REVIEW: MOLECULAR GENETIC MARKERS ASSOCIATED WITH BOAR TAINT – COULD MOLECULAR GENETICS CONTRIBUTE TO ITS REDUCTION?

Falková L. and Vrtková I.

Laboratory of Agrogenomics, Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Czech Republic

Abstract

Boar taint is an unpleasant meat odour or taste occurring in uncastrated male pigs usually. Naturally occurring compounds – androstenone, skatole and indole – and their accumulation in the adipose tissue of entire boars cause the perceptible boar taint. Individual levels or combination of these compounds lead to perception of boar taint observed during culinary process and pork consumption. The ban on surgical castration based on EU legislation makes it necessary to find a solution that enables our producers to adapt to these new conditions and ensure their competitiveness. Alternative options include the use of molecular genetic markers that affect the levels of androstenone and skatole in pig adipose tissue by Marker Assisted Selection (MAS). The aim of this review is to provide overview in recent facts in the field of molecular genetics and possibility in boar taint reduction solution.

Key Words: Boar taint, genomic markers, selection

Boar taint

Boar taint is an unpleasant odour or taste of meat from entire male pigs. The occurrence in adult pig males is connected with the hormone changes during maturation (Duijvesteijn et al., 2010). It can occur in 5-40% of uncastrated boars (Große-Brinkhaus et al., 2015).

There are three compounds responsible for boar taint: androstenone, skatole and indole (Grindflek et al., 2011). These substances individually or in combination lead to perception of boar taint. The concentration of these compounds varies according to the age and weight of the individual at slaughter. The main substances influencing boar taint are androstenone and skatole. Indole (produced during degradation of tryptophan in colon) is considered as less important cause of meat flavour and scent.

Androstenone is a testicular steroid metabolized in the liver. Non-metabolized part of androstenone is accumulated in adipose tissue and causing a urine like odour. Skatole is produced by bacterial degradation of tryptophan in the colon. The non-metabolized part of skatole accumulates in adipose tissue causing a faecal like odour. Mostly, the androstenone level are influenced by genetic factors and adolescence degree while in the skatole level besides genetic and age factor the nutrition and environmental factors play key role (Zamartskaia and Squires, 2009). Skatole occurs in both male and female pigs but in male ones three times more (Wesoly and Weiler, 2012).

The acceptance thresholds for androstenone and skatole differ between consumers and countries and range from 0,5 to 1 μg/g fat for androstenone and from 0,2 to 0,25 μg /g for skatole (Rowe et al., 2014). On the other hand Mörlein et al. (2012) describe the acceptance threshold for skatole lower – 0,15 μg /g fat. The detection ability of boar odour caused by androstenone is in human under genetic control: results of Lunde et al.´s (2012) study indicate that the Odorant Receptor OR7D4 has a major role in perceiving androstenone.

Surgical castration without anaesthesia

Surgical castration of boars is a common practice to reduce boar taint. Following the European Declaration on Alternatives to Surgical Castration of Pigs (European Commission, 2011), the Member States of the European Union committed themselves to ending surgical castration without anaesthesia by 2018. The declaration was signed by 33 parties representing

European farmers, the meat industry, retailers, scientists, veterinarians and animal welfare associations. The deadline has been expired and according to statistical data collected by the Federation of Veterinarians of Europe the surgical castration without anaesthesia is performed still in European countries commonly (De Briyne et al, 2016). Since 2016, surgical castration without anaesthesia has been limited in Sweden and since 2019 Germany has confirmed a ban on surgical castration.

Surgical castration without anaesthesia is no longer applicable in Denmark and Belgium, in Great Britain and Northern Ireland castration is not used traditionally. The Czech Republic has not yet set an exact deadline for the ban. Report on the ending of surgical castration of pigs (2012 – 2014) from the Expert Group (Brussels, October 2014) marks the Czech Republic together with Poland, Romania and Hungary as "Countries with little or no perceived sense of urgency".

