CONTROL OF BOAR TAINT BY USING GENETIC MARKERS: A SINGLE NUCLEOTIDE POLYMORPHISM IN THE CYP2E1 GENE PROMOTER AFFECTS SKATOLE CONTENT

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Abstract – The prevention of unpleasant boar taint is the main reason for castration of male piglets. This study aimed to investigate how the malodorous compound skatole is affected by a single nucleotide polymorphism (g.2412 C>T at -586 ATG) in the porcine cytochrome p450 II E1 (CYP2E1) gene. 119 boars of two commercial Duroc-sired crossbred populations were investigated. Skatole and androstenone in backfat averaged 114 ± 125 ng/g and 1206 ± 895 ng/g melted fat, respectively. The frequency of the genotypes CC, CT, and TT were 25, 52, and 23 %, respectively. CC boars had the highest average skatole levels (175 ng/g) compared to CT (92 ng/g) and TT (93 ng/g). Applying suggested sensory threshold levels for skatole (> 150 ng/g) and androstenone (> 2000 ng/g), 30 % of the carcasses may be unacceptably tainted while the proportion of tainted carcasses is significantly higher within genotype CC (56.7 %) compared to genotypes CT (24.3 %) and TT (14.8 %). Effective reduction of tainted carcasses appears feasible applying marker assisted selection.

Key Words – skatole; androstenone; cytochrome; CYP2E1; SNP; marker assisted selection, Duroc; crossbreds

I. INTRODUCTION

Surgical castration of male piglets without anesthesia is controversial for animal welfare reasons, and will be banned in the European Union by 2018. Castration effectively prevents the occurrence of the so called "boar taint" that is suggested to be mainly caused by the accumulation of the testicular steroid androstenone (5α-Androst-3ene-16one) and skatole (3-methylindole) in fat tissue unless they are metabolised by hepatic enzymes responsible for oxidation and sulfation (1). Skatole metabolism rather than intestinal skatole production is suggested to affect skatole levels in the backfat. Odor perception of androstenone and skatole is often described as urine-like and faecal-like, respectively. Varying consumer rejection thresholds for androstenone (500 to 1000 ng/g fat) and skatole (200 to 250 ng/g fat) have been reported previously (2). For skatole, recent studies suggest the acceptance thresholds to be considerably lower, i.e. 150 ng/g fat (3). Similarly, German (4) and French (5) consumers did not rate boar loin chops worse than meat of castrates and gilts as long as the skatole levels were low.

Thus, the primary goal is to reduce skatole levels to prevent consumer dissatisfaction along with the fattening of boars. Breed, diet composition, feeding strategies, feed intake, environmental factors, age at puberty and the genetic background were reported to affect the individual's level of skatole (6). Recently estimated heritabilities of about 0.5 support the hypothesis of a major genetic influence on skatole levels in pigs (7; 8). Quantitative trait loci (QTL) regions for skatole have been identified by genome scans with microsatellite markers (9) and (10) on sus scrofa chromosome (SSC) 6, 13 and 14. More recently genome wide association studies using Porcine 60K SNP bead chips were applied to map associations with skatole levels in fat on SSC6p in the initial 6 Mb of the chromosome (11). On the other hand, the functional approach comprises of investigations to estimate gene expression patterns (12; 13) and of association studies between single nucleotide polymorphisms (SNP) within candidate genes supposedly affecting backfat skatole levels (14; 15). Special emphasis is laid on genes coding for enzymes that are involved in the liver metabolism of skatole as a decreased skatole metabolism will finally lead to an accumulation of skatole in the fat (16; 17). There is ample evidence that different cytochrome P450 enzymes catalyze the turnover of skatole in phase I. The clearance of skatole in phase II is metabolized by phenol sulfotransferase (18).

The present study aimed to investigate the segregation of a SNP within the promoter of the CYP2E1 gene (14) in two commercial pig populations of entire males in Germany. Subsequently, its association with skatole levels should be analyzed and a potential selection strategy will be discussed.

II. MATERIALS AND METHODS

Two Duroc-sired crossbreds of entire male pigs (n = 119) with 50% and 75% Duroc blood, i.e. A_{Du50}: Duroc x (Large White x Land Race) and B_{Du75}: Duroc x (Duroc x (Large White x Land Race)), respectively, were raised at two commercial farms. Average hot carcass weight was 99.9 ± 7.1 kg. Mean backfat thickness was 16.8 ± 2.6 mm and lean meat percentage averaged 56 % as estimated by AutoFOM carcass grading.

Backfat samples excised from the neck region, approximately 10 cm from the usual carcass split line, were analysed for androstenone using gas-chromatography/mass spectrometry (GC-MS); for the quantification of skatole and indole a liquid chromatography (RP-HPLC) procedure with fluorescence detection was used as described in (19). Results are given as ng per g melted back fat.

Genomic DNA was extracted from adipose tissue using a modified phenol/chloroform method. SNP g.2412 C>T (at -586 ATG) in CYP2E1 was analyzed by direct sequencing on an ABI-PRISM 3100® capillary analyzer. Gene-specific primers FOR (5'-CCC TTA ATT TTC TAC AGT AA) and REV (5'-GCA ACC CCA GTG GTA C) were created based on the available sequence (GenBank Accession Number AJ697882). The PCR product covered 209 bp of the promoter region from position 2310 to 2518 of the reference sequence.

Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, USA). Effects of crossbred type (A_{Du50}, B_{Du75}), CYP2E1 genotype (CC, TT, CT) as well as their interaction effects on carcass traits and boar taint compounds were assessed with a fixed effect model (ANOVA) using PROC GLM.

