ABSTRACT

KHANAL, PIUSH. Genomic Selection of Meat Quality and Carcass Composition Traits of Crossbred Swine. (Under the direction of Dr. Christian Maltecca).

Meat quality and carcass traits are becoming important in swine industry because of their high economic value. Understanding the genetic basis of these traits is necessary to include these traits in breeding objectives. Therefore, the overall objective of this study was to estimate the genetic parameter and prediction accuracy of meat quality and carcass traits utilizing the pedigree information, genomic information and gut microbiome information. In the first study, two different datasets were obtained from The Maschhoffs LLC (TML) and Smithfield Premium Genetics. The TML datasets consists of 1,254 crossbred pigs genotyped with 60K SNP chip and phenotyped for meat quality and carcass traits. The SPG population included over 35,000 crossbred pigs phenotyped for meat quality and carcass traits. In the first study, genetic and phenotypic parameters for meat quality and carcass traits were estimated using pedigree and genomic based analysis. Heritabilities [high posterior density interval] of meat quality traits ranged from 0.08[0.03, 0.16] for pH and 0.08[0.03, 0.1] for Minolta b* to 0.27[0.22, 0.32] for marbling score, except intramuscular fat with the highest estimate 0.52[0.40, 0.62]. Heritabilities of primal yield traits were higher than that of primal weight traits and ranged from 0.17[0.13, 0.25] for butt yield to 0.45[0.36, 0.55] for ham yield. The genetic correlations of meat quality and carcass traits ranged from moderate to high in both directions. Second and third study were conducted from the TML population, from which fecal 16S microbial sequences were obtained at three different stages: weaning (Wean: 18.64± 1.09days), 15 weeks after weaning (Mid-test: 118.2±1.18 days) and end of test (Off-test: 196.4±7.80 days). Microbiome information was modeled using microbial relationship matrix among individuals. In the second study, data were analyzed to estimate microbiability and microbial correlation to investigate their effect on heritability and genetic

correlation between meat quality and carcass traits at different stages of production. The contribution of microbiome to all traits was significant with the effects increasing over time, from Wean to Off-test. Microbiome did not affect the estimates of heritability of meat quality traits. However, decrease in heritability was observed for majority of carcass traits at Off-test and Mid-test when microbiome information was added in the model. The decrease in heritability was up to 10% for fat depth. High microbial correlations were found among majority of the traits, particularly with traits related to fat deposition. Decreased genomic correlation was observed among those traits which has high microbial correlation suggesting that, decreased portion of genomic correlation was due to genetic covariance among the microbiome composition affecting those traits. In the third study, genomic and microbial prediction of meat quality and carcass traits were evaluated and contribution of host-microbiome interactions to the predictive ability was assessed. The inclusion of microbiome yielded higher predictive ability for fat related traits (fat depth, belly weight, intramuscular fat and subjective marbling score), Minolta color related traits (Minolta a*, Minolta b* and Minolta L*) and carcass average daily gain. The proportion of variance explained by host genome-microbiome interaction was largest for fat depth (~20% at Mid-test and Off-test) and shearing force (~20% consistently across Wean, Mid-test and Off-test). Microbiome information collected at later stages of life was better predictor of meat quality and carcass traits compared to earlier measures. The genome-microbiome interaction yielded higher predictive ability only for fat depth and belly weight. Overall, results suggested that gut microbial composition can contribute to the improvement of complex traits in swine.

Genomic Selection of Meat Quality and Carcass Composition Traits of Crossbred Swine

by
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DEDICATION

To my parents who showed me the value in education.

BIOGRAPHY

Piush Khanal was born and raised on Chitwan, Nepal. After graduating from high school, Piush started his undergraduate degree in Bachelor of Veterinary Science and Animal Husbandry (B.V.Sc. & A. H.) in Institute of Agriculture and Animal Science, Rampur Campus at Tribhuvan University. In fall of 2014, Piush came to United States to pursue his further education on animal science and joined Tennessee State University. He conducted his research under the supervision of Dr. Richard Browning Jr. in the area of crossbreeding of goat and selection of replacement female goat. Piush graduated with Master of Science (MS) degree in Animal Science on August 2016. Then, Piush joined North Carolina State University (NCSU) for PhD program in Animal breeding and genetics starting from fall 2016, also obtaining Masters of Statistical genetics. At NCSU he has been working for his doctoral research in genomic selection of meat quality and carcass composition traits of crossbred swine. His dissertation project involved the elucidation of genomic control of pig host on abundance and composition of gut microbiome. He was involved in construction of predictive models which incorporate the genomic and microbial information in the model used for genetic evaluation.

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CHAPTER 1: Literature Review

1.1 Introduction

In the past decade, the pork industry has been more focused on production traits like leanness, feed efficiency, growth, and backfat thickness (Ciobanu et al., 2011). Recently, meat quality and carcass composition traits are becoming important in the swine industry because of higher demand for quality meat by the consumers (Dransfield et al., 2005). Consumers are interested in juiciness, tenderness, flavor, and color of meat (Ciobanu et al., 2011), and different primal parts of meat (Miar et al., 2014a). As a result, the pork industry is moving towards the grading of different primal cuts, and the producers are being paid based on different primal cuts, color, and marbling score. Meat quality and carcass traits are affected by the genetics of pig, muscle physiology and characteristics, slaughter practices, and post-mortem conditions (Schaeffer, 2006). Variation in carcass and meat quality traits was significantly affected by the genetic difference between breed and within breed (Plastow et al., 2005). So, the selection based on meat quality and carcass traits is essential. However, measurement of different primal cuts and meat quality traits on a routine basis is expensive and difficult since these traits can only be measured post-mortem and the selection becomes difficult. Genomic selection could be useful for those traits (Lee et al., 2015). Genomic selection provides an increased rate of genetic gain per unit time with improved selection accuracy without costly and time-consuming progeny testing (van der Werf, 2013). The costs associated with measurement of carcass quality and increased preference of consumers towards meat quality and primal cuts warrant the estimation of genetic parameters associated with meat quality and carcass traits. A novel strategy should be implemented to estimate the genetic parameters, prediction accuracy of pork quality and carcass traits in order to implement selection program that focus on meat quality and carcass traits.

1.2 Genomic selection

Marker-assisted selection using molecular marker data has been proposed in the past as a way to increase the accuracy of selection in pigs. Nonetheless its application remained limited since markers typically capture only a limited proportion of total genetic variance. This can be overcome by using high-density markers across the entire genome to capture total genetic variance for a particular trait of interest. This method was termed genomic selection by Meuwissen et al. (2001). Genomic selection exploits linkage disequilibrium. Since the markers are in LD with QTL, it is assumed that the effect of the chromosome segments will be same across the population. Hence, the marker density must be sufficiently high to ensure that all QTL are in LD with a marker or haplotype of markers Meuwissen et al. (2001). Genomic selection has become possible with the availability of thousands of markers and high throughput genotyping technology.

Genomic selection needs a reference population that has genotypic and phenotypic information to derive a prediction equation of genomic estimated breeding values (GEBV) for selection candidates that have marker information without phenotypic record (Goddard and Hayes, 2009). To predict GEBV for animals with genotypes but no phenotypes, the effect of the chromosome segments they carry can be summed across the genome:

$$\text{GEBV} = \sum_{i}^{n} X_i \quad \widehat{g_i}$$

where n is the number of chromosome segments across the genome, $\boldsymbol{X_i}$ is a design matrix allocating marker effects at segment i and $\widehat{g_i}$ is the vector of effects of markers within chromosome segment i. Subsequently, this GEBV is applied to the population with genotypic information but without phenotypic information (selection population) to predict GEBV for each individual to select the best animals for future breeding (Goddard and Hayes, 2009). Genomic selection helps to predict the genetic potential of animal at early age even without phenotypic records. It helps to

increase in rate of genetic gain by decreasing the generation interval especially for animals having long generation interval like cattle and reducing the genotyping cost. Although the generation interval in swine is not large as compared to cattle, it is useful for meat quality traits, which could be measured only after the pigs are slaughtered. The accuracy of selection will be increased with selecting the animal with genotypes instead of selecting the animals with only phenotypic information and information from their relatives using traditional BLUP (Meuwissen et al., 2001).

1.2.1 Genomic Best Linear Unbiased Prediction (GBLUP) method

The genomic best linear unbiased prediction (GBLUP) method utilizes the genomic relationships to estimate the genetic merit of an individual. For this method, genomic relationship matrix is used, which is estimated from DNA marker information (Clark and van der Werf, 2013). This method was first proposed by Nejati-Javaremi et al. (1997). They compared the method of estimating breeding values with the use of total allelic relationship to the pedigree-derived additive genetic relationship. They concluded that using total allelic relationship gives more accurate breeding values than using pedigree-derived additive genetic relationship matrix because it accounts the average measures of relationship and identity in state of alleles, which eventually reduces the sources of variance of prediction. The model for GBLUP sums the SNP effect and estimate GEBV as the sum of these effects with the assumption that the markers are normally distributed as Hayes and Goddard, (2010). The model could be written as:

$$y = 1_n \mu + Zg + e$$

where y is a vector of phenotypes, μ is the mean, 1_n is a vector of ones, n is the number of observations, **Z** is a design matrix allocating records to breeding values, g is a vector of breeding values and e is a vector of residual error with assumption of N (0, σ_e^2). Here, g = **W**u where u_j is the SNP effect of the jth SNP and variance V(g) = **WW**$^{'}$ σ_u^2. Each element of matrix **W** are w_{ij} for

i[th] animal and j[th] SNP, where w_{ij} = 0 - $2p_j$ if the animal is homozygous AA at j[th] SNP, 1 - $2p_j$ if the animal is heterozygous, and 2 - $2p_j$ if the animal is homozygous aa at the j[th] SNP. In GBLUP the breeding values could be predicted as shown:

$$[\hat{g}] = \left[Z'Z \quad G^{-1}\frac{\sigma_e^2}{\sigma_g^2}\right]^{-1} [Z'y]$$

where **G** is genomic relationship matrix, σ_e^2 is error variance and σ_g^2 is genetic variance and other elements are as described above. This method is useful for the populations without good pedigree records where the genomic relationship matrix captures the relationship information among the genotyped individual. VanRaden et al. (2009) reported the increase in breeding value accuracies of 20-50% with the use of genomic relationship matrix in comparison to use of pedigree relationship matrix in dairy cattle.

1.2.2 Ridge regression

In order to overcome the problem of over-estimation of segment effects in marker assisted selection, Whittaker et al. (2000) proposed ridge regression for the prediction in quantitative genetics. Ridge regression assumes that all marker effects are normally distributed with equal variance. In ridge regression, estimates of marker effect are shrunk towards zero to avoid the over-estimation of these effects. Ridge regression uses the mixed model equation with the shrinkage parameter (λ) to the diagonal elements of least squares equation as shown in equation:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + I\lambda \end{bmatrix} \begin{bmatrix} \beta \\ b \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix}$$

where y is a vector of phenotypic observation, **X** is the design matrix of fixed effects, **Z** is the design matrix for the random effects, **R** is the covariance matrix for the residual errors, **I** is the identity matrix and λ is a vector of shrinkage parameter.

Ridge regression is not appropriate to estimate the SNP effects for QTL mapping because the same magnitude of shrinkage is applied to all SNPs (Xu, 2003). Since, all of the loci in genome did not contribute equally to the phenotypes, those SNPs which have least to almost no contribution should be penalized more in the analysis. This idea leads to development of current Bayesian methods (Meuwissen et al., 2001).

1.2.3 Bayesian methods

The data were modeled in two levels in bayesian methods. The first is at the level of data and second is at the level of variance of chromosome segments. The first one at level of data is shown below:

$$y = \mu 1_n + X_i g_i + e$$

where y is the data vector, μ is the overall mean, 1_n is a vector of n (n= number of records) ones, g_i represents the genetic effects of the haplotypes at the ith segments with $g_i \sim (0, \sigma^2_{gi})$. The second level of model is at the variances of chromosome segments. Meuwissen et al. (2001) described that many QTL in the chromosome segments have small effect and few with large effect. The prior distribution was scaled inverted chi-square distribution, prior $(\sigma^2_{gi}) \sim \chi^{-2}(\upsilon, S)$, where S is the scale parameter and υ is the number of degrees of freedom. Different bayesian methods differ in the hypotheses of distributions of marker effects. Three different bayesian methods have been used frequently in animal breeding and genetics: Bayes A, Bayes B and Bayes C. Bayes A and Bayes B were proposed by Meuwissen et al. (2001) and Bayes C was proposed by Kizilkaya et al. (2010). Bayes A approach assumes that variance of marker effects differs among different loci and this approach considers all markers in the model (Su et al. 2010). So, this method is computational less efficient in comparison to other Bayesian approaches because of large number of effects in the model and no marker is assumed to have zero variance. Bayes B approach assumes different

variances of SNP effects, with many SNP having contribution zero effect and few contribute large effect on a trait (Meuwissen et al., 2001). It considers a proportion π of the markers having zero effect. The distribution of variances of the effect of markers could be written as: $\sigma_{gi}^2 = 0$ with probability π and $\sigma_{gi}^2 \sim \chi^{-2}(\upsilon,S)$, with probability (1- π). This approach needs less computational time than Bayes A because it excludes the loci with no effect on quantitative trait of interest.

The Bayes C method was developed by Kizilkaya et al. (2010). New approaches of Bayes Cπ and Bayes Dπ have been described by Habier et al. (2011) as extension of Bayes C approach. The Bayes C method differs from Bayes B by assuming common variance for all markers. In Bayes C, the probability π that a SNP has a nonzero effect is assumed to be known. The model assumed homogenous variance of effects on all loci: $\sigma_{gi}^2 = 0$ with probability 1- π; $\sigma_{gi}^2 \sim \chi^{-2}(\upsilon, S)$. In Bayes C, the probability π that a SNP has a nonzero effect is assumed to be known. Habier et al. (2011) modified the Bayes C and Bayes D where the probability π of has been estimated. The prior distribution of π becomes uniform over [0,1]. Similar to Bayes C, the SNP model is: $P(g_i|\pi, \sigma_{gi}^2) = 0$ with a probability of 1 – π; $P(g_i|\pi, \sigma_{gi}^2) \sim N(0, \sigma_{gi}^2)$ where $P(\sigma_{gi}^2) \sim \chi^{-2}(\upsilon, S)$ with probability π. Bayes R proposed by Erbe et al. (2012) used a mixture of normal distributions as the prior for SNP effects, including one distribution that set SNP effects to zero. Brøndum et al. (2012) proposed Bayes RS approach where prior estimates of the proportion of variance from different chromosome segments were used to weight the priors for each segment. Bayes RC approach was developed for multi-population genomic prediction (MacLeod et al., 2016), which used the same approach as Bayes R except that a priori independent biological information was used to allocate each variant to a specific class. Generally, two or more classes are assigned to the variant that are enriched for QTL.

1.2.4 Single step versus multiple step BLUP for genomic selection

Misztal et al. (2009) proposed single step method for genomic evaluation in which pedigree-based relationship matrix was integrated with genomic relationship matrix into single matrix (H) matrix. The joint relationship matrix based on pedigree relationship and genomic relationship was proposed by Legarra et al. (2009). Single step method is simple, faster, more accurate with less error and applicable to complicated model complicated to multiple step method (Aguilar et al., 2010). Multi step procedure involve evaluation using traditional pedigree BLUP, extraction of pseudo-observations for genotyped individuals, estimation of allelic effect for each SNP and combination of parent average with the genomic prediction (VanRaden, 2008a). However, single step method utilizes phenotypic records, genotype and pedigree simultaneously to derive GEBV (Misztal et al., 2013). Single step method is faster and less prone to errors. So, Single step GBLUP has been successfully implemented in large scale analyses of dairy (Tsuruta et al., 2011; VanRaden, 2012) and pigs (Forni et al., 2011; Christensen et al., 2012) with less inflation of GEBV than multi steps procedure.

1.2.5 Factors affecting the genomic selection

Major factors affecting genomic selection are as follows:

i) Number of markers required

Number of markers is one of the important factors affecting accuracy (Hayes and Goddard, 2010). The number of markers required depends on the linkage disequilibrium (LD) of species. The haplotype or single markers must be in enough LD with the QTL such that the prediction of QTL effects across population is possible. If there is not enough LD between SNPs and QTL, more SNPs are needed to increase the power of QTL detection. On the simulation study, Calus et al.

(2008) evaluated the effect of the average LD between adjacent marker pairs on the accuracy of genomic selection.

ii) Number of phenotypic records in the reference population

The accuracy of genomic selection depends on the number of haplotype effects at the chromosome segments, and the number of phenotypic records per unique haplotype, of per marker allele if single markers are used. Higher accuracy is obtained if more phenotypic records are available because of the presence of more number of observations per haplotype (Meuwissen et al., 2001). Furthermore, Meuwissen et al. (2001) reported that accuracy of least squares, BLUP and Bayes B increased with more phenotypic records.

1.3 Genetics of meat quality and carcass traits

Estimation of genetic parameters such as heritability, genetic correlation and variance components are necessary for the improvement of meat quality and carcass traits. Various factors like population size, breeds, genomic information, pedigree, environmental factors and statistical model affect the variance components (Khanal et al., 2019). Estimates of heritability of meat and carcass quality traits and genetic correlation among these traits has recently received attention (Miar et al., 2014a). The published estimates of heritability of meat quality and carcass composition traits are presented in Table 1. All of these studies were conducted only with pedigree information. To the best of my knowledge, studies on estimation of heritabilities and genetic correlation with the inclusion of both pedigree and genomic information is lacking, particularly in crossbred swine.

In brief, carcass composition traits exhibit moderate to high heritability which range from 0.28 to 0.54 and meat quality traits exhibit low to moderate heritability which range from 0.16 to 0.29. Correlations between growth rate and meat quality traits were generally small and negative,

however their magnitude depends upon the breed (Ducos et al., 1993; De Vries et al., 1994). The range of correlations among meat quality and carcass traits were wide in both positive and negative direction (Van Wijk et al., 2005; Miar et al., 2014a; Miar et al., 2014b). Marbling and color traits are important traits as these could be visually seen by the consumers (Wilson et al., 2017). Marbling score had high correlation with intramuscular fat. Intramuscular fat had high correlation with color traits (Hermesch et al., 2000) indicating the pale color of meat when the intramuscular fat is high which implied that the pale color might be due to intramuscular fat in pork. pH, another important meat quality traits had high positive correlation with color traits (Hovenier et al., 1992). The genetic correlations among carcass traits were high (Lo et al., 1992; Hermesch et al., 2000; Van Wijk et al., 2005; Miar et al., 2014a; Miar et al., 2014b). Van Wijk et al. (2005) reported that fat depth was negatively correlated with primal cuts and selection of lean meat did not affect the meat quality traits. Furthermore Van Wijk et al. (2005) also reported that the subjective or objective color traits were favorably correlated with lean meat. Favorable correlation of meat quality traits and primal cuts were found by Van Wijk et al. (2005) and (Miar et al. (2014a). There were no significant correlations between meat quality traits and average daily gain and loin depth (De Vries et al., 1994; Suzuki et al., 2005; Miar et al., 2014b). However, Van Wijk et al. (2005) reported that average daily gain has a detrimental effect on pork quality. To the best of my knowledge, correlations of meat quality traits and primal yield traits (proportion of primal cuts to total hot carcass weight) has not been studied.

1.4 Microbiome

The gut microbiome has been defined as the community of microorganisms, bacteria, viruses, protozoa, and fungi present in the gastrointestinal tract (Nowland et al., 2019). It is the complex interactive community of organisms whose functions are the result of activities of all

microbial components. In early time, microbes have been viewed as pathogens affecting health. However, the understanding of microbiome is increased as a concept of animal "flora" in the past few decades due to advancement in genomic sequencing technologies and "omics" (e.g., proteomics, transcriptomics, metabolomics) (Mach et al., 2015a; Zhao et al., 2015).

1.4.1 Development of gut microbiome in pigs

The gastrointestinal tract of piglet is sterile during parturition. Gut microbiota starts developing rapidly. Initially the gut microbes in newborn piglets come from sows or environment (Ducluzeau, 1983) and there is possibility of having similar gut microbiota to that of sows as they are in close contact with sows through different means (Nowland et al., 2019). Then the gut microbes shift remarkably in early age (Wang et al., 2019) and become unique and characteristic to each individual (Nowland et al., 2019), which is governed by various internal and external factors as host genotype, diet, antibiotics. The growth of bacteria is followed by different successional step where abundant bacteria become predominant in the gut. The succession continues until there is stable bacteria (Zoetendal et al., 2001; Thompson et al., 2008). Initially, the suckling piglets were represented by aerobes and facultative anaerobes from the sow and environment. In very early age, *Escherichia coli*, *Lactobacilli*, *Streptococci* and *Shigella* constituted as the dominant bacterial species in gut (Swords et al., 1993; Fouhse et al., 2016). As time passes, these bacteria consume oxygen and creates the anaerobic environment. *Lactobacilli* and *Streptococci* become more dominant. The anaerobic bacteria are diversified in the distal part of gut where predominant species are *Bacteroides*, *Eubacterium*, *Peptostreptococcus* and *Clostridium* (Tannock, 1990; Dicksved et al., 2015). The number of bacteria in pig colon has been estimated to be 10^{10}-10^{11} per gram of the gut content (Guevarra et al., 2019). Guevarra et al. (2019) further reported that the weaning transition caused great influence in gut microbiome because of

the change in diet and due to the stress. The weaning transition affects the overall health and growth performance of the individual throughout the life and is critical to understand the dynamic of gut microbiome at this phase of life (Lu et al., 2018).

1.4.2 Diversity of gut microbiota

There are different ways to measure the microbial diversity in pigs. Whittaker (1960) proposed the terminology alpha, beta and gamma diversity. Two most common measure of diversity in pigs are alpha (α) and beta (β) diversity. Alpha diversity is the measure of diversity within sample. Alpha diversity can be measured by Shannon, Simpson and Chao1 indices. These indices are unitless and only useful to compare samples or group of samples. Alpha diversity measures the species richness and species diversity. The term "species richness" was first introduced by (McIntosh, 1967) and is estimated as dividing the number of species by the geographical area (Simpson, 1964). Species diversity is a measure of diversity which accounts for both the number of species present and relative abundance of each species. Species richness is easier to measure than species diversity, as measuring diversity requires measurement of species richness, species evenness and species abundance (Sanjit and Bhatt, 2005). The Shannon diversity index was measured as:

$$-\sum_{i=1}^{n} p_i \, ln(p_i)$$

where p_i was the proportional abundance of i^{th} OTU (Ortiz-Burgos, 2016). This is always a positive value, ranging from zero to log (number of species) and is considered as the best method to estimate the diversity (Kim et al., 2012). Higher value of Shannon index indicates greater diversity. Simpson's diversity index was measured as:

$$\frac{\sum n(n-1)}{N(N-1)}$$

where n is the total number of organisms of species of each type and N is the total number of organisms of all species (Simpson, 1949; Lemos et al., 2011). It varies from 0 to 1 and the index increases as the diversity increases. Chao 1 richness estimator is the non-parametric that calculates the minimal number of OTUs present in the sample (Chao, 1984). Chao 1 was estimated as:

$$S_{Chao\ 1} = S_{obs} + \frac{F_1(F_1-1)}{2(F_2+1)}$$

where S_{obs} is the number of observed species, F_1 and F_2 are the count of singletons and doubletons, respectively (Chao, 1984). Chao richness estimator gives more weight to singleton and doubletons to estimate the number of missing species. So, this index is particularly used for data skewed towards the low-abundance species (Hughes et al., 2001). The abundance-based coverage estimator (ACE) is a nonparametric method for estimating the number of species using sample coverage, which is derived as:

$$S_{ACE} = S_{abund} + \frac{S_{rare}}{C_{ACE}} + \frac{F_1}{C_{ACE}}\gamma_{ACE}^2$$

where S_{abund} and S_{rare} are the number of abundant and rare OTUs respectively, C_{ACE} is the sample abundance coverage estimator, F_1 is the frequency of singletons and γ_{ACE}^2 is the estimated coefficient of variation for rare OTUs (Chao and Lee, 1992; Kim et al., 2017).

Beta diversity is the measure of a degree to which samples differ from each other and can expose some important aspect of microbial ecology that are not apparent from visualizing the composition of individual samples. Beta diversity metrics are robust to issues such as low sequence counts and noise (Goodrich et al., 2014). Beta diversity metrics that are popular in microbiome studies include Jaccard, Bray-Curtis, Moristita- Horn and Sorenson (Lozupone and Knight, 2008). Some of the metrics take abundance into account (e.g., diversity: Bray Curtis, weighted UniFrac) and some of them only calculate the diversity based on presence-absence (e.g., richness: Jaccard, unweighted UniFrac) (Lozupone et al., 2007). Principal component and co-ordinate plots are commonly used

to estimate beta diversity. The best way to visualize beta-diversity is by non-metric multidimensional scaling (nMDS). This is similar to principle component analysis. However, nMDS is statistically more robust, with multiple iterations (Kruskal, 1964).

Alpha diversity is related to better gut health in human (Le Chatelier et al., 2013) and higher alpha diversity helps to predict the disease condition in human (Prehn-Kristensen et al., 2018). There was increase in alpha diversity with the increase in age (Kim et al., 2011; Looft and Allen, 2012; Frese et al., 2015; Odamaki et al., 2016; Chen et al., 2017). Variation in gut microbe diversity has been found in different breeds of pig (Yang et al., 2014) and in different stages of production (Lu et al., 2018) and different location of intestine (Isaacson and Kim, 2012).

As the host genetics affects the gut microbiome to some extent the diversity in microbiome is treated as separated traits (Camarinha-Silva et al., 2017) and different studies (Dou et al., 2017; Lu et al., 2018) have explained the relationship of alpha diversity with the complex phenotypes . Sandoval-Motta et al. (2017) reported that the microbial diversity of intestine accounted for significant amount of phenotypic variation for any trait in human and should be accounted when assessing the heritability not only in human but also in plants and livestock. Lu et al. (2018) reported that alpha diversity is strongly correlated with back fat thickness and average daily gain in swine. Higher alpha diversity has been associated with reduced susceptibility to post-weaning diarrhea (Dou et al., 2017).

1.4.3 Microbiome genomics and bioinformatics

Detailed characterization of entire gut microbiome has been done mainly by two techniques: i) amplicon sequencing and ii) metagenomics. The amplicon sequencing generates taxonomic compositional microbiota profiles at lower cost than that of metagenomics which is beneficial to run large scale project on microbial analysis. The metagenomic sequencing provides

more comprehensive sampling of all genes in all organisms present in a given complex sample. This method enables us to evaluate bacterial diversity and detect the abundance of microbes in various environments.

i) Microbiome profiling by amplicon sequencing

Amplicon sequencing is the most widely used method for characterizing the diversity of microbiota. In this method, DNA is extracted from all cells in the sample. It is a targeted approach that allows researchers to analyze-genetic variation in specific genomic regions which is useful for the discovery of rare somatic mutations in complex samples (Fricker et al., 2019). It depends on the selective binding of the primers that is common to all the organism to the highly conserved regions within the genomes of microbiome and sequencing of the resulting PCR products (Fricker et al., 2019). The most commonly used target amplicon for microbial sequencing is bacterial 16S rRNA gene across multiple species for phylogeny and taxonomy studies, but universal primer pairs have also been described for archaeal and eukaryotic small subunit ribosomal RNA genes, internal transcribed spacer (ITS) of the fungal and other ribosomal RNA operons and other conserved genomic loci (Kittelmann et al., 2013). Numerous primer combinations have been used to amplify different hypervariable region (Fricker et al., 2019) within 16S rRNA gene. The 16S rRNA sequences generated from microbiomes are traditionally clustered into operation taxonomic units (OTU) at a few distance levels to determine species richness, diversity, composition, and community structure. Operational taxonomic units are defined at a phylogenetic distance (0.03 – species, 0.05- genus, 0.10- family) based on full-length 16S rRNA gene sequences (Kim et al., 2011). Generally, V1-V4 regions can provide more accurate estimates than other regions sequences (Kim et al., 2011). For the formation of OTU, similar sequences (even slight difference) are clustered into the same taxon assuming that they share common biological origin. Fungal

microbiota analysis with the help of ITS amplicon sequencing does not look like exactly the same sequence but grouped based on similar sequence, there could be loss of accuracy in determining the biological variation among different sequences. To differentiate the biological and technical sequence variation, Dada2 (Callahan et al., 2016) and Deblur (Amir et al., 2017) has been implemented in QIIME2 (Caporaso et al., 2010), a software package for 16S rRNA analysis. Dada2 and Deblur provides the sensitive means to assess ecological patterns driven by differentiation of closely related taxa. Briefly, these tools generate error profiles of amplicon sequence and use this profile to resolve the sequencing pair. Recently, it was found that amplicon sequence variants (ASV) provides more sensitivity and specificity and reduced the problem of falsely identifying OTU due to error in clustering (Callahan et al., 2017).

ii) Microbiome profiling by shotgun metagenomics sequencing

Shotgun metagenomic sequencing is an alternative approach to study the microbiota. Shotgun metagenomics uses the whole-genome shot gun method to fragment and sequence the entire genome instead of 16S rRNA. In this method DNA sequences of microbiome is aligned to various genomic locations of all genomes present in sample, including non-microbes. The reads are sampled from taxonomically informative loci and some reads are sampled from general coding sequences (Sharpton, 2014). Shotgun metagenomics help to analyze the unculturable microorganism that are difficult or impossible to analyze and produces high-complexity datasets with millions of short-reads (Quince et al., 2017). Despite its benefit, this approach is being less implemented since this is expensive than 16S rRNA, it requires greater coverage depth and the data analysis is more complex (Knight et al., 2012). Most genomes are not represented by reads because of diversity of microbial communities in samples. With this method, identification of the group could be difficult because matching of specific genes to taxonomic groups is hard.

1.4.4 Data processing of microbiome count data

High throughput sequencing technologies described in previous sections has been advantageous for understanding the microbial communities. However, processing and interpretation of data is complicated because of several statistical challenges (Weiss et al., 2017). After quality control steps for sequencing error, microbial sequencing data is organized into table where column represents sample and row represents counts of clustered sequences known as operational taxonomic units (OTUs). Most often vast difference in total number of sequences per sample is found even though the samples are sequenced in the same sequencing machine at same time. Difference in the counts may be due to the differential sequencing efficiency of machine rather than true biological variation. OTU table could be sparse due to insufficient sequencing depth to detect some sample and some organisms are found in only a small percentage of samples (Paulson et al., 2013). Total number of reads does not represent the absolute number of microbes per sample as the sample is only a part of total environment (Weiss et al., 2017). To address these issues, normalization of the data is done via different methods prior to downstream analysis. Normalization is the process which identify and remove the systematic technical differences between samples that occur in data and ensure minimal impact on results by technical bias (González-Recio et al., 2014). For normalization an appropriate baseline is chosen and the OTU counts are expressed relative to that baseline. Weiss et al. (2017) and McMurdie and Holmes (2014) described different normalization methods which are:

i) Rarefy: Rarefying is most common method of normalization of OTU count data. During rarefaction minimum library size is selected which is known as rarefaction level. The samples that have fewer reads than the rarefaction level is discarded, and each column is subsampled to equal depth without replacement. Rarefying is not ideal method of normalization because it might reduce

the statistical power as some data is removed and this does not address the challenge of compositional microbiome data (Gloor et al., 2017).

ii) Cumulative Sum Scaling: Cumulative sum scaling enables scaling of sample by specific percentile of its count distribution and counts are log transformed. This method scales the segment of each sample's count distribution that is relatively similar across samples. This method is useful to remove the influence of larger count values in the OTU table.

iii) Variance stabilization: In this method, a scaling factor for each OTU is calculated by dividing OTU count by geometric mean across all samples. Then, all reads of each sample are divided by the median of scaling factors for that sample. Using the scaled count for OTUs, negative binomial distribution general linear model is used to adjust the matrix counts such that the variance in an OTU's counts across samples is independent of mean (Weiss et al., 2017).

iv) Trimmed Mean of M-values (TMM): Robinson and Oshlack (2010) proposed this method. The scaling factor of trimmed mean is the average by removing the upper and lower counts. This minimizes the log-fold change between samples for most OTUs. The TMM scaling factors are usually around 1. The normalization factors for each sample are the product of the TMM scaling factor and the original library size. This method assumes that most microbes are not differentially abundant and even if those are, there is an approximately balanced amount of change in abundance. So, these methods are not appropriate for highly diverse microbes.

1.4.5 Function of gut microbiome in pig

There is symbiotic relationship between pigs and gut microbes (Crespo-Piazuelo et al., 2018) where the pig provides a spacious, anaerobic and nutritious environment and different composition of gut microbes plays important role in immunological, physiological and nutritional functions of pigs (Fouhse et al., 2016). In principle the function of gut microbiome is to get energy

from undigested feed components from the gut through fermentation and providing the basis for a barrier that prevents pathogenic bacteria from invading through gut epithelium, perform protective function together with host immune system (Salminen et al., 1998). The two major functions are:

i) Nutritional function

Gut microbiota metabolizes various foods and provide nutritional compound to the host in the form of fermentation end-products and other by-products such as short chain fatty acid (SCFAs), amino acids, vitamin B and K, and indole derivatives (Feng et al., 2018). Den Besten et al. (2013) reported that bacteria generate SCFAs like acetate, propionate, butyrate, formate, valerate, capronate etc. SCFAs play important role in maintaining the morphology and functions of epithelial cells. Specifically, SCFAs reduce the luminal pH and enhance the nutrient absorption by gut epithelial cell proliferation and differentiation, which result in increased intestinal villus height (Khan et al., 2016). Shibata et al. (2017) reported that SCFAs help in inhibition of pathogenic bacteria of host. Short chain fatty acids act as a specific G protein coupled receptor signaling molecule which help in the process of gluconeogenesis (Den Besten et al., 2013; Khan et al., 2016). Other by-products of fermentations like vitamins, indole derivatives help in growth and development of the intestine and improves the absorption of nutrients (Khan et al., 2016).

ii) Immune function

Gut microbiota interacts with epithelial cells, which is essential for immune system development, maturation and maintenance of homeostasis (Nowland et al., 2019). The interaction of microbes with mucosa is complex phenomenon which involves the cross-talk between the microbes, and microbes and host (Jha and Berrocoso, 2015; Corfield, 2018). The microbiota degradation of mucin polymeric glycoprotein to various monosaccharides like N-acetylglucosamine and fucose, which in turn is used for growth of microbiota itself in intestine

(Hooper et al., 2002). Gut microbiota acts on intestinal permeability, glycosylation of the intestinal cell layer, which helps in resistance to infection and expression of microbiocidal and angiogenins (Pickard et al., 2017). Hooper et al. (2001) reported that gut microbiota influences the gene expression of the epithelium which could be responsible for the immunity. “Mucosal firewall” has been termed by Macpherson et al. (2009) for the combined action of the epithelial cells, mucus, IgA, immune cells. Generally, the “mucosal firewall” is beneficial but some interactions of bacteria and host mucosa might lead to diseased conditions like Crohn’s disease or ulcerative colitis (Joossens et al., 2011). The hematopoietic and non-hematopoietic cells of innate immune system are located on host-microbiome interface, which has the ability to sense the metabolic products that translates the signal to the physiological response of host (Thaiss et al., 2016). Tang et al. (2019) reported that the loss of gut microbiome alters the immune system. Smaller quantities of Peyer’s patch, mesenteric lymph node, gut-associated lymphoid tissue is found in germ-free animals (Iebba et al., 2012). Furthermore, previous researchers (Turnbaugh et al., 2009; Cahenzli et al., 2013; Markle et al., 2013) reported that those animals with disrupted microbiota or reduced microbial diversity are with increased risk of obesity, cardiovascular disease, asthma and autism.

1.4.6 Effect of microbiome on host

Due to the advancement in “omics” technologies in recent years, research interest about the role of gut microbiome in animal health and different phenotypes has been increased (Park et al., 2014; Guevarra et al., 2019). Few researches has been conducted to estimate the effect of gut microbiome on host phenotypes although gut microbiome has been considered as second genome (Zhu et al., 2010; Sommer and Bäckhed, 2013). Dąbrowska and Witkiewicz (2016) linked the host genetics profile to the different gut composition in human although the cause and consequence of

those relationship are unknown. The gut microbiome of all animals influence the physiology and fitness of host such as growth and development (Gould et al., 2018).

The correlation between gut microbiome and different phenotypes has been studied by different authors in human (Goodrich et al., 2014; Chen et al., 2018), cattle (Difford et al., 2018) and swine (He et al., 2016; Ramayo-Caldas et al., 2016; Camarinha-Silva et al., 2017; Xiao et al., 2018). Gut microbiome has direct effect on obesity of human (Backhed et al., 2004; Ley, 2010). Chen et al. (2018) identified 81 and 67 microbial taxa with heritability greater than 0.15 in fecal and cecum luminal samples respectively. Goodrich et al. (2014) reported that 5.3% of bacterial taxa had heritability estimate of greater than 0.2 in stool samples of dizygotic and monozygotic twins in human. Difford et al. (2016) termed "microbiability" as the proportion of total variance explained by microbiome for performance trait in dairy cattle. Difford et al. (2018) reported that the variation in methane emission in dairy cattle was partly due to the ruminal microbiome (15%) and the authors found that the model containing the microbial composition and genetic component as random effect is significantly better than the model containing only genomic information in dairy cattle. Camarinha-Silva et al. (2017) reported that microbial prediction accuracy was better than genomic prediction accuracy for daily gain, feed conversion and feed intake respectively. However, it is difficult to determine the prediction accuracy through microbiome because microbiome change through the environment, diet etc (Pajarillo et al., 2014; Mach et al., 2015b). Furthermore, Camarinha-Silva et al. (2017) highlighted the importance of incorporating the microbiome information in addition to GBLUP for the prediction of complex phenotypes of swine. He et al. (2016) reported that gut microbiome absorbed 2.73% of total phenotypic variance of abdominal fat weight and provided new insight on effect of gut microbes on porcine fatness traits. Guo et al. (2008) reported that body fat is correlated with *Bacteroidetes* division of gut microbiota

in pigs. Fang et al. (2017) reported that the gut microbiota is associated with intramuscular fat in swine. The authors also suggested the possibility of improving the intramuscular fat by altering the gut microbiota. Wang et al. (2019) reported that swine health and production could be improved by governing gut microbiome at different stages of life. All of these studies show that gut microbiome affect the complex phenotypes, which indicated that microbiome information should be included in the statistical model for genomic evaluation. Ross et al. (2013) and Rothschild et al. (2018) also reported that for the prediction of complex traits which are associated with microbiome require the metagenomic information in addition to genomic information for the maximization of prediction accuracy in bovine and human respectively.

Genetic variation of the host is responsible in shaping the composition of gut microbiome in human indicating the presence of interaction between microbiome and genetics (Blekhman et al., 2015). The evidence of host-microbiome interaction has been reported in human, dairy cattle and mice (Kostic et al., 2013; Ross et al., 2013), which highlighted the possibility of host-by-microbiome interaction in swine. All the previous literatures supported the hologenome theory of evolution which considers the individual animal or plant as a "holobiont"- the combination of host plus microbes. From the hologenome theory it could be inferred that variation in microbiome and genome are the source of variation for complex traits on which artificial, natural selection and genetic drift can act (Zilber-Rosenberg and Rosenberg, 2008; Bordenstein and Theis, 2015).

1.5 Conclusion

The following chapter will address methods to estimate the genetic parameters, prediction accuracy utilizing the pedigree information, genomic information and gut microbiome information through the following objectives:

- Chapter 2: The objectives of this chapter were to estimate the heritabilities for different carcass composition traits and meat quality traits, and to estimate the genetic and phenotypic correlations between the meat quality, carcass composition, and growth traits in two large commercial crossbred swine populations using genotypes and phenotypes data.
- Chapter 3: The objectives of this study were to estimate the microbiabilities for different meat quality and carcass composition traits; to investigate the impact of gut microbiome on heritability estimates; to estimate the correlation between microbial diversity and meat quality and carcass composition traits; and to estimate the microbial correlation between the meat quality and carcass composition traits in commercial swine population.
- Chapter 4: The objectives of this study were to i) evaluate genomic and microbial predictions of meat quality and carcass traits and ii) to evaluate the effect of host-microbiome interaction on prediction of meat quality and carcass traits in swine.
- Chapter 5: Conclusions and future recommendations.

Table 1.1. Heritability of meat quality and carcass composition traits.

