly low *p*-values, which indicates that the relationship shown is true based on the predictors and responses, not false positives or merely trends. Only predicted ω -

6:ω -3 has a p-value > 0.05, at 0.07, which shows a trend towards significance, but is not statistically significant. The relationship noted by the R^2_{adj} for this phenotype must be evaluated carefully, as the values are potentially due to error. These results indicate that our model may have some limitations in predicting backfat FA content, despite positive results in associated literature, including the paper from which the training samples were obtained (Prieto *et al*, 2017). As such, the predicted phenotypes for backfat would not be recommended for decision making in selection-based programs such as genetic selection or breeding programs.

Limitations

The most likely reason our prediction values are not very promising is not due to an inability of the NIR rather to do with the samples used in this study; the study samples were too dissimilar (frozen storage, breed collected from) to the training samples used to calibrate the prediction algorithm. The calibrations and standards used for sample evaluation were developed with a specific population and may not have been ideal for the study samples. Incorporation of training samples from different pig populations may improve the results. Additionally, the study samples were collected from the larger carcass and subsequently frozen and thawed prior to scanning. Each sample was a portion of the entire subcutaneous backfat layer. The training samples, however, were fresh and consisted of the entire length of the fat depot (Prieto *et al*, 2017). By only having a subsample of the subcutaneous fat at our disposable for the study, we may not have been able to get a true representative scan of the larger fat depot. Additionally, though seemingly imperceptible, there was some amount of moisture loss from freezing and thawing the study samples; no adjustments were made for moisture in the backfat samples and predictions as was done with the loin samples and this may have impacted the accuracy of prediction from spectra and final measurable effect.

Finally, many of the study samples were quite thin, and despite efforts to maximise surface area, it is possible that scanning was negatively affected. Without duplicate scans and although the spectra were cleaned, normalised and checked for significant outliers, it is possible that some imperfect spectra were used in the analysis and negatively impacted results. In the future, the ability to exclude imperfect spectra without excluding the sample entirely would be beneficial. For research studies, taking two scans per sample is recommended, however for processing speed at abattoirs this is not feasible. All predicted values for the study samples, GC results, along with statistics can be found in the Supplemental Workbook Dataset B (Appendix A).

3.4 Conclusions

Lean meat IMF content

NIR spectroscopy was used to predict the IMF content in loin samples from purebred Duroc hogs backfat samples. The results were promising, as we saw a p-value <0.001, R^2_{adj} value of 0.78, and RMSEP of 0.183 for the predicted phenotypes when compared to the proximate analysis validation, indicating strong positive relationship. Based on the high coefficient of variation, however, we understand that the study had some limitations. The two primary limitations are 1) the inability to completely correct for moisture loss in the study samples and 2) comparison of previously frozen samples to standards calibrated from fresh samples; using calibrations developed from different population is not best practice. In future studies, these limitations must be accounted for in order to predict the most accurate phenotypes. Based on the discussed limitations and high CV, it is not recommended to use the predicted phenotypes from this study as phenotypes for future GWAS', GS or breeding programs. The adjusted phenotypes, however, were significantly closer to the calculated values

from proximate analysis. If the adjustment factor was applied to all predicted values in order to obtain new, corrected phenotypes, these could be used confidently in further studies. We are able to reject the null hypothesis; there is an accurate, high-positive relationship ($p < 0.0001$) between predicted NIR phenotypes and those obtained via the reference method.

Backfat

NIR spectroscopy was used to predict full fatty acid profiles for purebred Duroc hog backfat samples, which were further used to calculate IV as a basic measure of fat quality. Based on the low *p*-values associated with each predicted phenotype and, we understand that NIR is a feasible and useful technology for the rapid prediction of fat composition. However, we also saw low coefficients of determination and high RMSEP values, which indicate poor relationships with the GC results. Due to this, we reject the null hypothesis that no relationship exists, however we understand that due to the previously described limitations that these phenotypes are of low quality. Many of the study samples were thin, which negatively impacted the scanning efficiency and accuracy of probe placement. Spectra returned were compared to standards calibrated from fresh samples, which also affected the final predicted phenotypes. Moving forward, the NIR phenotypes generated from this study are not recommended for use in any additional studies (GWAS, genetic selection or breeding programs). In future studies, the discussed limitations should be addressed. Despite our disappointing results, NIR is a reliable option for the collection of data for fat composition analysis in subcutaneous pork fat, as based on the large body of literature behind this method.

Supplementary information 3.0

Table S3.1 Proximate analysis results with moisture corrections for lean meat samples from a purebred Duroc population

Sample ID[A]	Moisture (%)[B]	Fat (%)[C]	Chop Initial (g)[D]	Chop Final (g)[E]	Purge loss (%)[F]	Water Purge (g)[G]	Water weight chop final (g)[H]	Total water weight(g)[I]	Final moisture (%)[J]	Total fat in final sample (%)[K]	Fat in initial sample (%)[L]
7684	73.04	2.67	144.64	137.80	4.73	6.84	100.65	107.49	74.31	3.67	2.54
7940	74.35	1.97	155.79	152.44	2.15	3. 35	113.34	116.69	74.90	3.00	1.92
7773	72.98	2.39	159.01	148.65	6.51	10.36	108.48	118.84	74.74	3.55	2.23
7961	73.33	2.63	143.44	123.26	14.07	20.18	90.39	110.57	77.08	3.24	2.26
7687	72.86	2.76	129.68	122.43	5.59	7.25	89.20	96.45	74.38	3.37	2.60
7014	73.68	1.43	137.54	125.02	9.10	12.52	92.11	104.63	76.08	1.78	1.30
7003	73.72	2.41	169.17	160.96	4.85	8.21	118.66	126.87	75.00	3.87	2.29
7167	73.00	2.31	183.04	164.49	10.13	18.55	120.07	138.61	75.73	3.79	2.07
7025	73.09	2.18	140.57	129.57	7.82	11.00	94.70	105.69	75.19	2.82	2.01
7568	74.10	2.22	193.11	187.76	2.77	5.35	139.12	144.47	74.81	4.17	2.16
6056	73.72	1.97	139.00	130.35	6.22	8.65	96.09	104.74	75.35	2.56	1.84
5957	73.70	1.79	137.45	132.40	3.67	5.05	97.58	102.63	74.67	2.36	1.72
5800	74.34	1.85	178.68	173.56	2.86	5.12	129.02	134.13	75.07	3.20	1.79
5053	71.49	2.33	156.70	132.09	15.71	24.61	94.42	119.04	75.96	3.08	1.96
5374	73.24	2.07	170.81	155.29	9.09	15.52	113.73	129.25	75.67	3.21	1.88
5530	71.80	2.19	182.21	153.32	15.86	28.89	110.08	138.98	76.27	3.35	1.84
5222	73.00	2.19	211.49	193.22	8.64	18.27	141.05	159.32	75.33	4.24	2.00
2868	73.80	2.47	132.36	126.95	4.08	5.40	93.68	99.09	74.87	3.13	2.36
4622	73.20	1.81	175.90	156.57	10.99	19.33	114.60	133.93	76.14	2.83	1.61
2839	72.08	2.86	150.88	137.06	9.16	13.82	98.79	112.61	74.64	3.91	2.59
2720	73.49	1.86	166.73	157.84	5.33	8.89	115.99	124.88	74.90	2.94	1.76
2825	72.13	2.87	160.13	142.15	11.23	17.98	102.53	120.50	75.25	4.08	2.55
3262	72.42	2.03	119.02	104.61	12.11	14.41	75.76	90.17	75.76	2.12	1.78
3135	72.06	2.82	153.79	138.71	9.81	15.08	99.95	115.03	74.80	3.91	2.54
4307	74.11	1.70	139.34	130.69	6.21	8.65	96.85	105.51	75.72	2.22	1.59
4772	72.95	2.34	144.82	129.28	10.73	15.54	94.31	109.85	75.85	3.02	2.08
3609	73.09	2.03	145.94	139.04	4.73	6.90	101.62	108.52	74.36	2.82	1.93
4350	73.88	1.91	127.18	118.77	6.62	8.41	87.75	96.17	75.61	2.26	1.78
3620	74.14	1.90	154.08	151.26	1.83	2.82	112.14	114.95	74.61	2.87	1.87
4217	72.93	1.78	136.66	126.98	7.09	9.68	92.61	102.29	74.85	2.26	1.65
6329	73.13	2.49	136.92	131.17	4.20	5.75	95.92	101.67	74.26	3.26	2.38
6179	74.03	1.72	200.92	194.90	3.00	6.02	144.28	150.30	74.81	3.35	1.67
2993	73.99	1.73	130.09	125.68	3.39	4.41	92.98	97.40	74.87	2.17	1.67
3119	72.21	2.48	128.37	121.44	5.40	6.93	87.69	94.62	73.71	3.01	2.34
1472	72.54	3.28	139.47	135.81	2.63	3.66	98.51	102.17	73.26	4.45	3.19
9886	73.34	2.86	169.71	166.16	2.09	3.55	121.87	125.42	73.90	4.75	2.80
1624	72.86	2.17	133.02	124.18	6.64	8.84	90.47	99.31	74.66	2.69	2.02
1623	73.73	1.96	121.46	117.10	3.59	4.36	86.33	90.69	74.67	2.30	1.89
9881	74.08	1.63	145.75	140.82	3.38	4.93	104.32	109.26	74.96	2.30	1.57

A Unique pig ID associated with each sample.
B Moisture result for lean meat sample returned from proximate analysis.
C Fat result for lean meat sample returned from proximate analysis.
D The weight of the lean meat sample pre 48-hour drip loss.
E The weight of the lean meat sample after thawing, just prior to NIR scanning.
F The difference between the initial and final chop weights, divided by the initial weight, multiplied by 100 to obtain the purged %.
G The difference between the initial chop weight and final chop weight, reported in grams of water.
H The final water weight in the chop, calculated by the final chop weight (g) multiplied by the moisture, divided by 100.
I The water purge (g) plus the water weight chop final (g)
J The total water weight divided by the initial chop weight.
K Total fat in the final sample, post 48-hour drip loss and thaw purge loss. Calculated by the fat percentage returned from proximate analysis multiplied by the final chop weight.
L Total fat in initial sample, pre 48-hour drip loss and freezing. Calculated by the fat in the final sample divided by the initial chop weight, multiplied by 100.

Table S3.2 Corrected IMF content from proximate analysis ,predicted IMF content from NIR and calculated adjusted value with correction factor for lean meat samples from a commercial crossbred purebred Duroc population.

Sample ID	Fat in initial sample (%) actual[A]	Predicted Fat (%)[B]	Difference between predicted & actual[C]	Adjusted Values (%)[D]
1472	3.19	4.21	-1.01	2.94
1623	1.89	3.34	-1.45	2.08
1624	2.02	3.22	-1.20	1.96
2720	1.76	3.16	-1.40	1.89
2825	2.55	3.90	-1.36	2.64
2839	2.59	3.79	-1.19	2.52
2868	2.36	3.68	-1.32	2.42
2993	1.67	2.75	-1.09	1.49
3119	2.34	3.67	-1.33	2.41
3135	2.54	3.94	-1.39	2.67
3262	1.78	3.43	-1.65	2.17
3609	1.93	3.23	-1.29	1.96
3620	1.87	3.24	-1.37	1.97
4217	1.65	2.66	-1.01	1.40
4307	1.59	3.05	-1.46	1.79
4350	1.78	2.97	-1.19	1.70
4622	1.61	2.97	-1.37	1.71
4772	2.08	3.48	-1.40	2.22
5053	1.96	3.26	-1.30	2.00
5222	2.00	2.96	-0.95	1.69
5374	1.88	2.74	-0.86	1.48
5530	1.84	2.95	-1.11	1.69
5800	1.79	2.59	-0.80	1.33
5957	1.72	3.19	-1.47	1.92
6056	1.84	3.42	-1.58	2.16
6179	1.67	3.04	-1.38	1.78
6329	2.38	3.68	-1.30	2.41
7003	2.29	3.62	-1.34	2.36
7014	1.30	2.49	-1.19	1.22
7025	2.01	3.28	-1.27	2.01

7167	2.07	3.48	-1.41	2.22	
7568	2.16	3.32	-1.16	2.06	
7684	2.54	3.55	-1.01	2.29	
7687	2.60	3.72	-1.11	2.45	
7773	2.23	3.62	-1.38	2.35	
7940	1.92	3.32	-1.40	2.06	
7961	2.26	3.78	-1.52	2.52	
9881	1.57	2.75	-1.17	1.48	
9886	2.80	3.91	-1.11	2.64	
	2.053	3.32	-1.26	2.053	Average

A Total fat in initial sample, pre 48-hour drip loss and freezing. Calculated by the fat in the final sample divided by the initial chop weight, multiplied by 100, as described in table S3.1. The average is found at the bottom of the column.

B The predicted IMF content from NIR spectra. The average is found at the bottom of the column.

C the individual sample differences from actual and predicted values. The average value of all the differences, given at the bottom of this column, is the adjustment factor subsequently applied to all predicted values.

D The adjusted values for predicted phenotypes, calculated by applying the average difference between the actual and predicted values, -1.26, to all predicted phenotypes.

Table S3.3 Iodine values calculated by gas chromatography (GC) and predicted by NIR algorithm for backfat samples obtained from a purebred Duroc population

Sample ID	GC iodine value[A]	Predicted iodine value[B]
1351	73.10	88.20
1920	75.11	89.38
1947	75.45	89.44
2082	76.49	82.53
2222	79.08	95.61
2349	73.90	88.23
2378	73.40	86.23
2449	84.93	95.75
2586	75.58	93.66
2724	78.47	97.31
2859	75.98	89.42
3571	79.45	96.78
3596	75.93	90.30
3606	76.36	99.16
3627	79.77	94.52
3630	77.61	93.42
4306	78.11	96.20
4622	83.51	103.05
4624	81.01	92.85
4631	77.19	90.16
4945	76.84	90.32
5062	77.27	93.39
5341	74.39	89.52
5533	80.21	93.39
5659	80.58	92.26
5953	80.15	102.26
5957	76.82	91.53
6078	79.02	97.02
6310	74.42	88.57
6321	75.64	94.31
6325	80.52	85.80
7385	76.26	90.23
7569	77.11	89.30
7789	79.80	93.37

8268	78.07	89.26
8277	78.00	95.39
8509	74.27	90.11
8533	72.64	83.35
8536	74.75	79.00
8690	70.20	86.91
8921	70.93	85.56
8952	72.40	90.88
8971	72.98	87.86
9253	72.63	84.15
9441	73.53	87.16
9573	80.24	91.83
9705	72.08	90.02
9760	78.38	87.28
9936	71.57	82.24
9940	74.38	86.03
AVERAGES:	**76.53**	**90.81**

A The iodine values for 5% of the total samples, calculated from gas chromatography results
B The iodine values for 5% of the total samples, calculated from phenotypes predicted with NIR spectra

Table S3.4 Values for stearic acid and total polyunsaturated fatty acids by gas chromatography (GC) and predicted by NIR in backfat samples from a purebred Duroc population.

Sample ID	Stearic Acid (C18:0) Gas Chromatography	PUFA Gas Chromatography	PUFA Predicted by NIR
1351	11.6594	22.03	29.03
1920	11.2942	22.41	28.16
1947	10.9742	22.84	28.06
2082	9.5728	21.01	22.08
2222	10.8336	25.65	31.07
2349	11.6168	21.73	27.41
2378	11.4535	21.05	25.55
2449	10.2593	30.51	32.77
2586	10.8180	23.00	30.25
2724	9.3507	24.15	30.57
2859	11.4563	23.48	26.94
3571	9.5247	25.29	31.00
3596	10.2693	22.50	28.58
3606	11.0650	23.59	35.30
3627	11.4363	26.96	31.62
3630	10.3400	23.74	30.56
4306	11.3728	25.51	32.13
4622	9.4890	28.61	34.88
4624	10.6416	28.22	29.54

4631	11.1839	24.40	28.89
4945	11.5615	25.57	29.41
5062	11.5289	25.36	31.96
5341	11.3514	23.12	30.00
5533	11.3895	27.76	32.06
5659	10.8383	29.36	31.23
5953	9.4702	25.41	36.72
5957	11.1413	23.83	29.01
6078	11.7544	27.28	33.29
6310	11.3942	23.45	27.62
6321	12.3582	24.16	31.25
6325	10.5700	27.80	28.24
7385	10.9909	23.82	29.59
7569	11.8945	25.39	29.26
7789	11.8948	27.22	31.18
8268	10.7008	25.77	28.35
8277	10.5377	23.80	30.36
8509	10.3623	21.31	28.15
8533	10.9138	20.27	23.89
8536	11.8807	23.28	21.25
8690	12.8812	19.52	26.22
8921	12.0533	19.72	26.93
8952	11.6521	20.21	29.32
8971	11.1270	20.33	26.82
9253	10.9329	19.67	24.29
9441	11.6227	21.03	26.47
9573	11.4100	27.77	29.42
9705	10.8040	19.93	28.56
9760	9.8655	24.39	27.19
9936	10.9334	20.21	25.39
9940	11.2473	22.54	26.06

Figure S3.1

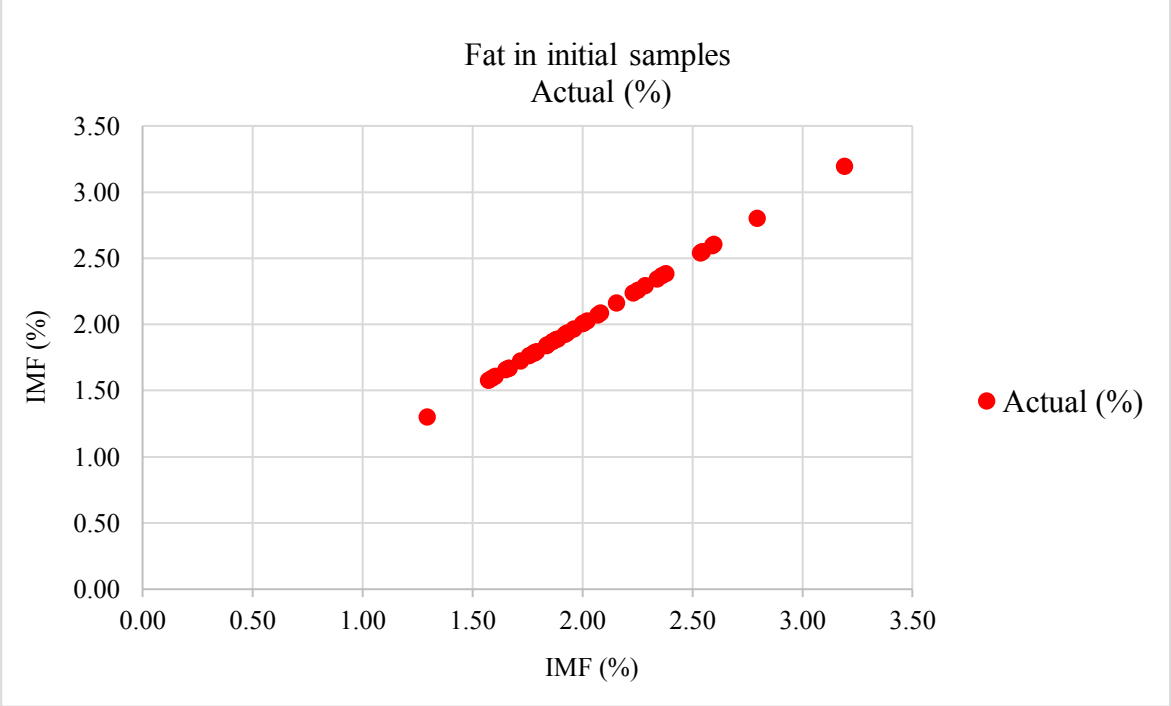

Figure S3.1 Graph of the IMF values in the initial, pre drip loss sample, all values presented have been corrected for moisture. These values served as the actual values for comparison and validation of the predicted phenotypes from NIR spectral data.

Figure S3.2

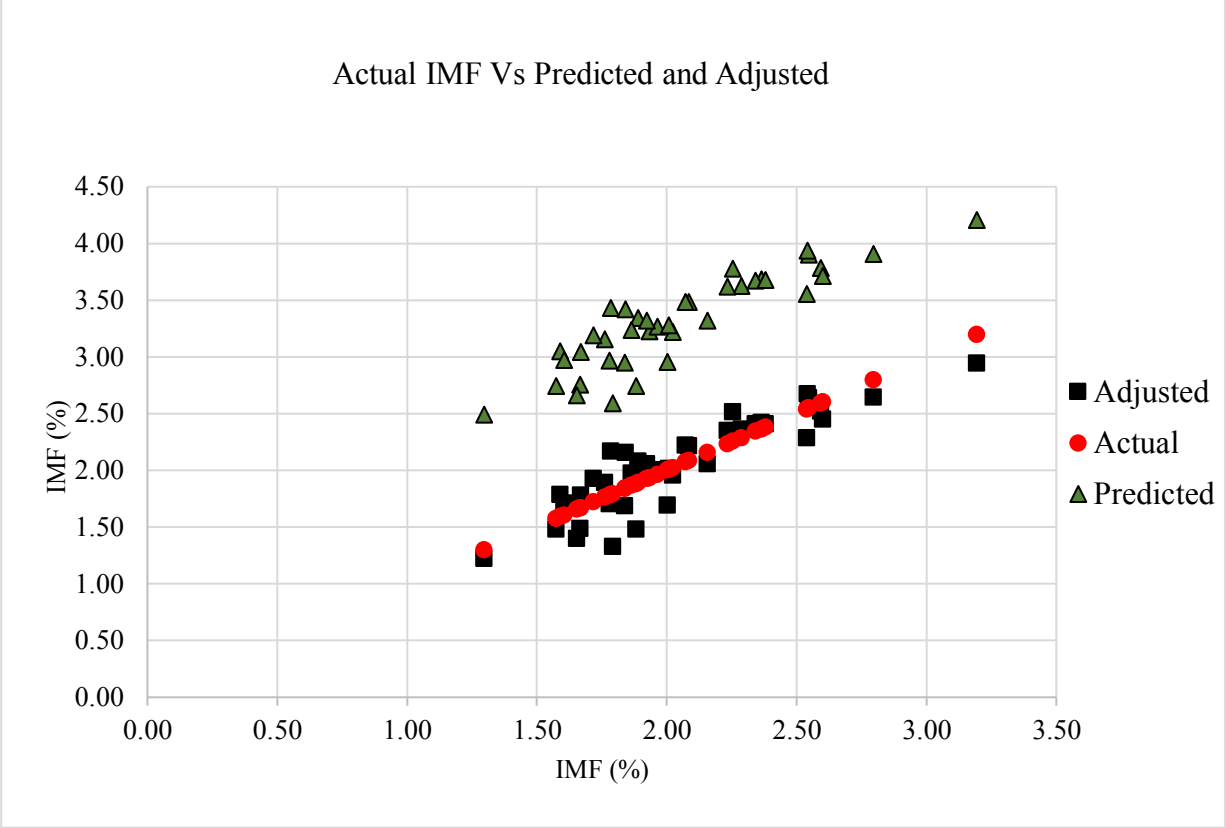

Figure S3.2 Graph of the IMF values initial sample (Actual, red circles) calculated from the proximate analysis data and corrected for moisture (See table S3.1) against the predicted phenotypes from spectral data (Predicted, green triangles) and values which have a uniform correction factor applied (Adjusted, black squares). All samples displayed in this graph are those which underwent proximate analysis so that the predicted and adjusted values can be validated through the actual values.

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Chapter 4.0 Genetic and phenotypic parameter estimations for several fatness traits measured by various methods in a purebred Duroc and commercial crossbred population

4.1 Introduction

The focus of swine breeding in previous decades has been to increase lean yield and carcass leanness through reduction of backfat thickness. This carcass leanness was rapidly achieved, however, positively genetically correlated traits such as intramuscular fat (IMF) also experienced significant reductions, resulting in undesirable decreases in meat quality. When using a selection index[9], the availability and application of genetic correlations enables estimates of responses of more than a single trait, which is invaluable for breeders (Hill, 2013a). Of particular importance to the hog industry is the relationship of backfat depth (BFD) and IMF, as they are positively genetically correlated (0.28 ± 0.03- 0.38 ± 0.005) (Suzuki *et al*, 2005; Hernández-Sánchez *et al*, 2013). Due to this relationship, the selection for decreased backfat led to poorer product quality, significantly due to the unintended decrease of IMF; IMF is known to be extremely important for excellent eating quality of pork (Wood *et al*, 1999; Fernández *et al*, 1999; Cannata *et al* 2010; Lim *et al,* 2016). Due to consumers' demands for better quality pork, the importance of improving meat and carcass quality is growing (Dransfield *et al*, 2005). Understanding the genetic correlations of economically relevant pork quality traits and carcass characteristics is necessary in order to implement a successful breeding program in which the emphasis is on product quality.

