Animal Science Papers and Reports vol. 34 (2016) no. 2, 107-128
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Boar taint – the effects of selected candidate genes associated with androstenone and skatole levels – a review*

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(Accepted Jauary 20, 2016)

Androstenone and skatole are the two main components causing the so-called boar taint as well as the unpleasant taste of pork meat from boars. These substances increasingly accumulate in the adipose tissue after reaching sexual maturity. For this reason, boars are less frequently used for pork production. The most commonly used method for boar taint elimination is the castration of male pigs shortly after birth. Another way how to reduce the boar taint is the identification of candidate genes and SNPs (single nucleotide polymorphisms) that affect the androstenone and skatole levels in the adipose tissue of pigs. The aim of this review is to present and systematize the current knowledge about the genetic influences on androstenone and skatole levels. In recent years several studies focused on the identification of potential candidate genes have been carried out and a study which purpose was to verify whether these genes or their mutations may have a significant effect on androstenone and skatole levels in pigs. Several genes and their SNPs that significantly influence the levels of androstenone (*CYP17A, CYB5, CYP21, SULT2A1, SULT2B1, HSD3B*) and skatole (*CYP2E1, CYP2A6, SULT1A1*) were identified. In addition, there are other candidate genes (*LH, TEAD3*) whose association with the components of boar taint has not been clearly confirmed yet. Several studies also mentioned the possibility of interactions between genes themselves as well as the possibility of negative effects of the tested genes on important pig production characteristics

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This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6046070901).

as fertility and carcass value. It is therefore clear that thorough studies on the effect of individual genes on different components of boar taint are desirable.

KEY WORDS: boar taint / androstenone / skatole / candidate genes / QTL / SNPs

Fattening of the entire male pigs, when compared with other pig categories (barrows and gilts), appears to be more profitable, mainly due to better growth intensity, feed conversion and improved carcass leanness. This is caused by the production of male sex hormone androstenone [Kim *et al*. 2013, Lundström and Zamaratskaia 2006, Moe *et al*. 2007b, Peacock *et al*. 2008, Strathe *et al*. 2013]. Non-castrated male pigs are already being used for the production of pork meat in some countries, however the occurrence of so called "boar taint" in this meat represents a significant problem [Babol *et al*. 1999]. Boar taint is a strong, faecal- and urine-like odour found in heat treated pork meat [Lee *et al*. 2005, Strathe *et al*. 2013]. In order to eliminate this odour, in many countries the male pigs are castrated very soon after being born [Moe *et al*. 2008, Duijvesteijn *et al*. 2010]. The methods of castration represent a problem from an ethical point of view and also endanger the welfare of animals in general [Gray and Squires 2013], because the castration of young male pigs less than 7 days old is performed without the use of any anesthetics [Engelsma *et al*. 2007]. Because this process is painful, frightening and can lead to a health status decline [Lundström and Zamaratskaia 2006], there are continuous efforts to change the way of pig castration, which is being supported by EU legislation as well. There is a new recommendation not to perform the surgical castration of male pigs without narcosis, which will come into effect in 2018 [Mörlein *et al*. 2012]. Considering all of these points, it is necessary to find alternatives to removing the boar taint and preventing the devaluation of pork meat [Engelsma *et al*. 2007, Grindflek *et al*. 2011b].

Boar taint

Boar taint is primarily caused by substances produced in pigs in relation with reaching sexual maturity [Duijvesteijn *et al*. 2010]. The main substances responsible for the unpleasant odour are androstenone (5α-androst-16-en-3-on), skatole (3-methylindole) and indole (2,3-benzopyrol) [Grindflek *et al*. 2011b]. It has also been shown that a minor part in taint production is played by 3α-androstenol (3α-OL) and 3β-androstenol (3β-OL) [Xue and Dial 1997] which are created during the metabolism of androstenone [Zamaratskaia and Squires 2009]. Acceptable levels of the two main substances differ between consumers and range between 0.5 - 1 μg/g for androstenone and 0.2 – 0.25 μg/g for skatole. However, acceptable skatole levels can be as low as 0.15 μg/g [Mörlein *et al*. 2012]. A study published by Bonneau *et al*. [2000] states that on average 6.5% of consumers can detect the unpleasant odour of boar meat, while about 3% of consumers can taste the unpleasant taste, although the published results varied for different countries.

