INTERFACE BETWEEN MOLECULAR GENETICS AND SWINE NUTRITION

Scott C. Fahrenkrug, Ph.D.
Department of Animal Science
University of Minnesota
St. Paul, MN

The efficiency of swine production ([P]henotype) is influenced by genetic [G], and environmental [E] factors (P = G x E). The preeminent environmental influences relevant to swine production relate to animal health, comfort and nutrition. Environmental cues are responded to by changes in the expression of genes, and by alterations in the proteins they encode. These responses result in the generation of physiological signals that influence animal reproduction, tissue development, and growth. Half a century of biochemistry, genetics and comparative biology have identified some of the most important gene products in these processes. Diagnostics tests based on these molecular players are trickling their way into the marketplace, and have begun to fulfill their promise as important tools in animal production and breeding. Completion of the Human Genome Sequencing Project, and the ongoing efforts to sequence livestock genomes provides a platform for a dramatic increase in the rate we can discover the genes important to swine production.

Genetic regulatory networks control nutrient digestion, absorption, and metabolism. They also control nutrient partitioning, biosynthesis and waste production. We have coined the term "nutriome" to refer to that portion of the animal genome directly involved with these metabolic processes. The nutriome influences the profitability, as well as the environmental impact of swine production. However, little investment has yet been directed towards optimizing animal genetics with regard to nutrition and waste production. Undigested and unabsorbed protein in animal feed is lost value to the producer. It is also degraded by anaerobic bacteria into toxic and malodorous compounds. Excess tryptophan is converted to phenol and p-cresol, excess tyrosine degrades to indole and skatole, excess methionine and cysteine are converted to sulfides (Gottschalk 1986; Mackie et al., 1998). The atmospheric release of these volatile organic compounds in manure gases, along with ammonia, hydrogen sulfide, and other yet uncharacterized odor components, has raised concern in increasingly vocal communities near pig confinement facilities. With continued urban sprawl, the issues surrounding swine waste are likely to become more serious.

Although there is little evidence that manure malodor is detrimental to human health, some manure and manure-odor components do have the potential to damage the environment and decrease the quality of life around production facilities. Ammonia is not only a noxious respiratory irritant, but also contributes to an increase in soil pH, and eutrophication of nearby lakes, rivers, and streams. Likewise, hydrogen sulfide, which smells like rotten eggs, is a chemical asphyxiant in large concentrations. Manure run-off results in the transfer of phosphorous (P) and nitrates (N) into the soil and water table and provides a potential point of pathogen introduction. This later point is especially troublesome given the potential for the selection for antibiotic-resistant pathogens by an industry that relies on sub-clinical doses of antibiotics as growth promotants (Cromwell, 2002). Effective December 1998, European

authorities have prohibited the supplementation of feed with antibiotics. Comparable U.S. regulations are likely to follow. Thus, market and legislative forces argue for the development of pigs that can be produced under antibiotic-free conditions with optimal FCE, decreased waste production, and lower levels of objectionable compound emission.

GENETIC VARIATION IN SWINE METABOLISM

DNA sequences from two individuals of the same species are highly similar – differing by only about 1 nucleotide in 900 in domestic pig breeds (Fahrenkrug et al., 2002). The types of DNA differences (polymorphisms) include single nucleotide polymorphisms (SNP), insertion/deletions (indels), or differences in the length of DNA repeats. By far the majority of these differences have no effect on animal performance due to the fact that they are not in genes. Some of the polymorphisms are in genes but influence the encoded proteins in subtle ways. Such differences may only be revealed when acting in concert with polymorphisms at other loci. It is only in rare cases that a polymorphism (mutation) has a dramatic effect, such as disease.