[9] A selection index is a method of artificial selection in which several economically relevant traits are simultaneously selected for, enabling breeders to make balanced selection decisions based on the emphasis of the breeding program (Hill, 2013b).

In addition to genetic correlations, the heritability of a trait can significantly influence how a trait will be expressed in the progeny of any given breeding animal. Estimates of heritability are not only specific for the trait, but also for the population from which they were calculated. This is firstly due to the environmental influence and secondly due to differing genetic variance seen in different populations (Oldenbroek and van der Waaij, 2014). Heritability has been reported in the literature for BFD measured by ultrasound (UBFD) (Li and Kennedy, 1994; Miar *et al*, 2014) and IMF measured via ultrasound (UIMF), subjective scoring (SUBIMF), near-infrared technology (NIR) (NIRIMF) and proximate analysis (Hermesch *et al*, 2000; Newcom *et al*, 2005; Miar *et al*, 2014) in various pig populations. However, it has been shown that heritability is not a fixed value and regular recalculation for any given trait is wise, particularly given a change in environment or for different populations.

The objectives of this study were to first estimate the genetic and phenotypic correlations of BFD measured *post-mortem* with a ruler or *ante-mortem* via ultrasound with IMF measured in a variety of methods (NIR, ultrasound, subjective) in both a commercial pig and purebred Duroc populations. This was done in order to assess their relationships as well as identify any differences between the IMF and BFD measurement methods in terms of accuracy and repeatability. Additionally, the second objective was to estimate the heritabilities of BFD, ultrasound-backfat fat depth (UBFD), near-infrared IMF (NIRIMF) (purebred only), ultrasound IMF (UIMF) and IMF assessed subjectively (SUBIMF) in these same populations in order to compare and add these to the literature as well as provide industry with valuable information for selection indices. All methods for t1rait measurement are described in section 4.2 (Material and methods).

Ultimately, this work helps to obtain a greater understanding of the genetic relationships of backfat and IMF in both a commercial and purebred pig population as well as

the various, common measurement techniques for these traits. Based on the well documented relationship between IMF and BFD found in the literature we expected to identify moderate-high positive correlations between these traits, as well as moderate – high heritabilities for these traits. Our null hypothesis is that there is no relationship between the traits and that estimated heritabilities are outside the range of those previously reported.

4.2 Materials and methods

Ethics statement

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 (Canadian Council on Animal Care, 1993).

Animals

Two separate pig populations were investigated in this study: a crossbred population and a purebred population. Both populations have a common sire breed (Duroc).

Crossbred pigs

A subsample of 916 animals from a total of 1098 commercial crossbred pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study. Animals without phenotypes available for all traits studied were removed; this subsample are animals with phenotypic data available. The pigs were a three-way cross between a Duroc sire and F1 hybrid (Landrace × Large White) dams. The majority of commercial pigs for slaughter in Canada are a result of this terminal cross (Miar *et al*, 2014). The animals used in the pedigree has 100% of the parents known and included 64 sires and 113 dams over 1 generation. Feeding, raising, and slaughter protocols have been described in

previous studies (Miar *et al*, 2014; Zhang *et al*, 2015; Yang *et al*, 2017). The commercial population was 53.7% male (barrows) and 46.3% females (gilts).

Purebred pigs

A total of 997 purebred Duroc pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study, all had phenotypic data available (no missing phenotypes). The animals used in the pedigree had 100% of the parents known and included 83 sires and 292 dams over 1 generation. Pigs were all female as slaughter took place at a commercial facility where no intact males are accepted and the nucleus farm from which the animals were sourced does not contain barrows as they are breeding facilities. The Duroc breed was chosen as this is the typical sire line in the Canadian commercial cross, and they are known for having higher IMF content than other common breeds (Schwab *et al,* 2006) and also higher growth and feed efficiency than fatter breeds such as the Berkshire; growth and feed efficiency are important to ensure profitability of the final carcass (Fahmy *et al*, 1987; Alfonso *et al*, 2010; Choi *et al*, 2014). Feeding, raising, and slaughter protocol have been described in previous studies (Miar *et al*, 2014; Yang *et al*, 2017; Zhang *et al*, 2015).

Phenotypes

The traits used in this investigation were backfat depth (BFD), ultrasound backfat depth (UBFD), ultrasound intramuscular fat (UIMF), near-infrared intramuscular fat (NIRIMF) and subjective marbling score (SUBIMF). BFD, in this study was defined as the depth of the fat 5 cm off the midline and perpendicular to the hide between the 3rd and 4th last ribs; this was measured within 24 hours post-mortem with a ruler and is reported in millimetres (mm). UBFD was assessed via ultrasound on all pigs (purebred and crossbred), using the BioQ station (Biotronics Inc, Iowa USA) equipped with the Exago ultrasound scanner (IMV imaging). Scans were taken two days prior to slaughter, before shipping, just off of the midline (P.

Charagu, personal communication, June 18, 2020)[10] . The proprietary algorithm used in the built-in software of the BioQ station (BioSoft Toolkox®, Biotronics Inc) provides an estimation of the BFD (mm). UIMF was assessed via ultrasound on each pig, both purebred and crossbred, also using the BioQ station (Biotronics Inc, Iowa USA). The proprietary algorithm used in the built-in software of the BioQ station provides an estimation of the IMF content in the muscle. Scans were done between the 3rd and 4th last rib, approximately 6 inches off of the midline at the apex of the loin and animals were scanned two days prior to slaughter, before shipping (P. Charagu, personal communication, July 22, 2019)[11]. They are reported as a percentage. SUBIMF was assessed on the *longissimus dorsi* (loin) muscle within 24 hours *post-mortem*, for both the purebred and crossbred animals, using the National Swine Improvement Federation (NSIF) marbling charts (NSIF, 1997). A single grader, Dr. Bob Mckay of Mckay-Genstat Consultants (Brandon, Manitoba) assessed all loin samples. They are reported as a single value, according to the following grades; 0 to 6: 0 = devoid, 1 = practically devoid, 2 = trace amount of marbling, 3 = slight, 4 = small, 5 = moderate, 6 = abundant. NIRIMF was available only for the purebred animals and phenotype generation is described in Chapter 3.0, section 3.2 (Materials and methods). The corrected NIR phenotypes, those which have the adjustment factor applied, were used in this study. They are reported as a percentage. The descriptive statistics for the 5 traits (UBFD, BFD, NIRIMF, UIMF and SUBIMF) including the standard mean, minimum, maximum and standard deviation were calculated in Microsoft Excel using the appropriate functions. Coefficient of variation (CV) was calculated with the following formula.

[10] Dr. P.Charagu, personal communication, June 18, 2020, describing the collection method for UBFD .

[11] Dr. P.Charagu, personal communication, July 22, 2019, describing the collection method for UIMF.

$$CV = \frac{SD}{\mu} \times 100\% \qquad (1)$$

Where SD is standard deviation and μ is mean of the phenotypes.

Bivariate analysis

Genetic and phenotypic correlations were calculated using a series of bivariate analyses in ASReml (Gilmour *et al*, 2015). The statistical model used was the animal model. The significance of fixed effects and covariates was determined in ASReml using the REML procedure. Fixed effects fitted for all pairwise analyses ($p < 0.05$) can be seen in Table 4.1 below.

Table 4.1 Significant effects (fixed and random) in bivariate analysis

Purebred Durocs		
Pairwise Analysis	**Fitted fixed effects***	**Fitted random effects***
BFD - NIRIMF	Sex, date of slaughter	Animal
BFD - UIMF	Sex, date of slaughter	Animal
BFD - SUBIMF	Sex, date of slaughter	Animal
NIR - SUBIMF	Sex	Animal
UIMF - SUBIMF	Sex, date of slaughter	Animal
NIRIMF - UIMF	sex	Animal
UBFD - BFD	Sex, date of slaughter	Animal
UBFD - NIRIMF	Sex, date of slaughter	Animal
UBFD - UIMF	Sex, date of slaughter	Animal
UBFD - SUBIMF	sex	Animal
Commercial crossbreds		
BFD - UIMF	Sex	Animal
BFD - SUBIMF	Sex	Animal
SUBJ - UIMF	Sex	Animal
UBFD - BFD	Sex, date of slaughter	Animal
UBFD - SUBIMF	Sex, date of slaughter	Animal
UBFD - UIMF	Sex, date of slaughter	Animal

*Significance level for fixed and random effects set at $p < 0.05$.

The animal model used for bivariate analysis includes random additive polygenic effects and is given by:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} \quad (2)$$

In this model where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are the vectors of phenotypic measurements for traits 1 and 2, $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ are the incidence matrices which relate the fixed effects to vectors $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$, respectively; $\boldsymbol{b_1}$ and $\boldsymbol{b_2}$ are the vectors of significant fixed effects for traits 1 and 2, respectively $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ are the incidence matrices relating the phenotypic observations to the vector of polygenic effects, $\boldsymbol{a_1}$and $\boldsymbol{a_2}$ for traits 1 and 2, respectively. $\boldsymbol{e_1}$ and $\boldsymbol{e_2}$ are the vectors of random residual effects for traits 1 and 2, respectively. Heritability was estimated using the variance components obtained from model (2) as:

$$\boldsymbol{h^2} = \frac{\boldsymbol{\sigma_a^2}}{\boldsymbol{\sigma_a^2 + \sigma_e^2}} \quad (3)$$

Where $\boldsymbol{\sigma_a^2}$ is the direct additive genetic variance and $\boldsymbol{\sigma_e^2}$ is the residual variance).In order to assess statistical significance of genetic correlations, confidence intervals (CI) were calculated for the correlations by the following formula (Hazra, 2017):

$$\boldsymbol{CI} = [\, \boldsymbol{r_g} + (\boldsymbol{se} \times \boldsymbol{1.96}), \boldsymbol{r_g} - (\boldsymbol{se} \times \boldsymbol{1.96})] \quad (4)$$

Where $\boldsymbol{r_g}$ is the genetic correlation and $\boldsymbol{se}$ is the standard error. The same formula was used for the phenotypic calculations, replacing $\boldsymbol{r_g}$ with $\boldsymbol{r_p}$. If the 95% confidence interval does not contain zero, then the genetic correlations are considered statistically different from zero at the 0.05 significance level ($p < 0.05$); if the confidence interval contains zero, the genetic correlations are not considered significantly different from zero at the 0.05 level.

Coefficients of correlation

The coefficient of correlation (r) for the phenotypes of NIRIMF x UIMF and UBFD x BFD were calculated in Microsoft Excel for the relevant population using the following formula:

$$r = \frac{Cov(1,2)}{\sigma_1 \sigma_2} \qquad (5)$$

Where r is the relationship coefficient, $Cov(1,2)$ is the population covariance of trait 1 and 2, σ_1 is the standard deviation of trait 1 and σ_2 is the standard deviation of trait 2.

4.3 Results and discussion

Phenotypic descriptive statistics

The means (μ), standard deviations (SD), number of records (n) , coefficients of variation (CV), minimum and maximum values for all investigated traits are presented below in Table 4.2A and Table 4.2B.

Table 4.2A Descriptive statistics for BFD and IMF traits* in a purebred Duroc population.

Purebred Durocs					
	SUB IMF	UIMF	NIRIMF	UBFD	BFD
Mean (μ)	1.5	2.89	2.07	10.5	10.3
Minimum	0	0.70	0.87	4.8	4
Maximum	3	4.90	3.84	20	19
Standard deviation (SD)	0.59	0.75	0.42	2.21	2.45
Coefficient of Variation (CV) (%)	40.5	25.9	20.2	21.0	23.7

* Traits all measured by different methods, outlined in section 4.2 (Materials and methods). UBFD = Ultrasound BFD (mm), BFD = backfat depth (mm) , UIMF = ultrasound intramuscular fat, NIRIMF = Near-infrared intramuscular fat, SUBIMF= subjectively scored intramuscular fat.

Table 4.2B Descriptive statistics for BFD and IMF traits* in a commercial pig population.

Commercial crossbreds				
	SUB IMF	UIMF	UBFD	BFD
Mean (μ)	2.9	2.89	15.9	15.5
Minimum	1	0.7	7.9	6
Maximum	6	6.6	33.7	32
Standard deviation (SD)	1.01	0.92	3.4	4.20
Coefficient of variation (CV) (%)	34.9	31.8	21.8	27.1
Commercial crossbreds (Female)				
	SUB IMF	UIMF	UBFD	BFD
Mean (μ)	2.6	2.70	14.5	14
Minimum	1	0.7	7.9	6
Maximum	5	6.4	25.4	27
Standard deviation (SD)	0.922	0.80	2.99	3.65
Coefficient of variation (CV) (%)	35.7	29.5	20.7	26.9

* Traits all measured by different methods, outlined in section 4.2 (Materials and methods). UBFD = Ultrasound BFD (mm), BFD = backfat depth (mm) , UIMF = ultrasound intramuscular fat, SUBIMF= subjectively scored intramuscular fat.

Interestingly, the average UIMF for both purebreds and crossbreds are the same. This is unexpected, as the Duroc breed, specifically selected as a sire line for commercial crossbreds due to their increased IMF (among other growth and carcass traits) is expected to have higher (Schwab *et al*, 2006) IMF content. All other IMF measurements indicate a lower average IMF content for the purebred animals compared to the crossbred animals. The commercial crossbred animals not only have higher average backfat thickness than the purebred Durocs (15.5 mm vs 10.3 mm), but also higher marbling scores; the subjective marbling score is approximately 1.5 grades higher for the crossbreds (1= practically devoid, 2 = trace, 3 = slight). It is known that BFD and IMF are positively genetically correlated traits (Wood, 1990; Rosenvold and

Anderson, 2003; Hernández-Sánchez *et al*, 2013; Miar *et al*, 2014); with the increased BFD seen in the commercial animals, higher IMF is not surprising, however, the quantity is higher than what is generally seen. The average IMF content of Canadian commercial hogs is just 1.5% (Meadus *et al*, 2018) making this population of crossbred animals over 1% above the national average. It is possible that this unexpected result is related to sex, as the purebreds were only females and the crossbreds were approximately half males and half females. Sex is an important factor for fat deposition, as barrows are fatter than gilts (Dunshea and D'Sousza, 2003). When comparing the purebred animals to only the female commercial animals (Table 4.2B), despite small reductions in all values due to the removal of barrows, the commercial animals still have greater UIMF, higher SUBIMF, UBFD and BFD. Reductions of these averages from the removal of barrows are not significant, but this does indicate that the highest values were from male animals.

Aside from sex or genetic factors, diet and environment can significantly influence fat deposition, including IMF. Approximately 75% of fat deposits in pigs are from *de novo* synthesis in the adipose tissue, the remainder are absorbed and incorporated into tissues, unchanged, from dietary sources (O'Hea and Leveille, 1969; Lawrence and Fowler, 2002). Both populations were raised separately, in different environments and were not fed identical rations, which may have influenced the phenotypic results noted in this study.

Genetic parameters

Heritabilities, genetic and phenotypic correlations for all investigated traits are presented in Table 4.3A (purebred Durocs) and Table 4.3B (commercial crossbreds).

Table 4.3A Estimates of genetic (below diagonal), phenotypic (above diagonal) correlations, heritabilities (diagonal) and their standard error of estimates among IMF and BFD for a purebred Duroc pig populations.

	Traits[1] Purebreds				
	UBFD	BFD	NIRIMF	UIMF	SUBIMF
UBFD	0.482 (0.085)	0.808 (0.0119)	0.396 (0.031)	0.4995 (0.027)	0.333 (0.0298)
BFD	0.946 (0.028)	0.44 (0.087)	0.37 (0.031)	0.407 (0.0297)	0.34 (0.03)
NIRIMF	0.319 (0.127)	0.299 (0.135)	0.491 (0.11)	0.504 (0.026)	0.483 (0.025)
UIMF	0.439 (0.122)	0.344 (0.140)	0.775 (0.085)	0.438 (0.090)	0.364 (0.029)
SUBIMF	0.359 (0.158)	0.397 (0.16)	0.930 (0.0901)	0.792 (0.146)	0.234 (0.073)

1 UBFD = Ultrasound BFD (mm), BFD = backfat depth (mm) , UIMF = ultrasound intramuscular fat, NIRIMF = Near-infrared intramuscular fat, SUBIMF= subjectively scored intramuscular fat.
2 No correlations were significantly different from zero based on 95% CI

Table 4.3B Estimates of genetic (below diagonal), phenotypic (above diagonal) correlations, heritabilities (diagonal) and their standard error of estimates among IMF and BFD for a and commercial crossbred pig populations.

	Traits[1] Crossbreds			
	UBFD	BFD	UIMF	SUBIMF
UBFD	0.543 (0.094)	0.738 (0.0192)	0.272 (0.0379)	0.219 (0.0391)
BFD	0.999 (0.018)	0.429 (0.078)	0.368 (0.033)	0.297 (0.035)
UIMF	0.430 (0.162)	0.428 (0.172)	0.241 (0.071)	0.228 (0.038)
SUBIMF	0.479 (0.157)	0.405 (0.138)	0.376 (0.129)	0.245 (0.073)

1 UBFD = Ultrasound BFD (mm), BFD = backfat depth (mm) , UIMF = ultrasound intramuscular fat, SUBIMF= subjectively scored intramuscular fat.
2 No correlations were significantly different from zero based on 95% CI

Heritabilities

Moderate to high heritability was obtained for all of the traits investigated in both populations. Estimates for purebred UBFD, BFD, NIRIMF, UIMF and SUBIMF were 0.48 (± 0.09), 0.44 (± 0.09), 0.49 (± 0.11), 0.44 (± 0.09) and 0.23 (± 0.07) and crossbred estimates for UBFD, BFD, UIMF and SUBIMF were 0.54 (± 0.09), 0.43 (± 0.08), 0.24 (± 0.07) and 0.25 (± 0.07). Almost all of these estimates are in line with previously reported heritability values. Heritability values from the literature for purebred Duroc BFD (0.44 ± 0.11; Newcom *et al*, 2005), commercial crossbred BFD (0.45 ± 0.07; Miar *et al,* 2014), purebred Duroc NIRIMF ($0.50 - 0.62 \pm 0.06$; Gjerlaug-Enger *et al*, 2010), purebred Duroc UIMF (0.48; Jung *et al,* 2015), commercial crossbred UIMF (0.26 ± 0.06; Miar *et al*, 2014) and as well as commercial crossbred subjective carcass marbling score (0.23 ± 0.05; Miar *et al*, 2014) are all very close to the estimates from this study. The heritability reported in this study for SUBIMF (0.23 ± 0.07) is lower than previously reported heritability for marbling scores in purebred Durocs by Cabling *et al*. (2005) of 0.79 ± 0.08, but higher than those reported by Lo *et al.* (1992) which reported this to be 0.16 ± 0.07 in F1 Landrace x Durocs. There are many factors which affect heritability estimates and subsequently the range seen across the literature for certain traits. These factors include population size, the effect of heterosis on crossbred populations, completeness of pedigree, and, as investigated here, the assessment technique (Koots *et al*, 1994).

Of particular importance, and the focus of this thesis, is marbling and IMF content. The predicted values from this study are in line with the many literature estimates; there is a range of reported heritability (0.13 - 0.31; Lo *et al*, 1992; Sonesson *et al,* 1998; Schwab *et al*, 2009) for UIMF. Marbling is an important visual assessment which consumers use in order to gauge

the quality of meat prior to purchase. The range of heritabilities indicate that deposition of IMF is dependent upon not only genetics, but external factors (environment) as well; the higher the estimate of heritability, the higher proportion of the trait is controlled by genetics (Falconer and Mackay, 1996). Additionally, all estimates of heritability are specific for the population it was calculated from, which is another possible explanation to the range of reported heritability estimates. NIRIMF reported higher heritabilities than other, more traditional methods of assessment such as ultrasound and subjective scoring, which is a consistent finding in the literature (Gjerlaug-Enger *et al*, 2010). The reference technique to quantify IMF of proximate analysis was not investigated in the present study but reported heritabilities for this value are moderate to high (0.51 $\pm$ 0.13 - 0.69 $\pm$ 0.12; Lo *et al*, 1992; Newcom *et al*, 2005). Inflated phenotypic variability due to inaccurate measurements (among other factors) can have negative effects on h^2 estimates, particularly by downwardly biasing the estimates (Ge *et al*, 2017). Therefore, it is possible that increased phenotypic accuracy may increase the accuracy of a heritability estimate values, given the same population.

Heritabilities are used to predict responses to selection and to help breeders choose the best method for trait improvement (selection or management) (Cassell, 2009). Those traits with moderate to high heritabilities can be improved using genomic technologies, because a large degree of the variation seen in the trait is due to genetics. Overall, this study estimated BFD and IMF content to have moderate to high heritabilities, which is consistent with current estimates from the literature.

Correlation estimates

Genetic and phenotypic correlations for BFD and IMF content are presented in Table 4.3A (purebreds) and 4.3B (crossbreds). No correlation estimates were found to be statistically significant ($p < 0.05$). This is possibly due to the relatively small sample sizes and

subsequently large standard errors (SE); because the SE is a function of the square root of the sample size, in order to decrease the SE by half, four times as many observations are required (Mcdonald, 2009). For the sake of the discussion, relevant correlations will be addressed. Additionally, phenotypic correlations are in general approximately equal to their corresponding r_g (Kominakis, 2003) and can, in some cases, be substituted for genetic correlations (r_g) if the r_g is not precise; a lack of precision is related to small sample sizes and resulting long CI's. The CI's in this study are due to the relatively large SE's (Chapter 4.0, model 4); increased length of a CI results in lower precision of the estimates (Nakagawa and Cuthill, 2007). In this case, since sample sizes are small, SE's are increased, CI length is increased and precision is reduced, if a genetic correlation significantly deviates from the literature, the phenotypic correlation will also be investigated.

Backfat depth measures (BFD and UBFD) were moderately correlated with all measures of IMF (UIMF, NIRIMF and SUBIMF) in both the purebred and crossbred animals. In the commercial crossbred animals, the estimated correlations for BFD x UIMF and SUBIMF are 0.43 (± 0.17) and 0.41 (± 0.14) respectively, UFBD x UIMF and SUBIMF are 0.43 (± 0.16) and 0.48 (± 0.16) respectively. In the purebreds, BFD x UIMF, NIRIMF, and SUBIMF are 0.34 (± 0.14), 0.30 (± 0.14) and 0.40 (± 0.16) respectively. UBFD x UIMF, NIRIMF and SUBIMF are 0.44 (± 0.12), 0.32 (± 0.13) and 0.36 (± 0.16). It is well known that backfat and IMF are positively genetically correlated traits, and estimated values from this study (UIMF and NIRIMF) are similar to those from the literature for both the commercial crossbreds (0.48 ± 0.19; Miar *et al*, 2014) and in the purebred animals (0.28 ± 0.03; Suzuki *et al*, 2005). Estimates from the literature were statistically significant. The estimated r_g for BFD and UBFD x SUBIMF, however, are higher in both the purebreds (0.40 ± 0.16, 0.36 ± 0.16)

and crossbreds (0.41 ± 0.14, 0.48 ± 0.16) than previous estimates. Miar *et al.* (2014) estimated a negative correlation of -0.16 ± 0.14 between carcass marbling score and UBFD in a crossbred pig population, as did Cabling *et al*. (2015) in a purebred Duroc population (-0.41). Interestingly, Willson *et al*, (2020) reported a positive genetic correlation for subjective marbling score and UBFD (0.30 ± 0.11) in a population of all female Duroc gilts. The estimate from Cabling *et al*. (2020), though similar to the genetic estimates from this study, is more similar to their phenotypic correlation estimates (phenotypic estimates for UBFD and BFD x SUBIMF in the commercial and purebred animals are 0.22 (± 0.04), 0.30 (± 0.04), 0.33 (± 0.03) and 0.34 (± 0.03)). Although none of the estimates in this study were statistically significant different from zero, it is evident that there is a large range of correlation estimates for BFD and SUBIMF than with other methods of IMF assessment. A wide range can indicate differences due to breed, sampling, method of collection (section 4.2, Materials and methods, phenotypes) or technician (Koots *et al*, 1994); particularly since this measure is subjective, a larger range is not surprising.