Main substances causing boar taint

Androstenone

Androstenone is a steroid hormone produced by the Leydig cells located in the testis, which produce also other steroid hormones, androgens and estrogens. It is stored in the fat tissue and salivary glands, from which it is then released into the air as a pheromone stimulating the willingness toward mating in female pigs [Moe *et al*. 2008, Robic *et al*. 2011b]. Apart from this, lower concentrations of androstenone have also been detected in the blood serum of barrows and gilts, which suggests possible androstenone production in the cortex of suprarenal glands [Claus *et al*. 1971]. The organic precursor used for the androstenone biosynthesis is pregnenolone. It is synthesized from pregnenolone with the use of andiene-β synthase system (Fig. 1). An important role in the biosynthesis of 5,16-androstadien-3β-ol is played by cytochrome P450C17 (CYP17A1) and cytochrome b5 (CYB5) [Davis and Squires 1999]. The production of androstenone and other testicular hormones is controlled by the neuroendocrine system, mainly through the effects of luteinizing hormone (LH)

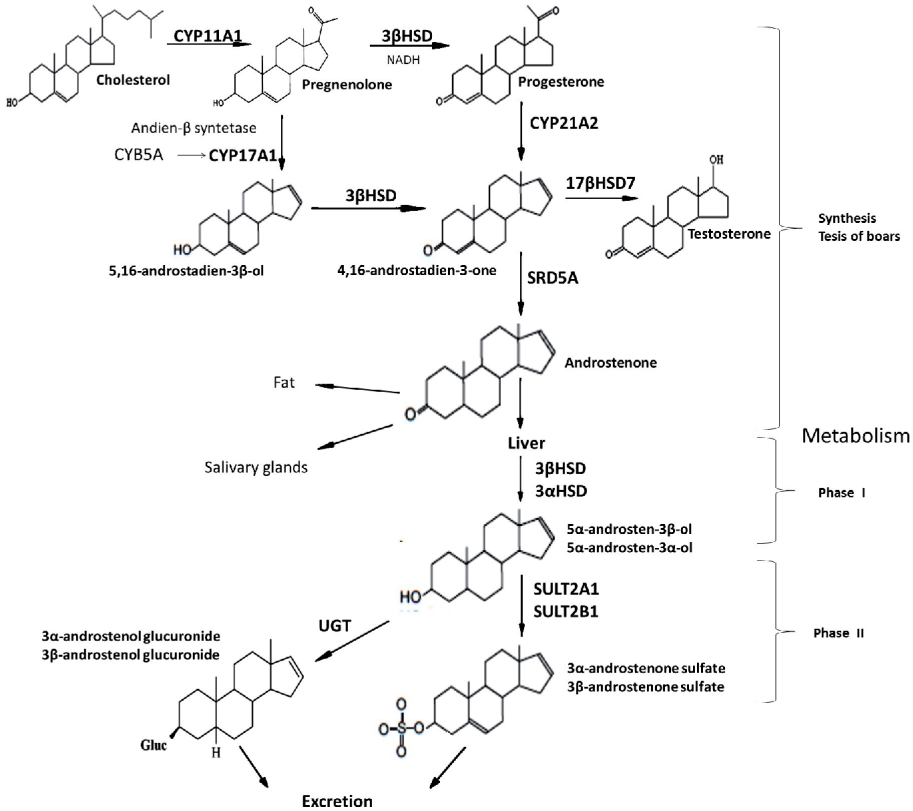

Fig. 1 Genes affecting androstenone synthesis and metabolism.

