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Possibilities for selection against boar taint

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Introduction

The amount of boar taint due to high levels of androstenone and skatole is affected by a number of factors including the degree of sexual maturity, environment, dietary and management factors, and genetics. These factors have been discussed in previous papers by B.B. Jensen (Factors affecting the level of skatole) and M. Bonneau (Factors affecting the level of androstenone). To briefly summarize, sexual maturity can affect levels of both androstenone and skatole, while skatole is more affected by diet and environment and management factors than androstenone, unless these factors also affect the degree of sexual maturity.

Both androstenone and skatole are affected by genetic factors, and distinct breed differences in the levels of these compounds have been identified in a number of studies. Between five to eight percent of purebred Hampshire, Yorkshire and Landrace boars have high concentrations of androstenone in fat, whereas 50 percent of Duroc intact males have high concentrations; fat skatole levels also differ between breeds [1-5]. Low levels of androstenone measured in some market weight boars may be a consequence of sexual immaturity, since the testis may not be producing peak levels of steroids. Low levels of skatole may be due to low production of skatole in the gut due to dietary and other factors and not due to a genetic predisposition to decreased boar taint. However, genetic selection for animals with low boar taint should be possible due to the relatively high heritability (range from 0.25 to 0.87) of fat androstenone [6]. Likewise, the heritability of skatole is 0.55 for Landrace and 0.23 for Duroc [7]. Tajet et.al [7] also reported a positive genetic correlation between skatole and androstenone of 0.36 for Landrace and 0.62 for Duroc. Thus, genetic selection for low levels of one boar taint compound may result in an overall decrease in boar taint compounds.

Previous attempts at selection against androstenone resulted in decreased performance and sexual maturation due to lower production of androgens and estrogens. For example, Willeke et al. [8] observed a delayed puberty in the gilts of a "low androstenone" line. Using a selection index associating androstenone and bulbo-urethral gland thickness [9] resulted in increased bulbourethral gland size and no reduction in androstenone due to inaccuracies in estimated genetic parameters for these traits. It is therefore desirable to identify animals that have a decreased genetic capacity to accumulate androstenone in fat while maintaining the normal levels of testicular steroids that are characteristic of intact males. The development of genetic markers to identify these pigs would allow the selection of pigs that are free of taint from androstenone but otherwise grow as normal boars.

Development of genetic selection tools

QTL Identification and Use for Selection

Genetic markers can be developed using a number of different experimental approaches. Two common approaches are the use of anonymous markers and the candidate gene approach. Quantitative trait loci (QTL), which are chromosomal regions that contain genes that affect a particular trait, can be identified by comparing the genotype of anonymous markers located throughout the chromosome to the phenotype or trait of interest. The QTL is then described by the position of the markers that are most closely associated with differences in the trait phenotype. Because these markers are located on the chromosome close to the gene responsible for the trait, they are "linked" or in linkage disequilibrium with this gene.

Candidate genes can be identified by examining genes located within a QTL region previously detected using anonymous markers (positional candidate gene approach) or by directly developing markers within genes expected to influence the phenotype of interest (functional candidate gene approach). The candidate gene approach is most effective when gene function is well characterized or if the QTL has been mapped to a very small region in which the identity of the genes is known.

Once a marker genotype has been associated with a preferred phenotype, the marker genotype can be used for making selection decisions, a process referred to as marker assisted selection. Individuals with the marker genotype that is linked to the preferred or improved phenotype are selected for their superior QTL genotype on the basis of their linked marker genotype. This process does not require knowing the gene or genes responsible for the QTL effect and for successful, multiple generation selection the marker should be tightly linked to the QTL in order to reduce the possibility of recombination events disrupting the marker-QTL association. Ultimately the best marker involves identifying the genetic change in the gene that directly affects the trait and using that polymorphism as the marker for marker assisted selection.

Several QTL's for androstenone have been reported. Quintanilla et al. [10], using a three generation experimental cross between Large White and Meishan pig breeds, found significant gene effects using two different statistical methods on chromosomes 3, 7, and 14. The QTL on chromosome 7, close to the major histocompatibility complex of the pig (swine leucocyte antigen system, SLA), showed the largest effects. Two candidate genes in this region were investigated, CYP21 and CYP11a, but found not to be responsible for the QTL. A dominant gene affecting fat androstenone has been described by Fouilloux et al. [11], but this gene is not associated with the SLA region. Varona et al. [12], using a commercial Landrace population, could not find any significant QTL for androstenone in the 10 chromosomal regions they analysed. However, they did find a significant QTL for fat skatole on chromosome 6. Lee et al [13], using a Large White × Meishan crossbred population, also found a QTL for skatole, but it was located on chromosome 14. They also found a QTL for androstenone and boar flavour on chromosome 6. The genes responsible for these QTL's have not yet been identified.

