GENETICS AND GENOMICS OF SWINE LEAN GROWTH AT THE INTERFACE BETWEEN HOST AND COMMENSAL GUT BACTERIA

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SUMMARY

The main objectives of this paper will be to quantify the impact of gut microbiome composition on lean growth in swine and to quantify the heritability of relative taxa abundance in swine. The gut microbiome absorbed a significant portion of the phenotypic variation, ranging from approximately 3% for microbiome composition at weaning on ADG15 to more than 65% for microbiome composition at week 15 on ADG15. Point estimates for the heritabilities of the 57 taxonomical families ranged from low to moderately high ranging from less than 5% to almost 50%, according to family and time point. Different patterns of h^2 (from low to high and vice versa) were observed across time for different families possibly reflecting the overall abundance of a particular family across the trial.

INTRODUCTION

Efficiency of producing saleable meat products is largely determined by costs associated with feed and by the amount of and quality of lean meat produced(Hoque et al., 2009)·(Hoque et al., 2008)·(McGlone and Pond, 2003). Utilizing feed resources more efficiently has become a clear challenge that faces the livestock industry. Recent efforts have been devoted to identify and exploit the genomic variability of individual pigs in increasing feed efficiency(Jiao et al., 2014a)·(Jiao et al., 2014b) (Howard et al., 2015). While partially successful this approach presents limitations. First, feed efficiency is not a directly measurable trait. Instead it must be obtained from its components and it includes all traits associated with the efficiency of feed utilization, typically feed conversion ratio (FCR) or its reciprocal (feed:gain ratio) or RFI (Koch et al., 1963). These commonly used measures have inherent flaws (Arthur and Herd, 2008). More importantly, a continued effort concentrating only on the pig variability for efficiency will inevitably result in diminished marginal gains, incurring in concomitant losses of overall fitness and diversity over time(Colleau and Tribout, 2008). The amount and type of bacteria present in the gut of individuals represent a key part of all mammalian organisms (Gill et al., 2006). The makeup of the microbiome represents a vast pool of genomic diversity that contributes to the individual physiology and health (Pflughoeft and Versalovic, 2012). Particularly, the intestinal microbiome directly affects the degradation of carbohydrates, provides short chain fatty acids, mitigates and alter the effect of potential toxic compounds and produce essential vitamins(Gill et al., 2006). Different composition of the gut population in humans has been linked to the ability of degrading enzymes, maintain a certain population balance and influence the overall health status(Cho and Blaser, 2012). Relatively few full microbiome sequencing studies have been conducted in swine to date (Isaacson and Kim, 2012), while many studies have focused on either humans or model organisms. There is nonetheless a striking physiological similarity between the human and the swine intestine such that the second is currently successfully employed as model for the first (Odle et al., 2014)· (Heinritz et al., 2013)· (Zhang et al., 2013). Several studies comparing different geographical populations of humans and studies comparing different animal species have found that host genetic differences play a significant role in the composition of the microbiome. One study of tilapia, toads, geckos, quail, and mice tested changes in the microbiota of the colon and cecum after periods of fasting (Kohl et al., 2014). The study found that in most species, there was

more genetic diversity in the colon microbiome during a fast compared to a regular diet (Kohl et al., 2014). This suggests how environmental factors such as diet are not the only features keeping the microbiota balance but that other factors are at play most likely related to genetic. In the same study, results from the cecum found that in tilapia and toads, although there were initial changes in genetic diversity after the start of the fast, the microbiotic species returned to normal later in the fast (Kohl et al., 2014), again suggesting how the microbiota seem able to "self-regulate" without input from the environment. In the same study, mice showed no changes in the microbiotic composition during fasting (Kohl et al., 2014). In this case it appears that the microbiota might be completely controlled by the host genetic. Similar studies have been conducted in humans. For example Goodrich et al.(Goodrich et al., 2014a) found that twins' fecal samples have a more similar microbiota composition than unrelated individuals, with monozygotic twins having a more similar composition than dizygotic. This again suggests that genetics might play a significant role in the microbiome composition. A study of samples of Columbian gut microbiome found that samples of people with a higher BMI had less *Firmicutes* while European gut microbiome did not show decreased *Furmicutes* (Escobar et al., 2014). Differences in microbiome between individuals of different BMIs seem to indicate a direct genetic influence. A study of data from a twin study (Goodrich et al., 2014b) further linked human genotype and the composition of the gut microbiome. The study identified *Christensenellaceae* group as central to a network of co-occurring heritable microbes that has been associated with lean body mass index (BMI)(Ley, 2015). Numerous studies of rodents suggest that the gut microbiota populations are sensitive to genetic, and can produce or influence signals that directly or indirectly impact energy balance (weight gain or loss) and energy stores (Parks et al., 2013). Thus, the microbiota is certainly implicated in the development of obesity, and with tissue deposition in general. There are compelling arguments for the existence of a genetic control over the abundance of taxa in different species and the link of these with energy balance and growth. Currently some evidence has been presented in pigs.