Overall, despite being not significantly different from zero, the correlation estimates provided in this study for backfat thickness and IMF content indicate that selection for increased IMF will also increase BFD, and vice versa. This relationship is well understood based on previously reported positive genetic correlations (Wood, 1990; Rosenvold and Andersen, 2003; Newcom *et al*, 2005; Miar *et al*, 2014, Hernández-Sánchez *et al*, 2013) as well as the known biological processes of fat deposition. In order to deconstruct this correlation and independently select traits which are positively correlated, increased understanding of the traits' genetic architecture is important. This understanding is also important for the independent manipulation of these traits, which is of particular importance for IMF and BFD as the ultimate goal is to improve IMF without increasing BFD; it is believed that with such

increased understanding incorporated into GS models that BFD and IMF can be independently manipulated (Rosenvold and Anderson, 2003; Ros-Friexedes *et al*, 2013).

Genetic and phenotypic correlations were investigated for traits which provided estimates for the same trait by different methods (NIRIMF, UIMF, SUBIMF and UBFD, BFD) in order to explore which traits may be good substitutes for one another. Ultrasound is a commonly used method for IMF assessment as it can be done on live animals prior to shipping (Jung *et al*, 2015), however this may not be feasible in all situations. NIR technology is primarily used *post-mortem* on carcasses or meat samples (Dixit *et al*, 2017). Subjective marbling score is an inherently less precise measure than NIR or ultrasound (Cheng *et al*, 2015), can only be assessed *post-mortem* but also requires destruction of the *longissimus dorsi* in order to expose the surface of the muscle to assessment. In a previous study by Jung *et al.* (2015) who investigated UIMF and IMF by proximate analysis, they found that a genetic and phenotypic correlation of 0.75 was appropriate to substitute UIMF for proximate analysis values.

In the purebreds, genetic and phenotypic correlations for NIRIMF x UIMF were 0.78 ($\pm$ 0.09) and 0.50 ($\pm$ 0.03), NIRIMF x SUBIMF 0.93 ($\pm$0.09), 0.48 ($\pm$ 0.03), and finally for UIMF x SUBIMF 0.79 ($\pm$ 0.15) and 0.36 ($\pm$0.03). Interestingly, SUBIMF has the higher genetic correlation to NIRIMF and UIMF than NIRIMF x UIMF, however, this pair has higher phenotypic correlation than those with SUBIMF. The high genetic correlations seen with the SUBIMF are unlikely as visual measures are less precise and are at a disadvantage when compared to wet chemistry techniques due to the subjective nature of the assessment (Cheng *et al*, 2015). As such, these values are not dependable; despite the high estimate genetic correlation, SUBIMF is not a suitable substitution as it does not provide the precision needed for accurate phenotypes. Though none were statistically significant, the CI for the genetic

correlation of NIRIMF x UIMF was the smallest of all pairwise analyses, and the second smallest phenotypic CI, so based on these estimates and their SE, NIRIMF and UIMF would be suitable substitutes for one another.

In the crossbreds, NIRIMF was unavailable, therefore only UIMF and SUBIMF were investigated. The genetic and phenotypic correlations for this pair were 0.376 (± 0.13) and 0.23 (± 0.04), respectively. These are low correlation values and indicate that these are not appropriate substitutes for one another; further, substituting a subjective grade for a scientific, algorithm-based estimate is not an appropriate switch.

UBFD and BFD were estimated to have high genetic and phenotypic correlations in both the purebreds (0.95 (±0.03) and 0.81 (±0.01), respectively) and crossbreds (0.99 (±0.02), 0.74 (±0.02), respectively). Backfat thickness is an extremely relevant and economically important trait as it influences the valuation of carcasses and can also provide information about carcass fatness based on the previously described relationships of BFD and IMF. Ultimately, accurate phenotypic records are imperative to the success of a GS program and based on these estimates UBFD phenotypes could be confidently substituted for BFD. UBFD is a non-invasive method which could be assessed on selection candidates themselves, which offers many benefits for a GS program. Moving forward with genetic selection programs to improve IMF it is important to choose accurate phenotypes for two main reasons. First, training the GS model with phenotyped (and genotyped) individuals will foster favourable outcomes through improved accuracy of GEBVs. Phenotypes and genotypes are needed for the first (training) step in GS to estimate SNP effects, and the increased accuracy/precision of phenotypes will help to more accurately estimate these SNP effects; accurate marker effects are important for accurate GEBVs. The routine integration of new phenotypic information is

important as populations and environments are constantly changing. Secondly, these phenotypes may be used in further genetic studies, such as a GWAS, in order to uncover some of the genetic architecture of important traits, which can further be used to increase the accuracy of GEBVs through the integration of these identified functional gene variants into GS programs. With these, it is believed that we may be better able to estimate the overall genetic effects, additive and non-additive, of a trait which may be difficult or expensive to measure (IMF). Based on this study, UBFD or BFD, UIMF or NIRIMF are all appropriate phenotypes to use when available. This provides flexibility for breeders and based on available equipment, technician training, and feasibility in given various situations (pre-slaughter, abattoir, samples, live animals).

Coefficients of correlation

In order to compare the different methods of phenotyping the same trait: 1) NIRIMF and UIMF (purebred population), the two methods which estimate the percentage of IMF content and 2) UBFD and BFD (crossbreds and purebreds), the two methods which estimate depth of backfat, their correlation coefficients (r) were investigated. These were also chosen for investigation based on their pairwise analysis results, given that they were shown to be appropriate substitute measures for one another. The correlation coefficient obtained for NIRIMF x UIMF was 0.504, which is a moderate positive linear correlation; this indicates that these two variables are directly related, and have a moderate association to one another (Mukaka, 2012). This is shown in Figure S4.1 in Supplementary information 4.0. This is not unexpected, as it is understood that the method of collection for a phenotype can significantly impact its estimated values. Both NIR and ultrasound provide estimates based on algorithms, not precise values via wet chemistry, so it is not expected that they will be identical. Additionally, measuring IMF with these methods can be complex. When using NIR for

collection of these phenotypes, four scans were used to obtain a single averaged value for each ground loin sample; IMF it is not evenly distributed throughout the muscle and it is still possible that homogenisation was not perfect which would affect the accuracy of estimation. Increased heterogeneity of a sample decreases the accuracy of prediction (Dixit *et al*, 2017). The accuracy of phenotypic estimates for meat quality traits, such as IMF, also depends upon the cleanliness of the probe used – if there is residual meat left on the window between samples and probing, this will negatively affect the accuracy of the estimate (Alexandrakis, 2012). Finally, NIR measurements are sensitive to environmental factors including ambient light, temperature and spectrophotometer temperature (Marković *et al*, 2014); because not all samples were measured on the same day, it is possible that environmental factors impacted the accuracy of some estimates.

Ultrasound technology also has some drawbacks; collagen and fatty acid profile may affect the accuracy of prediction (Maignel *et al,* 2010), as the black and white nature of the image does not detect the nuances that are present. Visually, intramuscular collagen can appear as 'noise' in the pictures, and it has been shown that a higher degree of unsaturation in the IMF can reduce the correlation between ultrasound assessment and subsequent proximate analysis (Maignel *et al,* 2010). Shadows and reflections may also be inaccurately determined (Miller, 2000). Accuracy of ultrasound IMF measurements have been reported to be quite high, despite these limitations (Jung *et al*, 2015).

The correlation coefficient calculated for BFD and UBFD in from the purebred Duroc pigs and the crossbred pigs were 0.78, and 0.75, respectively; these are high positive correlations. These are shown in Figures S4.2 and S4.3 in Supplementary information 4.0. The depth of backfat is a simpler trait to estimate via ultrasound, as it is less heterogenous than IMF and is more suited to a black and white image. Therefore, the aforementioned limitations of

shadows and 'noise' would be less of a hindrance. These estimated relationships are not perfect, however, as there would still be the influential factors that cannot be accounted for via algorithm. Different technicians operating the ultrasound, animal movement and subsequent picture quality can all affect the final estimated value (Jung *et al*, 2015).

Limitations

Despite the promising results obtained from the estimation of variance components for these two pig populations, there was one primarily limitation associated with this study. In both the purebreds and the crossbreds, the data spanned only one generation and had a small sample size of animals. Increasing the size of the data set through inclusion of additional generations and animals will help to reduce the size of the confidence intervals via reduction of SE (Hill, 2013a); large datasets are important for the accuracy of variance component estimates and the accurate description of relationships between traits in a given population as well as obtain an accurate relationship value between the animals in the data file. A second limitation which affected only the purebred analysis is the composition of the population; only gilts were used in this study. There is a well understood relationship between sex and fatness. Gilts tend to be fatter than boars (Farnworth and Kramer, 1987; Kouba and Sellier, 2011), and barrows fatter than gilts (Dunshea and D'Sousza, 2003). Future studies should have a mixed population including both sexes in order to more wholly represent the phenotypic variance.

4.4 Conclusions

Based on these results, we are unable to reject the null hypothesis because no correlations were significantly different from zero. It is the result of a type II error that we cannot reject the null hypothesis, as the relationship between BFD and IMF is well understood. The lack of significant results is likely due to the aforementioned limitation of small sample

size and single generational data used in this study. Due to this, these variance component estimates must be interpreted cautiously.

The improvement of meat quality is important for the pork industry, particularly the improvement of IMF in order to enhance eating quality. IMF is a polygenic trait with complex genetic architecture, but it is known to have a positive genetic correlation to backfat thickness. Genetic improvement of IMF is possible through selection. However, knowledge of related traits and their genetic parameters are important to make informed decisions and accurately predict the outcome of selection. The accuracy of genetic correlations and heritabilities depend upon the populations they are calculated from and are not static values and therefore the periodic recalculation of these parameters is wise. The understanding of these genetic parameters is helpful for the implementation of selection programs on which the emphasis is improvement of IMF. The selection for increased IMF in the purebreds has been shown to also increase BFD as both heritabilities are moderate-high, and their genetic correlation is also high. As such, parameters calculated would be valuable for designing a breeding program, and the moderate-high heritabilities indicate that BFD and IMF (particularly NIRIMF and UIMF) can be improved using genomic technologies. The calculated coefficient of correlation between the UIMF and NIRIMF indicates a moderate positive correlation and that between BFD and UBFD in both the commercial and purebred animals indicates a high positive relationship. Finally, based on their variance components NIRIMF and UIMF would be suitable substitute measurements as would UBFD and BFD. Moving forward with additional genetic work, such as genome-wide association studies (GWAS'), either phenotype would be appropriate to use, though it is important to be mindful that they are not identical and based of their different methods of measurement may provide different results. Overall, increased understanding of

how traits are related on a genetic level can be valuable and beneficial, particularly when attempting to predict the consequences of selecting for one trait on other traits.

Supplementary information 4.0

Figure S4.1

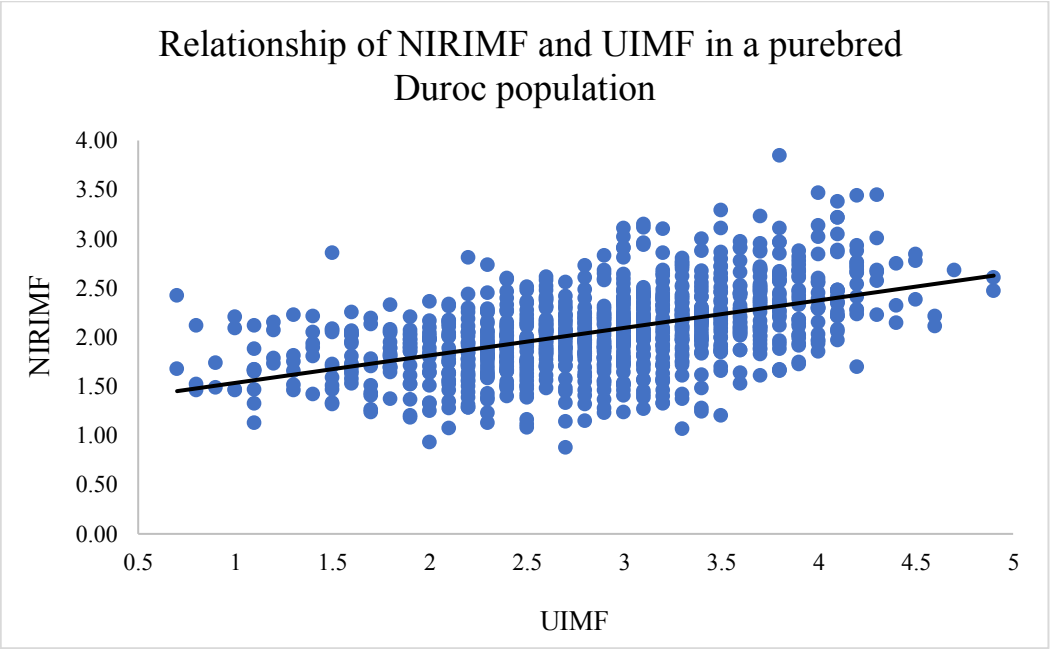

Figure S4.1 depicting the relationship of intramuscular fat phenotypes measured by NIR (NIRIMF) (y-axis) and ultrasound (UIMF) (x-axis) in the same purebred Duroc population. r = 0.504

Figure S4.2

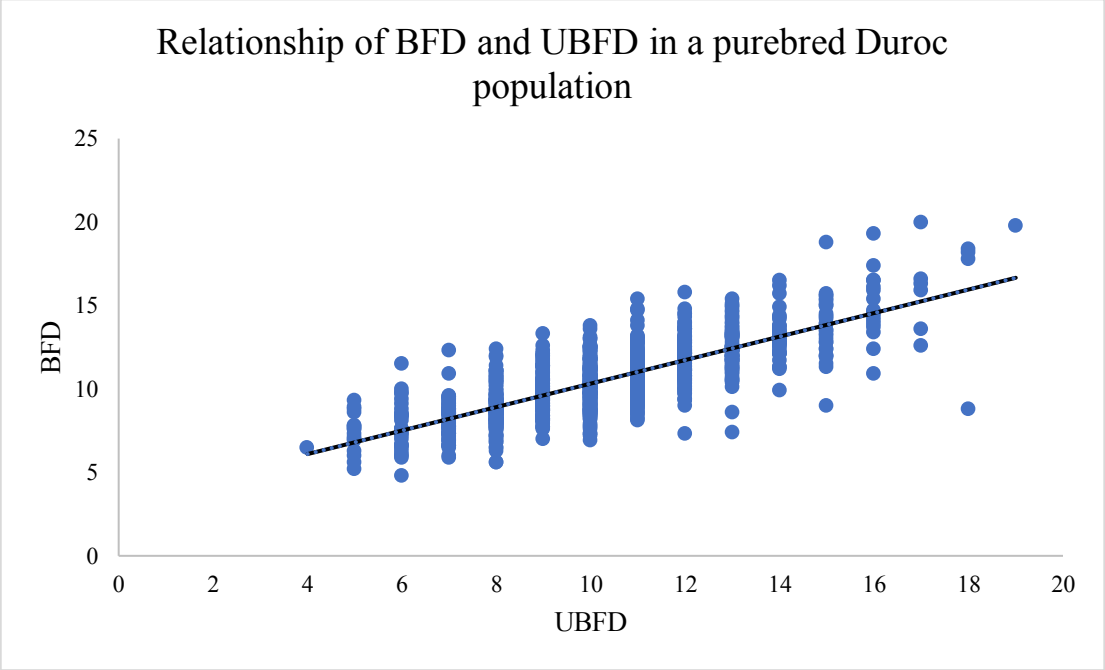

Figure S 4.2 depicting the relationship of backfat depth phenotypes measured by ruler (BFD) (y-axis) and ultrasound (UBFD) (x-axis) in the same purebred Duroc population. r = 0.779

Figure S4.3

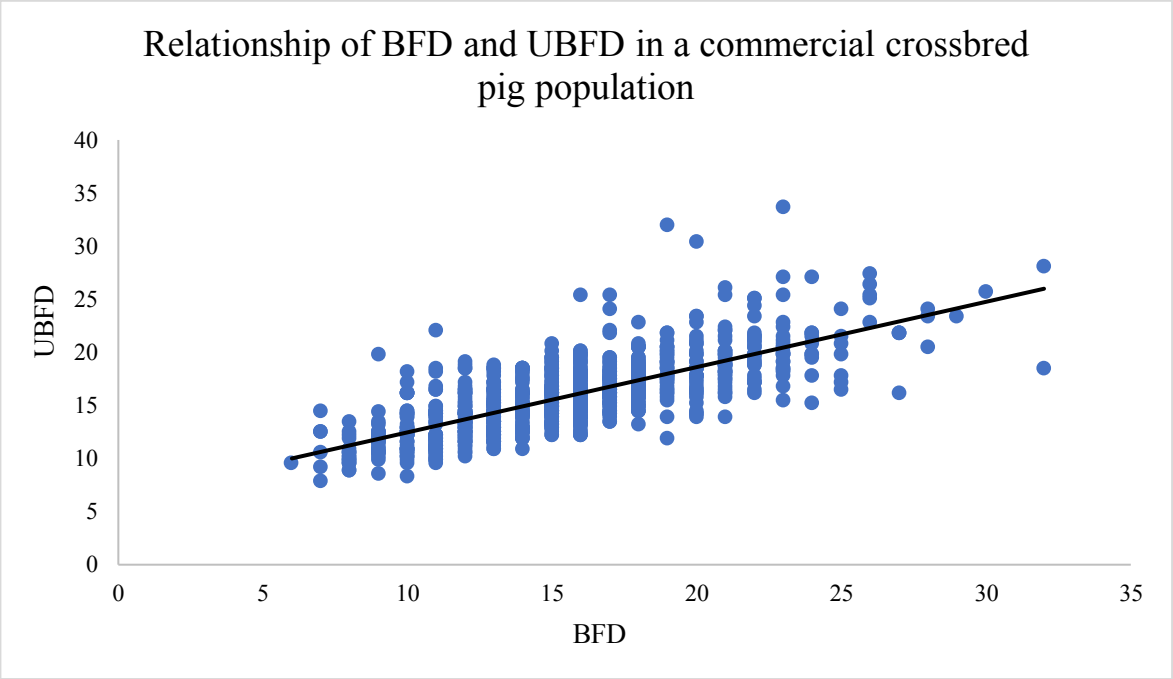

Figure S4.3 depicting the relationship of backfat depth phenotypes measured by Ultrasound (UBFD) (y-axis) and ruler (BFD) (x-axis) in the same commercial crossbred population. r = 0.748

4.5 Literature Cited

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Chapter 5.0 A Genome-wide association study (GWAS) for intramuscular fat (IMF) in purebred Duroc pigs

5.1 Introduction

Genome-wide association studies (GWAS') are a commonly used technique to identify QTL which are significantly associated with a trait of interest, providing insights to the genetic architecture behind a trait (Korte and Farlow, 2013). Through the principle of linkage disequilibrium (LD) at the population level, specifically between genotyped single nucleotide polymorphisms (SNPs) and ungenotyped causal variants (Visscher *et al*, 2012), a GWAS is able to identify SNPs that are common in the population and are in association with causal variants (Goddard *et al*, 2016). To capture these SNPs, animals are genotyped with a SNP panel (Chips); animals are genotyped with a SNPChip, which is then used as their genotype for a GWAS. The genotype of an animal is used in conjunction with their phenotypic record in order to evaluate the results. The results of a GWAS can be used to help identify biological pathways for complex traits (Goddard *et al,* 2016), to better understand the genomic architecture of a trait, predict future phenotypes, or to identify the causal variants (Goddard *et al,* 2016) responsible for trait variation. This identification may be done by not only the detection of the causative mutation itself, but also by investigation of the regions surrounding the identified SNPs and subsequent biological pathway analysis. GWAS' have been successful in the identification of quantitative trait loci (QTL) responsible for observed variation in economically important traits in many livestock species. In fact, tens of thousands of QTL have been reported for pigs alone on the Pig QTL database for hundreds of different traits including performance, disease, and carcass traits (Hu *et al*, 2005).

The improvement of intramuscular fat (IMF) in pigs has become of particular interest recently and a GWAS is a practical method to investigate the underpinnings of such meat quality traits. Total IMF content, or marbling, present in pork is significantly correlated with tenderness upon consumption (Wood *et al,* 1999; Cannata, *et al,* 2010; Lim *et al,* 2016), which is widely considered as the most important attribute for eating (Fortin *et al*, 2004; Teye *et al,* 2005). In the 1980's, breeding goals focused on the reduction of backfat thickness in order to create a lean carcass, which was fitting with the consumer demands of the time (La Berge, 2008). Unfortunately, this also reduced IMF content significantly, leading to a dry product with poor eating quality. Consumer acceptance is critical to the pork industry; both the initial and re-purchasing of products is key to its longevity and prosperity (Lim *et al*, 2016). Due to this demand for better quality pork, industry's focus has more recently shifted to improving meat quality and related traits (Van Wijk *et al*, 2005; Papanagiotou *et al*, 2012) with high importance placed on attributes which relate to eating experience. Uncovering the biological factors related to IMF is necessary to the practical application of genetic selection programs; as we uncover more QTL and genes associated with IMF, further and more targeted work to identify the causative mutations in these genes can be done. Identified variants which have significant association with IMF can be used in animal breeding programs to aide in the selection for superior purebred breeding stock.

Due to the pyramidal structure of hog industry, genetic improvement achieved in purebred herds directly affects the rate of genetic improvement in the commercial animals; a high rate of genetic progress in the purebreds will lead to a higher genetic gain in the commercial crossbreds. The study of purebreds has been shown to be able to accurately predict the phenotypes of crossbred progeny, given that the genetic correlation of the crossbred and purebred animals for those traits is high (Bichard, 1971); meat quality traits have been shown

to have a high genetic correlation between crossbred and purebred animals (0.99) (Brandt and Täubert, 1998). Total carcass fatness and fat tissue accumulation is significantly influenced by breed effects (Kociucka *et al,* 2016); the Duroc breed, the typical sire in the Canadian commercial cross [(Duroc × (Landrace × Large White)] (Miar *et al*, 2014) is therefore a relevant and logical choice for investigation of the genetic underpinnings of IMF. Additionally, Durocs are known to have higher IMF content than other potential sire breeds such as the Pietrain (Schwab *et al,* 2006), but also higher growth and feed efficiency than fatter breeds such as the Berkshire; growth and feed efficiency are important to ensure profitability of the final carcass (Fahmy *et al*, 1987; Alfonso *et al*, 2010; Choi *et al*, 2014) Through the improvement of IMF content in purebred Duroc pigs, the performance of crossbred progeny under commercial conditions will benefit from breed complementarity by maximising the combinations of favourable alleles. We expect this under the assumption that the identified alleles associated with increased IMF are to be fixed in the Duroc and that they are additive in nature. IMF has a wide range (low to high) of reported heritability ($0.26 \pm 0.06 - 0.48$) (Miar *et al*, 2014; Jung *et al*, 2015) and moderate correlation to backfat thickness (0.38 ± 0.005) (Switonski *et al,* 2010; Hernández-Sánchez *et al,* 2013) which suggests that it can be manipulated separately from backfat (Rosenvold and Andersen, 2003; Miar *et al,* 2014). Based on these factors, IMF appears to be a good candidate for genomic analysis and marker-based breeding and selection programs. In this study, we hoped to identify SNPs with a significant association with IMF content of the *longissimus dorsi* and further, to compare the results to those from the literature and in the Pig QTL database (Hu *et al*, 2005). For this, we chose to perform two GWAS' focusing on IMF content with phenotypes measured by a different method (ultrasound and NIR).We hypothesised that we will identify a SNP which has a large effect size that explains a sizable amount of the phenotypic variance seen in IMF; in the event

there are no single SNPs with large effect size we expect to identify SNPs with a smaller effect size which still pass the threshold of significance. We have no null hypothesis for the GWAS'

5.2 Material and methods

Ethics statement

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 (Canadian Council on Animal Care, 1993).

