Title: Large scale SNP association analyses of feed efficiency and longevity – **NPB #08-190**

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Industry Summary

Many traits financially important to hog producers are either expensive or time-consuming to measure or difficult to successfully select animals for based on low heritability of the targeted traits. Feed efficiency and sow longevity are examples of such traits. If genetic markers can be used successfully in marker-assisted selection (MAS) to improve these traits, producers would see a large impact on their profits.

The objectives of this study were to: 1) use the newly developed porcine SNP chip to genotype animals from the NPB funded feed efficiency and longevity trials; 2) complete association analyses to determine which markers are associated with the traits of interest; and 3) inform producers of how to utilize this genetic information in selection programs.

Feed efficiency and its relationship to growth and backfat were assessed in this genetic marker association study by analyzing residual feed intake (RFI – a measure of the difference between what an animal actually consumes and the average amount of feed required for that animal's level of maintenance and growth), average daily feed intake (ADFI), average daily gain (ADG), and backfat (BF). Sow longevity depends significantly on the structural soundness of sows' feet and legs. Many feet and leg structure traits, such as overall leg action and front leg pastern conformation, were analyzed to address this objective.

All traits were analyzed in Yorkshire and/or Landrace x Yorkshire crossbreds with a total of 1550 animals genotyped, 250 more animals than planned in the original proposal. The SNP chip genotyped 64,232 genetic

markers (almost 15,000 more than originally planned) in each animal at a cost of approximately 0.3 cents per genotype. Genetic effects of these markers were estimated using an advanced statistical method developed by Drs. Dorian Garrick and Rohan Fernando at Iowa State University.

Many well-known and newly discovered regions of significance were identified throughout the pig genome during analyses for each of these traits. An example of a well-known gene is *MC4R* which was again found to be associated with ADFI, ADG, and BF in the current study. Hundreds of new regions of significance were also identified which will require more in-depth research to confirm their effects. More detailed results are given in the final reports on NPB grants #08-011 and #08-012. Overall, the findings from this study look very promising. Validation of these results may prove that utilization of some of these markers in MAS can decrease production costs by several dollars per pig in the near future.

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Scientific Abstract

Many traits financially important to hog producers are either expensive or time-consuming to measure or difficult to successfully select animals for based on low heritabilities. Feed efficiency and sow longevity are examples of such traits. If genetic markers can be used efficiently in marker-assisted selection (MAS) to improve these traits, producers would see a large impact on their profits. Feed efficiency and its relationship to growth and backfat were assessed in this genetic marker association study by analyzing residual feed intake (RFI), average daily feed intake (ADFI), average daily gain (ADG), and backfat (BF). Sow longevity depends significantly on the structural soundness of sows' feet and legs. Many feet and leg structure traits, such as overall leg action and front leg pastern conformation, were analyzed in the present study to address this objective. All traits were analyzed in Yorkshire and/or Landrace x Yorkshire crossbreds with a total of 1550 animals genotyped. Genetic effects were fitted using a Bayesian model averaging approach (Bayes-C) that simultaneously fitted various combinations of 250-325 SNPs (approximately 0.5% of the SNPs segregating in the population). Many well-known and newly discovered regions of significance were identified based on build 9 of the porcine genome during analyses for each of these traits. An example of a well-known gene is *MC4R* which was found again to be associated with ADFI, ADG, and BF in the current study. Hundreds of new regions of significance were also identified which will require more in-depth research to confirm their effects. Overall, results look promising for delivering markers for marker-assisted selection to benefit hog producers.

Introduction

The recent development of the PorcineSNP60 BeadChip was a large feat of collaborative science. This new single nucleotide polymorphism (SNP) chip is used to genotype more SNPs (64,232) at a cheaper cost per chip than initially anticipated. The chip serves as a great tool for genome-wide association analyses due to the high speed and low cost of genotyping and the high-quality genotypes that result. Traits that are especially important to analyze with the new chip are those which are expensive or difficult to measure and those with low heritability which respond poorly to traditional selection methods. Marker-assisted selection has the potential to greatly increase genetic gains for these types of traits. Examples of such traits include feed efficiency and sow longevity.

The high genetic correlation between growth and feed intake has increased feed requirements as pigs have been selected for increased growth rate. The largest variable cost in swine production today is feed. Boggess *et al.* (2009) claimed that around $500 million dollars annually could be saved by swine producers in the US by reducing the average feed:gain ratio from 2.75 to 2.45. Significant variability exists between pigs in the amount of feed intake required to achieve the same rate of growth. Residual feed intake (RFI) is a measure of the difference between what an animal actually consumes and the average amount of feed required for that animal's levels of maintenance and growth. It is believed that animals can be successfully selected for both increased growth and decreased RFI, to reduce the feed costs per-unit gain.