The Society for Inborn Errors of Metabolism (http://www.ssiem.org.uk/ssiemj.html) currently lists more than 100 human genetic diseases of metabolism. Despite centuries of selective breeding, genetic diversity influencing livestock metabolic potential also remains. This is clearly illustrated by the presence of inborn errors of metabolism (IEM) in livestock populations. For example, the Ossabaw pigs were left on a Georgia island by Spanish galleons, and have for 500 years been subject to natural selection for a feast-or-famine subsistence on a diet of acorns and salt water (Martin, 1973). These pigs have in common with American Pima Indians, a predisposition to type II or non-insulin-dependent diabetes mellitus (NIDDM; Marx, 2002). Citrullinemia in Australian Friesian cattle is due to a mutation in the argininosuccinate synthetase gene. This results in ammonia intoxication and accumulation of citrulline in serum, spinal fluid, and urine (Dennis et al., 1989). Bovine Maple Syrup Urine disease, results in mental and physical retardation, feeding problems, and a maple syrup odor in urine due to the accumulation of keto acids of leucine, isoleucine and valine. In this case a mutation in the gene encoding a branched chain acetyl transferase results in a block in oxidative decarboxylation (Mitsubuchi et al., 1991). Another bovine IEM causes the accumulation of orotic acid and embryonic lethality around 40 days gestation due to a mutation in the uridine monophosphate synthase gene (Schwenger et al., 1993). The Wisconsin hypoalpha mutant (WHAM) chicken displays hypercatabolism by the kidney of lipid-poor apoA-I, due to a mutation in the ABCA1 gene (Attie et al., 2002). Another line of chickens (rd) presented with riboflavinuria due to a mutation in the riboflavin-binding protein gene (MacLachlan et al., 1993). Given that most metabolic variants do not result in disease, they quite often go undetected. For example, a fishy off-flavor occasionally observed in cow's milk is caused by elevated trimethylamine levels, and is due to a mutation in the flavin-containing mono-oxygenase 3 gene. With no obvious outward phenotype, this IEM remained undiscovered until very recently. Production practices, the bulk collection of milk, further obscured the genetic component so completely that this bothersome mutation is currently maintained at high frequency in some cattle breeds (Lunden et al., 2003). These examples are clear evidence of genetic diversity with profound effects on animal metabolism. For these and many yet undiscovered genetic variants, the development of molecular markers and diagnostic tools provides the opportunity for optimization of livestock metabolic potential.

Nutritional Targets for Optimization

Feed conversion efficiency (FCE) directly affects the profitability of the livestock industry and indirectly affects the environment through the output and chemical composition of waste. Most efforts to improve swine FCE have focused on dietary manipulation. Although FCE is clearly influenced by genetics, this strictly dietary approach treats all pigs as having the same genetic potential. Genetic potential segregating in production populations has been estimated as moderately to highly heritable for a number of feed conversion traits including; average daily gain, daily feed intake, FCE, and FCE during the entire growing period (Bereskin 1986; Clutter and Brascamp, 1998; Hermesch et al., 2000). Perhaps to be expected, there are correlations between feed conversion traits and performance traits such as back leg weight, ham weight, and average back fat thickness (Bereskin 1986; Clutter and Brascamp, 1998; Hermesch et al., 2000). Although intermediate to high heritability provides an excellent rationale for targeted genetic improvement, traditional approaches would require a breeding program dependent on precise monitoring of individual animal feed intake and performance in the production setting. A marker assisted selection program could provide a much more cost-effective approach for optimizing these traits by requiring only an experimental resource population be phenotyped.

Attempts to control the odor of swine waste by dietary manipulation have met with mixed results. Dietary nonstarch polysaccharides have reproducibly been demonstrated to influence the partitioning of excretory nitrogen between urine and feces, ammonia emission, and the pH of urine, feces, and manure (Cahn et al., 1997, 1998a, b; Mroz et al., 2000). Reducing urinary pH by dietary supplementation with adipic acid was also found to reduce ammonia emission from both urine and manure (van Kempen 2001). Replacing dietary crude protein with amino acids has shown some success in reducing the emission of ammonia from pig waste, and in some cases, the production of volatile fatty acids (Sutton et al., 1999; Hayes et al., 2004; Otto et al., 2003). Obrock-Hegel (1997) found lower levels of indole, skatole and p-cresol in the air above manure from animals fed an amino acid manipulated diet. The nutriome is clearly capable of responding to dietary manipulation in a manner that influences waste composition. But is there heritable genetic potential for improvement in these traits currently segregating in swine populations? Can any genetic improvement be made?