Animals

A subsample of 891 animals from a total of 997 purebred Duroc pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study; animals used had both phenotype and genotype data available. All pigs were female; this was primarily because slaughter took place at a commercial facility where no intact males are accepted and the nucleus farm does not contain barrows, as they are breeding facilities. Additionally, females were used because IMF content can only be definitively measured *post-mortem* and cannot be measured on selection candidates themselves; the males have greater potential of being used for commercial breeding and therefore their female siblings are used in this study. Feeding, raising, and slaughter protocol have been described in previous studies (Miar *et al*, 2014; Zhang *et al*, 2015; Yang *et al*, 2017). The Duroc breed was chosen as this is the typical sire line in the Canadian commercial cross, and they are known for having higher IMF content than other common breeds (Schwab *et al,* 2006)

Genotypes

Tissue samples were collected from the ear on all pigs using Biopsytec samplers which were then sent to the Hendrix Genetics DNA laboratory in Plougragan, France. Genomic DNA was isolated from tissue using the Thermo Fisher Scientific Ltd (Ottawa, ON, Canada) DNA extraction instruction manual and genotyping was done by Geneseek Laboratory in Nebraska, USA with an Illumina 50K Porcine beadchip (Illumina, Inc., San Diego, CA, USA). SNPs with the following features were excluded during quality control procedures of 50K; minor allele frequency (MAF) < 0.01, genotype call rate < 0.95, and departure from Hardy-Weinberg > 0.15. Any SNPs which were unmapped were also excluded, as were those on the sex chromosomes; non-autosomal SNPs were removed as the recombination landscape of the sex chromosomes is known to be different than on autosomes, which can cause distortion in the subsequent analysis (Bosse *et al*, 2012; Zhang *et al*, 2018). Imputation of any missing genotypes was done with FImpute version 2.2 (Sargolzaei *et al,* 2014). Finally, 35,809 SNPs and 891 animals remained from the original of 45, 436 SNPs and 997 animals on 18 autosome chromosomes for the GWAS.

Phenotypes

Two methods of IMF assessment were investigated: 1) IMF was assessed via ultrasound on each pig using the BioQ station (Biotronics Inc, Iowa USA). The proprietary algorithm used in the built-in software of the BioQ station provides an estimation of the IMF content in the muscle (UIMF). Scans were done between the 3rd and 4th last rib, approximately 6 inches off of the midline at the apex of the loin. Animals were scanned two days prior to slaughter. 2) IMF was assessed via NIR on ground *longissimus dorsi* samples (NIRIMF). Phenotype generation is described in Chapter 3.0, section 3.2 Materials and methods. The corrected NIR phenotypes, those which have the adjustment factor applied, were used in this study. All phenotypes were corrected in ASReml using the following model (1) (Gilmour *et al*,

2015) to obtain residuals (adjusted phenotypes), which were further plotted to evaluate the distribution.

$$\boldsymbol{y}^* = \boldsymbol{y} - \boldsymbol{\tau} \qquad (1)$$

Where $\boldsymbol{y}$ is the vector of uncorrected phenotypes and $\boldsymbol{\tau}$ is the p x 1 vector of fixed effects (Gilmour *et al*, 2015). $\boldsymbol{y}^*$ is the vector of adjusted (corrected) phenotypes for fixed effects. The residuals (adjusted phenotypes) are presented in Figures S5.1 and S5.2 in the Supplementary information 5.0. For UIMF, the only fixed effect with significance at $p < 0.05$ was date of slaughter. Fixed effects with significance at $p < 0.05$ for NIRIMF were sex and date of slaughter. Heritability of both traits in the purebred population was also calculated in ASReml (Gilmour *et al*, 2015), as described in chapter 4.0, section 4.2 materials and methods. The descriptive statistics for UIMF and NIRIMF were calculated in Microsoft Excel using the standard mean, minimum, maximum and standard deviation functions. CV was calculated using the following formula:

$$CV = \frac{SD}{\mu} \times 100\% \qquad (2)$$

Where SD is standard deviation and μ is mean of the phenotypes.

Genome-wide association study (GWAS)

Using Rstudio software (RStudio team, 2018) and the RRBLUP package (Endelman, 2019), two single-SNP association analyses were performed. The following model was used:

$$\hat{\boldsymbol{y}} = \boldsymbol{Zu} + \boldsymbol{Sr} + \boldsymbol{e} \qquad (3)$$

Where $\hat{\boldsymbol{y}}$ is the vector of observations (phenotypes) adjusted for fixed effects in ASReml (see above), the only significant fixed effect was date of slaughter ($p < 0.05$). $\boldsymbol{Z}$ is the design matrix which associates random animal genetic effects to the observations; $\boldsymbol{u}$ is the vector of genetic values for all animals (random animal effects). $\boldsymbol{r}$, which models the additive SNP effect as a

fixed effect, is a vector containing the SNP genotype indicator variable coded as -1 (AA), 0 (AB), and 1 (BB). $\boldsymbol{S}$ is the design matrix corresponding to $\boldsymbol{r}$ and $\boldsymbol{e}$ is a vector of random residual effects. It is assumed that animal effects and residual effects are normally distributed as: $\boldsymbol{u} \sim N(0, \boldsymbol{G}\sigma_u^2)$ and $\boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2)$ where σ_u^2 and σ_e^2 are the genetic and residual variances, respectively. $\boldsymbol{G}$ is the genomic relationship matrix based on genotypes (Van Raden, 2008) using the following model;

$$\boldsymbol{G} = \frac{\boldsymbol{W}\acute{\boldsymbol{W}}}{2\sum p_i(1-p_i)} \qquad (4)$$

where $\boldsymbol{W}$ is the matrix of SNP genotypes corrected for expected genotype frequencies. p_i is the allele frequency at the *i*th SNP. Allele frequencies of the purebred population are used to make $\boldsymbol{G}$. Multiple testing was controlled for through the use of false discovery rate (FDR). FDR was calculated using the following formula (Van Den Oord, 2007).

$$FDR(t) = \frac{mt}{\#\{P_i \leq t\}} \qquad (5)$$

In this model, FDR is calculated by dividing the estimated number of false positives (mt) by the total number of *p*-values smaller than *t* (significant markers) ($\#\{Pi \leq t\}$). In other words, FDR is the number of false positives in all of the rejected hypotheses. Genome-wide significance level was set at 5% type 1 error and suggestive significance was declared at 10%. FDR was used to avoid false positives from multiple testing. Significant SNPs identified in the GWAS are expected to be in high linkage disequilibrium (LD) with SNPs that are located physically nearby. In the purebred Duroc pig population, LD (r^2) has been found to be 0.23-0.26 for distances up to 0.5Mbp (Badke *et al,* 2012; Veroneze *et al,* 2014). With this in mind, QTLs were defined as 1 Mbp window around any SNP identified as significant by the GWAS: 0.5 Mbp upstream and 0.5 Mbp downstream. Any loci which are further than 0.5 Mbp in this

purebred population have a low chance of being in LD with a significant SNP and are unlikely to be connected to the studied trait. The pairwise LD (r^2) between the identified significant SNPs was calculated using PLINK software (Purcell *et al*, 2007) using the following equation:

$$r^2 = \frac{D^2}{P_A(1-P_A)P_B(1-P_B)} \quad (6)$$

where *A* and *B* are two alleles, and *p* is the frequency of the alleles (A, B). D^2 is the squared result of $D_{AB} = p_{AB} - p_A p_B$ where p_A *and* p_B are the population frequencies of alleles A and B, respectively. When a population is large, mates randomly, and is not affected by selection or migration then, $p_{AB} = p_A p_B$. However, if a population is affected by any of those factors or by genetic drift then, $p_{AB} \neq p_A p_B$ (Balding *et al,* 2019). Each pair of alleles has its own *D* value (Slatkin, 2008; Song and Song, 2007). The LD was plotted using Haploview software (Barrett *et al*, 2005).

The first two principle components were plotted against one another to visualise any population stratification, all animals originated from the same farm and breeding population (Figure S5.3 in Supplementary information 5.0). Additionally, a scree plot with eigenvectors was also created. Each eigenvector corresponds to an eigenvalue; the magnitude of this eigenvalue indicates how much of the variability seen in the data will be explained by its eigenvector (Richardson, 2009). Both the scree plots and principle component analysis were done using the Factoextra package (Kassambrara and Mundt, 2020) using Rstudio software (RStudio team, 2018).

Post GWAS analysis

Any identified significant SNPs were investigated using BIOMART in Ensembl with a 1 Mbp window and subsequent identified genes within this region were searched in the Ensembl Genome Browser (https://www.ensembl.org), the National Center for Biotechnology

Information Database (NCBI) (https://www.ncbi.nlm.nih.gov), and in the pigQTL database (Hu *et al,* 2005). To obtain additional functional information, the candidate genes were also searched in the Human Gene Database (https://www.genecards.org).

Minor allele frequencies and genetic variance

Minor allele frequency was calculated using PLINK software (Purcell, 2007). SNP variances were computed based on the estimated allele substitution effects and allele frequencies as:

$$\boldsymbol{\sigma^2_{SNP} = 2pq(\alpha)^2} \qquad (7)$$

where $\boldsymbol{p}$ is the minor allele frequency and $\boldsymbol{q}$ is the major allele frequency SNP, and $\boldsymbol{\alpha}$ is the allele substitution SNP (SNP effect). Furthermore, the proportion of total phenotypic variance explained by additive genetic variance was computed as:

$$\boldsymbol{\sigma^2_g = (\sigma^2_{SNP}/\sigma^2_P) \times 100} \qquad (8)$$

where $\boldsymbol{\sigma^2_P}$ is the phenotypic variance, as calculated in ASReml.

5.3 Results and discussion

Phenotypic descriptive statistics

The mean UIMF, was 2.88% with a standard deviation of 0.75, maximum of 4.90%, and minimum of 0.7% and a coefficient of variation (CV) of 25.84%. Mean NIRIMF 2.07% with a standard deviation of 0.42, maximum of 3.84%, minimum of 0.87% and CV of 20.3%.

Principle component analysis

Figure S5.3 in Supplementary information 5.0 shows the first two principle components plotted with no outliers or stratification observed, as well as a scree plot showing eigenvalues up to ten. In order to determine the number of PCAs to retain, the scree plots were investigated. From this, one PCA was chosen to include in each GWAS, as it captured the most variation

and the inclusion of additional eigenvectors would not significantly increase variation captured.

Variance component estimation

The genetic variance of UIMF and NIRIMF in this purebred population, as calculated by ASReml at $p < 0.05$, were 0.25 ($\pm$ 0.06) and 0.07 ($\pm$0.02), with residual variance of 0.32 ($\pm$ 0.05) and 0.07 ($\pm$0.02) and estimated heritability of 0.44 $\pm$0.09 and 0.49 $\pm$ 0.11, respectively. The heritability of IMF from various collection methods, including ultrasound, proximate analysis and NIR has been widely discussed in the literature. UIMF in the purebred Duroc has previously been found to be a moderately heritable trait, reported at 0.48 (Jung *et al*, 2015). NIRIMF heritability in the purebred Duroc has been previously reported to range from 0.50 to 0.62 $\pm$ 0.06 (Gjerlaug-Enger *et al*, 2010).

GWAS 1 (for UIMF)

In GWAS 1, which used UIMF phenotypes, five significant SNPs located on SCC1 were elucidated from this investigation (Table 5.1). The Manhattan plot and quantile-quantile (Q-Q) plots showing the results from GWAS 1 are below in Figure 5.1 and 5.2, respectively.

Figure 5.1 Manhattan plot showing results of a single-SNP association analysis of IMF content measured by ultrasound (UIMF) n a Purebred Duroc population.

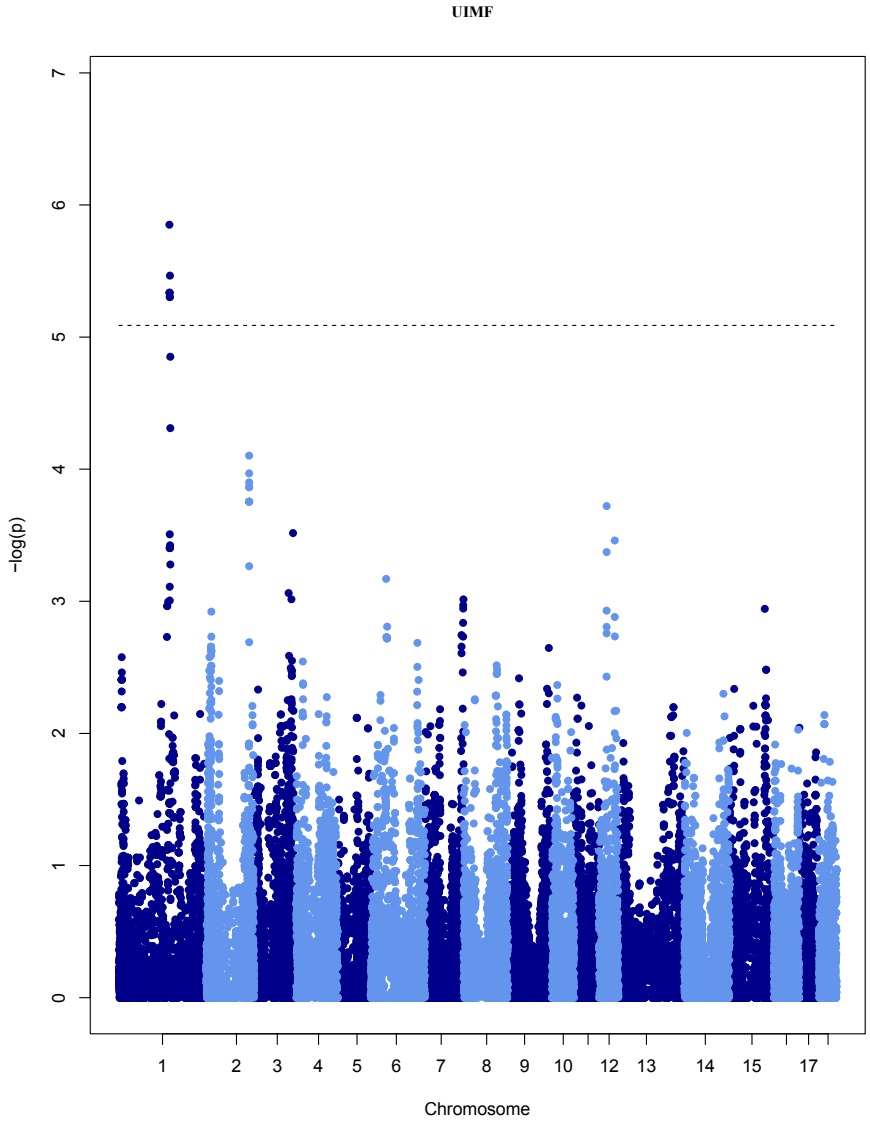


Figure 5.1. Manhattan plot associated with GWAS 1. 5 SNPs surpassed the threshold of significance of 5% denoted by the horizontal dotted line. The Manhattan plot shows the chromosome on the x-axis and the -log10 p-values on the y-axis. FDR was used to control for multiple testing.

Figure 5.2 Quantile-quantile plot showing the results of a single-SNP association analysis of IMF content measured by ultrasound (UIMF) in a Purebred Duroc population.

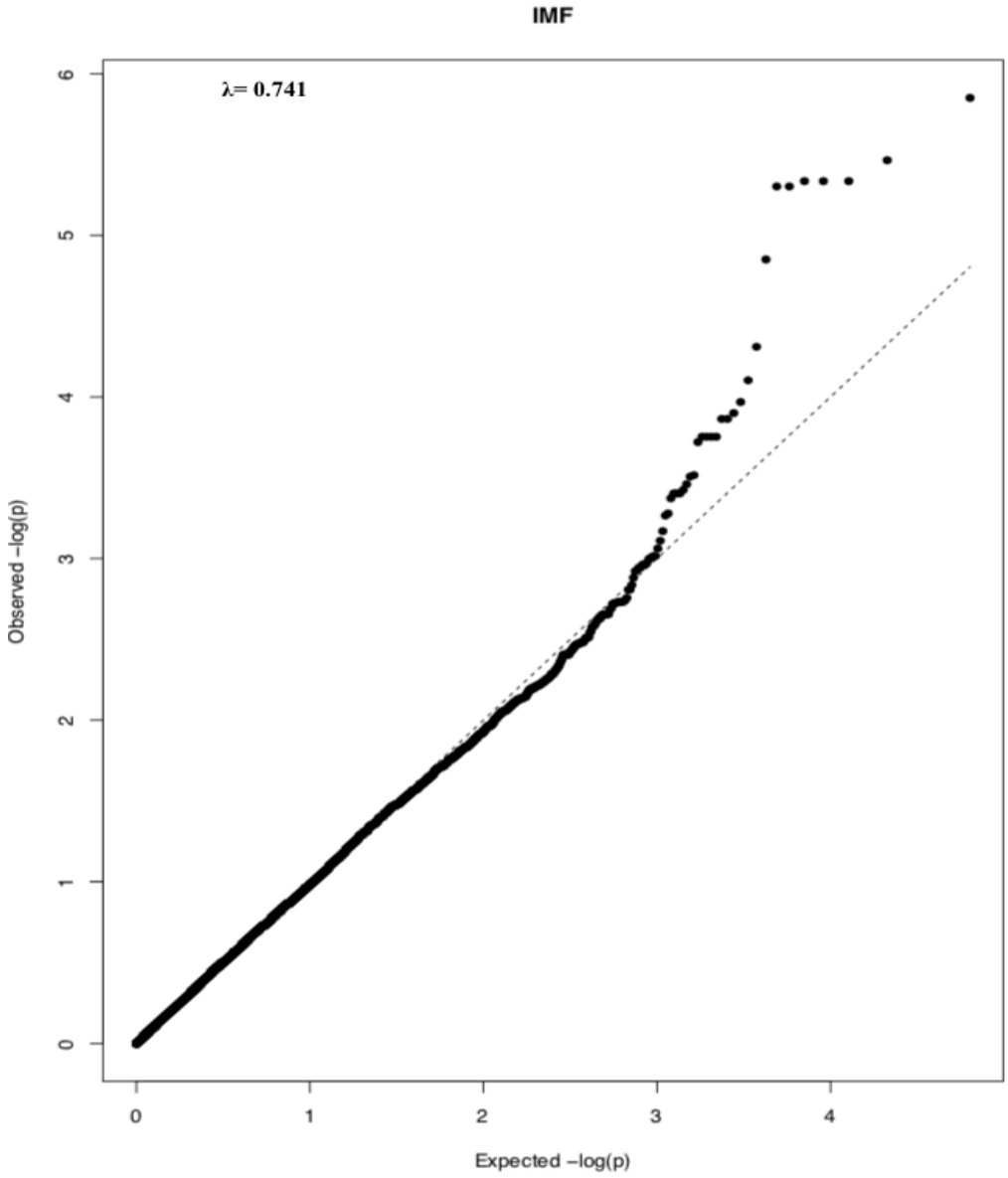


Figure 5.2 Quantile-quantile (Q-Q) plot associated with GWAS 1 (UIMF). The Q-Q plot shows the expected null distribution of -log10 (p-values) (dashed line) compared to the actual distribution (black line and dots). Genomic inflation factor ($\boldsymbol{\lambda}$) is shown on the Q-Q -plot, $\boldsymbol{\lambda = 0.741}$.

In order to investigate the relationship between the significant SNPs and those surrounding them, the pairwise LD between the significant SNPs and all SNPs within a 1 Mbp window (Table S5.1) was plotted and shown below in Figure 5.3.

Figure 5.3 Linkage disequilibrium (LD) map structure of the 5 significant SNPs found on SSC1 from a single-SNP association analysis in purebred Durocs for UIMF and all surrounding SNPs in a 1 Mbp window.

Figure 5.3 Linkage disequilibrium (LD) map structure of the 5 significant SNPs found on SSC1 from a single-SNP association analysis in purebred Durocs for UIMF content and all surrounding SNPs in a 1 Mbp window. Haploview plot shows pairwise LD (r^2) for 36 SNPs depicted by block colour. White blocks indicate r^2 = low LD grey indicates r^2 = moderate LD and black indicates r^2 = high LD. The numbers inside the blocks are the LD measurements (r^2) on a scale of 0 to 100%. The five significant SNPs in this plot are underlined in yellow. They are numbers 10 (MARC0075909), 12 (ALGA0006602), 14 (H3GA0003104), 26 (ASGA0004988), and 33 (12784636). The other 31 SNPS are those within a 1 Mbp window surrounding the significant SNPS, 0.5 Mbp each side.

Table 5.0 Summary of pairwise LD for the 5 significant SNPs found on SSC1 from a single-SNP association analysis in purebred Durocs for UIMF and all surrounding SNPs in a 1 Mbp window.

Significant SNPs	Adjacent SNP	LD
Block 2		
10; **MARC0075909**	8, H3GA0003096	$r^2 = 0.474$
	9, ASGA0004978	$r^2 = 0.352$
	11, ASGA0004980	$r^2 = 0.958$
	12, ALGA0006602	$r^2 = 0.958$
12; ALGA0006602	**10, MARC0075909**	$r^2 = 0.958$
	11, ASGA0004980	$r^2 = 1.0$
	13, H3GA0003111	$r^2 = 0.337$
	14, H3GA0003104	$r^2 = 0.337$
14; H3GA0003104	**12; ALGA0006602**	$r^2 = 0.337$
	13, H3GA0003111	$r^2 = 1.0$
	15, ALGA0006599	$r^2 = 1.0$
	16, WU_10.2_1_177201808	$r^2 = 1.0$
Block 3		
26; ASGA0004988	24, ASGA0004992	$r^2 = 0.405$
	25, ASGA0004994	$r^2 = 0.405$
	27, ALGA0006621	$r^2 = 1.0$
	28, INRA0004954	$r^2 = 0.405$
Block 4		
33; 12784636	31, INRA0004955	$r^2 = 1.0$
	32, WU_10.2_1_178486722	$r^2 = 1.0$
	34, INRA0004964	$r^2 = 0.083$
	35, ALGA0006632	$r^2 = 0.075$

Significant SNPs identified by the single-SNP association analysis for UIMF are bolded

GWAS' exploits the LD between SNPs and ungenotyped causal mutations, therefore, we rely on LD information to determine if a SNP is truly explaining a large part of the trait variation. With this, it is understood that SNPs which are in high LD are truly informative SNPs affecting the trait/phenotype, such as those identified in this study. Informative SNPs may aide in improving the accuracy of genomic prediction, if they are fit into genomic selection models. By investigating the entire 1 Mbp regions surrounding the identified significant SNPs, we are able to narrow down a subregion which could be further investigated for a detailed analysis (LD mapping). Any causal mutations or causal loci are most likely where the pairwise LD for adjacent marker peaks (Dorak, 2016). Though this investigation is

beyond the scope of this study, the initial investigation done by plotting the LD of these SNPs (Figure 5.3) has identified three areas of interest. There are a number of additional regions which see strong LD, but this study will focus on those containing the significant SNPs identified by the GWAS. Table 5.0 contains the summary of pairwise LD analysis for the 5 significant SNPs identified in these three areas (block 2, block 3, block 4).

Block 2 (Figure 5.3) contains three of the five significant SNPs identified by single-SNP analysis (MARC0075909, ALGA0006602 and H3GA0003104). SNPs MARC0075909 and ALGA0006602 are in perfect LD with one another ($r^2 = 1$) and are in high LD with their adjacent SNPs ($0 < r^2 < 1$). Perfect LD ($r^2 = 1$) occurs if two SNPs have the same allele frequencies and have not been separated by recombination. SNP H3GA0003104 is in perfect LD with its two adjacent SNPs and is in high LD with the two aforementioned significant SNPs. Block 2, all 15 SNPs, has no linkage equilibrium[12] (LE) seen; based on the high level of LD with surrounding SNPs, it is possible that a causal mutation associated with higher UIMF is present in this subregion as the closer a SNP is to a causal variant, the stronger the LD will be between them. Additionally, block 3 (Figure 5.3) which contains significant SNP ASGA0004988 and nine adjacent SNPs may also be a subregion of interest based on the strong LD noted, many in perfect LD or in strong LD (>0.90) with one another. Finally, block 4, containing 3 SNPs, one of which is 12784636, are in perfect LD with one another. All three of these regions warrant further investigation for a causal variant though LD mapping; this is done by screening this area for additional polymorphisms and sequencing to identify a causal SNP (Dorak, 2016). The pairwise SNPs which have longer distances between them generally

[12] Two or more alleles are said to be in linkage equilibrium when they occur randomly in a population; $r^2 = 0$, the alleles are in LE (Slatkin, 2008).

are closer to LE (r^2=0) than those which are closer together – this is shown to be generally accurate in this study. All SNPs were quite physically close (Table 5.1, Table 5.2) and by investigating in a 1 Mbp window around significant SNPs, many SNPs in chronological order upstream and downstream were included. In fact, four of the five SNPS with the lowest *p*-values from GWAS 2 were included in this LD investigation as two were identified as significant in GWAS 1, (12784636 and MARC0075909) and two were within the 1Mbp window of the significant SNPS (WU_10.2_1_178188861 and ALGA0006623) (Table S5.1, Supplementary information 5.0). The final SNP with the lowest *p*-value from GWAS 2 (MARC0013872) was approximately 0.5 Mbp upstream from the window of SNP 12784636 (1.602 – 1.612) and was subsequently not included in the LD investigation. .

