DEVELOPMENT OF GENETIC MARKER TECHNOLOGY I: QUANTIFYING BOAR TAINT

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Abstract—**As a first step in the development of genetic lines of pigs with decreased levels of boar taint, the present study investigates the distribution in levels of androstenone and skatole of 300 uniquely identified entire boars, slaughtered at a weight greater than 110 kg and less then 200 days of age. The boars comprised of 50 each from 6 different lines of pigs (3 sire lines A, B, C and 3 dam lines X, Y, Z). Back fat was collected from each animal and the levels of androstenone were analysed by ELISA and levels of skatole analysed by HPLC. The C line had the lowest amount of boar taint, as determined by average levels of both androstenone and skatole. The highest androstenone levels were in the A breed line, with 100% of the animals above the acceptable cutoff level for androstenone. The highest skatole levels were found in the Z line with 20% of this line over the cutoff level for skatole. The ultimate goal of this two stage project is to identify genetic markers that are effective in reducing boar taint in these lines. For the next stage of research, we will genotype all animals for our SNP marker set and then carry out association analysis to determine the effectiveness of each marker in the six different lines. These markers can then be used in breeding programs to develop lines of pigs that are free of boar taint, but otherwise grow as normal boars. This will significantly improve the profitability of pork production and address animal welfare concerns about castration, that are already of increasing concern in several EU countries**.

Index Terms— **Androstenone, Boar Taint, Dam Line, Skatole, Sire Line.**

I. INTRODUCTION

Boar taint is an unpleasant odour that is associated with the fatty tissue and, more precisely with the nonsaponifiable fraction (Bonneau, 1982) of boar meat. In 1959 Craig and Pearson carried out the first attempts to identify the compound(s) responsible (Bonneau, 1982) for the taint. However, it was suggested by Sink (1967) that steroids which have a musk-like odour could be responsible for boar taint. Two main compounds were identified as being the cause of boar taint. Androstenone was isolated as being a sex odour in the fat of the entire male. Skatole was also identified as being a prime cause of boar taint. However, skatole is not unique to the pig and can be found in all mammals. There are many other compounds that may contribute to the odour and flavour of meat, however it is the levels androstenone and skatole that are the main contributing factors towards boar taint.

Androstenone is fat-soluble and can be used to predict the presence of boar taint in backfat. Skatole is useful for determining levels of boar taint in both fat and lean meat since it is both fat and water soluble (Lundström *et al*, 1988). It has been suggested that there may be an interaction between the effects of skatole and androstenone so that the levels of one compound may determine the importance of levels of the other (Warriss, 2010). Although it has been observed that sensitivity to androstenone varies between men and women, it is thought that there is no variation between sexes for skatole sensitivity. Due to the natutral variation in sensitivity, it has been suggested that, for meat acceptability, the maximum concentrations in fat should be: Androstenone: 1.0 μg g^{-1} and Skatole 200 ng g^{-1} (Warriss, 2010).

The susceptibility of individual pigs to express boar taint is partially determined by the pig's genetics. Pigs that produce less of these compounds are less susceptible to taint, and pigs that are able to efficiently break down and excrete these compounds are also less susceptible to taint (Zamaratskaia and Squires, 2009). Male pigs are normally castrated to prevent boar taint, but this reduces feed efficiency, lean gain and has a negative impact on animal welfare. Alternatives to surgical castration are immunocastration (Pauly *et al*. 2009) and the use of genetic markers to select pigs that have reduced propensity to produce boar taint (Zamaratskaia and Squires, 2009). It has been posited that genetic markers within the affected pathways can be used as tools to identify those pigs with a greater chance of developing boar taint, and removing those pigs from the breeding pool. This approach could potentially result in a lasting solution for boar taint. In terms of production efficiencies, it is anticipated that the use of entire male pigs will improve profits per pig by more than $5, which is based on analyses that were conducted previously by de Lange and Squires (1995) and adjusted to 2010 economic conditions in Canada.

To date, several genetic markers have been identified that have a significant impact on androstenone and skatole (Squires and Schenkel, 2010). Certain combinations of markers have been found to represent a solution to the boar taint problem in certain lines of pigs. On-going research will work toward completing a 'tool-box' of markers that will be useful to all lines of pigs. The heritability of both androstenone and skatole is moderate to high, but previous attempts to select for pigs with low boar taint have resulted in reproductive problems (reviewed in Zamaratskaia and Squires, 2009). The development of specific genetic markers for boar taint would minimize these negative effects on reproduction. It has been postulated that genetic markers within the affected pathways can be used as tools to identify those pigs with a greater chance of developing boar taint, and removing those pigs from the breeding pool (Squires and Schenkel, 2010).

