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1 **Microbiability of meat quality and carcass composition traits in swine**

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24 **Abstract**

25 The impact of gut microbiome composition was investigated at different stages of
26 production (Wean, Mid-test, and Off-test) on meat quality and carcass composition traits
27 of 1,123 three-way-crossbred pigs. Data were analyzed using linear mixed models which
28 included the fixed effects of dam line, contemporary group and gender as well as the
29 random effects of pen, animal and microbiome information at different stages. The
30 contribution of the microbiome to all traits was prominent although it varied over time,
31 increasing from weaning to Off-test for most traits. Microbiability estimates of carcass
32 composition traits were greater compared to meat quality traits. Adding microbiome
33 information did not affect the estimates of genomic heritability of meat quality traits but
34 affected the estimates of carcass composition traits. High microbial correlations were
35 found among different traits, particularly with traits related to fat deposition with
36 decrease in genomic correlation up to 20% for loin weight and belly weight. Decrease in
37 genomic heritabilities and genomic correlations with the inclusion of microbiome
38 information suggested that genomic correlation was partially contributed by genetic
39 similarity of microbiome composition.

40 **Keywords**: microbiome, microbiability, heritability, microbial diversity, meat quality and
41 carcass composition traits

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47 **Introduction**

48 The mammalian gastrointestinal tract is a home of a diverse microbiota population which
49 serve various biological functions of the host (Frese et al., 2015). Gut microbiota has
50 recently been the target of many research efforts resulting from the rapid development in
51 molecular technologies and led to a vast influx of “omics” studies (Guevarra et al., 2019)
52 . The importance of gut microbiota is widely accepted (Kim et al., 2011), with
53 commensal bacteria often being called the “forgotten organ” of the host (O’Hara and
54 Shanahan, 2006), impacting hosts in a multitude of ways. For example, microbial
55 composition helps in promoting the gastrointestinal health through metabolites, postnatal
56 development, degradation of short chain fatty acids and stimulation of immune system
57 (Mann et al., 2014; Pedersen et al., 2013; Stappenbeck & Herbert, 2016).

58 Gut microbiome constitutes a portion of the whole genome (Sommer & Bäckhed,
59 2013; Xiao et al., 2016) and has the potential to affect numerous biological activities that
60 the hosts lack (Pajarillo et al., 2014). Different researchers reported that microbiome has
61 considerable effect on human health and traits (Clemente et al., 2012; Dave et al., 2012;
62 Huttenhower et al., 2012). For example, differences in bacterial species diversity and
63 gene counts between lean and obese individuals have been found (Le Chatelier et al.,
64 2013). The microbial diversity of intestine accounted for significant amount of
65 phenotypic variation for any trait in human and should be accounted when assessing the
66 heritability not only in human but also in plants and livestock (Sandoval-Motta et al.,
67 2017) . In livestock, Difford et al. (2016) termed “microbiability” the proportion of total
68 variance explained by microbiome for performance traits of dairy cattle. Difford et al.
69 (2018) reported the effect of microbiota variation in methane production in dairy cows

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70 while, Mach et al. (2015) reported the impact of gut microbiome at early life on
71 phenotypes of pig. Gut microbiome also has a significant impact on porcine fatness (He
72 et al., 2016). Camarinha-Silva et al. (2017) reported the presence of a significant effect
73 of microbial composition on daily gain, feed intake and feed conversion rate in swine.
74 Until recently, selection of different traits in pigs has been done with the use of pedigree
75 and genomic information, yet the advantage of incorporating microbial information in the
76 genetic evaluation processes has not been assessed. Few studies have described the
77 relationship of microbial diversity and host e.g. (Guevarra et al., 2019; McCormack et al.,
78 2018), however these were mostly from a nutritional perspective.

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

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89 **Materials and methods**

90 Animal welfare approval was not needed for this study since all data came from animals
91 raised in a commercial setting by The Maschhoffs, LLC (Carlyle, IL, USA). All pigs

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92 were harvested in commercial facilities under the supervision of USDA Food Safety and
93 Inspection Service.

94 ***Animals and sample collection***

95 Data were collected from crossbred individuals that were obtained from 28 funding
96 Duroc sires and 747 commercial $F_1$ sows composed of Yorkshire × Landrace or Landrace
97 × Yorkshire. The pigs were weaned at 18.64 ± 1.09 days old and were moved to nursery-
98 finishing facility. Pigs were kept in 334 single-sire single-sex pens with 20 pigs per pen.
99 The test period began the day that pigs were moved to the nursery-finishing facility.
100 During the nursery, growth and finishing period all pigs were fed a standard pelleted feed
101 based on sex and live weight. Details of diet and their nutritional values are provided (see
102 additional File 1). The pigs received a standard vaccination and medication routine. (see
103 additional File 2). End of test (**Off-test**) was reached when the average weight of pigs of
104 each pen reached 138 kg. The average age at off-test was 196.4 ± 7.80 days. Fecal
105 samples for 16S rRNA sequencing were collected as follow. Rectal swabs were collected
106 from all pigs at three stages: weaning (**Wean**), 15 weeks post weaning (**Mid-test**; average
107 118.2 ± 1.18 days), and off-test. Four pigs from each pen were selected as detailed by
108 Wilson et al. (2016) and their rectal swabs were used for subsequent microbial
109 sequencing. There were 1,205, 1,295 and 1,273 samples for weaning, Mid-test and Off-
110 test respectively. Distribution of samples across families, time points and sex are
111 provided (see additional file 3).

