RESEARCH REPORT
ANIMAL SCIENCE

Title: The effects of PRRSV infection in commercial pigs on growth performance, energy and nutrient digestibility – NPB #12-151

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Industry Summary: The research herein is an extension of the PRRS Host Genetics Consortium **(**PHGC), which is a national effort to identify genomic markers and host response pathways in response to PRRSV infection. The typical trial includes 200 pigs that are followed through 42 days post-infection (dpi) of PRRSV, with blood (serum and RNA tempus), oral fluids, and weights collected for phenotypic analysis. DNA recovered from each pig is then genotyped using the Porcine SNP60 Bead chip. The pigs in the PHGC model have shown wide variation in weight gain, with some pigs gaining weight at a relatively normal rate, while others failed to thrive during the 42 day infection period. Furthermore, weight gain following PRRSV infection was moderately heritable (0.3) and a substantial proportion of the genetic variation in weight gain following challenge was mapped to chromosome 4 (SSC 4), indicating a role for host genetics. Therefore, this research paper took advantage of two PHGC trials 13 and 14, and investigated the individual digestibility of dry matter and energy in the face of PRRSV infection. Total tract feces was collected and pooled from individual infected (n=376) pigs during peak virus load (9-14 dpi). Total tract apparent digestibility coefficients on 122 pigs were then used to estimate genetic parameters and to perform a genome-wide association study. This novel study is the first to provide genetic parameters for digestibility traits in PRRSV infected pigs. Heritability estimates were low to moderate. Digestibility was not phenotypically correlated with weight gain and viral load, but their genetic correlation was moderate, indicating that some of the same genes may control these traits. Several QTL for digestibility were identified, jointly explaining over 8% of the genetic variance in dry matter and energy digestibility. Many of these regions have been previously associated with growth or feed intake related traits and harbor genes that are involved in important mechanisms related to energy and nutrient usage. Albeit small, this data set allowed us to identify one SNP controlling variation in digestibility of dry matter and energy, and provided further information on the effect of the WUR SNP on PRRSV infected pigs.

Keywords: Porcine Reproductive and Respiratory Syndrome, genome-wide association study, genetic parameters

Scientific Abstract. Digestibility data on 122 growing pigs infected with the porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) were used to estimate genetic parameters and to perform a genome-wide association study. The digestibility traits (energy and dry matter) had moderate to low heritabilities and moderate genetic correlations with weight gain and viral load. Quantitative trait locus (QTL) for the digestibility traits were found on *Sus scrofa* chromosome (SSC) SSC1, SSC4, SSC6, and SSC13, jointly explaining over 8% of the genetic variance. Most of these regions have been previously associated with growth- or feed intake- related traits, and harbor genes that are involved in important mechanisms related to energy and nutrient usage. In addition, we identified one Single-nucleotide polymorphism (SNP) on SSC1 that was associated with dry matter and energy digestibility in PRRSV infected growing pigs, as well as provided associations with the WUR10000125 SNP on SSC4, which has previously been associated with the response to PRRSV infection.

Introduction. Porcine respiratory and reproductive syndrome (PRRS) causes major economic losses to the pork industry across the globe, including the United States (Holtkamp et al. (2013)). These losses come, for example, in the form of a reduction in the number of pigs born alive and efficiency of gain in growing pigs. We have previously shown in growing pigs that continuous immune system stimulation with lipopolysaccharide results in attenuated apparent total tract digestibility (ATTD) of nitrogen and organic matter (Rakhshandeh et al. 2012). However, the impact that PRRS virus (PRRSV) infection has on ATTD of nutrients and energy is not well understood.

Without an effective vaccination program, efforts have been taken to better understand the host response to PRRSV infection. Boddicker et al. (2012) found a major QTL on *Sus scrofa* chromosome (SSC) 4 associated with PRRSV response and weight gain in growing pigs, with one SNP accounting for the vast majority of the genetic variation in the region (WUR10000125; WUR).