[Zamaratskaia and Squires 2009]. Androstenone is degraded in the liver and testis [Andresen 2006]. A large part of androstenone is immediately sulfoconjugated by the hydroxysteroid sulfotransferases SULT2A1 [Sinclair *et al*. 2006] and SULT2B1 [Moe *et al*. 2007a]. The metabolic degradation of androstenone is divided into two phases. During the phase I, androstenone is degraded by the 3β-hydroxysteroid dehydrogenases (3βHSD), using the NADH and NADPH cofactors [Doran *et al*. 2004]. The level of androstenone metabolism is directly related to the level of hepatic 3βHSD expression [Andresen 2006]. High levels of androstenone in the fat tissue correspond with low levels of 3βHSD [Dasgupta *et al*. 2007]. The concentration of androstenone in fat tissue also increases with the age of boars [Xue and Dial 1997]. Although the liver is capable of producing metabolites of both of these phases, its main role is in the phase II – sulfoconjugation. The key enzyme of the phase II is SULT2A1 [Sinclair *et al*. 2006]. Androstenone is cut into two main metabolites during its degradation: 5α-androst-16-en-3a-ol and 5β-androst-16-en-3b-ol [Zamaratskaia and Squires 2009], which represent about 68% of all the phase II metabolites [Sinclair *et al*. 2005] (Fig. 1).

Skatole

Skatole is formed from the amino acid L-tryptophan during the degradation of proteins. It is produced by the bacteria commonly found in the large intestine of monogastric animals [Lee *et al*. 2005]. It is responsible for giving the meat a faecal-like odour. A part of it is removed from the body by feces, another part is metabolised by the liver and a rest is stored in the kidneys as well as in the fat tissues due to its lipophilic character [Babol *et al*. 1998b]. Contrary to monogastric animals, ruminants are sensitive to skatole which is toxic for them and causes ABPE (acute bovine pulmonary edema and emphysema). Thus, high concentrations of skatole have no adverse effects on pigs [Deslandes *et al*. 2001]. Skatole synthesis is a two-step process which requires the assistance of intestinal bacteria. *Escherichia coli* and *Clostridium spp.* (*C. dýfficile, C. sticklandii, C. lituseburense, C. subterminale* and *C. putrefaciens*) transform tryptophan into indole-3-acetic acid [Yokoyama and Carlson 1979], which is then metabolised by *Lactobacillus* and *Clostridium* into skatole [Zamaratskaia and Squires 2009]. Skatole is metabolised within the liver tissues by enzymes P450 2E1 (CYP2E1), P450 2A6 (CYP2A6) [Diaz *et al*. 1999] and phenol sulfotransferase (SULT1A1) [Lin *et al*. 2004b]. The degradation of skatole consists of two phases – oxidative, followed by conjugation reactions. Phase I involves mainly the cytochrome P450 (CYP) family. The main enzymes are considered to be CYP2E1 [Babol *et al*. 1998b], CYP2A [Diaz and Squires 2000] as well as aldehyde oxidase (AO) [Lanthier *et al*. 2007]. Phase I results in an attachment of a hydroxyl group, which is then used to create a conjugate in phase II [Zamaratskaia and Squires 2009]. Diaz *et al*. [1999] describe the total of 7 intermediate products of phase I (see Fig. 2). These metabolites are then used as substrates for the reactions of phase II [Diaz and Squires 2003]. Phase II then includes sulfoconjugation with the use of sulfotransferases (SULT1A1),

glucuronic acid [Babol *et al*. 1998b] and uridine-diposphate-glucuronosyltransferase (UDP) [Diaz and Squires 2003]. For the final products of skatole degradation (Fig. 2) are then eliminated from the organism via urine [Xue and Dial 1997].