The main objectives of this paper will be to quantify the impact of gut microbiome composition on lean growth in swine and to quantify the heritability of relative taxa abundance in swine.

MATERIALS AND METHODS

From a Duroc closed-nucleus population 28 boars were selected to be sires of the individuals used in this trial. Sires were mated to crossbred sows to generate terminal-cross piglets. These were weaned at an average of 19 days of age and grouped in single-sire-gender pens (groups). During the nursery, growth and finish period, all pigs will be fed standard diets. End of test was declared on a pen-specific basis, entire pens of pigs were taken off test and sent for harvest at a pen mean live weight of 304.6 ± 5.51 lb.

Live weight measurements were taken on individual pigs at the start (weaning) and end of the study and weeks 15, 18 and 22 post-weaning. Ultrasound back-fat depth and *Longissimus* muscle depth and area at approximately the 10th rib were measured on the right side of the pig on a transverse ultrasound scan taken at weeks 15, 18 and 22 post-weaning and at the end of the study. Fecal samples were taken for a total 1300 individual pigs at three time points. After editing, there were 3,783 fecal samples collected, including 15-24 days of age (1205 individuals), 115-124 days old (1295 individuals), and 180-217 days old (1283 individuals). Microbiome composition was obtained by amplifying the V6-V8 region of the 16S rRNA genes of the stool samples through pyrotag sequencing. After sequence processing and QC, there were 10,000 sequence reads per sample. Reads were organized into 2,026 phylotypes (operational taxonomic units, OTUs). Any taxonomic identifier with a confidence score below 80% was grouped as "unassigned". The 2,026 phylotypes were classified into 14 known phyla, 57 families, 112 genera and 213 species.

The bacterial composition of 3,783 samples was determined in each taxonomic level according to the read counts of the 2,026 taxonomically-annotated OTUs in each sample. The 3,783 samples were statistically compared according to their age range using the Kruskal-Wallis test. Further discrimination of grouping of the different taxonomical units was performed through principal component analysis (PCA).

To investigate the divergence of microbial community at taxonomic species level, samples were clustered by age group and sex. All taxonomic units with unassigned genus and/or species were removed, leaving a total of 380 OTUs for this analysis.

To highlight potential association between particular taxa combinations and growth/composition phenotypes, pseudo-enterotypes were obtained for growth and carcass composition through clustering of individuals and families.

The overall contribution of microbiome to phenotypic variability was investigated through linear mixed models. Two traits were considered, average daily gain at market weight (ADGM), as well as average daily gain at 15 weeks (ADG15). For each of the traits a model that included fixed effects of sex, dam-line, contemporary group, back fat at market weight and random effects of permanent environmental effect, animal additive genetic effect (A), and residual, were fitted. This base line model was compared to a model that a random Microbiome (M) effect. Three microbiome compositions were fitted separately to the models representing the populations present at weaning 15 weeks of age and off-test.

The host genetic control over microbiome composition was investigate at the family level. Second-degree polynomial random regression models utilizing 57 family abundance as the dependent variable were fitted. The models included time and sex and their interaction as fixed effect and random regression on animal and permanent environmental effects. All models were run with ASREML v.4.0.