Table 5.1 Identified significant SNPs from single-marker association analyses performed in RRBLUP package for UIMF content in a purebred Duroc pig population.

Name	**Position** (SSC (chromosome), Genomic position in bp)	**Investigation window**	**-Log10 *p*- value**	**Minor allele frequency (MAF)**	**Genetic Variance explained (% of phenotypic variance)**
MARC0075909	SSC1, 159238083	1.587 - 1.597	5.877	0.097	0.91
12784636	SSC1, 160773437	1.602 –1.612	5.591	0.210	1.55
ALGA0006602	SSC1, 159538854	1.590 -1.600	5.477	0.363	2.15
H3GA0003104	SSC1, 159619891	1.591 –1.601	5.477	0.1762	1.36
ASGA0004988	SSC1, 159881634	1.593 –1.603	5.477	0.147	1.16

The SNPs are all physically quite close together, and as such, the 1 Mbp investigation windows of all SNPs overlapped slightly. The windows of SNPs MARC0075909, ALGA0006602, H3GA0003104 and ASGA0004988 overlapped quite notably (Table 5.1), as they are physically the closest. For investigation of the regions around the SNPs, the

exploration windows were not changed due to the SNPs as LD (r^2) has been previously found to be 0.23-0.26 for distances up to 0.5 Mbp in Duroc populations (Badke *et al,* 2012; Veroneze *et al,* 2014). For discussion purposes, any identified genes in the 1 Mbp window of a SNP will be discussed once, regardless of the number of windows a SNP was identified in**.** In general, our findings in this subset of SNPs in this region appear to have stronger LD than this at larger distances. It is unlikely that this is due to the small population, as r^2 does not inflate due to small sample size (Dorak, 2016). The strong LD noted in this region may indicate that not very much time has elapsed since a potential causative mutation has arisen (Slatkin, 2008). In order to confidently identify a new mutation, this entire region should be sequenced in animals with the apparent mutation and subsequently compared to the genome assembly for this region; other statistical methods for fine mapping also exist (Hormozdiari, 2014). This warrants further study.

GWAS 2 (for NIRIMF)

In GWAS 2, which used NIRIMF phenotypes, no significant SNPs were identified. For the sake of investigation, the top 5 SNPs from the Manhattan plot with the lowest *p*-values were investigated in order to compare to the results from GWAS 1, these are presented in Table 5.2. The Manhattan plot and quantile-quantile plot (Q-Q) showing the results of this study are presented in Figure S5.4 and Figure S5.5 in Supplementary information 5.0

Table 5.2 SNPs with lowest *p*-values from single-marker association analyses performed in RRBLUP package for NIRIMF content in a purebred Duroc pig population.

Name	Position (SCC (chromosome), Genomic position in bp)	Investigation window	-Log10 *p*- value
*12784636	SSC1, 160773437	1.602 – 1.612	5.318
ALGA0006623**	SSC1, 160347188	1.598 - 1.608	5.226
WU_10.2_1_178188861**	SSC1, 160447734	1.599 – 1.609	5.226
*MARC0075909	SSC1, 159238083	1.587- 1.597	5.169
MARC0013872	SSC1, 161824864	1.613 – 1.623	5.001

*: Indicates a SNP which was identified as statistically significant in GWAS 1 with UIMF phenotypes.
**: Indicates a SNP which was included in the LD investigation for GWAS 1 based on its physical location.

The *p*-values seen for the (non-significant SNPs) in Table 5.2 are very close to those shown Table 5.1 (significant SNPs), showing similar results between the two phenotypes. The method of collection for NIRIMF and UIMF are quite different (section 5.2, Material and methods, phenotypes), however, it believed that all measurements of traits have some inherent errors and that this minor imprecision will not significantly affect the results of a GWAS.

The genetic and phenotypic correlations for NIRIMF x UIMF, investigated in Chapter 4, were 0.775 ($\pm$0.085) and 0.504 ($\pm$0.026) Chapter 4, section 4.3 Results and discussion); based on these estimates it is believed that NIRIMF and UIMF would be suitable substitutes for one another. Additionally, the correlation coefficient obtained for NIRIMF x UIMF was 0.504 (Chapter 4, section 4.3 Results and discussion), which is a moderate positive linear correlation; this indicates that these two variables are directly related, and have a moderate association to one another (Mukaka, 2012). Although the results were similar, they were not identical; it is possible that the quality of the NIRIMF phenotypes was marginally lower than

the UIMF based on the observed results as high-quality phenotypes are important to ensure accuracy of a GWAS (Barendse, 2011). The algorithm used to predict UIMF from the live animals may have been more accurate than the one used to predict NIRIMF from the ground samples; it is possible there was less measurement error associated with the UIMF phenotypes as compared to the NIRIMF phenotypes based on the results of the GWAS.

QTL investigation

Table S5.2 in Supplementary information 5.0 contains the exhaustive list of identified genes in all investigation windows for GWAS 1. Genes were identified through investigation of a 1Mbp window around each SNP (Tables 5.1 and 5.2) and subsequently searching on the Pig QTL database, the National Center for Biotechnology Information Database (NCBI) and the Ensembl genome browser. Table S5.3 in the Supplementary information 5.0 contains identified genes as from the five SNPs from GWAS 2 with the lowest *p*-values. Genes identified in the regions from GWAS 2 will not be referred to as candidate genes, as the SNPs were not found to be statistically significant, however, based on the results of the LD investigation and their physical proximity, their investigation may offer some insight into the architecture of the trait.

Eight unique candidate genes were identified in the SNP windows from GWAS 1; five of them have been identified in previous studies with relationships to fatness and feeding behaviour in pigs. Of the most interest, two genes, *RNF152* and *PMAIP1*, have been previously identified as correlated with increased IMF (Rothschild *et al*, 2014; Silva *et al*, 2019). *RNF152* was identified in the investigation windows of SNP MARC0075909 (1.587-1.597 Mbp), ALGA0006602 (1.590-1.600 Mbp), H3GA0003104 (1.591-1.601 Mbp) and ASGA0004988 (1.593-1.603 Mbp), and *PMAIP1* was only noted in the window of SNP 12784636. A study by Rothschild *et al.* (2014), which utilised purebred Durocs, identified a region on SSC1 which

contained *RNF152* and *PMAIP1,* among other genes not identified in this study. This region also contains the gene *MC4R,* located at 36 428 488 bp downstream of SNP MARC0075909's window; *MC4R* was identified in the SNP windows of ALGA0006623 (1.598-1.608 Mbp) and WU_10.2_1_178188861 (1.587-1.597 Mbp) from GWAS 2. Interestingly, both of these SNPs were included in the LD investigation as SNP 29 and 30; both were found to be in high LD (99%) with SNP MARC0075909. *MC4R* has been identified as associated with increased IMF in Duroc pigs; it plays a large role in the regulation of food intake, energy balance and body weight in mammals (Wang *et al*, 2013; Rothschild *et al*, 2014; Silva *et al*, 2019). Based on the polygenic nature of IMF, it is understood that no single gene explains a major amount of the phenotypic variance seen, and therefore the variance is attributed to the interaction of many genes. It is thought that this entire region on SSC1 is associated with increased IMF content, including this study's identified genes of *RNF152* and *PMAIP1*. This is supported by the strong LD noted in the region surrounding SNP MARC0075909 (Figure 5.3). *RNF152* and *PMAIP1* were again identified by Silva *et al.* (2019) which investigated feed-efficiency related traits in crossbred pigs. It is promising that these candidate genes have been identified in the current purebred study as well as in a crossbred GWAS by Silva *et al*. (2019). This could indicate that the genetic efforts to improve IMF in the purebred breeding stock through fixing of relevant alleles may be being translated to the commercial progeny. Additionally, the identification of SNPs with a relationship to higher IMF in crossbreds can be helpful for further improving accuracy of GS in the purebred animals. This is more thoroughly discussed in Chapter 6, however, briefly, it has been shown that using crossbred information (genomic information most favourably) in combination with purebred information will improve the accuracy of selection (Wei and Van der Steen, 1991; Xiang *et al*, 2017; Sewell *et al*, 2018, Sevillano *et al*, 2019).

Average daily gain (ADG) and average feed intake (AFI) were the two traits noted to be associated with *RNF152* and *PMAIP1.* As discussed in Chapter 3.0, the dietary manipulation of IMF has been explored, as diet can play a significant role in the development of fat depots, including IMF (Hocquette *et al*, 2010). Increased ADG coupled with increased caloric consumption due to higher AFI may lead to IMF being deposited at earlier in the growth and finishing phase and result in increased amounts of this fat depot at slaughter. IMF is a late maturing fat depot, and therefore if other, earlier maturing fat depots have been deposited due to caloric excess from ADG and AFI, it may provide the opportunity for IMF to begin maturation earlier. *TNFRSA11a* and *PIGN,* which are in the same region on SSC1, only 0.130 Mbp from one another*,* have been identified in feeding behaviour studies, particularly with increased daily feed intake and daily feeder occupation time (Reyer *et al*, 2017). Increased daily intake and longer occupation of the feeder discussed by Reyer *et al.* (2017) coincide nicely with the feed-efficiency traits discussed by Silva *et al.* (2019) of ADG and AFI. Additionally, in a previous GWAS conducted by Kogelman *et al.* (2017) using a purpose bred F2 pig population (Duroc × Göttingen Minipig and Yorkshire × Göttingen Minipig) investigated the genetic underpinnings of obesity and lean mass percentage. This study identified a number of significant SNPs which mapped to the vicinity of *CDH20 (*SSC1, 1.59-1.60 Mbp). A separate GWAS by Reyer *et al.* (2017), which also investigated obesity-related genes in pigs (Pietrain), found that their most significant SNP, ASGA0004992 (SSC1, 1.602 Mbp), mapped to a region near *CDH20* and the previously discussed *MC4R (*SSC1, 1.60 - 1.60 Mbp). This SNP, though not identified as significant in the current study, was included in the LD investigation (SNP 24, Table S5.1) and was found to be in moderate LD with identified significant SNP ASGA0004988 (dark grey, $0 < r^2 < 1$, $r^2 = 0.405$) (Table 5.0, Figure 5.3). Additionally, *CHD20* was identified in in the SNP windows of ALGA0006623 (1.598 - 1.608

Mbp) and WU_10.2_1_178188861 (1.587-1.597 Mbp) from GWAS 2. The region containing gene *CDH20* and significant SNP ASGA0004988 overlaps with the region identified for lean mass percentage in pigs from Kogelman *et al.* (2017), and although our study did not identify *MC4R* from a significant SNP investigation window, these are still encouraging results. Obesity in pigs, however, is slightly more difficult to investigate as in commercial hog barns, ad libitum feeding and subsequent consumption to the point of obesity is not realistic, as feed comprises approximately 70% of costs associated with growing pigs; this makes any fat deposition expensive (Martinsen *et al,* 2015). It is possible that animals with causative mutations in the same region which *CDH20* is mapped to may be more likely to consume more than their allotted share of feed, making them calorically able to deposit an excess of fat. Increased subcutaneous fat, also known as increased backfat thickness, often associated with obesity, is positively correlated to increased IMF (r = 0.33-0.49) in pigs (Bahelka *et al*, 2007; Jacyno *et al*, 2015). Additionally, Schwab *et al* (2009) showed that the long-term selection for IMF in Duroc pigs resulted in increased backfat thickness. Finally, though *TNFRSA11a*, *PIGN* and *CDH2O* have not been specifically identified as associated with increased IMF in the literature, they are in close physical proximity to *RNF152* and *PMAIP1* and have been linked to feeding behaviours. These connections have not been studied or confirmed but do warrant further investigation.

The three remaining genes identified in the 1 Mbp windows of the significant SNPs from GWAS 1, *RELCH*, *GAD1* and *ZCCHC2*, have not been found in the literature with relationships to fatness or related traits in pigs. The investigation window of SNP MARC0013872 (1.613 - 1.623 Mbp) from GWAS 2 contained nine genes (Table S5.3, Supplementary information 5.0), one of which (*LMAN1*) had been previously identified with a

connection to fatness and feeding behaviour in pigs. *LMAN1* was identified to have association daily feed intake and daily feeder occupation time in a study by Reyer *et al.* (2017).

With further investigation, the identified region on SSC1 would contribute to the overall objective of this study through increased understanding of the genetic architecture of IMF. The incorporation of this biological information into GS programs will aide in the independent manipulation of IMF from BFD.

Genetic variance

The proportion of total phenotypic variance explained by SNP variance of the 3 investigated SNPs was 7.13% (Table 5.1). This is comparable to other studies which have identified significant SNPs for fat deposition and fat-related traits in pigs. Reyer *et al.* (2017) identified a number of SNPs in their GWAS investigating obesity-related genes in pigs using Maxgro terminal boars (primarily Pietrain) which are within the 1 Mbp windows of a number of significant SNPs identified in this study. In fact, these were included in the LD investigation. From the GWAS performed by Reyer *et al.* (2017) SNPs ASGA0004992 (SSC1, located at 1.602 Mbp), INRA0004895 (SSC1, located at 1.588 Mbp), ALGA0006623 (SSC 1, located at 1.603 Mbp), ALGA0006621 (SSC1, located at 1.603 Mbp), INRA0004955 (SSC 1, located at 1.605 Mbp) were identified as significant and explained 0.89%, 3.64%, 2.55%, 1.44% and 0.86%, respectively, of the genetic variance.

Limitations

Although we were able to identify 5 significant SNPs and a number of potentially meaningful genes from these GWAS and subsequent investigation, some limitations exist, and improvements which should be implemented for future studies. Primarily, the sample size of 891 animals is low for a genetic study. A larger sample size can aide with the detection of SNPs with small effects and they may be more likely to exceed the stringent threshold and the

statistical power associated with increasing the sample size is significant (Visscher *et al*, 2012). It was shown that to obtain 80% statistical power when investigating human disease, which is the recommended level to avoid false negative associations that 248 cases were required to test a single SNP (Hong and Park, 2012). Of course, the model assumptions when studying a quantitative trait such as IMF are different, but the understanding that increasing sample size helps to increase power is consistent (Hong and Park, 2012). Additionally, we utilised a 50K panel for genotyping followed by imputation in order to obtain a higher density marker map. It is possible that with this lower sample size and the high number of markers that the 'big n little p' problem ($n << p$) affected the analysis. This disproportionate increase in the number of variants in the GWAS compared to the smaller number of phenotypes may have deteriorated the statistical power (Chang *et al*, 2018); the causal mutations are estimated with error, and the larger effect of causal mutations may be then distributed over many SNPs (Heidaritabar, 2016, PhD thesis). However, proper variant filtration may help with removing the false positive or uninformative SNPs. Finally, it is generally thought that small differences in the measurement of a trait will not significantly affect the results of a GWAS as differences between samples or between methodologies will not affect the location of significant associations (Barendse, 2011). However, in a 2011 study by Barendse, it was found that measuring subcutaneous fat on cattle carcasses with two methods did alter the outcome of the GWAS performed; there was an overlap in the significant SNPs and the chromosomes with the largest number of significant SNPs were not the same. In the present study, the identification of two SNPs in both GWAS 1 and 2 (12784636 and MARC0075909, significant in GWAS 1, non-significant in GWAS 2), it is evident that the method of phenotype collection impacted the results of the study. This shows that a GWAS may be sensitive to differences when mapping quantitative traits and as such, increased effort should be taken to improve the precision of trait measurement (Barendse,

2011). For future studies, additional measures can be taken to ensure the accuracy of the GWAS. This includes examination of phenotype collection methodology for repeatability prior to the GWAS by independent collectors (ideal repeatability is $r \geq 0.95$) and that animals to used (n) in the GWAS only be genotyped and included if their phenotype is accurately measured (Barendse, 2011).

5.4 Conclusions

In this study, two genome-wide single-SNP association analyses were performed in order to elucidate SNPs associated with IMF content in a purebred Duroc population. The results of the first GWAS with UIMF phenotypes identified 5 significant SNPs in a region with multiple candidate genes. A number of genes in this region have been previously found to be associated with increased IMF. The candidate genes of *RNF152, PMAIP1, TNFRSA11a, PIGN,* and *CDH2O,* all found within 0.5 Mbp of the 5 significant SNPs on SSC1, have previously been associated with IMF, fatness, and feed-efficiency related traits in pigs. GWAS 2, with NIRIMF phenotypes, did not reveal any significant SNPs, however, five SNPs with the lowest *p*-values were investigated. These revealed two SNPs common to both GWAS 1 and 2 and, additionally, the genes *MC4R* and *CHD20,* previously identified as being associated with increased IMF, were within 1 Mbp windows of two other SNPs (0.5 Mbp downstream and upstream). These results, consistent with the current literature, help to build upon this body of evidence and can be used within breeding programs in order to improve IMF in the purebred animal, and subsequently the performance of the crossbred; we are able to accept the hypothesis based on the identification of five statistically significant SNPs which explain 7.13% of the total phenotypic variance seen in IMF. Interestingly, the method of phenotype collection used gave similar, but still different results, which is an important factor to consider

in futures studies. In order to better understand the genetic underpinnings of IMF and its expression and to improve selection accuracy the study of crossbreds in addition to the purebreds is important. In the next Chapter we will focus on commercial crossbred animals with UIMF phenotypes, as these were shown to have less measurement error.

Supplementary information 5.0

Figure S5.1

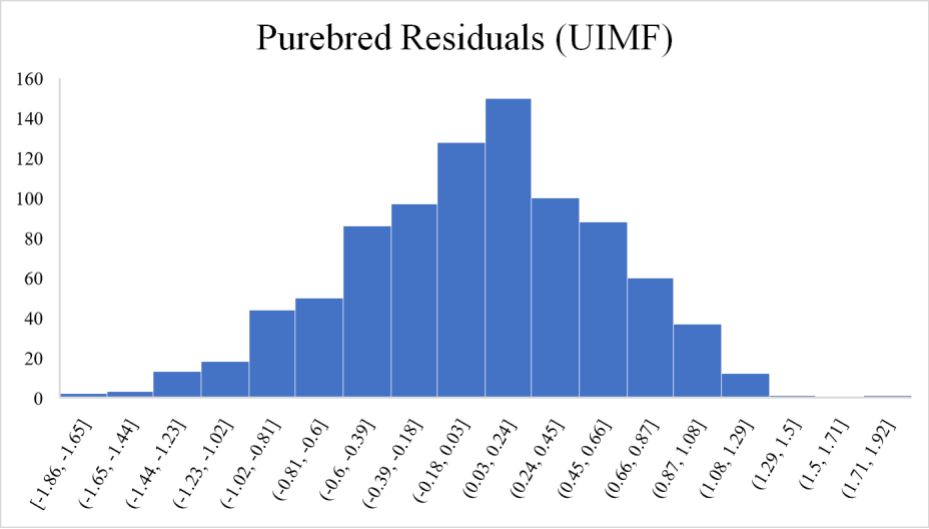


Figure S5.1. Distribution of ultrasound IMF phenotypes which have been corrected using ASReml. Residuals (corrected phenotypes) follow an approximately normal distribution.

Figure S5.2

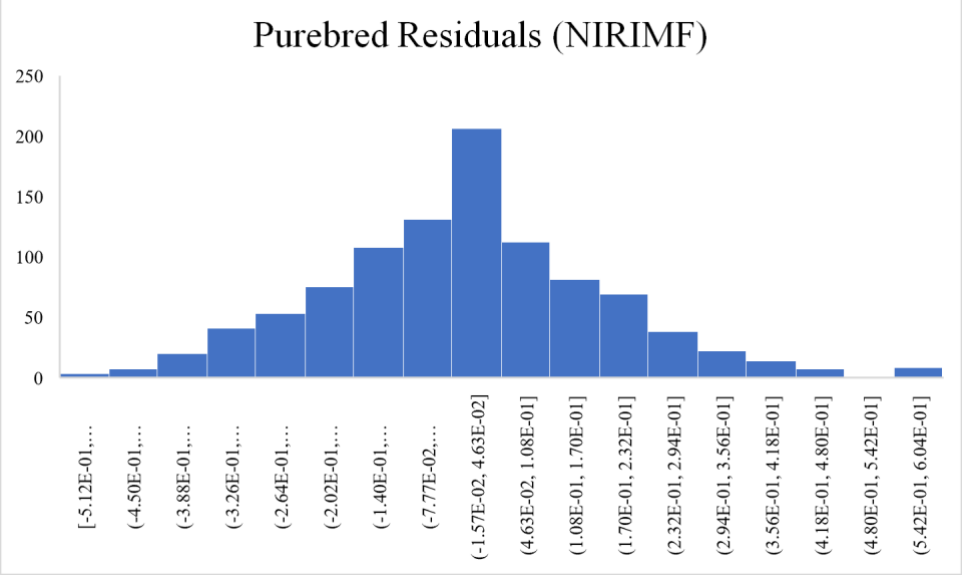
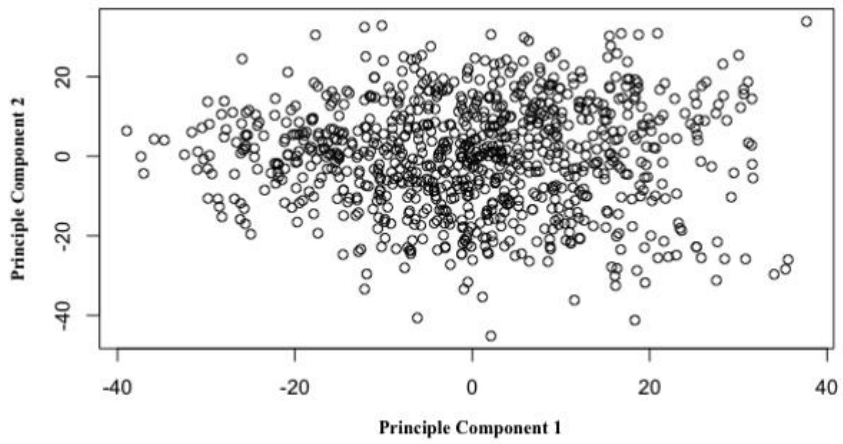


Figure S5.2. Distribution of NIRIMF phenotypes which have been corrected using ASReml. Residuals (corrected phenotypes) follow an approximately normal distribution.

Figure S5.3



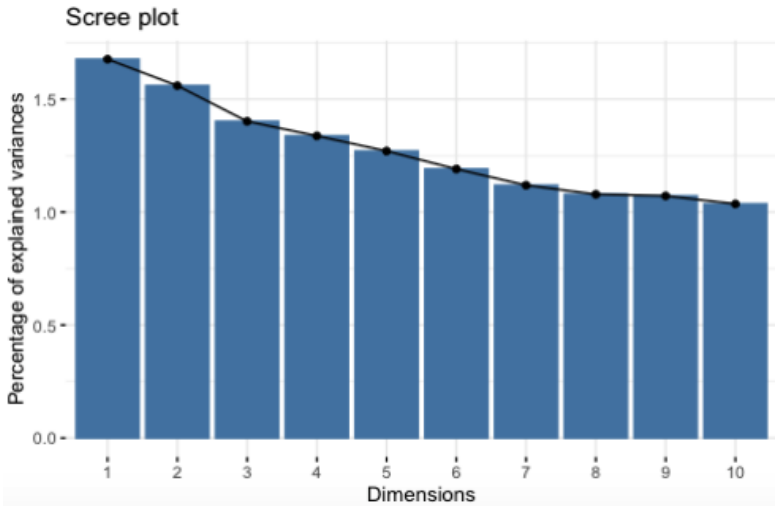


Figure S5.3. The top figure shows the principle component analysis (PCA) of SNP genotypes in a purebred Duroc pig population. Principle components 1 and 2 were plotted against one another to visualize potential subpopulations (population stratification); none were noted. The scree plot (lower) shows the eigenvalues and the amount for variation each one accounts for. To determine the number of principle components which should be retained in a study, we choose the number of eigenvectors which captures the most amount of variance. From this, 1 PCA was chosen to include in the study, as it captured the most variation and additional eigenvalues would not significantly add to variation captured.