112 ***Illumina amplicon sequencing***

113 DNA extraction, purification, illumina library preparation and sequencing were done as
114 described by Lu et al. (2018). Briefly, total DNA (gDNA) was extracted from each rectal

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115 swab by mechanical disruption in phenol: chloroform. The DNA was purified using a
116 QIAquick 96 PCR purification kit (Qiagen, MD, USA). Purification was performed per
117 the manufacturer's instruction with the following minor modifications: (i) sodium acetate
118 (3 M, pH 5.5) was added to Buffer PM to a final concentration of 185 mM to ensure
119 optimal binding of genomic DNA to the silica membrane; (ii) crude DNA was combined
120 with 4 volumes of Buffer PM (rather than 3 volumes); and (iii) DNA was eluted in 100
121 μL Buffer EB (rather than 80μL). All sequencing was performed at DNA Sequencing
122 Innovation Laboratory at the Center of Genome Sciences and Systems Biology at
123 Washington University in St. Louis. Phased, bi-directional amplification of the v4 region
124 (515-806) of the 16S rRNA gene was employed to generate indexed libraries for Illumina
125 sequencing as described in Faith et al. (2013). Sequencing was performed on an Illumina
126 MiSeq instrument (Illumina, Inc. San Diego, USA), generating 250 bp paired-end reads.

127 ***16S rRNA gene sequencing and quality control of data***

128 Pairs of 16S rRNA gene sequences were first merged into a single sequence using
129 FLASH v1.2.11 (Magoc and Salzberg, 2011) with a required overlap of at least 100 and
130 less than 250 base pairs in order to provide confident overlap. Sequences with a mean
131 quality score below Q35 were then filtered out using PRINSEQ v0.20.4 (Schmieder and
132 Edwards, 2011). Sequences were oriented in the forward direction and any primer
133 sequences were matched and trimmed off. Mismatch was allowed up to 1. Sequences
134 were subsequently demultiplexed using QIIME v1.9 (Caporaso et al., 2010). Sequences
135 with greater than 97% nucleotide sequence were clustered into operational taxonomic
136 units (**OTU**) using QIIME with the following settings: `max_accepts = 50,`
137 `max_rejects = 8, percent_subsample = 0.1 and -`

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138 `suppress_step4`. A modified version of GreenGenes (Ley et al., 2006; Schloss and
139 Handelsman, 2006) was used as reference database. Input sequences that had 10% of the
140 reads with no hit to the reference database were then clustered de novo with UCLUST
141 (Schloss and Handelsman, 2006) to generate new reference OTU to which the remaining
142 90% of reads were assigned. The most abundant sequence in each cluster was used as
143 representative sequence for the OTU. Sparse OTU were then filtered out by requiring a
144 minimum total observation count of 1,200 for an OTU to be retained, the resulting OTU
145 table was rarefied to 10,000 counts per sample and after data processing and quality
146 control 1,755 OTU were retained for further analysis.

147 ***Genotyping***

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

152 ***Phenotypic data***

153 Phenotypic data collection was done as described by Wilson et al. (2016). Meat
154 quality traits (intramuscular fat content (**IMF**), Minolta a* (**MINA**), Minolta b* (**MINB**),
155 minolta L* (**MINL**), ultimate pH (**PH**), subjective color score (**SCOL**), subjective
156 marbling score (**SMARB**), subjective firmness score (**SFIRM**), shearing force (**SSF**))
157 and carcass composition traits (Belly weight (**BEL**), ham weight (**HAM**), loin weight
158 (**LOIN**), fat depth (**FD**), loin depth (**LD**) and carcass average daily gain (**CADG**)) were
159 used for the current analysis. All the traits were measured as described by Khanal et al.
160 (2019). A summary of traits used in current analysis is reported in Table 1.

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161 ***Statistical analysis***

162 The data were analyzed using ASREML v4.1 (Gilmour et al., 2014). Univariate analyses
163 were conducted to estimate heritabilities, microbiabilities and variance components for
164 each trait. Single trait models were fitted as:

165 $$y_{ijklmn} = \mu + dl_i + cg_j + sex_k + animal_l + pen_{m(j)} + e_{ijklmn} \quad (1)$$

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

174 $$\mathbf{G} = \frac{(\mathbf{M}-\mathbf{P})(\mathbf{M}-\mathbf{P})'}{2\sum_{j=1}^{m} p_j(1-p_j)}$$

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

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

184 $y_{ijklmno} = \mu + dl_i + cg_j + sex_k + animal_l + microbiome_m + pen_{n(j)} + e_{ijklmno}$ (2)

185 Where *dl*, *cg*, *sex*, *animal*, *pen* and *e* were as previously described and $microbiome_m$
186 was the random effect of the animal microbiome. The effect of the microbiome was
187 assumed normally distributed with mean 0 and variance $\mathbf{O}\sigma_m^2$ in which **O** was a microbial
188 correlation matrix among individuals and $\sigma_m^2$ was the microbiome variance. The matrix **O**
189 was created following Camarinha-Silva et al. (2017). Briefly, **O** was obtained as $\mathbf{O} =$
190 $\frac{1}{q}\mathbf{XX^T}$, with matrix **X** of dimension of $n \times q$, where $n$ is the number of animals and $q$ is
191 the number of OTU. **X** was constructed from **S**, a matrix of equivalent dimensions $n \times q$.
192 Each element of the **S** matrix, $S_{jk}$, was the relative abundance of OTU k in animal j. The
193 elements of X were calculated as:

194 $$X_{ij} = \frac{log\,(S_{jk}) - \overline{logS_k}}{sd(logS_k)}$$

195 where $S_k$ is the vector of the k$^{th}$ column of **S.** The **O** matrix was created for each stage
196 (Wean, Mid-test and Off-test) separately and fitted in each model separately. The
197 contribution of the microbiome to the overall variance (microbiability) was calculated as:
198 $m^2 = \frac{\sigma_m^2}{\sigma_P^2}$ (Difford et al., 2016). The total variance $\sigma_P^2$ was in this case obtained as $\sigma_P^2 =$
199 $\sigma_a^2 + \sigma_m^2 + \sigma_{pen}^2 + \sigma_e^2$.