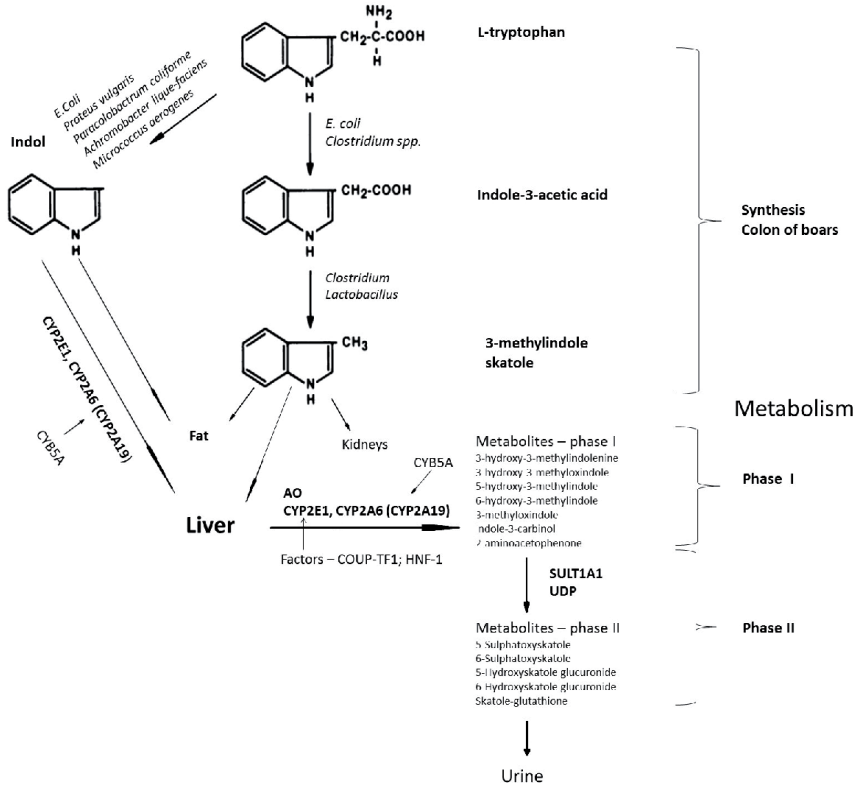

Fig. 2 Synthesis and metabolism of skatole; genes affecting skatole metabolism

Indole

Similarly to skatole, indole (2,3-benzopyrol) is produced by the degradation of L-tryptophan in the gastrointestinal tract (GIT) of monogastric animals. Indole is absorbed by blood and metabolised by the liver tissue. L-tryptophan can be either degraded in the GIT to indole or transformed to indole-3-acetic acid, which is the starting point in skatole synthesis as described above [Yokoyama and Carlson 1979]. As opposed to skatole, the degradation process resulting in indole uses a wide range of bacteria (figure 2) [Deslandes *et al*. 2001]. Similarly to skatole, indole production is influenced by the cytochrome CYP2A6 expression, on which both indole and skatole have the stimulating effect. It has also been found that in the case of indole this stimulating effect is a lot more significant than in skatole [Chen *et al*. 2008]. Indole too is accumulated in the fat tissue in pigs and its levels can be affected by other than

genetic influences, such as pen dirtiness or feed mixture composition. Indole appears to influence the quality of pork meat to a lower extent than skatole and it mostly just intensifies the unpleasant odour caused by skatole [Yokoyama and Carlson 1979].

Elimination of boar taint using genetic markers

During the recent years there has been a strong effort to minimalize the occurrence of the main substances causing boar taint in the pork meat. There are several approaches to study the genetic basis of boar taint: identification of QTL, genome-wide association study (GWAS), study of candidate genes and polymorphisms thereof (SNPs). The identification of suitable SNPs causing boar taint could help to accelerate its elimination through crossbreeding and thus remove the need for male pig castration [Duijvesteijn *et al*. 2010]. The reduction of boar taint through genetic selection seems to be a promising solution, because the levels of androstenone and skatole concentrations show medium heritability [Strathe *et al.* 2013]. Androstenone and skatole heritabilities range between 0.25-0.87 [Oskam *et al*. 2010] and 0.19-0.54 [Robic *et al*. 2008], respectively. Tajet *et al*. [2006] also report a positive gene correlation between the skatole and androstenone levels (0.36-0.62).

Quantitative trait loci (QTL) contain the genes affecting specific traits and can be identified by comparing the genotypes of unknown markers scattered along the chromosome with the phenotype or a specific trait under study. Quintanilla *et al.* [2003] and Lee *et al.* [2005] identified several QTL for androstenone and skatole in an experimental population of crossbred pigs (Meishan and Landrace). Lee *et al.* [2005] described the location with most skatole and indole affecting QTL to be the chromosome 14 (SSC14 – *Sus scrofa* chromosome 14). The locations with QTL for androstenone are SSC2, SSC4, SSC6, SSC7 and SSC9. However, only the SSC6 carries the QTL for causing the unpleasant ”boar taste“ of meat. Quintanilla *et al.* [2003] in their work concerning the PORQTL project identified QTL controlling the androstenone levels in the fat tissue on SSC3, SSC4, SSC7, SSC14 and also at the ends of short arms of SSC6 and SSC9. All these findings suggest that the androstenone and skatole levels are controlled by a large number of genes. Duijvesteijn *et al*. [2010] also mentioned SSC1 and SSC6 as the locations of genes affecting the androstenone levels.