Identification of Candidate Genes from Metabolic Studies

Another approach to developing genetic markers is to investigate polymorphisms, usually Single Nucleotide Polymorphisms (SNP), in candidate genes. Candidate genes can code for key enzymes in the metabolic pathway of boar taint compounds and ideally should not involve other pathways, such as anabolic steroid metabolism. A number of key enzymes involved in the metabolism of both androstenone and skatole have been identified to date.

Genes for Androstenone

We have identified cytochrome b5 as a key protein regulating the synthesis of androstenone in the testis. Androstenone and the sex steroid hormones are produced from pregnenolone by the andien-β synthase enzyme complex, which consists of cytochrome b5, CYP17, and reductase enzymes. We isolated each of these proteins from pig testis and studied the synthesis of the 16-androstene steroids (precursors of androstenone) and the sex steroids using an in vitro reconstitution system. When only CYP17 was present, only the sex steroids were produced, but when cytochrome b5 was added, the 16-androstene steroids were made [14]. Levels of cytochrome b5 in the testis were also correlated with fat androstenone levels and 16-androstene steroid synthesis rates in vitro [15]. Recently, we have reported a G/T polymorphism at -8bp upstream from the translation start site that dramatically affects androstenone synthesis and accumulation in fat [16].

For the metabolism of androstenone, Doran et al. [17] reported that the conversion of androstenone to 3β-androstenol was greater in liver microsomes from Large White compared to Meishan pig breeds. The expression of 3β-hydroxysteroid dehydrogenase (3βHSD) mRNA was also higher in the Large White breed, which is characterized by lower androstenone levels than the Mieshan breed. They have thus suggested that 3βHSD could be a key enzyme involved in the metabolism of androstenone. No polymorphisms in 3βHSD have yet been reported.

We have recently reported on the role of hydroxysteroid sulfotransferase (SULT2A1) in the formation of androstenone-sulfate and the effect on androstenone accumulation in fat [18-20]. SULT2A1 activity was negatively correlated with 5α-androstenone concentrations in fat. Animals with high concentrations of 5α-androstenone in fat and low SULT2A1 activity had corresponding low levels of SULT2A1 protein. Real-time PCR analysis indicated that the expression of the SULT2A1 mRNA was increased 3.5-fold in animals with high levels of the protein. A mutation was identified within the porcine SULT2A1 coding region; however, this did not affect the amino acid sequence. These findings suggest that the accumulation of 5α-androstenone in fat is influenced by the proportion of the sulfoconjugated forms of 5α-androstenone present in the circulation. Low SULT2A1 activity will result in decreased levels of the sulfoconjugated form of 5α-androstenone and thus more of the unconjugated form that can accumulate in adipose tissue in high boar taint pigs.

Genes for Skatole

The metabolism of skatole in the liver is an important factor regulating skatole accumulation in the carcass. Gilts and barrows can efficiently metabolise and clear skatole, while some boars have low levels of the enzymes important in skatole metabolism and produce carcasses tainted with high levels of skatole. We have studied the metabolism of skatole with liver cell fractions to identify the metabolites produced [21] and the enzymes important in this metabolism using specific inhibitors against the enzymes. The enzymes CYP2E1 [22,23,5], CYP2A6 [24], aldehyde oxidase [25] and phenol sulfotransferase (SULT1A1) [26,27] are related to skatole metabolism and clearance. The molecular cloning and functional characterization of CYP2A6 and SULT1A1 have been reported. CYP2A6 was cloned and sequenced from pig liver and a deletion mutation was found in the coding region which caused a complete lack of enzyme activity [28]. For SULT1A1, a SNP was identified at 546bp within the coding region that caused a significant decrease in enzyme activity [29].

Microarray and Proteomics Approaches

A major limitation of studying metabolic pathways to identify candidate genes is that you can only find those genes that are directly involved in the particular pathway being studied. A much broader approach is to conduct transcriptional profiling using DNA microarrays, in which the expression of thousands of genes (the 'transcriptome') is compared between animal with two different phenotypes. We have conducted preliminary studies using human DNA microarrays to compare gene expression profiles between pigs with low or high levels of steroidogenesis [30]. We are continuing this work to identify genes related to the accumulation of skatole and androstenone in pig carcasses.

In addition to transcriptome analysis, proteomic approaches examine the levels of different proteins that are expressed between animals with two extremes of a trait. This involves separation of the proteins by 2-D electrophoresis or chromatographic methods, followed by quantification of the proteins and identification by mass spectrometry. Taken together, these analyses can identify differences in the expression of genes that may be responsible for the trait of interest.

Conclusion

Boar taint due to high levels of skatole and androstenone is highly heritable and not all market weight entire males have boar taint; it should thus be possible to select for pigs which do not have boar taint. A number of candidate genes for boar taint have been identified and work is continuing to develop genetic markers for low boar taint based on SNP's in these genes.

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