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

202 $$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix}\begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} K_1 & 0 \\ 0 & K_2 \end{bmatrix}\begin{bmatrix} o_1 \\ o_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix}\begin{bmatrix} p_1 \\ p_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \quad (3)$$

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203 where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ were the vectors of phenotypic measurements for trait 1 and trait 2
204 respectively; $\boldsymbol{X_1}$ and $\boldsymbol{X_2}$ were the incidence matrices relating the fixed effects to vector
205 $\boldsymbol{y_1}$ and vector $\boldsymbol{y_2}$ respectively; $\boldsymbol{b_1}$ and $\boldsymbol{b_2}$ were the vector of fixed effect for trait 1 and
206 trait 2 respectively; $\boldsymbol{Z_1}$ and $\boldsymbol{Z_2}$ were the incidence matrices relating the phenotypic
207 observations to the vector of random animal effects for trait 1 and trait 2 respectively; $\boldsymbol{a_1}$
208 and $\boldsymbol{a_2}$ were the vectors of random animal effect for trait 1 and trait 2 respectively; $\boldsymbol{K_1}$
209 and $\boldsymbol{K_2}$ were the incidence matrices relating the phenotypic observations to the vector of
210 random microbiome effect for trait 1 and trait 2 respectively; $\boldsymbol{o_1}$ and $\boldsymbol{o_2}$ were the vectors
211 of random microbiome effect for trait 1 and trait 2 respectively; $\boldsymbol{W_1}$ and $\boldsymbol{W_2}$ were the
212 incidence matrices relating the phenotypic observations to the vector of random pen
213 effects for trait 1 and trait 2 respectively; $\boldsymbol{p_1}$ and $\boldsymbol{p_2}$ were the vector of random pen effect
214 for trait 1 and trait 2 respectively; and $\boldsymbol{e_1}$ and $\boldsymbol{e_2}$ were the vectors of random residuals for
215 trait 1 and trait 2 respectively. The fixed effects and random effects were the same as
216 fitted in the univariate analyses.

217 The additive effects were again assumed normally distributed with means 0 and
218 variance $\mathrm{Var}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} = \mathbf{C} \otimes \mathbf{G}$; where $\mathbf{C} = \begin{bmatrix} \sigma^2_{a1} & \sigma_{a12} \\ \sigma_{a21} & \sigma^2_{a2} \end{bmatrix}$. The elements of the covariance
219 matrix $\mathbf{C}$ were defined as: $\sigma^2_{a1}$, the genetic variance for trait 1, $\sigma^2_{a2}$ , the genetic variance
220 for trait 2, $\sigma_{a12} = \sigma_{a21}$, the additive genetic covariance between trait 1 and trait 2. Similar
221 assumptions were made for the microbiome effect for which the covariance structure was
222 assumed $\mathrm{Var}\begin{bmatrix} o_1 \\ o_2 \end{bmatrix} = \mathbf{Q} \otimes \mathbf{O}$; with $\mathbf{Q} = \begin{bmatrix} \sigma^2_{m1} & \sigma_{m12} \\ \sigma_{m21} & \sigma^2_{m2} \end{bmatrix}$. The elements $\mathbf{Q}$ were: $\sigma^2_{m1}$ , the
223 microbiome variance for trait 1, $\sigma^2_{m2}$ , the microbiome variance for trait 2 and $\sigma_{m12} =$
224 $\sigma_{m21}$ the microbiome covariance between trait 1 and trait 2. The pen (co)variance

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225 structure was $\text{Var}\begin{bmatrix} p_1 \\ p_2 \end{bmatrix} = \mathbf{W} \otimes \mathbf{I}$; with $\mathbf{W} = \begin{bmatrix} \sigma_{p1}^2 & 0 \\ 0 & \sigma_{p2}^2 \end{bmatrix}$ and **I** an identity matrix. The **W**

226 matrix elements were: $\sigma_{p1}^2$, and $\sigma_{p2}^2$ being the pen variance for trait 1 and 2, respectively.

227 Pen effects were in this case was assumed uncorrelated among traits. The residual

228 variance was given by $\text{Var}\begin{bmatrix} e_1 \\ e_2 \end{bmatrix} = \mathbf{R} \otimes \mathbf{I}$; where $\mathbf{R} = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e12}^2 \\ \sigma_{e21}^2 & \sigma_{e2}^2 \end{bmatrix}$ and **I** was an identity

229 matrix. The components of **R** were defined as: $\sigma_{e1}^2$ was the residual variance for trait 1,

230 $\sigma_{e2}^2$ was the residual variance for trait 2, $\sigma_{e21}^2 = \sigma_{e12}^2$ was the residual covariance among

231 the two traits. Preliminary analyses (data not shown), showed how correlations were not

232 estimable for the traits with estimated of microbiome variance of less than 3%. Microbial

233 correlations were therefore estimated among traits for which microbiome explained at

234 least 3% of total phenotypic variance. In all cases microbial correlations were not

235 estimated for weaning since microbiome accounted for less than 3% of total variance for

236 all traits.