To date few of studies have examined genetic factors of swine waste output and chemical composition. A study by Crocker and Robison (2002) measured groups of animals in the only attempt I am aware of to evaluate the genetic factors of swine waste quantity and composition. Their study has shown that significant differences exist in swine excreta output, as well as chemical composition between the white breeds (Large White x Landrace) and a Duroc x Hampshire cross. In addition, significant differences exist in the waste chemical composition between two Duroc lines with different testosterone levels (Crocker and Robison 2002). The Large White x Landrace cross had smaller quantities of all measured nutrients and chemical compounds and excreted significantly less P, Ca, Cu, Zn, and Fe than the Duroc x Hampshire cross. Within the Duroc breed, a line selected for high testosterone had a greater output of P, Ca and Cu, and larger quantities of all other nutrients except NH_3N and Fe.

Pig waste composition is not only a product of nutritional regimen and pig genetics, but also of the concerted activity of bacteria present in the pig's gastrointestinal tract (GIT). The bacterial community in the pig GIT is very complex (summarized by Gaskins et al., 2002), with several hundred species, and countless strains of bacteria being present. Mounting evidence suggests that the microflora present in the swine GIT is itself influenced by animal nutrition and pig genetics. For example, the interaction of bacteria with the host can clearly be influenced by variation in genetically encoded epitopes. Gaskins et al., (2002) noted that; "In the proximal small intestine the rate of digesta flow, and therefore bacterial washout, exceeds the maximal growth rate of most bacterial species. Accordingly, this intestinal region is colonized typically by bacterial that adhere to the mucus layer or epithelial cell surface". There are many publications that refer to bacteria interacting with specific glycoconjugates (receptors) on intestinal epithelial cells (Bock et al., 1988). One of the most highly studied interactions is *Escherichia coli* K88 (F4) with its receptor in the swine GIT (Jin and Zhao 2000). There are three alleles for this receptor. The receptor binds this strain of *E. coli* and permits colonization of the small intestine, causing diarrhea in neonatal and weaned piglets. Pigs with specific alleles of the receptors are not affected by this strain of *E. coli*. Although numerous studies have mapped and fine mapped the K88 receptor the identity and gene of the receptor remains to be determined (Jorgensen et al., 2003). Another porcine receptor binds to *E. coli* strain O139:K12(B):H1:F18ab serotype (Vogeli et al., 1996). What was thought to be the receptor was mapped to SSC6 using an epithelial cell-binding assay. The gene responsible has now been identified as the α (1,2) fucosyltransferase 1 gene (*FUT1*; Meijerink et al., 2000). Interestingly, this gene turned out not to be the bacterial receptor, but instead an enzyme that regulates the fucoslyation status of the receptor. These examples reveal that host genetics affects bacterial colonization of the swine GIT. Given the role of bacteria in waste composition, this raises the concept for an indirect role for the host genome in waste composition by its control over bacterial colonization and argues for the analysis of the relationship between pig and microbe genomes.

MOLECULAR GENETIC OPTIMIZATION OF SWINE NUTRITION

For many of the traits relevant to livestock nutrition, phenotyping of many nucleus herds or production populations is cost-prohibitive. Marker assisted selection (MAS) promises a powerful method for breeders to identify and propagate germplasm with enhanced production value (Dekkers and Hospital, 2002). Using this technology, breeding decisions are made using molecular diagnostics that indicate whether superior genetic alleles will be transmitted to the next generation. Data about animal genetics would also enable the development of production practices tailored to metabolic potential. In order to implement a MAS program, genetic loci that segregate with the desired variation in performance must first be identified. Such genetic loci can be identified by a co-segregation analysis of animal performance and genetic markers. A "genome scan" is conducted to find genetic loci currently causing reproducible variation in animal performance, and to map them to specific locations in the animal genome. The idea behind this approach is to find pieces of the genome that most often segregate through a structured pedigree along with animals that show variation in the trait of interest (Kruglyak and Lander, 1995 a,b; Darvasi, 1998). This involves a molecular tracking of genome segments, a systematic measure of traits, and the application of powerful statistical tools to determine linkage. This permits the identification of the chromosomal location(s), and ultimately the genes