Sow longevity in the herd is also an important component of profitability on a pig farm. According to the PigCHAMP™ 2008 annual report, the culling rate of breeding females was over 46% and the sow mortality rate was almost 9% in the US (www.pigchamp.com/ summary_archives.html). Several factors are involved in culling decisions, including reproduction problems and structural insufficiencies of the sow. Structural unsoundness was estimated to account for 10-40% of culling decisions (Stalder et al., 2004; Kirk et al., 2005). The low to moderate heritability of structural soundness traits makes them difficult to select animals for using traditional selection methods. Consequently, the goal of this study was to identify markers for these structural soundness traits and the feed efficiency traits mentioned earlier using the new SNP chip technology.

Stated Objectives from original proposal

The objectives of this research are to:

1) Use the porcine 50K SNP chip and genotype animals from the NPB funded feed efficiency and longevity trials.

2) Complete association analyses to determine which markers are associated with the traits of interest.

3)   Recommend use of genetic markers associated with traits to be used in breeding programs and disseminate information to swine breeders, company and genotyping services.

Materials & Methods

Genotyping

Genotyping of each population of animals was completed as described in NPB final reports on projects #08-011 and #08-012. The PorcineSNP60 BeadChip was used to genotype 730 Yorkshire animals from the feed efficiency project and 820 Yorkshire and Landrace x Yorkshire crossbred animals from the longevity study.

Statistical analyses

Quality control included the removal of all SNPs which were fixed in the entire population or had a quality control (QC) score less than 0.4 in greater than 20% of the population. Over 55,000 SNPs remained for analysis. Bayes C model averaging, as implemented in GenSel (http://bigs.ansci.iastate.edu) was used for data analyses. The regression model used was: $Y = \mathbf{X}\beta + \mathbf{Z}u + e$, where $\mathbf{X}$ is an incidence matrix for fixed effects and $\mathbf{Z}$ is a matrix of SNP genotypes fitted as random effects. For the feed efficiency population, fixed effects included line, sex, on-test group, pen fitted within group, and on-test age as a covariate, except for BF analysis where on-test age was replaced with off-test weight. For the longevity population, fixed effects included mean, genetic line and farrowing season for all traits. For the leg structure and body composition traits, scorer and date measured were additionally fit as fixed effects. The prior probability that a SNP in $\mathbf{Z}$ has zero effect was set to 0.995, corresponding to about 300 non-zero SNP effects fitted in any particular realization of the Monte-Carlo Markov Chain (MCMC) used for the Bayesian analysis. Following a burn-in period of at least 5,000 iterations, at least 40,000 additional MCMC iterations were run. Results were obtained in the form of a post burn-in posterior distribution for the effect of every SNP fitted simultaneously with other informative SNPs. The posterior mean effect of each SNP across the chains was used to predict the genomic breeding value of every chromosomal fragment consisting of 5 contiguous loci. Each such chromosomal fragment's contribution to the additive genetic variance in the population was then derived, a statistic that has a multi-locus analogy to $2pq\hat{\alpha}^2$, the gene frequency specific contribution to genetic variance of the substitution effect of a single locus. That variance was divided by an estimate of the total genetic variance.

The most significant regions of the genome were examined for genes based on build 9 of the porcine genome. Gene positions were taken from Ensembl (www.ensembl.org) and identified by proximity to the most informative individual SNPs or 5-SNP windows and relation of the gene's function to the trait being analyzed.

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Results

Objective 1

The SNP chip developed by PI, Rothschild, and collaborators included almost 15,000 more SNPs than originally planned. Using this chip, the average genotyping call rate for the feed efficiency population was over 99.6%, which is exceptional, and all animals had a call rate over 97%. In the longevity population, the genotyping results were similar except for 5 samples with lower call rates.