237 ***Diversity analysis and its correlations with traits***

238 The diversity analysis performed in this paper was aimed at investigating the distribution

239 of alpha diversities at Wean, Mid-test and Off-test. The R package "vegan" (Oksanen et

240 al., 2019) was used to calculate alpha diversity at each stage. The diversity was measured

241 using Shannon index, and was computed as: $-\sum_{i=1}^{n} p_i \, ln(p_i)$ where $p_i$ was the

242 proportional abundance of $i^{th}$ OTU. To estimate the correlation among different traits and

243 alpha diversity at weaning (**alpha_w**), week 15 (**alpha_mid**) and end of test (**alpha_off**),

244 bivariate analyses were conducted using ASREML v.4.1 (Gilmour et al., 2014) by

245 removing the effect of microbiome from model (3) and fitting diversity as the dependent

246 variable.

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247

248 **Result and discussions**

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

251 Mean and standard deviation for each meat quality and carcass composition trait are
252 provided in Table 1. There were 9 meat quality and 6 carcass composition traits. The
253 number of individual samples with complete genotypic, phenotypic and microbiome
254 information at each stage was 1,123, which was used for further analyses. The
255 distribution of OTU at weaning, Mid-test and Off-test is given in Figure. 1A. Of a total
256 1,755 OTU, there were 1,580 OTU in common between weaning, Mid-test and Off-test.
257 There were 1,685 OTU in common between Mid-test and Off-test, while between
258 weaning and Mid-test were 1,626 and between weaning and Off-test were 1590.

259 Alpha diversity is a measure of within-sample diversity. It measures the richness
260 of species and is measured as the number of species in a sample of standard size
261 (Whittaker, 1972). Distribution of alpha diversity among weaning, Mid-test and Off-test
262 is given in Figure 1B. Mean alpha diversity at Off-test, Mid-test and Wean was 4.63 ±
263 0.01, 4.53 ± 0.01 and 3.85 ± 0.02 respectively. Results from Man-Whitney tests showed
264 that alpha diversity at all stages were different ($P < 0.001$) to each other. This was in
265 accordance with similar studies in pigs and other organisms (Frese et al., 2015; Guevarra
266 et al., 2019; Lu et al., 2018). The increase in alpha diversity with age was similar to what
267 previously found by different authors (Chen et al., 2017; Kim et al., 2011; Looft and
268 Allen, 2012; Thompson et al., 2008). The change in diet in piglet from sow's milk to
269 complete feed-based diet partially explains the shift in microbial diversity after weaning.

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270 Different researchers (Frese et al., 2015; Konstantinov et al., 2006) reported that change
271 in the diet impact significantly the microbiota composition in the gut. Different types of
272 diet at different stage might explain the difference in alpha diversity at each stage. Piglets
273 are exposed to a large number of stressors during weaning which triggered the
274 physiological change in structure and function of intestine (Guevarra et al., 2019). This
275 change caused the microbial shift after weaning transition (Kim and Isaacson, 2015) and
276 microbial succession continues until microbiota composition reaches to climax
277 community (Chen et al., 2017) which consists of microbes that are stable in composition.
278 Further higher granularity results on the characterization of the microbial composition in
279 the individuals of the current study can be found in Lu et al. (2018).

280 ***Microbiability estimates***

281 The proportion of variance explained by each random term for meat quality and carcass
282 composition traits is presented in Figure 2 and Figure 3, respectively. The estimates of
283 microbiability and variance components along with their respective standard errors are
284 provided (see additional File 4). The results identified several traits with significant
285 microbiability.

286 The microbiability of carcass composition traits were higher than those of meat
287 quality traits. In all cases microbiabilities for both meat quality and carcass composition
288 traits at weaning were negligible and ranged from zero for several traits to a maximum of
289 0.06 ± 0.03 (estimate ± SE) for CADG. Three of the 9 meat quality traits investigated
290 shown significant microbiability at Mid-test, with estimates of 0.07±0.03 for SMARB,
291 0.08±0.03 for SFIRM and 0.10±0.04 for MINB. At Off-test, 4 meat quality traits had
292 significant microbiability, with estimates of 0.06±0.02 for IMF, 0.09±0.04 for MINA,

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293 0.11±0.04 for MINB and 0.13±0.04 for SFIRM. For carcass composition traits, we found
294 that 5 out of 6 traits were significantly affected by microbiome at Mid-test and Off-test.
295 The microbiability of carcass composition traits at Mid-test ranged from 0.12 ± 0.04 for
296 LOIN and FD to 0.20 ± 0.04 for BEL. The microbiability of carcass composition traits at
297 Off-test ranged from 0.13 ± 0.05 for LOIN to 0.29 ± 0.05 for BEL. In our study, the
298 microbiome did not contribute significantly to loin depth variability. In most of the cases
299 microbiome at weaning did not contribute to trait variation, however, microbiome at Mid-
300 test and Off-test contributed significantly to trait variation. This might have several
301 causes including the sudden change of microbiome composition shortly after the diet
302 switch occurring at weaning as well as other environmental factors like, stress. To our
303 knowledge this is the first attempt to obtain microbiability estimates for meat quality and
304 carcass composition traits. We did not find any literature to compare the estimates with
305 previous research. Our results suggest that later measures of microbial composition might
306 be more informative for selection purposes, but further research would be needed to
307 clarify this aspect.