RESULTS AND DISCUSSION

The bacterial composition of 3,783 samples was determined in each taxonomic level according to the read counts of the 2,026 taxonomically-annotated OTUs in each sample. The 3,783 samples were statistically compared according to age range. The Kruskal-Wallis test for differences in bacterial composition among the three age groups showed that 55 out of 57 bacterial families had significantly different (P<0.005) abundance counts between 15-24d and the rest of the samples. The proportion of the 10 most different families is presented in Figure 1. Similarly, the 115-124d and 180-217d groups were significantly different by 45 out of 57 families. The bacteria proportion of the 6 most different families is shown in Figure 2. At 15-24 d, the fecal bacteria were presented by three main phyla, *Firmicutes* (39.38%), *Bacteroidetes* (29.93%) and *Proteobacteria* (22.16%). Over time, the proportion of bacteria in the two phyla *Bacteroidetes* and *Proteobacteria* decreased, while the proportion of bacteria in the phylum *Firmicutes* pronouncedly increased to 72.71% and 77.26% at 115-124 d and 180-217 d, respectively. Our findings agree well with the reports by Kim et al. (2011), Ivarsson et al. (2011), Dicksved et al. (2015).
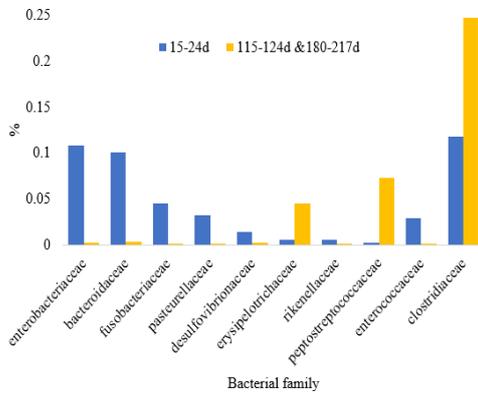
Figure 1. Bacteria proportion of the 10 most different families between 15-24d and 115-124d & 180-217d
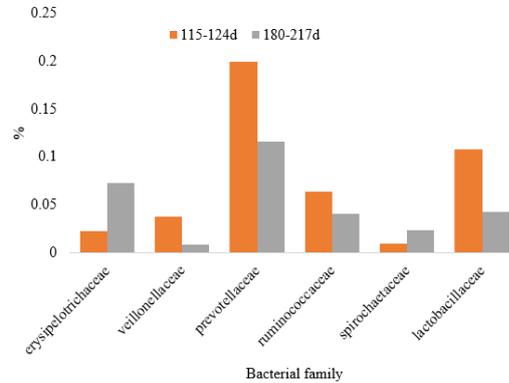
Figure 2. Bacteria proportion of the 6 most different families between 115-124d and 180-217d groups

Using principal component analysis (PCA), family-level bacterial composition data of 3,783 samples over 3 time points were decomposed into two factors that explained 44.03% of the variance (Figure 3). Principal component 1 (PC1), which explained 31.26% of the variance, was heavily negatively loaded with *Enterobacteriaceae, Bacteroidaceae, Fusobacteriaceae, Enterococcaceae, and Pasteurellaceae.* Principal component 2 (PC2) was heavily loaded with *Clostridiaceae* and *Enterobacteriaceae*, and negatively loaded with *Prevotellaceae* and *Fusobacteriaceae.*

Figure 3. Principal component analysis of bacterial families and the 10 largest loadings of bacterial families for PC1 and PC2.

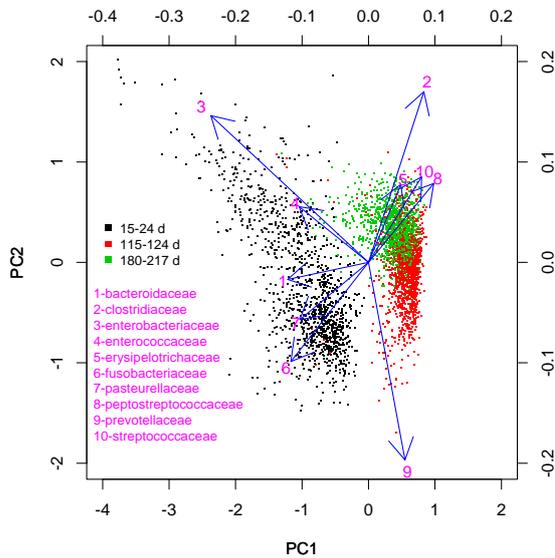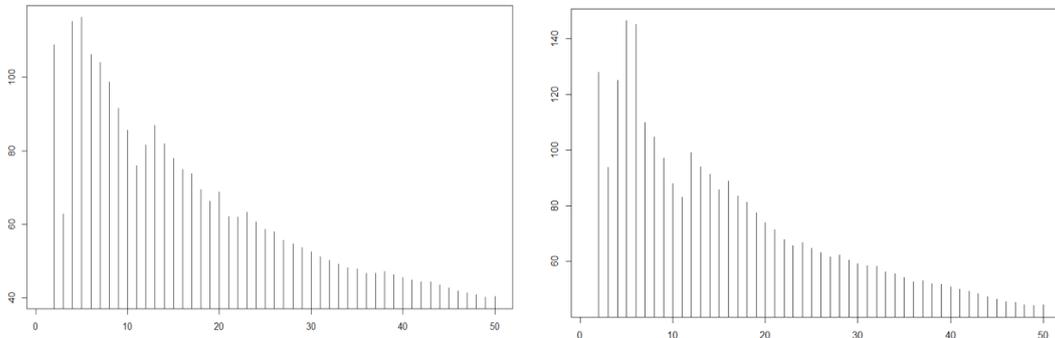**Figure 4. Calinski-Harabasz indexes for number of clusters of samples from 115-124 d males (left) and 180-217 d males (right). Number of clusters on X-axis. The index on Y-axis.**

Samples of 115-124 d and 180-217 d female pigs clustered best into 3 groups whereas the male samples fit best into 5 groups as shown in Figure 4. Though the number of clusters by sex was similar between the 2 age groups, animals that grouped together during 115-124 d did not appear to remain in the same group in the later stage.