Traits	Range	Average	Reference
Carcass composition traits			
Fat depth	0.44-0.61	0.50	Akanno et al. (2013); Hovenier et al., (1992); Lo et al. (1992); Miar et al. (2014a)
Loin depth	0.13-0.38	0.30	Van Wijk et al. (2005); Miar et al. (2014a); Maignal et al. (2010)
Average Daily Gain	0.20-0.67	0.36	Lo et al. (1992); Van Wijk et al. (2005); Chimonyo and Dzama (2007); Jiao et al. (2014); Cabling et al. (2015)
Hot carcass weight	0.28	0.28	Miar et al. (2014a)
Loin Weight	0.29-0.63	0.49	Newcom et al., (2002); Van Wijk et al. (2005); Miar et al. (2014a)
Ham Weight	0.46- 0.66	0.54	Singh et al. (2001); Van Wijk et al. (2005); Miar et al. (2014a)
Butt weight	0.17 - 0.29	0.32	Newcom et al., (2002); Miar et al. (2014a)
Picnic weight	0.21-0.44	0.33	Newcom et al., (2002); Miar et al. (2014a)
Meat quality traits			
Intramuscular fat	0.26-0.61	0.47	Hovenier et al., (1992); Newcom et al., (2002); de Vries et al. (1994); Gjerlaug-Enger et al., (2010); Hermesch et al. (2000); Jiao et al. (2014); (Miar et al., 2014b)

Table 1. (continued)

pH	0.14-0.21	0.16	Hovenier et al., (1992); de Vries et al. (1994); Hermesch et al. (2000); Ciobanu et al. (2011)
Shear force	0.04-0.39	0.21	de Vries et al. (1994); Cabling et al. (2015); Lee et al. (2015)
Firmness score	0.20-0.29	0.25	Lo et al. (1992); Van Wijk et al. (2005)
Subjective marbling score	0.16 – 0.31	0.24	Lo et al. (1992); Van Wijk et al. (2005)
Color score	0.11-0.22	0.17	Lo et al. (1992); Van Wijk et al. (2005)
Minolta L*	0.18 -0.44	0.29	De Vries et al. (1994); Van Wijk et al. (2005); Miar et al. (2014a); Cabling et al. (2015)
Minolta b*	0.15-0.64	0.28	Van Wijk et al. (2005); Miar et al. (2014a); Cabling et al. (2015)
Minolta a*	0.21-0.68	0.42	Van Wijk et al. (2005); Miar et al. (2014a); Cabling et al. (2015)
Subjective marbling score	0.16-0.23	0.21	Lo et al. (1992); Sonesson et al. (1998) Van Wijk et al. (2005); Miar et al. (2014a)
Subjective color score	0.19-0.29	0.24	Hovenier et al., (1992); Lo et al. (1992); Hermesch et al. (2000);
Subjective firmness score	0.17-0.20	0.19	Lo et al. (1992); Van Wijk et al. (2005)

Chapter 2: Genetic Parameters of Meat Quality, Carcass Composition and Growth Traits in Commercial Swine

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2.1 Abstract

Swine industry breeding goals are mostly directed towards meat quality and carcass traits due to their high economic value. Yet, studies on meat quality and carcass traits including both phenotypic and genotypic information remain limited, particularly in commercial crossbred swine. The objectives of this study were: to estimate the heritabilities for different carcass composition traits and meat quality traits; and to estimate the genetic and phenotypic correlations between meat quality, carcass composition and growth traits in two large commercial swine populations: The Maschhoffs LLC (**TML**) and Smithfield Premium Genetics (**SPG**), using genotypes and phenotypes data. The TML dataset consists of 1,254 crossbred pigs genotyped with 60K SNP chip and phenotyped for meat quality, carcass composition and growth traits. The SPG population included over 35,000 crossbred pigs phenotyped for meat quality, carcass composition and growth traits. For TML datasets, the model included fixed effects of dam line, contemporary group (**CG**), gender, as well as random additive genetic effect and pen nested within CG. For the SPG dataset, fixed effects included parity, gender and CG, as well as random additive genetic effect and harvest group. Analyses were conducted using BLUPF90 suite of programs. Univariate and bivariate analyses were implemented to estimate heritabilities and correlations among traits. Primal yield traits were uniquely created in this study. Heritabilities [high posterior density interval] of meat quality traits ranged from 0.08[0.03,0.16] for pH and 0.08[0.03,0.1] for Minolta b* to 0.27[0.22,0.32] for marbling score, except intramuscular fat with the highest estimate of 0.52[0.40,

0.62]. Heritabilities of primal yield traits were higher than that of primal weight traits and ranged from 0.17[0.13, 0.25] for butt yield to 0.45[0.36, 0.55] for ham yield. The genetic correlations of meat quality and carcass composition traits with growth traits ranged from moderate to high in both directions. High genetic correlations were observed for male and female for all traits except pH. The genetic parameter estimates of this study indicate that a multi-trait approach should be considered for selection programs aimed at meat quality and carcass composition in commercial swine populations.

2.2 Introduction

The demand for higher quality meat by consumers has been steadily growing. Both meat quality and carcass composition traits are becoming important in swine breeding programs because of their increased economic value (Dransfield et al., 2005). Until the recent past, producers were paid for the weight of carcass as opposed to the weight of each primal cut (Miar et al., 2014a). Now, the swine industry has focused its attention towards meat quality traits (Dransfield et al., 2005), and producers are aiming at improving intramuscular fat, color, firmness, and different primal cuts (Wijk et al., 2005; Miar et al., 2014a) to meet consumer demand.

Estimation of genetic parameters for pork quality traits and their correlations with carcass composition and production traits are necessary for successful breeding programs that focus on meat quality. Carcass composition traits exhibit moderate to high heritability, while meat quality traits exhibit low to moderate heritability (Ciobanu et al., 2011; Miar et al., 2014a). Hence, selection for meat quality traits is challenging and makes the use of genomic selection particularly appealing (Lee et al., 2015). Genomic selection provides more genetic gain per unit time by increasing selection accuracy without costly and time-consuming progeny testing (van der Werf, 2013). Previous studies on meat quality and carcass traits on purebred swine (Cameron, 1990;

Gilbert et al., 2007; Cabling et al., 2015) and crossbred swine (Van Wijk et al., 2005; Miar et al., 2014a; Miar et al., 2014b) were conducted only with pedigree information. The results from these studies indicated a wide range of heritability and correlation estimates. Estimates of genetic parameters of meat quality and carcass composition traits which include both phenotypic and genotypic information remain limited, particularly in crossbred swine. The objectives of this study were, therefore: to estimate the heritabilities for different carcass composition traits and meat quality traits; and to estimate the genetic and phenotypic correlations between the meat quality, carcass composition and growth traits in two large commercial crossbred swine populations using genotypes and phenotypes data.

2.3 Materials and Methods

Animal welfare approval was not needed for this study because recorded data came from animals raised in commercial facilities under routine circumstances. Pigs were slaughtered in commercial facilities under the supervision of USDA Food Safety and Inspection Service. Two large commercial crossbred swine populations: **The Maschhoffs LLC** and **Smithfield Premium Genetics** were available for this study and analyzed separately.

The Maschhoffs LLC (TML)

Animals

Data were collected from 1,254 terminal cross pigs raised in a commercial setting managed by The Maschhoffs LLC (Claryle, IL, USA), and were obtained from the cross of 28 Duroc sires and 806 commercial F_1 sows composed of Yorkshire × Landrace or Landrace × Yorkshire sows. The weaned pigs (n = 6,642) were moved to 334 single-sex single-sire pens with approximately 20 pigs per pen. The experiment was repeated 6 times, each of which comprised of 2 pens (1 pen of female pig and one pen of castrated male pig). Pigs that came together in 1 replicate were put

in 1 contemporary group. Five pigs were selected from each pen for the collection of detailed phenotypic data. The selected pigs represented a pig with BW 1 SD above and a pig with BW 1 SD below the average BW of the pen, a pig with BW 2 SD above and a pig with BW 2 SD below the average BW of the pen and a pig with BW closest to the average BW of the pen. The 1,254 selected pigs were genotyped with the PorcineSNP60 v2 BeadChip (Illumina, Inc., San Diego, CA). Quality control procedures were applied by removing the SNPs that had call rate less than 0.90 and minor allele frequency less than 0.05, respectively. The final number of SNPs after quality control was 42,529. The Duroc sires had pedigrees traced back for nine generations, while no pedigree information was available for the F_1 dams. Parity information was not available for the F_1 dams. Transportation of pigs to the slaughterhouse was done as detailed by (Wilson et al., 2016). Pigs were slaughtered when the average body weight of each pen was 138kg. Pigs were immobilized via carbon dioxide stunning and killed by exsanguination.

Data collection

After the commercial slaughter procedures, fat depth (**FD**) and loin depth (**LD**) were measured using a Fat-O-Meater probe (SFK Technology A/S, Herlev, Denmark) at approximately 10^{th} rib location before measurement of hot carcass weight (**HCW**). Hot carcass weight was recorded immediately after animals were stunned, exsanguinated and dressed. Carcasses were then split, and blast chilled for approximately 90 min and different meat quality and carcass traits were measured. Carcasses were separated at approximately 22h post mortem into primal cuts, and different primal weights were measured: ham (**HAM**), butt (**BUTT**), picnic (**PICN**), sparerib (**SRIB**), total loin (**LOIN1**) and belly (**BEL1**) weight. Skin-on belly weight was recorded. The proportion of primal cuts, i.e., ham yield (**HAMY**), loin yield (**LOINY1**), belly yield (**BELY1**), sparerib yield (**SRIBY**), picnic yield (**PICNY**) and butt yield (**BUTTY**) were calculated by

dividing the respective primal cut weight by HCW. The total primal yield (**PRIMY**) was calculated as the proportion of sum of all primal cuts to HCW. Carcass average daily gain (**CADG**) was calculated by dividing the difference of HCW and birth weight by the age when that pig was sent to market. Loins were separated from the shoulder between the second and third ribs and separated from the ham 2.79 to 3.81 cm anterior to symphysis pubis bone and made-up boneless, vacuum-packaged and sent to the University of Illinois Meat Science Laboratory and preserved as described by (Wilson et al., 2017).

Boneless loins were aged for 14 days post mortem at 4ºC. After 14 days, loins were removed from the packaging and measured for pH, and mechanically sliced into 2.54 cm thick chops as described by Wilson et al. (2016). Ultimate pH (**PH**) was measured on the ventral side of the *longissimus dorsi* muscle using handheld MPI pH-meter fitted with a glass electrode (Meat Probes Inc., Topeka, KS). After slicing, a minimum of 30 min was allowed for the oxygenation of myoglobin before the subjective measurements, and instrumental color measurements were recorded. The subjective color score (**SCOL**) and subjective marbling score (**SMARB**) were recorded on the basis of 5 color categories (2.0, 2.5, 3.0,3.5, and 4.0) and 6 marbling categories (<1.5, 2.0, 2.5, 3.0, 3.5, and >4.0) as reported by Wilson et al. (2017) where higher color score represents the darker chop and the higher marbling score represents the greater extractable lipid content of each chop. Subjective firmness score (**SFIRM**) was measured using the scale from 1 to 5 where 1 was the softest, and 5 was the firmest. Instrumental L* (**MINL**), a* (**MINA**) and b* (**MINB**) color scores measured the lightness (greater L* indicates a lighter as opposed to darker color), redness (greater a* indicates a more red color as opposed to green), and yellowness (greater b* indicates a more yellow color as opposed to blue), respectively with a Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan). The sliced chops were then kept frozen. The 2.54

cm thick chops were taken out from frozen storage (-41°C) 24 hours prior to analysis and allowed to thaw completely. Secondary muscles and excess subcutaneous muscles were trimmed. Then the slice shear force (**SSF)** was determined as described by Wilson et al. (2017). Intramuscular fat percent (**IMF**) was based on the extractable lipid content of each chop as described by Wilson et al. (2017).

Statistical analysis

The data were analyzed using the single step genomic BLUP (Legarra et al., 2009) using BLUPF90 (Misztal et al., 2015). Univariate analyses were conducted to obtain a first estimate of heritabilities and variance components. Heritabilities and genetic correlations between male and female for different traits were also estimated in similar fashion. Single trait models were fitted as:

$$y_{ijklm} = \mu + D_i + C_j + G_k + a_l + p_{m(j)} + e_{ijklm}$$

where y_{ijklm} was the trait measured, μ was the intercept, D_i was the i^{th} effect of the dam line (2 levels), C_j was the j^{th} effect of contemporary group (6 levels); G_k was the k^{th} effect of the gender (2 levels), a_l was the random additive genetic effect, $p_{m(j)}$ was the random effect of pen nested in contemporary group and e_{ijklm} was the random residual error. Covariance matrices of the pen and residuals were equal to $\boldsymbol{I}\sigma_p^2$ and $\boldsymbol{I}\sigma_e^2$, where $\boldsymbol{I}$ was an identity matrix. Initially, the model also included the random litter effect and random permanent environmental effect. The variance absorbed by litter and permanent environmental effect was close to zero. So, these two effects were removed from all analyses for all traits. The random effect of animal was given by covariance matrix of $\boldsymbol{H}\sigma_l^2$ in which **H** was blended genomic and pedigree relationship matrix (Forni et al., 2011). $\mathbf{H^{-1}}$ was calculated as follows:

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where $\mathbf{G}^{-1}$ was the inverse of the genomic relationship matrix, $\mathbf{A}^{-1}$ was the inverse numerator relationship matrix (**A**) and $\mathbf{A}_{22}^{-1}$ was the inverse of pedigree-based relationship matrix for genotyped animals (Forni et al., 2011). All the individuals with detailed phenotypes (n =1,254) had genomic information available. **G** was created according to VanRaden (VanRaden, 2008b) :

$$G = \frac{(M-P)(M-P)'}{2\sum_{j=1}^{m} p_j(1-p_j)}$$

where **M** is a matrix of marker alleles with *m* columns (*m* = total number of markers) and *n* rows (*n* = total number of genotyped individuals), and **P** is a matrix containing the frequency of the second allele (p_j), expressed as $2p_j$. $\mathbf{M_{ij}}$ was 0 if the genotype of individual i for SNP j was homozygous for the first allele, 1 if heterozygous, or 2 if the genotype was homozygous for the second allele. Bivariate analyses were conducted to estimate the genetic and phenotypic correlations. Multi trait models were of form:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} p_1 \\ p_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where y_1 and y_2 were the vector of phenotypic measurements for traits 1 and 2 respectively; X_1 and X_2 were the incidence matrices relating the fixed effects to vector y_1 and y_2 respectively; b_1 and b_2 were the vector of fixed effect for trait 1 and trait 2 respectively; Z_1 and Z_2 were the incidence matrices relating the phenotypic observations to the vector of random animal effects for trait 1 and trait 2 respectively; a_1 and a_2 were the vector of random animal effect for trait 1 and trait 2 respectively; W_1 and W_2 were the incidence matrices relating the phenotypic observations to the vector of random pen effects for trait 1 and trait 2 respectively; p_1 and p_2 were the vector of random pen effect for trait 1 and trait 2 respectively; and e_1 and e_2 were the vectors of random residuals for trait 1 and trait 2 respectively. The fixed effects and random effects were the same as the ones fitted in the univariate analyses.

The additive variance was assumed to be $\mathrm{Var}\begin{bmatrix}a_1\\a_2\end{bmatrix}$ = **C** ⊗ **H**; where **C** = $\begin{bmatrix}\sigma^2_{g1} & \sigma_{g21}\\ \sigma_{g12} & \sigma^2_{g2}\end{bmatrix}$. The components of covariance matrix **C** were defined as: σ^2_{g1} was the additive genetic variance for trait 1, σ^2_{g2} was the additive genetic variance for trait 2, $\sigma_{g1} = \sigma_{g12}$ was the additive genetic covariance between trait 1 and trait 2. The pen variance was assumed to be $\mathrm{Var}\begin{bmatrix}p_1\\p_2\end{bmatrix}$ = **P** ⊗ **I**; where **P** = $\begin{bmatrix}\sigma^2_{p1} & 0\\ 0 & \sigma^2_{p2}\end{bmatrix}$ and **I** was the identity matrix. The components of **P** matrix were defined as: σ^2_{p1} was the pen variance for trait 1 and σ^2_{p2} was the pen variance for trait 2. From a preliminary analysis (data not shown) it was found that variance for the pen effect on PH, HCW and CADG was zero. Therefore, the variance of pen effect for those traits was fixed to zero for the subsequent analyses.

The residual variance was given by $\mathrm{Var}\begin{bmatrix}e_1\\e_2\end{bmatrix}$ = **R** ⊗ **I**; where **R** = $\begin{bmatrix}\sigma^2_{M1} & 0 & \sigma_{M12} & 0\\ 0 & \sigma^2_{F1} & 0 & \sigma_{F12}\\ \sigma_{M2} & 0 & \sigma^2_{M2} & 0\\ 0 & \sigma_{F21} & 0 & \sigma^2_{F2}\end{bmatrix}$ and **I** was the identity matrix. The components of **R** were defined as: σ^2_{M1} was the residual variance associated with male for trait 1, σ^2_{F1} was the residual variance associated with female for trait 1, σ^2_{M2} was the residual variance associated with male for trait 2, σ^2_{F2} was the residual variance associated with female with trait 2, $\sigma_{M1} = \sigma_{M2}$ was the residual covariance associated with male for trait 1 and trait 2 and $\sigma_{F12} = \sigma_{F21}$ was the residual covariance associated with female for trait1 and trait 2.

Smithfield Premium Genetics (SPG)

Animals

Data were collected from over 35,000 Duroc-sired terminal crossbred swine raised by Smithfield Premium Genetics (Roseville, NC, USA). Pedigree for all sires (n = 3,446) were traced

back for ten generations and 1,156 sires were genotyped. Pedigree information was not available for dams. Genotypes were obtained for 29 animals from GGP PorcineSNP60 BeadChip (GeneSeek, Neogen Corp.), 793 animals from PorcineSNP80 BeadChip (GeneSeek, Neogen Corp.), 2 animals from Genomic Profiler 10k BeadChip (GeneSeek, Neogen Corp.), 52 animals from Infinium PorcineSNP60v1 BeadChip (Illumina, Inc., San Diego, CA) and 280 animals from Infinium PorcineSNP60 v2 BeadChip (Illumina, Inc., San Diego, CA). All animals were imputed to have a set of 45,747 SNP on GGP Porcine Beadchip (GeneSeek, Neogen Corp.) using FImpute 2.2 (Sargolzaei et al., 2014). Quality control procedures for SPG were the same as in TML. The number of SNPs was 35,186 after quality control. Genotypes for crossbred pigs were not available in this population. The phenotypic data were collected from 2008 to 2017 from 7 different farms. Data were collected for different traits from different contemporary groups ranging from 27 to 71 depending on traits. Contemporary groups were created by concatenation of harvest year, pig farm and sex of pigs. All the males were castrated.

Data collection

Pigs were immobilized via carbon dioxide stunning and slaughtered by exsanguination. Fat depth and LD were measured by Fat-O-Meater probe (Carometec A/S, Denmark) between the 9th rib and the last rib in the carcass before measuring the HCW. Hot carcass weight was recorded as the same as TML. Then the carcass was transferred to the blast, which helped to cool down the carcass quickly and contained the fans blowing air towards the carcass at -26^{o}C, however the room temperature was maintained at -14^{o}C. Following the blast, carcasses were placed at the cooler (-1^{o}C to 2^{o}C) for 24 hours. After 24 hours post-mortem, the temperature of carcass was around 2^{o}C. Then center cut boneless loin and belly weights (**LOIN2** and **BEL2**, respectively) were recorded. The LOIN2 was measured in such a way that all rib end, bones, and belly strap with less than one

square inch were removed. Furthermore, fat was also trimmed from it so as not to exceed one-eighth inch. Skin-less and skin-on belly weight were recorded from two different farms. The genetic correlation [high posterior density interval] of skin-less and skin-on belly weight was 0.96 [0.91, 0.99]. So, it was treated as single trait as BEL2. All the further traits were measured on LOIN2 at same time. The 6 subjective color (**SCOL**) categories (1 = pale pinkish gray to white, 2 = grayish pink, 3 = reddish pink, 4 = dark reddish pink, 5 = purplish red, and 6 = dark purplish red) were classified using the guidelines declared by the National Pork Producers Council (1999). The subjective marbling (**SMARB**) scores were measured on the basis of 10 marbling categories (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10), which were classified using the guidelines as described by the National Pork Producers Council (1999) where 10 represented abundant fat and 1 represented devoid of fat. Subjective firmness score (**SFIRM**) was measured using the scale from 1 to 3 where 1 was the softest and 3 was the firmest. Ultimate pH (**PH**) was measured on boneless loin by portable pH-meter 1140 (Mettler-Toledo LCC, Columbus, OH, United States). Loin yield (**LOINY2**), belly yield (**BELY2**), and carcass average daily gain (**CADG**) were calculated with the same procedure as for TML.

Statistical analysis

Single and multi-trait analyses for estimation of heritability, variance components, genetic and phenotypic correlations were again conducted with BLUPF90. Fixed effects included 6 levels of parity of dam, 2 levels of gender and contemporary group ranging from 28 to 71 levels depending on the trait. Fixed effects and random effects were the same for both single and multi-trait models. Single trait model had form:

$$y_{ijklm} = \mu + P_i + G_j + C_k + a_l + h_m + e_{ijklm}$$

where y_{ijklm} was the trait measured, μ was the intercept, P_i was the effect of i^{th} parity, G_j was the effect of j^{th} gender, C_k was the effect of k^{th} contemporary group, a_l was the random animal effect, h_m was the random effect of harvest group, e_{ijklm} was the residual error. Covariance matrices of the harvest batch and residuals were equal to $\boldsymbol{I}\sigma_h^2$ and $\boldsymbol{I}\sigma_e^2$, where $\boldsymbol{I}$ was an identity matrix. The random effect of animal was given by covariance matrix of $\boldsymbol{H}\sigma_l^2$ in which **H** was estimated following the same process as TML. Initially, the model also included the random litter effect and random permanent environmental effect in addition to other random effects. The variance absorbed by litter and permanent environmental effect was close to zero. So, these two effects were removed from all analyses for all traits. Multi-trait model was similar to that of TML except random effect of pen was replaced by random effect of harvest group. Harvest group was defined as a group of pigs harvested together at same date.

The harvest group variance was assumed to be $\text{Var}\begin{bmatrix} h_1 \\ h_2 \end{bmatrix} = \mathbf{P} \otimes \mathbf{I}$; where $\mathbf{P} = \begin{bmatrix} \sigma_{h1}^2 & 0 \\ 0 & \sigma_{h2}^2 \end{bmatrix}$ and **I** was the identity matrix. The components of **P** matrix were defined as: σ_{h1}^2 was the harvest group variance for trait1 and σ_{h2}^2 was the harvest group variance for trait 2. From the preliminary analyses it was found that harvest group variance for PH, LD, SLAGE, HCW and CADG was zero, so the variance for this effect was fixed to zero in every analysis for those traits. The zero variance of harvest group was due to same harvest date.

Heritabilities and correlations

For both populations, a Gibbs Sampling algorithm was used with 120,000 iterations, with the first 20,000 samples discarded as burn-in. Samples were saved every 20 iterations for posterior calculations. The posterior mean was used as the estimate of (co)variance components. Lower and upper bounds of 95% highest posterior density interval for variance components, heritabilities,

genetic and phenotypic correlations were estimated from the stored samples. Heritabilities for TML were calculated as: $h^2 = \frac{\sigma_a^2}{\sigma_a^2+\sigma_p^2+\sigma_e^2}$ and heritability for SPG were calculated as: $h^2 = \frac{\sigma_a^2}{\sigma_a^2+\sigma_h^2+\sigma_e^2}$, where σ_e^2 was the residual variance and calculated as: $\sigma_e^2 = \frac{n_m\sigma_m^2 + n_f\sigma_f^2}{n_t}$. Here n_m was the number of males in population, n_f was the number of females in population, σ_m^2 was the residual variance for male, σ_f^2 was the residual variance for female and $n_t$was the total number of populations. Genetic correlations were calculated as $r_g = \frac{\sigma_{g12}}{\sqrt{\sigma_{g1}^2 * \sigma_{g2}^2}}$.

2.4 Results and Discussions

Data summary

Means, standard deviations, number of measurements per trait, and minimum and maximum values for each meat quality and carcass composition traits of TML and SPG are given in Table 2.1 and Table 2.2 respectively. There were 14 carcass traits, 3 growth traits and 9 meat quality traits for TML, and 5 carcass traits, 4 growth traits and 4 meat quality traits for SPG. The relevant fixed and random effects fitted in the mixed model analysis for carcass, growth and meat quality traits of TML and SPG are given in Table S.A1 and Table S.A2 respectively.

Genetic material, model and experimental setup for TML

This study was conducted on three-way-crossbred pigs obtained from 1 sire line (Duroc) and F1 sows (Yorkshire × Landrace and Landrace × Yorkshire). Genomic and phenotypic information was collected from terminal cross, but genomic information of F1 sows was not available. Different authors (Christensen et al., 2015; Sevillano et al., 2017) employed models with partial genetic effect tracing the breed-of-origin of alleles of crossbred individuals when predicting crossbreds. This model could not be used in this study because of the lack of genomic information for F1 sows and for three purebred populations, which is one limitation of this study.

Heritability estimates

Heritabilities and variance components estimates of growth, meat quality and carcass traits (primal weights and primal yields) are presented in Table 2.3 for TML, and Table 2.4 for SPG. All the remaining estimates along with their respective high posterior density intervals are provided in Table S.A3 and Table S.A4 for TML and SPG, respectively.

For TML, heritability estimates [high posterior density interval] for growth traits were moderate except for LD, which was 0.15[0.09, 0.21]. Moderate heritabilities of growth traits were also reported in previous studies (Lo et al., 1992; Clutter, 2011; Miar et al., 2014a). Miar et al. (2014b), Jiao et al. (2014) and Hicks et al. (1998) reported a moderate heritability of ADG, which agreed with present study, but less than that of Cabling et al. (2015) who reported an estimate of 0.67. This higher estimate might be due to differences in the measurement of ADG than that of our study. The present study measured the carcass average daily gain, however Cabling et al. (2015) measured the average daily gain of live weight. The heritability of FD in this study agreed with previous studies (Stewart and Schinkel, 1989; Clutter and Brasscamp, 1998; Sonenson et al., 1998; van Wijk et al., 2005; Ciobanu et al., 2011 and Miar et al., 2014b). The heritability of LD in this study agreed with Van Wijk et al. (2005) and de Campos et al. (2015). However, the heritability of LD was less than some of the previous studies (Stewart and Schinckel, 1991; Ducos, 1994; Magniel et al., 2010 and Miar et al., 2014a).

The heritability estimates of meat quality traits for TML ranged from 0.08[0.03, 0.1] for PH to 0.27[0.22,0.32] for SMARB except for IMF, which had the heritability estimate of 0.52[0.40,0.62]. This agreed with previous studies (Lo et al., 1992; de vries et al.,1994; Sellier, 1998; Lee et al., 2015). Newcom et al. (2005) mentioned that IMF was the important physicochemical trait of meat quality, which affected the sensory characteristics of pork and was

associated with other traits like marbling and color. Marbling score is related to IMF. The heritability of SMARB was moderate. This was in concordance with the range (0.14 - 0.31) of previous studies (Lo et al., 1999; Sonenson et al., 1998; van Wijk et al., 2005; Miar et al., 2014a).

Heritabilities of instrumental color ranged from 0.08[0.03, 0.16] for MINB to 0.20[0.14, 0.29] for MINL. These values were in range as reported by van Wijk et al. (2005) and Lee et al. (2015). The SCOL was moderately heritable, which was in the range as reported by previous studies (Cameron, 1990, Hovenier et al., 1992; Lo et al., 1992; Hermesch et al., 2000a). The heritability of PH in this study was low. The heritability of PH was in the range of 0.07 to 0.39 as reported by previous authors (Cameron, 1990; Hovenier et al., 1992; De Vries 1994; Suzuki et al., 2005; Van Wijk et al., 2005; Gjerlaug-Enger et al., 2010; Miar et al., 2014a and Lei et al., 2018). The wide range of heritability in these studies could be due to differences in time of recording pH, genetic origin of sampled populations, nutrition of pigs and differences in pre-slaughter and post-slaughter procedures (Cannon et al. 1994). The SFIRM was lowly heritable which was lower than that of 0.20 and 0.29 as reported by van Wijk et al. (2005) and Lo et al. (2015) respectively. Shear force is related to tenderness. The SSF was moderately heritable which agreed with the previous estimates reported by Lo et al. (1992) and De Vries et al. (1994) but lower than the value (0.39) reported by Miar et al. (2014a) and value (0.30) as reported by the average of several studies by Ciobanu et al. (2011). The effect of heterosis in crossbred population could have affected the variance components and heritability estimates (Miar et al., 2014a) in the present study. The differences among the heritability estimates might also have been due to the difference in composition of population (purebred vs. crossbred), and models (using pedigree information vs. inclusion of genomic information) used in statistical analysis (Lei et al., 2018).

Heritability of HAM in this study was less than that of the range (0.40 to 0.63) previously reported estimates (Newcom et al., 2002; van Wijk et al., 2005; Gilbert et al., 2007; Miar et al. 2014a). Loin weight was moderately heritable which was lower than the range of estimates (0.29-0.51) reported by van Wijk et al. (2005), Gilbert et al. (2007) and Newcom et al. (2002). Belly weight was moderately heritable which agreed with Gilbert et al. (2007) but lower than the estimate (0.51) as reported by Newcom et al. (2002). Moderate heritabilities were found for SRIB and BUTT, which agreed with Miar et al. (2014a). Picnic weight was lowly heritable, which was lower than the estimate of 0.44 as reported by Miar et al. (2014a) and 0.21 as reported by Newcom et al. (2002). The difference in previous studies may be due to constant slaughter weight using different carcass measurement techniques and statistical model used for (co)variance estimation. Furthermore, this could be due to the existence of maternal heterosis or heterotic effect itself in in the crossbred population and incorporation of genomics in the study. Limited studies on heritability of primal and sub primal cuts weights especially for PICN, BUTT and SRIB make it difficult for comparison with other studies.

The heritabilities of primal yield traits were higher than that of primal weight traits and ranged from 0.17[0.13, 0.25] for BUTTY to 0.45[0.36, 0.55] for HAMY (Table 2.3). To our knowledge, no literature had been published relative to the heritability of proportion of primal weight traits to compare with previous studies. Further study is warranted. The heritability estimates for primal yield traits were the highest among the study. The high estimate suggested that the selection could be done based on the primal yield traits.

For SPG, the heritability estimates of growth traits were moderate (Table 2.4) and ranged from 0.42[0.41, 0.43] for CADG to 0.59[0.58, 0.62] for SLAGE except LD which has heritability of 0.20[0.18, 0.22]. The low heritability of LD and moderate heritability of CADG and FD were

in concordance with TML. The heritability estimate of slaughter age agreed with Singh et al. (2001). The literature of heritability of SLAGE is limited in swine for further comparison. The heritability estimates of growth traits were moderate in both populations, which further validate the results and suggested that these traits should respond to selection directly.

Moderate heritability of meat quality traits was found for SPG (Table 2.4) ranging from 0.24[0.22, 0.26] for SCOL, SFIRM and PH to 0.33[0.31, 0.35] for SMARB. These values agreed with TML except for PH. This difference could be due to difference in time of recording of PH, statistical model used for estimation of co(variance) estimation. The heritability of primal yield traits was less than that of primal weight traits in SPG, which was in contrast with results from TML. The difference could be related to constant endpoint of TML, difference in fixed and random effects in statistical model, differences in sample size, differences in measurement techniques of primal weight traits and different management system.

Correlation among traits

In the discussion of genetic correlations, we will only focus on relevant results and estimates of widely reported combination will not be discussed unless useful in the comparison with the present study.

Correlation among meat quality traits

The genetic and phenotypic correlations among meat quality traits of TML and SPG are presented in Fig 2.1A and 2.1B respectively. All the estimates of genetic and phenotypic correlations for TML and SPG are provided in Table S.A5 and Table S.A6, respectively. Most of the genetic and phenotypic correlations were significant. Marbling and color are important characteristic of meat that could be seen visually by consumers and used for juiciness and tenderness (Lonergan et al., 2007; Wilson et al., 2017). High marbling category and darker meat

had high buying probability of pork by consumers (Brewer et al., 1999; Brewer et al., 2001). Marbling and IMF represent the content of fat between muscle. High correlation of 0.74[0.59,0.89] was found between IMF and SMARB. Correlations of IMF with minolta color measurements ranged from 0.34[0.11,0.57] between IMF and MINA to 0.78[0.55,0.99] between IMF and MINB. Higher genetic correlations of IMF with color measurements indicated that higher intramuscular fat is associated with pale meat where pale color was due to high intramuscular fat content (Hermesch et al., 2000b). Subjective marbling score was moderately correlated (0.45[0.03,0.87]) to SFIRM. Subjective color was highly negatively correlated with MINB (-0.93[-1.00, -0.73]) and MINL (-0.96[-1.00, -0.83]), and moderately positively correlated with MINA (0.45[0.13,0.74]). Minolta a* measurement had non-significant correlation with MINB and MINL. However, Miar et al., (2014a) reported a significant correlation of Minolta a* with Minolta b* (0.46) and Minolta l* (-0.40). The high positive correlation (0.86[0.62,0.99]) of MINB and MINL in present study agreed with Miar et al. (2014a) and van Wijk et al. (2005). The results indicated that light colored meat was more yellow. The MINA is related to the amount of myoglobin in muscle, which underpin the red color in meat (Mancini and Hunt, 2005). This could explain the positive moderate correlation of MINA and SCOL. pH had a high positive correlation (0.87[0.56,1.00]) with SCOL, in agreement with Hovenier et al. (1992). Strong negative correlations were found between PH with MINB (-0.64[-1.00, -0.73]) and MINL (-0.90[-1.00, -0.83]). This agreed with Hermesch et al. (2000b). Meat color and pH are highly related. Low pH is caused by lactic acid buildup, which results in anaerobic breakdown of glucose and glycogen, which loosens the myofibril and scatter more light making the muscle pale (Walters, 1975). Furthermore, low pH causes the myoglobin to be readily oxidized into metmyoglobin, which causes the discoloration of muscle (Mancini and

Hunt, 2005). Shear force is a measure of tenderness, and in this study was uncorrelated with other meat quality traits, in agreement with what reported by Miar et al. (2014a).

For SPG, subjective color was weakly correlated to SFIRM (0.05[0.01,0.09]) and PH (0.14(0.02,0.19]). This was in concordance with the TML results.

Correlations among meat quality with growth traits

The genetic correlations between meat quality traits with growth traits are presented in Fig 2.1C and 2.1D for TML and SPG respectively. All the estimates of genetic and phenotypic correlations for TML are presented in Table S.A7 and Table S.A8, respectively. All the estimates of genetic and phenotypic correlations for SPG are presented in Table S.A9 and Table S.A10, respectively. There were no significant correlations between meat quality traits and CADG and LD suggesting that meat quality traits are genetically independent of CADG and LD. This agreed with previous studies (De Vries et al., 1994; Hermesch et al., 2000b; Suzuki et al., 2005; Miar et al. 2014b). However, Van wijk et al. (2005) reported that selection for ADG has a detrimental effect on meat quality. The difference in magnitude of correlations from present study could be due to the use of carcass ADG in the present study rather than live weight measurement in ADG in previous studies. This result suggests that genetic improvement in meat quality had no change in CADG and LD. Fat depth was moderately and positively correlated with meat quality traits except SSF which had moderate negative correlation (-0.48[-0.70, -0.25]) with FD, so that selection for lean meat lead to decrease in tenderness. We found no significant correlation of FD with PH similarly to the results by Miar et al. (2014a). The correlations of FD with MINA (0.38[0.13,0.61]), MINB (0.55[0.22,0.88]) and MINL (0.26[0.01,0.51]) were favorable albeit of moderate magnitude. Consumers preference is towards lean meat with low fat depth. Selection for low FD would therefore results in moderate yellow, moderate light and moderate red which is the

desired color combination. In the present study, unfavorable correlations of FD with SMARB (0.29[0.06,0.50]) and IMF (0.37[0.19,0.55]) were found. This agreed to previous studies (Cameron, 1990; Hovenier et al., 1992; Hermesch et al., 2000b). Selection for SMARB and IMF would increase FD which might not be desirable from the producers' point of view.

For the SPG and similarly to TML we did not find significant correlations between meat quality and CADG and LD. The SLAGE trait was unique for SPG. Slaughter age was positively correlated with SCOL (0.38[0.27,0.43]), SFIRM (0.47[0.35,0.56]) and SMARB (0.33[0.22,0.43]), so that an increase in SLAGE would result in dark red meat, firmer and with more marbling. The dark color of meat of pigs slaughtered at old age was also found by Virgili et al. (2003).

Correlations among meat quality and carcass traits

Genetic correlations among meat quality and primal yield traits are presented in Fig 2.1E and 2.1F for TML and SPG, respectively. All the estimates of genetic and phenotypic correlations for TML and SPG are provided in Table S.A11, Table S.A12, Table S.A13 and Table S.A14, respectively. All the estimate for both populations. Moderate negative correlations were found between IMF and HAMY, LOINY1 and PRIMY (-0.31[-0.50, -0.14], -0.36[-0.57, -0.16] and -0.27[-0.52, -0.01], respectively). Similarly, moderate negative correlations were found between SMARB with HAMY (-0.26[-0.48, -0.03]) and LOINY1 (-0.31[-0.57, -0.06]). Intramuscular fat and SMARB was moderately positively correlated with BELY1 (0.37[0.15, 0.59] and 0.45[0.19,0.73], respectively) and SRIBY (0.29[0.09,0.49] and 0.28[0.02,0.53], respectively). The correlations of SSF with PICNY (0.36[0.08, 0.62]) and PRIMY (0.55[0.22,0.86]) were moderate and positive. Similar correlations were found between SFIRM and BELY1 (0.40[0.02,0.77]) while we found moderate to high negative correlations of SFIRM with PRIMY (-0.43[-0.83,0.02]) and PICNY (-0.64[-1.00, -0.29]). No significant correlations were found between color traits and

primal yield traits. The results suggest that genetic improvement for PRIMY and PICNY could result in less tender, firm and intramuscular fat. The results also suggest that selection of higher BELY1 and SRIBY is needed to improve the intramuscular fat which is one of the most important traits for pork quality.

In SPG and contrary to the TML dataset, a low positive correlation was found between SCOL and BELY2 (0.15[0.03,0.20]). The TML was at constant weight at slaughter whereas SPG has variation in slaughter weight. At SPG, when pigs get older, they accumulate more fat and their meat were darker in color. The correlations of SMARB with BELY2 and LOINY2 agreed with TML. Subjective marbling score were positively and moderately correlated to BELY2 (0.32[0.13,0.49]) in SPG. This is due to fact that white fat is visually distinct in dark red pork compared to pale pork.

Correlation among carcass traits

All the phenotypic correlations and most of the genetic correlations were significant for primal weight traits (see Table S.A15 and Table S.A16) for both TML and SPG. Moderate to high genetic correlations were found among primal weight traits and ranged from 0.39[0.10, 0.65] between HAM and BUTT to 0.79[0.66, 0.91] between BEL1 and SRIB with few being non-significant.

The genetic and phenotypic correlations among the primal yield traits for TML are presented in Fig 2.2A. All the estimates of genetic and phenotypic correlations for TML are presented in Table S.A17. Ham yield was negatively and moderately correlated to BUTTY (-0.34[-0.64, -0.03]), BELY1 (-0.59[-0.74, -0.43]) and SRIBY (-0.45[-0.61, -0.27]), while HAMY was positively and moderately correlated with PICNY (0.58[0.40,0.75]) and PRIMY (0.60[0.43,0.79]). This suggested that selection of HAMY, PICNY and PRIMY would decrease BUTTY, BELY1

and SRIBY. Loin yield was highly unfavorably correlated (-0.70[-0.85, -0.54]) to BELY1 but positively favorably correlated (0.39[0.13, 0.65]) to PRIMY. Belly yield was moderately and favorably correlated (0.41[0.17, 0.64]) with SRIBY but moderately and unfavorably correlated (-0.46[-0.71, -0.18]) with PRIMY. Picnic yield was highly and favorably correlated (0.68[0.47, 0.86]) with PRIMY. This implies that selection of BELY1 would happen at the expense of PRIMY. This could be due to fat deposition in belly muscles. The inverse relationship of SRIBY and BELY1 with LOINY1 and HAMY might be due to disproportionate growth. Lean pigs have more loin and hams whereas fat pigs have deep belly and spare ribs. The non-significant correlations among primal yield traits indicated that different genes could affect the growth and differentiation of muscles in different cut parts. For SPG, no significant correlation was found between BELY2 and LOINY2 which was different from TML. The exact reason for this different relationship could not be tested in our study. This could be due to difference in sample size and statistical models employed. To our knowledge no literature has been published regarding the correlation of primal yield traits. The cost of measuring primal weight traits might have limited the amount of literature available for more comparison. Thus, future work is needed in this area.

Correlation among carcass with growth traits

Primal weight traits have relationships with growth traits similar to previously published studies. All the estimates of genetic and phenotypic correlations of primal weight and yield traits with growth traits for TML and SPG are presented in Table S.A18, Table S.A19, Table S.A20 and Table S.A21, respectively. The genetic correlations among primal yield traits and growth traits for TML are presented in Fig 2.2B. Primal yield traits were both positively and negatively correlated with average daily gain. Moderate negative correlations were found between CADG with HAMY, LOINY1, PICNY and PRIMY (-0.54[-0.73, -0.33], -0.46[-0.67, -0.20], -0.43[-0.66, -0.14] and -

0.47[-0.60, -0.24], respectively), while moderate positive correlations were found with BELY1 and SRIBY (0.47[0.24,0.70] and 0.48[0.23,0.72],respectively), indicating how fast-growing pigs would accumulate muscle and fat more rapidly on belly and ribs compared to other primal cuts. Fat depth was favorably correlated with HAMY, PICNY, LOINY1 and PRIMY (-0.46[-0.60, -0.31], -0.57[-0.76, -0.38], -0.64[-0.79, -0.48] and -0.52[-.80, -0.34], respectively). However, FD was unfavorably correlated (0.68[0.54,0.80]) with BELY1. Moderate negative genetic correlation was found between LD with BELY1 and PRIMY (-0.42[-0.80, -0.03] and -0.44[-0.78, -0.10], respectively). For SPG, LOINY2 was unfavorably and moderately correlated to FD (-0.36[-0.56, -0.16]). This agreed with TML. Belly yield had moderate correlation (0.52[0.46,0.58]) with SLAGE, indicating that the pigs that are slaughtered at later age are fatter. Very limited literature estimates are available regarding the correlation of proportion of cut weight with growth traits.

Heritability estimates and genetic correlations between male and female among different traits

Different papers described differences in heritability between males and females for different traits in human as well as model organism (Wang et al., 2006; Ober et al., 2008). However, literature on heritability estimates and genetic correlations between male and female for different traits in livestock remains limited. Here, we report heritability estimates for castrated male and female, and genetic correlation between castrated male and female for different traits. Results are presented in Table 2.5 and estimates with confidence interval are provided in Table S.A22. For growth traits, heritability for male and female were fairly similar and genetic correlations were high. Non-significant effect of sex on growth rate was presented by Franco et al. (2014) and confirmed the high correlation of ADG in present study among castrated males and females.

For meat quality traits there were some discrepancies among heritabilities and genetic correlations. For IMF, the heritability of male was lower than that of the female, possibly due to the fact that deposition of intramuscular fat in male and female occurs at different rates. More fat deposition in female might have caused more variation, which resulted in higher heritability. This result is in concordance to Serrano et al. (2009) and Franco et al. (2014). The difference of IMF between male and female could be explained biologically as testosterone (male hormone) plays important role in lipolysis and lipoprotein lipase activity inhibition. Lipoprotein lipase helps to remove fatty acids from blood to cell and hence the fats are deposited in cells (Power and Schulkin, 2008).

Minolta a* had higher heritability for females than males. Cui et al. (2014) identified a significant sexually dimorphic locus on chromosome 6 associated with Minolta a* values, suggesting a different genetic architecture for the trait across sexes. Heritability of LOINY1 for female was higher than that for male, while non-significant correlation among male and female was found for PH. The exact reason for this is unknown. For MINL, SCOL, SSF, BUTT, SRIBY and BUTTY, males and females had lower correlation (< 0.9). This could be due to the sex specific effect on these traits. The results from this study indicated that for some traits (MINA, IMF and LOINY1) it is beneficial to fit model with heterogenous variance.