that are affecting the phenotype. The precision with which this variation can be detected and mapped is determined by the heritability, the number of genes involved, the magnitude of phenotypic variation, and population size. The accuracy of phenotyping and genotyping are also critical. With a sufficient population size and a map with significantly dense markers, a genome scan can narrow the search for important genes to 10-50 centiMorgans (1 centiMorgan ~ 1 million base pairs; Da et al., 2000). Fine mapping (increasing marker density around detected loci) can further resolve the candidate locus to a chromosomal segment containing several hundred genes. Sequencing of livestock genomes will increasingly enhance the efficiency and precision with which important chromosomal segments, and the genes contained therein, can be identified.

Several studies have attempted to genetically map loci associated with swine reproduction (Rathje et al., 1997; Rohrer et al., 1999; Wilkie et al., 1999; Cassady et al., 2001; Braunschweig et al., 2001) and growth & body composition (Andersson et al., 1994; Wang et al., 1998; Rohrer and Keele, 1998a, 1998b; Marklund et al., 1999; Paszek et al., 1999; De Koning et al., 1999; Perez-Enciso et al., 2000; Rohrer et al., 2000; Malek et al., 2001a). A limited number of studies have also been undertaken to identify loci associated with meat quality (Andersson-Eklund et al., 1998; Milan et al., 1998; Wang et al., 1998; Moser et al., 1998; Yu et al., 1999; De Koning et al. 2000a, 2000b; Malek et al., 2001b; Thomsen et al, 2004). Nearly every pig chromosome has been found to harbor at least one locus that influences growth, carcass or meat quality traits. The resource population for most of these studies were F-2 crosses between Western pig breeds and exotic breeds (Chinese or Wild Boar). Though many of the loci identified in these exotic crosses have not yet been replicated in domestic swine breeds, a notable few do segregate in Western and Continental breed crosses. These include loci on pig chromosome 1 (SSC1), SSC7 and SSCX (Rohrer and Keele, 1998a & b; Malek et al., 2001a & b; Nezer et al., 2002). The regions described above are the subject of intensive verification and marker development in a diversity of commercial pig breeds and populations, and may permit for the selection for superior alleles in production programs around the world. Though seminal in nature, these first generation swine genome scans have for the most part ignored phenotypes relating to animal nutrition, feed conversion and waste production. It should be noted that it is not possible to determine what the antibiotic status of the diets the pigs in these studies were fed, although we might assume that the Swedish studies did not use antibiotics as these have been banned in that country since 1986 (Stein 2002). It makes good sense that gains sought in carcass composition and meat quality should be accompanied by more efficient feed conversion and decreased environmental impact.

Without financial or temporal constraint it could be argued that a genome scan for nutrition and waste traits ought to await the determination of heritability and breed variation. This information *would* help to design an ideal resource population with maximal phenotypic variation, one that would likely involve exotic germplasm. However, this is by no means optimal for technology transfer to the pork industry. The translation of data from these exotic breed crosses first requires the risky business of verifying that variation in the locus is present in industrial breeds, or potentially, introgression of germplasm from pigs that may be inferior for other production traits. Using germplasm already known to harbor some genetic variation in waste composition traits seems a superior approach (Crocker and Robison 2002). Relying on a population comprised of Yorkshire, Landrace, and Duroc breeds would also account for by far the majority of US commercial swine waste production.