The genome-wide association study (GWAS) is often used for detection of QTL and SNPs. The Illumina Porcine SNP60 Genotyping BeadChip is the most comprehensive genome-wide genotyping array for the porcine genome [de Campos *et al.* 2015, Ernst and Steibel 2013, Lukić *et al.* 2015, Rowe *et al.* 2014]. Große-Brinkhaus *et al.* [2015] identified 33 SNPs, of which 31 significantly associated with concentration in fat of one of boar taint compounds (androstenone or skatole). Only one SNP was significantly associated with concentration of both compounds. Lukić *et al.* [2015] used the Illumina SNP60 porcine BeadChip for detecting of 62 153 SNPs. The accuracy of predicting phenotypes was assessed based on the genotypes by cross-validation using six different evaluation methods: genomic best linear unbiased

prediction (GBLUP) and five Bayesian regression methods (Bayes Lasso, Bayes A, Bayes B, Bayes C, Bayes SSVS). In addition, this was compared with the accuracy of predictions using only QTL that showed genome-wide significance. The methods of GWAS gave greater accuracy than the QTL analysis. The aim of the de Campos *et al.* [2015] study was the comparison of two different Genome Wide Selection (GWS) methods (Ridge Regression BLUP - RR-BLUP and Bayesian LASSO - BL) to predict the genomic estimated breeding values of four phenotypes, including two boar taint compounds. Another method within the GWAS for study of candidate genes and SNPs associated with androstenone and skatole is next generation sequencing (NGS) [Grindflek *et al*. 2011a]. RNA-Seq is a recently developed NGS technology for transcriptome profiling which enables identification of new and less expressed transcripts. Association analysis identified boar taint candidate genes in flavin monooxygenease family, cytochrome P450 family and hydroxysteroid dehydrogenase family. Moreover, polymorphisms associated with boar taint were revealed in *IRG6*, *MX1*, *IFIT2*, *CYP7A1*, *FMO5* and *KRT18* genes. Sahadevan *et al.* [2015] studied gene co-expression clusters in liver tissue from three pig populatoins with low and high androstenone levels in backfat. The examined populations were Duroc sire line, Duroc × F_2 boars and Norwegian Landrace The RNA-seq and microarrays were used. Based on the results they assumed that low androstenone cluster 2 might be a signature co-expression cluster for androstenone metabolism in animals with low levels of androstenone in backfat.

Another possible method of identifying the suitable genes is to study candidate gene polymorphisms. These are most commonly found as SNPs. Several published studies have already focused on SNPs associated with skatole [Lin *et al.* 2004a, 2006] or androstenone [Lin *et al.* 2005a, Kim *et al.* 2013]. Ramos *et al.* [2011] also found several SNP markers affecting skatole levels at the distal end of SSC6p. These SNP markers are grouped into 3 individual clusters. Marked differences were observed mainly between the homozygotes in each group. Single SNP markers can account for up to 22% of the phenotypic variability [Grindflek *et al.* 2011a].

Genes for androstenone

The main genes involved in androstenone synthesis are *CYP17A1, CYB5A* [Davis and Squires 1999] and *LHB* – gene coding the β-chain of LH – luteinizing hormone [Duijvesteijn *et al.* 2010]. Lin *et al.* [2005a] stated that mutations in the *CYB5A* gene result in a decrease of androstenone levels in pigs. Quintanilla *et al.* [2003] also mentioned the possible effect of *CYP21A2* and *CYP11A1*, located on the chromosome SSC7. Phase I of androstenone metabolism is controlled mainly by 3βHSD, while phase II is under the control of sulfotransferases – SULT2A1, SULT2B1 [Duijvesteijn *et al.* 2010]. Robic *et al.* [2011a] studied crossbred pigs of Large White and Meishan genotypes and focused on several other potential genes which could have an effect on androstenone levels (*C6ORF106, C6ORF81, CLPS, SLC26A8, SRPK1, MAPK14* and

TEAD3). However, the study proved no significant direct effect of any of these genes. Gregersen *et al*. [2012] identified also gene *SRD5A2* (5α-reductase) as a candidate gene for androstenone. According to Moe *et al*. [2009], the *CYP2C49, CYP2D6, NGFIB* and *CTNND1* genes are connected with the androstenone levels in fat tissue in pigs.