Table S5.1 Genomic positions of significant SNPs identified from a single-SNP association analysis for UIMF content in purebred Durocs and surrounding SNPs within a 1 Mbp window used for linkage disequilibrium (LD) analysis.

SNP number in Haploview LD plot	SNP Name[1]	SSC	Genomic position (Mbp)
1	ALGA0006564	1	1.584
2	ALGA0006572	1	1.586
3	ASGA0004970	1	1.586
4	ASGA0004971	1	1.586
5	MARC0034873	1	1.587
6	INRA0004895	1	1.588
7	INRA0004898	1	1.588
8	H3GA0003096	1	1.588
9	ASGA0004978	1	1.589
10	**MARC0075909**	**1**	**1.592**
11	ASGA0004980	1	1.595
12	**ALGA0006602**	**1**	**1.595**
13	H3GA0003111	1	1.596
14	**H3GA0003104**	**1**	**1.596**
15	ALGA0006599	1	1.597
16	WU_10.2_1_177201808	1	1.597
17	ASGA0101718	1	1.598
18	DIAS0000206	1	1.598
19	H3GA0003114	1	1.599
20	ASGA0106369	1	1.599
21	ASGA0004988	1	1.599
22	ASGA0004989	1	1.600
23	WU_10.2_1_177662261	1	1.600
24	ASGA0004992	1	1.602
25	ASGA0004994	1	1.602
26	**ASGA0004998**	**1**	**1.603**
27	ALGA0006621	1	1.603
28	INRA0004954	1	1.603
29	ALGA0006623	1	1.603
30	WU_10.2_1_178188861	1	1.604
31	INRA0004955	1	1.605
32	WU_10.2_1_178486722	1	1.607

33	**12784636**	**1**	**1.608**
34	INRA0004964	1	1.609
35	ALGA0006632	1	1.611
36	MARC0056620	1	1.612

[1]Significant SNPs from single-SNP association analysis (GWAS 1) are presented in bold text (SNP#s 10, 12, 14, 26, 33)

Figure S5.4

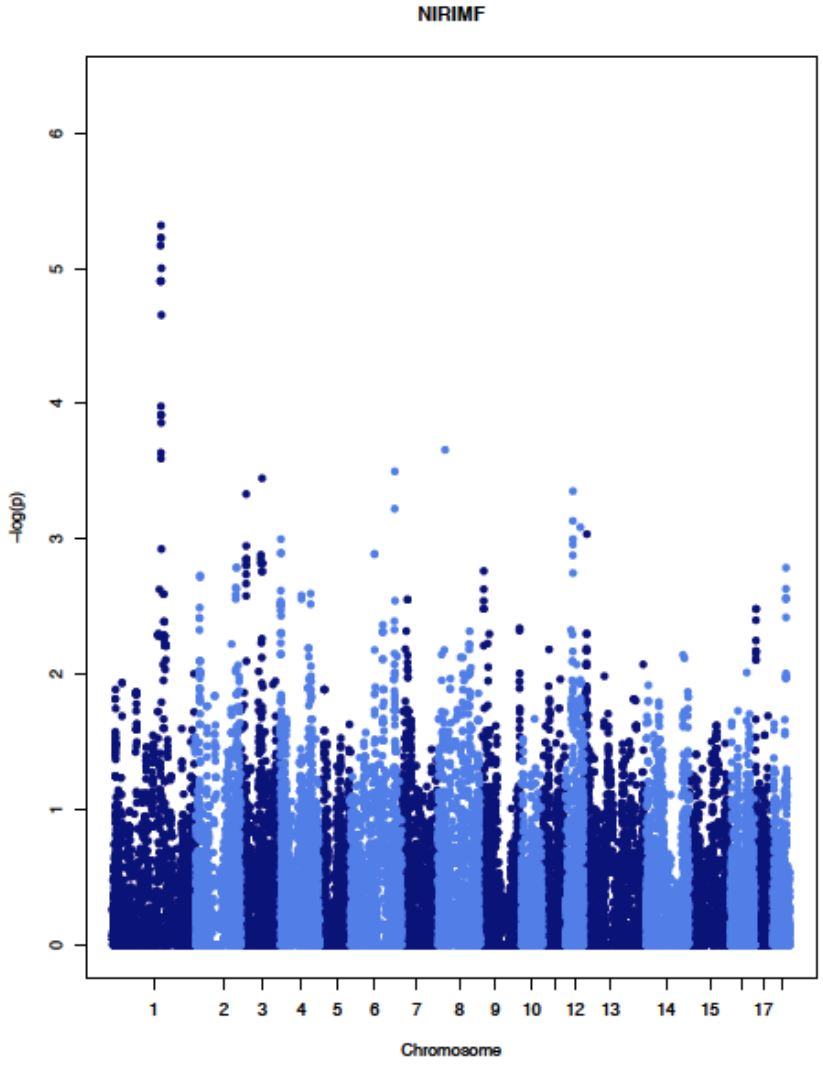


Figure S5.4. Manhattan plot associated with GWAS 2. No SNPs surpassed the threshold of significance of 5%. The Manhattan plot shows the chromosome on the x-axis and the -log10 p-values on the y-axis.

Figure S5.5

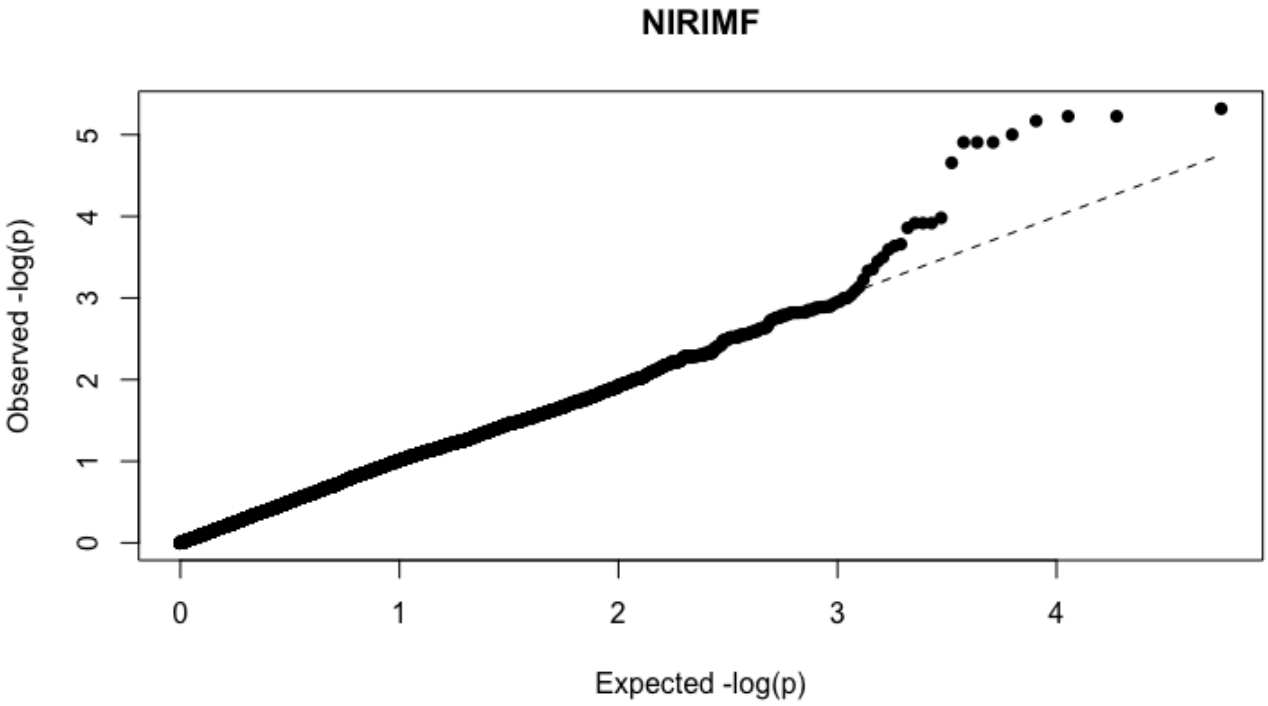


Figure S5.5 Quantile -quantile (Q-Q) plot associated with GWAS 2. The Q-Q plot shows the expected null distribution of -log10 (p-values) (dashed line) compared to the actual distribution (black line, dots). Genomic inflation factor (λ) is shown on the Q-Q -plot, $\lambda = 0.73$.

Table S5.2 Genes identified in a 1 Mbp investigation window surrounding significant SNPs elucidated from a single-SNP association analysis for UIMF content in a purebred Duroc pig population.

[1]SNP Position (chromosome, genomic position in bp)	SNP ID	RefSNP ID[2]	Gene[3]	Gene function	Reference[4]	Gene position	Gene Distance from identified SNP
SSC1: 159238083	MARC0075909	rs80820997	*ZCCHC2*	Protein coding gene, annotations related include nucleic acid binding and phosphatidylinositol binding. Associated with increased boar taint in Yorkshire boars (*p <0.05)*	Leung *et al*, 2010.	1.589-1.590	starts 275572 bp downstream
			*TNFRSF11a****	Protein coding gene, protein encoded is a member of TNF-receptor superfamily. Involved of during the activation of NFKB during the initiation of uterine receptivity during the estrous cycle and early pregnancy in pigs. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Ross *et al* 2010., Reyer *et al*, 2017	1.591-1.591	Starts 110045 bp downstream
			*RELCH***	RELCH promotes non-vesicular cholesterol transport from recycling endosomes to the trans-Golgi network through membrane tethering.	Sobajima *et al*, 2018.	1.592-1.593	Starts 30467 bp downstream
			*PIGN****	This gene encodes a protein that is involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis; the GPI-anchor is a	Alessandri *et al* 2018; Reyer *et al*, 2017	1.593-1.594	Starts 79651 bp upstream

				glycolipid found on blood cells and anchors proteins to the cell surface. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.			
			*RNF152***	Regulates mTORC1 signaling and plays a role in the cellular response to amino acid availability. This gene has been previously identified to be correlated with increased intramuscular fat in pigs.	Silva *et al*, 2019; Rothschild *et al*, 2014.	1.595-1.596	Starts 292540 bp upstream
SSC1, 160773437	12784636	Not identified in Ensembl BIOMART. Window was viewed based on position.	*GAD1- like*	Protein coding gene which produced an uncharacterised transcript. Not identified in literature. GAD1 in pigs is found on SSC15. In humans, GAD1 encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes.	NCIB Database, *GAD1, Homo sapiens,* 2020.	1.606-1.607	Starts 144217 bp downstream
			PMAIP1 *** *(* Synonym: *TN3)*	Promotes activation of caspases and apoptosis. Promotes mitochondrial membrane changes and efflux of apoptogenic proteins from the mitochondria. Competes with BAK1 for binding to MCL1 and can displace BAK1 from its binding site on MCL1, by similarity. This gene has been previously identified to be correlated with increased intramuscular fat in pigs.	Silva *et al*, 2019 ; Rothschild *et al*, 2014	1.611-1.611	Starts 409431 bp upstream

SSC1: 159538854	ALGA0006602	rs80854621	*TNFRSF11a****	Protein coding gene, protein encoded is a member of TNF-receptor superfamily. Involved of during the activation of NFKB during the initiation of uterine receptivity during the estrous cycle and early pregnancy in pigs. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Ross *et al* 2010., Reyer *et al*, 2017	1.591-1.591	Starts 410816 bp downstream

	*RELCH***	RELCH promotes non-vesicular cholesterol transport from recycling endosomes to the trans-Golgi network through membrane tethering.	Sobajima *et al*, 2018	1.592-1.593	Starts 331238 bp downstream
	*PIGN****	This gene encodes a protein that is involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis; the GPI-anchor is a glycolipid found on blood cells and anchors proteins to the cell surface. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Alessandri *et al* 2018; Reyer *et al*, 2017	1.593-1.594	Starts 221120 bp downstream
	*RNF152****	Regulates mTORC1 signaling and plays a role in the cellular response to amino acid availability. This gene has been previously identified to be correlated with increased intramuscular fat in pigs.	Silva *et al*, 2019; Rothschild *et al*, 2014.	1.595-1.596	Starts 8231 bp downstream

			*CDH20****	Protein coding gene. encoded membrane protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats cadherins are considered prime candidates for tumor suppressor genes. Has been shown to in a genomic region with associations to variation in growth rates and lean mass percentage of pig. SNPs identified as highly significant in previous GWAS for obesity in pigs mapped to the vicinity of CDH20.	Reyer *et al*, 2017, Kogelman *et al,* 2014.	1.598-1.600	Starts 278703 bp upstream
SSC1: 159619891	H3GA0003104	rs80861000	*TNFRSF11A**** .	Protein coding gene, protein encoded is a member of TNF-receptor superfamily. Involved of during the activation of NFKB during the initiation of uterine receptivity during the estrous cycle and early pregnancy in pigs. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Ross *et al* 2010, Reyer *et al*, 2017.	1.591-1.591	Starts 491853 bp downstream

	*RELCH***	RELCH promotes non-vesicular cholesterol transport from recycling endosomes to the trans-Golgi network through membrane tethering.	Sobajima *et al*, 2018	159,207,616-159,317,641	Starts 412275 bp downstream
	*PIGN****	This gene encodes a protein that is involved in glycosylphosphatidylinositol (GPI)-anchor biosynthesis; the GPI-anchor is a glycolipid found on blood cells and anchors proteins to the cell surface. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Alessandri *et al* 2018; Reyer *et al*, 2017	159,317,734-159,417,333	Starts 302157 bp downstream
	*RNF152****	Regulates mTORC1 signaling and plays a role in the cellular response to amino acid availability. This gene has been previously identified to be correlated with increased intramuscular fat in pigs.	Silva *et al*, 2019; Rothschild *et al*, 2014.	159,530,623-159,601,981	Starts 89268 bp downstream

			*CDH20****	Protein coding gene. encoded membrane protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats cadherins are considered prime candidates for tumor suppressor genes. Has been shown to in a genomic region with associations to variation in growth rates and lean mass percentage of pig. SNPs identified as highly significant in previous GWAS for obesity in pigs mapped to the vicinity of CDH20.	Reyer *et al*, 2017; Kogelman *et al,* 2014.	159,817,557-160,024,988	Starts 197666 bp upstream
SSC1: 159881634	ASGA0004988	rs80900421	*RNF152****	Regulates mTORC1 signaling and plays a role in the cellular response to amino acid availability. This gene has been previously identified to be correlated with increased intramuscular fat in pigs.	Silva *et al*, 2019; Rothschild *et al*, 2014.	159,530,623-159,601,981	Starts 351011 bp downstream

	*CDH20****	Protein coding gene. encoded membrane protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats cadherins are considered prime candidates for tumor suppressor genes. Has been shown to in a genomic region with associations to variation in growth rates and lean mass percentage of pig. SNPs identified as highly significant in previous GWAS for obesity in pigs mapped to the vicinity of CDH20.	Reyer *et al*, 2017; Kogelman *et al,* 2014.	159,817,557-160,024,988	Starts 64077 bp downstream

1 Note: Chromosomal positions are according to the pig genome assembly Sscrofa11.1. obtained vis Ensembl (https://uswest.ensembl.org/index.html).

2 The RefSNP is the rs name of the SNPs.

3 Any gene that is identified in more than 1 window is denoted with **.
Candidate genes with a previously identified association to intramuscular fat or feeding behaviour are denoted by ***.

4 Information on function gathered from identified sources in addition to GeneCards. (https://www.genecards.org) and NCIB database (https://www.ncbi.nlm.nih.gov).

Table S5.3 Genes identified in a 1 Mbp investigation window surrounding top five SNPs with lowest p-values from single-SNP association analysis for NIRIMF content in a purebred Duroc pig population.

[1]SNP Position (chromosome, genomic position in bp)	SNP ID	RefSNP ID[2]	Gene[3]	Gene function	Reference[4]	Gene position	Gene Distance from identified SNP
SSC1: 160347188	ALGA0006623	rs80877507	*MC4R***	Expressed primarily in the nervous system, plays a large role in the regulation of food intake, energy balance and body weight in mammals.	Rothschild *et al*, 2014, Silva *et al*, 2019; Wang *et al*, 2013	1.607-1.607	Starts 424825 bp upstream
			*GAD1-like***	Protein coding gene which produced an uncharacterised transcript. Not identified in literature. *GAD1* in pigs is found on SSC15. In humans, *GAD1* encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes.	NCIB Database, GAD1, *Homo sapiens*, 2020.	1.606-1.6078	Starts 282032 bp upstream
			CHD20 **	Protein coding gene. encoded membrane protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats cadherins are considered prime candidates for tumor suppressor genes. Has been shown to in a genomic region with associations to variation in growth rates and lean mass percentage of pig. SNPs identified as highly significant in previous GWAS for obesity in pigs mapped to the vicinity of CDH20.	Reyer *et al*, 2017; Kogelman *et al,* 2014.	1.598-1.600	Ends 322200 bp downstream

SSC1: 160447734	WU_10.2_1_178188861	rs341619787	***MC4R***	Expressed primarily in the nervous system, plays a large role in the regulation of food intake, energy balance and body weight in mammals.	Rothschild *et al*, 2014, Silva *et al*, 2019; Wang *et al*, 2013	1.607-1.607	Starts 324279 bp upstream
			*GAD1-like***	Protein coding gene which produced an uncharacterised transcript. Not identified in literature. *GAD1* in pigs is found on SSC15. In humans, *GAD1* encodes one of several forms of glutamic acid decarboxylase, identified as a major autoantigen in insulin-dependent diabetes.	NCIB Database, *GAD1*, Homo sapiens, 2020.	1.606-1.607	Starts 181486 bp upstream
			CHD20	Protein coding gene. encoded membrane protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats cadherins are considered prime candidates for tumor suppressor genes. Has been shown to in a genomic region with associations to variation in growth rates and lean mass percentage of pig. SNPs identified as highly significant in previous GWAS for obesity in pigs mapped to the vicinity of CDH20.	Reyer *et al*, 2017; Kogelman *et al,* 2014.	1.598-1600	Ends 422746 bp downstream
SSC1; 161824864	MARC0013872	rs81284646	*ALPK2*	ALPK2 (is a Protein Coding gene. Involved with ATP binding and protein kinase activity. This gene has been found to be downregulated in longissimus dorsi muscle tissue in a traditional Chinese breed, Wannanhua pigs, as compared to leaner, more heavily	Li *et al*, 2015.	1.621-1.623	Starts 319607 bp upstream

				selected, Yorkshire pigs.			
			MALT1 paracaspase	This gene encodes a caspase-like protease that plays a role in BCL10-induced activation of NF-kappaB. Has been postulated to be associated with survival traits such as resistance to disease	Joaquim *et al*, 2019.	1.620-1.621	Starts 252087 bp upstream
			ZNF532	Involved with nucleic acid binding	NCBI database, ZNF532, 2020.	1.618-1.619	Starts 52710 bp upstream
			SEC11C	A homolog of sec11 . SEC11 is the only essential factor for signal peptide processing, plays an important role in protein processing, localization, and secretion. The lack of SEC11 will cause serious growth defects .Identified in a GWAS for growth traits in pigs (Duroc, Yorkshire, Landrace, and Pietrain)	Böhni *et al*, 1988; Tang *et al*, 2019.	1.617-1.617	Ends 55338 bp downstream
			GRP	Encodes a number of peptides which regulate numerous functions of the gastrointestinal and central nervous systems, including release of gastrointestinal hormones, smooth muscle cell contraction, and epithelial cell proliferation.	NCBI database, GRP, 2020.	1.617-1.617	Ends 103082 bp downstream

	RAX	Encodes a homeobox-containing transcription factor for eye development. Rax is expressed early in the eye primordia and is required for retinal cell fate determination.	NCBI database, RAX, 2020.	1.616-1.616	Ends 141804 bp downstream
	CPLX4	Part of the complexin family l encodes a protein which may be involved in synaptic vesicle exocytosis.	Genecards, CPLX4, 2020.	1.616-1.616	Ends 149134 bp downstream
	***LMAN1	Protein encoded by this gene is a Mannose-specific lectin. Which may recognize sugar residues of glycoproteins, glycolipids, or glycosylphosphatidyl inositol anchors. May be involved in the sorting or recycling of proteins, lipids, or both. Has been found to be associated with feed efficiency and feeding behaviours, namely daily feed intake and daily feeder occupation time.	Reyer *et al*, 2017	1.616-1.616	Ends 193214 bp downstream
	CCBE1	Encodes a protein that is found in the lattice of extracellular proteins and contributes to lymphatic vascular development. Identified in a GWAS for Body Composition and Structural Soundness in commercial crossbred pigs as a gene of interest.	Bos *et al*, 2011; Fan *et al*, 2011.	1.613-1.615	Ends 268327 bp downstream

1 Note: Chromosomal positions are according to the pig genome assembly Sscrofa11.1. obtained vis Esembl (https://uswest.ensembl.org/index.html)
2 The RefSNP ID is the rs name of SNP from Ensembl genome browser
3 Any gene that is identified in more than 1 window is denoted with **.
Genes with a previously identified association to intramuscular fat, feeding behaviour or growth are denoted by ***.
4 Information on function gathered from identified sources in addition to GeneCards. (https://www.genecards.org) and NCIB database (https://www.ncbi.nlm.nih.gov).

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Chapter 6.0 A Genome-wide association study (GWAS) for intramuscular fat (IMF) in commercial crossbred pigs

6.1 Introduction

The pyramidal system used by the Canadian hog industry performs selection and testing for desirable traits primarily in the nucleus herds (top of the pyramid); any genetic improvement achieved at the commercial level is directly dependent upon on the rate of the genetic improvement achieved in the nucleus herd (Bichard, 1971; See, 1995). Despite this, the study and investigation of crossbred animals to identify SNPs and QTLs with relationships to relevant traits is routinely done; why? It has been shown that using genomic information from crossbred animals in combination with purebred information will improve the accuracy of selection (Wei and Van der Steen, 1991; Xiang *et al*, 2017; Sewell *et al*, 2018, Sevillano *et al*, 2019). Since crossbred genomes are a mixture of their parental breeds, the breed origin from which a SNP-allele was inherited is thought to influence their effect (Sevillano *et al*, 2019). These different allele effects arise for multiple reasons: 1) a QTL may be in LD with different SNPs, depending on which parental breed the QTL was inherited from (Lopes, 2016), and as such, 2) different quantitative trait nucleotide (QTN) may be underlying a QTL in the different parental breeds, (Wientjes *et al*, 2015), and finally 3) the parental breeds may experience different epistatic interactions (Mackay, 2014). Some of these relate primarily to the initial identification of a QTL (1,3), however the outcome is impacted by which QTN underlies a QTL.

The heavy focus on carcass leanness for increased lean yield in the last four decades has placed primary emphasis on the reduction of backfat thickness (Dunshea and D'Souza, 2003), which has resulted in overall carcass fatness reduction, including intramuscular fat (IMF) due to their positive genetic correlation (Huff-Lonergan *et al*, 2002; Hernández-Sánchez

et al, 2013). IMF is a key meat quality factor which affects consumer eating experience, therefore its improvement in the commercial crossbred animals is of particular importance as they are the final product. IMF is significantly correlated with tenderness, the trait widely considered the most important for eating enjoyment (Wood *et al,* 1999; Teye *et al*, 2005; Cannata *et al*, 2010; Lim *et al*, 2016; Won *et al,* 2018). Additionally, genetics play a significant role in the development, ultimate quantity and composition of IMF; IMF has a reported heritability between 0.26 ($\pm$ 0.06) - 0.50, depending upon the technique utilised for assessment (Rosenvold and Andersen, 2003; Miar *et al*, 2014; Jung *et al*, 2015) which indicates that a significant proportion of IMF expression is related to an animal's genotype.