Objective 2

Many significant statistical associations between SNPs and traits were found for all of the traits examined in this study. Results included genes that were previously known to have effects on some of the traits as well as genes not previously known to be associated with any of these traits. More details are provided in the final reports from grants #08-011 and #08-012, but some highlights for each trait follow.
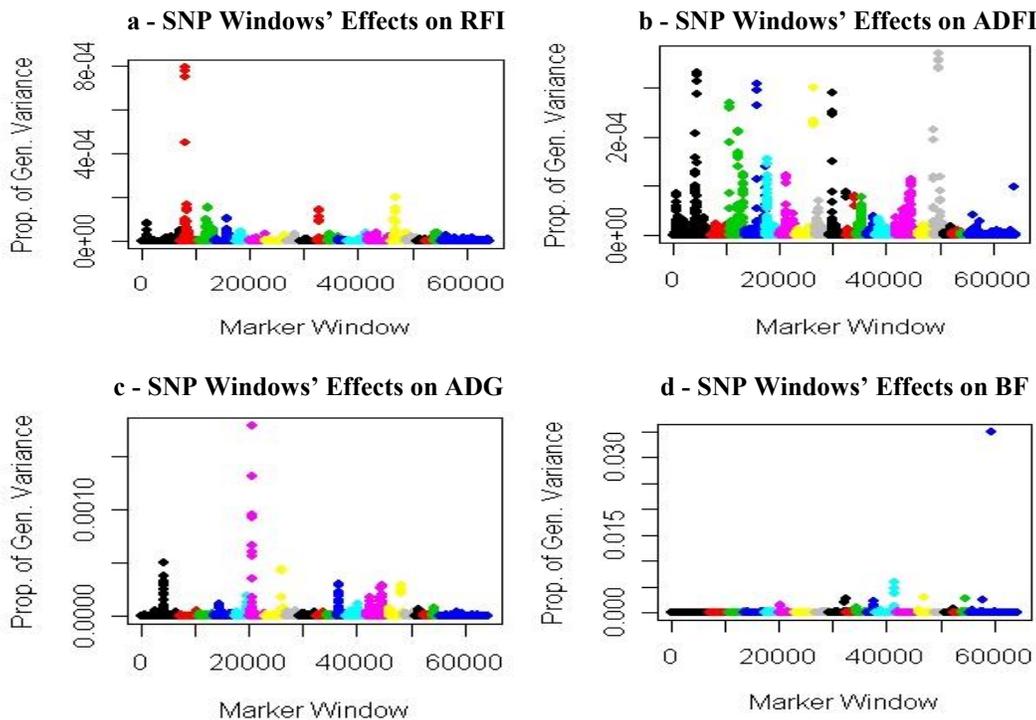

Figure 1: Proportion of genetic variance explained by each window of 5 consecutive markers in the genome for each trait. Each chromosome is a different color with SSC1 on the left, SSC18 in red on the right, followed by SSCX in green and unmapped markers in blue. Note: y-axes differ in scale.

Traits analyzed on the feed efficiency pigs included RFI, ADFI, ADG, and BF. The vast majority of additive genetic variance was explained by the sets of 250-300 markers for each of these traits. For RFI, SNPs near *SLC5A12* on *Sus scrofa* chromosome (SSC) 2 and *SUCLG1* on SSC3 showed some of the largest associations (Figure 1a). Markers on chromosomes 10 and 15 also had significant effects on RFI. Markers with large effects on ADFI were found on chromosomes 1, 3, 4,

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7, 9, and 16 (Figure 1b). Markers on chromosomes 1, 6, 7, 12, 14, and 15 had the largest effects on ADG (Figure 1c). The gene *MC4R*, located near the significant markers on chromosome 1 for ADFI and ADG, was previously known to have large effects on both traits. A currently unmapped marker had the largest effect for BF (Figure 1d), which accounted for over 3% of the genetic variance in the trait. The markers with the largest effects on BF and with known positions in the genome were on chromosomes 9, 12, 13, and 15.
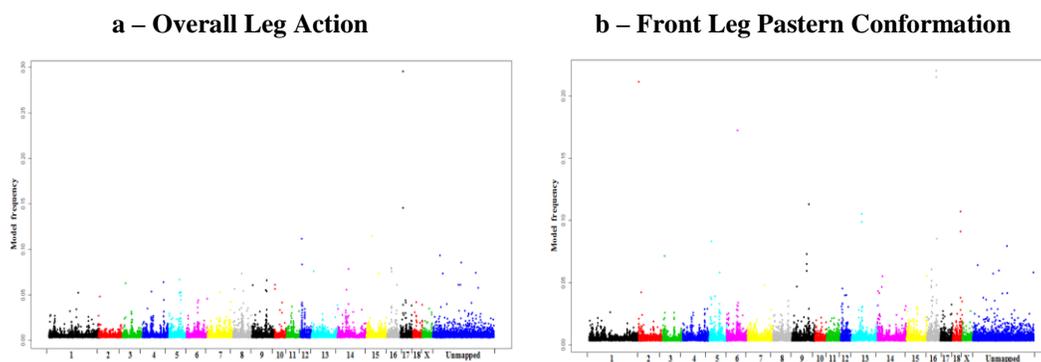
a – Overall Leg Action   **b – Front Leg Pastern Conformation**

Figure 2: Plots of model frequency of SNPs associated with overall leg action (a) and front leg pastern conformation (b). Higher values indicate more association with the trait. Each chromosome is a different color with SSC1 on the left, SSC18 in red on the right, followed by SSCX in green and unmapped markers in blue. Note: y-axes differ in scale.