2.5 Conclusions

This study utilized pedigree and genomic information for the estimation of genetic parameters in commercial crossbred swine. For meat quality traits heritabilities ranged from low to moderate whereas moderate to high heritabilities were found for carcass composition traits. The genetic correlations of meat quality and carcass composition traits with growth traits ranged from moderate to high in both directions. The genetic parameter estimates indicated that a multi-trait

approach should be considered for selection programs aimed at carcass quality and composition in commercial crossbred swine population. Although, there was general opinion that selection for growth traits decreased the pork quality, we have shown here that growth traits namely, CADG and LD did not deteriorate the pork quality. In addition, fat depth has both favorable and unfavorable relationship with meat quality traits. Hence, caution should be taken while selecting against fat depth. Selection for CADG will improve the primal weight. Primal yields could be improved by selection for growth traits with caution due to varied relationship. Identification of sex specific genetic parameter on some traits indicated the advantage of selection of male and female separately for those traits in crossbred swine. Estimated parameters provide a reference value to establish the efficient breeding program that focuses on meat quality in United States.

2.6 Tables

Table 2.1. Descriptive statistics for growth, meat quality, primal weight and primal yield traits: abbreviations, number of animals per trait (n), mean, SD, minimum (Min) and maximum (Max) values of TML.

Traits	Acronym	n	Mean	SD	Min	Max
Growth traits						
Loin depth, mm	LD	4,894	66.78	6.91	91.44	226.06
Back fat depth, mm	FD	4,893	22.62	4.88	25.4	129.54
Carcass average daily gain, g/day	CADG	5,124	560.00	70.00	32.00	950.00
Meat quality						
Intra muscular fat, %	IMF	1,227	2.71	0.93	0.44	7.23
Minolta a*	MINA	1,241	3.79	1.10	0.68	7.89
Minolta b*	MINB	1,236	-0.15	0.91	-2.45	3.43
Minolta L*	MINL	1,241	45.30	3.10	35.98	56.58
Ultimate pH	PH	1,171	5.64	0.18	5.03	6.91
Subjective color	SCOL	1,237	2.72	0.47	2.0	4.00
Subjective marbling	SMARB	1,237	3.10	0.83	1.00	6.00
Subjective firmness	SFIRM	1,237	3.04	0.99	1.00	5.00
Slice shear force, kg	SSF	1,227	15.90	3.65	9.06	39.93
Primal weight trait						
Ham weight, kg	HAM	1,254	25.19	2.34	18.09	32.07
Loin Weight, kg	LOIN1	1,254	23.04	2.16	16.92	30.98
Belly weight, kg	BEL1	1,254	18.28	2.78	10.70	26.71
Sparerib weight, kg	SRIB	1,254	5.00	0.67	3.08	7.17
Picnic weight, kg	PICN	1,254	7.03	1.18	7.85	15.20
Butt weight, kg	BUTT	1,254	9.51	1.09	6.26	12.93
Hot carcass weight, kg	HCW	5,124	103.11	11.51	60.33	186.43
Primal yield trait						
Ham yield, %	HAMY	1,242	24.37	1.01	19.42	27.61
Loin yield, %	LOINY1	1,242	22.34	1.08	18.79	26.74
Belly yield, %	BELY1	1,242	17.59	1.41	12.61	22.40
Sparerib yield, %	SRIBY	1,242	4.83	0.36	3.70	6.16
Picnic yield, %	PICNY	1,242	11.20	0.73	8.45	15.27
Butt yield, %	BUTTY	1,242	9.19	0.68	6.46	12.51
Primal yield, %	PRIMY	1,242	89.48	1.22	82.96	96.02

Table 2.2. Descriptive statistics for growth, meat quality, primal weight and primal yield traits: abbreviations, number of animals per trait (n), mean, SD, minimum (Min) and maximum (Max) values of SPG.

Traits	Acronym	n	Mean	SD	Min	Max
Growth traits						
Fat depth, mm	FD	42,783	20.52	4.74	2.00	36.00
Loin depth, mm	LD	40,951	58.10	7.07	45.00	75.00
Slaughter age, days	SLAGE	320,545	186.00	13.69	152.00	225.00
Carcass average daily gain, g/day	CADG	320,545	520.00	60.00	250.00	810.00
Meat Quality traits						
Subjective color	SCOL	51,168	3.24	0.62	1.00	6.00
Subjective firmness	SFIRM	49,323	2.19	0.69	1.00	3.00
Subjective marbling	SMARB	51,246	2.51	1.02	1.00	10.00
Ultimate pH	PH	24,713	5.69	0.16	4.50	6.90
Primal weight traits						
Belly weight, kg	BEL2	37,539	7.16	0.57	4.79	8.40
Loin weight, kg	LOIN2	46,892	3.17	0.44	1.96	4.27
Hot carcass weight, kg	HCW	320,468	96.27	10.15	53.64	135.91
Primal yield traits						
Belly yield, %	BELY2	37,539	7.44	0.76	4.17	12.8
Loin yield, %	LOINY2	46,892	3.29	0.44	2.3	4.20

Table 2.3. Heritabilities (h^2), genetic variance (σ^2_a), pen variance (σ^2_p), residual variance of female (σ^2_f), residual variance of male (σ^2_m) of growth, meat quality, primal weight and primal yield traits of TML

Traits[1]	h^2	σ^2_a	σ^2_p	σ^2_f	σ^2_m
Growth traits					
LD	0.15	7.33	1.64	37.60	39.88
FD	0.47	10.67	0.65	9.48	13.01
CADG	0.44	2.43	0.03	2.65	3.06
Meat quality					
IMF	0.52	0.004	0.0004	0.002	0.004
MINA	0.19	0.22	0.16	0.73	0.80
MINB	0.08	0.0006	0.001	0.004	0.005
MINL	0.20	1.96	1.43	6.02	6.44
PH	0.08	0.003	0.01	0.02	0.02
SCOL	0.26	0.06	0.01	0.16	0.15
SMARB	0.27	0.18	0.09	0.44	0.40
SFIRM	0.12	0.12	0.05	0.89	0.72
SSF	0.21	0.37	0.08	1.48	1.10
Primal weight trait					
HAM	0.14	1.71	0.05	10.70	10.25
LOIN1	0.18	1.80	0.05	8.81	8.11
BEL1	0.22	3.78	0.06	14.41	11.85
SRIB	0.30	0.31	0.005	0.72	0.71
PICN	0.12	0.35	0.02	2.85	2.63
BUTT	0.20	0.52	0.02	2.17	2.06
HCW	0.39	42.9	0.09	65.49	68.86
Primal yield trait					
HAMY	0.45	4.38	0.44	4.60	4.78
LOINY1	0.33	3.73	0.56	7.22	7.13
BELY1	0.33	6.22	0.30	13.44	11.33
SRIBY	0.35	7.85	1.05	13.36	13.63
PICNY	0.32	1.58	0.33	3.29	2.84
BUTTY	0.17	0.74	0.23	3.68	3.41

Table 2.3. (continued)

PRIMY	0.21	2.98	0.34	9.37	12.35

[1]LD = Loin depth; FD = Fat depth; CADG = Carcass average daily gain; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; SSF = Slice shear force, HAM = Ham weight; LOIN1 = Loin weight; BEL1 = Belly weight; SRIB = Spare rib weight, PICN = Picnic weight; BUTT = Butt weight; HCW = Hot carcass weight; HAMY = Ham yield; LOINY1 = Loin yield; BELY1 = Belly yield; SRIBY = Spare rib yield; PICNY = Picnic yield; BUTTY = Butt yield; PRIMY = Primal yield

Table 2.4. Heritabilities (h^2), genetic variance (σ^2_a), variance of harvest batch (σ^2_h), residual variance of female (σ^2_f), residual variance of male (σ^2_m) of growth, meat quality, primal weight and primal yield traits of SPG.

Traits[1]	h^2	σ^2_a	σ^2_h	σ^2_f	σ^2_m
Growth traits					
FD	0.45	7.26	0.92	6.64	9.64
LD	0.20	4.68	NC[2]	18.40	20.13
SLAGE	0.59	50.07	NC	65.28	69.55
CADG	0.42	0.10	NC	0.10	0.20
Meat Quality traits					
SCOL	0.24	0.07	0.05	0.17	0.29
SFIRM	0.24	0.11	0.02	0.31	0.31
SMARB	0.33	0.28	0.07	0.41	0.56
PH	0.24	0.004	0.01	0.01	0.02
Primal weight traits					
BEL2	0.22	0.06	0.06	0.16	0.17
LOIN2	0.39	0.03	0.01	0.03	0.03
HCW	0.30	21.90	NC	50.88	52.67
Primal yield traits					
BELY2	0.19	0.09	0.06	0.22	0.25
LOINY2	0.28	0.04	0.02	0.05	0.07

[1]LD = Loin depth; FD = Fat depth; CADG = Carcass average daily gain; SLAGE = Slaughter age; PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; LOIN2 = Loin weight; BEL2 = Belly weight; HCW = Hot carcass weight; LOINY2 = Loin yield; BELY2 = Belly yield

Table 2.5. Genetic correlation (r_g) and heritability estimate for male (h^2_m) and female (h^2_f) for growth, meat quality, primal weight and primal yield traits of TML.

Traits[1]	h^2_m	h^2_f	r_g
Growth traits			
LD	0.11	0.15	**0.97***
FD	0.24	0.28	**0.99**
CADG	0.38	0.35	**0.99**
HCW	0.33	0.33	**0.99**
Meat quality			
IMF	0.38	0.60	**0.97**
MINA	0.14	0.29	**0.95**
MINB	0.07	0.16	**0.76**
MINL	0.21	0.20	**0.92**
PH	0.11	0.12	0.20
SCOL	0.32	0.22	**0.84**
SMARB	0.23	0.30	**0.94**
SFIRM	0.10	0.14	**0.79**
SSF	0.18	0.27	**0.89**
Primal weight trait			
HAM	0.10	0.19	**0.93**
LOIN1	0.13	0.25	**0.96**
BEL1	0.21	0.25	**1.00**
SRIB	0.27	0.31	**0.98**
PICN	0.10	0.14	**0.93**
BUTT	0.19	0.20	**0.89**
Primal yield trait			
HAMY	0.45	0.45	**0.94**
LOINY1	0.23	0.39	**0.95**
BELY1	0.33	0.31	**0.96**
SRIBY	0.38	0.32	**0.89**
PICNY	0.31	0.30	**0.93**
BUTTY	0.20	0.15	**0.80**
PRIMY	0.20	0.20	**0.91**

[1]LD = Loin depth; FD = Fat depth; CADG = Carcass average daily gain; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; SSF = Slice shear force, HAM = Ham weight; LOIN1 = Loin weight;

Table 2.5 (continued)

BEL1 = Belly weight; SRIB = Spare rib weight, PICN = Picnic weight; BUTT = Butt weight; HCW = Hot carcass weight; HAMY = Ham yield; LOINY1 = Loin yield; BELY1 = Belly yield; SRIBY = Spare rib yield; PICNY = Picnic yield; BUTTY = Butt yield; PRIMY = Primal yield

*Significant correlations are highlighted in bold.

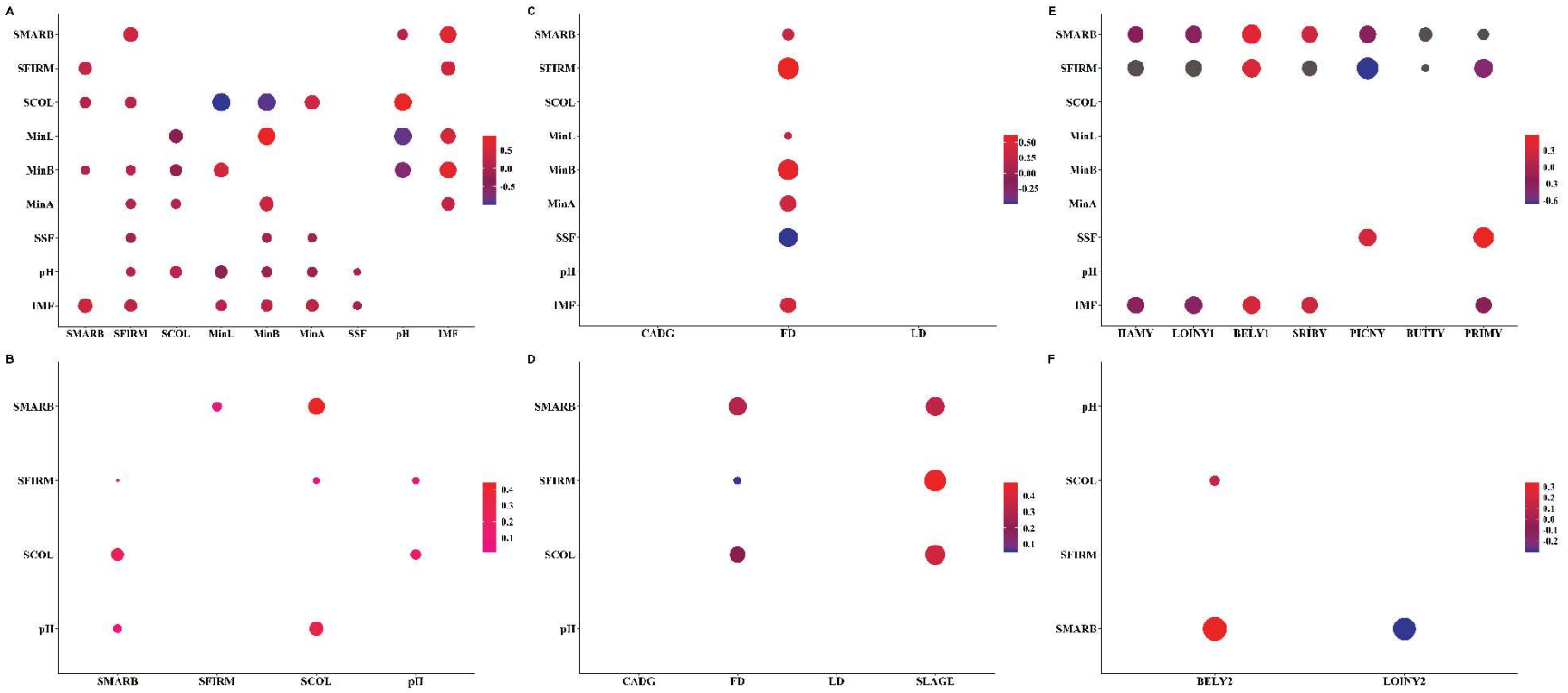

Figure 2.1. Genetic correlation (upper diagonal) and phenotypic correlation (lower diagonal) among meat quality traits for TML (A) and SPG (B), Genetic correlations of meat quality trait with growth traits for TML (C) and SPG (D), Genetic correlations of meat quality traits with carcass traits for TML (E) and SPG (F). Blank spaces represent non-significant correlation. Size of point represents the strength of correlation and color represents the direction of correlation.

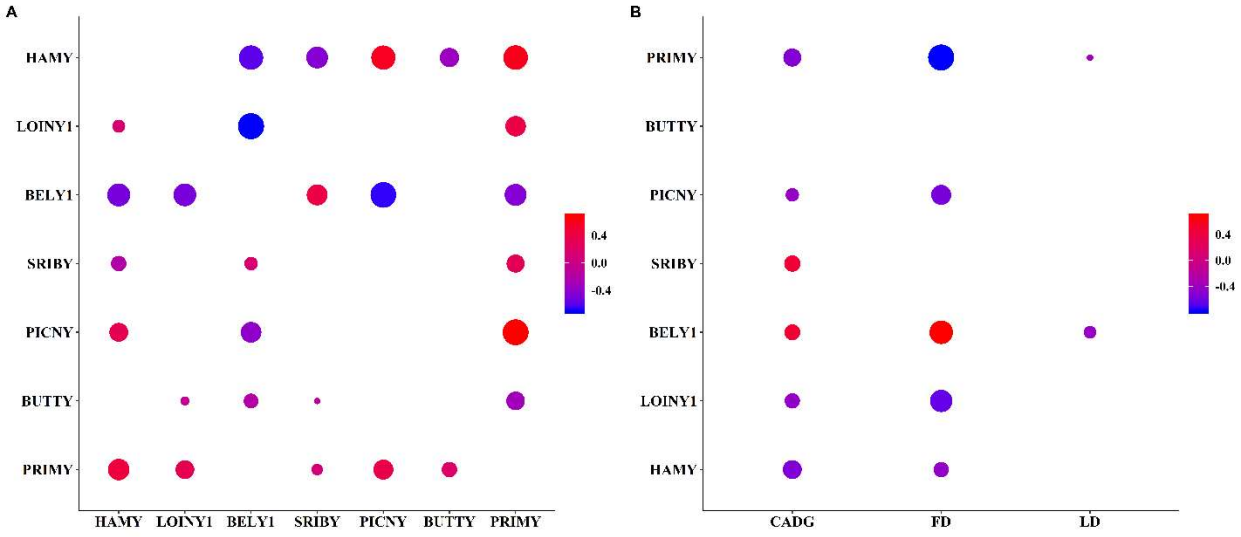

Figure 2.2. Genetic correlation (upper diagonal) and phenotypic correlation (lower diagonal) among carcass traits for The Maschhoffs population (A), Genetic correlation among carcass traits and growth traits (B). Blank spaces represent non-significant correlation. Size of point represents the strength of correlation and color represents the direction of correlation.

Chapter 3: Microbiability of meat quality and carcass composition traits in swine

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3.1 Abstract

The impact of gut microbiome composition was investigated at different stages of production (Wean, Mid-test, and Off-test) on meat quality and carcass composition traits of 1,123 three-way-crossbred pigs. Data were analyzed using linear mixed models which included the fixed effects of dam line, contemporary group and gender as well as the random effects of pen, animal and microbiome information at different stages. The contribution of the microbiome to all traits was prominent although it varied over time, increasing from weaning to Off-test for most traits. Microbiability estimates of carcass composition traits were greater than that of meat quality traits. Adding microbiome information did not affect the estimates of genomic heritability of meat quality traits but affected the estimates of carcass composition traits. High microbial correlations among several traits suggested that genomic correlation was partially contributed by genetic similarity of microbiome composition.

3.2 Introduction

The mammalian gastrointestinal tract is a home of a diverse microbiota population which serve various biological functions of the host (Frese et al., 2015). Gut microbiota has recently been the target of many research efforts resulting from the rapid development in molecular technologies and led to a vast influx of "omics" studies (Guevarra et al., 2019) . The importance of gut microbiota is widely accepted (H.B. Kim et al., 2011), with commensal bacteria often being called the "forgotten organ" of the host (O'Hara and Shanahan, 2006), impacting hosts in a multitude of

ways. For example, microbial composition helps in promoting the gastrointestinal health through metabolites, postnatal development, degradation of short chain fatty acids and stimulation of immune system (Mann et al., 2014; Pedersen et al., 2013; Stappenbeck & Herbert, 2016).

Gut microbiome constitutes a portion of the whole genome (Sommer & Bäckhed, 2013; Xiao et al., 2016) and has the potential to affect numerous biological activities that the hosts lack (Pajarillo et al., 2014). Different researchers reported that microbiome has considerable effect on human health and traits (Jose C Clemente et al., 2012; Dave et al., 2012; Huttenhower et al., 2012). For example, differences in bacterial species diversity and gene counts between lean and obese individuals have been found (Le Chatelier et al., 2013). The microbial diversity of intestine accounted for significant amount of phenotypic variation for any trait in human and should be accounted when assessing the heritability not only in human but also in plants and livestock (Sandoval-motta, Aldana, & Martínez-romero, 2017). In livestock, Difford, Lassen, & Løvendahl (2016) termed "microbiability" the proportion of total variance explained by microbiome for performance traits of dairy cattle. Difford et al. (2018) reported the effect of microbiota variation in methane production in dairy cows while, Mach et al. (2015) reported the impact of gut microbiome at early life on phenotypes of pig. Gut microbiome also has a significant impact on porcine fatness (He et al., 2016). Camarinha-Silva et al. (2017) reported the presence of a significant effect of microbial composition on daily gain, feed intake and feed conversion rate in swine. Until recently, selection of different traits in pigs has been done with the use of pedigree and genomic information, yet the advantage of incorporating microbial information in the genetic evaluation processes has not been assessed. Few studies have described the relationship of microbial diversity and host e.g. (Guevarra et al., 2019; McCormack et al., 2018), however these were mostly from a nutritional perspective.

Specifically, the contribution of microbial composition to the phenotypic variation of meat quality and carcass composition traits in pigs has yet to be explored and no studies to date have been conducted on the effect of microbial composition at different stages of production on growth and carcass composition. Therefore, the objectives of this study are to estimate the microbiabilities for different meat quality and carcass composition traits; to investigate the impact of intestinal microbiome on heritability estimates; to estimate the correlation between microbial diversity and meat quality and carcass composition traits; and to estimate the microbial correlation between the meat quality and carcass composition traits in a commercial swine population.

3.3 Materials and Methods

Animal welfare approval was not needed for this study since all data came from animals raised in a commercial setting by The Maschhoffs, LLC (Carlyle, IL, USA). All pigs were harvested in commercial facilities under the supervision of USDA Food Safety and Inspection Service.

Animals and sample collection

Data were collected from crossbred individuals that were obtained from 28 funding Duroc sires and 747 commercial F_1 sows composed of Yorkshire × Landrace or Landrace × Yorkshire. The pigs were weaned at 18.64 ± 1.09 days old and were moved to nursery-finishing facility. Pigs were kept in 334 single-sire single-sex pens with 20 pigs per pen. The test period began the day that pigs were moved to the nursery-finishing facility. During the nursery, growth and finishing period all pigs were fed a standard pelleted feed based on sex and live weight. Details of diet and their nutritional values are provided (see Appendix B). The pigs received a standard vaccination and medication routine. (see Appendix B). End of test (**Off-test**) was reached when the average weight of pigs of each pen reached 138 kg. The average age at off_test was 196.4 ± 7.80 days.

Fecal samples for 16S rRNA sequencing were collected as follow. Rectal swabs were collected from all pigs at three stages: weaning (**Wean**), 15 weeks post weaning (**Mid-test**; average 118.2 ± 1.18 days), and off_test. Four pigs from each pen were selected as detailed by Wilson et al. (2016) and their rectal swabs were used for subsequent microbial sequencing. There were 1,205, 1,295 and 1,273 samples for weaning, Mid-test and Off-test respectively. Distribution of samples across families, time points and sex are provided (see Appendix B).

Illumina amplicon sequencing

DNA extraction, purification, illumina library preparation and sequencing were done as described by Lu et al. (2018) and Maltecca et al. (2019). Briefly, total DNA (gDNA) was extracted from each rectal swab by mechanical disruption in phenol: chloroform. The DNA was purified using a QIAquick 96 PCR purification kit (Qiagen, MD, USA). Purification was performed per the manufacturer's instruction with the following minor modifications: (i) sodium acetate (3 M, pH 5.5) was added to Buffer PM to a final concentration of 185 mM to ensure optimal binding of genomic DNA to the silica membrane; (ii) crude DNA was combined with 4 volumes of Buffer PM (rather than 3 volumes); and (iii) DNA was eluted in 100 µL Buffer EB (rather than 80µL). All sequencing was performed at DNA Sequencing Innovation Laboratory at the Center of Genome Sciences and Systems Biology at Washington University in St. Louis. Phased, bi-directional amplification of the v4 region (515-806) of the 16S rRNA gene was employed to generate indexed libraries for Illumina sequencing as described in Faith et al. (2013). Sequencing was performed on an Illumina MiSeq instrument (Illumina, Inc. San Diego, USA), generating 250 bp paired-end reads.

16S rRNA gene sequencing and quality control of data

Pairs of 16S rRNA gene sequences were first merged into a single sequence using FLASH v1.2.11 (Magoc and Salzberg, 2011) with a required overlap of at least 100 and less than 250 base pairs in order to provide confident overlap. Sequences with a mean quality score below Q35 were then filtered out using PRINSEQ v0.20.4 (Schmieder and Edwards, 2011). Sequences were oriented in the forward direction and any primer sequences were matched and trimmed off. Mismatch was allowed up to 1. Sequences were subsequently demultiplexed using QIIME v1.9 (Caporaso et al., 2010). Sequences with greater than 97% nucleotide sequence were clustered into operational taxonomic units (**OTU**) using QIIME with the following settings: `max_accepts = 50, max_rejects = 8, percent_subsample = 0.1 and -suppress_step4`. A modified version of GreenGenes (Ley et al., 2006; Schloss and Handelsman, 2006) was used as reference database. Input sequences that had 10% of the reads with no hit to the reference database were then clustered de novo with UCLUST (Schloss and Handelsman, 2006) to generate new reference OTU to which the remaining 90% of reads were assigned. The most abundant sequence in each cluster was used as representative sequence for the OTU. Sparse OTU were then filtered out by requiring a minimum total observation count of 1,200 for an OTU to be retained, the resulting OTU table was rarefied to 10,000 counts per sample and after data processing and quality control 1,755 OTU were retained for further analysis.

Genotyping

All pigs were genotyped with the PorcineSNP60 v2 BeadChip (Illumina, Inc., San Diego, CA). Quality control procedures were applied by removing the SNPs that had call rate less than 0.90 and minor allele frequency less than 0.05. After quality control the number of SNPs remaining for further analyses was 42,529.

Phenotypic data

Phenotypic data collection was done as described by Wilson et al. (2016). Meat quality traits (intramuscular fat content (**IMF**), Minolta a* (**MINA**), Minolta b* (**MINB**), minolta L* (**MINL**), ultimate pH (**PH**), subjective color score (**SCOL**), subjective marbling score (**SMARB**), subjective firmness score (**SFIRM**), shearing force (**SSF**)) and carcass composition traits (Belly weight (**BEL**), ham weight (**HAM**), loin weight (**LOIN**), fat depth (**FD**), loin depth (**LD**) and carcass average daily gain (**CADG**)) were used for the current analysis. All the traits were measured as described by Khanal, Maltecca, Schwab, Gray, & Tiezzi. (2019). A summary of traits used in current analysis is reported in Table 3.1.

Statistical analysis

The data were analyzed using ASREML v4.1 (Gilmour, 2015). Univariate analyses were conducted to estimate heritabilities, microbiabilities and variance components for each trait. Single trait models were fitted as:

$$y_{ijklmn} = \mu + dl_i + cg_j + sex_k + animal_l + pen_{m(j)} + e_{ijklmn} \quad (1)$$

where μ was the overall mean, dl_i was the ith fixed effect of dam line (2 levels), cg_j was the jth fixed effect of the contemporary group (6 levels), sex_k was the kth fixed effect of sex (2 levels), $animal_l$ was the random animal genetic effect, $pen_{m(j)}$ was the random effect of pen nested within contemporary group and e_{ijklmn} was the random residual. Pen and residuals were assumed normally distributed with mean zero and with variances $\mathbf{I}\sigma^2_{pen}$ and $\mathbf{I}\sigma^2_e$, respectively, where **I** was an identity matrix. The random effect of animal was assumed normally distributed with mean 0 and variance $\mathbf{G}\sigma^2_a$ where **G** was a realized genomic relationship matrix obtained according to VanRaden (VanRaden, 2008) as:

$$\mathbf{G} = \frac{(\mathbf{M}-\mathbf{P})(\mathbf{M}-\mathbf{P})'}{2\sum_{j=1}^{m} p_j(1-p_j)}$$

where **M** is a matrix of marker alleles with *m* columns (*m* = total number of markers) and *n* rows (*n* = total number of genotyped individuals), and **P** is a matrix containing the frequency of the second allele (p_j), expressed as $2p_j$. $\mathbf{M_{ij}}$ was -1 if the genotype of individual i for SNP j was homozygous for the first allele, 0 if heterozygous, or 1 if the genotype was homozygous for the second allele. Narrow sense heritabilities were estimated as $h^2 = \frac{\sigma_a^2}{\sigma_P^2}$, with $\sigma_P^2 = \sigma_a^2 + \sigma_{pen}^2 + \sigma_e^2$.

We added the microbiome information to model (1) in order to estimate the changes in heritability due to the incorporation of microbiome information at each collection stage. Model (2) was then:

$$y_{ijklmno} = \mu + dl_i + cg_j + sex_k + animal_l + microbiome_m + pen_{n(j)} + e_{ijklmno} \quad (2)$$

Where *dl*, *cg*, *sex*, *animal*, *pen* and *e* were as previously described and $microbiome_m$ was the random effect of the animal microbiome. The effect of the microbiome was assumed normally distributed with mean 0 and variance $\mathbf{O}\sigma_m^2$ in which **O** was a microbial correlation matrix among individuals and σ_m^2 was the microbiome variance. The matrix **O** was created following Camarinha-Silva et al. (2017). Briefly, **O** was obtained as $\mathbf{O} = \frac{1}{q}\mathbf{X}\mathbf{X}^{\mathbf{T}}$, with matrix **X** of dimension of $n \times q$, where *n* is the number of animals and *q* is the number of OTU. **X** was constructed from **S**, a matrix of equivalent dimensions $n \times q$. Each element of the **S** matrix, S_{jk}, was the relative abundance of OTU k in animal j. The elements of X were calculated as:

$$X_{ij} = \frac{log(S_{jk}) - \overline{log_{\circ k}}}{sd(logS_{\circ k})}$$

where $S_{\circ k}$ is the vector of the k[th] column of **S.** The **O** matrix was created for each stage (Wean, Mid-test and Off-test) separately and fitted in each model separately. The contribution of the microbiome to the overall variance (microbiability) was calculated as: $m^2 = \frac{\sigma_m^2}{\sigma_P^2}$ (Difford et al., 2016). The total variance σ_P^2 was in this case obtained as $\sigma_P^2 = \sigma_a^2 + \sigma_m^2 + \sigma_{pen}^2 + \sigma_e^2$.

Bivariate analyses were subsequently conducted to estimate genomic and microbial correlations among traits. Bivariate models were of form:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{K_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{K_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{o_1} \\ \boldsymbol{o_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{W_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{p_1} \\ \boldsymbol{p_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} \quad (3)$$

where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ were the vectors of phenotypic measurements for trait 1 and trait 2 respectively; $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ were the incidence matrices relating the fixed effects to vector $\boldsymbol{y_1}$ and vector $\boldsymbol{y_2}$ respectively; $\boldsymbol{b_1}$ and $\boldsymbol{b_2}$ were the vector of fixed effect for trait 1 and trait 2 respectively; $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ were the incidence matrices relating the phenotypic observations to the vector of random animal effects for trait 1 and trait 2 respectively; $\boldsymbol{a_1}$ and $\boldsymbol{a_2}$ were the vectors of random animal effect for trait 1 and trait 2 respectively; $\boldsymbol{K_1}$ and $\boldsymbol{K_2}$ were the incidence matrices relating the phenotypic observations to the vector of random microbiome effect for trait 1 and trait 2 respectively; $\boldsymbol{o_1}$ and $\boldsymbol{o_2}$ were the vectors of random microbiome effect for trait 1 and trait 2 respectively; $\boldsymbol{W_1}$ and $\boldsymbol{W_2}$ were the incidence matrices relating the phenotypic observations to the vector of random pen effects for trait 1 and trait 2 respectively; $\boldsymbol{p_1}$ and $\boldsymbol{p_2}$ were the vector of random pen effect for trait 1 and trait 2 respectively; and $\boldsymbol{e_1}$ and $\boldsymbol{e_2}$ were the vectors of random residuals for trait 1 and trait 2 respectively. The fixed effects and random effects were the same as fitted in the univariate analyses.

The additive effects were again assumed normally distributed with means 0 and variance $\text{Var}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} = \mathbf{C} \otimes \mathbf{G}$; where $\mathbf{C} = \begin{bmatrix} \sigma^2_{a1} & \sigma_{a1} \\ \sigma_{a21} & \sigma^2_{a2} \end{bmatrix}$. The elements of the covariance matrix **C** were defined as: σ^2_{a1}, the genetic variance for trait 1, σ^2_{a2} , the genetic variance for trait 2, $\sigma_{a1} = \sigma_{a2}$, the additive genetic covariance between trait 1 and trait 2. Similar assumptions were made for the microbiome effect for which the covariance structure was assumed $\text{Var}\begin{bmatrix} o_1 \\ o_2 \end{bmatrix} = \mathbf{Q} \otimes \mathbf{O}$; with **Q** =

$\begin{bmatrix} \sigma^2_{m1} & \sigma_{m1} \\ \sigma_{m21} & \sigma^2_{m2} \end{bmatrix}$. The elements **Q** were: σ^2_{m1} , the microbiome variance for trait 1, σ^2_{m2} , the microbiome variance for trait 2 and $\sigma_{m12} = \sigma_{m2}$ the microbiome covariance between trait 1 and trait 2. The pen (co)variance structure was $\text{Var}\begin{bmatrix} p_1 \\ p_2 \end{bmatrix} = \mathbf{W} \otimes \mathbf{I}$; with $\mathbf{W} = \begin{bmatrix} \sigma^2_{p1} & 0 \\ 0 & \sigma^2_{p2} \end{bmatrix}$ and **I** an identity matrix. The **W** matrix elements were: σ^2_{p1}, and σ^2_{p2} being the pen variance for trait 1 and 2, respectively. Pen effects were in this case was assumed uncorrelated among traits. The residual variance was given by $\text{Var}\begin{bmatrix} e_1 \\ e_2 \end{bmatrix} = \mathbf{R} \otimes \mathbf{I}$; where $\mathbf{R} = \begin{bmatrix} \sigma^2_{e1} & \sigma^2_{e1} \\ \sigma^2_{e21} & \sigma^2_{e2} \end{bmatrix}$ and **I** was an identity matrix. The components of **R** were defined as: σ^2_{e1} was the residual variance for trait 1, σ^2_{e2} was the residual variance for trait 2, $\sigma^2_{e21} = \sigma^2_{e12}$ was the residual covariance among the two traits. Preliminary analyses (data not shown), showed how correlations were not estimable for the traits with estimated of microbiome variance of less than 3%. Microbial correlations were therefore estimated among traits for which microbiome explained at least 3% of total phenotypic variance. In all cases microbial correlations were not estimated for weaning since microbiome accounted for less than 3% of total variance for all traits.

Diversity analysis and its correlations with traits

The diversity analysis performed in this paper was aimed at investigating the distribution of alpha diversities at Wean, Mid-test and Off-test. The R package "vegan" (Oksanen et al., 2019) was used to calculate alpha diversity at each stage. The diversity was measured using Shannon index, and was computed as: $-\sum_{i=1}^{n} p_i \, ln(p_i)$ where p_i was the proportional abundance of ith OTU. To estimate the correlation among different traits and alpha diversity at weaning (**alpha_w**), week 15 (**alpha_mid**) and end of test (**alpha_off**), bivariate analyses were conducted using ASREML v.4

(Gilmour, 2015) by removing the effect of microbiome from model (3) and fitting diversity as the dependent variable.

3.4. Result and discussions

Data summary, distribution of alpha diversities and variance contributed by each sample

Mean and standard deviation for each meat quality and carcass composition trait are provided in Table 3.1. There were 9 meat quality and 6 carcass composition traits. The number of individual samples with complete genotypic, phenotypic and microbiome information at each stage was 1,123, which was used for further analyses. The distribution of OTU at weaning, Mid-test and Off-test is given in Figure. 3.1A. Of a total 1,755 OTU, there were 1,580 OTU in common between weaning, Mid-test and Off-test. There were 1,685 OTU in common between Mid-test and Off-test, while between weaning and Mid-test were 1,626 and between weaning and Off-test were 1590.

Alpha diversity is a measure of within-sample diversity. It measures the richness of species and is measured as the number of species in a sample of standard size (Whittaker, 1972). Distribution of alpha diversity among weaning, Mid-test and Off-test is given in Figure 1B. Mean alpha diversity at Off-test, Mid-test and Wean was 4.63 ± 0.01, 4.53 ± 0.01 and 3.85 ± 0.02 respectively. Results from Man-Whitney tests showed that alpha diversity at all stages were different ($P < 0.001$) to each other. This was in accordance with similar studies in pigs and other organisms (Frese et al., 2015; Lu et al., 2018; Guevarra et al., 2019). The increase in alpha diversity with age was similar to what previously found by different authors (Thompson et al., 2008; Kim et al., 2011; Looft and Allen, 2012; Chen et al., 2017). The change in diet in piglet from sow's milk to complete feed-based diet partially explains the shift in microbial diversity after weaning. Different researchers (Konstantinov et al., 2006; Frese et al., 2015) reported that change in the diet

impact significantly the microbiota composition in the gut. Different types of diet at different stage might explain the difference in alpha diversity at each stage. Piglets are exposed to a large number of stressors during weaning which triggered the physiological change in structure and function of intestine (Guevarra et al., 2019). This change caused the microbial shift after weaning transition (Kim and Isaacson, 2015) and microbial succession continues until microbiota composition reaches to climax community (Chen et al., 2017) which consists of microbes that are stable in composition. Further higher granularity results on the characterization of the microbial composition in the individuals of the current study can be found in Lu et al. (2018).

Microbiability estimates

The proportion of variance explained by each random term for meat quality and carcass composition traits is presented in Figure 3.2 and Figure 3.3, respectively. The estimates of microbiability and variance components along with their respective standard errors are provided (see Appendix B). The results identified several traits with significant microbiability.

The microbiability of carcass composition traits were higher than those of meat quality traits. In all cases microbiabilities for both meat quality and carcass composition traits at weaning were negligible and ranged from zero for several traits to a maximum of 0.06 ± 0.03 (estimate ± SE) for CADG. Three of the 9 meat quality traits investigated shown significant microbiability at Mid-test, with estimates of 0.07±0.03 for SMARB, 0.08±0.03 for SFIRM and 0.10±0.04 for MINB. At Off-test, 4 meat quality traits had significant microbiability, with estimates of 0.06±0.02 for IMF, 0.09±0.04 for MINA, 0.11±0.04 for MINB and 0.13±0.04 for SFIRM. For carcass composition traits, we found that 5 out of 6 traits were significantly affected by microbiome at Mid-test and Off-test. The microbiability of carcass composition traits at Mid-test ranged from 0.12 ± 0.04 for LOIN and FD to 0.20 ± 0.04 for BEL. The microbiability of carcass composition

traits at Off-test ranged from 0.13 ± 0.05 for LOIN to 0.29 ± 0.05 for BEL. In our study, the microbiome did not contribute significantly to loin depth variability. In most of the cases microbiome at weaning did not contribute to trait variation, however, microbiome at Mid-test and Off-test contributed significantly to trait variation. This might have several causes including the sudden change of microbiome composition shortly after the diet switch occurring at weaning as well as other environmental factors like, stress. To our knowledge this is the first attempt to obtain microbiability estimates for meat quality and carcass composition traits. We did not find any literature to compare the estimates with previous research. Our results suggest that later measures of microbial composition might be more informative for selection purposes, but further research would be needed to clarify this aspect.

Among meat quality traits, microbial variance explained a larger proportion of phenotypic variance than additive genetic for SFIRM and MINB at Off-test (Figure 3.2). Among carcass composition traits, BEL, HAM, and CADG at Off-test had higher proportion of phenotypic variation explained by microbiome than by additive genetic (Figure 3). These results indicated that a significant proportion of total variance is explained by the microbiome, in some cases larger than the additive genetics and that prediction for these traits could be improved by accounting the effect of variability in gut microbiome composition. The variation in gut microbiome could be fitted as the systematic environmental effect in model.

In the current study we observed a decrease in genomic heritability for most of the carcass composition traits at Off-test when microbiome information was added. The decrease in heritability ranged from 0% for LD to about 10% for FD. At Mid-test, the decrease in heritability ranged from 0% for CADG, BEL, HAM and LOIN to 4% for FD. No change in genomic heritability were observed at weaning. The decrease in heritability for FD was similar to what

found by Lu et al. (2018) for similar traits. He et al. (2016) also reported the significant contribution of microbiome in porcine fatness. These results suggested that part of the resemblance among individuals captured by genetic effects in breeding values prediction, might be in fact a resemblance among microbial composition and genetic parameters might not be accurate.

In contrast, for most of the meat quality traits considered, the inclusion of microbial composition did not affect the estimates of genomic heritability, thus suggesting that at least for meat quality traits, gut microbial composition is mostly an environmental factor. The decrease in genomic heritability as we included the microbiome composition in the models was previously observed by Sandoval-Motta et al. (2017) who reported the possibility of overestimation of heritability values with the use of genetic similarities by kinship information. The authors also suggested that inclusion of genetic diversity of individual microbiome will most likely increase the accuracy of heritability of various traits. The heritability and microbiability estimation of daily gain, feed intake and feed conversion ratio in swine by Camarinha-Silva et al. (2017) and methane emission in cattle by Difford et al. (2018) strongly suggested the significant contribution of microbiome in the total variation in the complex phenotypes of livestock. In human, Richards et al. (2018) reported that host genes are affected by the microbiome and are involved in the complex traits. These previous studies agreed with our results. Our results also agreed with Zilber-Rosenberg & Rosenberg, (2008) who reported the concept of "hologenome" of evolution, where the animal or plant along with associated microorganisms are the unit of selection in evolution.

Correlation of meat quality and carcass composition traits with alpha diversity at different stages

Host genetics plays a major role in shaping the intestinal microbiota of mice and humans (Büsing and Zeyner, 2015; Hancox et al., 2015; Dąbrowska and Witkiewicz, 2016). Chen et al. (2018), Kubasova et al. (2018) and Lu et al. (2018) reported the impact of host genetics on development

of gut microbiota in pigs. So, the alpha diversities at weaning, Mid-test and Off-test were considered as separate phenotypic records and genetic correlations were estimated between different alpha diversities and other traits measured. The results are presented in Table 3.2 suggesting very weak correlations for alpha_w for all traits measured. Weak correlations were estimated between also with alpha_mid with the exception of MINA (-0.45±0.19) where greater alpha diversity seems linked to a paler red color of meat given that MINA is related to the amount of myoglobin in muscle. We obtained weak correlations between alpha_mid and carcass composition traits except for CADG (-0.43±0.19), suggesting that an increase in microbial diversity would decrease average daily gain. This was in contrast with general opinion that the diversity will increase the metabolite production from different microbiota (Kim and Isaacson, 2015; Yan et al., 2017) and increase the weight of host. However, this was in agreement with what found by Lu et al. (2018). Alpha diversity could then be used as a potential indicator trait in CADG selection. In all cases correlations of alpha_off with growth, carcass and meat quality traits were weak (Table 3.2).