$y_{ijkl} = \mu + \text{crossbred type}_i + SNP_j + \text{abattoir}_k + (\text{crossbred type x } SNP)_{ij} + e_{ijkl}$

Due to the skewed nature of the androstenone, skatole, and indole values, data were transformed with natural logarithm before ANOVA to achieve normality. Type III sum of squares were used to calculate F-ratios.

Effects of crossbred type, CYP2E1 genotype and their interaction effect on frequency of putatively tainted carcasses were estimated using the GLIMMIX procedure of SAS applying a generalized linear model:

$$Logit\left(\frac{\pi_{rs}}{1-\pi_{rs}}\right) = \eta_{rs} = \varphi + \alpha_r + \beta_s + \alpha\beta_{rs}$$

where π_{rs} is the probability of exceeding the threshold level (e.g. skatole > 150 ng/g), ϕ is the overall mean effect, α_r is the fixed effect of genotype (levels: CC, CT, TT); β_s is the fixed effect of crossbred type (levels: A_{Du50}, B_{Du75}); $\alpha\beta_{rs}$ is the fixed effects of interaction of genotype and crossbred type.

III. RESULTS AND DISCUSSION

Average concentrations of skatole, indole, and androstenone in backfat were 114 ± 125 ng/g, 72 ± 50 ng/g, and 1206 ± 895 ng/g melted fat, respectively. With respect to putative sensory rejection thresholds for androstenone (> 2000 ng/g) and skatole (> 150 ng/g) about 30 percent of the carcasses in this study may be unacceptably tainted as either of the thresholds was exceeded. Considerable inter-laboratory differences for determination of malodorous compounds have been reported (20). The proportion of potentially tainted carcasses may thus not safely be estimated and compared to previous studies; conclusions from relative differences due to breed or SNP effects as intended in the present investigation are feasible though.

In total, nearly 22 % of the boars exceeded skatole levels of 150 ng/g; this was significantly more pronounced ($X^2 = 9.34$; $p < 0.002$) in breed type B_{Du75} (33 %) compared to breed type A_{Du50} (10 %).

While about half of the tested boars were genotyped as heterozygous for both crossbred types, they differed, however, with respect to the corresponding homozygous genotypes. CC was more frequent within breed type B_{Du75} (37 %) compared to A_{Du50} (14 %). Correspondingly, genotype TT was observed more often in breed type A_{Du50} (37 %) compared to B_{Du75} (8 %).

Skatole (and indole), but not androstenone is significantly affected by CYP2E1 genotype. Highest skatole values were determined in genotype CC (120 ng/g), whereas CT (79 ng/g) and TT (73 ng/g) genotypes exhibited significantly lower skatole values in backfat. With respect to untransformed skatole values, least squares means correspond to 175 ng/g, 92 ng/g, and 93 ng/g for genotypes CC, CT, and TT, respectively.

Compared to genotype CC (50.0 %), the frequency of putatively skatole-tainted carcasses is considerably lower for genotypes CT (12.9 %) and TT (11.1 %). When putative consumer rejection is based not only on skatole but also androstenone (> 2000 ng/g melted fat) 56.7 % of the CC carcasses are suggested to be tainted (figure 1). The proportion of tainted carcasses is considerably lower within genotypes CT (24 %) and TT (14.8 %) suggesting an effective reduction of consumer complaints applying marker assisted selection strategies.

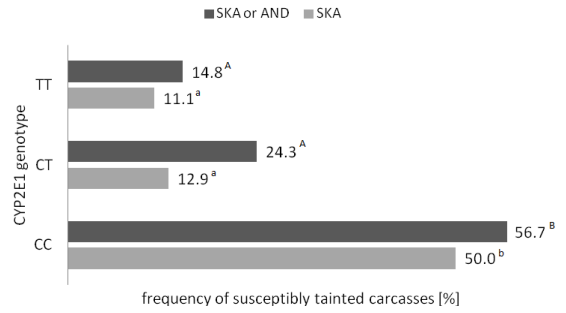

Figure 1: Frequency of carcasses exceeding the putative sensory rejection threshold for skatole (150 ng/g melted fat; light bars) and for either skatole (150 ng/g melted fat) or androstenone (2000 ng/g melted fat; dark bars) with respect to CYP2E1 genotypes (AB, ab: means with different letters are significantly different between genotypes within sorting criteria). SKA = skatole; AND = androstenone

Our findings are in line with previous studies, where *CYP2E1* was found to be associated with skatole but not androstenone levels (15). In this study, the effect was more pronounced in Duroc than in Norwegian Landrace boars. It was suggested, that the effects of different polymorphisms vary among different lines and breeds likely because some alleles may be fixed in different lines (1).

Breed type as well as husbandry and feeding were reported to affect boar taint compounds (1). However, the present study did not aim at directly investigating these effects but at segregation of the SNP and its effects on boar taint compounds in two commercial populations. Management factors such as feeding, husbandry or hygiene were not standardized in the present study.

IV. CONCLUSION

The previously detected SNP g.2412 C>T (at -586 ATG) located in the promoter of *CYP2E1* segregates in two commercial Duroc-sired crossbred populations in Germany. Its allele frequencies varied with the proportion of Duroc blood. Significant associations of the CYP2E1 genotype were found for the indolic boar taint compounds. Genotype CC was significantly higher in skatole and indole but no effect was found for androstenone. Subsequently, the proportion of putatively tainted carcasses was significantly lower within CT and TT genotypes. As *CYP2E1* is associated with skatole but not androstenone, it could be implemented in breeding strategies in order to reduce skatole levels without compromising reproductive traits. Further studies need to evaluate whether this SNP is fixed in Duroc populations.

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