The objective of this paper is to report on the first stage of development of low boar taint pigs based on the use of genetic markers, that is the identification of average levels and variation of both androstenone and skatole in 6 different breed lines.

II. MATERIALS AND METHODS

Samples description: 300 backfat samples were collected from uniquely identified entire boars, slaughtered at a weight greater than 110 kg and less the 200 days of age. The samples comprised of 50 pigs, each from 6 different lines (3 sire lines A, B, C and 3 dam lines X, Y, Z). Blood samples (5mL in standard EDTA tubes) were collected from all boars prior to slaughter and DNA stored on Whatman FTA cards. Where possible, samples of DNA on Whatman FTA cards from each pigs' dam and each pigs' sire were also provided.

The samples of backfat (5g), with a small piece of muscle attached, were obtained from the loin on the day of slaughter and frozen the same day. The individually identified samples were then shipped on dry ice to the University of Guelph for analysis. All phenotypic, performance, genetic and lineage infromation was also provided with the corresponding animal ID number.

Data: Fat samples were analyzed for 5α-androstenone by Enzyme Linked Immunoassay (ELISA) (Squires and Lundström, 1997) and for skatole by high performance liquid chromatography (HPLC) with fluorescence detection (Lanthier et al., 2007). Animals with low levels of androstenone were only included in the study if they had plasma oestrone sulphate greater than 20 ng/ml (Sinclair *et al*., 2001), in order to control for low androstenone levels due to sexual immaturity.

III. RESULTS AND DISCUSSION

Overall from the original 300 samples supplied, 9 were rejected due to being non viable samples and of the remaining 291 samples, 288 were analyzed for androstenone and 286 were analysed for skatole. The number of samples was reduced due to some of the samples not being able to yield results. The results are summarized in Table 1 below.

Table 1: Total samples received and analyzed organized by breed.

		Samples Analyzed:	
Breed line	**Total Samples**	**Androstenone**	**Skatole**
Line A	52	52	52
Line B	57	57	57
Line C	44	44	43
Line X	41	38	38
Line Y	47	47	46
Line Z	50	50	50
Total	291	288	286

The average and median levels of androstenone and skatole in the different breeds and the number of animals with levels above the acceptable cutoff values are summarized in Table 2. The overall average for the two boar taint compounds in fat was 1.572 ug/g for androstenone and 69.4 ng/g for skatole. Accepted cutoff levels for the presence of detectable boar taint are 1.0 ug/g for androstenone and 200 ng/g for skatole.

Of the tested animals, 37.5% overall were above the 1.0 ug/g cutoff for androstenone, and 7.5% were above the 200 ng/g cutoff for skatole. The C breed line had the lowest amount of boar taint, as determined by both androstenone and skatole average levels and the percentage of animals above the acceptable cutoff values. The highest androstenone

levels were in the A breed line, with 100% of the animals having unacceptable levels. The highest skatole levels were found in the Z breed line, with 20% of this breed over the cutoff level for skatole.

Table 2: Results of Androstenone and Skatole analysis.

Breed line	Androstenone average (ug/g)	Androstenone Median (ug/g)	% Above 1ug/g	Skatole Average (ng/g)	Skatole Median (ng/g)	% Above 200ng/g
Line A	4.602±0.411	3.365	100.0	67.7±9.67	37.6	7.7
Line B	1.036±0.135	0.754	29.8	49.4±6.69	32.1	3.5
Line C	0.456±0.045	0.358	4.5	39.2±2.52	39.0	0
Line X	0.813±0.213	0.512	13.2	63.8±7.47	45.2	2.6
Line Y	1.070±0.185	0.581	29.8	63.1±8.37	39.6	6.5
Line Z	0.961±0.121	0.687	32.0	129.5±13.69	87.9	20.0
Overall	1.572±0.124	0.702	37.5	69.4± 4.06	43.6	7.5

The levels of androstenone and skatole for each breed are further illustrated in Figure 1 and Figure 2. The breed lines B, X and Y have very similar levels and distributions of both androstenone and skatole. The B breed line has slightly lower skatole levels, and the Y breed line has slightly lower androstenone levels. The range of androstenone in each breed line varies. The A breed line has the largest range as well as the highest average in androstenone levels, and the Z line has the largest range as well as the highest average for skatole levels. The C line has the smallest range for both androstenone and skatole levels.