To estimate the heritability of nutritional and waste composition traits, and eventually to pursue the important genes, requires the analysis of feed intake, animal performance and excretae from a large number of individuals (500 – 1000). Although electronic feeders are well established for monitoring individual feed intake in a group setting (Eissen et al., 1999; Hyun et al., 1997, 2001, 2002; Bruininx et al., 2001), individual penning is more consistent with the collection of individual waste. The technologies for determining the composition of feces, urine and manure also already exist. Fecal elemental composition analyses of Ca, Cl, Cu, P, K, Mg, Mn, Na, Zn, and Fe can be determined by inductive coupled plasma emission spectrometry. Fecal Carbon, Nitrogen and Sulfur can be assessed by combustion analysis (CNS). Fecal indole, (C_8H_7N), skatol (C_9H_9N), p-cresol (C_7H_80), ammonium (NH_4), and hydrogen sulfide (H_2S) can be assessed by solid phase micro-extraction (SPME). The analysis of the composition of urine, feces, and manure could all be informative, although collection of urine separately would probably require animals be catheterized, an approach that is probably unrealistic given the population size.

Metabolic profiles are standard in the human clinical setting and could be extremely valuable in a screen for loci that influence pig nutrition. A typical metabolic profile would examine carbohydrate-, lipid- and protein- metabolism, muscle-, bone- and fat- catabolism/metabolism ratios, liver-, kidney-, pituitary, and adrenal- function, hydration, potassium, electrolyte and fluid balances, acid-base balance, sepsis, stress, and hemolysis. Of particular interest are the small, low-molecular-weight compounds that serve as substrates and products in various metabolic pathways. These small molecules, estimated to number about 3000, include compounds such as lipids, sugars, and amino acids that can provide important clues about the individual's health and performance. The metabolome—the collection of all metabolites in a cell at a point in time—reveals much about that cell's physiological state at the time of sampling (Schmidt, 2004). As in the other "-omics," metabolomics data are generated with high throughput methods; nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS) using robotic automation are the dominant analytical techniques. Tremendous advances in technology have expanded the number of analytes that can be quantified simultaneously, and it is often as simple to measure many compounds as to measure one.

Finding the genes and capturing the value

With phenotypic data in hand a statistical analysis to estimate phenotypic and genetic parameters of feed conversion and waste traits could be conducted. Phenotypic and genotypic parameters of importance include the phenotypic mean and variance of each trait, additive and dominance variances, heritabilities in the narrow and broad sense for each trait, and phenotypic and genetic correlations among the feed efficiency, production traits and manure traits. Restricted maximum likelihood estimation (Patterson and Thompson, 1972) would permit estimates of variance and covariance components required by the heritabilities and genetic correlations. There are essentially two forms of genetic mapping. Linkage mapping exploits within pedigree associations between marker alleles and putative trait-influencing alleles based on co-segregation and recombination data (Lander and Botstein, 1989). Linkage disequilibrium mapping (LD), unlike linkage mapping, exploits both within-pedigree and across-pedigree associations between marker alleles and locus alleles (Schork et al., 2000). While linkage analysis depends on the co-segregation of a gene (locus) and a phenotype within a pedigree, association analysis, or LD

mapping, depends on measuring deviation from the random occurrence of alleles in a haplotype across pedigrees. As is being effectively demonstrated in human medicine, the construction of a dense LD map can facilitate the identification and positional cloning of genes underlying both simple and complex traits. Semiparametric methods are being developed for finding loci linked to complex traits, as well as for estimating the degree of LD between markers (Wu and Zeng, 2001; Zhao et al., 1998). These methods have the power to simultaneously capture the information about marker linkage (as measured by recombination fraction) and the degree of marker linkage disequilibrium (characterized as segments of identical-by-decent [IBD]).