This study is the first to estimate the genetic and phenotypic correlation between alpha diversity, and carcass and meat quality traits. Our results suggested that diversity at weaning might not be an accurate predictor of growth, carcass and meat quality traits which agreed with Huttenhower et al. (2012). Alpha diversity was reported to be associated with gut health of animal and associated with the normal physiology of host animals (Guevarra et al., 2019). The major role could include the normal function of gut, enhance immune response and play active role in digestion and utilization of nutrients. Our results presented the varied range of correlation in terms of magnitude and direction at different stages. So, for routine use of the alpha diversity as indicator trait, further investigation of alpha diversity after weaning of piglets is warranted.

Correlation among traits

In the discussion of correlation, we only focus on microbial correlations. Genomic correlations are only discussed if the genomic correlations changed due to inclusion of microbiome information in the model. The genomic correlations without inclusion of microbiome in the model are presented (see Appendix B).

Correlations among meat quality and carcass composition traits at Mid test

Overall there were 3 meat quality traits and 5 carcass composition traits having variance of microbiome composition greater than 3%. Microbial correlations among meat quality traits at Mid-test are presented in Table 3.3. Most of the microbial correlations were significant. Subjective marbling score was moderately positively correlated (0.46±0.24) with FD. This suggested that shifting of microbiota for high marbled meat would results in higher fat depth. Shear force is the measure of tenderness. In this study, the microbial composition of SSF was highly negatively correlated with SMARB, SFIRM, FD, CADG, LOIN and BEL which ranged from -0.93±0.11 for SSF and SFIRM to -0.50±0.25 for SSF and LOIN. High positive correlations of SFIRM were found with CADG, HAM, LOIN and BEL which ranged from 0.58±0.26 between SFIRM and LOIN to 0.87±0.16 between SFIRM and BEL. We did not find any other estimates to compare with our values of microbial correlation between meat quality and carcass composition traits. There were moderate to high correlations of microbial composition of FD with CADG, HAM, LOIN and BEL which ranged from 0.44±0.21 between FD and LOIN to 0.74±0.11 between FD and BEL. High positive correlations were found between CADG and HAM, LOIN and BEL. Belly weight was highly positively correlated with HAM (0.96±0.03) and LOIN (0.94±0.06).

Correlation between meat quality traits and carcass composition traits at the end of test

There were 6 meat quality traits and 5 carcass composition for which variance of microbiome composition was greater than 3%. The microbial and genomic correlations among meat quality traits at Off-test are presented in Table 3.4. pH had high positive microbial correlation (0.90±0.25) with SCOL and SFIRM (0.73±0.35). This is in partial agreement with results from Ratzke & Gore, (2018) that reported how there are specific bacteria which build lactic acid in the muscle resulting in the anaerobic breakdown of glucose and glycogen, which eventually loosens the myofibril, thus scattering more light making the muscle pale (Walters, 1975). Furthermore, increasing pH causes swelling of myofibrils (Huff-Lonergan and Lonergan, 2005) which ultimately makes the muscle firmer. High positive microbial correlation was found between IMF and SFIRM (0.91±0.17), MINA (0.55±0.28) and MINB (0.75±0.27). This agrees with Fang, Xiong, Su, Huang, & Chen. (2017) who reported that gut bacteria involving in energy metabolism and intramuscular fat content in pig also regulate the muscle composition and muscle fibers. Higher microbial correlation of IMF with minolta color measurements and SFIRM indicated that microbial composition increasing IMF would make the muscle pale and firmer. High microbial correlation of MINA and MINB (0.78±0.16) suggests that microbiota responsible for redness of meat also contribute to the yellowness in the meat. This agreed with Kim et al. (2010) who reported the positive correlation of yellowness and redness in the muscle of pig.

The microbial and genomic correlations among carcass composition traits at Off-test are presented in Table 3.5. The microbial composition of carcass composition traits were highly and positively correlated to each other ranging from 0.55±0.17 between FD and LOIN to 0.97±0.02 between CADG and HAM. McCormack et al. (2018) reported a positive correlation between gut microbiota and feed efficiency in swine. Gut microbiota has considerable effect on feed intake,

final body weight (Kubasova et al., 2018) and growth traits (Ramayo-Caldas et al., 2016). All these studies suggested that microbial composition has considerable effects on many carcass composition traits, with positive correlations between them. This high correlation indicated that all the traits could be simultaneously improved through the same microbial composition.

The microbial correlations for meat quality traits and carcass composition traits at Off-test are presented in Table 3.6. Intramuscular fat was highly correlated with FD (0.90±0.14) and BEL (0.73±0.18). Firmness score was positively correlated with BEL (0.50±0.18). Moderate positive correlation was found between MINA and BEL (0.41±0.21) and high positive correlation was found between MINA and FD (0.53±0.18), and MINA and CADG (0.66±0.17). Minolta b* has moderate positive correlation with FD (0.43±0.19) and high positive correlation with CADG (0.58±0.18): suggesting that increase in microbiota for lean meat and high daily gain of carcass would make the meat more yellowish.

Change in genomic correlation with the inclusion of microbiome information

In this study, we observed a decrease in genomic correlation for the majority of the carcass composition traits when microbiome information was included in the model. At Mid-test, the decrease in genomic correlation ranged from 0% for majority of meat quality traits to 18% for BEL and LOIN. The genomic correlation of BEL with FD and HAM decreased by 5% and 16%, respectively. The genomic correlation of FD with SMARB and SSF decreased by 7% and 4%, respectively.

At Off-test, the genomic correlation between PH and SCOL (0.91±0.29), SFIRM and IMF (0.36±0.15), FD and CADG (0.27±0.13), and BEL and HAM (0.58±0.19) became non-significant with the inclusion of microbiome. Among carcass traits, the decrease in genomic correlation ranged from 1% between BEL and CADG to 30% between BEL and LOIN. The genomic

correlation of BEL with FD, CADG with HAM, CADG with LOIN, FD with IMF, FD with MINB, BEL with IMF, and BEL with SFIRM decreased by 13%, 4%, 2%, 9%, 6%, 13% and 8%, respectively. Among meat quality and carcass traits, the decrease in genomic correlations ranged from 1% for FD and SFIRM to 9% for BEL and IMF. We observed the decrease in genomic correlations with the inclusion of microbiome, particularly of any other traits with fat related traits e.g. (BEL, FD, IMF). This could be due to the greater influence of gut microbiome on fat deposition. Furthermore, we observed that there was a decrease in genomic correlation for those traits which had higher microbial correlation. High microbial correlations among different traits suggested that genomic correlations among traits are partially contributed by the correlations among the gut microbiota composition. The covariance among microbiome for different traits might have contributed to the genetic covariance and hence the genomic correlation. We observed that the decrease in the genomic correlation was higher at Off-test than at Mid-test. This was due to high variability accounted by microbiome composition at Off-test in comparison to Mid-test.

This is the first study to evaluate the variance accounted by microbiome and estimate the microbial correlations for meat quality and carcass traits in swine. So, we have explored the model sequentially, first with inclusion of genomic information and then addition of microbiome information at different stages. Variance component estimates of different random effects with inclusion of interaction of genotype-by-microbiome in the model is recommended for future studies.

3.5 Conclusions

This study was conducted on crossbred pigs to investigate the impact of intestinal microbiota through different stages (weaning, Mid-test and Off-test) of production. To our knowledge this study is the first attempt to investigate the impact of microbiome on the meat

quality and carcass composition traits at a large scale in swine. The contribution of microbiome to all traits was significant although it varied over time with an increase from weaning to Off-test for most of the traits. Adding microbiome information did not affect the estimates of genomic heritability of meat quality traits but changed the estimate of carcass composition traits suggesting that portion of genomic variance was contributed by gut microbiome. Alpha diversity at Mid-test was strongly correlated with carcass average daily gain and minolta a* color score. A better understanding of microbial composition could aid the improvement of complex traits, particularly the carcass composition traits in swine by inclusion of microbiome information in the genetic evaluation process. High microbial correlations were found among different traits, particularly with traits related to fat deposition. Adding microbiome information decreased the genomic correlation for those traits which had higher microbial correlation suggesting that portion of genomic correlation was due to genetic covariance among microbiome composition affecting those traits. Based on the results we can conclude that microbial composition could be altered to improve a given trait. To obtain optimum microbial composition, manipulation of gut microbiota could be done using specific bacterial composition as probiotics or increasing the relative abundance through prebiotics, feed additives supplements and fecal microbiota transplantation could also be done. The estimated parameters provide a reference value for further research on gut microbial contribution to complex phenotypes in pigs. These results may lead to establish a newer approach of genetic evaluation process through the addition of gut microbial information.

3.6 Tables

Table 3.1. Descriptive statistics of carcass composition and meat quality traits: acronym, means, standard deviation (SD) values.

Traits	Acronym	Mean	SD
Carcass composition traits			
Loin depth, mm	LD	67.99	7.21
Back fat depth, mm	FD	22.07	5.24
Carcass average daily gain, g/day	CADG	552.90	73.93
Ham weight, kg	HAM	25.19	2.34
Loin Weight, kg	LOIN	20.01	1.88
Belly weight, kg	BEL	15.88	2.55
Meat quality			
Intra muscular fat, %	IMF	2.71	1.01
Minolta a*	MINA	3.77	1.16
Minolta b*	MINB	-0.16	0.87
Minolta L*	MINL	45.37	5.76
Ultimate pH	PH	5.64	0.22
Subjective color	SCOL	2.72	0.57
Subjective marbling	SMARB	3.10	0.91
Subjective firmness	SFIRM	3.05	1.04
Slice shear force, N	SSF	156.96	41.99

Table 3.2. Genetic correlation of carcass composition traits and meat quality traits with alpha diversity at weaning (alpha_w), week 15 (alpha_mid) and end of test (alpha_off).

Traits[1]	alpha_w	alpha_mid	alpha_off
Carcass composition			
FD	0.54±0.39	-0.22±0.15	-0.30±0.19
LD	0.16±0.48	-0.15±0.24	-0.30±0.29
CADG	0.36±0.39	**-0.43±0.19**	-0.25±0.24
HAM	-0.13±0.50	-0.13±0.22	0.04±0.26
LOIN	-0.65±0.60	0.16±0.20	0.13±0.24
BEL	0.02±0.43	-0.31±0.20	-0.41±0.23
Meat quality			
SCOL	0.31±0.44	-0.09±0.17	-0.25±0.21
SFIRM	0.50±0.42	-0.21±0.22	-0.22±0.27
SSF	0.01±0.39	0.11±0.18	0.10±0.22
IMF	0.14±0.32	-0.13±0.15	0.001±0.18
SMARB	0.17±0.37	-0.15±0.18	-0.21±0.21
MINA	0.78±0.76	**-0.45±0.19**	-0.30±0.25
MINB	0.66±0.48	-0.03±0.27	0.50±0.31
MINL	-0.22±0.46	0.05±0.19	0.14±0.23
PH	0.88±0.60	0.007±0.33	0.43±0.39

[1]LD = Loin depth; FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight, SCOL = Subjective color score, SFIRM = Subjective firmness score, SSF = Slice shear force, IMF = Intramuscular fat percent, SMARB = Subjective marbling score, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; Numbers in bold are significant.

Table 3.3. Estimates of microbial correlation (above diagonal) and genomic correlation (below diagonal) at Mid-test among meat quality and carcass composition traits.

	[1]SMARB	SFIRM	SSF	FD	CADG	HAM	LOIN	BEL
SMARB		0.39±0.33	**-0.72±0.28**	**0.46±0.24**	-0.21±0.28	-0.27±0.29	-0.34±0.32	-0.02±0.26
SFIRM	**0.42±0.18**		**-0.93±0.11**	NC[2]	**0.86±0.17**	**0.62±0.24**	**0.58±0.26**	**0.87±0.16**
SSF	0.08±0.16	-0.23±0.21		**-0.70±0.21**	**-0.68±0.22**	-0.45±0.25	**-0.50±0.25**	**-0.55±0.24**
FD	**0.22±0.11**	NC	**-0.44±0.13**		**0.68±0.15**	**0.50±0.19**	**0.44±0.21**	**0.74±0.11**
CADG	0.02±0.17	0.03±0.23	0.19±0.18	0.21±0.15		**0.98±0.02**	**0.95±0.03**	**0.98±0.01**
HAM	-0.13±0.18	0.11±0.24	0.27±0.20	0.01±0.15	**0.67±0.11**		NE[3]	**0.96±0.03**
LOIN	-0.09±0.17	0.10±0.23	0.11±0.18	-0.14±0.15	**0.69±0.09**	**0.53±0.11**		**0.94±0.06**
BEL	0.31±0.17	0.35±0.23	0.18±0.15	**0.57±0.11**	**0.79±0.06**	**0.42±0.17**	**0.42±0.15**	

[1]SMARB = Subjective marbling score, SFIRM = Subjective firmness score, SSF = Slice shear force, FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight;

[2]Not Converged; [3]Not estimable; Numbers in bold are significant.

Table 3.4. Estimates of microbial correlation (above diagonal) and genomic correlation (below diagonal) at end of test among meat quality traits.

	[1]SCOL	IMF	SFIRM	MINA	MINB	PH
SCOL		-0.28±0.57	0.07±0.31	0.29±0.44	-0.26±0.39	**0.90±0.25**
IMF	-0.22±0.13		**0.91±0.17**	**0.55±0.28**	**0.75±0.27**	0.10±0.47
SFIRM	0.18±0.19	0.29±0.17		0.26±0.27	0.12±0.26	**0.73±0.35**
MINA	**0.45±0.16**	**0.29±0.14**	-0.53±0.28		**0.78±0.16**	0.33±0.36
MINB	**-0.94±0.22**	**0.78±0.16**	-0.03±0.32	-0.10±0.27		0.38±0.38
PH	0.13±0.50	-0.18±0.25	0.44±0.36	-0.04±0.33	-0.47±0.42	

[1]SCOL = Subjective color score, SFIRM = Subjective firmness score, IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, PH = Ultimate pH;

Numbers in bold are significant.

Table 3.5. Estimates of microbial correlation (above diagonal) and genomic correlation (below diagonal) at end of test among carcass composition traits.

	[1]FD	CADG	HAM	LOIN	BEL
FD		**0.71±0.11**	**0.59±0.16**	**0.55±0.17**	**0.94±0.05**
CADG	0.14±0.15		**0.97±0.02**	**0.91±0.05**	**0.94±0.03**
HAM	-0.10±0.17	**0.63±0.13**		[2]NE	**0.87±0.06**
LOIN	-0.13±0.15	**0.67±0.10**	**0.54±0.19**		**0.82±0.08**
BEL	**0.49±0.13**	**0.78±0.07**	0.34±0.19	**0.40±0.16**	

[1]FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight;

[2]Non estimable; Numbers in bold are significant.

Table 3.6. Estimates of microbial correlation between meat quality traits and carcass composition traits at Off test.

	[1]FD	CADG	HAM	LOIN	BEL
SCOL	-0.29±0.37	-0.09±0.35	0.16±0.38	-0.25±0.35	-0.32±0.37
IMF	**0.90±0.14**	0.43±0.33	0.29±0.27	0.21±0.30	**0.73±0.18**
SFIRM	NE[2]	0.31±0.19	0.18±0.24	-0.01±0.20	**0.50±0.18**
MINA	**0.53±0.18**	**0.66±0.17**	0.11±0.27	0.08±0.30	**0.41±0.21**
MINB	**0.43±0.19**	**0.58±0.18**	0.12±0.25	-0.13±0.28	0.35±0.20
PH	0.17±0.31	0.27±0.35	NC[3]	NC	0.11±0.32

[1]FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight, LOIN = Loin weight, BEL = Belly weight;

[2]Non estimable; [3]Not converged; Numbers in bold are significant.

3.7 Figures

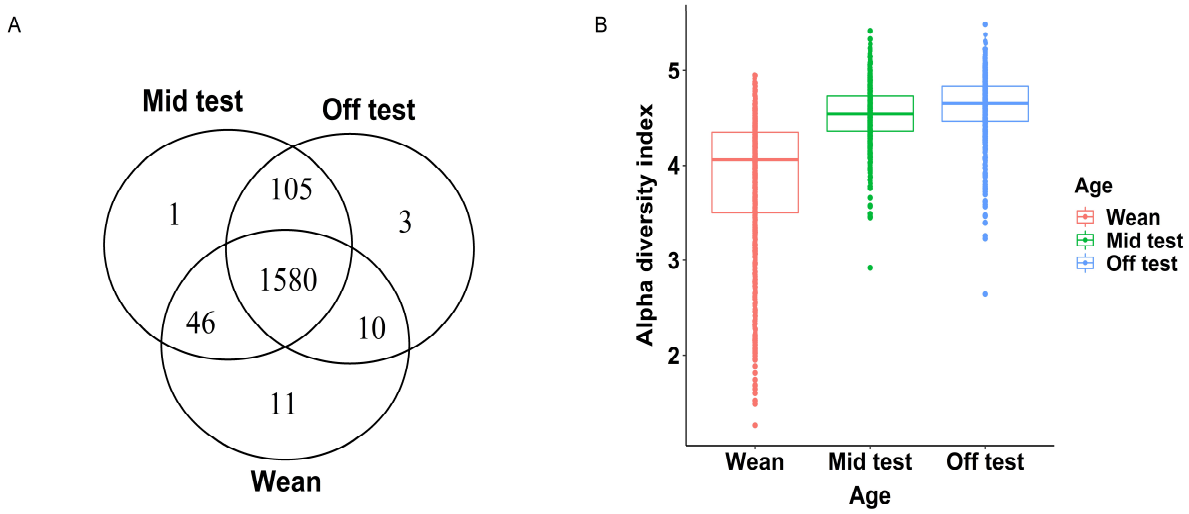

Figure 3.1. (A) Venn diagram with the numbers of common operational taxonomic units (OTU) among weaning, mid test and off test. (B) Distribution of alpha diversity index among weaning, mid test and off test. X- axis represents the different age group and Y-axis represent the alpha diversity index of each sample for each group.

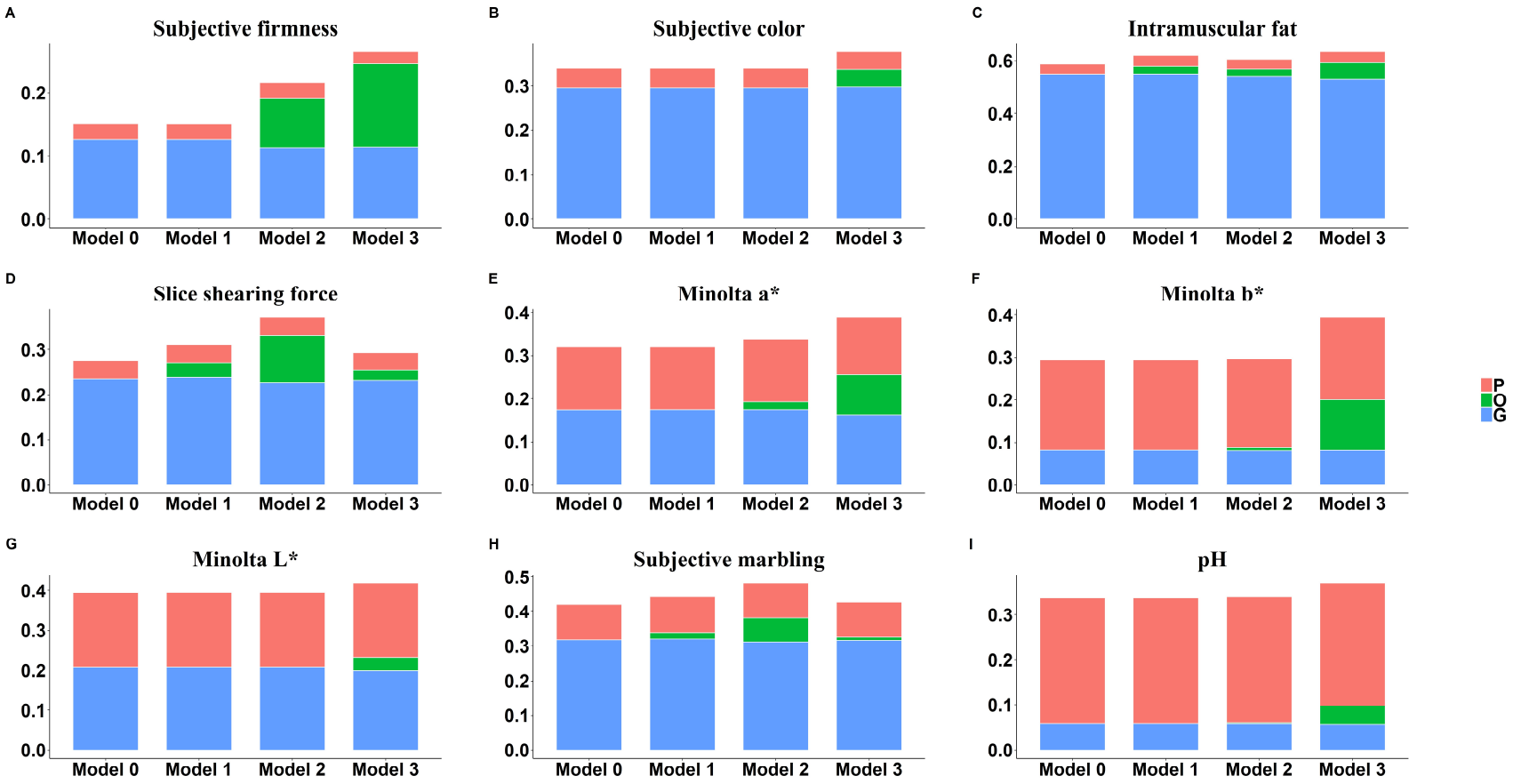

Figure 3.2. Proportion of variance explained by microbiome relationship matrix (**O**), genomic relationship matrix (**G**) and pen (**P**) for meat quality traits. Model 0 contains **G** matrix and pen effect as random effect, Model 1, Model 2 and Model 3 contains **O** matrix at weaning, Mid-test and Off-test in addition to **G** matrix and pen effect.

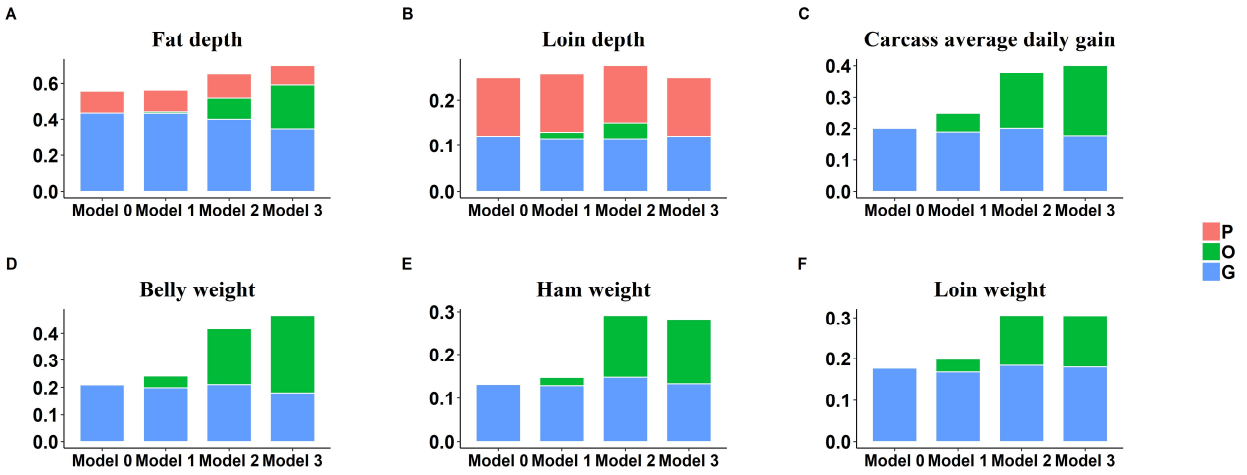

Figure 3.3. Proportion of variance explained by microbiome relationship matrix (**O**), genomic relationship matrix (**G**) and pen (**P**) for carcass composition traits. Model 0 contains **G** matrix and pen effect as random effect, Model 1, Model 2 and Model 3 contains **O** matrix at weaning, Mid-test and Off-test in addition to G matrix and pen effect.

Chapter 4: Modeling Host by Microbiome Interaction for the Prediction of Meat Quality and Carcass Composition Traits in Swine

4.1. Abstract

The objectives of this study were to evaluate genomic and microbial prediction and to test the contribution of host-microbiome interactions to the predictive ability for meat quality and carcass traits in swine. Data were collected from Duroc sired three-way crossbred individuals (n = 1,123) genotyped with 60k SNP chips. Fecal 16S rRNA microbial sequences for all individuals were obtained at three different stages: (Wean: 18.64±1.09 days; Mid-test 118.2±1.18 days; and Off-test: 196.4±7.80 days). A 4-fold cross validation was used, with animals grouped based on sire relatedness. Analyses were conducted with the *BGLR* package in R. Five different models were ran for each model complexity (full, informatively reduced and randomly reduced) to assess their predictive ability. The null model included the fixed effects of dam line, sex and contemporary group and the random effect of pen. The other four models were constructed sequentially including only genomic information, only microbiome information, both genomic and microbiome information, and microbiome, genomic information and their interaction. Full contained whole marker and whole operational taxonomic units (**OTU**) information, informatively reduced and randomly reduced contained informatively reduced and randomly selected markers and OTU information, respectively. The inclusion of microbiome yielded higher predictive ability for fat related traits (fat depth, belly weight, intramuscular fat and subjective marbling), Minolta color related traits (Minolta a*, Minolta b* and Minolta L*) and carcass daily gain. The proportion of variance explained by host genome-microbiome interaction was largest for fat depth (~ 20% at Mid-test and Off-test) and shearing force (~20% consistently across Wean, Mid-test and Off-test). Microbiome information collected at later stages of life was a better predictor of carcass

composition compared to earlier measures. The genome-microbiome interaction yielded higher predictive ability only for fat depth and belly weight. The selection of OTU to include in the model generally did not increase the predictive ability.

4.2 Introduction

Carcass composition and meat quality are economically important traits in pig industry and have been one of the major objectives in pig breeding program (Dransfield et al., 2005; Moeller et al., 2010). Besides genetics, meat quality and carcass traits are affected by various factors like nutrition and environment (Millet et al., 2004; Lebret, 2008; Lee et al., 2014). Genetic improvement of meat and carcass traits requires understanding of genetic basis of these traits to implement selection program that focus on meat quality and carcass. Recent efforts have been devoted to exploiting the genomic variability of individual pigs for meat quality and carcass traits (Miar et al. 2014a; Khanal et al. 2019; Miar et al. 2014b). The microbiome is the key component of the animal and can contribute in variation of phenotype (He et al., 2016). Pig genome contains almost half the number of genes in second genome of host which is called as microbiome (Xiao et al., 2016) has not been explored yet.

The gastrointestinal tract of pigs contain complex microbial ecosystem, which interacts with the host and contributes in several of its biological functions particularly related to health and well-being (Marchesi et al., 2016; Valdes et al., 2018). Previous studies (Jose C. Clemente et al., 2012; Sandoval-Motta et al., 2017) have reported that variability of microbiota should be accounted to better understand health and disease in human. With the advent of efficient and cheap sequencing technologies, research interests in the role of the gut microbiome in animal health and performance has increased (Dowd et al., 2008; Park et al., 2014; Robin B Guevarra et al., 2019). In swine, Lu et al. (2018) and Camarinha-Silva et al. (2017) showed how microbiome contributes

significantly to phenotypic variation of growth traits suggesting that microbiome composition is useful in predicting complex traits in swine.

Prediction of performance in pigs is now routinely performed with the inclusion of genomic information, yet the advantage of including microbiome information is yet to be fully assessed. Few studies (Camarinha-Silva et al., 2017; Maltecca et al., 2019) have been conducted to estimate the accuracy of microbial predictions in swine. However, estimation of prediction ability with the inclusion of both genomic and microbiome information has not been fully explored. Particularly for meat quality and carcass traits the effect of including microbial and host-microbiome interactions on prediction has not been studied at large scale and through several stages of the production life of the pig. The objectives of this study were to i) evaluate genomic and microbial predictions of meat quality and carcass traits and ii) to evaluate the effect of host-microbiome interaction on prediction of meat quality and carcass traits in swine.

4.3. Materials and method

Animal welfare approval was not needed for this study since all data came from animals raised in a commercial setting by The Maschhoffs LLC (Carlyle, IL, USA) under routine circumstances. All pigs were harvested in commercial facilities under the supervision of USDA Food Safety and Inspection Service.

Animals and sample collection

The study pigs consisted of 1,123 three-way cross individuals obtained from 28 purebred Duroc sires and 747 commercial F_1 sows composed of Yorkshire × Landrace or Landrace × Yorkshire. The pigs were weaned at 18.64 ± 1.09 days old and subsequently moved to a nursery-finishing facility. At this facility, weaned pigs were kept in single-sire single-sex pen with 20 pigs per pen. The test period began the day that pigs were moved to the nursery-finishing facility.

During the nursery, growth and finishing period all pigs were fed a standard pelleted feed based on sex and live weight. The diet and their nutritional values are provided (see Appendix B). Standard vaccination and medication protocols (see Appendix B) were followed. End of test was reached when the average weight of pigs of each pen reached 138 kg. Rectal swabs were collected from all pigs at three stages of production (referred to "stage" hereafter): weaning (**Wean**, 15 weeks post weaning (**Mid-test**; average 118.2 ± 1.18 days), and Off-test (**Off-test**, average 196.4 ± 7.80 days). Four pigs from each pen were selected as described by Wilson et al. (2016) and their rectal swabs were used for subsequent microbial sequencing. There were 1,205, 1,295 and 1,273 samples for Wean, Mid-test and Off-test respectively. Distribution of samples across families, time points and sex are provided (see Appendix B).

Meat quality traits (intramuscular fat content (**IMF**), Minolta a* (**MINA**), Minolta b* (**MINB**), minolta L* (**MINL**), ultimate pH (**PH**), subjective color score (**SCOL**), subjective marbling score (**SMARB**), subjective firmness score (**SFIRM**), shearing force (**SSF**)) and carcass composition traits (Belly weight (**BEL**), ham weight (**HAM**), loin weight (**LOIN**), fat depth (**FD**), loin depth (**LD**) and carcass average daily gain (**CADG**)) were used for the current analysis and data were collected as described by Khanal et al. (2019). A summary of traits used in current research is reported in Table 4.1.

Illumina amplicon sequencing

DNA extraction, purification, Illumina library preparation and sequencing were done as described by Lu et al. (2018). Briefly, total DNA (gDNA) was extracted from each rectal swab by mechanical disruption in phenol: chloroform. The DNA was purified using a QIAquick 96 PCR purification kit (Qiagen, MD, USA). Purification was performed per the manufacturer's instruction with the following minor modifications: (i) sodium acetate (3 M, pH 5.5) was added to Buffer PM

to a final concentration of 185 mM to ensure optimal binding of genomic DNA to the silica membrane; (ii) crude DNA was combined with 4 volumes of Buffer PM (rather than 3 volumes); and (iii) DNA was eluted in 100 µL Buffer EB (rather than 80µL). All sequencing was performed at DNA Sequencing Innovation Laboratory at the Center of Genome Sciences and Systems Biology at Washington University in St. Louis. Phased, bi-directional amplification of the v4 region (515-806) of the 16S rRNA gene was employed to generate indexed libraries for Illumina sequencing as described in Faith et al. (2013). Sequencing was performed on an Illumina MiSeq instrument (Illumina, Inc. San Diego, USA), generating 250 bp paired-end reads.

16S rRNA gene sequencing and quality control of data

Pairs of 16S rRNA gene sequences were first merged into a single sequence using FLASH v1.2.11 (Magoc and Salzberg, 2011) with a required overlap of at least 100 and less than 250 base pairs in order to provide confident overlap. Sequences with a mean quality score below Q35 were then filtered out using PRINSEQ v0.20.4 (Schmieder and Edwards, 2011). Sequences were oriented in the forward direction and any primer sequences were matched and trimmed off. Mismatch was allowed up to 1. Sequences were subsequently demultiplexed using QIIME v1.9 (Caporaso et al., 2010). Sequences with greater than 97% nucleotide sequence were clustered into operational taxonomic units (**OTU**) using QIIME with the following settings: `max_accepts = 50, max_rejects = 8, percent_subsample = 0.1 and -suppress_step4`. A modified version of Green Genes (Ley et al., 2006) was used as reference database. Input sequences that had 10% of the reads with no hit to the reference database were then clustered de novo with UCLUST (Schloss and Handelsman, 2006) to generate new reference OTU to which the remaining 90% of reads were assigned. The most abundant sequence in each cluster was used as representative sequence for the OTU. Sparse OTU were then filtered out by requiring a

minimum total observation count of 1,200 for an OTU to be retained, the resulting OTU table was rarefied to 10,000 counts per sample and after data processing and quality control 1,755 OTU were retained for further analyses.

Genotyping

All pigs were genotyped with the PorcineSNP60 v2 BeadChip (Illumina, Inc., San Diego, CA). Quality control procedures were applied by removing SNPs with a call rate of less than 0.90 and minor allele frequency of less than 0.05. After quality control the number of SNPs remaining for further analyses was 42,529.

Statistical analyses

Univariate analyses were conducted to estimate the variance components explained by genomic and microbiome information. Single trait models had form:

$$y_{ijklms} = \mu + dl_i + cg_j + sex_k + a_s + pen_{l(j)} + e_{ijklms} \quad (1)$$

where y_{ijklms} was the trait measured, μ was the overall mean, dl_i was the ith fixed effect of dam line (2 levels), cg_j was the jth fixed effect of contemporary group (6 levels), sex_k was the kth fixed effect of sex (2 levels), $pen_{l(j)}$ was the random effect of pen nested within contemporary group and a_s was the random additive genetic effect. The random effect of the animal was assumed normally distributed with mean 0 and variance $\mathbf{G}\sigma_a^2$ where σ_a^2 was total genomic variance and **G** was the genomic relationship matrix built on marker information as described in first method by VanRaden (VanRaden, 2008). Pen and residuals were assumed normally distributed with mean zero and variances $\mathbf{I}\sigma_{pen}^2$ and $\mathbf{I}\sigma_e^2$, respectively, where **I** was an identity matrix, σ_{pen}^2 was pen variance and σ_e^2 was the error variance.

Microbiome information was added to model (1) to estimate the variance explained by the microbiome at each growth stage. Model (2) was written as:

$$y_{ijklmsn} = \mu + dl_i + cg_j + sex_k + a_s + o_m + pen_{l(j)} + e_{ijklmn} \quad (2)$$

where *dl*, *cg*, *sex*, *a*, *pen* and *e* remained the same as previously described and o_m was the random effect of the animal microbiome. The random effect of the microbiome was assumed normally distributed with mean 0 and variance $\mathbf{M}\sigma_m^2$ in which **M** was a microbial correlation matrix among individuals and σ_m^2 was the microbiome variance. The matrix **M** was created following Camarinha-Silva et al. (2017). Briefly, **M** was obtained as $\mathbf{M} = \frac{1}{q}\mathbf{X}\mathbf{X}^{\mathbf{T}}$, with **X** of dimension of $n \times q$, where n was the number of animals and q was the number of OTU. **X** was constructed from **S**, a matrix of equivalent dimensions $n \times q$. Each element of the **S** matrix, S_{ij}, was the relative abundance of OTU j in animal i. The elements of **X** were calculated as:

$$X_{ij} = \frac{log(S_{ij}) - \overline{logS_{\circ j}}}{sd(logS_{\circ j})}$$

where $S_{\circ k}$ was the vector of the j[th] column of **S**. The **M** matrix was created for each stage (Wean, Mid-test and Off-test) separately and fitted in each model separately.

Modeling of all possible interactions between markers and OTU is computationally impossible, however, interactions can be modeled through covariance functions. Jarquín et al. (2014) showed that covariance function is simply the Hadamard (cell-by-cell) product of genomic and environmental relationship matrices. The model was the expanded as following:

$$y_{ijklmsn} = \mu + dl_i + cg_j + sex_k + pen_{l(j)} + o_m + a_s + ao_{ms} + e_{ijklmsn} \quad (3)$$

where *y*, μ, *dl*, *cg*, *sex*, *pen*, *a*, *o* and *e* were the same as defined above. ao_{ms} was the random interaction of m[th] OTU and s[th] animal. The random effect of interaction *ao* was again assumed ~ N (0, [**G°M**] σ_{am}^2), where σ_{am}^2 was the variance of the genomic by microbiome interaction and **G°M** indicates the Hadamard product of **G** and **M**.

Predictive ability

Models employed

The baseline model (**NULL**) consisted of all fixed effects and only pen as random effect. The model was defined as:

$$y_{ijklm} = \mu + dl_i + cg_j + sex_k + pen_{l(j)} + e_{ijklm} \quad (4)$$

where μ, dl_i, cg_j, sex_k, $pen_{l(j)}$ and e_{ijklm} were the same as described before. All of these elements will be collectively called as "*else*" hereafter. The second model (**M_mod**) included the microbiome information in addition to the effects in the NULL model:

$$y_{ijklmn} = else + o_m \quad (5)$$

The third model (**G_mod**) included the genomic information in addition to the NULL model:

$$y_{ijkls} = else + a_s \quad (6)$$

The fourth model (**GM_mod**) included both microbiome and genomic information in addition to NULL model:

$$y_{ijklmn} = else + o_m + a_s \quad (7)$$

Finally, the fifth model (**G×M_mod**) included all other terms plus the interaction between microbiome and genomic information.

$$y_{ijklmsn} = else + o_m + a_s + ao_{ms} \quad (8)$$

All the analyses were performed using Bayesian reproducing kernel Hilbert space (RKHS) regression models (Gianola and van Kaam, 2008; Morota and Gianola, 2014) implemented in the BGLR package (Pérez and de los Campos, 2014) in R. The Markov chain-Monte Carlo algorithm was run for 120,000 iterations with 20,000 iterations discarded as burn-in and thinning interval of 5 iterations. Parameters' estimates were obtained from the mean of the samples. Convergence of

the models was checked by visual inspection of trace plots of variance components and post-Gibbs analyses using CODA package (results not shown) in R (Plummer et al., 2006).

Reduced Models

To test the possibility of increasing predictive ability (**PA**) of the models by using a reduced number of significant markers and OTUs, a series of alternative models were tested. All the models presented in the previous section were run with three different sets of predictors (referred to as "complexity" hereafter): full (**FULL**), informatively reduced (**IR**) and randomly reduced (**RR**). Full denoted the use of all available markers and OTU to calculate **G** and **M**, while **IR** denoted the use of the top 10% markers and OTU associated with a particular trait and **RR** the use of a random sample of OTU and SNP, respectively. The threshold of 10% was in this case set arbitrarily for ease of comparison.

The association between each marker/OTU with the phenotypes was established using a single marker linear regression model with SNP allele count and OTU count as linear covariate, and the available performance traits as responses. The model was:

$$y_{ijklmno} = else + S_m + \beta X_n + e_{ijklmno} \quad (9)$$

where *else* remained the same as previously describe. S_m was the m^{th} fixed effect of sires (28 levels), X_n is the n^{th} marker/OTU and β represented the marker/OTU effects. The markers and OTU with *P*-value < 0.05 after Bonferroni correction, were considered significant (referred to "informatively reduced markers/OTU" hereafter) and included in the subsequent calculations of **G** and **M** predictions.

Training and testing sets

A stratified 4-fold cross validation scheme was used to split the data into training (~ 75% of observations) and testing (~25% of observations) sets, in which the individuals were grouped

based on the relatedness of the 28 sires in the trial. In each fold, progenies from 21 sires were allocated to the training set while progenies from the remaining 7 sires were put testing set. A pictorial representation of overall experimental design is presented in Figure 4.1.

Different sets of markers and OTU were selected concomitantly with the four folds of cross-validation scheme. The pictorial representation of selection of markers and OTU is presented on Figure 4.2. Briefly, for IR, a unique set of significant markers was selected from each training set. We had different sets of OTU counts for each stage (Wean, Mid-test and Off-test). So, a unique set of significant OTU was selected for each stage from each training set. The numbers of significant markers and OTU obtained from each training set is presented (see Appendix C; Table S.C1 and S.C2). For RR, a unique set of the markers was randomly selected from each training set and 3 unique sets of OTU were randomly selected from each training set. The resulting SNPs and OTU were then used to construct **G** and **M** matrix for fitting in different models (M_mod, G_mod, GM_mod and G×M_mod) in order to obtain predictive ability.

The PA of different models was assessed by comparing the observed phenotype values (y) with predicted phenotype values ($\widehat{y_{test}}$) in the testing set using Pearson's product-moment correlation coefficient and the mean squared error (MSE) of prediction calculated as MSE = (y-$\widehat{y_{test}}$)^2.

Post analysis

In order to provide a comprehensive assessment of all factors in the experimental design, we conducted a post-analysis of the results. All predictive abilities of trait, stage, complexity and model were pooled into a single dataset. The following linear model was then fitted:

$$y_{ijklmn} = T_i + S_j + M_k + C_l + F_m + SM_{jk} + SC_{jl} + MC_{jk} + TS_{ij} + TM_{ik} + TC_{il} + e_{ijklmn} \quad (10)$$

where y_{ijklmn} was the prediction ability of each trait/ stage/ model/complexity/fold combination; T_i was the fixed effect of traits measured (15 levels of traits), S_i was the fixed effect of stage of production (3 levels: Wean, Mid-test and Off-test), M_j was the fixed effect of model (5 levels: NULL, M_mod, G_mod, GM_mod and G×M_mod), C_k was the fixed effect of complexity of model (3 levels: Full, IR and RR), F_m was the fixed effect of each fold of cross-validation (4 level: Fold 1, Fold 2, Fold 3 and Fold 4). $SM_{jk}, SC_{jl}, MC_{jk}, TS_{ij}, TM_{ik}, TC_{il}$ were the pairwise interactions of the main effects. The 'lm' function of R was used to fit the model (Chambers and Hastie, 1992). The type III ANOVA table was obtained from the 'Anova' function of R package *car* (Fox and Weisberg, 2019) and, lsmeans and pairwise contrasts were obtained from *emmeans* (Searle et al., 1980) package in R.

4.4 Results

Data summary

Mean and standard deviation of each meat quality and carcass composition trait are provided in Table 4.1. There were 9 meat quality and 6 carcass composition traits. The numbers of individual samples with complete genotypic, phenotypic and microbiome information at each stage was 1,123, which were used for further analyses. The distribution of taxonomic abundances at three stages (Wean, Mid-test, Off-test) in the current animals was reported in Lu et al. (2018). Briefly, at three stages, there were 14, 21, 29, 54, 106, and 202 identified phyla, classes, orders, families, genera, and species, respectively. Over the three stages, 95.79-97.80% of the OTU were classified into six phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Spirochaetes*, and *Actinobacteria*. *Firmicutes* represented the largest proportion of OTU followed by *Bacteroides*. *Bacteroides* and *Firmicutes* accounted for 73.61, 95.35, and 93.03% of all reads at weaning, Mid-test and Off-test respectively.