We investigated the relationship between clusters of the OTUs and 180-217d animals with regard to fat depth measures. Animals and OTUs were clustered into 5 and 20 groups, respectively, as shown in Figure 5. Average estimated breeding values (EBV) was calculated for each animal cluster. The relative abundance of OTUs in groups 3, 5, 6, 8, 13, 16, 17 and 19 appeared to be significantly correlated with fat depth EBV.
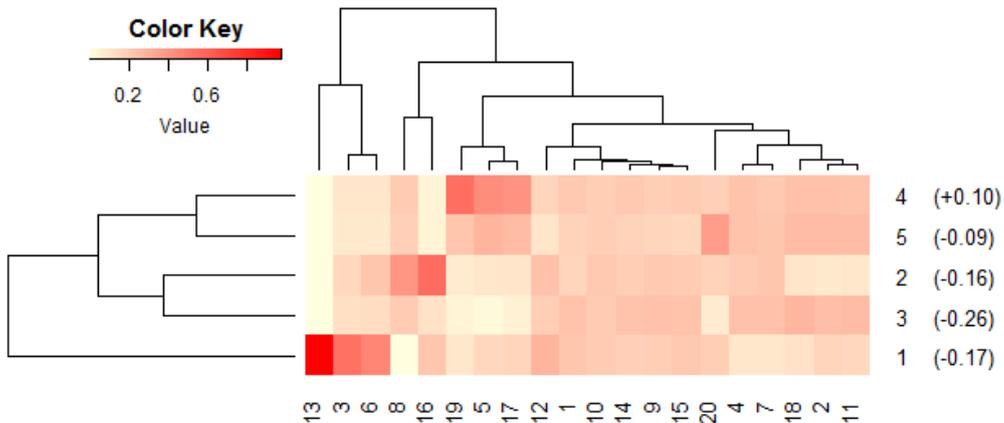
Figure 5. A heatmap of relative abundance of bacterial clusters within pig's fecal microbiome. Five animal clusters, 1 – 5, with animal counts of 10, 153, 180, 174, 92 respectively. Average breeding value of fat depth for animals within each animal cluster is presented in parentheses next to cluster number. The 380 taxonomic units were clustered into 20 groups. Within each OUT cluster, level of redness shows average OTU count relative to other animal clusters.

Microbiome contribution to the overall daily gain variability is reported on tables 1 and 2. For both traits measured (ADGM and ADG15) the gut microbiome absorbed a significant portion of

the phenotypic variation, ranging from approximately 3% for microbiome composition at weaning on ADG15 to more than 65% for microbiome composition at week 15 on ADG15. In both cases the largest amount of variance was absorbed when the composition at week 15 was fit. The microbiome effect eroded the most variance from the residual effect and PE, while a significant portion was also absorbed from the animal genetic effect.

Table.1 Proportion of variances estimated for ADGM

	A	A+M $^{(1)}$	A+M $^{(2)}$	A+M $^{(3)}$
AIC	9855.71	9856.75	9842.15	9842.95
PE (%)	11.52	10.47	7.15	6.54
A (%)	13.20	12.65	6.07	7.29
M (%)	N/A	6.26	57.21	46.64
Residual (%)	75.28	70.62	29.57	39.53

N/A: Not available.

AIC: Akaike information criterion.

$^{(1), (2), and (3)}$, representing microbial data at weaning, 15 weeks of age, and off-test, respectively.

Table.2 Proportion of variances estimated for ADG15

	A	A+M $^{(1)}$	A+M $^{(2)}$	A+M $^{(3)}$
AIC	10217.89	10219.40	10182.36	10215.58
PE (%)	15.36	14.40	7.20	12.88
A (%)	11.11	10.90	3.51	9.60
M (%)	N/A	2.74	66.14	13.68
Residual (%)	73.53	71.96	23.15	63.84

N/A: Not available.

AIC: Akaike information criterion.

$^{(1), (2), and (3)}$, representing microbial data at weaning, 15 weeks of age, and off-test, respectively.

Point estimates for the heritabilities of the 57 taxonomical families ranged from low to moderately high ranging from less than 5% to almost 50%, according to family and time point. Different patterns of h^2 (from low to high and vice versa) were observed across time for different families possibly reflecting the overall abundance of a particular family across the trial. A plot of the h^2 for a sample of the families fitted is presented in figure 6.

Figure 6. h^2 for a sample of the 57 microbiome taxonomical families represented

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