In the longevity population, analyzed traits included BF, loin muscle area (LMA), body depth, body length, body width, overall leg action, several foot and leg structure traits, total number of born (TNB) and number born alive (NBA) over the first three parities, lifetime total number born (LTTNB) and lifetime number born alive (LTNBA). A total of 18 chromosomal regions associated with BF were identified. These included regions around *MC4R* on SSC1 and the largest effect on SSC18. Markers on chromosomes 2, 13, 16, and 17 had the largest effects on LMA. The *BMP2* gene was associated with body depth, body length, body width, and several foot and leg structure traits (e.g. front leg pastern conformation plotted in Figure 2b). For overall leg action, the most significant SNPs were located on chromosomes 2, 6, 9, 13, 16, and 18 (Figure 2a). The HOX gene family (SSC18), *TWIST1* (SSC9), and *SP4* (SSC9) were the most likely candidate genes for leg action. For the reproduction traits, results were similar for TNB and NBA within parity, but significant genes differed between the first three parities. Significant genes included *MEF2C* for parity 1, *PTX3* for parity 2, and *ITG6* for TNB and *GDF8* for NBA for parity 3. Finally, for the lifetime reproduction traits, markers on chromosomes 2 and 16 had the largest effects.

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Objective 3

So far citations including results from this grant have included:

Fan, B., S.K. Onteru, D. Garrick, K.J. Stalder, and M.F. Rothschild. 2009. A genome-wide association study for pig production and feet and leg structure traits using the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, 2009, Hinxton, UK. Abstract No. 6.

Gorbach, D.M., W. Cai, J.C.M. Dekkers, J.M. Young, D.J. Garrick, R.L. Fernando, and M.F. Rothschild. 2009. Whole-genome analyses for genes associated with residual feed intake and related traits utilizing the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, 2009, Hinxton, UK. Abstract No. 11.

Onteru, S.K., B. Fan, D. Garrick, K.J. Stalder, and M.F. Rothschild. 2009. Whole genome analyses for pig reproductive traits using the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, 2009, Hinxton, UK. Abstract No. 5.

Fan, B., S.K. Onteru, D. Garrick, K.J. Stalder, and M.F. Rothschild. 2010. Whole-genome association analysis on body composition and feet and leg structure soundness traits in the pig. The International Plant & Animal Genome XVIII Conference. January 9-13, 2010, San Diego, California. Abstract No. P605.

Gorbach, D.M., W. Cai, J.C.M. Dekkers, J.M. Young, D.J. Garrick, R.L. Fernando, and M.F. Rothschild. 2010. Genetic analysis of residual feed intake and its components based on the PorcineSNP60 BeadChip. The International Plant & Animal Genome XVIII Conference. January 9-13, 2010, San Diego, California. Abstract No. P614.

Onteru, S.K., B. Fan, D.J. Garrick, K.J. Stalder, and M.F. Rothschild. 2010. Genome-wide analyses for pig reproductive traits using the PorcineSNP60 BeadChip. The International Plant & Animal Genome XVIII Conference. January 9-13, 2010, San Diego, California. Abstract No. P615.

Rothschild, M.F., D.M. Gorbach, B. Fan, S.K. Onteru, Z.-Q. Du, D.J. Garrick, R.L. Fernando, K.J. Stalder, and J.C.M. Dekkers. 2010. Applications of new porcine genomic tools to trait discovery and understanding genomic architecture. The International Plant & Animal Genome XVIII Conference. January 9-13, 2010, San Diego, California. Abstract No. W614.

Information was also included in presentations at:
- The National Swine Improvement Federation (NSIF) conference in Nashville, TN in December 2009
- The Iowa Feed and Nutrition Seminar in Des Moines, Iowa in January 2010.

An article was also published in the February 15th, 2010 issue of the National Hog Farmer. Further publication plans include presentation of results at the International Society of Animal Genetics (ISAG) conference in July 2010, the World Congress on Genetics Applied to Livestock Production (WCGALP) in August 2010, and submission of multiple larger papers to major journals.

Discussion

The new SNP chip is a wonderful tool for quickly and cheaply evaluating over 60,000 genetic markers throughout the pig genome. Use of the SNP chip allows for successful analyses of all the phenotypes gathered on a population. For both populations studied in the current project, each animal was phenotyped for a variety of traits. This large amount of phenotypic data facilitated association analyses of many production traits in the maternal pig lines. The large amount of results produced in this study mean there are many interesting genomic regions which need further analyses. Currently, these results show significant promise for use in MAS by producers. Validation of these results may prove that utilization of some of these markers in MAS will decrease production costs by several dollars per pig in the near future.

References

Boggess, M. 2009. A pork industry perspective. Pig Genome III Conference. November 2-4, 2009, Hinxton, UK.

Kirk, R.K., B. Svensmark, L.P. Ellegarrd, and H.E. Jensen. 2005. Locomotive disorders associated with sow mortality in Danish pig herds. J Vet Med A. 52: 423-428.

Stalder, K.J., M. Knauer, T.J. Baas, M.F. Rothschild, and J.W. Mabry. 2004. Sow longevity. Pig News and Information. 25: 53N-74N.