308 Among meat quality traits, microbial variance explained a larger proportion of
309 phenotypic variance than additive genetic for SFIRM and MINB at Off-test (Figure 2).
310 Among carcass composition traits, BEL, HAM, and CADG at Off-test had higher
311 proportion of phenotypic variation explained by microbiome than by additive genetic
312 (Figure 3). These results indicated that a significant proportion of total variance is
313 explained by the microbiome, in some cases larger than the additive genetics and that
314 prediction for these traits could be improved by accounting the effect of variability in gut

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315 microbiome composition. The variation in gut microbiome could be fitted as the
316 systematic environmental effect in model.
317 In the current study we observed a decrease in genomic heritability for most of
318 the carcass composition traits at Off-test when microbiome information was added. The
319 decrease in heritability ranged from 0% for LD to about 10% for FD. At Mid-test, the
320 decrease in heritability ranged from 0% for CADG, BEL, HAM and LOIN to 4% for FD.
321 No change in genomic heritability were observed at weaning. The decrease in heritability
322 for FD was similar to what found by Lu et al. (2018) for similar traits. He et al. (2016)
323 also reported the significant contribution of microbiome in porcine fatness. These results
324 suggested that part of the resemblance among individuals captured by genetic effects in
325 breeding values prediction, might be in fact a resemblance among microbial composition
326 and genetic parameters might not be accurate.
327 In contrast, for most of the meat quality traits considered, the inclusion of
328 microbial composition did not affect the estimates of genomic heritability, thus
329 suggesting that at least for meat quality traits, gut microbial composition is mostly an
330 environmental factor. The decrease in genomic heritability as we included the
331 microbiome composition in the models was previously observed by Sandoval-Motta et al.
332 (2017) who reported the possibility of overestimation of heritability values with the use
333 of genetic similarities by kinship information. The authors also suggested that inclusion
334 of genetic diversity of individual microbiome will most likely increase the accuracy of
335 heritability of various traits. The heritability and microbiability estimation of daily gain,
336 feed intake and feed conversion ratio in swine by Camarinha-Silva et al. (2017) and
337 methane emission in cattle by Difford et al. (2018) strongly suggested the significant

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338 contribution of microbiome in the total variation in the complex phenotypes of livestock.
339 In human, Richards et al. (2018) reported that host genes are affected by the microbiome
340 and are involved in the complex traits. These previous studies agreed with our results.
341 Our results also agreed with Zilber-Rosenberg & Rosenberg, (2008) who reported the
342 concept of "hologenome" of evolution, where the animal or plant along with associated
343 microorganisms are the unit of selection in evolution.

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

346 Host genetics plays a major role in shaping the intestinal microbiota of mice and humans
347 (Büsing and Zeyner, 2015; Dąbrowska and Witkiewicz, 2016; Hancox et al., 2015). Chen
348 et al. (2018), Kubasova et al. (2018) and Lu et al. (2018) reported the impact of host
349 genetics on development of gut microbiota in pigs. So, the alpha diversities at weaning,
350 Mid-test and Off-test were considered as separate phenotypic records and genetic
351 correlations were estimated between different alpha diversities and other traits measured.
352 The results are presented in Table 2 suggesting very weak correlations for alpha_w for all
353 traits measured. Weak correlations were estimated between also with alpha_mid with the
354 exception of MINA (-0.45±0.19) where greater alpha diversity seems linked to a paler
355 red color of meat given that MINA is related to the amount of myoglobin in muscle. We
356 obtained weak correlations between alpha_mid and carcass composition traits except for
357 CADG (-0.43±0.19), suggesting that an increase in microbial diversity would decrease
358 average daily gain. This was in contrast with general opinion that the diversity will
359 increase the metabolite production from different microbiota (Kim and Isaacson, 2015;
360 Yan et al., 2017) and increase the weight of host. However, this was in agreement with

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361 what found by Lu et al. (2018). Alpha diversity could then be used as a potential indicator
362 trait in CADG selection. In all cases correlations of alpha_off with growth, carcass and
363 meat quality traits were weak (Table 2).

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

374 ***Correlation among traits***

375 In the discussion of correlation, we only focus on microbial correlations. Genomic
376 correlations are only discussed if the genomic correlations changed due to inclusion of
377 microbiome information in the model. The genomic correlations without inclusion of
378 microbiome in the model are presented in additional file 5.

379 ***Correlations among meat quality and carcass composition traits at mid test***

380 Overall there were 3 meat quality traits and 5 carcass composition traits having variance
381 of microbiome composition greater than 3%. Microbial correlations among meat quality
382 traits at Mid-test are presented in Table 3. Most of the microbial correlations were
383 significant. Subjective marbling score was moderately positively correlated (0.46±0.24)

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384 with FD. This suggested that shifting of microbiota for high marbled meat would results
385 in higher fat depth. Shear force is the measure of tenderness. In this study, the microbial
386 composition of SSF was highly negatively correlated with SMARB, SFIRM, FD, CADG,
387 LOIN and BEL which ranged from -0.93±0.11 for SSF and SFIRM to -0.50±0.25 for SSF
388 and LOIN. High positive correlations of SFIRM were found with CADG, HAM, LOIN
389 and BEL which ranged from 0.58±0.26 between SFIRM and LOIN to 0.87±0.16 between
390 SFIRM and BEL. We did not find any other estimates to compare with our values of
391 microbial correlation between meat quality and carcass composition traits. There were
392 moderate to high correlations of microbial composition of FD with CADG, HAM, LOIN
393 and BEL which ranged from 0.44±0.21 between FD and LOIN to 0.74±0.11 between FD
394 and BEL. High positive correlations were found between CADG and HAM, LOIN and
395 BEL. Belly weight was highly positively correlated with HAM (0.96±0.03) and LOIN
396 (0.94±0.06).