Alternative methods

Worldwide increased pressure on ban of surgical castration creates alternative methods request. The surgical castration with anaesthesia is one of them. On the other hand, there are risks of loss, health impact, economic and organisational difficulties caused by it. Immunocastration is another alternative: vaccination stimulates the production of antibodies against Gonadotropin Releasing Hormone (GnRH) (Yamsakul et al. 2017). The method is safe for animals but final consumer perceives it negatively. Another possibility is to change the fattening of boars: boars can be fattened to a lower slaughter weight (80 kg), while their meat is still free of boar taint. The declared disadvantage is the untapped growth potential of animals (Grauer, 2014). Finally, a highly effective method seems to be the Marker Assisted Selection (MAS) associated with boar taint.

Molecular genetics markers – SNPs influencing traits

Heritability of androstenone and skatole level gives a high potential of genetic selection. Its range is 0,5 – 0,7 and 0,3 – 0,5 androstenone and skatole respectively (Große-Brinkhaus et al. 2015).

Quantitative trait loci (QTL) and candidate genes associated with boar taint are most important for genetic selection. QTLs are parts of the chromosomes involving genes that affect the traits. Candidate genes encode development of receptors and enzymes involved in the metabolism of androstenone and skatole. There are gene polymorphisms (Single Nucleotide Polymorphism – SNP – mostly) changing the specific phenotype of the trait (Squires, 2006).

The QTLs for androstenone and skatole are spread on many chromosomes mainly on SSC6, 7, 10, 14 (SSC - *Sus scrofa* chromosome). Significant association have also been identified on SSC1, 12, 16 and 17 (Große-Brinkhaus et al., 2015). Thus let us supposed the androstenone and skatole levels are influenced by a lot of markers/ genes involved in their biosynthesis and metabolic pathways. There are differences in expression of candidate genes (connected with androstenone and skatole level) between individuals with high and low levels of boar taint and among breeds (Enssenger, 2015). Mathur et al. (2013), they found a higher level of boar taint in the Landrace and Yorkshire dam lines than in the Pietrain sire line. Therefore it is necessary to work with the dam lines too when we use genetic selection on boar odour.

The SNPs are gene polymorphisms, and in boar taint problem, studies have focused on genes of metabolic pathways associated with boar taint compounds. The first association research by Moe et al. (2009) compares high number of SNPs with boar taint in Duroc and Norwegian Landrace: 1102 Duroc and 1726 Norwegian Landrace boars were genotype in 135 SNPs in 57 genes. They found significant markers with effects on androstenone in Duroc, but not in Landrace, and significant markers with effects on fat skatole in both breeds. In Duroc breed they discovered 4 SNPs in NGFIB, CYP2D6, CYP2C49 and CTNNDIS genes associated with androstenone level in adipose tissue, significantly. In Norwegian Landrace breed these SNPs was not associated with androstenone in adipose tissue. The skatole level in fat tissue was associated with 5 SNPs within genes CYP21 and CYP2E1 in Duroc, and 3 SNPs from CYP2E1 gene in Norwegian Landrace, significantly. Seven SNPs in CYP21 and CYP2E1 in Duroc and 5 SNPs in CYP2E1 in Norwegian Landrace was associated with indole level in adipose tissue.

Individual markers explained 2,5-16,3% of the total variation of the traits. Haplotype analyses identified 5 genes – CYP21, CYP2D6, CYP2E1, CTNNDI, NGFIB in Duroc and 5 genes – BAPI, CYP2D6, CYP2E1, HYAL2 and SRD5A2 in Norwegian Landrace. Results of haplotype analyses CYP2E1 were significant in levels of skatole and indole in both breeds and can explain up to 6% of the phenotypic variance for skatole and up to 12% of the phenotypic variance for indole. Significant effects were also found in BAP1, HYAL2 and SRD5A2 influencing androstenone level in Norwegian Landrace. Their non-association with testosterone or estrogen means that they are attractive for genetic selection in reducing boar taint because they have no effect on reproductive traits. Mörlein et al. (2012) found SNP in CYP2E1 gene in two commercial Duroc-sire crossbred populations. The CC genotype indicated higher level of skatole and indole than the other genotypes, significantly. Duijevesteijn et al. (2010) revealed 37 SNPs influencing androstenone level in fat tissue significantly; they are located on SSC1 and SSC6 mainly. They confirmed already known candidate genes – CYP2A19, SULT2A1 and SULT2B1 and described other genes influencing pathways of metabolism of androstenone (CYP2A6 and HSD17B14).