Improved understanding of the genetic architecture of complex traits through the identification of markers in crossbred animals with high IMF can be done through genome-wide association studies (GWAS) and subsequent fine mapping. GWAS are a commonly used technique to identify QTL which are significantly associated with a trait of interest, providing insights to the genetic architecture behind a trait (Korte and Farlow, 2013). Identified markers can be incorporated into GS programs in order to improve the accuracy of GEBV predictions, increase the rate of genetic improvement and can better estimate the overall genetic effects, additive and non-additive, of a trait such as IMF which may be difficult or expensive to measure. Additionally, the generation of this new information can be used to create novel genomic tools for the improvement of Canadian selection programs and breeding. Any identified SNPs in this study will be compared to the those from the literature, investigated in the Pig QTL database (Hu *et al*, 2005) as well as the results from the purebred GWAS conducted in Chapter 5.0. Therefore, the goal of this study was to identify SNPs and potential candidate genes which contribute to IMF content in commercial crossbred pigs (Duroc sire $\times$ F1 (Large White $\times$ Landrace) dam). We hypothesised that through the GWAS we will identify

a SNP which has a large effect size that explains a sizable amount of the phenotypic variance seen in IMF; in the event there are no single SNPs with large effect size we expect to identify SNPs with a smaller effect size which still pass the threshold of significance. We have no null hypothesis for the GWAS'.

6.2 Materials and methods

Ethics statement

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 (Canadian Council on Animal Care, 1993.

Animals

A subsample of 808 animals from a total of 1098 commercial crossbred pigs originating from a Canadian breeding company (Hypor Inc. Regina, SK, Canada) were used for this study; animals used were those with both phenotype and genotype data available. The pigs were a three-way cross between a Duroc sire and an F1 dam (Landrace × Large White). The majority of commercial pigs for slaughter in Canada are a result of this terminal cross (Miar *et al*, 2014). Feeding, raising, and slaughter protocol have been described in previous studies (Miar *et al*, 2014; Zhang *et al*, 2015; Yang *et al*, 2017).

Phenotypes

IMF was assessed via ultrasound on each pig using the BioQ station (Biotronics Inc, Iowa USA). The proprietary algorithm used in the built-in software of the BioQ station provides an estimation of the IMF content in the muscle and is described as ultrasound IMF (UIMF). Scans were done between the 3rd and 4th last rib, approximately 6 inches off of the

midline at the apex of the loin. Animals were scanned two days prior to slaughter. Phenotypes were corrected using the animal model in ASReml (Gilmour, 2015) to obtain residuals (adjusted phenotypes), which were further plotted to evaluate the distribution:

$$\boldsymbol{y}^* = \boldsymbol{y} - \boldsymbol{x_1 b} \qquad (1)$$

where $\boldsymbol{y}^*$ are the residuals (phenotypes adjusted for fixed effects), $\boldsymbol{y}$ are the uncorrected phenotypes, **b** is the vector is fixed effects, sex and date of slaughter, and $\boldsymbol{x_1}$ is the design matrix associated with **b** (Yang *et al*, 2017). The residuals (adjusted phenotypes) are presented in Figure S6.1 in the Supplementary information 6.0.The mean, standard deviation, minimum, maximum and coefficient of variation (CV) for the phenotypes were calculated using Microsoft Excel. CV was calculated with the following formula:

$$CV = \frac{SD}{\mu} X\ 100\% \qquad (2)$$

Where SD is standard deviation and μ is mean of the phenotypes.

Genotypes

DNA extraction and genotyping details were previously described by Zhang *et al.* (2015). Briefly, genomic DNA was isolated from tissue using the Thermo Fisher Scientific Ltd (Ottawa, ON, Canada) DNA extraction instruction manual and genotyping was done by Delta Genomics using an Illumina PorcineSNP60 V2 beadchip (Illumina, Inc., San Diego, CA, USA). SNPs with the following features were excluded during quality control procedures; minor allele frequency (MAF) < 0.01, genotype call rate < 0.95, and departure of heterozygous from Hardy-Weinberg > 0.15 (i.e. if the difference between observed and expected frequency of heterozygotes was > 0.15). Any unmapped SNPs were also excluded, as were those on the sex chromosomes; non-autosomal SNPs were removed as the recombination landscape of the sex chromosomes is known to be different than on autosomes, which can cause distortion in

the subsequent analysis (Bosse *et al*, 2012; Zhang *et al*, 2018). Imputation of any missing genotype was done with FImpute version 2.2 (Sargolzaei *et al,* 2014). Finally, 40,438 SNPs and 808 animals remained from the original of 61,565 SNPs and 1,098 animals on 18 autosome chromosomes for GWAS analysis.

Genome-wide association study (GWAS)

Using ASReml software (Gilmour *et al*, 2015), a single-SNP association analysis utilising a genomic relationship matrix was performed. The following generalised linear mixed model was used:

$$\boldsymbol{y}^* = \mathbf{1}\mu + \mathbf{Z}\boldsymbol{g} + \boldsymbol{v}\boldsymbol{\alpha} + \boldsymbol{e} \qquad (3)$$

where $\boldsymbol{y}^*$ denotes the vector of corrected phenotypes; $\mathbf{1}$ is simply a vector of 1's, and μ is the population mean. In this study, significant fixed effects were slaughter date and sex ($p < 0.001$) (phenotype corrections for fixed effects were described previously). $\mathbf{Z}$ is a design matrix; associating random animal genetic effects to the observations and $\boldsymbol{g}$ is the vector of genetic values of all animals (random animal effects). The animal effects are assumed to have a normal distribution, where; $\mathbf{g} \sim \mathrm{N}\left(0, \mathbf{G}\sigma^2_{\mathbf{g}}\right)$ and where $\sigma^2_{\mathbf{g}}$ is the additive genetic variance. SNP genotypes are presented in vector $\boldsymbol{v}$ as 0, 1, or 2, fitted as a fixed effect, and $\boldsymbol{\alpha}$ is the additive SNP effect. Finally, $\boldsymbol{e}$ is a vector of random residual effects. These effects are also assumed to be normally distributed where; $\mathbf{e} \sim \mathrm{N}(0,\ \mathbf{I}\sigma^2_{\mathrm{e}})$ and σ^2_{e} is the residual variance. $\boldsymbol{G}$ is the realized genomic relationship matrix and **I** is the identity matrix. The **G**-matrix was created using GCTA software (Yang *et al,* 2011). The **G-**Matrix utilizes elements of the realized section of the genome which two individuals share (Mendelian sampling term) (Lee *et al*, 2015). The formula of the **G-**Matrix is described as follows:

$$\boldsymbol{G} = \frac{1}{N}\sum \left(X_A - 2p_j\right)\left(X_A - 2p_j\right)/2p_j\,(1 - p_j) \qquad (4)$$

In this model, where N is the number of SNPs, X_A is coded as 0, 1, or 2 for genotypes AA, AB, and BB, respectively; p_j is the observed allele frequency at the jth SNP in the population (Yang *et al*, 2011). A Manhattan plot was created using the -log10 p-values of the SNPs. These were plotted with regard to their genomic position. A quantile-quantile (Q-Q) plot was also created to visualise the distribution of the -log10 p-values to the expected null distribution. The genomic inflation factor, or lambda (λ), was calculated by dividing the median of observed χ^2 test statistics by the expected median of the corresponding χ^2 with 1 degree of freedom. Both the Q-Q and Manhattan plots were constructed using the qqman package in R (Turner, 2018).

Multiple testing was controlled for through the use of false discovery rate (FDR). FDR was calculated model (5) as described in Chapter 5.0. Additionally, the first two principle components were plotted against one another to visualise any population stratification, all animals originated from the same farm and breeding population.

Significant SNPs identified in the GWAS are expected to be in high LD with SNPs that are located physically nearby. In the Canadian commercial pig population (Duroc X (Landrace X Large White), LD (r^2) has been found to be 0.15 for distances up to 0.5 Mbp (Badke *et al,* 2012; Grossi *et al,* 2017). With this in mind, a QTL can be defined as 1 Mbp window around any significant identified SNP: 0.5 Mbp upstream and 0.5 Mbp downstream. This was used to investigate any significant SNP identified by the GWAS. Any associations detected are assumed to be as a result of the significant SNP or a nearby SNP which is correlated – any loci which are further than 0.5 Mbp in this crossbred population have a low chance of being in LD

with a significant SNP and are unlikely to be connected to the studied trait. In general, as the distance increases between SNPs, the linkage also decreases.

Subsequently, since no QTL were identified from the single-SNP association analysis (GWAS), least absolute shrinkage and selection operator (LASSO) and adaptive LASSO (ADALASSO) were conducted on the residual values (corrected phenotypes) using the glmnet package (Friedman *et al,* 2010) and the parcor package (Kraemer and Schaefer, 2014) in R-studio (R-Studio Team, 2015). Two rounds were performed for both operators at 10 iterations followed by 20 iterations of the model. A multiple linear model was used for the association analysis. The general linear mixed model, as below, was then fitted using a general LASSO.

$$\boldsymbol{y}^* = \boldsymbol{X_2}c + \boldsymbol{Z}s + \varepsilon \qquad (5)$$

Where $\boldsymbol{y}^*$is the adjusted phenotype described above, $\boldsymbol{c}$ is the source of the animals (Hypor Inc.), **s** is the vector of SNP effects . $\boldsymbol{X_2}$ is the design matrix associated with c, $\boldsymbol{Z}$ is the design matrix associated with s and finally, ε is the random residual in the model (Yang *et al*, 2017). The LASSO estimators are described in great details in Wu *et al.* (2009) and Zou *et al.* (2012).

QTL detection

Any identified SNPs from the single marker GWAS or LASSO analyses were investigated using BIOMART in Ensembl Genome Browser with a 1 Mbp window and subsequent identified genes within this region were searched in the Ensembl Genome Browser (https://www.ensembl.org), the National Center for Biotechnology Information Database (NCBI) (https://www.ncbi.nlm.nih.gov), and in the pigQTL database (Hu *et al,* 2005). To obtain additional functional information, the candidate genes were also searched in the Human Gene Database (https://www.genecards.org), as the translation to livestock is relatively well established. Regions identified in this study which overlap with previously identified genomic

regions with IMF associations can be helpful in the provision of evidence for any noteworthy observations.

Genetic variance

Minor allele frequency was calculated using PLINK software (Purcell, 2007). The SNP variances were computed based on the estimated allele substitution effects and allele frequencies , using models (7) and (8) as described in Chapter 5.0.

6.3 Results and discussion

Phenotypic descriptive statistics

The average UIMF for those animals included in the study was 2.88% with a standard deviation of 0.917, maximum of 6.6%, minimum of 0.7% and CV of 31.79%.

Principle component analysis

Figure S6.2 in Supplementary information 6.0 shows the first two principle components plotted with no outliers or stratification observed. From this, no PCA were chosen to include in the GWAS.

Variance component estimation

The genetic variance of UIMF in this crossbred population, as calculated by ASReml at $p < 0.05$, was 0.18 ± 0.06 with residual variance of 0.55 and estimated heritability of 0.24 ± 0.07. UIMF has previously been reported to be a moderately heritable trait, ranging from 0.26 ± 0.06 to 0.48 (Miar *et al*, 2014; Jung *et al*, 2015).

GWAS and LASSO

No significant SNPS were identified by single-SNP GWAS; all tested SNPs failed to reach the threshold of significance (FDR 5% or 10%). The Manhattan plot and quantile-quantile (Q-Q) plots showing the GWAS results are provided in Figure S6.3, Supplementary

information 6.0. However, in order to further explore the data, an alternative Bayesian method (LASSO) was employed, which resulted in the identification of three SNPs with measurable effect size (non-zero effect). These SNPs are presented in Table 6.1 below.

Table 6.1 Identified non-zero SNPs from LASSO for UIMF content in a commercial crossbred pig population.

SNP Name	Position [1]	Investigation window (Mbp)	SNP effect or allele substitution effect	Minor allele frequency (MAF)	Genetic variance explained (% of phenotypic variance)
DRGA0003711	SSC2 146949742	1.46-1.47	-0.134	0.294	0.563
ALGA0032074	SSC5 58601394	0.58 - 0.59	-0.349	0.0396	0.128
MARC0057051	SSC7 101752523	1.01-1.02	0.009	0.287	0.002

1 Position is presented in Chromosome (SSC) followed by genomic position in bp.

The LASSO is useful as it utilises a double exponential, non-normal distribution for the SNP effects (Tibshirani, 1996). This is done by putting a constraint on the model parameters which causes the regression coefficients for some variables to be shrunk towards zero; any variable with a regression coefficient that is zero after this shrinkage process is not included in the final model and variables with non-zero regression coefficients are those which are most strongly associated with the trait (Tibshirani, 1996). In this way, we are able to identify those SNPs which may not have met a stringent threshold of significance for single-SNP GWAS, but still explain a proportion of the variance seen. The single-SNP GWAS detects the association of hundreds of thousands of genetic variants and a trait through a regression analysis in order to find association between a single variant and the phenotype. So, many statistical tests are done, and a very stringent threshold is needed to identify an SNP as significant. Therefore, only the SNPs that explain a relatively large amount of variation (SNPs with large effects) will

exceed the threshold, and SNPs with small effects which explain only a small proportion of genetic variance often do not reach stringent significance thresholds. This is particularly noted when the sample size is relatively small, as it is in this study; the power to detect QTL decreases with a decreasing sample size. While LASSO, alternatively, allows us to jointly model the relationship between all genetic variants and the phenotype (Arbet *et al*, 2017). These three SNPs explain 0.693% of the total phenotypic variance; the calculated genetic variance explained by each SNP is quite small, as are their minor allele frequencies (MAF). GWAS' are inherently designed to detect frequent genetic variants with MAF > 5% and have often failed in the detection of rare variants (MAF <1%); therefore, the contribution of SNPs with low MAF to genetic variation are unlikely to be detected (Visscher *et al*, 2017). This is true in the present study, as these SNPs were undetected by the GWAS, only the LASSO was able to identify them. Additionally, low MAF may indicate that the alleles with low MAF may be un-fixed. It is possible then that the SNP with low MAF, identified by the LASSO, resulted from random favourable allele combinations. The average IMF of these crossbred animals is over 1% above the national average of 1.5% (Meadus *et al*, 2018), which indicates there are favourable allele combinations occurring. The small effect sizes (Table 6.1) of the identified non-zero SNPs by LASSO and the lack of results from the single-SNP GWAS may indicate that that some of these favourable allele combinations were achieved by chance as a result of the crossbred nature rather than fixing of favourable alleles in the purebreds for IBD inheritance. The breed of origin for these alleles could be determined in order to further investigate this (Sevillano *et al*, 2018). The low heritability calculated (0.241 ± 0.071) is consistent with the low genetic variance explained by each SNP, as h^2 is the variance in the trait which is explained by genetics. The identified non-zero SNPs were not found in the coding sequence of any genes, and therefore surrounding regions were investigated. The genes

found within the 1 Mbp window of the identified SNPs were investigated and are shown in Table S6.1 in Supplementary information 6.0.

QTL investigation

Of the genes identified, *ATF7IP*, from the investigation window of SNP ALGA0032074 (0.58 to 0.59 Mbp), has been previously identified in the literature with a connection to IMF. In a study by Pena *et al*. (2013) the differential gene expression of F2 backcrossed [Iberian (25%) x Landrace (75%)] pigs were investigated as these breeds exhibit both high and low levels of IMF. They analysed the total IMF content of the *longissimus thoracis* muscle and selected animals which exhibited high and low IMF extreme phenotypes. In total, 219 differentially expressed probes were identified and further assigned to 283 unique genes based on their expression level in either group. Gene *ATF7IP* was one of just seven genes to be commonly expressed between both groups. Due to the minimal overlap seen in the transcriptome for the two groups we can infer that common genes may perform essential functions and additionally may interact differently with the other genes given an animal's unique genotype. *ATF7IP* is a multifunctional nuclear protein that associates with heterochromatin. It can act as a transcriptional coactivator or corepressor depending upon its binding partners (Liu *et al*, 2009). It is assumed that the effects of this gene are additive, so when present with the transcriptome of a higher IMF animal, it is possible it contributes to a greater deposition of IMF.

In the 1 Mbp window for SNP MARC0057051 (1.01 to 1.02 Mbp), the gene *NEUREXIN 3* was identified. Though this gene has no noted connection in the literature to IMF in pigs, it has been associated with waist circumference, obesity and energy balance in humans (Heard-Costa *et al*, 2009). In commercial hog barns, consumption to the point of obesity is not realistic as feed comprises approximately 70% of costs associated with growing

pigs, making such fat deposition expensive (Martinsen *et al,* 2015). However, the underlying motivations of reward and addictive behaviour may increase aggression associated with group feeding, which is common practice in hog barns. Animals with the identified mutation in this gene may be more likely to consume more than their allotted feed portion, making them more likely to deposit an increased proportion of fat (Heard-Costa *et al*, 2009). This connection has not been studied or confirmed but does warrant further investigation in relation to IMF. Additionally, this gene was investigated on the Pig QTL database; a connection with carcass length was been identified by Sato *et al.* (2016). Carcass length and UIMF have been previously found to have a low positive genetic correlation of 0.15 ± 0.23 and phenotypic correlation of 0.04 ± 0.05 (Miar *et al,* 2014). In the same study, the genetic and phenotypic correlations between carcass length and ultrasound fat depth, a trait with known high genetic correlation to IMF (Solanes *et al*, 2009; Hernández-Sánchez *et al*, 2013), were found to be 0.05 ± 0.14 and 0.09 ± 0.04, respectively. All reported correlations were non-significant, save for the phenotypic correlation for carcass length and fat depth, and as such cannot be fully trusted. However, they are of interest particularly for selection indices as the understanding of all correlated traits to those under selection are important to ensure that breeding goals are met to optimise profitability and desirability of the final carcass.

Finally, from the investigation window of SNP DRGA0003711 (1.46 - 1.47 Mbp) gene *SH3RF2* was investigated. This gene has not been identified in the literature to have an association to porcine IMF content, but its described function and identification in other species warrants further investigation for a connection in pigs. *SH3RF2* affects growth via regulation of appetite; IMF can be significantly affected by nutrition (Hocquette *et al*, 2010; Turner *et al*, 2014). Additionally, this gene was recently identified in a study by Jerez-Timaure *et al.* (2019) where they investigated differential gene expression in the *longissimus thoracis*

muscle of beef animals with either normal or high PHU; 53 differentially expressed transcripts were identified and then evaluated for up or down regulation in the groups. *SH3RF2* was identified as having a significant, negative fold change in expression (downregulation) in the high PHU group. This may indicate that in samples of meat with normal pH, the expression of this gene is normalised, being neither up - or down regulated and low pH may have an upregulation of this gene. High pH is undesirable for meat quality; in pork, high pH is associated with dark firm and dry (DFD) meat and low pH is associated with pale, soft and exudative (PSE) meat. Both of these extremes are detrimental to pork quality and often result in rejection of the carcass (Rosenvold and Andersen, 2003). The influence of ultimate pH and IMF content on pork tenderness was discussed by Van Laack *et al.* (2001). Both IMF and pH have a profound impact on tenderness of the final pork, which as discussed, is widely considered the most important trait associated with positive eating quality, though their impact is achieved through different mechanisms. Interaction between PHU and IMF and any resulting effect on meat is unclear. pH of meat is significantly affected by pre-slaughter environment (stress) and *post-mortem* handling (temperature/climate control) (Van de Perre *et al*, 2010; Kim *et al*, 2016). IMF, however, is not affected by post-slaughter handling, but environment does impact deposition; both pH and IMF are polygenic, and therefore some interaction within the many genes which contribute to both IMF and pH may be present, though such an interaction has not been identified yet. Investigation of this potential interaction warrants further inquiry.

Genetic variance

The proportion of total phenotypic variance explained by SNP variance of the 3 investigated SNPs was 0.69% (Table 6.1). This is less than the significant SNPs investigated in Chapter 5 (7.13%), however, because the SNPs in the present study were not identified as significant by GWAS, their lower proportion of genetic and phenotypic variance explained is

not unexpected. The values calculated in this study are consistent with previous literature findings; in a study by Jiang *et al.* (2018) which investigated growth and fatness traits in Yorkshire pigs identified two groups of SNPs (6 and 5) which explained 2.09% and 0.52% of additive genetic variance of the backfat thickness and days to 100 kg (growth rate).

Limitations

In order to improve upon our results in the commercial pig, there are a number of limitations that must be addressed in future studies. Firstly, I would suggest increasing the sample size. Our sample size of 808 is small in terms of genetic studies and as such any signal may have been too weak to detect. This is supported by the LASSO results, MAF, and calculated genetic variance explained by each SNP (Table 6.1). With low MAF and low effect size, the likelihood of repetition of these results is low, particularly with small sample sizes; increasing the sample size would help to increase the power of GWAS and thus to detect association regions. A larger sample size can aide with the detection of SNPs with small effects and they may be more likely to exceed the stringent threshold and the statistical power associated with increasing the sample size is significant (Visscher *et al,* 2012). Secondly, I suggest using a higher density panel or even using the whole-genome sequence (WGS). WGS may help identify a causal variant responsible for trait variation, and therefore, with WGS, the power of detecting the causative variant increases, as QTL detection does not depend on LD between the causal mutations and other SNPs. It is important, however, to be cautious of the detriments that using significantly increased density can cause. This is discussed in Chapter 5.0, section 5.3, Results and discussion. Briefly, it is possible that the 'big n little p' problem ($n << p$) contributed to the results; that the causal mutations were estimated with error, and the larger effect of causal mutations was distributed over many SNPs (Heidaritabar, 2016, PhD thesis).

6.4 Conclusions

A single-SNP association analysis utilising a genomic relationship matrix was performed with the UIMF phenotypes form a commercial crossbred pig population, followed by a LASSO. Due to the stringent threshold needed to identify an SNP as significant, the GWAS was unable to detect any significant SNPs. The LASSO, however, was able to identify three non-zero SNPs. This is because of the SNP distribution and constraints put on the model, which allows us to shrink the coefficients for some variables towards zero, and subsequently not include any zero coefficients in the final model; non-zero regression coefficients are those which are most strongly associated with the trait. Even though these SNPs did not meet the threshold of significance for the GWAS, they still explain a proportion of the variance seen. We are unable to accept our hypothesis as the GWAS was unable to identify any SNPs with measurable effect size, only after the application of the LASSO were three SNPs with measurable effect identified.

The polygenic nature and subsequent complex genetic architecture of IMF has been shown through many studies, including ours, to be complex and controlled by many genes. The SNPs identified in this study explain only a small portion of the total variance seen. Because we obtained different results in the crossbreds as compared to the purebreds (Chapter 5), even when using the same phenotype collection method (UIMF), there may be underlying reasons (LD with QTL based on inheritance from different parental breeds, different causative mutations underlying in different parental breeds, epistatic interactions) which warrant investigation. It is understood that the interaction of the 3 breeds the commercial Canadian crossbred pig adds a layer of complexity because IMF is expressed differently in each breed.

As such, the SNPs identified in this study should be further investigated in both purebred animals and additional crossbred studies.

Supplementary information 6.0

Figure S6.1

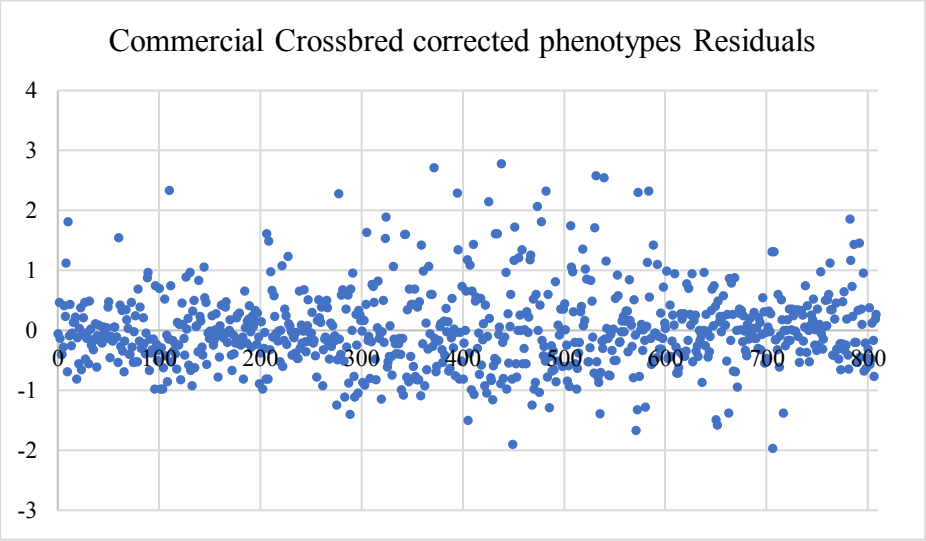

Figure S6.1. Distribution of ultrasound IMF phenotypes which have been corrected using ASReml (Residuals). Residuals are randomly dispersed around the horizontal axis, indicating that the use of a linear model is appropriate. Graph shows the approximate value of the residual on the Y-axis and the X-axis shows the number of animals (n=808).