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

398 There were 6 meat quality traits and 5 carcass composition for which variance of
399 microbiome composition was greater than 3%. The microbial and genomic correlations
400 among meat quality traits at Off-test are presented in Table 4. pH had high positive
401 microbial correlation (0.90±0.25) with SCOL and SFIRM (0.73±0.35). This is in partial
402 agreement with results from Ratzke & Gore, (2018) that reported how there are specific
403 bacteria which build lactic acid in the muscle resulting in the anaerobic breakdown of
404 glucose and glycogen, which eventually loosens the myofibril, thus scattering more light
405 making the muscle pale (Walters, 1975). Furthermore, increasing pH causes swelling of
406 myofibrils (Huff-Lonergan and Lonergan, 2005) which ultimately makes the muscle

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407 firmer. High positive microbial correlation was found between IMF and SFIRM
408 (0.91±0.17), MINA (0.55±0.28) and MINB (0.75±0.27). This agrees with Fang, Xiong,
409 Su, Huang, & Chen. (2017) who reported that gut bacteria involving in energy
410 metabolism and intramuscular fat content in pig also regulate the muscle composition and
411 muscle fibers. Higher microbial correlation of IMF with minolta color measurements and
412 SFIRM indicated that microbial composition increasing IMF would make the muscle pale
413 and firmer. High microbial correlation of MINA and MINB (0.78±0.16) suggests that
414 microbiota responsible for redness of meat also contribute to the yellowness in the meat.
415 This agreed with Kim et al. (2010) who reported the positive correlation of yellowness
416 and redness in the muscle of pig.

417 The microbial and genomic correlations among carcass composition traits at Off-
418 test are presented in Table 5. The microbial composition of carcass composition traits
419 were highly and positively correlated to each other ranging from 0.55±0.17 between FD
420 and LOIN to 0.97±0.02 between CADG and HAM. McCormack et al. (2018) reported a
421 positive correlation between gut microbiota and feed efficiency in swine. Gut microbiota
422 has considerable effect on feed intake, final body weight (Kubasova et al., 2018) and
423 growth traits (Ramayo-Caldas et al., 2016). All these studies suggested that microbial
424 composition has considerable effects on many carcass composition traits, with positive
425 correlations between them. This high correlation indicated that all the traits could be
426 simultaneously improved through the same microbial composition.

427 The microbial correlations for meat quality traits and carcass composition traits at
428 Off-test are presented in Table 6. Intramuscular fat was highly correlated with FD
429 (0.90±0.14) and BEL (0.73±0.18). Firmness score was positively correlated with BEL

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430 (0.50±0.18). Moderate positive correlation was found between MINA and BEL
431 (0.41±0.21) and high positive correlation was found between MINA and FD (0.53±0.18),
432 and MINA and CADG (0.66±0.17). Minolta b* has moderate positive correlation with
433 FD (0.43±0.19) and high positive correlation with CADG (0.58±0.18): suggesting that
434 increase in microbiota for lean meat and high daily gain of carcass would make the meat
435 more yellowish.

436 ***Change in genomic correlation with the inclusion of microbiome information***

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

443 At Off-test, the genomic correlation between PH and SCOL (0.91±0.29), SFIRM
444 and IMF (0.36±0.15), FD and CADG (0.27±0.13), and BEL and HAM (0.58±0.19)
445 became non-significant with the inclusion of microbiome. Among carcass traits, the
446 decrease in genomic correlation ranged from 1% between BEL and CADG to 30%
447 between BEL and LOIN. The genomic correlation of BEL with FD, CADG with HAM,
448 CADG with LOIN, FD with IMF, FD with MINB, BEL with IMF, and BEL with SFIRM
449 decreased by 13%, 4%, 2%, 9%, 6%, 13% and 8%, respectively. Among meat quality and
450 carcass traits, the decrease in genomic correlations ranged from 1% for FD and SFIRM to
451 9% for BEL and IMF. We observed the decrease in genomic correlations with the
452 inclusion of microbiome, particularly of any other traits with fat related traits e.g. (BEL,

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453 FD, IMF). This could be due to the greater influence of gut microbiome on fat deposition.
454 Furthermore, we observed that there was a decrease in genomic correlation for those
455 traits which had higher microbial correlation. High microbial correlations among
456 different traits suggested that genomic correlations among traits are partially contributed
457 by the correlations among the gut microbiota composition. The covariance among
458 microbiome for different traits might have contributed to the genetic covariance and
459 hence the genomic correlation. We observed that the decrease in the genomic correlation
460 was higher at Off-test than at Mid-test. This was due to high variability accounted by
461 microbiome composition at Off-test in comparison to Mid-test.

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

468

469 **Conclusions**

470 This study was conducted on crossbred pigs to investigate the impact of intestinal
471 microbiota through different stages (weaning, Mid-test and Off-test) of production. To
472 our knowledge this study is the first attempt to investigate the impact of microbiome on
473 the meat quality and carcass composition traits at a large scale in swine. The contribution
474 of microbiome to all traits was significant although it varied over time with an increase
475 from weaning to Off-test for most of the traits. Adding microbiome information did not

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476 affect the estimates of genomic heritability of meat quality traits but changed the estimate
477 of carcass composition traits suggesting that portion of genomic variance was contributed
478 by gut microbiome. Alpha diversity at Mid-test was strongly correlated with carcass
479 average daily gain and minolta a* color score. A better understanding of microbial
480 composition could aid the improvement of complex traits, particularly the carcass
481 composition traits in swine by inclusion of microbiome information in the genetic
482 evaluation process. High microbial correlations were found among different traits,
483 particularly with traits related to fat deposition. Adding microbiome information
484 decreased the genomic correlation for those traits which had higher microbial correlation
485 suggesting that portion of genomic correlation was due to genetic covariance among
486 microbiome composition affecting those traits. Based on the results we can conclude that
487 microbial composition could be altered to improve a given trait. To obtain optimum
488 microbial composition, manipulation of gut microbiota could be done using specific
489 bacterial composition as probiotics or increasing the relative abundance through
490 prebiotics, feed additives supplements and fecal microbiota transplantation could also be
491 done. The estimated parameters provide a reference value for further research on gut
492 microbial contribution to complex phenotypes in pigs. These results may lead to establish
493 a newer approach of genetic evaluation process through the addition of gut microbial
494 information.