The genetic basis of digestibility in swine has only minimally been studied. Noblet et al. (2013) indicated that sire origin can contribute to variation in both nutrient and energy digestibility. Nevertheless, genetic

parameter estimates for digestibility traits in healthy, and in particular in infected pigs, are scarce. One of our hypotheses was that the reduced weight gain observed post PRRSV infection is due to reduced nutrient and energy digestibility in the host. This warrants exploration of host variation as part of the quest to develop the best feeding and management programs for PRRSV infected pigs. The objectives of this study were to estimate genetic parameters and to perform a genome-wide association study (GWAS) for ATTD traits in growing pigs during a PRRSV infection. Specific attention focused on potential effects of the WUR SNP.

Materials & Methods. *Data.* Data were obtained from 376 commercial crossbred nursery pigs (7.5±1.5 kg, initial weight) from trials 13 and 14 of the PRRS Host Genomics Consortium (Boddicker et al. 2012) that were experimentally infected with PRRSV isolate KS 06-72109 at 28.8±4.1 days of age. Blood samples and body weights were collected weekly through 42 days post infection (dpi). Viral load (VL) was calculated as area under the curve for q-PCR viremia through 21 dpi. Weight gain (WG) was defined as the difference between body weight at 21 and 0 dpi.

Pigs were fed ad libitum a standard corn-soy diet containing the digestibility marker titanium dioxide at 0.4% of the diet. Individual fecal grab samples were collected daily during the 6-week study. Over the peak viremia period, 9-16 dpi, all pigs that had 3 or more fecal grab samples were selected and samples were pooled within pig. On this basis, 122 pigs were used for the analysis. Titanium dioxide concentrations, ATTD of energy (En%) and dry matter (DM%) were measured and calculated on each pooled pig sample, as previously described (Htoo et al. 2008 and Kerr et al. 2009). Additional data included dam and sire identification, trial (2 levels), pen (5 levels), sex (2 levels), and dam parity (1 to 7). The pedigree included 202 animals.

Genotype data. All 122 piglets were genotyped using the Illumina PorcineSNP60 BeadChip. Of the 61,565 SNPs, 10,049 were removed due to poor quality (SNP call rate < 80% and MAF < 3%). The remaining 51,516 SNPs had a total genotyping call rate of 99.96%.

Genetic parameters and statistical analysis. Heritabilities and genetic and phenotypic correlations were estimated for En%, DM%, VL, and WG using ASReml 3.0 (Gilmour et al. 2009). Models included fixed effects

of trial, parity, sex, and initial age (covariate), and random effects of litter, pen within trial, and a polygenic effect. Heritabilities were estimated using univariate models; correlations with bivariate models. The effects of VL, WG, and the WUR genotype on En% and DM% were assessed. Only six animals had the BB genotype for the WUR SNP, and since it has been shown that the B allele has complete dominance over the A allele (Boddicker et al. (2012)), BB animals were combined with AB animals. Any other SNPs obtained from the GWAS results (see below) were also fitted in the full model as fixed effects.

Genome-wide association study. Associations of SNPs with digestibility traits were performed using Bayesian genomic selection methods. The Bayes-B method (Meuwissen et al. 2001) was used for the association analyses in a model that included the fixed effects of trial, parity of dam, age, and sex, using a π (proportion of SNPs with zero effects) of 0.999. All SNP association analyses were performed using GenSel version 4.4 (Fernando and Garrick 2009), which provides estimates of the total genetic variance explained by the markers (TGVM) and of the genetic variance explained by each non-overlapping 1-Mb SNP window across the genome (Wolc et al. 2012). SNP windows that accounted for over 0.8% of the TGVM (%TGVM) were reported.

Results and Discussion. Genetic parameter estimates are presented in Table 1. With the exception of WG, which had a high heritability (0.66±0.49), all traits had moderate to low heritabilities, ranging from 0.15 to 0.17. Heritability estimates obtained in this study for WG and VL differed from those reported by Boddicker et al. (2012). In our study, we had greater estimates for WG and lower for VL. These differences were likely due to the much smaller data set used in this study. To our knowledge, these heritability estimates for the digestibility traits En% and DM% are the first reported in the literature for growing pigs infected with PRRSV.