Variance explained by G, M and G × M interaction

Variance estimates, as well as heritabilities and microbiabilities for meat quality and carcass traits are presented (see Appendix B). Heritabilities and microbiabilities have already been described by Khanal et al. (2019). Briefly, the proportion of variance explained by microbiome (microbiability) was higher for carcass composition traits than for meat quality traits. The microbiabilities of both meat quality and carcass composition traits at weaning were negligible. Three meat quality traits had significant microbiability at Mid-test, with estimates of 0.07±0.03 for SMARB, 0.08±0.03 for SFIRM and 0.10±0.04 for MINB. At Off-test, 4 meat quality traits had significant microbiability, with estimates of 0.06±0.02 for IMF, 0.09±0.04 for MINA, 0.11±0.04 for MINB and 0.13±0.04 for SFIRM. Majority of carcass traits were significantly affected by microbiome at Mid-test and Off-test. The microbiability of carcass composition traits at Mid-test ranged from 0.12 ± 0.04 for LOIN and FD to 0.20 ± 0.04 for BEL. The microbiability of carcass composition traits at Off-test ranged from 0.13 ± 0.05 for LOIN to 0.29 ± 0.05 for BEL. For most of the carcass traits, additive genetic variance was eroded when incorporating microbiome information in the model, particularly at Mid-test and Off-test suggesting a possible microbiome-host interaction. In this study this component was included in the models explicitly.

The proportion of variance explained by different effects for models G_mod and G×M_mod at different stages and complexity is reported in Figure 4.3 for carcass traits and Figure 4.4 for meat quality traits. At FULL complexity, the interaction between host genetics and microbiome was sizable for FD, LD, BEL, SMARB, MINA, MINB, MINL, SFIRM and pH, where the proportion of variance explained by G×M ranged from ~2% for LD at Mid-test to ~28% for pH at Off-test. With the inclusion of **G×M** in the model, there was no decrease in the genomic

heritability compared to model containing genomic and microbiome information separately except for pH and MINL where the decrease in genomic heritability was ~2%.

For most of the traits, the proportion of variance explained by the **G×M** was highest for IR than FULL complexity. At IR complexity, the magnitude of interaction was higher at Off-test followed by Mid-test and Wean except for LD where the trend was reversed, and the proportion of variance explained by **G×M** was highest at weaning and least for Off-test. Among carcass composition traits, the proportion of variance explained by **G×M** was largest for fat depth (~ 20% at Mid-test and Off-test), while virtually null for CADG, HAM and LOIN. Among meat quality traits, the proportion of variance explained by **G×M** was largest for SSF (~20% consistently across time points) and almost null for SCOL. At IR complexity, FD, pH and SSF had greater proportion of variance explained by **G×M** than the proportion of variance explained by the microbiome itself. There was ~10%, ~8% and ~11% more variance explained by **G×M** than **M** itself for SSF at Wean, Mid-test, and Off-test respectively. Similarly, ~6%, ~10% and ~13% more variance was explained by **G×M** than **M** itself for pH at Wean, Mid-test, and Off-test respectively. Approximately 10% and 5% more variance were explained by **G×M** than **M** itself for FD at Mid-test and Off-test respectively.

For RR complexity, proportion of variance explained by **G×M** was almost null for majority of traits except FD, SSF, MINA and PH, where FD had highest magnitude at Mid-test (~19%) followed by pH at Off-test (~11%), SSF at weaning (~8%) and MINA at weaning (~4%). Use of informatively reduced markers and OTU had greater proportion of variance explained by **G×M** compared to randomly reduced markers and OTU.

Predictive ability

We have investigated the effectiveness of various prediction models under different complexities (FULL, IR and RR). Predictive ability of different models for carcass composition traits and meat quality traits for each stage and complexity is reported in Figure 4.5 and Figure 4.6 respectively. All of the PA of each fold of cross-validation for carcass composition and meat quality traits are reported (see Appendix C). Histograms in the figures represent the average predictive ability (over the 4 folds) and their respective standard deviation. The dashed line represents the predictive ability of the NULL model. Mean squared errors (MSE) of various models at Full, IR and RR complexities are reported in Figure 7 and Figure 8 for carcass traits and meat quality traits respectively.

At FULL complexity (panel A of Figure 4.5 and Figure 4.6), majority of traits had greater PA for all models compared to NULL models. Model M_mod, GM_mod and G×M_mod had greater PA compared to G_mod for majority of carcass traits, specially at Mid-test and Off-test. Model M_mod outperformed model G_mod for BEL, CADG, HAM and LOIN, especially at Mid-test and Off-test by 4% and 8%; 3% and 6%; 4% and 6%; and 2% and 3%, respectively. The predictive ability of FD, BEL, CADG, HAM, LOIN, IMF, SSF, MINA and MINB for model GM_mod and G×M_mod was greater compared to G_mod. Among all the traits having greater PA for model GM_mod than model G_mod, BEL had the greatest increase in PA (~7% averaged over stage). Similarly, BEL also had greater increase in PA for G×M_mod compared to G_mod (~7.5% averaged over stage). The predictive ability of all traits had greater PA for GM_mod and G×M_mod compared to M_mod. The greatest increase in PA for GM_mod and G×M_mod was ~14% (averaged over each stage) for SCOL. The predictive abilities of M_mod, GM_mod and G×M_mod for majority of the traits at were substantially larger at Mid-test and Off-test compared

to weaning. The predictive ability of G×M_mod did not improve compared to GM_mod for majority of traits except BEL, where the PA increased by ~1% for Mid-test and ~2% at Off-test. Regarding predictive ability measured in terms of MSE, for FD, CADG, HAM, IMF and MINB, model G×M_mod at Off-test had lower MSE compared to other models.

At IR complexity (panel B of Figure 4.5 and 4.6), majority of traits had greater PA for all models compared to NULL models. Model M_mod outperformed the model G_mod for BEL, CADG and LOIN at Mid-test and Off-test by 8% and 10%; 10% and 8%; 9% and 1%, respectively. The PA was greater for model that contains microbiome and genomic information separately (G_mod and M_mod). However, if we include the genomic and microbiome information together (GM_mod and G×M_mod), PA decreased for all traits except BEL where the PA of model G×M_mod was greater than model G_mod at Off-test and at Mid-test by 3% and 5%. The model GM_mod and G×M_mod performed similar for all meat quality and carcass traits.

Informatively reduction of markers and OTU increased the PA of all traits for G_mod and M_mod in comparison to FULL complexity. The MSE of G_mod was smaller compared to G_mod of FULL complexity. However, the MSE of M_mod for majority of traits was greater compared to M_mod of FULL complexity (Figure 4.5 and 4.6). For majority of traits, Model GM_mod and G×M_mod did not perform better in terms of PA except for BEL, where the PA of IR complexity was ~4% and ~5% greater compared to FULL. However, the MSE of both models were greater compared to FULL complexity. Regarding predictive ability measured in terms of MSE, models with IR complexity had greater MSE compared to FULL complexity except for G_mod, which had lower MSE with informatively selected markers (panel A and B of Figure 4.7 and 4.8).

Informatively reduction of markers and OTU increased the PA for G_mod and M_mod compared to RR complexity. The models with both genomic and microbiome information

(GM_mod and G×M_mod) for FD, CADG, BEL, HAM, LOIN, IMF and MINL performed better in terms of both prediction accuracy. However, both models did not perform better in terms of MSE (panel B and C of Figure 4.7 and 4.8).

Post analysis

To evaluate the overall influence of all the factors in the experimental design to PA, we conducted post-analysis of the cross-validation study. Results of this analysis are reported in Table 4.2 and Figure 4.9. Table 4.2 presented the Type III ANOVA table of overall design. All the factors were highly significant except the interaction between Stage and Trait. Figure 4.9 presented the least square means of significant main effects and their interactions. All models performed better than Null model. Genomic prediction was better than microbial prediction (averaged over all other factor). However, microbial prediction was better for some traits (FD, BEL, CADG and LOIN). Averaging over all the factors in the design, models G_mod, GM_mod and G×M_mod performed similar in predictive performance. However, for BEL and FD, models GM_mod and G×M_mod performed similar predictive performance and better than G_mod model. Of the three stages of production, Mid-test and Off-test performed similar and were higher than weaning. Among individual traits, predictive performance at Mid-test and Off-test was heterogenous. However, both were always better than at weaning except for LD, where PA at weaning was better than that of Mid-test and Off-test. Informatively reduced markers and OTU had better PA than Full and RR complexity. Fat related traits (FD, IMF, BEL) and CADG can be best predicted while LD, MINL, SCOL and pH was least accurately predicted. The interaction of stage and model restated that at Mid-test and Off-test, G_mod, GM_mod and G×M_mod performed similar but at weaning G_mod performed better than GM_mod and G×M_mod. The interaction of complexity

and model showed that G_mod at IR had high predictive performance and Null model at all stages had least predictive performance.

4.5 Discussion

Previous studies have explored host-microbiome interactions in swine (Crespo-Piazuelo et al., 2018; Han et al., 2018). However, these studies did not involve the genomic information of the host. These studies explained the effect of microbiota on various physiological processes. In the current study, we have evaluated the effect of host genome, gut microbiome and interaction of genome-by-microbiome on prediction of meat quality and carcass traits in swine. For this purpose, we employed various models with different sets of markers and OTU to provide a better understanding of the contribution of the different components in the prediction of meat quality and carcass traits.

Genomic by microbiome interaction

In the current study, the **G×M** contribution was higher for IR complexity of models. This result suggested that using markers and OTU with a putative biological role helps to understand the mechanism of interaction of host and microbiome rather than simply accounting for genomic relationships and microbial relationships with all or randomly selected markers and OTU respectively. The magnitude of interaction was sizable for fat related traits (FD, BEL, SMARB, IMF), lower for objective color traits (MINA, MINB, MINL), pH and SSF. Significant contribution of interaction of host genetics and gut microbiome to obesity has been demonstrated by different authors (Turnbaugh et al. 2009; Kinross et al., 2011; Kelly et al., 2015; Ussar et al., 2016) in human. Gut microbes produce short chain fatty acids which regulate the host body energy homeostasis (Gill et al., 2011; Mann et al., 2014) and deposition of fat in body and muscle. Fang et al. (2017) reported that gut bacteria was involved in energy metabolism and subsequently in the

intramuscular fat content of muscle in pig. Prominent G×M interaction of objective color measures and pH suggests that the genetic response to color and pH of different animals varies among different microbiome composition. High **G×M** for SSF suggested that genetic response to SSF of different host is affected by different gut microbiome altering the shear force. The higher proportion of variance explained by **G×M** for different traits suggests that pigs have different trait values at different microbial composition, which could create an opportunity to improve these traits with alteration of gut microbiome. To the best of our knowledge, this study is the first attempt to investigate the effect of host-microbiome interaction in meat quality traits of swine. This study could be the basis of genome-by-microbiome study in the future.

Predictive ability

To the best of our knowledge this study is the first to investigate the predictive ability of meat quality and carcass traits with the inclusion of genome-by-microbiome components in swine. In this study, PA of most of the models increased and MSE of most models decreased with the inclusion of microbiome. These results agreed with Maltecca et al. (2019). Our results showed that the predictive ability was greater when including microbiome data collected at Off-test rather than other timepoints for majority of traits. Higher PA at later-stage microbiome information is in agreement with Maltecca et al. (2019). The greater PA of fat related traits (FD, BEL, IMF) suggests us to use the microbiome information in the model for greater accuracy. Generally, the prediction at young age is important in swine industry. So, further evaluation of predictive models with the microbiome information at early age should be done because the DNA marker is fixed and OTU could be affected by various factors like environment, individual samples and bioinformatic machinery used to obtain taxonomical units.

At FULL complexity, for most of the trait (for eg. BEL, FD, CADG, LOIN, HAM, SSF, SFIRM, MINL) models GM_mod and G×M_mod had higher PA than G_mod. However, GM_mod and G×M_mod have similar PA. Within FULL complexity, the MSE of prediction was lower for GM_mod and G×M_mod for some of traits at Mid-test and Off-test compared to other models. For example, MSE of FD, CADG, HAM, IMF and MINB for model G×M_mod was lower than that of models G_mod and GM_mod (Figure 7 and Figure 8). González-Recio, et al. (2014) reported that MSE is preferred to select models because it considers both prediction bias and variability, whereas the predictive ability provides only a measure of association. Furthermore, the authors also reported that predictive correlations are bounded between 0 and 1, while MSE can move from 0 to infinity. This property of MSE suggested that G×M_mod could be used for prediction with more accuracy for FD, CADG, HAM, IMF, MINA and MINB. However, post analysis showed that models G_mod, GM_mod and G×M_mod have similar predictive ability. To get better predictive ability for model GM_mod and G×M_mod, study with larger population size could be done. Similar concept of increase in study sample for increment of predictive ability was reported by Lado et al. (2016) in wheat breeding.

Informatively reduced markers had greater prediction compared to whole or random set of SNPs. This suggested that the predictive performance of traits is governed by the markers with putative biological role rather than simply accounting for genomic relationship among different individuals. Previous studies have also reported that SNP subsets with larger effect achieved higher predictive performance than that obtained from all SNPs (Weigel et al. 2009; Tiezzi and Maltecca, 2015). However, the models with informatively selected OTU did not outperform the PA of model GM_mod and G×M_mod for FULL complexity in terms of both correlation and MSE (Figure 4.5, 4.6, 4.7 and 4.8). This suggested that reducing the dimension of OTU did not perform better on

predicting phenotypes. This could be because the effect of microbiome on various traits is distributed equally on all OTU, more than what happens with markers on the host genome. It could be concluded that the traits are not only polygenic in nature but also polymicrobial in nature. This agreed with Camarinha-Silva et al. (2017) who reported that many of the OTU present in the study explain the small portion of variability for daily gain, feed intake and feed conversion in pigs. We should conclude that the predictive ability exhibited by the significant OTU is not driven by their biological roles but rather accounting for microbiome relationships. It is likely that a better understanding of biology of each trait and OTU affecting each trait can provide new opportunities in prediction through microbiome. To our knowledge, this is the first study to investigate the PA by reducing the dimension of OTU. We believe this should be further investigated with a larger number of individuals and OTU than it is available in the current study.

4.6 Conclusion

This study was conducted to investigate the effect of host-microbiome interaction on meat quality and carcass composition traits in crossbred swine at different stages of production (Wean, Mid-test and Off-test). To the best of our knowledge, this is the first attempt to investigate the effect of host-microbiome interaction on meat quality and carcass traits. Inclusion of microbiome increased the prediction ability for majority of traits although it varied over time. Microbiome information at later stage of life had greater prediction ability. With the inclusion of microbiome, higher predictive ability was found for fat related traits (fat depth, belly weight, intramuscular fat and subjective marbling), objective color measures (Minolta a, Minolta b and Minolta L) and carcass daily gain. Informatively selected SNPs showed better predictive ability but reducing the number of OTU did not improve prediction ability. This suggested that to employ the benefit of microbiome information in prediction, all OTU should be used. There was sizable proportion of

variance explained by **G×M** for fat depth and belly weight suggesting that fat related traits performed different across different composition of gut microbiota. Further research on identification of bacterial composition that contribute to lean meat production could be done in future.

4.7 Tables

Table 4.1. Descriptive statistics of carcass composition and meat quality traits: acronym, means, standard deviation (SD) values.

Traits	Acronym	Mean	SD
Carcass traits			
Loin depth, mm	LD	67.99	7.21
Back fat depth, mm	FD	22.07	5.24
Carcass average daily gain, g/day	CADG	552.90	73.93
Ham weight, kg	HAM	25.19	2.34
Loin Weight, kg	LOIN	20.01	1.88
Belly weight, kg	BEL	15.88	2.55
Meat quality			
Intra muscular fat, %	IMF	2.71	1.01
Minolta a*	MINA	3.77	1.16
Minolta b*	MINB	-0.16	0.87
Minolta L*	MINL	45.37	5.76
Ultimate pH	PH	5.64	0.22
Subjective color	SCOL	2.72	0.57
Subjective marbling	SMARB	3.10	0.91
Subjective firmness	SFIRM	3.05	1.04
Slice shear force, N	SSF	156.96	41.99

Table 4.2. Type III ANOVA of post-analysis of experimental design

	Sum Square	Mean Square	F value	Pr (> F)
Complexity	0.49	0.25	57.94	0.000***
Complexity:Trait	0.40	0.01	3.35	0.000***
Fold	1.43	0.48	112.14	0.000***
Model	3.08	0.77	181.44	0.000***
Model:Complexity	1.03	0.13	30.50	0.000***
Model:Stage	0.12	0.02	3.57	0.000***
Model:Trait	1.45	0.03	6.09	0.000***
Stage	0.12	0.06	13.88	0.000***
Stage:Complexity	0.05	0.01	2.83	0.023*
Stage:Trait	0.09	0.00	0.80	0.77
Trait	26.66	1.90	449.20	0.000***

Model (5 levels: Null, M, G, M+G, M+G+MG), Stage (3 levels: Wean, On test and Off test), Complexity (3 levels: Full, IR (Informatively reduced), RR (Randomly reduced)), Trait (15 levels: FD, CADG, LD, HAM, LOIN, BEL, IMF, SMARB, MINA, MINB, MINL, pH, SSF, SFIRM). All elements with (:) represents pairwise interactions.

4.8 Figures

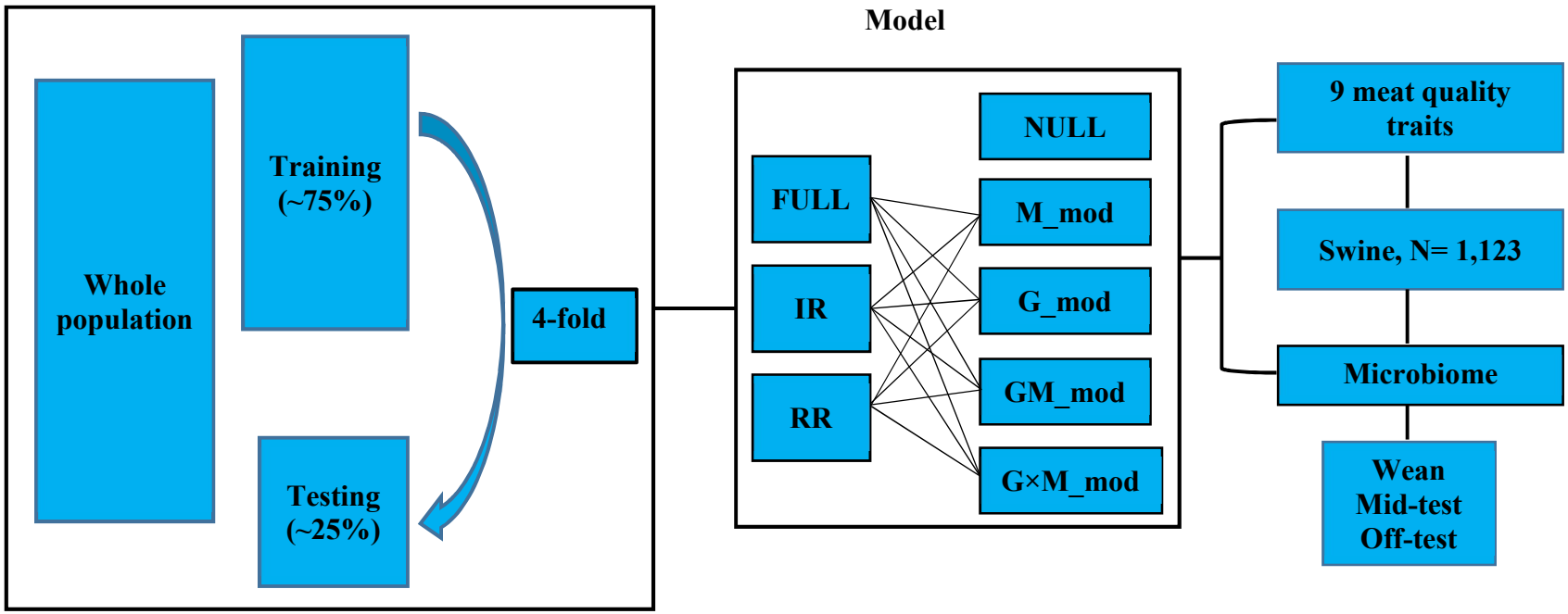

Figure 4.1. Overall experimental design. FULL contains all available markers and operational taxonomic units (OTU), IR contain informatively reduced markers and OTU, RR contains randomly reduced markers and OTU, NULL is base line model, M_mod contains microbiome information in addition to NULL model, G_mod contains genomic information in addition to NULL model, GM_mod contains microbiome and genomic information in addition to NULL model and G×M_mod contains genomic, microbiome and interaction of genome-by-microbiome information in addition to NULL model.

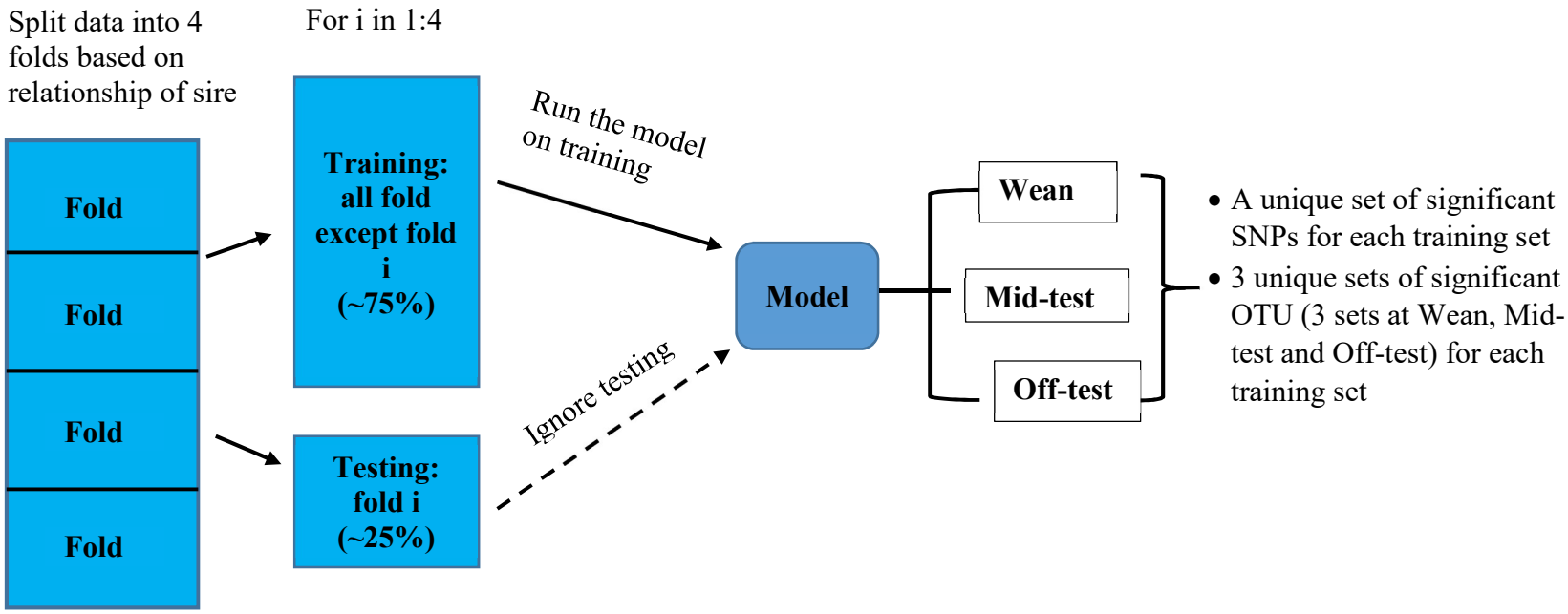

Figure 4.2. Pictorial representation of analysis to obtain informatively reduced markers and operational taxonomic units (OTU).

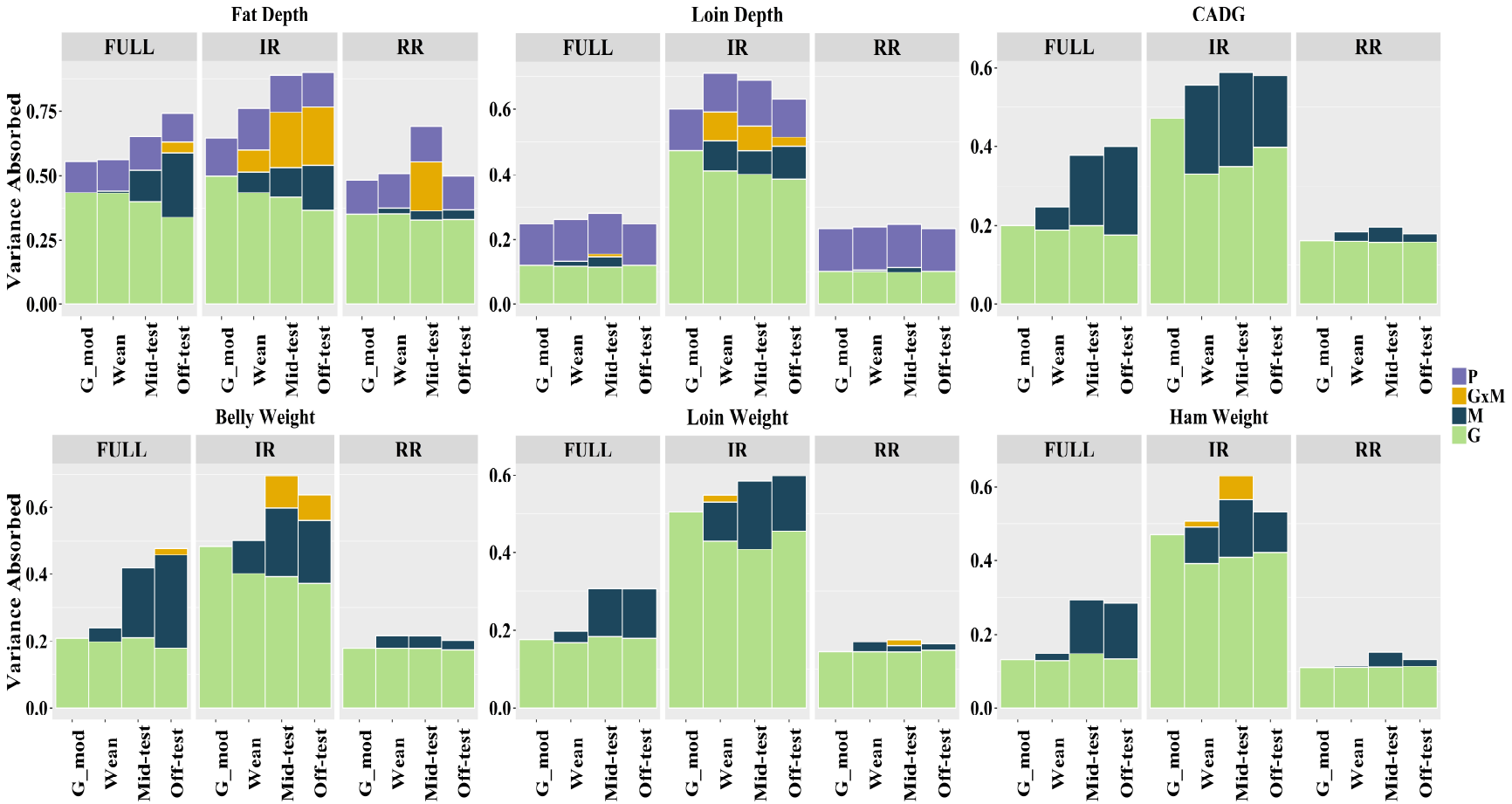

Figure 4.3. Variance components estimates of carcass traits for model containing only genomic information (G_mod) and microbiome, genomic and genotype-by-microbiome interaction (G×M_mod) at different stages of production (Wean, Mid-test and Off-test) with different sets of markers and operational taxonomic units (OTU) counts (FULL: contain all available markers and OTU, IR: contain informatively reduced markers and OTU, and RR: contain randomly reduced markers and OTU). Each bar shows different model with the proportion of variance explained by microbiome relationship matrix (**M**), genomic relationship matrix (**G**) and pen (**P**). G_mod contains **G** matrix and pen effect as random effect; Wean, Mid-test and Off-test contains **M** matrix and interaction of **M** and **G** at weaning, Mid-test and Off-test in addition to **G** matrix and pen effect.

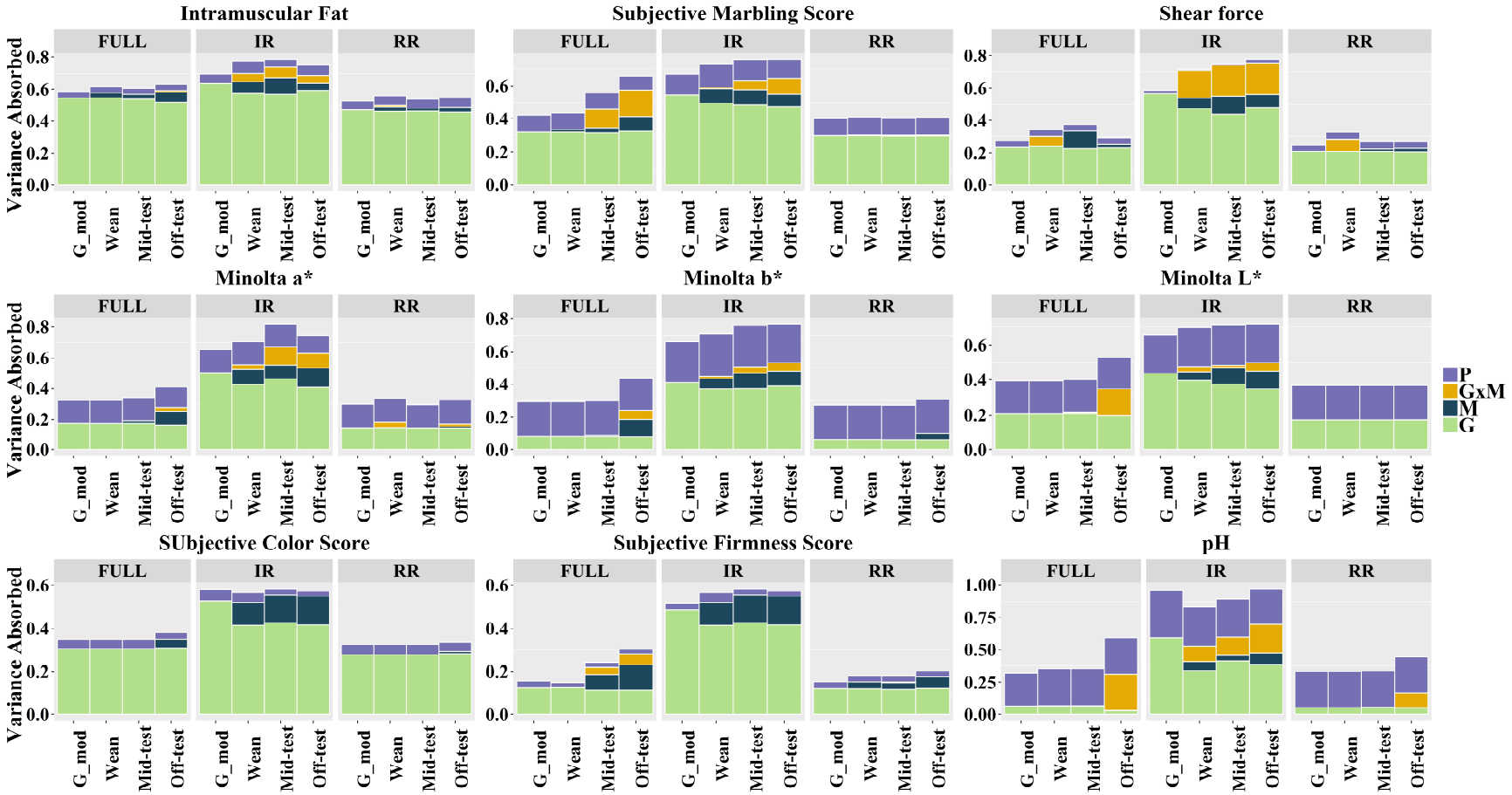

Figure 4.4. Variance components estimates of meat quality traits for model containing only genomic information (G_mod) and microbiome, genomic and genotype-by-microbiome interaction (G×M_mod) at different stages of production (Wean, Mid-test and Off-test) with different sets of markers and operational taxonomic units (OTU) counts (FULL: contains all available markers and OTU, IR: contains informatively reduced markers and OTU, and RR: contain randomly reduced markers and OTU). Each bar shows different model with the proportion of variance explained by microbiome relationship matrix (**M**), genomic relationship matrix (**G**) and pen (**P**). G_mod contains **G** matrix and pen effect as random effect; Wean, Mid-test and Off-test contains **M** matrix and interaction of **M** and **G** at weaning, Mid-test and Off-test in addition to **G** matrix and pen effect.

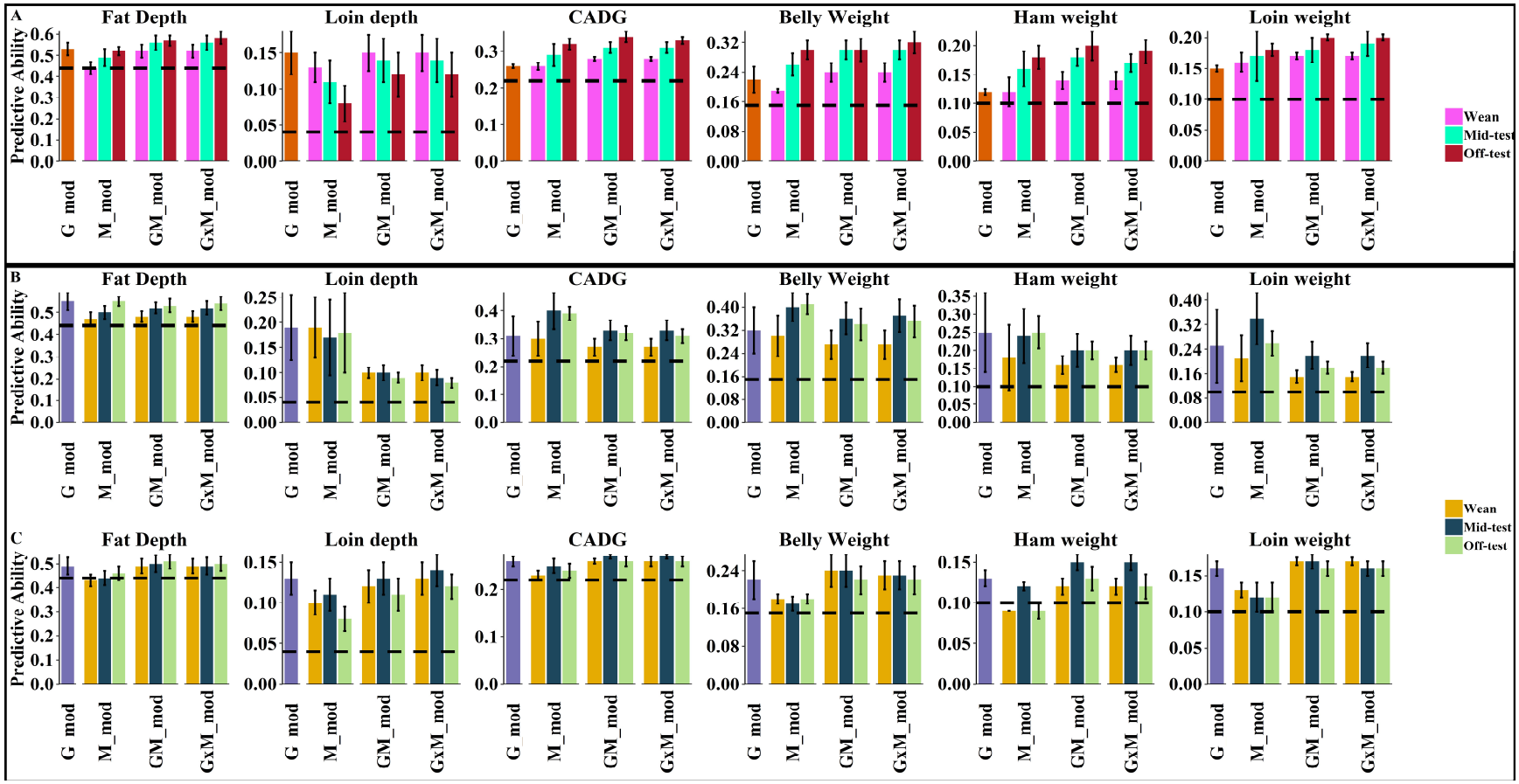

Figure 4.5. Predictive ability of carcass traits. Each bar shows different model with standard deviation at different complexities (Full (Panel A) with all markers and operational taxonomic units (OTU), Informatively reduced (Panel B) with significant markers and OTU and Randomly reduced (Panel C) with randomly selected markers and OTU). Dashed line represents the prediction accuracy of NULL model which contains only random effect as pen. G_mod contains genomic relationship matrix in addition to NULL model. M_mod contains microbiome relationship matrix at Wean, Mid-test and Off-test in addition to NULL model. GM_mod contains genomic contains genomic relationship matrix and microbiome relationship matrix at Wean, Mid-test and Off-test in addition to NULL model. G×M_mod contains both the main effect of microbiome relationship matrix and genomic relationship matrix and interaction between them in addition to NULL model.

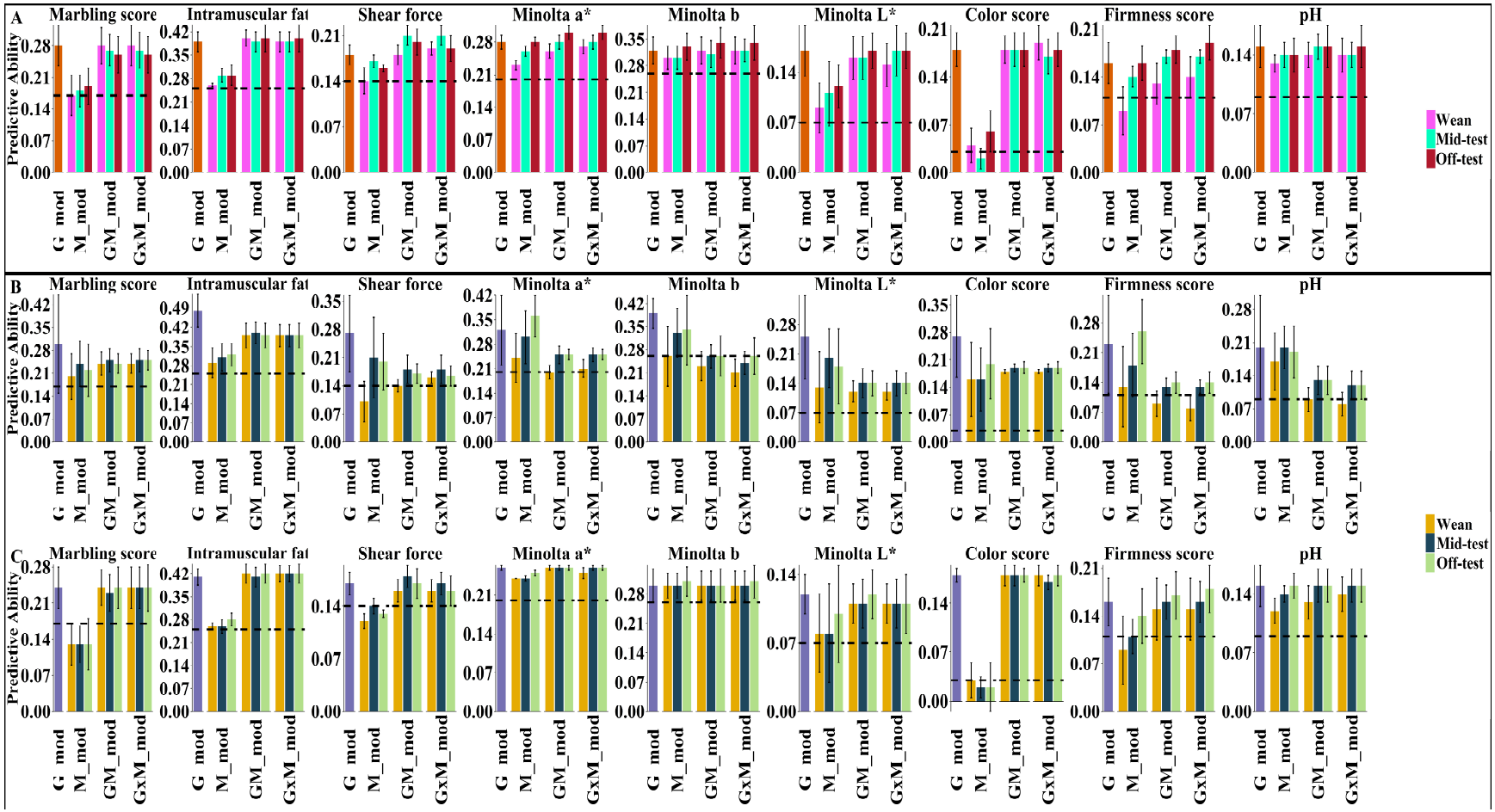

Figure 4.6. Predictive ability of meat quality traits. Each bar shows different model with standard deviation at different complexities (Full (Panel A) with all markers and operational taxonomic units (OTU), Informatively reduced (Panel B) with significant markers and OTU and Randomly reduced (Panel C) with randomly selected markers and OTU). Dashed line represents the prediction accuracy of NULL model which contains only random effect as pen. G_mod contains genomic relationship matrix in addition to NULL model. M_mod contains microbiome relationship matrix at Wean, Mid-test and Off-test in addition to NULL model. GM_mod contains genomic relationship matrix and microbiome relationship matrix at Wean, Mid-test and Off-test in addition to NULL model. G×M_mod contains both the main effect of microbiome relationship matrix and genomic relationship matrix and interaction between them in addition to NULL model.