495

496 **Acknowledgements**

497 We would like to thank Jessica Hoisington-Lopez from the DNA sequencing Innovation
498 Lab at the Center for Genome Sciences and Systems Biology at Washington University

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499 in St. Loius for her sequencing expertise and Nicholas S. Grohmann for phenotype and
500 sample collection.

501

502 **Funding**

503 This study is a part of the project "Re-defining growth efficiency accounting for the
504 interaction between host genome and commensal gut bacteria" funded by The National
505 Pork Board Association and part of the project "From Host to Guest and back" funded by
506 The Maschhoffs, LLC and North Carolina State University.

507

508 **Author Contributions**

509 PK designed and carried all analyses, as well as interpreted the results and drafted the
510 manuscript. CS and JF were involved in designing the experiment and helped in
511 interpretation of the results. CM and FT were involved in designing the experiment and
512 providing the consultation for the analyses. All co-authors provided comments for
513 manuscript. All authors have read and approved the final manuscript.

514

515 **Competing interests**

516 The authors declare that they have no competing interests.

517

518 **Contribution to the field statements**

519 This manuscript describes about the impact of gut microbiome composition at different
520 stages of production on meat quality and carcass composition traits. Until recently,
521 selection of different traits in pigs has been done with the use of pedigree and genomic

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522 information, yet the advantage of incorporating microbial information in the genetic
523 evaluation processes has not been assessed. So, this study evaluates the variance
524 accounted by microbial composition and its effect on heritability and genomic
525 correlation. Adding microbiome information did not affect the estimates of genomic
526 heritability of meat quality traits but affected the estimates of carcass composition traits.
527 We found high microbial correlations among several traits which suggested that genomic
528 correlation was partially contributed by genetic similarity of microbiome composition.
529 Since this is one of the earlier studies in determining the effect of microbiome
530 composition in heritability and genomic correlation of meat quality and carcass traits in
531 swine, we believe that these parameters will provide a reference value to for the
532 researchers in future who wants to conduct research on effect of gut microbiome in
533 complex phenotypes of swine.
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734 Table 1. Descriptive statistics of carcass composition and meat quality traits: acronym,

735 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 |

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744 Table 2. Genetic correlation of carcass composition traits and meat quality traits with
745 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 |

746 [1]LD = Loin depth; FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham
747 weight; LOIN = Loin weight; BEL = Belly weight, SCOL = Subjective color score,
748 SFIRM = Subjective firmness score, SSF = Slice shear force, IMF = Intramuscular fat

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749 percent, SMARB = Subjective marbling score, MINA = Minolta a*, MINB = Minolta

750 b*, MINL = Minolta L*, PH = Ultimate pH;

751 Numbers in bold are significant

752 Table 3. Estimates of microbial correlation (above diagonal) and genomic correlation (below diagonal) at Mid-test among meat quality

753 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** | |

754 [1]SMARB = Subjective marbling score, SFIRM = Subjective firmness score, SSF = Slice shear force, FD = Fat depth,

755 CADG = Carcass average daily gain, HAM = Ham weight; LOIN = Loin weight; BEL = Belly weight;

756 [2]Not Converged; [3]Not estimable; Numbers in bold are significant

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757 Table 4. Estimates of microbial correlation (above diagonal) and genomic correlation
758 (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 | |

759 [1]SCOL = Subjective color score, SFIRM = Subjective firmness score, IMF =
760 Intramuscular fat percent, MINA = Minolta a*, MINB = Minolta b*, PH = Ultimate pH;
761 Numbers in bold are significant.

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763 Table 5. Estimates of microbial correlation (above diagonal) and genomic correlation
764 (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** | |

765 [1]FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight; LOIN =
766 Loin weight; BEL = Belly weight;
767 [2]Non estimable; Numbers in bold are significant.

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769 Table 6. Estimates of microbial correlation between meat quality traits and carcass
770 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 |

771 [1]FD = Fat depth, CADG = Carcass average daily gain, HAM = Ham weight, LOIN =
772 Loin weight, BEL = Belly weight;
773 [2]Non estimable; [3]Not converged; Numbers in bold are significant.
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787 **Figure legends**