Squires and Schenkel (2010) found approximately 80 SNPs in 28 candidate genes in 1300 animals representing 8 different lines, comprising 6 breeds (Duroc, Hampshire, Landrace, Large White, Pietrain and Yorkshire). The number of significant SNPs across lines ranged from 5 to 17 and from 3 to 16 for skatole and androstenone, respectively. A large proportion of effective SNPs were associated with both skatole and androstenone (65%) across lines. The use of markers to selection of homozygotes carrying the favourable alleles would reduce fat androstenone by 26-61% and fat skatole by 20-54% depending on the pig line. No negative effects on production traits were found in these markers. Grindflek et al. (2010) examined 15 candidate genes connected with the androstenone level in adipose tissue in 2560 boars of Duroc and Norwegian Landrace. Five SNPs in the candidate genes CYB5A, CYP11A1, HSD3 and NCOA4 were detected. One SNP located in position -8 of the CYB5A gene was associated with androstenone level in Duroc significantly, but not in Landrace. Ramos et al. (2011) detected 16 SNPs created 3 clusters on SSC6 associated with skatole in Duroc. GG genotype in the second cluster indicates twice more level of skatole then TT genotype. In the third cluster the GG genotype exceeded skatole level beside AA genotype. The best variation of haplotype was T-G-A in 3 SNPs. Gregersen et al. (2012) identified 25 haplotypes and 3 single markers with effects in 923 Duroc, Landrace and Yorkshire boars. The haplotype with the strongest effect on androstenone level occurred on SSC1 around the CYB5A gene and accounted for 16,8% of phenotypic variation. Other candidate genes for androstenone are CYB5A, SRD5A2, LOC100518755 and CYP21A2. For skatole and indole, SULTA1 and CYP2E1 are reported. Various CYP21A2 expressions have been reported in boars with high and low levels of androstenone. The effect on androstenone levels was found only in the Landrace population (Grindflek et al., 2010).

Gunawan et al. (2013a) described mutations in genes IRG6, MX1, IFIT2, CYP7A1, FMO5 and KRT18 as possible candidate markers for androstenone. Mutations in genes ATP5B, KRT8, PGM1, SLC22A7 and IDH1 as candidate markers for skatole are written in Gunawan et al. (2013b). Kim et al. (2013) identified 8 SNPs in the HSD3B gene in 147 Duroc boars. Individuals carrying the GG genotype in SNP5 (g.165262G>A) had significantly lower androstenone concentrations then individuals with the GA and the AA genotypes. They recommended the GG genotype in SNP5 in the HSD3B gene as selection marker suitable for MAS for low androstenone level. The effect of this gene is also different for different breeds e.g. the Meishan breed exhibited lower expression levels than the Large White breed (Doran et al., 2004). Rowe et al. (2014) detected SNPs in RD45 and RDH16 genes influencing androstenone level in Landrace. Große-Brinkhaus et al. (2015) revealed by genome-wide association analyses 33 SNPs significantly associated with at least one component of boar odour in 600 Pietrain sire crossbred boars. The results suggest strong population-specific, breed-specific and crossbreed -specific effects on boar taint trait.

Holly (2016) analysed 120 SNPs in 3730 boars in Duroc, Landrace and Yorkshire and identified 13 SNPs associated with levels of boar taint compounds. These 13 SNPs found in 10 genes are significantly associated with metabolism of boar taint compounds (AKR1C4, ALD1β1, CYB5A, CYB5R3, CYP2E1, ENO3,

EPHX2, INPP5K and SULT2A1). Eleven SNPs were found to be significantly associated with androstenone metabolism, while four SNPs were significantly associated with skatole.