Figure S6.2

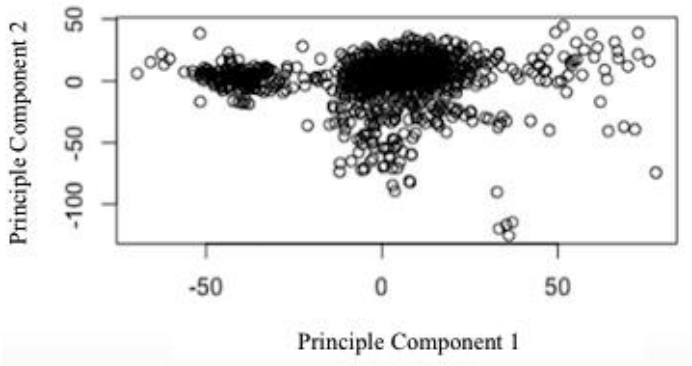

Figure S6.2. Principle Component Analysis (PCA) in our commercial crossbred pig population. principle components 1 and 2 were plotted against one another to visualize potential subpopulations; none were noted. Two main clusters (left, middle) with a third, less dense cluster (right) can be seen, which indicate the crossbred nature of the population (Duroc X (Landrace X Large white).

Figure S6.3

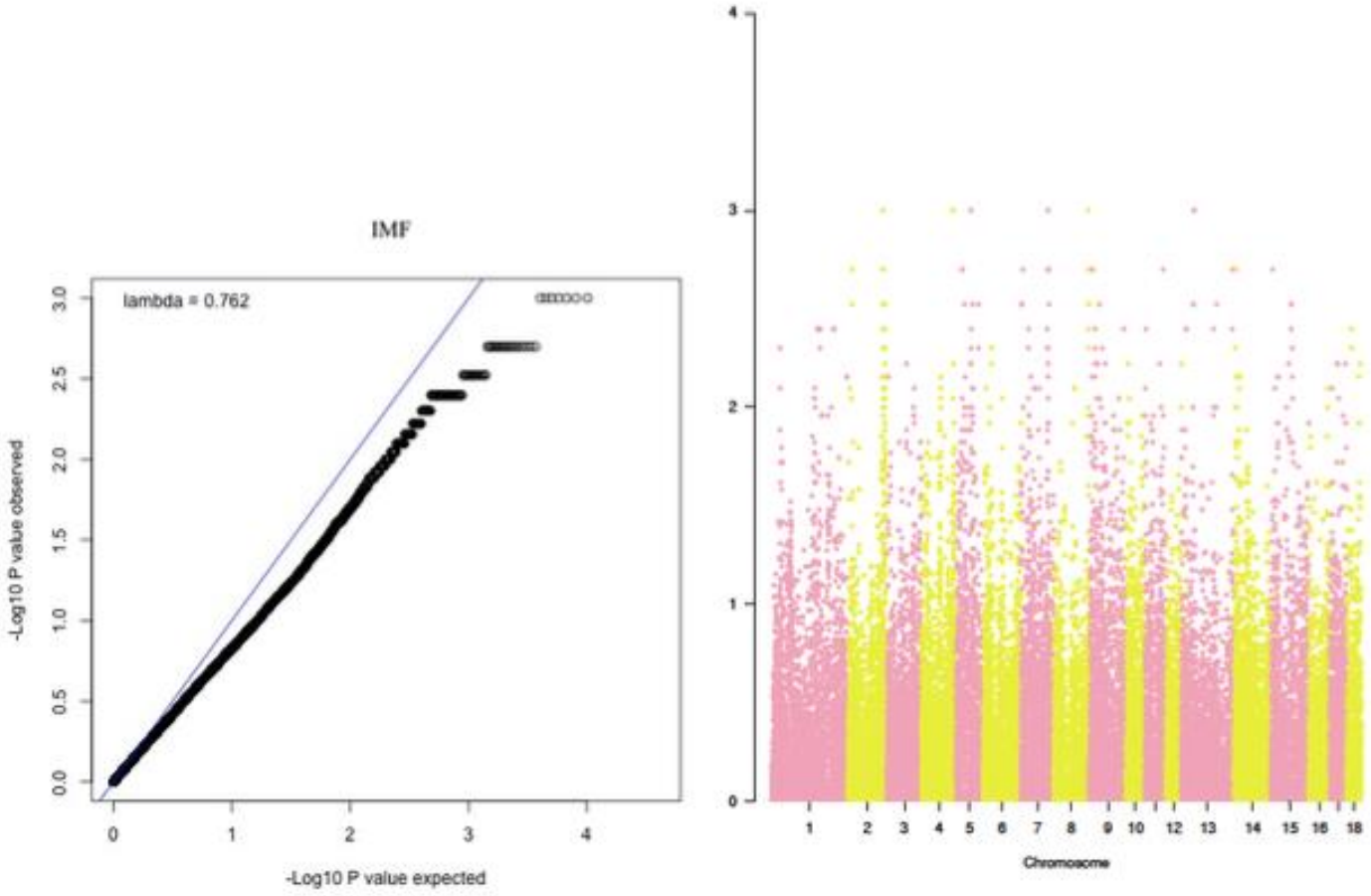

Figure S6.3. The quantile-quantile (Q-Q) (left) and Manhattan plot (right) from a GWAS for UIMF on a commercial crossbred pig population. The Q-Q plot shows the expected null distribution of -log10 (p-values) (solid blue line) compared to the actual distribution (dotted black line). The Manhattan plot 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. FDR was used to control for multiple testing, with *p*-values of 0.05 (-log10 = 5.91), and 0.10 (-log10 = 5.61).The genomic inflation factor (λ) is displayed on the Q-Q plot (0.76).

Table S6.1 Genes found within a 1 Mbp window of SNPs identified by LASSO in a commercial crossbred pig population

[1]SNP Position	SNP ID	RefSNP ID[2]	Candidate Gene	Gene function	Reference	Gene position	Candidate gene Distance from identified SNP
SSC2: 146949742	DRGA0003711	rs81294208	*PRELID2*	Involved in mouse embryogenesis during mid-late gestation	Gao *et al,* 2009	1.471-1.471	Starts upstream 159844 bp
			GRXCR2	Glutaredoxin and cysteine rich domain containing 2. Associated with hereditary hearing	Carpena and Lee, 2018	1.472-1.472	Starts upstream 272801 bp
			SH3RF2	Affects growth via regulation of appetite	Andersson, 2012	1.472-1.474	Starts upstream 342435 bp
SSC5: 58601394	ALGA0032074	rs80787531	*GRIN2B*	Detection of mechanical stimulus involved in sensory perception of pain and behavioral defense response.	Huashui *et al.,* 2014	0.584-0.589	Starts downstream 121203 bp
			ATF7IP	ATF7IP is a multifunctional nuclear protein that associates with heterochromatin. It can act as a transcriptional coactivator or corepressor depending upon its binding partners	Liu *et al*, 2009	0.580-0.582	Starts downstream 578661 bp
SSC7: 101752523	MARC0057051	rs80925093	*NEUREXIN 3*	Has been associated with human obesity and energy balance	Heard-Costa *et al,* 2009	1.016-1.019	Starts Upstream 147954 bp

1 Note: Chromosomal positions are according to the pig genome assembly Sscrofa11.1. obtained vis Ensembl (https://uswest.ensembl.org/index.html)
2 The RefSNP ID of the SNP identified by adaptive LASSO.

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Chapter 7.0 General discussion and conclusion

7.1 Summary and general discussion

Excellent pork meat quality is increasingly desired by consumers, both domestically and abroad, and the increased understanding of the underlying genetics which affect pork quality can help to satisfy this demand. Subsequently, the hog industry as a whole has begun shifting breeding focus for commercial animals from primarily lean yield and growth rate towards the improvement of meat and carcass quality and traits (Papanagiotou *et al*, 2012). Further, the use of genetic technologies has become increasingly common in order to meet these demands (Dransfield *et al*, 2005). The use of GS provides an opportunity to select for pigs with superior meat quality. GS typically follows a two-step procedure in which 1) the animals in a reference population are genotyped and phenotyped, these are referred to as the training population. The effects of each SNP genotype are then estimated and subsequently all the QTL effects are summed in order 2) to obtain a GEBV for potential breeding animals (selection candidates) (Goddard and Hayes, 2007). Prediction methods can be used for estimation of GEBV to predict the genotypic value of selection candidates (validation population) which are not necessarily phenotyped but are genotyped.

Fat plays a unique role in the acceptance and desirability of pork, particularly intramuscular fat (IMF) or visual marbling, and backfat thickness or depth (BFD), as these traits are positively genetically correlated. Backfat thickness is also integral to the valuation of an entire carcass, based on lean yield, and goes on for further processing in value added products, making it an economically relevant trait. The reduction of backfat thickness during the pursuit of lean yield and efficient lean growth lead to the reduction of IMF due to their positive genetic correlation (Solanes *et al*, 2009; Hernández-Sánchez *et al*, 2013) which

resulted in a dry, flavourless product and decreased consumer satisfaction with pork. Therefore, the focus of this thesis is on IMF and backfat quality in two pig populations with the goal of identifying genetic correlations and heritabilities of these two traits as well the identification of SNPs and potential candidate genes which contribute to the variance seen in IMF in a purebred Duroc and commercial crossbred pig population. The ultimate goal for these findings, though out of the scope of this thesis, is for incorporation into breeding programs to improve the accuracies of GEBVs, and the development of novel genomic tools for the improvement of Canadian selection programs and breeding through the ability to independently manipulate IMF from backfat. Many methods for GS exist, but most common is genomic best linear unbiased prediction (GBLUP), or single-step GBBLUP (ssGBLUP). Most methods differ based on their underlying assumptions; the GBLUP methods assume that each SNP contributes a small, equal effect on a complex trait. Though this assumption is beneficial to the practical implementation of GS, as the identification of a specific gene or causative mutation is not necessary, it does not incorporate any genetic or biological information known for the trait. Some alternative methods which do consider biological information have been developed (MacLeod *et al*, 2016). However, these are limited by a lack of understanding of the genetic architecture of many traits. As such, there has been an increase research focusing on these underlying genetic factors which affect relevant traits; this is made possible by the widespread availability of many SNPs across the genome of many species which allow high-density SNP genotyping. One such method of study are GWAS', and these have been a primary tool used to determine underlying complex traits. GWAS' use single SNPs spread across the entire genomes to detect regions of the genome associated with traits of interest, or QTL. Numerous GWAS' have been performed in pigs for meat and carcass quality traits and many and subsequently many QTL effects have been identified (Hu *et al*, 2005). These GWAS

results can help to progress the understanding of meat quality traits, particularly from this thesis, fat IMF deposition. Overall, this will be important for the future implementation of GS methods for improving IMF in pigs independently from genetically correlated traits, like BFD.

The objective of the first study in Chapter 3 was to generate phenotypes using NIR spectroscopy for IMF content of the loin as well as for backfat quality for a purebred Duroc population with the goal to build on the body of literature validating this as a rapid method for reliable phenotype generation and to utilise these phenotypes in further genetic studies. The results of this study showed two significant limitations to the accuracy of the generated phenotypes, 1) that the impact moisture loss from freezing and thawing of samples significantly affects the final composition and subsequent spectra and 2) the use of calibrations/standards which have been developed from populations which differ from the sample population will reduce accuracy of the prediction algorithm (fresh samples, varying breeds). The relationship between the backfat quality phenotypes from NIR as compared to gas chromatography, represented by calculated iodine value (IV), was poor (R^2_{adj} = 0.4, RMSEP = 2.81, *p*-value <0.0001); the phenotypes were not appropriately accurate enough for use in further studies. The IMF phenotypes, however, were more promising, based on the R^2_{adj} (0.78) value, low RMSEP value (0.183) and low *p*-values (<0.0001), all which indicated a high degree of correlation between the NIR generated phenotypes and the reference method of proximate analysis. After the application of the additional correction factor (-1.26) , these phenotypes were appropriately accurate, based on comparison to the phenotypes obtained via the reference method of proximate analysis, for further use.

The objectives of the second study in Chapter 4 were to 1) estimate the heritabilities for IMF and backfat thickness measured by various methods (NIR, ultrasound, subjective and

traditional) and 2) phenotypic and genotypic correlations among these traits in both a purebred Duroc and commercial crossbred population. All estimated heritabilities were consistent with the current literature for purebred Duroc BFD (0.44 ± 0.11; Newcom *et al*, 2005), commercial crossbred BFD (0.45 ± 0.07; Miar *et al,* 2014), purebred Duroc NIRIMF (0.50 - 0.62 ± 0.06; Gjerlaug-Enger *et al*, 2010), purebred Duroc UIMF (0.48 ; Jung *et al,* 2015), commercial crossbred UIMF (0.26 ± 0.06; Miar *et al*, 2014) and as well as commercial crossbred subjective carcass marbling score (0.23 ± 0.05; Miar *et al*, 2014) are all very close to the estimates from this study. From our study, estimates for purebred UBFD, BFD, NIRIMF, UIMF and SUBIMF were 0.48 ($\pm$ 0.09), 0.44 ($\pm$ 0.09), 0.491 ($\pm$ 0.11), 0.44 ($\pm$ 0.09) and 0.23 ($\pm$ 0.07) and crossbred estimates for UBFD, BFD, UIMF and SUBIMF were 0.54 ($\pm$0.09), 0.43 ($\pm$0.08), 0.24 ($\pm$ 0.07) and 0.25 ($\pm$ 0.07). Additionally, the results of the variance component estimations showed a high positive genetic correlation between IMF and backfat thickness ($\mu = 0.40 \pm 0.145$), regardless of measurement method or population, which is consistent with the current literature. This indicates that selection for one of these two traits, without any additional investigation (increased understanding of genetic architecture), would result in the increase of the other; they are good indicator traits for one another. There has been a proposed strategy to utilise crossbred animals are used as the training population in order to predict the best purebred animals to breed for optimal crossbred performance (Dekkers *et al*, 2007; Hidalgo *et al*, 2016). A high r_g is necessary between crossbred and purebred animals for the desired trait is necessary in order for this strategy to have high accuracy. Based on the results of this the present study, I think this proposed strategy of GS may be feasible for the improvement of IMF in commercial crossbred pigs. The genetic correlations between the same traits measured by different methods was high for NIRIMF – UIMF (0.78 ($\pm$ 0.085)) in the

purebred Durocs, indicating that these two phenotypes would be appropriate replacements for one another. Additionally, BFD and UBFD were estimated to have high genetic and phenotypic correlations in both the purebreds (0.95 ($\pm$0.03) and 0.81 ($\pm$0.01)) and crossbreds 0.99 ($\pm$0.02), 0.74 ($\pm$0.02), indicating that these two methods of phenotype collection would be appropriate replacements for one another. Although none of the correlations were significantly different from zero, resulting in the failure to reject the null hypothesis, based on the assessment of previous literature these values can be interpreted cautiously but optimistically. In future studies, to address the primary limitation of this study, increased generational data should be included. Large datasets which span multiple generations can help decrease error, help obtain an accurate relationship value between the animals in the data file and increase the accuracy of the predicted variance components. The ability to confidently choose between methods of phenotyping is beneficial, particularly for genetic studies, as phenotyping often represents a large cost of a study due to the large number of animals generally required. A certain methodology may be more practical or feasible for phenotype collection in different environments with different populations (ultrasound is low cost and can be done on live animals, whereas NIR or physical measurement must be conducted on carcasses / samples), and when collected properly, the understanding is that both will provide similar results in any further studies, such as GWAS.

The objectives of Chapters 5 and 6 were to, investigate IMF in a purebred Duroc population and a commercial crossbred population, respectively, using GWAS. GWAS' are a commonly used technique to identify SNPs which are significantly associated with a trait of interest, which can provide insights to the genetic architecture behind a trait (Korte and Farlow, 2013). In the purebreds, five SNPs were identified as significant using UIMF phenotypes (GWAS 1) and no SNPs were identified as significant from the NIRIMF phenotypes (GWAS

2), however the *p*-values were very close to those identified as significant. Since a GWAS exploits the LD between SNPs and ungenotyped causal mutations, we rely on LD information to determine if a SNP is truly explaining a large part of the trait variation (truly informative SNP). The LD was investigated in a 1 Mbp window around all SNPs identified as significant, and strong (> 90%) r^2 was found in the pairwise significant SNPs, as well as between many surrounding SNPs. By investigating the entire 1 Mbp regions surrounding the identified significant SNPs, we were able to narrow down to a number of subregions which could be further investigated for a detailed analysis (LD mapping). This is important, as any causal mutations or causal loci are most likely where the pairwise LD for adjacent markers peaks (Dorak, 2016); the regions of high LD surrounding the significant SNPS identified by GWAS warrant further investigation. In addition to the LD investigation, a 1Mbp window surrounding each significant SNP from GWAS 1, and the 5 SNPs with the lowest *p*-values from GWAS 2 were investigated for candidate genes. All SNPs were within close proximity on SSC 1. A number of interesting genes were identified, including *RNF152*, *PMAIP1, CDH20,* and *MC4R;* all of these genes have been previously identified with fatness (IMF) and related traits, including average daily gain and daily feed intake. Additionally, the significant SNPs from GWAS 1 were found to explain 7.13% of the total phenotypic variance. From chapter 6, the GWAS performed using UIMF phenotypes in a commercial crossbred population (GWAS 3) yielded no significant SNPs, however after a LASSO was performed which resulted in the identification of three SNPs with measurable effect size (non-zero effect). A 1 Mbp window around these SNPs were investigated for candidate genes, which revealed a number of interesting findings. Genes *ATF7IP*, *NEUREXIN 3* and SH3RF2 were identified in these windows; only *ATF7IP* has previous connections to IMF in the literature, however the described functions of *NEUREXIN 3* and *SH3RF2* warrant further investigation for potential

connections to IMF content. Finally, the proportion of total phenotypic variance explained by SNP variance of the 3 investigated SNPs was 0.69%.

Upon comparison of the crossbred and purebred GWAS results, significantly different outcomes are seen. The comparison of results from the GWAS between populations will aide in the investigation of candidate genes in the purebred animal for improved crossbred performance. The primary differences are, in GWAS 1, five significant SNPs on SSC 1 are identified which explain 7.13% of the total phenotypic variance and GWAS 3 no significant were SNPs are identified, but 3 non-zero SNPs from SSC 2, 5 and 7 are identified through LASSO which explain 0.69% of the total phenotypic variance. There are a number of reasons that different results were seen from these differing populations. Inconsistency in results between studies are often in part due to the breed/population investigated, sample size, and methods of analysis (Marees *et al,* 2018). The power of a GWAS increases with increasing sample size and testing a higher number of markers also requires increased sample size in order to obtain enough instances to be detected. In order to overcome this limitation in future studies the study-design must account for number of markers being tested and increase the sample size coincidingly. Other factors which can affect the outcome of the GWAS are 1) allele frequency of SNPs, 2) the effect size of a SNP, 3) differences in SNP effects in different populations, and 4) selection pressure.

Allele frequency is integral to GWAS: the strength of statistical associations between alleles at different loci depends upon their allele frequencies (Visscher *et al*, 2012). If a SNP has very low allele frequency (rare variant) it is unlikely to be detected by GWAS, particularly because SNP chips used for genotyping are comprised primarily of common SNPs with MAF typically > 0.05 (Visscher *et al*, 2012), as rare variants (MAF < 0.01) will have low LD. Genetic drift and selection can also change allele frequencies, which subsequently change the

genetic architecture of traits; this is because the average effects at the loci which underlie the trait and the contribution of these loci to the genetic variance are altered (Wientjes *et al*, 2019). Populations which are under selection pressure will see changes in the genetic architecture of traits, particularly at loci that are important for the selection indices (Wientjes *et al*, 2019). Additionally, the effect size of a SNP is important; SNPs with large effects are found at lower allele frequencies than alleles with small effects, this is because negative selection acts more strongly to reduce the allele frequency of large-effect SNPs as compared to those with small-effects (Josephs *et al*, 2017). Different populations also result in different SNP effects, as different QTN may underly a certain QTL in different breeds, (Wientjes *et al*, 2015), and different epistatic or dominance interactions may be at play in different breeds as well (Mackay, 2014). Additional methods of GWAS should be conducted with both the purebred and crossbred populations in order to explore any non-additive genetic control of IMF; this would be beneficial to aide in the understanding of how IMF deposition and content differs in these populations would be useful for the application of GS programs. I believe that through use of alternative statistical models, such as those which employ non-infinitesimal SNP distribution models, and those which incorporate biological information (BayesRC) would be beneficial to capturing novel information in regard to the genetic architecture of IMF.

The ultimate goal of these results is for their incorporation into a breeding program in order to aide in the selection of superior animals (improved accuracy of GEBVs); the independent selection of IMF from positively genetically correlated traits (primarily backfat thickness). This incorporation and practical application of GS in pig breeding can be done by weighting markers in the GEBV based on their effect on the trait of interest (Knol *et al*, 2016). Additionally, the development of novel genomic tools, such as new custom SNP panels, can be used to identify animals with the desired genotype in early life.

7.2 General conclusions and implications

Increased understanding of IMF and backfat, how they are genetically related, heritabilities and the genetic architecture of IMF will help in the pursuit of superior meat quality; particularly, the ability to independently manipulate IMF from backfat in order to increase this fat depot separate from increasing overall carcass fatness. We were able to accept our hypothesis' for the first (phenotypic generation) and third (purebred GWAS) studies. The null hypothesis failed to be rejected for the second study (variance component) due to type II error and finally the hypothesis was not accepted for study four (commercial GWAS).

Estimates of variance components for IMF and backfat, generated in chapter 4, in the purebred and crossbred pigs will help provide insight to the traits, their relationships and also serve as a reference to aide in the development of genetic improvement programs. The correlations calculated between the same trait measured by different methods (NIRIMF – UIMF; UBFD-BFT) will also provide excellent opportunity for future research programs; the ability to choose the most suitable phenotypic measurement for the situation while maintaining similar accuracy of final results (GWAS 1 and 2). In addition, the moderate to high heritabilities of IMF and backfat (all methods of measure) show that these traits can be improved through genomic selection; their high genetic correlation also indicates that increased understanding of the genetic architecture of IMF is necessary in order to be independently manipulated. The single-SNP GWAS and LASSO (crossbreds) performed aimed to investigate this; a number of candidate genes were identified in both the purebreds and crossbreds which can be investigated further through subsequent biological pathway analysis. In the purebreds, a number of subregions of high LD were identified, and it is understood any causal mutations or causal loci are most likely where the pairwise LD for adjacent markers peaks (Dorak, 2016).

The commercial crossbred GWAS and LASSO results indicated that there may be non-additive effects at play, which must be validated by future research.

Given that genetic improvement is generally done from the 'top' (nucleus herd) down, to be realised in the commercial animals; any genetic progress gained in the purebreds is moved into the commercial animals through crossbreeding; the continued study of both purebreds and crossbreds is important, as the expression of IMF differs between breeds. There is high genetic correlation (0.99) (Tusell *et al*, 2016) between crossbred and purebred pigs for meat quality traits which indicates that this genetic improvement in the purebreds will indeed be realised in the crossbreds. The incorporation of crossbred information with purebred information into GS programs helps to increase the accuracy of GEBVs and increase the rate of genetic improvement.

7.3 Future recommendations

1) For the generation of NIR phenotypes, calibrations and standards should be created from populations which match the study population (breed, sample collection method and storage). This would ensure greater accuracy of the algorithm, thereby increasing the accuracy of predicted phenotypes.
2) Biological pathway analysis for the candidate genes identified, as well as LD mapping for the subregions identified in GWAS 1 would provide an interesting research opportunity. This would also help increase the understanding of the biological basis of IMF and aide in its independent manipulation.
3) Additive genetic models were used; favourable breed combinations/ breed complementarity is a large factor in the swine industry which contributes to not only the overall profitability of commercial swine operations but is imperative to realising

genetic improvement. Extending the models to include dominance effects would be interesting.

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Appendix A

Supplemental Workbook Dataset A: **Supplemental Workbook Dataset A Hyperlink**

Supplemental Workbook Dataset B: **Supplemental Workbook Dataset B Hyperlink**