The identification of economically valuable loci in swine in the future will rely on both linkage and LD mapping. The development of dense LD maps will require the discovery and mapping of many more markers than is currently available and will be enhanced with the parallel sequencing of the pig genome. Current animal genotyping technology relies on the use of simple sequence length repeats (SSRs) that are distributed across the swine genome every 10 to 100 kilobase pairs (1 kilobase pairs = 1000 base pairs). Although they have been instrumental in generating livestock genetic maps, SSRs are not presently amenable to high-throughput genotyping platforms. Additionally, SSRs identical-by-state (IBS) are not always IBD, further complicating the analysis of genotypic information. Unlike SSRs, single nucleotide polymorphisms (SNPs) are abundant (1 heterozygous position per 1000 base pairs in humans (Chakravarti, 1999) and amenable to high-throughput genotyping-platforms. In addition, SNPs are extremely stable, arising at a rate of only 1 mutation in $2x10^{-8}$ nucleotides in humans (Sachidanandam et al., 2001). Because the rate of back mutation is equally rare, IBS of SNPs almost exclusively represents IBD. SNP-based marker systems are therefore ideal for establishing the genetic identity and composition of livestock animals. The efficiency of LD analysis is restricted by the number of alleles per marker, the frequency of marker alleles and marker density (Kruglyak, 1997). While in most cases each SNP has only two alleles, they occur with such great abundance over the genome, groups of neighboring SNPs have alleles that show distinctive patterns of LD and as such create a haplotypic diversity that can be exploited in direct linkage and direct association studies (Nickerson et al., 1992). More advanced algorithms examine the association of traits and particular haplotype frequencies (Fallin et al., 2001). In addition, because SNP genotyping provides a mechanism for the identification of chromosomal regions inherited by descent, genome scans can be extended across pedigrees of distantly related animals. This will enhance the power of detection, as well as reveal specific populations and individuals suitable for fine-mapping efforts aimed at identifying economically valuable genes.

Functional genomics for optimizing swine nutrition

Functional Genomics is an emerging discipline focused on identifying and understanding the molecular and genetic networks that enable the genome to sense and respond to the environment. What distinguishes functional genomics as a discipline, is its reliance on the global analysis and targeted manipulation of gene expression. Recently developed cDNA and oligonucleotide-based microarray (gene-chip) technologies provide the technical capacity to simultaneously examine the expression of the entire genome in a single experiment. Microarrays come in essentially two flavors, those based on cDNAs (DNA copies of messenger RNA, mRNA) and those based on oligonucleotides. For most species, the application of cDNA arrays has preceded that of oligo arrays because probes for cDNA arrays may be derived from the abundance of available cDNA

clones, whereas oligo probes must be designed from the available sequence data. This oligo design process generally requires a large collection of expressed sequence tags (EST), snippets of sequence from cDNA clones. This data is clustered using algorithms that compare DNA sequences, and oligos are designed based on an intensive evaluation of potential oligos relative to a series of molecular parameters. There are few well designed studies that have compared the performance of cDNA and oligo arrays, however, these are beginning to emerge and the early evidence seems to indicate that the two platforms perform similarly. Wang et al., (2003) found that both perform equally well at consistently detecting 2-fold changes in rare mRNA levels and that both platforms were highly reproducible ($r>0.95$) when replicate slides were hybridized with targets generated from different batches of RNA.

Interest in oligo-based arrays is stimulated by several factors including the ease of managing probe synthesis and flexibility of probe design. The public availability of more than 284,853 porcine EST sequences, the availability of the human genome sequence, and the impending completion of sequencing of the bovine and porcine genomes will allow the flexibility to design probes to distinguish among gene family members, and to detect alternately spliced mRNAs. A very attractive feature is the ease of extensibility of the probe set; probes may readily be added, removed or exchanged. The US swine functional genomics community has completed the synthesis of a first generation >13,000 element 70-mer oligo array and distribution of this array to the research community has commenced. This microarray is already providing valuable, reproducible results at the University of Minnesota in the analysis of gene expression in pig endocrine and immune tissues (Fahrenkrug and Murtaugh, unpublished).