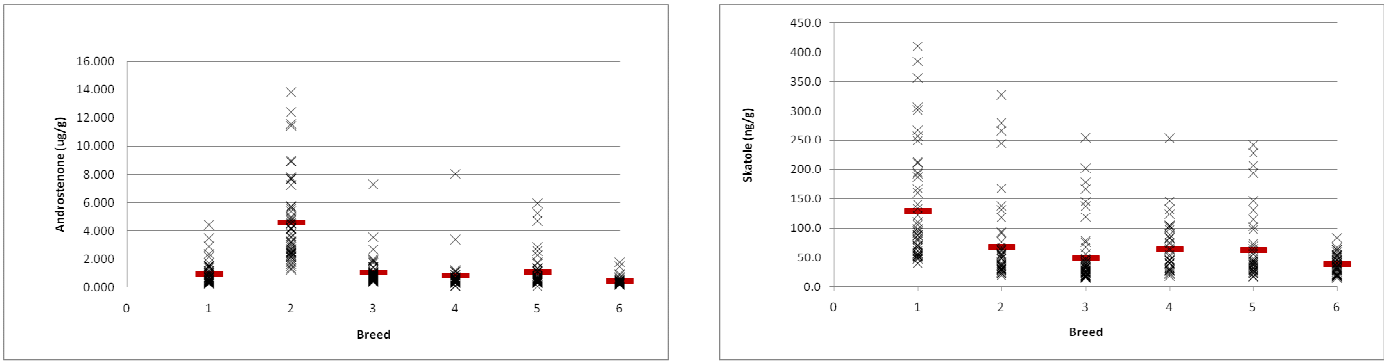

Figure 1: Androstenone and skatole levels in boar fat samples organized by breed line. (1) Line Z, (2) Line A, (3) Line B, (4) Line X, (5) Line Y, (6) Line C. Red lines indicate the average for each breed line.

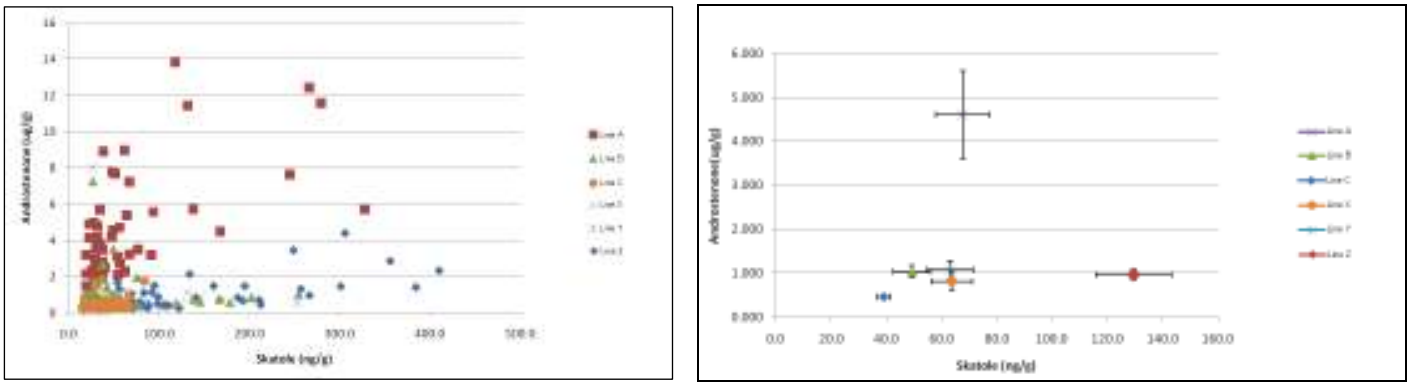

Figure 2: A comparison of the individual (left) and average (right) of androstenone and skatole levels in analyzed fat samples arranged by breed line.

We have previously calculated that the application of our current marker set to lines of pigs, to produce pigs that were homozygous for the favourable SNP alleles, would decrease the average (geometric mean) fat skatole levels by 20-53% and fat androstenone by 26-61%, depending on the line (Squires and Schenkel, 2010). We now need to determine the effectiveness of these markers in the breed lines.

IV. CONCLUSIONS

Castration to prevent boar taint limits productivity and increases animal welfare concerns of commercial pork production, so alternative strategies for controlling taint are needed. The development of low boar taint lines of pigs by using genetic markers would provide a long term solution to the problem. This will improve pork quality and consistency, profitability, environmental impact and animal welfare in pork production by eliminating the need for castration of male piglets. The ultimate goal of this two stage project is to identify effective SNPs for boar taint in the sire and dam lines. This next stage of research will require SNP genotypes to be identified for each animal and then carry out association analyses to validate the SNP markers and determine their individual effectiveness in the lines. The markers can then be used in breeding programs to develop lines of pigs that are free of boar taint but otherwise grow as normal boars. This will significantly improve the profitability of pork production and address animal welfare concerns about castration that are now a hot topic in several EU countries.

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