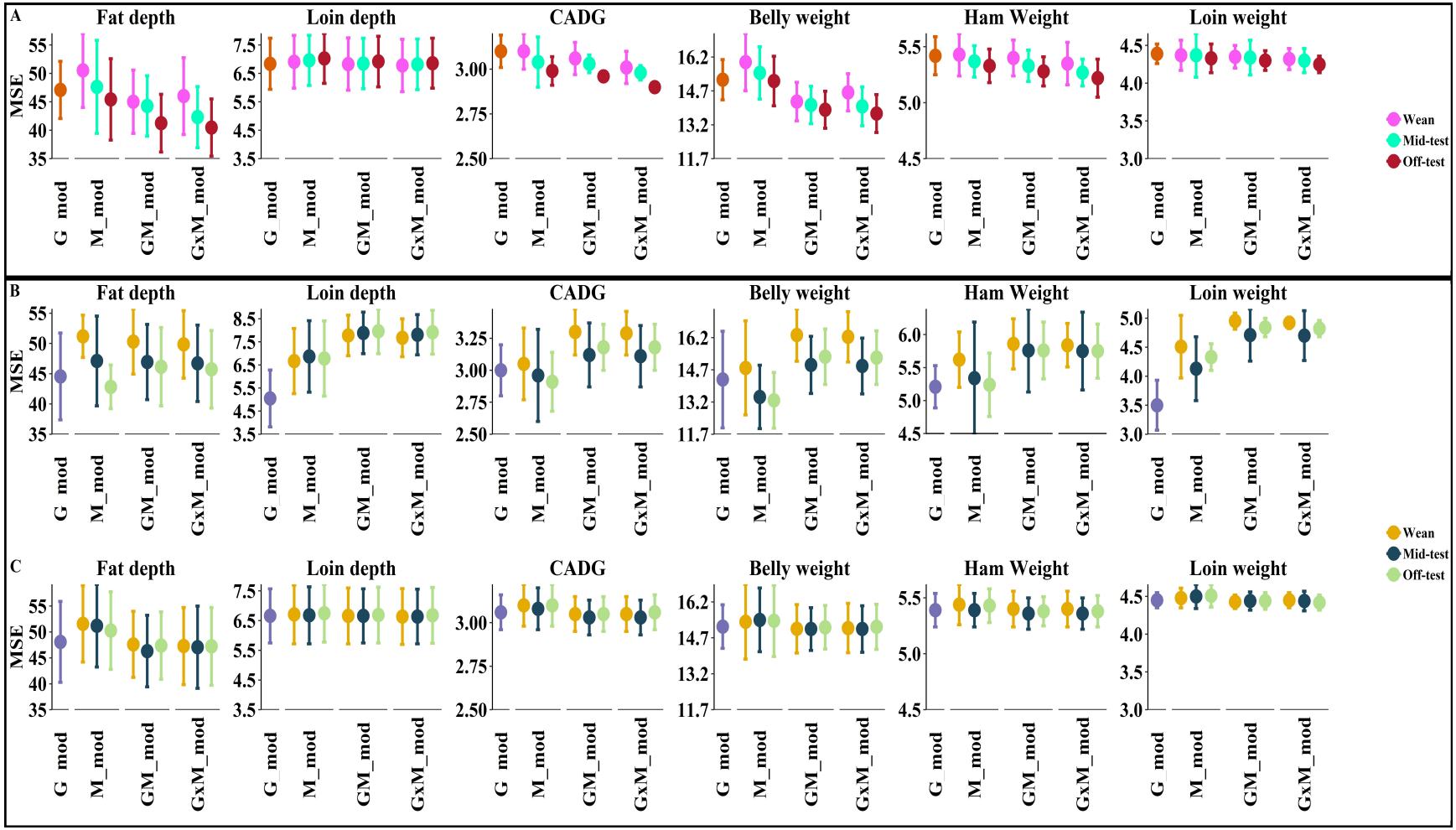

Figure 4.7. Mean squared error of prediction of carcass traits. Each point shows average mean squared error of different model with standard deviation at different complexity (Full (Panel A) with all markers and operational taxonomic units (OTU), Informatively reduced (Panel B) with significant markers and OTU and Randomly reduced (Panel C) with randomly selected markers and OTU). M_mod contains microbiome relationship matrix at Wean, Mid-test and Off-test. G_mod contains genomic relationship matrix. GM_mod contains genomic relationship matrix and microbiome relationship matrix at Wean, Mid-test and Off-test. G×M_mod contain both the main effect of microbiome relationship matrix and genomic relationship matrix and interaction between them.

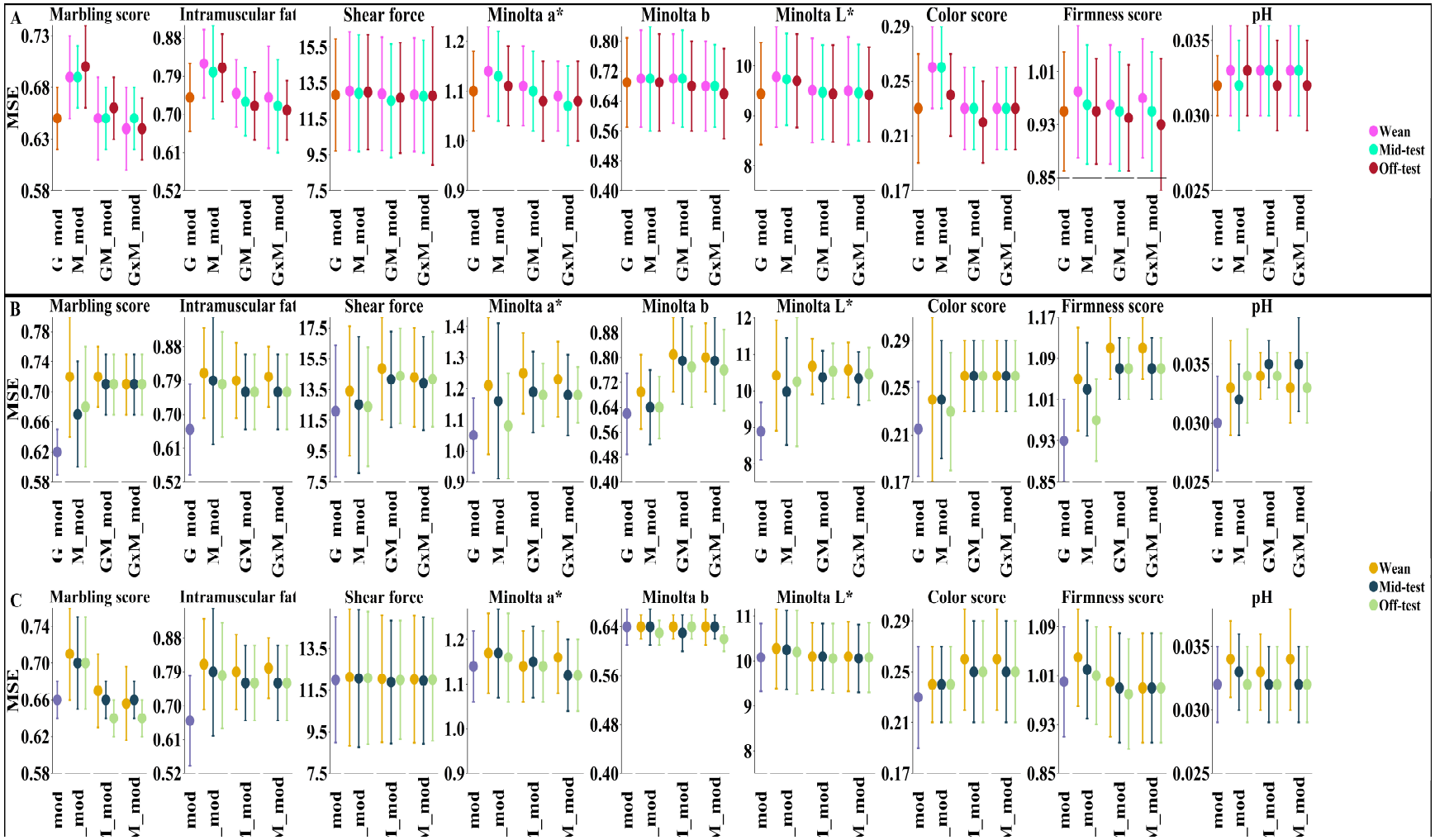

Figure 4.8. Mean squared error of prediction of meat quality traits. Each point shows average mean squared error of different model with standard deviation at different complexity (Full (Panel A) with all markers and operational taxonomic units (OTU), Informatively reduced (Panel B) with significant markers and OTU and Randomly reduced (Panel C) with randomly selected markers and OTU). M_mod contains microbiome relationship matrix at Wean, Mid-test and Off-test. G_mod contains genomic relationship matrix. GM_mod contains genomic relationship matrix and microbiome relationship matrix at Wean, Mid-test and Off-test. G×M_mod contain both the main effect of microbiome relationship matrix and genomic relationship matrix and interaction between them.

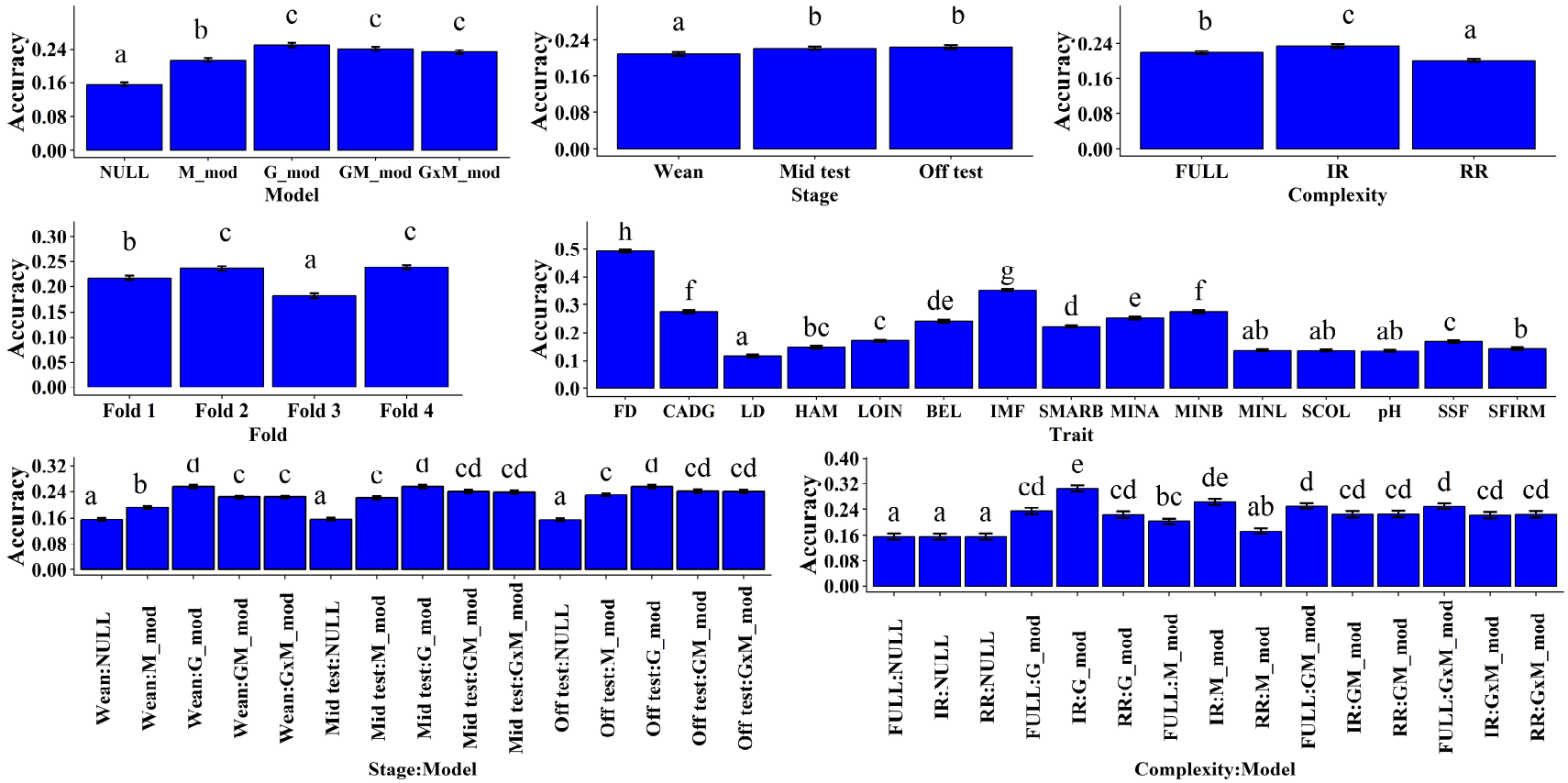

Figure 4.9. Least Square Means and standard error (SE) for main effects and interactions for the post analysis of the experimental design. Model (5 levels: Null, M_mod, G_mod, GM_mod, G×M_mod), Stage (3 levels: Wean, Mid test and Off test), Complexity (3 levels: FULL, IR (Informatively reduced), RR (Randomly reduced)), Trait (15 levels: FD, CADG, LD, HAM, LOIN, BEL, IMF, SMARB, MINA, MINB, MINL, pH, SSF, SFIRM). All elements with (:) represents pairwise interactions.

Chapter 5: Conclusions and Future Directions

5.1 Conclusions

The demand for higher quality meat by consumer has been increased. So, the swine industry breeding goals are mostly directed toward meat quality and carcass traits due to their high economic value. Despite their importance, measurement of meat quality and carcass traits is difficult and expensive as it could be measured only after slaughter of pigs. Therefore, selection of purebreds based on crossbred performance is necessary to improve the meat and carcass quality of crossbred descendants under field conditions. Genetic parameters are necessary to understand the genetic basis of traits which helps in genetic improvement. The results from past studies indicated a wide range of correlation and heritability estimates for meat quality and carcass traits and has been conducted with only pedigree information, particularly for crossbred pigs. The focus of these studies was to obtain a better understanding of the genetic relationships of meat quality and carcass traits with incorporation of genomic information, microbiome information and explore the prediction accuracy with different models which is essential to develop efficient genetic improvement program for meat quality and carcass traits.

The widespread use of genomic information in livestock genetic evaluation allows the opportunity of incorporating the genomic information in addition to the pedigree information which increase the accuracy of the genetic parameter estimates. In Chapter 2 genetic parameters were estimated with the use of genotype and phenotype information. It was found that heritabilities of meat quality traits ranged from low to moderate whereas moderate to high heritabilities were found for carcass composition traits. Moderate to high genetic correlations were found in both directions. Based on the estimates of genetic parameter it can be concluded that multi-trait approach could be used to improve meat quality and carcass traits. The relationship among the

growth traits and meat quality traits in this study indicated that selection for growth traits did not deteriorate the pork quality which contrasts with the general opinion that selection of growth traits decreased the pork quality. The genetic parameters and variance components obtained in this study not only provide the valuable information to understand the biology of traits but also a reference value to establish the efficient breeding program that focuses on meat quality in United States.

Due to the rapid development in molecular technologies, gut microbiota has recently been target of many research effort. The importance of gut microbiota in prediction of complex phenotypes has been widely accepted in other species and human. In Chapter 3 and Chapter 4 we have added microbiome information in addition to the genomic information for the estimation of genetic parameters and prediction accuracy. To the best of my knowledge, this study is the first to investigate the impact of microbiome on meat quality and carcass traits in commercial swine at a large scale. In Chapter 3, microbiability of different traits were estimated which suggests that significant portion of total phenotypic variation was absorbed by gut microbiome. Adding microbiome information in the model did not affect the heritability estimates of meat quality traits but decrease the heritability of carcass traits. High microbial correlation was found among the majority of traits, especially with fat related traits (FD, BEL, IMF, SMARB) and there was decrease in genetic correlation with the inclusion of microbiome for those traits. This suggests that portion of genetic variance and covariance was explained by gut microbiome. Based on the results, we can conclude that microbial composition could be altered to improve a given trait. Manipulation of gut microbiome could be done by using a specific bacterial composition as probiotics or increasing the relative abundance through prebiotics, fecal microbiota transplantation.

In Chapter 4, interaction of genome-by-microbiome was modeled to evaluate the prediction ability across different models. Inclusion of microbiome information increased the prediction

ability although it varied over time. The study revealed that the prediction could be done better at latter stage of life compared to early stage. However, the prediction at earlier age could be interesting to the producers. Since gut microbial sequences could be affected heavily by the environment of sampling and bioinformatics machinery, further research on earlier stage is warranted. Higher predictive ability was obtained for fat related traits (fat depth, subjective marbling score, intramuscular fat) and instrumental color traits and carcass average daily gain. Informatively reduction of dimension of markers improved the prediction ability. However, informatively reduction of operational taxonomic units did not improve the prediction ability compared to the use of full operational taxonomic units. This suggested that the complex traits of swine are polymicrobial in nature. In conclusion, the results in this dissertation provide novel insight on using microbial information for the prediction of meat quality and carcass traits. These results may lead to establish a newer approach of genetic evaluation process through the addition of gut microbial information.

5.2 General recommendations

a) Heterosis has significant effect on crossbred performance. So, the single step BLUP could be extended to include the dominance effects on the model. Due to the lack of genomic information of F1 sows and for all purebred populations we could not fit the models with partial genetic effect tracing the breed-of origin of alleles of crossbred individuals. Inclusion of all these effects in the model would be an interesting topic in future.

b) This study has shown that microbiome explained a portion of genetic variance of traits. An opportunity exists to remove the genetic component of gut microbiome to visualize the effect of microbiome only on various traits. Identification of such microbiome could aid in improvement of traits through alteration of gut microbiome.

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APPENDICES

Appendix A

Table S.A1. Significance of the fixed and random effects included in the models for the analysis of growth, meat quality, primal weight and primal yield traits of TML

Trait	Damline	CG	Gender	Animal	Pen
Growth traits					
LD	NS	*	***	√	√
FD	***	***	***	√	√
CADG	***	***	***	√	NS
Meat quality					
IMF	NS	**	***	√	√
MINA	*	***	***	√	√
MINB	NS	***	***	√	√
MINL	NS	**	NS	√	√
PH	NS	*	***	√	NS
SCOL	NS	*	NS	√	√
SMARB	*	**	**	√	√
SFIRM	NS	***	**	√	√
SSF	NS	NS	***	√	√
Primal weight trait					
HAM	***	***	***	√	√
LOIN1	NS	*	***	√	√
BEL1	*	***	*	√	√
SRIB	NS	NS	*	√	√
PICN	NS	**	NS	√	√
BUTT	NS	*	NS	√	√
HCW	**	NS	*	√	NS
Primal yield trait					
HAMY	***	***	***	√	√
LOINY1	*	NS	***	√	√
BELY1	***	***	NS	√	√
SRIBY	NS	NS	*	√	√
PICNY	***	***	***	√	√
BUTTY	NS	***	*	√	√
PRIMY	***	**	***	√	√

[1]*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$; NS = $P > 0.05$ and √ = significant

Table S.A2. Significance of the fixed and random effects included in the models for the analysis of growth, meat quality, primal weight and primal yield traits of SPG

Trait	Parity[1]	CG	Gender	Animal	Harvest group
Growth traits					
FD	***	***	***	√	√
LD	***	***	*	√	NS
SLAGE	***	***	***	√	NS
CADG	***	***	NS	√	NS
Meat quality traits					
SCOL	**	***	***	√	√
SFIRM	***	***	NS	√	√
SMARB	***	***	*	√	√
PH	NS	***	*	√	NS
Primal weight traits					
BEL2	**	***	***	√	√
LOIN2	***	***	***	√	√
HCW	***	***	*	√	NS
Primal yield traits					
BELY2	***	**	NS	√	√
LOINY2	*	***	NS	√	√

[1]*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$; NS = $P > 0.05$ and √ = significant

Table S.A3. Heritabilities (h^2), genetic variance (σ^2_a), pen variance (σ^2_p), residual variance of female (σ^2_f), residual variance of male (σ^2_m) of growth, meat quality, primal weight and primal yield traits of TML

Traits[1]	h^2	σ^2_a	σ^2_p	σ^2_f	σ^2_m
Growth traits					
LD	0.15 (0.09, 0.21)	7.33(4.64,10.45)	1.64(0.93,2.50)	37.61(34.36,40.48)	39.88(36.98,43.16)
FD	0.47(0.39,0.54)	10.67(8.39,12.69)	0.65(0.35,0.97)	9.48(8.08,11.04)	13.01(11.31,14.66)
CADG	0.44(0.36,0.51)	2.43(1.73,2.72)	0.03(0.002,0.06)	2.65(2.29,3.00)	3.06(2.68,3.40)
Meat quality					
IMF	0.52(0.40,0.62)	0.004(0.003,0.005)	0.0004(0.00002,0.0006)	0.002(0.001,0.003	0.004(0.003,0.005)
MINA	0.19(0.09,0.27)	0.22(0.11,0.32)	0.16(0.09,0.22)	0.73(0.59,0.84)	0.80(0.66,0.92)
MINB	0.08(0.03,0.16)	0.0006(0.00005,0.0009)	0.001(0.0008,0.002)	0.004(0.003,0.005)	0.005(0.004,0.006)
MINL	0.20(0.14,0.29)	1.96(1.04,2.89)	1.43(0.83,1.99)	6.02(5.06,7.16)	6.44(5.34,7.57)
PH	0.08(0.03,0.1)	0.003(0.0006,0.004)	0.01(0.006,0.017)	0.02(0.017,0.025)	0.02(0.017,0.023)
SCOL	0.26(0.16,0.36)	0.06(0.04,0.08)	0.01(0.0001,0.02)	0.16(0.13,0.19)	0.15(0.12,0.18)
SMARB	0.27(0.22,0.32)	0.18(0.10,0.26)	0.09(0.05,0.13)	0.44(0.35,0.52)	0.40(0.32,0.46)
SFIRM	0.12(0.05,0.21)	0.12(0.04,0.21)	0.05(0.001,0.09)	0.89(0.75,1.00)	0.72(0.61,0.84)
SSF	0.21(0.14,0.31)	0.37(0.21,0.53)	0.08(0.01,0.16)	1.48(1.26,1.74)	1.10(0.90,1.27)
Primal weight trait					
HAM	0.14(0.06,0.23)	1.71(0.66,2.88)	0.05(0.0004,0.15)	10.70(9.21,12.26)	10.24(8.71,11.72)
LOIN1	0.18(0.09,0.27)	1.80(0.91,2.79)	0.05(0.0002,0.13)	8.81(7.41,10.09)	8.11(6.88,9.22)
BEL1	0.22(0.12,0.32)	3.78(1.88,5.55)	0.06(0.0003,0.20)	14.41(12.29,16.79)	11.85(9.73,13.60)
SRIB	0.30(0.20,0.41)	0.31(0.20,0.43)	0.005(0.0004,0.01)	0.72(0.60,0.84)	0.71(0.60,0.84)
PICN	0.12(0.04,0.21)	0.35(0.05,0.62)	0.02(0.001,0.06)	2.85(2.47,3.25)	2.63(2.25,3.02)
BUTT	0.20(0.10,0.28)	0.52(0.27,0.76)	0.02(0.0001,0.04)	2.17(1.90,2.54)	2.06(1.76,2.39)
HCW	0.39(0.31,0.46)	42.90(31.54,52.83)	0.09(0.001,0.25)	65.49(56.38,72.78)	68.86(60.68,76.62)
Primal yield trait					
HAMY	0.45(0.36,0.55)	4.38(3.21,5.65)	0.44(0.01,0.78)	4.60(3.69,5.60)	4.78(3.85,5.93)
LOINY1	0.33(0.23,0.44)	3.73(2.27,5.07)	0.56(0.03,0.90)	7.22(5.92,8.66)	7.13(5.86,8.49)

Table S.A3. (continued)

BELY1	0.33(0.22,0.43)	6.22(4.15,8.67)	0.30(0.01,0.80)	13.44(10.95,15.72)	11.33(9.02,13.32)
SRIBY	0.35(0.25,0.45)	7.85(5.22,10.38)	1.05(0.06,2.00)	13.36(10.84,15.64)	13.63(11.04,16.29)
PICNY	0.32(0.22,0.41)	1.58(0.97,2.11)	0.33(0.10,0.58)	3.29(2.65,3.83)	2.84(2.28,3.41)
BUTTY	0.17(0.13,0.25)	0.74(0.40,1.17)	0.23(0.006,0.41)	3.68(3.14,4.20)	3.41(2.84,3.97)
PRIMY	0.21(0.13,0.29)	2.98(1.72,4.29)	0.34(0.004,0.82)	9.37(7.64,10.70)	12.35(10.60,14.31)

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Table S.A4. Heritabilities (h^2), genetic variance (σ^2_a), variance of harvest batch (σ^2_h), residual variance of female (σ^2_f), residual variance of male (σ^2_m) of growth, meat quality, primal weight and primal yield traits of SPG.

Traits[1]	h^2	σ^2_a	σ^2_h	σ^2_f	σ^2_m
Growth traits					
FD	0.45(0.42,0.46)	7.26(6.85,7.64)	0.92(0.69,1.20)	6.64(6.36,6.91)	9.64(9.33,10.01)
LD	0.20(0.18,0.22)	4.68(4.11,5.20)	NC[2]	18.40(17.92,18.95)	20.13(19.60,20.71)
SLAGE	0.59(0.58,0.62)	50.07(48.56,51.56)	NC	65.28(64.28,66.22)	69.55(67.33,71.22)
CADG	0.42(0.41,0.43)	0.10(0.02, 0.18)	NC	0.10(0.04,0.17)	0.20(0.16,0.25)
Meat Quality traits					
SCOL	0.24(0.22,0.26)	0.07(0.06,0.08)	0.05(0.04,0.058)	0.17(0.16,0.18)	0.29(0.28,0.295)
SFIRM	0.24(0.22,0.26)	0.11(0.10,0.12)	0.02(0.015,0.024)	0.31(0.30,0.32)	0.31(0.30,0.32)
SMARB	0.33(0.31,0.35)	0.28(0.25,0.29)	0.07(0.06,0.078)	0.41(0.39,0.43)	0.56(0.54,0.58)
PH	0.24(0.20,0.27)	0.004(0.003,0045)	0.01(0.08,0.014)	0.01(0.008,0.015)	0.02(0.009,0.032)
Primal weight traits					
BEL2	0.22(0.19,0.24)	0.06(0.05,0.07)	0.06(0.05,0.07)	0.16(0.15,0.17)	0.17(0.16,0.18)
LOIN2	0.39(0.36,0.40)	0.03(0.029, 0.031)	0.013(0.011,0.015)	0.03(0.028,0.032)	0.03(0.028,0.031)
HCW	0.30(0.29,0.31)	21.90(21.07,22.80)	NC	50.88(50.24,51.52)	52.67(51.97,53.30)
Primal yield traits					
BELY2	0.19(0.17,0.21)	0.09(0.01,0.14)	0.06(0.001,0.09)	0.22(0.19,0.24)	0.25(0.23,0.27)
LOINY2	0.28(0.26,0.31)	0.04(0.01,0.07)	0.02(0.01, 0.04)	0.05(0.03,0.06)	0.07(0.06,0.08)

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Table S.A5. Estimates of Genetic correlation (above diagonal) and phenotypic correlation (below diagonal) for meat quality traits of TML.

	IMF	MINA	MINB	MINL	PH	SCOL	SFIRM	SMARB	SSF
IMF		0.34(0.11,0.57)	0.78(0.55,0.99)	0.54(0.31,0.76)	-0.19(-0.62,0.21)	-0.12(-0.39,0.13)	0.47 (0.22, 0.76)	0.74(0.59,0.89)	-0.11(-0.37,0.15)
MINA	0.24(0.18,0.30)		0.02(-0.43,0.47)	-0.21(-0.56,0.14)	-0.27(-0.98,0.31)	0.45(0.13,0.74)	-0.10(-0.28, 0.35)	0.02(-0.31,0.36)	-0.18(-0.50, 0.14)
MINB	0.22(0.16,0.27)	0.42(0.37,0.47)		0.86(0.62,0.99)	-0.64(-1.00,-0.01)	-0.93(-1.00,-0.73)	-0.05(-0.65,0.52)	0.17(-0.27,0.60)	-0.34(-0.72,0.06)
MINL	0.15(0.31,0.76)	0.03(-0.03,0.08)	0.49(0.45,0.53)		-0.90(-1.00,-0.66)	-0.96(-1.00,-0.83)	-0.07(-0.48,0.38)	0.11(-0.22,0.41)	-0.07(-0.39,0.26)
PH	-0.04(-0.1,0.03)	-0.12(-0.18,-0.66)	-0.14(-0.21,-0.10)	-0.27(-0.32,-0.22)		0.87(0.56,1.00)	0.47(-0.02,0.97)	0.13(-0.33,0.57)	0.04(-0.43, 0.50)
SCOL	-0.02(-0.08,0.05)	0.10(0.04,0.15)	-0.21(-0.26,-0.15)	-0.36(-0.41,-0.31)	0.22(0.17,0.27)		0.06(-0.35,0.44)	0.09(-0.22,0.40)	0.06(-0.25, 0.36)
SFIRM	0.24(0.19, 0.30)	0.11(0.07,0.18)	0.09(0.04,0.15)	0.01(-0.05,0.07)	0.08(0.02,0.13)	0.18(0.12, 0.24)		0.45(0.03, 0.87)	-0.32(-0.67,0.06)
SMARB	0.44(0.39, 0.50)	0.05(-0.02,0.10)	0.06(0.01,0.11)	0.04(-0.02,0.10)	0.05(-0.01,0.11)	0.16(0.10,0.22)	0.31(0.25,0.36)		0.02(-0.32, 0.32)
SSF	-0.06(-0.14, -0.01)	-0.07(-0.12,-0.01)	-0.08(-0.14,-0.03)	-0.05(-0.11,0.01)	-0.05(-0.12,-0.01)	0.04(-0.01,0.10)	-0.10(-0.015,-0.04)	-0.01(-0.06,0.06)	

Table S.A6. Estimates of Genetic correlation (above diagonal) and phenotypic correlation (below diagonal) for meat quality traits of TML.

	SCOL	**SFIRM**	**SMARB**	**PH**
SCOL		0.05(0.01,0.09)	0.43(0.34,0.51)	0.14(0.02,0.19)
SFIRM	0.002(-0.007,0.009)		0.11(0.07,0.15)	0.06(0.02,0.09)
SMARB	0.22(0.20,0.23)	0.02(-0.01,0.04)		0.05(-0.01,0.11)
PH	0.3(0.26,0.33)	0.02(-0.001,0.04)	0.09(0.07,0.10)	

Table S.A7. Genetic correlation of meat quality and growth traits for TML

Traits	CADG	FD	LD
IMF	0.21(-0.06,0.44)	0.37(0.19,0.55)	-0.001(-0.34,0.37)
MINA	0.14(-0.21,0.51)	0.38(0.13,0.61)	-0.26 (-0.70,0.17)
MINB	0.20(-0.29,0.69)	0.55(0.22,0.88)	-0.07 (-0.64,0.54)
MINL	0.26(-0.08,0.55)	0.26(0.01,0.51)	0.35 (-0.02,0.73)
PH	-0.02(-0.56,0.50)	-0.22(-0.86, 0.28)	0.21 (-0.67,0.99)
SCOL	0.01(-0.30,30)	0.08(-0.17,0.32)	-0.29 (-0.99,0.09)
SMARB	0.08(-0.22,0.40)	0.29(0.06, 0.50)	-0.30 (-0.99,0.08)
SFIRM	0.20(-0.27,0.58)	0.59(0.30, 0.91)	0.31 (-0.22,0.84)
SSF	0.16(-0.17,0.47)	-0.48(-0.70, -0.25)	-0.30 (-0.74,0.15)

Table S.A8. Phenotypic correlation of meat quality and growth traits for TML

Traits	CADG	FD	LD
IMF	0.10(0.04,0.16)	0.27(0.21,0.33)	0.01(-0.06,0.07)
MINA	0.09(0.03,0.14)	0.13(0.08,0.20)	-0.001(-0.06,0.06)
MINB	0.10(0.05,0.16)	0.14(0.08,0.19)	0.06(0.01,0.11)
MINL	0.06(0.01,0.12)	0.06(0.01,0.12)	0.07(0.01,0.12)
PH	-0.01(-0.07,0.04)	0.01(-0.04,0.07)	-0.02(-0.07,0.04)
SCOL	-0.01(-0.08,0.05)	0.01(-0.06,0.07)	-0.01(-0.07,0.04)
SMARB	0.02(-0.05,0.07)	0.11(0.05,0.18)	-0.02(-0.08,0.03)
SFIRM	0.16(0.09,0.20)	0.20(0.14,0.25)	0.13(0.07,0.18)
SSF	-0.16(-0.23,-0.11)	-0.20(-0.26,-0.15)	-0.12(-0.18,-0.07)

Table S.A9. Genetic correlation of meat quality and growth traits for SPG

	CADG	FD	LD	SLAGE
PH	NC	NC	NC	NC
SCOL	0.41(-0.03,0.61)	0.2(0.10,0.28)	-0.04(-0.27,0.15)	0.38(0.27,0.43)
SFIRM	0.05(-0.01,0.11)	0.06(0.01,0.10)	NC	0.47(0.35,0.56)
SMARB	0.06(-0.02,0.10)	0.3(0.19,0.37)	-0.07(-0.03,0.0.12)	0.33(0.22,0.43)

Table S.A10. Phenotypic correlation of meat quality and growth traits for SPG

	CADG	FD	LD	SLAGE
PH	NC	NC	NC	NC
SCOL	0.07(0.01,0.11)	0.08(0.02,0.16)	-0.05(-0.08,-0.007)	0.01(0.006,0.012)
SFIRM	0.07(0.006,0.11)	0.19(0.05,0.24)	NC	0.01(0.006,0.014)
SMARB	0.04(0.001,0.08)	0.22(0.10,0.29)	-0.07(-0.01,-0.13)	-0.01(-0.008,0.006)

Table S.A11. Genetic Correlation of meat quality and primal yield traits for TML

	HAMY	LOINY1	BELY1	SRIBY	PICNY	BUTY	PRIMY
IMF	-0.31(-0.50,-0.14)	-0.36(-0.57,-0.16)	0.37(0.15,0.59)	0.29(0.09,0.49)	-0.21(-0.44,0.01)	0.03(-0.25,0.36)	-0.27(-0.52,-0.01)
MINA	-0.06(-0.14,0.02)	-0.25(-0.57,0.06)	0.24(-0.06,0.55)	0.24(-0.06,0.53)	-0.08(-0.41,0.22)	-0.09(-0.46,0.33)	-0.21(-0.64,0.17)
MINB	-0.25(-0.63,0.11)	-0.28(-0.69,0.19)	0.15(-0.29,0.64)	0.23(-0.17,0.66)	-0.03(-0.48,0.43)	-0.3(-0.59,0.54)	-0.35(-0.89,0.09)
MINL	-0.15(-0.39,0.12)	-0.12(-0.44,0.17)	0.16(-0.14,0.47)	0.06(-0.24,0.34)	-0.05(-0.37,0.25)	-0.09(-0.48,0.27)	-0.17(-0.48,0.16)
PH	0.01(-0.52,0.48)	0.38(-0.05,0.99)	-0.36(-0.99,0.10)	-0.11(-0.64,0.39)	0.03(-0.58,0.58)	0.24(-0.32,0.99)	-0.04(-0.59,0.47)
SCOL	0.03(-0.04,0.04)	-0.05(-0.33,0.25)	0.17(-0.13,0.45)	0.01(-0.28,0.27)	-0.13(-0.4,0.16)	-0.12(-0.47,0.29)	0.07(-0.26,0.37)
SFIRM	-0.29(-0.69,0.10)	-0.31(-0.67,0.09)	0.40(0.02,0.77)	0.24(-0.11,0.58)	-0.64(-1.00.-0.29)	-0.04(-0.56,0.43)	-0.43(-0.83,-0.02)
SMARB	-0.26(-0.48,-0.03)	-0.311(-0.57,-0.06)	0.45(0.19,0.73)	0.28(0.02,0.53)	-0.32(-0.59,-0.01)	0.16(-0.19,0.50)	-0.08(-0.41,0.23)
SSF	0.18(-0.08,0.41)	0.11(-0.12,0.42)	-0.24(-0.51,0.05)	0.09(-0.19,0.38)	0.36(0.08,0.62)	0.19(-0.16,0.52)	0.55(0.22,0.86)

Table S.A12. Phenotypic correlation of meat quality and primal yield traits for TML

	HAMY	LOINY1	BELY1	SRIBY	PICNY	BUTY	PRIMY
IMF	-0.22(-0.29,-0.16)	-0.211(-0.27,-0.15)	0.23(0.16,0.29)	0.11(0.05,0.18)	-0.11(-0.18,-0.04)	-0.02(-0.08,0.04)	-0.14(-0.19,-0.01)
MINA	-0.12(-0.18,0.05)	-0.07(-0.12,-0.004)	0.10(0.04,0.16)	0.04(-0.02,0.09)	-0.07(-0.13,-0.01)	-0.04(-0.09,0.02)	-0.07(-0.14,0.00)
MINB	-0.08(-0.14,-0.02)	-0.08(-0.14,-0.02)	0.06(0.01,0.11)	0.02(-0.03,0.07)	-0.01(-0.07,0.04)	-0.06(-0.11,0.00)	-0.08(-0.14,0.02)
MINL	-0.04(-0.11,0.01)	-0.09(-0.16,-0.04)	0.05(-0.01,0.10)	0.01(-0.05,0.06)	-0.02(-0.08,0.04)	-0.04(-0.09,0.02)	-0.08(-0.14,-0.01)
PH	-0.01(-0.05,0.05)	0.07(0.01,0.13)	-0.01(-0.07,0.05)	-0.01(-0.06,0.04)	-0.07(-0.13,-0.02)	-0.03(-0.09,0.02)	-0.01(-0.06,0.05)
SCOL	-0.01(-0.04,0.02)	0.03(-0.04,0.09)	0.05(-0.01,0.11)	0.02(-0.04,0.08)	-0.09(-0.15,-0.03)	0.01(-0.05,0.06)	0.03(-0.03,0.08)
SFIRM	-0.15(-0.21,-0.09)	-0.14(-0.2,-0.08)	0.20(0.14,0.25)	0.06(0.01,0.11)	-0.09(-0.15,-0.03)	0.03(-0.03,0.08)	-0.04(-0.09,0.02)
SMARB	-0.12(-0.18,-0.06)	-0.14(-0.20,-0.08)	0.14(0.08,0.19)	0.08(0.03,0.14)	-0.08(-0.14,-0.03)	0.03(-0.03,0.09)	-0.06(-0.12,-0.001)
SSF	0.15(0.09,0.21)	0.01(0.001,0.12)	-0.15(-0.21,-0.09)	-0.01(-0.07,0.05)	0.11(0.05,0.17)	0.12(0.07,0.17)	0.14(0.01,0.19)

Table S.A13. Genetic correlation of meat quality and primal yield traits for SPG

	BELY2	LOINY2
SMARB	0.32(0.13,0.49)	-0.29(-0.12, -0.43)
SFIRM	NC	NC
SCOL	0.15(0.03,0.20)	NC
PH	0.27(-0.13,0.48)	NC

Table S.A14. Phenotypic correlation of meat quality and primal yield traits for SPG

	BELY2	LOINY2
SMARB	0.06(-0.08,0.19)	-0.01(-0.10,0.09)
SFIRM	NC	NC
SCOL	0.002(0.0008,0.004)	NC
PH	-0.21(-0.32,0.31)	NC

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Table S.A15. Genetic correlation (above diagonal) and phenotypic correlation (below diagonal) of primal weight traits of TML

	HAM	LOIN1	BEL1	SRIB	PICN	BUTT
HAM		0.5(0.32,0.79)	0.45(0.10,0.74)	0.52(0.21,0.74)	0.62(0.25,0.88)	0.39(0.10,0.65))
LOIN1	0.81(0.78,0.82)		0.47(0.18,0.71)	0.74(0.57,0.88)	0.41(-0.06,0.76)	0.61(0.35,0.82)
BEL1	0.75(0.71,0.77)	0.70(0.67,0.73)		0.79(0.66,0.91)	0.02(-0.92,0.50)	0.68(0.45,0.85)
SRIB	0.75(0.72,0.77)	0.72(0.69,0.75)	0.77(0.74,0.79)		0.61(0.34,0.86)	0.69(0.51,0.85)
PICN	0.80(0.78,0.82)	0.69(0.66,0.72)	0.64(0.60,0.68)	0.70(0.67,0.73)		0.38(-0.07,0.73)
BUTT	0.69(0.66,0.72)	0.65(0.61,0.68)	0.67(0.63,0.7)	0.64(0.60,0.67)	0.60(0.57,0.64)	

Table S.A16. Genetic correlation (above diagonal) and phenotypic correlation (below diagonal) of primal weight and primal yield traits of SPG

	LOIN2	BEL2	LOINY2	BELY2
LOIN2		0.4(0.23, 0.56)	-	-
BEL2	0.65(0.55,0.75)		-	-
LOINY2	-	-		-0.1(-0.17,0.10)
BELY2	-	-	0.32(0.31,0.33)	

Table S.A17. Genetic correlation (above diagonal) and phenotypic correlation (below diagonal) of primal yield traits of TML

	HAMY	LOINY1	BELY1	SRIBY	PICNY	BUTTY	PRIMY
HAMY		0.16(-0.05,0.37)	-0.59(-0.74,-0.43)	-0.45(-0.61,-0.27)	0.58(0.40,00.75)	-0.34(-0.64,-0.03)	0.60(0.43,0.79)
LOINY1	0.14(0.08,0.21)		-0.70(-0.85,-0.54)	-0.08(-0.33,0.16)	0.25(-0.02,0.50)	-0.04(-0.36,0.29)	0.39(0.13,0.65)
BELY1	-0.51(-0.56,-0.47)	-0.50(-0.55,-0.46)		0.41(0.17,0.64)	-0.67(-0.84,-0.45)	-0.17(-0.50,0.15)	-0.46(-0.71,-0.18)
SRIBY	-020(-0.26,-0.14)	-0.18(-0.23,-0.12)	0.15(0.09,0.22)		-0.04(-0.28,0.22)	-0.05(-0.35,0.24)	0.29(0.01,0.55)
PICNY	0.32(0.27,0.38)	0.04(-0.02,0.09)	-0.40(-0.45,-0.35)	-0.01(-0.08,0.05)		-0.10(-0.44,0.20)	0.68(0.47,0.86)
BUTTY	-0.03(-0.09,0.03)	-0.08(-0.14,-0.02)	-0.18(-0.24,-0.12)	-0.07(-0.14,-0.02)	-0.04(-0.10,0.02)		-0.31(-0.69,0.07)
PRIMY	0.44(0.39,0.49)	0.32(0.27,0.38)	-0.02(-0.08,0.04)	0.11(0.04,0.17)	0.37(0.32,0.42)	0.20(0.14,0.25)	

Table S.A18. Genetic correlation of primal weight and primal yield traits with growth traits of TML

Traits	CADG	FD	LD
Primal weight			
HAM	0.69 (0.44,0.86)	0.11 (-0.20, 0.30)	0.22 (-0.21, 0.44)
LOIN1	0.73 (0.54, 0.86)	-0.02(-0.11, 0.19)	0.43 (0.16, 0.69)
BEL1	0.82 (0.76, 0.90)	0.62 (0.46, 0.78)	-0.1(-0.25, 0.24)
SRIB	0.82 (0.75, 0.92)	0.23 (0.11, 0.35)	0.16 (-0.1, 0.32)
PICN	0.56 (0.40,0.70)	-0.23 (-0.44, 0.36)	0.04(-0.12, 0.08)
BUTT	0.85 (0.80,0.90)	0.28 (0.10, 0.44)	0.26 (-0.14, 0.18)
Primal yield			
HAMY	-0.54(-0.73,-0.33)	-0.46(-0.60,-0.31)	-0.04(-0.38,0.28)
LOINY1	-0.46(-0.67,-0.20)	-0.64(-0.79, -0.48)	0.19(-0.26,0.64)
BELY1	0.47(0.24,0.70)	0.68(0.54,0.8)	-0.42(-0.80,-0.03)
SRIBY	0.48(0.23,0.72)	0.01(-0.2,0.22)	-0.03(-0.34,0.31)
PICNY	-0.43(-0.66,-0.14)	-0.57(-0.76,-0.38)	-0.08(-0.47,0.29)
BUTTY	0.22(-0.20,0.65)	-0.12(-0.40,0.18)	0.02(-0.45,0.56)

Table S.A19. Phenotypic correlation of primal weight and primal yield traits with growth traits of TML

Traits	CADG	FD	LD
Primal weight			
HAM	0.73 (0.52, 0.88)	0.16 (0.10, 0.22)	0.42 (0.38, 0.46)
LOIN1	0.82 (0.66, 0.94)	0.17 (0.13, 0.21)	0.44 (0.40, 0.48)
BEL1	0.85 (0.74, 0.92)	0.48 (0.43, 0.53)	0.27 (0.25, 0.29)
SRIB	0.8 (0.72, 0.88)	0.25 (0.20, 0.30)	0.31 (0.28, 0.34)
PICN	0.78 (0.74, 0.82)	0.09 (0.07, 0.11)	0.36 (0.33, 0.39)
BUTT	0.75 (0.70, 0.80)	0.22 (0.19, 0.23)	0.33 (0.30, 0.35)
Primal yield			
HAMY	-0.36(-0.42,-0.31)	-0.49(-0.54,-0.44)	-0.04(-0.09,0.02)
LOINY1	-0.37(-0.42,-0.32)	-0.39(-0.44,-0.33)	0.01(-0.05,0.07)
BELY1	0.48(0.43,0.52)	0.53(0.49,0.57)	0.03(-0.03,0.09)
SRIBY	0.23(0.18,0.28)	0.01(-0.05,0.07)	0.02(-0.04,0.08)
PICNY	-0.24(-0.29,-0.17)	-0.39(-0.44,-0.34)	-0.08(-0.14,-0.03)
BUTTY	-0.10(-0.16,-0.05)	-0.12(-0.18,-0.06)	-0.01(-0.10,0.01)
PRIMY	-0.20(-0.26,-0.14)	-0.40(-0.41,-0.36)	-0.05(-0.11,0.01)

Table S.A20. Genetic correlation of primal weight and primal yield traits with growth traits of SPG

Traits	FD	LD	SLAGE	CADG
Primal weight				
BEL2	0.14(0.10,0.18)	0.14 (0.11, 0.18)	NC	0.20 (-0.18, 0.40)
LOIN2	0.02(-0.06, 0.08)	NC	NC	NC
Primal yield				
BELY2	0.02 (0.01, 0.03)	NC	0.52(0.46, 0.58)	NC
LOINY2	-0.36(-0.56, -0.16)	NC	NC	-0.38(-0.50, -0.26)

Table S.A21. Phenotypic correlation of primal weight and primal yield traits of SPG

Traits	FD	LD	SLAGE	CADG
Primal weight				
BEL2	0.02 (0.01, 0.03)	0.005(0.003, 0.006)	NC	0.1 (-0.08, 0.14)
LOIN2	-0.01(-0.03, -0.002)	NC	NC	NC
Primal yield				
BELY2	0.001(-0.01, 0.01)	NC	0.08(0.06, 0.10)	NC
LOINY2	-0.001(-0.006, -0.0001)	NC	NC	-0.006(-0.10, -0.002)

Table S.A22. Genetic correlation among male and female, heritability estimates for male and female for growth, meat quality, primal weight and primal yield traits of TML.