A change in the expression of a gene in response to specific environmental conditions implicates it as a member of a regulatory network important for that specific physiological response. Most traits are due to the action of many genes (i.e. complex), although usually dominated by the action of a few "master" genes. Zeroing in on the most important genes in any given tissue remains a challenge due to the hundreds of genes that can be seen to change under varying conditions. Distinguishing between regulator, effector and bystander tissues and genes, requires a detailed understanding of trait physiology and gene function. Alternatively, the field of candidates detected by expression analysis can be narrowed to those few that are detected as positional candidates from genetic studies. The "comparative genomics" approach leverages data generated by decades of well-funded basic research related to human health and physiology. It also makes use of data from model organisms, whose genomes are completely sequenced and have been subject to exhaustive genetic analysis. A hypothesized functional role for a gene-product in livestock tissues would be bolstered by its observed importance in homologous tissues of humans or model organisms.

Marker assisted selection

The point of finding genes with significant roles in metabolic, performance, and waste traits is to enable the development of highly accurate diagnostic tests for enhanced animal breeding and for application to production practices tailored to animal potential. Besides the single-gene IEM discussed above, few molecular diagnostics have thus far been implemented in livestock breeding programs. Application of MAS to introgression provides a potential method to increase the recovery of desired genetic alleles, while minimizing the admixture of new and old genetics

at other loci. If many loci are known, and favorable alleles are present in different lines or breeds, genotype-building strategies can be designed to combine favorable alleles at many loci (Dekkers and Hospital, 2001). In the future, MAS will be most useful when the costs of molecular diagnostics are offset by savings in phenotype evaluation, such as those associated with the traits emphasized in this paper.

Retooling the swine genome

Modification of the livestock genome by genetic engineering has many potential applications to agriculture and medicine. Genetic engineering includes both the addition of exogenous genetic material, and the modification/manipulation of the endogenous genome. Before cloning (B.C.), the generation of transgenic livestock was primarily limited to pronuclear injection of DNA expression constructs into ova (Gordon et al., 1980; Wagner et al., 1981 a,b; Harbers et al., 1981; Brinster et al., 1981; Gordon & Ruddle, 1981) and was concerned with the production of desired protein products in animal tissues or body fluids. Although pronuclear injection remains the primary method for mouse transgenesis, low rates of germ-line transmission (0.31%-1.73% of microinjected ova) and expensive husbandry costs have impeded the widespread application of genetic engineering to livestock (Pursel et al., 1990). However, one notable case of pig transgenesis using this approach has bearing on pig metabolism and waste composition.

Golovan et al. (2001) generated transgenic pig lines with the *E. coli appA* phytase gene under control of the parotid secretory protein promoter (PSP/APPA). These pigs were generated to counter the fact that pigs are unable to digest plant phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), which accounts for up to 80% of phosphorus in common cereal grains, oil seed meals, and by-products (Jongbloed and Kemme, 1990; Kornegay, 2001). Pig diets are commonly supplemented with bioavailable mineral phosphate, a practice that is nutritionally successful but environmentally counterproductive. PSP/APPA directed salivary phytase production in these pigs provides essentially complete digestion of dietary phytate phosphorus, eliminates the requirement for inorganic phosphate supplements, and reduces fecal phosphorus output by up to 75% (Golovan et al., 2001).

Cultured-cell transgenesis in tandem with somatic cell nuclear transfer (SCNT, or cloning) is now the preferred method for generating transgenic pigs. For example, porcine fibroblasts have been demonstrated as suitable for transgenesis or homologous recombination prior to SCNT, and have permitted the generation of transgenic and knockout pigs for potential utility to xenotransplantation (Lai et al., 2002; Dai et al., 2002; Ramsoondar et al., 2003). Using this approach, additional manipulation of the pig genome to improve the composition of waste may be possible. However, despite the promise and some clear success, it remains to be seen whether consumers will accept genetically modified pigs into the food chain, whatever the product quality and positive environmental benefits.

CONCLUSIONS

The future interface of swine nutrition and molecular genetics shows great promise. Identifying genetic variation in swine nutrient utilization, performance, and waste composition provides an excellent starting point for the development of technologically advanced approaches to

improving animal germplasm, optimizing nutritional regimens, and to implementing environmentally sound production practices for long-term sustainability of swine agriculture. Bringing the economic and intellectual resources of producers, nutritionists and molecular biologists to the table is critical to a successful collaboration.

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