The association studies have revealed many genetic markers and candidate genes encoding key enzymes of androstenone and skatole metabolism. However, their number varies from study to study. According to several studies, Engesser (2015) listed candidate genes for androstenone in dissertation thesis: AKR 1C4, CYB 5A, CYP 11A1, CYP 17A1, DHR S4, FTL, HSD 17B4, STAR, SULT 2A1 and SULT 2B. The CYP2E1 gene seems to be the most suitable candidate gene for skatole and indole. Son et al. (2017) focused on the 74,7-80,5 Mb region of SSC7. They found 18 SNPs within genes GZMB, GZMH, STXBP6 and intergenic regions of 78,9-80,1 Mb in Duroc. The GZMB gene is indirectly associated with tryptophan metabolism rate. The effect of skatole metabolites on sintaxal genes is supposed. This region, in Duroc, was connected with androstenone and indole level too. Unlike Duroc, in Landrace there were identified haplotypes associated with skatole level. The haplotypes explained 2,3% of phenotypic variability in skatole level. Founded SNPs were not associated with sex steroids and thus these markers are attractive and relevant for selection purposes.

In the Czech Republic, the genetics of boar taint issue is studied e.g. by Zadinova et al. research group and the genes and their SNPs that significantly affect androstenone and skatole levels are reviewed in Zadinova et al. (2016). For androstenone level: CYP17A, CYB5A, CYP21, SULT2A1, SULT2B1, HSD3B and for skatole: CYP2E1, CYP2A6, SULT1A1, are described. All these markers are important for the synthesis or degradation of skatole or androstenone in pigs. Apparently, the CYP2A6 and CYP2E1 genes are more important in skatole metabolic regulation. The CYP2E1 gene plays an essential role in the metabolism of skatole and besides skatole level it influences indole level too. Zadinova et al. (2017) have analysed 4 SNPs (g.2412C > T, c.1422C > T, c.1423G > A and c.*14G > T) in this gene in commercial crossbreeds Czech Large White x Czech Landrace. They confirmed that these SNPs are connected with indole level. The T, C and A alleles have been determined as preferred. Also, these alleles were associated with skatole level but not significantly. They did not find the connection with androstenone. Individuals with high activity of CYP2A6 gene have low skatole level and low activity of this gene is connected with high accumulation of skatole in fat tissue (reviewed in Zadinova et al, 2016).

Kubesova et al. (2018) examined CYB5A gene in which G>T polymorphism (MN_001001770.1: c-8G> T) is positively correlated with fat levels of androstenone in pigs (Grindflek et al., 2010). They analysed 4 SNPs (g.165901487delG, g.165901767T> C, g.165902078C> T, AFO16388: c.-G> T) in the CYB5A gene promoter region in boars of the commercial Large White x Landrace (sow) x Duroc. No significant associations with skatole and androstenone or indole were found, but this could be due to the small number of analysed animals (40 boars). However, they showed variability of all four SNPs in Czech commercial hybrid pigs. One of the most recent research studies on boar taint molecular genetics in the Czech Republic is the work of Kubesova et al. 2019: it provides more comprehensive view of selected genes involved in the metabolism of skatole or androstenone in the liver of the commercial breeds. Results contribute to understanding how castration is related to expression of these genes.

Breeding against boar taint – commercial application

The boar taint reduction has been a breeding and marketing goal of breeding companies for many years. E.g. Company Topigs Norsvin has achieved significant success in its breeding to reduce boar taint. They claim on their website that they have managed to reduce boar taint by more than 50 % over the past ten years by breeding in their lines. Topigs Norsvin has included the reduction of boar taint in all its lines. This trait is part of the breeding goal not only for paternal lines but also for maternal lines (topigsnorsvin.cz).

Conclusion

The use of genetic markers in the production of boar taint-free but growing like normal boars pigs is a long-term solution to the use of non-castrated pigs for pork production. The aim of genomic selection is to select individuals with a high frequency of favourable alleles associated with a particular phenotype. The selection of boars genetically predisposed for a lower

content of boar taint compounds seems to be useful for boar taint reduction. Some studies have shown high genetic correlations of boar taint compounds with testosterone and estrogens. Thus, it is necessary to choose boar taint markers with negative correlation with production and reproduction traits. Also, the frequency of QTLs varies among breeds and the effect of individual markers differs among breeds too. However, in order to confirm the markers already described and to find a new one for each pig breed, further verification is necessary in order to use them in breeding programmes for a selection against boar taint.

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Corresponding Address:
Mgr. et Mgr. Lenka Falková
Mendel University in Brno
Department of Animal Morphology, Physiology
and Genetics
Laboratory of Agrogenomics
Zemědělská 1, 61300 Brno
Czech Republic
E-mail:lenka.falkova@mendelu.cz

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