Traits	Heritability male	Heritability female	Genetic correlation
Growth			
LD	0.11 (0.05, 0.18)	0.15 (0.06, 0.22)	0.97 (0.91, 0.99)
FD	0.24 (0.14, 0.34)	0.28 (0.18, 0.40)	0.99 (0.97, 1.00)
CADG	0.38 (0.26, 0.48)	0.35 (0.25, 0.46)	0.99 (0.99, 1.00)
Meat quality			
IMF	0.38 (0.25, 0.51)	0.6 (0.46, 0.74)	0.97 (0.86, 1.00)
MINA	0.14 (0.05, 0.23)	0.29 (0.16, 0.42)	0.95 (0.79, 1.00)
MINB	0.07 (0.01, 0.14)	0.16 (0.04, 0.28)	0.76 (0.10, 0.98)
MINL	0.21 (0.11, 0.31)	0.2 (0.10, 0.31)	0.92(0.67, 1.00)
PH	0.11(0.01, 0.20)	0.12(0.03, 0.23)	0.2(-0.85, 0.90)
SCOL	0.32(0.18, 0.46)	0.22(0.10, 0.34)	0.84(0.54, 0.99)
SMARB	0.23(0.11, 0.38)	0.3(0.17, 0.45)	0.94(0.87, 0.98)
SFIRM	0.1(0.02, 0.20)	0.14(0.05, 0.25)	0.79(0.25, 1.00)
SSF	0.18(0.08, 0.29)	0.27(0.15, 0.40)	0.89(0.69, 1.00)
Primal weight			
HAM	0.1(0.02, 0.18)	0.19(0.05, 0.33)	0.93(0.66, 1.00)
LOIN1	0.13(0.05, 0.21)	0.25(0.12, 0.41)	0.96(0.81, 1.00)
BEL1	0.21(0.12, 0.30)	0.25(0.14, 0.37)	1 (0.99, 1.00)
SRIB	0.27(0.15, 0.38)	0.31(0.18, 0.43)	0.98(0.88, 1.00)
PICN	0.1(0.02, 0.18)	0.14(0.03, 0.26)	0.93(0.69, 1.00)
BUTT	0.19(0.08, 0.30)	0.2(0.09, 0.34)	0.89(0.54, 1.00)
HCW	0.33(0.22, 0.43)	0.33(0.22, 0.43)	0.99(0.99, 1.00)
Primal yield			
HAMY	0.45(0.31, 0.60)	0.45(0.32, 0.60)	0.94(0.77, 0.99)
LOINY1	0.23(0.10, 0.35)	0.39(0.25, 0.54)	0.95(0.78, 1.00)
BELY1	0.33(0.19, 0.48)	0.31(0.17, 0.48)	0.96(0.83, 1.00)
SRIBY	0.38(0.22, 0.53)	0.32(0.16, 0.46)	0.89(0.67, 0.99)
PICNY	0.31(0.16, 0.46)	0.3(0.14, 0.45)	0.93(0.74, 1.00)
BUTTY	0.2(0.07, 0.33)	0.15(0.02, 0.29)	0.8(0.36, 0.99)
PRIMY	0.2(0.01, 0.32)	0.2(0.05, 0.33)	0.91(0.61, 1.00)

Table S.A22. (continued)

[1]LD = Loin depth; FD = Fat depth; CADG = Primal average daily gain; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; SSF = Slice shear force, HAM = Ham weight; LOIN1 = Loin weight; BEL1 = Belly weight; SRIB = Spare rib weight, PICN = Picnic weight; BUTT = Butt weight; HCW = Hot primal weight; HAMY = Ham yield; LOINY1 = Loin yield; BELY1 = Belly yield; SRIBY = Spare rib yield; PICNY = Picnic yield; BUTTY = Butt yield; PRIMY = Primal yield; BEL2 = Belly weight; LOIN2 = Loin weight; BELY2 = Belly weight; LOINY2 = Loin yield.

Numbers in parenthesis are confidence intervals.

- Not estimated

Appendix B

Table S.B1. Diet formulae and their nutritional values

	Nursery 3	Nursery 4	GF-1	GF-2		GF-3		GF-4		GF-5		GF-6		GF-7
	Barrow/Gilt	Barrow/Gilt	Barrow/Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow/Gilt
Ingredient														
Corn	660.60	861.88	800.62	1020.46	1013.36	1236.71	1204.82	1382.01	1335.94	1481.33	1435.26	1530.99	1499.03	1534.50
Corn germ meal	48.01	341.84	564.17	464.72	467.93	366.89	381.32	301.16	322.00	256.23	277.07	233.76	248.22	232.18
Soybean meal	326.70	594.00	490.79	394.42	397.53	299.63	313.60	235.93	256.12	192.39	212.59	170.62	184.63	169.09
Fat - yellow grease (post-pellet)			64.46	47.08	47.64	29.99	32.51	18.50	22.14	10.65	14.29	6.72	9.25	6.45
Limestone		30.20	27.99	25.81	25.88	23.66	23.97	22.21	22.67	21.23	21.69	20.73	21.05	20.70
Pelleting aid			10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
L-Lysine HCl (98%)		8.91	9.65	8.17	8.22	6.71	6.93	5.74	6.05	5.07	5.38	4.73	4.95	4.71
Salt		11.17	9.14	9.13	9.13	9.12	9.13	9.12	9.12	9.11	9.12	9.11	9.11	9.11
Fat - yellow grease	41.55	13.86	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Monocalcium phosphate (21%)		18.13	5.51	4.52	4.55	3.54	3.69	2.89	3.09	2.44	2.65	2.21	2.36	2.20
HMTBa		2.26	4.50	3.29	3.33	2.09	2.27	1.29	1.55	0.74	1.00	0.47	0.65	0.45
L-Threonine (98%)		2.07	2.35	1.78	1.80	1.23	1.31	0.85	0.97	0.60	0.72	0.47	0.55	0.46
Trace mineral premix		1.98	2.00	1.87	1.87	1.73	1.75	1.64	1.67	1.58	1.61	1.55	1.57	1.55
Phytase 2500		1.90	0.80	0.76	0.76	0.73	0.73	0.70	0.71	0.68	0.69	0.67	0.68	0.67
Vitamin premix		0.99	0.60	0.57	0.57	0.55	0.55	0.53	0.53	0.52	0.52	0.51	0.51	0.51
Copper chloride (58%)		0.68	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Nursery basemix	791.99													
DDGS	111.15	76.52												
Mecadox 2.5 (g/lb)	20	20												
Zinc oxide (72%)		6.93												
Organic acidifier		5.94												
Carbohydrase		0.74												
Total:	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Nutrient Units														
Metabolizable energy Kcal/lb	1500	1519.371	1460.004	1460.18	1460.17	1460.35	1460.33	1460.47	1460.43	1460.55	1460.51	1460.59	1460.56	1460.59
Crude protein %	20.103	22.561	21.281	18.59	18.68	15.95	16.34	14.17	14.74	12.96	13.52	12.35	12.74	12.31
Cystine, Dig %	0.293	0.272	0.244	0.22	0.22	0.20	0.20	0.19	0.19	0.18	0.18	0.17	0.18	0.17
Isoleucine, Dig %	0.675	0.786	0.715	0.62	0.62	0.52	0.53	0.45	0.47	0.41	0.43	0.39	0.40	0.38
Lysine, Total %	1.405	1.521	1.454	1.23	1.24	1.01	1.05	0.87	0.91	0.77	0.81	0.72	0.75	0.71

Table S.B1 (continued)

Lysine, Dig %	1.25	1.34	1.27	1.07	1.08	0.87	0.90	0.74	0.78	0.65	0.69	0.61	0.64	0.60
Leucine, Dig %	1.469	1.586	1.439	1.31	1.31	1.18	1.20	1.09	1.12	1.03	1.06	1.00	1.02	1.00
Met + Cys, Dig %	0.707	0.765	0.725	0.62	0.62	0.51	0.53	0.44	0.47	0.40	0.42	0.37	0.39	0.37
Threonine, Dig %	0.76	0.804	0.762	0.65	0.65	0.54	0.55	0.46	0.48	0.41	0.43	0.38	0.40	0.38
Tryptophan, Dig %	0.223	0.232	0.216	0.18	0.18	0.15	0.16	0.13	0.14	0.12	0.12	0.11	0.11	0.11
Valine, Dig %	0.826	0.871	0.826	0.72	0.72	0.62	0.63	0.55	0.57	0.50	0.52	0.47	0.49	0.47
Phosphorus %	0.728	0.689	0.557	0.50	0.50	0.44	0.45	0.41	0.42	0.38	0.39	0.37	0.38	0.37
P, Available %	0.569	0.4	0.3	0.27	0.27	0.24	0.24	0.21	0.22	0.20	0.21	0.19	0.20	0.19
Calcium %	0.809	0.896	0.7	0.63	0.63	0.57	0.58	0.52	0.54	0.49	0.51	0.48	0.49	0.48
Moisture %	13.735	13.035	13.172	13.60	13.58	14.01	13.95	14.29	14.20	14.48	14.39	14.58	14.52	14.58
Crude fat %	5.476	3.03	5.458	4.82	4.84	4.18	4.28	3.76	3.89	3.47	3.60	3.32	3.42	3.31
Crude fiber %	2.117	3.18	3.486	3.17	3.18	2.85	2.90	2.64	2.71	2.50	2.57	2.43	2.47	2.42
ADF %	2.92	5.01	5.367	4.86	4.88	4.37	4.44	4.03	4.14	3.81	3.91	3.69	3.76	3.68
NDF %	6.507	12.75	15	13.58	13.63	12.19	12.40	11.26	11.55	10.62	10.91	10.30	10.50	10.28

Table S.B2. Vaccinations

Condition	Timing
Mycoplasma Hyopneumoniae	Processing (~4 days old)
Porcine Circovirus Type 2 (PCV2)	Weaning
Porcine Respiratory Syndrome (PRRS)	10-14 days post-weaning
Porcine Circovirus	10-17 days post PRRS vaccination
Mycoplasma Hyopneumoniae Ileitis Erysipelas	~6 weeks post-weaning

Table S.B3. Injectable Medications

Condition	Product	Timing
Respiratory, Diarrhea, Lameness	Excede	Weaning to 8 weeks post-weaning
Respiratory	Biomycin 200	8-14 weeks post-weaning
Respiratory	Lincocin 300	14 weeks post-weaning to end of study
Diarrhea, Lameness	Lincocin 300	8 weeks post weaning to end of study
Lameness	Dexamethasone	Weaning to 14 weeks post-weaning

Table S.B4. Water medications

Diarrhea	Product	Timing
Respiratory, Diarrhea	Neomycin	Weaning
Respiratory, Diarrhea, Lameness	Oxytetracycline (OTC) Denagard	As needed
	Linco Soluble	As needed

Table S.B5. Distribution of samples across families, sex, and time points

	Female			Male			Total
Family	Weaning	Mid-test	Off-test	Weaning	Mid-test	Off-test	
1	22	23	22	20	20	20	127
2	19	25	24	23	23	21	135
3	20	23	22	20	23	23	131
4	23	24	23	21	23	23	137
5	21	18	21	15	15	15	105
6	21	24	24	23	25	25	142
7	18	22	21	19	20	19	119
8	20	25	25	23	24	23	140
9	21	24	25	25	25	26	146
10	22	25	25	23	24	22	141
11	21	22	23	24	24	23	137
12	20	21	20	20	22	22	125
13	23	25	24	21	23	21	137
14	24	26	25	22	21	21	139
15	23	23	23	25	25	24	143
16	19	24	23	24	25	25	140
17	19	20	21	22	23	23	128
18	23	23	22	23	23	23	137
19	22	26	26	20	19	19	132
20	22	25	22	24	26	20	139
21	18	21	21	18	19	19	116
22	21	25	23	23	22	24	138
23	19	23	21	19	22	20	124
24	22	25	25	23	24	23	142
25	24	27	27	20	23	23	144
26	22	24	25	23	23	24	141
27	23	26	27	21	24	24	145
28	24	26	25	25	20	23	143

Table S.B6. Variance components explained by microbiome relationship matrix (O), genomic relationship matrix (G), pen (P), residual (R), microbiability (m^2) and heritability (h^2) in different models[1]

Traits[2]	Effects	Model 0	Model 1	Model 2	Model 3
LD	P	6.55±1.67	6.55±1.67	6.42±1.67	6.49±1.66
	O	-	0.29E-04±0.00	1.76±1.58	0.71±1.05
	G	6.15±2.94	6.15±2.93	5.88±2.36	5.88±2.36
	R	38.53±2.06	38.53±2.06	37.20±2.15	38.09±2.05
	m^2	-	0.00±0.00	0.03+0.03	0.01±0.02
	h^2	0.12±0.04	0.11±0.04	0.12±0.04	0.11±0.05
FD	P	2.78±0.62	2.37±0.60	2.90±0.67	2.77±0.65
	O	-	5.37±1.05	2.71±0.87	0.18±0.48
	G	9.84±1.55	7.61±1.34	9.00±1.48	9.80±1.56
	R	9.99±1.05	6.56±1.05	7.83±1.13	9.84±1.14
	m^2	-	0.25±0.04	0.12+0.03	0.01±0.02
	h^2	0.44±0.05	0.34±0.05	0.40±0.05	0.43±0.05
CADG	P	0.78E-05±0.00	0.59E-05±0.00	0.61E-05 ± 0.00	0.72E-0±0.00
	O	-	1.18±0.29	0.95±0.26	0.31±0.18
	G	1.05±0.23	0.94±0.27	1.07±0.29	0.98±0.08
	R	4.71±0.29	3.18±0.30	3.31±0.26	4.63±0.30
	m^2	-	0.22±0.05	0.18+0.04	0.06±0.03
	h^2	0.20±0.05	0.18±0.04	0.20±0.05	0.18±0.05
HAM	P	0.87E-07±00	0.73E-05±0.0	0.72E-05±0.00	0.85E-05±0.00
	O	-	0.83±0.29	0.80±0.25	0.10±0.16
	G	0.72±0.27	0.73±0.27	0.82±0.28	0.70±0.27
	R	4.71±0.29	3.96±0.33	3.96±0.33	4.65±0.32
	m^2	-	0.15±0.05	0.14+0.04	0.02±0.02
	h^2	0.13±0.05	0.13±0.04	0.13±0.05	0.13±0.04
LOIN	P	0.82E-05±0.0	0.70E-05±00	0.70E-05±00	0.80E-05±0.00
	O	-	0.44±0.18	0.43±0.16	0.10±0.16
	G	0.62±0.18	0.64±0.18	0.65±0.18	0.58±0.17
	R	2.87±0.18	2.45±0.21	2.46±0.21	2.79±0.19
	m^2	-	0.13±0.05	0.12+0.04	0.03±0.02
	h^2	0.18±0.05	0.18±0.04	0.18±0.05	0.17±0.05
BEL	P	0.79E-05±0.0	0.54E-05±0.00	0.59E-05±0.00	0.76E-05±0.00
	O	-	1.89±0.38	1.37±0.38	0.28±0.20
	G	1.34±0.36	1.18±0.33	1.39±0.36	1.28±0.35
	R	5.09±0.34	3.50±0.37	3.45±0.38	4.88±0.36
	m^2	-	0.29±0.05	0.20+0.04	0.04±0.03
	h^2	0.21±0.05	0.18±0.04	0.21±0.05	0.19±0.05

Table S.B6 (continued)

IMF	P	0.04 ± 0.02	0.04 ± 0.02	0.04± 0.02	0.04±0.02
	O	-	0.06±0.03	0.03±0.02	0.03±0.02
	G	0.55±0.08	0.53±0.08	0.54±0.08	0.55±0.08
	R	0.41±0.05	0.37±0.37	0.39±0.05	0.38±0.04
	m^2	-	0.06±0.02	0.03+0.02	0.03±0.02
	h^2	0.55±0.05	0.53±0.05	0.54±0.05	0.54±0.05
SMARB	P	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
	O	-	0.007±0.02	0.06±0.02	0.02±0.03
	G	0.26±0.08	0.26±0.06	0.26±0.06	0.26±0.06
	R	0.48±0.04	0.47±0.05	0.43±0.05	0.46±0.04
	m^2	-	0.01±0.02	0.07+0.02	0.02±0.02
	h^2	0.32±0.05	0.32±0.05	0.31±0.05	0.32±0.05
MINA	P	0.19 ± 0.04	0.17 ± 0.03	0.18 ± 0.03	0.18 ± 0.03
	O	-	0.12±0.05	0.02±0.04	0.23E-04±0.00
	G	0.22±0.06	0.22±0.06	0.22±0.06	0.22±0.06
	R	0.85±0.06	0.77±0.07	0.83±0.05	0.85±0.06
	m^2	-	0.09±0.02	0.02+0.02	0.00±0.00
	h^2	0.17±0.05	0.16±0.05	0.17±0.05	0.17±0.05
MINB	P	0.15 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	0.15 ± 0.03
	O	-	0.08±0.03	0.005±0.01	0.64E-05±0.00
	G	0.056±0.03	0.056±0.03	0.058±0.03	0.056±0.03
	R	0.49±0.03	0.42±0.04	0.48±0.04	0.49±0.04
	m^2	-	0.11±0.04	0.007+0.02	0.00±0.00
	h^2	0.08±0.04	0.08±0.04	0.08±0.04	0.08±0.04
MINL	P	6.15 ± 1.16	6.15 ± 1.16	6.15 ± 1.16	6.15 ± 1.16
	O	-	1.16±1.15	0.99E-05±0.00	0.61E-05±0.00
	G	6.90±1.82	6.57±1.78	6.91±1.82	6.91±1.82
	R	20.05±1.63	19.23±1.81	20.04±1.52	20.04±1.52
	m^2	-	0.03±0.03	0.00+0.00	0.00±0.00
	h^2	0.21±0.04	0.19±0.05	0.21±0.05	0.21±0.05
PH	P	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.013 ± 0.002
	O	-	0.002±0.002	0.001±0.007	0.26E-05±0.00
	G	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001
	R	0.031±0.002	0.031±0.002	0.031±0.002	0.031±0.002
	m^2	-	0.04±0.03	0.002+0.01	0.00±0.00
	h^2	0.06±0.04	0.06±0.04	0.06±0.04	0.06±0.04
SCOL	P	0.014 ± 0.006	0.013 ± 0.006	0.014 ± 0.006	0.013 ± 0.006
	O	-	0.012±0.011	0.40E-05±0.00	0.41E-05±0.00
	G	0.096±0.02	0.097±0.02	0.096±0.02	0.096±0.02

Table S.B6 (continued)

	R	0.216±0.018	0.204±0.019	0.215±0.018	0.215±0.018
	m^2	-	0.04±0.03	0.002+0.01	0.00±0.00
	h^2	0.30±0.06	0.30±0.06	0.30±0.06	0.30±0.05
SFIRM	P	0.026 ± 0.029	0.022 ± 0.028	0.028 ± 0.029	0.026 ± 0.029
	O	-	0.012±0.011	0.40E-05±0.00	0.41E-05±0.00
	G	0.134±0.050	0.122±0.046	0.120±0.044	0.134±0.050
	R	0.904±0.059	0.791±0.068	0.834±0.063	0.904±0.059
	m^2	-	0.13±0.04	0.08+0.03	0.00±0.00
	h^2	0.13±0.04	0.11±0.04	0.11±0.04	0.13±0.05
SSF	P	0.52±0.36	0.49±0.36	0.50±0.36	0.52±0.36
	O	-	0.30±0.36	1.38±0.59	0.41±0.32
	G	3.07±0.74	3.03±0.73	3.00±0.73	3.12±0.75
	R	9.42±0.70	9.20±0.66	8.27±0.78	8.99±0.77
	m^2	-	0.02±0.02	0.10+0.04	0.03±0.02
	h^2	0.24±0.05	0.23±0.05	0.22±0.05	0.24±0.05

[1] Model 0 contains **G** matrix and pen effect as random effect, Model 1, Model 2 and Model 3 contains **O** matrix at weaning, mid test and off test in addition to **G** matrix and pen effect.

[2]LD = Loin depth; FD = Fat depth; CADG = Carcass average daily gain; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; SSF = Slice shear force, HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight

Table S.B7. Estimates of genomic correlation (below diagonal) at end of test among meat quality traits.

	[1]SCOL	IMF	SFIRM	MINA	MINB	PH
SCOL						
IMF	-0.24±0.13					
SFIRM	0.16±0.19	**[2]0.36±0.15**				
MINA	**0.45±0.16**	**0.29±0.14**	-0.38±0.26			
MINB	**-0.94±0.22**	**0.78±0.16**	-0.06±0.31	-0.02±010		
PH	**0.91±0.29**	-0.18±0.25	0.42±0.35	-0.05±0.31	-0.53±0.42	

Table S.B8. Estimates of genomic correlation (below diagonal) at end of test among carcass composition traits.

	[1]FD	CADG	HAM	LOIN	BEL
FD					
CADG	**0.27±0.13**				
HAM	0.03±0.17	**0.67±0.13**			
LOIN	-0.11±0.15	**0.69±0.10**	**0.54±0.19**		
BEL	**0.62±0.11**	**0.79±0.07**	**0.58±0.19**	**0.70±0.03**	

Table S.B9. Estimates of genomic correlation between meat quality traits and carcass composition traits with inclusion of microbiome

	[1]FD	CADG	HAM	LOIN	BEL
SCOL	0.06±0.13	-0.10±0.17	-0.11±0.19	-0.04±0.16	-0.07±0.17
IMF	**0.23±0.11**	0.08±0.15	-0.05±0.16	-0.03±0.14	**0.28±0.14**
SFIRM	**0.27±0.13**	0.09±0.23	0.09±0.25	0.10±0.22	**0.46±0.23**
MINA	**0.27±0.13**	-0.10±0.21	-0.31±0.22	-0.24±0.21	-0.15±0.21
MINB	**0.42±0.21**	0.19±0.26	0.02±0.20	0.08±0.26	0.13±0.27
PH	-0.02±0.26	0.10±0.32	-0.19±0.28	0.09±0.20	-0.30±0.33

Table S.B10. Estimates of genomic correlation between meat quality traits and carcass composition traits without inclusion of microbiome

	[1]FD	CADG	HAM	LOIN	BEL
SCOL	-0.06±013	-0.01±0.17	-0.05±0.18	-0.02±0.17	-0.10±0.17
IMF	**0.26±0.10**	0.13±0.14	0.01±0.16	-0.02±0.14	**0.37±0.14**
SFIRM	**0.28±0.08**	0.13±0.28	0.12±0.24	0.02±0.22	**0.51±0.18**
MINA	**0.30±0.14**	0.09±0.17	-0.27±0.21	-0.28±0.20	-0.05±0.21
MINB	**0.46±0.19**	0.14±0.27	0.02±0.25	-0.02±0.27	0.16±0.27
PH	0.01±0.24	0.12±0.31	-0.28±0.36	0.06±0.31	-0.26±0.32

[1]FD = Fat depth; CADG = Carcass average daily gain; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight;

[2]Numbers in bold are significant.

Appendix C

Table S.C1. Number of significant OTU of each fold of cross-validation and the number of common OTU for each trait at each stage within each fold

Traits[1]	Stage	Fold 1	Fold 2	Fold 3	Fold 4	Common
LD	Wean	108	80	245	229	25
	Mid-test	79	83	84	80	30
	Off-test	89	71	97	79	19
FD	Wean	179	82	80	93	25
	Mid-test	340	381	393	375	207
	Off-test	273	308	359	379	156
CADG	Wean	276	110	144	108	36
	Mid-test	186	221	240	199	117
	Off-test	204	223	222	272	88
BEL	Wean	290	122	120	118	39
	Mid-test	118	297	264	129	46
	Off-test	182	266	267	279	99
HAM	Wean	133	91	103	81	28
	Mid-test	128	133	123	117	38
	Off-test	168	184	204	194	61
LOIN	Wean	112	83	92	95	23
	Mid-test	91	141	188	105	29
	Off-test	81	206	112	92	74
SCOL	Wean	73	60	61	76	19
	Mid-test	69	69	76	69	6
	Off-test	126	73	121	119	30
PH	Wean	88	77	83	86	18
	Mid-test	119	92	84	101	29
	Off-test	94	85	92	109	21
IMF	Wean	108	101	117	81	26
	Mid-test	320	292	227	142	71
	Off-test	168	188	151	147	53
MINA	Wean	60	68	76	53	17
	Mid-test	87	91	82	90	24
	Off-test	186	125	171	209	48
MINB	Wean	64	52	72	78	16
	Mid-test	94	94	74	101	25
	Off-test	121	93	94	121	35
MINL	Wean	88	61	59	53	17
	Mid-test	70	108	65	108	21
	Off-test	115	78	71	95	19

Table S.C1 (continued)

SFIRM	Wean	61	56	49	51	20
	Mid-test	278	206	114	280	45
	Off-test	184	143	121	156	38
SMARB	Wean	96	116	82	92	24
	Mid-test	114	185	166	142	38
	Off-test	98	101	112	104	30
SSF	Wean	97	69	86	91	23
	Mid-test	102	80	89	198	20
	Off-test	263	93	123	116	27

Table S.C2. Number of significant markers of each fold of cross-validation and the number of common markers for each trait at each stage within each fold

Traits[1]	Fold 1	Fold 2	Fold 3	Fold 4	Common
LD	2926	2492	2944	2739	477
FD	4610	4189	5091	4432	1101
CADG	3236	2944	3535	3560	598
BEL	3458	3158	3950	3861	722
HAM	2929	2905	3035	2917	477
LOIN	3709	2978	3099	3384	591
SCOL	3376	3504	2981	2978	561
PH	2466	2123	2425	2377	593
IMF	3660	3993	3887	3542	797
MINA	2917	3158	3240	2679	501
MINB	2533	2540	2679	2605	539
MINL	3322	3403	2889	3323	405
SFIRM	2960	3009	2618	2889	487
SMARB	3448	3577	3916	3207	586
SSF	3046	3085	3138	3008	481

[1]LD = Loin depth; FD = Fat depth; CADG = Carcass average daily gain; HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight; IMF = Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, MINL = Minolta L*, PH = Ultimate pH; SCOL = Subjective color score; SMARB = Subjective marbling score; SFIRM = Subjective firmness score; SSF = Slice shear force

Table S.C3. Predictive ability of different traits for each fold at different complexity, stage and model.

Complexity	Stage	Model[1]	Fold 1	Fold 2	Fold 3	Fold 4	Aver age	SD
LD								
Full	Wean	M_mod	0.15	0.08	0.12	0.16	0.13	0.04
Full	Mid-test	M_mod	0.14	0.04	0.09	0.17	0.11	0.06
Full	Off-test	M_mod	0.1	0.02	0.06	0.14	0.08	0.05
Full	Wean	G_mod	0.17	0.06	0.14	0.21	0.15	0.06
Full	Mid-test	G_mod	0.17	0.06	0.14	0.21	0.15	0.06
Full	Off-test	G_mod	0.17	0.06	0.14	0.21	0.15	0.06
Full	Wean	GM_mod	0.18	0.10	0.13	0.20	0.15	0.05
Full	Mid-test	GM_mod	0.17	0.07	0.12	0.21	0.14	0.06
Full	Off-test	GM_mod	0.15	0.05	0.10	0.20	0.12	0.06
Full	Wean	G×M_mod	0.18	0.09	0.13	0.20	0.15	0.05
Full	Mid-test	G×M_mod	0.17	0.07	0.12	0.21	0.14	0.06
Full	Off-test	G×M_mod	0.14	0.05	0.1	0.19	0.12	0.06
Informatively reduced	Wean	M_mod	0.12	0.29	0.06	0.29	0.19	0.12
Informatively reduced	Mid-test	M_mod	0.07	0.32	0.00	0.27	0.17	0.15
Informatively reduced	Off-test	M_mod	0.07	0.29	0.07	0.30	0.18	0.16
Informatively reduced	Wean	G_mod	0.16	0.11	0.15	0.32	0.19	0.13
Informatively reduced	Mid-test	G_mod	0.16	0.11	0.15	0.32	0.19	0.13
Informatively reduced	Off-test	G_mod	0.16	0.11	0.15	0.32	0.19	0.13
Informatively reduced	Wean	GM_mod	0.10	0.08	0.10	0.13	0.10	0.02
Informatively reduced	Mid-test	GM_mod	0.09	0.09	0.07	0.13	0.10	0.03
Informatively reduced	Off-test	GM_mod	0.07	0.07	0.10	0.11	0.09	0.02
Informatively reduced	Wean	G×M_mod	0.10	0.06	0.10	0.14	0.10	0.03
Informatively reduced	Mid-test	G×M_mod	0.09	0.07	0.07	0.14	0.09	0.03
Informatively reduced	Off-test	G×M_mod	0.07	0.06	0.09	0.11	0.08	0.02
Randomly reduced	Wean	M_mod	0.12	0.05	0.11	0.11	0.10	0.03
Randomly reduced	Mid-test	M_mod	0.13	0.06	0.13	0.13	0.11	0.04
Randomly reduced	Off-test	M_mod	0.10	0.04	0.08	0.08	0.08	0.03
Randomly reduced	Wean	G_mod	0.18	0.09	0.13	0.13	0.13	0.04
Randomly reduced	Mid-test	G_mod	0.18	0.09	0.13	0.13	0.13	0.04
Randomly reduced	Off-test	G_mod	0.18	0.09	0.13	0.13	0.13	0.04
Randomly reduced	Wean	GM_mod	0.17	0.07	0.13	0.13	0.12	0.04
Randomly reduced	Mid-test	GM_mod	0.18	0.08	0.13	0.13	0.13	0.04
Randomly reduced	Off-test	GM_mod	0.15	0.06	0.12	0.12	0.11	0.04
Randomly reduced	Wean	G×M_mod	0.17	0.08	0.13	0.13	0.13	0.04
Randomly reduced	Mid-test	G×M_mod	0.18	0.08	0.14	0.14	0.14	0.04
Randomly reduced	Off-test	G×M_mod	0.15	0.07	0.12	0.12	0.12	0.03
FD								
Full	Wean	M_mod	0.46	0.42	0.37	0.50	0.44	0.06
Full	Mid-test	M_mod	0.56	0.44	0.41	0.55	0.49	0.08

Table S.C3 (continued)

Full	Off-test	M_mod	0.58	0.52	0.48	0.51	0.52	0.04
Full	Wean	G_mod	0.55	0.51	0.47	0.57	0.53	0.06
Full	Mid-test	G_mod	0.55	0.51	0.47	0.57	0.53	0.06
Full	Off-test	G_mod	0.55	0.51	0.47	0.57	0.53	0.06
Full	Wean	GM_mod	0.61	0.52	0.43	0.57	0.52	0.06
Full	Mid-test	GM_mod	0.61	0.54	0.46	0.60	0.56	0.07
Full	Off-test	GM_mod	0.63	0.58	0.5	0.58	0.57	0.05
Full	Wean	G×M_mod	0.61	0.52	0.43	0.57	0.52	0.06
Full	Mid-test	G×M_mod	0.61	0.54	0.46	0.61	0.56	0.07
Full	Off-test	G×M_mod	0.61	0.58	0.49	0.61	0.584	0.06
Informatively reduced	Wean	M_mod	0.52	0.5	0.39	0.46	0.47	0.06
Informatively reduced	Mid-test	M_mod	0.58	0.52	0.42	0.48	0.5	0.06
Informatively reduced	Off-test	M_mod	0.61	0.59	0.51	0.59	0.55	0.04
Informatively reduced	Wean	G_mod	0.61	0.56	0.44	0.64	0.55	0.08
Informatively reduced	Mid-test	G_mod	0.61	0.56	0.44	0.64	0.55	0.08
Informatively reduced	Off-test	G_mod	0.61	0.56	0.44	0.64	0.55	0.08
Informatively reduced	Wean	GM_mod	0.46	0.53	0.41	0.51	0.48	0.05
Informatively reduced	Mid-test	GM_mod	0.52	0.55	0.44	0.56	0.52	0.05
Informatively reduced	Off-test	GM_mod	0.54	0.58	0.45	0.55	0.53	0.06
Informatively reduced	Wean	G×M_mod	0.47	0.52	0.41	0.52	0.48	0.05
Informatively reduced	Mid-test	G×M_mod	0.52	0.55	0.43	0.56	0.52	0.06
Informatively reduced	Off-test	G×M_mod	0.55	0.58	0.45	0.56	0.54	0.06
Randomly reduced	Wean	M_mod	0.56	0.41	0.37	0.37	0.43	0.05
Randomly reduced	Mid-test	M_mod	0.59	0.42	0.37	0.37	0.44	0.06
Randomly reduced	Off-test	M_mod	0.6	0.44	0.39	0.39	0.46	0.06
Randomly reduced	Wean	G_mod	0.61	0.51	0.42	0.42	0.49	0.07
Randomly reduced	Mid-test	G_mod	0.61	0.51	0.42	0.42	0.49	0.07
Randomly reduced	Off-test	G_mod	0.61	0.51	0.42	0.42	0.49	0.07
Randomly reduced	Wean	GM_mod	0.54	0.52	0.44	0.44	0.49	0.06
Randomly reduced	Mid-test	GM_mod	0.57	0.52	0.43	0.47	0.5	0.07
Randomly reduced	Off-test	GM_mod	0.58	0.52	0.46	0.49	0.51	0.06
Randomly reduced	Wean	G×M_mod	0.55	0.52	0.44	0.44	0.49	0.06
Randomly reduced	Mid-test	G×M_mod	0.59	0.51	0.43	0.44	0.49	0.07
Randomly reduced	Off-test	G×M_mod	0.63	0.52	0.42	0.44	0.5	0.06
CADG								
Full	Wean	M_mod	0.27	0.24	0.24	0.28	0.26	0.02
Full	Mid-test	M_mod	0.35	0.22	0.28	0.32	0.29	0.06
Full	Off-test	M_mod	0.37	0.31	0.3	0.31	0.32	0.03
Full	Wean	G_mod	0.26	0.27	0.24	0.25	0.26	0.01
Full	Off-test	G_mod	0.26	0.27	0.24	0.25	0.26	0.01
Full	Mid-test	G_mod	0.26	0.27	0.24	0.25	0.26	0.01
Full	Wean	GM_mod	0.28	0.29	0.26	0.29	0.28	0.01

Table S.C3 (continued)

Full	Mid-test	GM_mod	0.34	0.28	0.3	0.32	0.31	0.03
Full	Off-test	GM_mod	0.36	0.35	0.3	0.33	0.34	0.03
Full	Wean	G×M_mod	0.28	0.29	0.26	0.29	0.28	0.01
Full	Mid-test	G×M_mod	0.34	0.28	0.29	0.32	0.31	0.03
Full	Off-test	G×M_mod	0.35	0.34	0.3	0.33	0.33	0.02
Informatively reduced	Wean	M_mod	0.20	0.40	0.18	0.41	0.3	0.12
Informatively reduced	Mid-test	M_mod	0.28	0.49	0.3	0.53	0.4	0.13
Informatively reduced	Off-test	M_mod	0.34	0.42	0.36	0.45	0.39	0.05
Informatively reduced	Wean	G_mod	0.21	0.29	0.22	0.52	0.31	0.14
Informatively reduced	Mid-test	G_mod	0.21	0.29	0.22	0.52	0.31	0.14
Informatively reduced	Off-test	G_mod	0.21	0.29	0.22	0.52	0.31	0.14
Informatively reduced	Wean	GM_mod	0.23	0.35	0.21	0.29	0.27	0.06
Informatively reduced	Mid-test	GM_mod	0.29	0.43	0.27	0.34	0.33	0.07
Informatively reduced	Off-test	GM_mod	0.28	0.39	0.28	0.31	0.32	0.05
Informatively reduced	Wean	G×M_mod	0.23	0.34	0.21	0.29	0.27	0.06
Informatively reduced	Mid-test	G×M_mod	0.29	0.43	0.27	0.34	0.33	0.07
Informatively reduced	Off-test	G×M_mod	0.28	0.39	0.28	0.30	0.31	0.05
Randomly reduced	Wean	M_mod	0.25	0.21	0.23	0.23	0.23	0.02
Randomly reduced	Mid-test	M_mod	0.29	0.22	0.24	0.24	0.25	0.03
Randomly reduced	Off-test	M_mod	0.28	0.21	0.23	0.23	0.24	0.03
Randomly reduced	Wean	G_mod	0.27	0.28	0.25	0.25	0.26	0.02
Randomly reduced	Mid-test	G_mod	0.27	0.28	0.25	0.25	0.26	0.02
Randomly reduced	Off-test	G_mod	0.27	0.28	0.25	0.25	0.26	0.02
Randomly reduced	Wean	GM_mod	0.26	0.28	0.25	0.25	0.26	0.01
Randomly reduced	Mid-test	GM_mod	0.29	0.28	0.26	0.26	0.27	0.01
Randomly reduced	Off-test	GM_mod	0.28	0.27	0.25	0.25	0.26	0.02
Randomly reduced	Wean	G×M_mod	0.27	0.28	0.25	0.25	0.26	0.02
Randomly reduced	Mid-test	G×M_mod	0.29	0.28	0.26	0.26	0.27	0.01
Randomly reduced	Off-test	G×M_mod	0.28	0.27	0.25	0.25	0.26	0.02
HAM								
Full	Wean	M_mod	0.09	0.10	0.10	0.19	0.12	0.05
Full	Mid-test	M_mod	0.18	0.09	0.14	0.22	0.16	0.06
Full	Off-test	M_mod	0.19	0.19	0.13	0.23	0.18	0.04
Full	Wean	G_mod	0.13	0.13	0.1	0.12	0.12	0.01
Full	Mid-test	G_mod	0.13	0.13	0.1	0.12	0.12	0.01
Full	Off-test	G_mod	0.13	0.13	0.1	0.12	0.12	0.01
Full	Wean	GM_mod	0.13	0.15	0.1	0.16	0.14	0.03
Full	Mid-test	GM_mod	0.20	0.14	0.16	0.21	0.18	0.03
Full	Off-test	GM_mod	0.21	0.22	0.13	0.23	0.20	0.05
Full	Wean	G×M_mod	0.12	0.15	0.11	0.17	0.14	0.03
Full	Mid-test	G×M_mod	0.19	0.14	0.15	0.20	0.17	0.03
Full	Off-test	G×M_mod	0.20	0.21	0.14	0.22	0.19	0.04
Informatively reduced	Wean	M_mod	0.02	0.27	0.04	0.38	0.18	0.18