Estimates of phenotypic correlations between traits were low, with the exception of the phenotypic correlation between En% and DM% (0.85±0.03). This high phenotypic correlation was expected since these traits reflect much of the same constituents of the diet. However, the genetic correlation between them was low (0.17±0.64). Interestingly, both digestibility traits had low phenotypic and moderate genetic correlations with

VL and WG. Although associated with large standard errors, these genetic correlation estimates are promising. Obtaining digestibility phenotypes is costly and labor intensive, and the identification of low-cost indicators traits could enhance selection for digestibility. Since WG has been previously reported to have moderate heritability (Boddicker et al. 2012), and in this study we obtained moderate genetic correlations between WG and En% and DM%, the relationship between WG and digestibility traits warrants further investigation.

The results from the GWAS for ATTD En% and DM% are presented in Table 2 and Figure 1. A total of 5 regions were associated (%TGVM > 0.8) with each digestibility trait. In general, the same genomic regions were associated with both En% and DM%, including regions on SSC1, SSC4, SSC6, and SSC13. Nearly all of these regions have previously been reported to be associated with average daily gain, body weight, or feed conversion rate.

The 1-Mb region on SSC6 at 119 Mb had the highest association in this study, accounting for 4.6% of the TGVM for DM%. This same region had the second highest association for En% (2.1% of the TGVM). This region harbors the lysophosphatidic acid receptor 3 gene (*LPAR3*), which is involved in lipid signaling (Chiang et al. 2011).

On SSC13, a 1-Mb region at 161 Mb was associated with En%, while the region at 43 Mb was associated with both En% and DM%. Genes associated with energy and nutrient utilization are located in this region, including the pyruvate dehydrogenase (lipoamide) beta gene (*PDHB*), which is involved in lipid biosynthesis and is a candidate gene for intramuscular fat in pigs (Serão et al. 2011) and the oncoprotein-induced transcript 1 gene (*OIT1*), a gut derived protein that is expressed and secreted in a nutritional and dietary fat dependent manner and may play a role in metabolic homeostasis (de Wit et al. 2012). The two regions on SSC4 (at 25 to 28, and at 89 Mb) accounted for 3.5% and 1.1% of the TGVM for En% and DM%, respectively. These two regions have been linked to small intestine length (Knott et al. 1998).

The GWAS also identified a region on SSC1 (at 50 Mb) that was associated with DM% and En%. This region has previously been associated with PRRSV antibody titer (Wimmers et al. 2009). In our study, further exploration of this region identified SNP ASGA0002573 (ASGA) to account for some of the variation in DM%

and En%. The effects of this SNP, as well as of the WUR genotype on SSC4 were tested for significant effects on digestibility by including the fixed effect of genotype in the model, as previously described. The results for these two SNPs are shown in Table 3. Genotype at ASGA was significantly associated (P=0.053) with both En% and DM%, with AA animals having an approximately 1% greater digestibility coefficient than AB animals. Interestingly, the WUR SNP was significantly associated with En% (P=0.036) but not with DM% (P>0.10). Furthermore, the favorable AB genotype for host response to PRRS (Boddicker et al. 2012) resulted in 1% lower digestibility coefficients than animals with the AA WUR genotype. This result was unexpected, but since feed intake was not measured, we were not able to determine why the WUR SNP acted in an opposite manner than expected.

In conclusion, this study offered new insights on the impact of PRRSV on the genetics of digestibility. This is the first study providing genetic parameters for digestibility traits in PRRSV infected pigs. Heritability estimates were low to moderate. Digestibility was not phenotypically correlated with weight gain and viral load, but their genetic correlation was moderate, indicating that some of the same genes may control these traits. The relationship between digestibility traits and weight gain should be further investigated. Several QTL for digestibility were identified, with regions on SSC1, SSC4, SSC6, and SSC13 jointly explaining over 8% of the genetic variance in dry matter and energy digestibility. Many of these regions have been previously associated with growth or feed intake related traits and harbor genes that are involved in important mechanisms related to energy and nutrient usage. Albeit small, this data set allowed us to identify one SNP (ASGA) controlling variation in digestibility of dry matter and energy, and provided further information on the effect of the WUR SNP on PRRSV infected pigs.