788 Figure 1. (A) Venn diagram with the numbers of common operational taxonomic units
789 (OTU) among weaning, mid test and off test. (B) Distribution of alpha diversity index
790 among weaning, mid test and off test. X- axis represents the different age group and Y-
791 axis represent the alpha diversity index of each sample for each group.
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793
794 Figure 2. Proportion of variance explained by microbiome relationship matrix (**O**),
795 genomic relationship matrix (**G**) and pen (**P**) for meat quality traits. Model 0 contains **G**
796 matrix and pen effect as random effect, Model 1, Model 2 and Model 3 contains **O** matrix
797 at weaning, Mid-test and Off-test in addition to **G** matrix and pen effect.
798
799
800 Figure 3. Proportion of variance explained by microbiome relationship matrix (**O**),
801 genomic relationship matrix (**G**) and pen (**P**) for carcass composition traits. Model 0
802 contains **G** matrix and pen effect as random effect, Model 1, Model 2 and Model 3
803 contains **O** matrix at weaning, Mid-test and Off-test in addition to G matrix and pen
804 effect.
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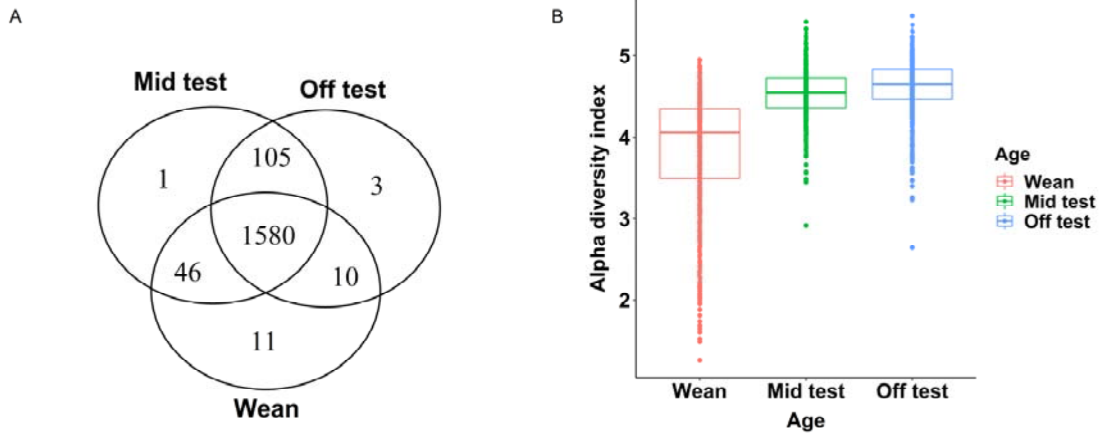
810

811 Figure 1

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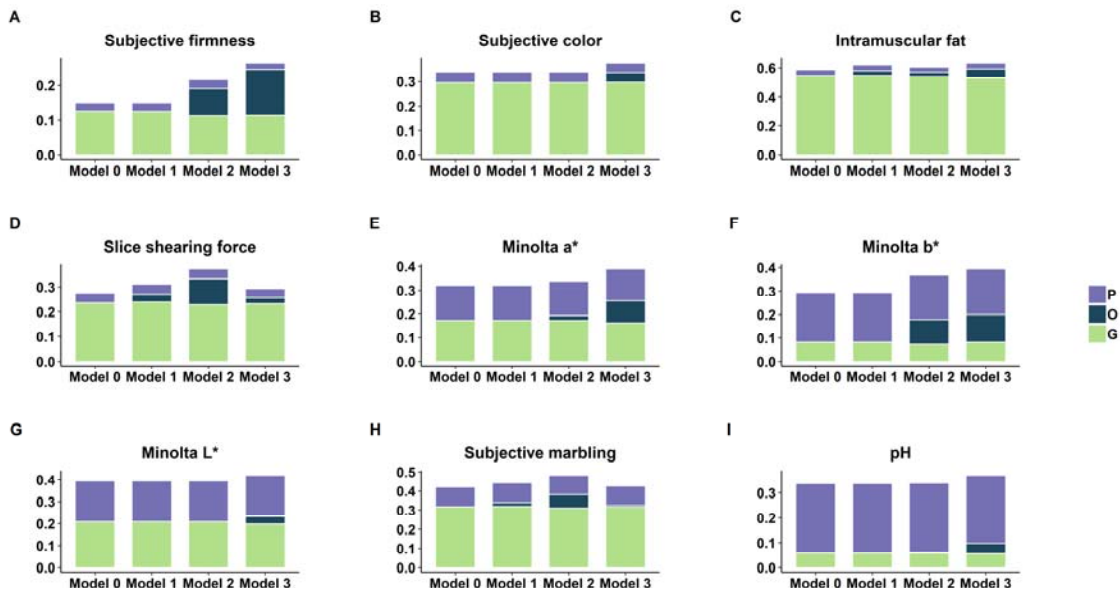
817 Figure 2

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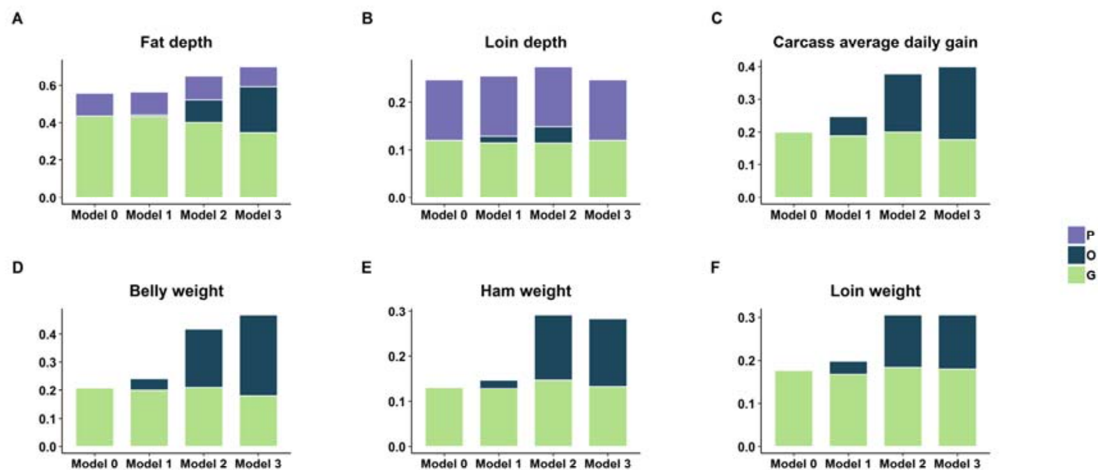
821 Figure 3

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