Table S.C3 (continued)

Informatively reduced	Mid-test	M_mod	0.09	0.37	0.14	0.38	0.24	0.15
Informatively reduced	Off-test	M_mod	0.16	0.35	0.19	0.3	0.25	0.09
Informatively reduced	Wean	G_mod	0.16	0.18	0.09	0.58	0.25	0.22
Informatively reduced	Mid-test	G_mod	0.16	0.18	0.09	0.58	0.25	0.22
Informatively reduced	Off-test	G_mod	0.16	0.18	0.09	0.58	0.25	0.22
Informatively reduced	Wean	GM_mod	0.16	0.22	0.1	0.17	0.16	0.05
Informatively reduced	Mid-test	GM_mod	0.17	0.33	0.13	0.17	0.2	0.09
Informatively reduced	Off-test	GM_mod	0.20	0.27	0.14	0.19	0.2	0.05
Informatively reduced	Wean	G×M_mod	0.16	0.21	0.10	0.16	0.16	0.04
Informatively reduced	Mid-test	G×M_mod	0.18	0.31	0.13	0.17	0.2	0.08
Informatively reduced	Off-test	G×M_mod	0.20	0.27	0.14	0.19	0.2	0.05
Randomly reduced	Wean	M_mod	0.09	0.08	0.09	0.09	0.09	0.00
Randomly reduced	Mid-test	M_mod	0.14	0.11	0.12	0.12	0.12	0.01
Randomly reduced	Off-test	M_mod	0.12	0.08	0.08	0.08	0.09	0.02
Randomly reduced	Wean	G_mod	0.15	0.14	0.11	0.11	0.13	0.02
Randomly reduced	Mid-test	G_mod	0.15	0.14	0.11	0.11	0.13	0.02
Randomly reduced	Off-test	G_mod	0.15	0.14	0.11	0.11	0.13	0.02
Randomly reduced	Wean	GM_mod	0.14	0.14	0.1	0.1	0.12	0.02
Randomly reduced	Mid-test	GM_mod	0.18	0.15	0.13	0.13	0.15	0.02
Randomly reduced	Off-test	GM_mod	0.17	0.14	0.1	0.1	0.13	0.03
Randomly reduced	Wean	G×M_mod	0.14	0.14	0.1	0.1	0.12	0.02
Randomly reduced	Mid-test	G×M_mod	0.17	0.15	0.13	0.13	0.15	0.02
Randomly reduced	Off-test	G×M_mod	0.16	0.14	0.1	0.1	0.12	0.03
LOIN								
Full	Wean	M_mod	0.19	0.14	0.14	0.19	0.15	0.03
Full	Mid-test	M_mod	0.24	0.07	0.15	0.22	0.17	0.08
Full	Off-test	M_mod	0.20	0.17	0.16	0.21	0.18	0.02
Full	Wean	G_mod	0.16	0.16	0.15	0.14	0.15	0.01
Full	Mid-test	G_mod	0.16	0.16	0.15	0.14	0.15	0.01
Full	Off-test	G_mod	0.16	0.16	0.15	0.14	0.15	0.01
Full	Wean	GM_mod	0.18	0.18	0.16	0.17	0.17	0.01
Full	Mid-test	GM_mod	0.23	0.13	0.19	0.19	0.18	0.04
Full	Off-test	GM_mod	0.20	0.22	0.20	0.20	0.20	0.01
Full	Wean	G×M_mod	0.18	0.18	0.16	0.17	0.17	0.01
Full	Mid-test	G×M_mod	0.23	0.14	0.19	0.19	0.19	0.04
Full	Off-test	G×M_mod	0.20	0.21	0.20	0.19	0.20	0.01
Informatively reduced	Wean	M_mod	0.10	0.27	0.08	0.40	0.21	0.15
Informatively reduced	Mid-test	M_mod	0.23	0.46	0.15	0.50	0.34	0.17
Informatively reduced	Off-test	M_mod	0.17	0.32	0.2	0.34	0.26	0.08
Informatively reduced	Wean	G_mod	0.11	0.16	0.12	0.60	0.25	0.24
Informatively reduced	Mid-test	G_mod	0.11	0.16	0.12	0.6	0.25	0.24
Informatively reduced	Off-test	G_mod	0.11	0.16	0.12	0.6	0.25	0.24
Informatively reduced	Wean	GM_mod	0.13	0.21	0.14	0.11	0.15	0.04

Table S.C3 (continued)

Informatively reduced	Mid-test	GM_mod	0.25	0.33	0.17	0.13	0.22	0.09
Informatively reduced	Off-test	GM_mod	0.15	0.24	0.18	0.14	0.18	0.04
Informatively reduced	Wean	G×M_mod	0.13	0.19	0.14	0.12	0.15	0.03
Informatively reduced	Mid-test	G×M_mod	0.25	0.31	0.18	0.12	0.22	0.08
Informatively reduced	Off-test	G×M_mod	0.15	0.24	0.18	0.14	0.18	0.04
Randomly reduced	Wean	M_mod	0.16	0.11	0.13	0.13	0.13	0.02
Randomly reduced	Mid-test	M_mod	0.18	0.09	0.11	0.11	0.12	0.04
Randomly reduced	Off-test	M_mod	0.17	0.09	0.1	0.1	0.12	0.04
Randomly reduced	Wean	G_mod	0.18	0.18	0.14	0.14	0.16	0.02
Randomly reduced	Mid-test	G_mod	0.18	0.18	0.14	0.14	0.16	0.02
Randomly reduced	Off-test	G_mod	0.18	0.18	0.14	0.14	0.16	0.02
Randomly reduced	Wean	GM_mod	0.18	0.18	0.16	0.16	0.17	0.01
Randomly reduced	Mid-test	GM_mod	0.20	0.17	0.15	0.15	0.17	0.02
Randomly reduced	Off-test	GM_mod	0.19	0.17	0.14	0.14	0.16	0.02
Randomly reduced	Wean	G×M_mod	0.19	0.18	0.16	0.16	0.17	0.01
Randomly reduced	Mid-test	G×M_mod	0.20	0.16	0.15	0.15	0.16	0.02
Randomly reduced	Off-test	G×M_mod	0.18	0.18	0.14	0.14	0.16	0.02
BEL								
Full	Wean	M_mod	0.19	0.21	0.19	0.18	0.19	0.01
Full	Mid-test	M_mod	0.33	0.2	0.22	0.28	0.26	0.06
Full	Off-test	M_mod	0.35	0.31	0.30	0.22	0.30	0.05
Full	Wean	G_mod	0.23	0.31	0.16	0.18	0.22	0.07
Full	Mid-test	G_mod	0.23	0.31	0.16	0.18	0.22	0.07
Full	Off-test	G_mod	0.23	0.31	0.16	0.18	0.22	0.07
Full	Wean	GM_mod	0.24	0.31	0.18	0.22	0.24	0.05
Full	Mid-test	GM_mod	0.36	0.30	0.23	0.30	0.30	0.05
Full	Off-test	GM_mod	0.37	0.38	0.27	0.26	0.30	0.06
Full	Wean	G×M_mod	0.24	0.31	0.19	0.22	0.24	0.05
Full	Mid-test	G×M_mod	0.36	0.3	0.23	0.29	0.30	0.05
Full	Off-test	G×M_mod	0.37	0.37	0.27	0.26	0.32	0.06
Informatively reduced	Wean	M_mod	0.18	0.43	0.17	0.41	0.30	0.14
Informatively reduced	Mid-test	M_mod	0.39	0.47	0.25	0.47	0.40	0.10
Informatively reduced	Off-test	M_mod	0.36	0.49	0.35	0.44	0.41	0.07
Informatively reduced	Wean	G_mod	0.22	0.35	0.18	0.53	0.32	0.16
Informatively reduced	Mid-test	G_mod	0.22	0.35	0.18	0.53	0.32	0.16
Informatively reduced	Off-test	G_mod	0.22	0.35	0.18	0.53	0.32	0.16
Informatively reduced	Wean	GM_mod	0.24	0.41	0.17	0.25	0.27	0.10
Informatively reduced	Mid-test	GM_mod	0.36	0.5	0.23	0.35	0.36	0.11
Informatively reduced	Off-test	GM_mod	0.34	0.49	0.22	0.32	0.34	0.11
Informatively reduced	Wean	G×M_mod	0.24	0.41	0.17	0.25	0.27	0.10
Informatively reduced	Mid-test	G×M_mod	0.35	0.51	0.23	0.35	0.37	0.11
Informatively reduced	Off-test	G×M_mod	0.33	0.49	0.22	0.32	0.35	0.11
Randomly reduced	Wean	M_mod	0.15	0.19	0.19	0.19	0.18	0.02

Table S.C3 (continued)

Randomly reduced	Mid-test	M_mod	0.22	0.17	0.15	0.15	0.17	0.03
Randomly reduced	Off-test	M_mod	0.21	0.16	0.18	0.18	0.18	0.02
Randomly reduced	Mid-test	G_mod	0.24	0.32	0.16	0.16	0.22	0.08
Randomly reduced	Off-test	G_mod	0.24	0.32	0.16	0.16	0.22	0.08
Randomly reduced	Wean	G_mod	0.24	0.32	0.16	0.16	0.22	0.08
Randomly reduced	Wean	GM_mod	0.23	0.33	0.19	0.19	0.24	0.07
Randomly reduced	Mid-test	GM_mod	0.27	0.31	0.18	0.18	0.24	0.07
Randomly reduced	Off-test	GM_mod	0.26	0.29	0.17	0.17	0.22	0.06
Randomly reduced	Wean	G×M_mod	0.22	0.32	0.19	0.19	0.23	0.06
Randomly reduced	Mid-test	G×M_mod	0.27	0.30	0.18	0.18	0.23	0.06
Randomly reduced	Off-test	G×M_mod	0.26	0.29	0.17	0.17	0.22	0.06
IMF								
Full	Wean	M_mod	0.25	0.24	0.26	0.27	0.26	0.01
Full	Mid-test	M_mod	0.31	0.23	0.28	0.32	0.29	0.04
Full	Off-test	M_mod	0.31	0.24	0.32	0.29	0.29	0.06
Full	Wean	G_mod	0.45	0.31	0.43	0.37	0.39	0.06
Full	Off-test	G_mod	0.45	0.31	0.43	0.37	0.39	0.06
Full	Mid-test	G_mod	0.45	0.31	0.43	0.37	0.39	0.06
Full	Wean	GM_mod	0.45	0.33	0.43	0.38	0.40	0.05
Full	Mid-test	GM_mod	0.45	0.31	0.42	0.39	0.39	0.06
Full	Off-test	GM_mod	0.48	0.29	0.45	0.38	0.40	0.08
Full	Wean	G×M_mod	0.44	0.32	0.43	0.38	0.39	0.06
Full	Mid-test	G×M_mod	0.45	0.31	0.42	0.39	0.39	0.06
Full	Off-test	G×M_mod	0.47	0.29	0.45	0.38	0.40	0.08
Informatively reduced	Wean	M_mod	0.23	0.37	0.17	0.39	0.29	0.11
Informatively reduced	Mid-test	M_mod	0.29	0.33	0.19	0.42	0.31	0.1
Informatively reduced	Off-test	M_mod	0.23	0.31	0.31	0.43	0.32	0.08
Informatively reduced	Wean	G_mod	0.47	0.38	0.43	0.65	0.48	0.12
Informatively reduced	Mid-test	G_mod	0.47	0.38	0.43	0.65	0.48	0.12
Informatively reduced	Off-test	G_mod	0.47	0.38	0.43	0.65	0.48	0.12
Informatively reduced	Wean	GM_mod	0.46	0.42	0.43	0.26	0.39	0.09
Informatively reduced	Mid-test	GM_mod	0.48	0.41	0.41	0.29	0.4	0.08
Informatively reduced	Off-test	GM_mod	0.47	0.4	0.43	0.27	0.39	0.09
Informatively reduced	Wean	G×M_mod	0.46	0.41	0.43	0.27	0.39	0.08
Informatively reduced	Mid-test	G×M_mod	0.47	0.4	0.41	0.29	0.39	0.08
Informatively reduced	Off-test	G×M_mod	0.47	0.39	0.43	0.27	0.39	0.09
Randomly reduced	Wean	M_mod	0.25	0.23	0.27	0.27	0.26	0.02
Randomly reduced	Mid-test	M_mod	0.27	0.21	0.29	0.29	0.26	0.04
Randomly reduced	Off-test	M_mod	0.28	0.23	0.31	0.31	0.28	0.04
Randomly reduced	Wean	G_mod	0.47	0.34	0.42	0.42	0.41	0.05
Randomly reduced	Mid-test	G_mod	0.47	0.34	0.42	0.42	0.41	0.05
Randomly reduced	Off-test	G_mod	0.47	0.34	0.42	0.42	0.41	0.05
Randomly reduced	Wean	GM_mod	0.47	0.34	0.44	0.44	0.42	0.06

Table S.C3 (continued)

Randomly reduced	Mid-test	GM_mod	0.46	0.33	0.43	0.43	0.41	0.06
Randomly reduced	Off-test	GM_mod	0.48	0.34	0.44	0.44	0.42	0.06
Randomly reduced	Wean	G×M_mod	0.46	0.35	0.43	0.43	0.42	0.05
Randomly reduced	Mid-test	G×M_mod	0.46	0.34	0.43	0.43	0.42	0.05
Randomly reduced	Off-test	G×M_mod	0.47	0.34	0.44	0.44	0.42	0.06
MINA								
Full	Wean	M_mod	0.22	0.22	0.22	0.26	0.23	0.02
Full	Mid-test	M_mod	0.26	0.25	0.24	0.28	0.26	0.02
Full	Off-test	M_mod	0.27	0.26	0.29	0.31	0.28	0.02
Full	Wean	G_mod	0.27	0.24	0.27	0.32	0.28	0.03
Full	Mid-test	G_mod	0.27	0.24	0.27	0.32	0.28	0.03
Full	Off-test	G_mod	0.27	0.24	0.27	0.32	0.28	0.03
Full	Wean	GM_mod	0.25	0.23	0.26	0.31	0.26	0.03
Full	Mid-test	GM_mod	0.28	0.25	0.27	0.32	0.28	0.03
Full	Off-test	GM_mod	0.28	0.27	0.31	0.33	0.30	0.03
Full	Wean	G×M_mod	0.25	0.24	0.26	0.31	0.27	0.03
Full	Mid-test	G×M_mod	0.28	0.25	0.27	0.32	0.28	0.03
Full	Off-test	G×M_mod	0.29	0.27	0.31	0.33	0.30	0.03
Informatively reduced	Wean	M_mod	0.13	0.41	0.12	0.29	0.24	0.14
Informatively reduced	Mid-test	M_mod	0.21	0.4	0.13	0.44	0.3	0.15
Informatively reduced	Off-test	M_mod	0.20	0.44	0.32	0.47	0.36	0.12
Informatively reduced	Wean	G_mod	0.21	0.23	0.22	0.62	0.32	0.20
Informatively reduced	Mid-test	G_mod	0.21	0.23	0.22	0.62	0.32	0.20
Informatively reduced	Off-test	G_mod	0.21	0.23	0.22	0.62	0.32	0.20
Informatively reduced	Wean	GM_mod	0.17	0.18	0.20	0.26	0.20	0.04
Informatively reduced	Mid-test	GM_mod	0.25	0.24	0.2	0.31	0.25	0.05
Informatively reduced	Off-test	GM_mod	0.22	0.25	0.26	0.28	0.25	0.03
Informatively reduced	Wean	G×M_mod	0.18	0.16	0.21	0.28	0.21	0.05
Informatively reduced	Mid-test	G×M_mod	0.25	0.23	0.21	0.30	0.25	0.04
Informatively reduced	Off-test	G×M_mod	0.22	0.22	0.26	0.29	0.25	0.03
Randomly reduced	Wean	M_mod	0.24	0.23	0.24	0.24	0.24	0.00
Randomly reduced	Mid-test	M_mod	0.24	0.25	0.24	0.24	0.24	0.01
Randomly reduced	Off-test	M_mod	0.24	0.25	0.25	0.25	0.25	0.01
Randomly reduced	Wean	G_mod	0.26	0.24	0.26	0.26	0.26	0.01
Randomly reduced	Mid-test	G_mod	0.26	0.24	0.26	0.26	0.26	0.01
Randomly reduced	Off-test	G_mod	0.26	0.24	0.26	0.26	0.26	0.01
Randomly reduced	Wean	GM_mod	0.26	0.24	0.27	0.27	0.26	0.01
Randomly reduced	Mid-test	GM_mod	0.26	0.25	0.26	0.26	0.26	0.01
Randomly reduced	Off-test	GM_mod	0.26	0.25	0.27	0.27	0.26	0.01
Randomly reduced	Wean	G×M_mod	0.26	0.23	0.26	0.26	0.25	0.02
Randomly reduced	Mid-test	G×M_mod	0.26	0.25	0.26	0.25	0.26	0.01
Randomly reduced	Off-test	G×M_mod	0.26	0.25	0.26	0.26	0.26	0.01
MINB								

Table S.C3 (continued)

Full	Wean	M_mod	0.26	0.39	0.26	0.30	0.30	0.06
Full	Mid-test	M_mod	0.26	0.39	0.28	0.28	0.30	0.06
Full	Off-test	M_mod	0.26	0.43	0.29	0.33	0.33	0.07
Full	Wean	G_mod	0.28	0.42	0.27	0.32	0.32	0.07
Full	Mid-test	G_mod	0.28	0.42	0.27	0.32	0.32	0.07
Full	Off-test	G_mod	0.28	0.42	0.27	0.32	0.32	0.07
Full	Wean	GM_mod	0.27	0.41	0.26	0.32	0.32	0.07
Full	Mid-test	GM_mod	0.27	0.41	0.27	0.3	0.31	0.07
Full	Off-test	GM_mod	0.28	0.45	0.29	0.35	0.34	0.08
Full	Wean	G×M_mod	0.28	0.41	0.26	0.32	0.32	0.07
Full	Mid-test	G×M_mod	0.28	0.41	0.28	0.30	0.32	0.06
Full	Off-test	G×M_mod	0.28	0.46	0.28	0.35	0.34	0.09
Informatively reduced	Wean	M_mod	0.1	0.47	0.12	0.34	0.26	0.18
Informatively reduced	Mid-test	M_mod	0.19	0.5	0.22	0.4	0.33	0.15
Informatively reduced	Off-test	M_mod	0.12	0.57	0.19	0.46	0.34	0.21
Informatively reduced	Wean	G_mod	0.32	0.36	0.35	0.51	0.39	0.09
Informatively reduced	Mid-test	G_mod	0.32	0.36	0.35	0.51	0.39	0.09
Informatively reduced	Off-test	G_mod	0.32	0.36	0.35	0.51	0.39	0.09
Informatively reduced	Wean	GM_mod	0.19	0.34	0.14	0.26	0.23	0.09
Informatively reduced	Mid-test	GM_mod	0.23	0.36	0.2	0.23	0.26	0.07
Informatively reduced	Off-test	GM_mod	0.18	0.42	0.15	0.28	0.26	0.12
Informatively reduced	Wean	G×M_mod	0.17	0.31	0.12	0.24	0.21	0.08
Informatively reduced	Mid-test	G×M_mod	0.21	0.34	0.18	0.21	0.24	0.07
Informatively reduced	Off-test	G×M_mod	0.19	0.41	0.16	0.28	0.26	0.11
Randomly reduced	Wean	M_mod	0.25	0.39	0.27	0.27	0.30	0.06
Randomly reduced	Mid-test	M_mod	0.26	0.39	0.28	0.28	0.30	0.06
Randomly reduced	Off-test	M_mod	0.26	0.41	0.29	0.29	0.31	0.07
Randomly reduced	Wean	G_mod	0.27	0.42	0.25	0.25	0.30	0.08
Randomly reduced	Mid-test	G_mod	0.27	0.42	0.25	0.25	0.30	0.08
Randomly reduced	Off-test	G_mod	0.27	0.42	0.25	0.25	0.30	0.08
Randomly reduced	Wean	GM_mod	0.26	0.41	0.26	0.26	0.30	0.07
Randomly reduced	Mid-test	GM_mod	0.27	0.41	0.26	0.26	0.30	0.07
Randomly reduced	Off-test	GM_mod	0.27	0.43	0.26	0.26	0.30	0.08
Randomly reduced	Wean	G×M_mod	0.27	0.41	0.26	0.26	0.30	0.07
Randomly reduced	Mid-test	G×M_mod	0.27	0.41	0.26	0.26	0.30	0.07
Randomly reduced	Off-test	G×M_mod	0.27	0.43	0.27	0.27	0.31	0.08
MINL								
Full	Wean	M_mod	0.12	0.16	0.00	0.07	0.09	0.07
Full	Mid-test	M_mod	0.21	0.14	0.01	0.08	0.11	0.09
Full	Off-test	M_mod	0.17	0.17	0.05	0.1	0.12	0.06
Full	Wean	G_mod	0.21	0.16	0.08	0.23	0.17	0.07
Full	Mid-test	G_mod	0.21	0.16	0.08	0.23	0.17	0.07
Full	Off-test	G_mod	0.21	0.16	0.08	0.23	0.17	0.07

Table S.C3 (continued)

Full	Wean	GM_mod	0.17	0.16	0.07	0.22	0.16	0.06
Full	Mid-test	GM_mod	0.22	0.15	0.08	0.20	0.16	0.06
Full	Off-test	GM_mod	0.20	0.17	0.1	0.21	0.17	0.05
Full	Wean	G×M_mod	0.16	0.16	0.07	0.22	0.15	0.06
Full	Mid-test	G×M_mod	0.22	0.16	0.07	0.21	0.17	0.07
Full	Off-test	G×M_mod	0.20	0.17	0.10	0.21	0.17	0.05
Informatively reduced	Wean	M_mod	-0.01	0.33	-0.01	0.22	0.13	0.17
Informatively reduced	Mid-test	M_mod	0.20	0.33	0.01	0.27	0.20	0.14
Informatively reduced	Off-test	M_mod	0.03	0.38	0.04	0.28	0.18	0.18
Informatively reduced	Wean	G_mod	0.21	0.13	0.11	0.54	0.25	0.20
Informatively reduced	Mid-test	G_mod	0.21	0.13	0.11	0.54	0.25	0.20
Informatively reduced	Off-test	G_mod	0.21	0.13	0.11	0.54	0.25	0.20
Informatively reduced	Wean	GM_mod	0.16	0.15	0.10	0.05	0.12	0.05
Informatively reduced	Mid-test	GM_mod	0.24	0.16	0.10	0.08	0.14	0.07
Informatively reduced	Off-test	GM_mod	0.16	0.2	0.12	0.07	0.14	0.06
Informatively reduced	Wean	G×M_mod	0.16	0.14	0.10	0.06	0.12	0.04
Informatively reduced	Mid-test	G×M_mod	0.23	0.15	0.10	0.09	0.14	0.06
Informatively reduced	Off-test	G×M_mod	0.16	0.19	0.12	0.07	0.14	0.05
Randomly reduced	Wean	M_mod	0.12	0.16	0.01	0.01	0.08	0.08
Randomly reduced	Mid-test	M_mod	0.18	0.14	-0.01	-0.01	0.08	0.10
Randomly reduced	Off-test	M_mod	0.17	0.2	0.01	0.01	0.10	0.10
Randomly reduced	Wean	G_mod	0.17	0.13	0.08	0.08	0.12	0.04
Randomly reduced	Mid-test	G_mod	0.17	0.13	0.08	0.08	0.12	0.04
Randomly reduced	Off-test	G_mod	0.17	0.13	0.08	0.08	0.12	0.04
Randomly reduced	Wean	GM_mod	0.16	0.13	0.08	0.08	0.11	0.04
Randomly reduced	Mid-test	GM_mod	0.17	0.12	0.07	0.07	0.11	0.05
Randomly reduced	Off-test	GM_mod	0.17	0.15	0.08	0.08	0.12	0.05
Randomly reduced	Wean	G×M_mod	0.15	0.14	0.07	0.07	0.11	0.04
Randomly reduced	Mid-test	G×M_mod	0.18	0.13	0.07	0.07	0.11	0.05
Randomly reduced	Off-test	G×M_mod	0.18	0.14	0.06	0.06	0.11	0.06
pH								
Full	Wean	M_mod	0.13	0.13	0.15	0.1	0.13	0.02
Full	Mid-test	M_mod	0.14	0.12	0.17	0.11	0.14	0.03
Full	Off-test	M_mod	0.19	0.12	0.15	0.09	0.14	0.04
Full	Wean	G_mod	0.22	0.1	0.14	0.14	0.15	0.05
Full	Mid-test	G_mod	0.22	0.1	0.14	0.14	0.15	0.05
Full	Off-test	G_mod	0.22	0.1	0.14	0.14	0.15	0.05
Full	Wean	GM_mod	0.17	0.1	0.15	0.12	0.14	0.03
Full	Mid-test	GM_mod	0.18	0.11	0.16	0.14	0.15	0.03
Full	Off-test	GM_mod	0.23	0.11	0.15	0.12	0.15	0.05
Full	Wean	G×M_mod	0.19	0.1	0.15	0.12	0.14	0.04
Full	Mid-test	G×M_mod	0.17	0.11	0.16	0.13	0.14	0.03

Table S.C3 (continued)

Full	Off-test	G×M_mod	0.22	0.11	0.16	0.12	0.15	0.05
Informatively reduced	Wean	M_mod	0.09	0.30	0.05	0.25	0.17	0.12
Informatively reduced	Mid-test	M_mod	0.11	0.29	0.13	0.27	0.20	0.09
Informatively reduced	Off-test	M_mod	0.15	0.29	0.06	0.26	0.19	0.11
Informatively reduced	Wean	G_mod	0.17	0.05	0.04	0.52	0.20	0.22
Informatively reduced	Mid-test	G_mod	0.17	0.05	0.04	0.52	0.20	0.22
Informatively reduced	Off-test	G_mod	0.17	0.05	0.04	0.52	0.20	0.22
Informatively reduced	Wean	GM_mod	0.16	0.10	0.03	0.08	0.09	0.05
Informatively reduced	Mid-test	GM_mod	0.17	0.10	0.06	0.18	0.13	0.06
Informatively reduced	Off-test	GM_mod	0.20	0.14	0.05	0.12	0.13	0.06
Informatively reduced	Wean	G×M_mod	0.16	0.07	0.03	0.08	0.08	0.05
Informatively reduced	Mid-test	G×M_mod	0.17	0.08	0.06	0.18	0.12	0.06
Informatively reduced	Off-test	G×M_mod	0.21	0.10	0.06	0.12	0.12	0.06
Randomly reduced	Wean	M_mod	0.16	0.1	0.12	0.12	0.12	0.03
Randomly reduced	Mid-test	M_mod	0.14	0.11	0.15	0.15	0.14	0.02
Randomly reduced	Off-test	M_mod	0.19	0.13	0.14	0.14	0.15	0.03
Randomly reduced	Wean	G_mod	0.20	0.09	0.15	0.15	0.15	0.05
Randomly reduced	Mid-test	G_mod	0.20	0.09	0.15	0.15	0.15	0.05
Randomly reduced	Off-test	G_mod	0.20	0.09	0.15	0.15	0.15	0.05
Randomly reduced	Wean	GM_mod	0.18	0.08	0.13	0.13	0.13	0.04
Randomly reduced	Mid-test	GM_mod	0.17	0.09	0.16	0.16	0.15	0.04
Randomly reduced	Off-test	GM_mod	0.21	0.1	0.15	0.15	0.15	0.04
Randomly reduced	Wean	G×M_mod	0.18	0.09	0.14	0.14	0.14	0.04
Randomly reduced	Mid-test	G×M_mod	0.17	0.09	0.16	0.16	0.15	0.04
Randomly reduced	Off-test	G×M_mod	0.20	0.11	0.14	0.14	0.15	0.04
SCOL								
Full	Wean	M_mod	0.06	0.11	0.00	0.00	0.04	0.05
Full	Mid-test	M_mod	0.02	0.06	0.05	0.04	0.02	0.03
Full	Off-test	M_mod	0.04	0.14	0.04	0.06	0.06	0.06
Full	Wean	G_mod	0.11	0.19	0.18	0.24	0.18	0.05
Full	Mid-test	G_mod	0.11	0.19	0.18	0.24	0.18	0.05
Full	Off-test	G_mod	0.11	0.19	0.18	0.24	0.18	0.05
Full	Wean	GM_mod	0.13	0.19	0.18	0.23	0.18	0.04
Full	Mid-test	GM_mod	0.11	0.19	0.17	0.24	0.18	0.05
Full	Off-test	GM_mod	0.13	0.21	0.16	0.24	0.18	0.05
Full	Wean	G×M_mod	0.12	0.2	0.19	0.23	0.19	0.05
Full	Mid-test	G×M_mod	0.11	0.18	0.16	0.24	0.17	0.05
Full	Off-test	G×M_mod	0.12	0.21	0.16	0.24	0.18	0.05
Informatively reduced	Wean	M_mod	0.01	0.42	0.05	0.2	0.16	0.19
Informatively reduced	Mid-test	M_mod	0.01	0.33	0.06	0.24	0.16	0.16
Informatively reduced	Off-test	M_mod	0.07	0.36	0.02	0.34	0.2	0.18
Informatively reduced	Wean	G_mod	0.17	0.15	0.18	0.58	0.27	0.21
Informatively reduced	Mid-test	G_mod	0.17	0.15	0.18	0.58	0.27	0.21

Table S.C3 (continued)

Informatively reduced	Off-test	G_mod	0.17	0.15	0.18	0.58	0.27	0.21
Informatively reduced	Wean	GM_mod	0.17	0.19	0.19	0.17	0.18	0.01
Informatively reduced	Mid-test	GM_mod	0.17	0.20	0.18	0.21	0.19	0.02
Informatively reduced	Off-test	GM_mod	0.17	0.24	0.19	0.17	0.19	0.03
Informatively reduced	Wean	G×M_mod	0.17	0.17	0.19	0.17	0.18	0.01
Informatively reduced	Mid-test	G×M_mod	0.17	0.19	0.18	0.21	0.19	0.02
Informatively reduced	Off-test	G×M_mod	0.17	0.23	0.19	0.17	0.19	0.03
Randomly reduced	Wean	M_mod	0.02	0.10	0.00	0.00	0.03	0.05
Randomly reduced	Mid-test	M_mod	0.01	0.06	0.00	0.00	0.02	0.03
Randomly reduced	Off-test	M_mod	0.01	0.12	0.02	0.02	0.02	0.07
Randomly reduced	Wean	G_mod	0.15	0.17	0.20	0.20	0.18	0.02
Randomly reduced	Mid-test	G_mod	0.15	0.17	0.20	0.20	0.18	0.02
Randomly reduced	Off-test	G_mod	0.15	0.17	0.20	0.20	0.18	0.02
Randomly reduced	Wean	GM_mod	0.15	0.17	0.21	0.21	0.18	0.03
Randomly reduced	Mid-test	GM_mod	0.14	0.16	0.2	0.2	0.18	0.03
Randomly reduced	Off-test	GM_mod	0.14	0.18	0.19	0.19	0.18	0.02
Randomly reduced	Wean	G×M_mod	0.15	0.17	0.21	0.21	0.18	0.03
Randomly reduced	Mid-test	G×M_mod	0.14	0.16	0.19	0.19	0.17	0.02
Randomly reduced	Off-test	G×M_mod	0.13	0.17	0.20	0.20	0.18	0.03
SMARB								
Full	Wean	M_mod	0.15	0.26	0.14	0.22	0.17	0.09
Full	Mid-test	M_mod	0.14	0.25	0.13	0.20	0.18	0.07
Full	Off-test	M_mod	0.18	0.25	0.15	0.16	0.19	0.08
Full	Wean	G_mod	0.23	0.36	0.17	0.35	0.28	0.09
Full	Mid-test	G_mod	0.23	0.36	0.17	0.35	0.28	0.09
Full	Off-test	G_mod	0.23	0.36	0.17	0.35	0.28	0.09
Full	Wean	GM_mod	0.23	0.35	0.19	0.35	0.28	0.08
Full	Mid-test	GM_mod	0.26	0.32	0.17	0.32	0.27	0.07
Full	Off-test	GM_mod	0.21	0.34	0.17	0.3	0.26	0.08
Full	Wean	G×M_mod	0.23	0.36	0.19	0.35	0.28	0.09
Full	Mid-test	G×M_mod	0.25	0.33	0.17	0.33	0.27	0.08
Full	Off-test	G×M_mod	0.21	0.34	0.17	0.3	0.26	0.08
Informatively reduced	Wean	M_mod	0.09	0.32	0.08	0.32	0.2	0.14
Informatively reduced	Mid-test	M_mod	0.16	0.44	0.12	0.25	0.24	0.14
Informatively reduced	Off-test	M_mod	0.13	0.43	0.07	0.24	0.22	0.16
Informatively reduced	Wean	G_mod	0.23	0.26	0.19	0.52	0.30	0.30
Informatively reduced	Mid-test	G_mod	0.23	0.26	0.19	0.52	0.30	0.30
Informatively reduced	Off-test	G_mod	0.23	0.26	0.19	0.52	0.30	0.30
Informatively reduced	Wean	GM_mod	0.25	0.32	0.16	0.23	0.24	0.07
Informatively reduced	Mid-test	GM_mod	0.24	0.35	0.18	0.23	0.25	0.07
Informatively reduced	Off-test	GM_mod	0.24	0.33	0.18	0.23	0.24	0.06
Informatively reduced	Wean	G×M_mod	0.25	0.32	0.17	0.24	0.24	0.06
Informatively reduced	Mid-test	G×M_mod	0.23	0.34	0.18	0.23	0.25	0.07

Table S.C3 (continued)

Informatively reduced	Off-test	G×M_mod	0.24	0.33	0.19	0.24	0.25	0.06
Randomly reduced	Wean	M_mod	0.08	0.25	0.09	0.09	0.13	0.08
Randomly reduced	Mid-test	M_mod	0.10	0.24	0.09	0.09	0.13	0.07
Randomly reduced	Off-test	M_mod	0.08	0.29	0.08	0.08	0.13	0.10
Randomly reduced	Wean	G_mod	0.24	0.36	0.18	0.18	0.24	0.08
Randomly reduced	Mid-test	G_mod	0.24	0.36	0.18	0.18	0.24	0.08
Randomly reduced	Off-test	G_mod	0.24	0.36	0.18	0.18	0.24	0.08
Randomly reduced	Wean	GM_mod	0.24	0.33	0.19	0.19	0.24	0.07
Randomly reduced	Mid-test	GM_mod	0.25	0.32	0.18	0.18	0.23	0.07
Randomly reduced	Off-test	GM_mod	0.24	0.35	0.18	0.18	0.24	0.08
Randomly reduced	Wean	G×M_mod	0.24	0.34	0.18	0.18	0.24	0.08
Randomly reduced	Mid-test	G×M_mod	0.25	0.34	0.18	0.18	0.24	0.08
Randomly reduced	Off-test	G×M_mod	0.24	0.36	0.17	0.17	0.24	0.09
SFIRM								
Full	Wean	M_mod	0.08	0.18	0.02	0.08	0.09	0.07
Full	Mid-test	M_mod	0.11	0.17	0.12	0.17	0.14	0.03
Full	Off-test	M_mod	0.11	0.23	0.15	0.15	0.16	0.05
Full	Wean	G_mod	0.16	0.25	0.10	0.13	0.16	0.06
Full	Mid-test	G_mod	0.16	0.25	0.10	0.13	0.16	0.06
Full	Off-test	G_mod	0.16	0.25	0.10	0.13	0.16	0.06
Full	Wean	GM_mod	0.13	0.21	0.08	0.11	0.13	0.06
Full	Mid-test	GM_mod	0.15	0.20	0.15	0.18	0.17	0.02
Full	Off-test	GM_mod	0.14	0.24	0.17	0.16	0.18	0.04
Full	Wean	G×M_mod	0.14	0.22	0.08	0.11	0.14	0.06
Full	Mid-test	G×M_mod	0.15	0.20	0.15	0.17	0.17	0.02
Full	Off-test	G×M_mod	0.15	0.26	0.17	0.16	0.19	0.05
Informatively reduced	Wean	M_mod	-0.05	0.33	-0.02	0.26	0.13	0.19
Informatively reduced	Mid-test	M_mod	0.05	0.35	0.06	0.26	0.18	0.15
Informatively reduced	Off-test	M_mod	0.10	0.41	0.18	0.37	0.26	0.15
Informatively reduced	Wean	G_mod	0.11	0.13	0.10	0.58	0.23	0.23
Informatively reduced	Mid-test	G_mod	0.11	0.13	0.10	0.58	0.23	0.23
Informatively reduced	Off-test	G_mod	0.11	0.13	0.10	0.58	0.23	0.23
Informatively reduced	Wean	GM_mod	0.06	0.18	0.08	0.03	0.09	0.06
Informatively reduced	Mid-test	GM_mod	0.1	0.19	0.12	0.12	0.13	0.04
Informatively reduced	Off-test	GM_mod	0.13	0.21	0.13	0.08	0.14	0.05
Informatively reduced	Wean	G×M_mod	0.06	0.16	0.09	0.03	0.08	0.06
Informatively reduced	Mid-test	G×M_mod	0.11	0.18	0.12	0.11	0.13	0.03
Informatively reduced	Off-test	G×M_mod	0.13	0.20	0.13	0.08	0.14	0.05
Randomly reduced	Wean	M_mod	0.10	0.23	0.01	0.01	0.09	0.10
Randomly reduced	Mid-test	M_mod	0.09	0.19	0.08	0.08	0.11	0.05
Randomly reduced	Off-test	M_mod	0.11	0.25	0.09	0.09	0.14	0.08
Randomly reduced	Wean	G_mod	0.18	0.25	0.10	0.10	0.16	0.07

Table S.C3 (continued)

Randomly reduced	Mid-test	G_mod	0.18	0.25	0.10	0.10	0.16	0.07
Randomly reduced	Off-test	G_mod	0.18	0.25	0.10	0.10	0.16	0.07
Randomly reduced	Wean	GM_mod	0.16	0.26	0.08	0.08	0.15	0.09
Randomly reduced	Mid-test	GM_mod	0.15	0.23	0.12	0.12	0.16	0.05
Randomly reduced	Off-test	GM_mod	0.16	0.27	0.13	0.13	0.17	0.07
Randomly reduced	Wean	G×M_mod	0.16	0.26	0.08	0.08	0.15	0.09
Randomly reduced	Mid-test	G×M_mod	0.15	0.24	0.12	0.12	0.16	0.06
Randomly reduced	Off-test	G×M_mod	0.16	0.28	0.13	0.13	0.18	0.07
SSF								
Full	Wean	M_mod	0.10	0.13	0.14	0.20	0.14	0.04
Full	Mid-test	M_mod	0.15	0.18	0.16	0.20	0.17	0.02
Full	Off-test	M_mod	0.16	0.14	0.16	0.17	0.16	0.01
Full	Wean	G_mod	0.22	0.15	0.16	0.20	0.18	0.03
Full	Mid-test	G_mod	0.22	0.15	0.16	0.20	0.18	0.03
Full	Off-test	G_mod	0.22	0.15	0.16	0.20	0.18	0.03
Full	Wean	GM_mod	0.21	0.16	0.16	0.21	0.18	0.03
Full	Mid-test	GM_mod	0.24	0.2	0.17	0.22	0.21	0.03
Full	Off-test	GM_mod	0.25	0.17	0.17	0.20	0.20	0.04
Full	Wean	G×M_mod	0.21	0.16	0.17	0.20	0.19	0.02
Full	Mid-test	G×M_mod	0.24	0.19	0.17	0.22	0.21	0.03
Full	Off-test	G×M_mod	0.24	0.16	0.17	0.20	0.19	0.04
Informatively reduced	Wean	M_mod	0.02	0.17	0.02	0.21	0.10	0.10
Informatively reduced	Mid-test	M_mod	0.10	0.34	-0.01	0.41	0.21	0.20
Informatively reduced	Off-test	M_mod	0.13	0.36	0.05	0.28	0.20	0.14
Informatively reduced	Wean	G_mod	0.20	0.15	0.17	0.56	0.27	0.19
Informatively reduced	Mid-test	G_mod	0.20	0.15	0.17	0.56	0.27	0.19
Informatively reduced	Off-test	G_mod	0.20	0.15	0.17	0.56	0.27	0.19
Informatively reduced	Wean	GM_mod	0.15	0.16	0.14	0.10	0.14	0.03
Informatively reduced	Mid-test	GM_mod	0.23	0.24	0.14	0.10	0.18	0.07
Informatively reduced	Off-test	GM_mod	0.22	0.19	0.16	0.10	0.17	0.05
Informatively reduced	Wean	G×M_mod	0.19	0.17	0.14	0.12	0.16	0.03
Informatively reduced	Mid-test	G×M_mod	0.24	0.22	0.14	0.10	0.18	0.07
Informatively reduced	Off-test	G×M_mod	0.22	0.18	0.16	0.10	0.165	0.05
Randomly reduced	Wean	M_mod	0.09	0.11	0.13	0.13	0.12	0.02
Randomly reduced	Mid-test	M_mod	0.11	0.14	0.15	0.15	0.14	0.02
Randomly reduced	Off-test	M_mod	0.14	0.13	0.12	0.12	0.13	0.01
Randomly reduced	Wean	G_mod	0.22	0.14	0.16	0.16	0.17	0.03
Randomly reduced	Mid-test	G_mod	0.22	0.14	0.16	0.16	0.17	0.03
Randomly reduced	Off-test	G_mod	0.22	0.14	0.16	0.16	0.17	0.03
Randomly reduced	Wean	GM_mod	0.20	0.14	0.15	0.15	0.16	0.03
Randomly reduced	Mid-test	GM_mod	0.22	0.16	0.16	0.16	0.18	0.03
Randomly reduced	Off-test	GM_mod	0.24	0.15	0.15	0.15	0.17	0.04
Randomly reduced	Wean	G×M_mod	0.20	0.14	0.16	0.16	0.16	0.03

Table S.C3 (continued)

Randomly reduced	Mid-test	G×M_mod	0.22	0.15	0.16	0.16	0.17	0.03
Randomly reduced	Off-test	G×M_mod	0.23	0.15	0.14	0.14	0.16	0.04

[1]M_mod = Model containing microbiome information only
G_mod = Model containing genomic information only
GM_mod = Model containing microbiome and genomic information
G×M_mod = Model containing microbiome, genome and microbiome-by-genome information