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Table 1. Genetic parameters[§] estimates for apparent total tract digestibility of dry matter (DM%) and energy (En%), viral load (VL) and weight gain (WG).

Trait	DM%	En%	VL	WG
DM%	**0.17±0.22**	0.17±0.64	0.53±0.82	0.36±0.50
En%	0.85±0.03	**0.15±0.23**	0.42±0.78	0.57±0.49
VL	0.02±0.10	0.04±0.10	**0.17±0.26**	-0.21±0.61
WG	0.11±0.11	0.02±0.11	-0.23±0.42	**0.66±0.49**

[§]Estimates of heritabilities on diagonal, genetic correlations above diagonal, and phenotypic below diagonal.

Table 2. Genomic regions associated with apparent total tract dry matter (DM%) and energy (En%) digestibility.

Trait	# of SNPs	%TGVM	PPI	SSC	Mb
DM%	23	4.6	0.27	6	119
	17	1.9	0.10	13	43
	20	1.1	0.09	4	89
	44	0.9	0.13	1	50
	16	0.9	0.09	13	161
En%	77	3.5	0.28	4	25
	23	2.1	0.16	6	119
	28	1.5	0.10	1	50
	19	1.3	0.07	1	77
	17	1.1	0.07	13	43

%TGVM, percentage of the total genetic variance explained by the window; PPI, posterior probability of inclusion of the window.

Table 3. Least square means for genotype at SNPs ASGA and WUR for apparent total tract dry matter (DM%) and energy (En%) digestibility.

	ASGA[1]			WUR[2]		
Trait	AA	AB	P-value	AA	AB	P-value
DM%	85.3	84.6	0.053	85.3	84.7	0.155
En%	83.9	82.9	0.053	83.9	82.9	0.036

[1]ASGA, SNP ASGA0002573 located on SSC1.
[2]WUR, SNP WUR10000125 located on SSC4.

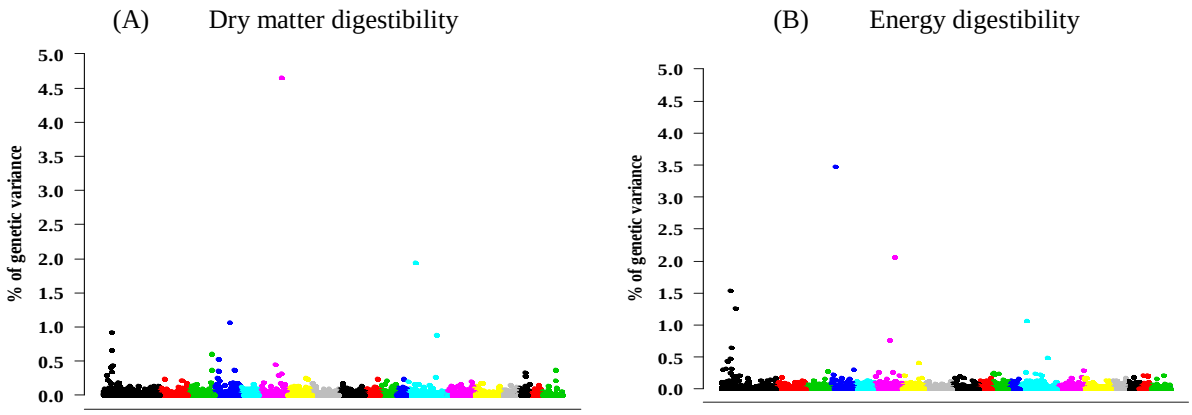

Figure 1. Manhattan plot of the genome-wide association analysis of apparent total tract digestibility of (**A**) dry matter and (**B**) energy. Each point in the Manhattan plot represents one non-overlapping 1-Mb SNP window, except for a 2-Mb and 4-Mb region on SSC1 and SSC4 for dry matter and energy digestibility, respectively. Consecutive windows that accounted for over 0.3% of the variance were combined. Windows are sorted by chromosome and position, from SSC1 through SSC18 and SSCX.