Genes controlling androstenone synthesis

Genes of the CYP (cytochrome P450) family

CYP11A1 (cytochrome P450, family 11, subfamily A, polypeptide 1): In pigs, the CYP11A1 gene (ENSSSCG00000025273) (Ensembl; http://www.ensembl.org/index.html) is located on SSC7 [Quintanilla *et al*. 2003] (Scaffold GL893363.2: 159,079-165,974 bp) (Ensembl) CYP11A1 is involved in the first step of biosynthesis of steroid hormones. It initializes the transformation of cholesterol to pregnenolone [Robic *et al*. 2008]. Except for pigs, this gene has also been found in mice, rats, humans and many other species. In humans, *CYP11A1* gene is located on chromosome 15q23-q24 (Ensembl). CYP11A1 is expressed mainly in the cortex of suprarenal glands, ovaries, testis and placenta. Its transcript can also be found in the central and peripheral nervous system [Payne and Hayle 2004]. In pig this gene has been considered a possible candidate gene affecting the androstenone levels, because it is involved at the beginning of its synthesis [Robic *et al.* 2011b]. Grindfleck *et al.* [2010] found different expression levels in boars with extremely high and low androstenone levels. However, Quintanilla *et al.* [2003] in their work excluded *CYP11A1* as a candidate gene for their pig population. Similarly, Robic *et al.* [2011b] were also not able to confirm the effect of *CYP11A1* (or its mutations) on androstenone accumulation in pigs.

CYP17A1 (cytochrome P450, family 17, subfamily A, polypeptide 1): This gene (ENSSSCG00000010591) is located on SSC14 (123,773,105-123,779,533 bp) in pigs (Ensembl). *CYP17A1* is also involved at the beginning of steroid hormone synthesis from pregnenolone. In humans, *CYP17A1* is found on chromosome 10q24.3 (Ensembl). Its expression is most pronounced in the testes and ovaries. However in some species such as humans and monkeys, some expression was observed in the placenta and suprarenal glands as well [Payne and Hales 2004]. CYP17 is important for the metabolism of androstenone in pigs [Davis and Squires 1999, Nakamura *et al.* 2011]. Lin *et al.* [2005b] reported a substitution mutation (T>A) in the nucleotide 1317, (NM_214428.1:c.1220T>A) which causes the change of amino acid leucine - Leu^{439} to histidine - His^{439} However, despite their expectations, this mutation had no apparent effect on the enzymatic activity of the resulting proteins involved in adrostenone biosynthesis. Because there is a possibility that the effect of individual genes can vary between breeds, Moe *et al.* [2007b] performed a study on two individual breeds, Landrase and Duroc. Neither of the studied breeds, however, showed any significant differences in the expression of *CYP17A1* gene in pigs with low and high androstenone levels.

CYP21A2 (cytochrome P450, family 21, subfamily A, polypeptide 2): CYP21A2 (ENSSSCG00000001428) is located on the SSC7 (27,722,776-27,725,977 bp) (Ensembl). This gene is also involved at the beginning of androstenone synthesis and the encoded protein acts as a catalyst in the progesterone hydroxylation process. Progesterone is a direct substrate for CYP21A2 and this reaction does not require any expression of CYP17. The *CYP21A2* expression was found exclusively in the cortex of suprarenal glands [Payne and Hales 2004]. Human *CYP21A2* is located on chromosome 6p21.3. It has been proved that this gene has a very strong influence on sow fertility [Grindflek *et al.* 2010]. Grindflek *et al.* [2011a] reported different *CYP21A2* expressions in boars with high and low androstenone levels. However, in their study [Grindfleck *et al.* 2010], they were able to confirm the effect of *CYP21A2* on androstenone levels only in Landrace breed populations.

Gene of the CYB (cytochrome b) family

CYB5A (cytochrome P450, family 21, subfamily A, polypeptide 2): In pigs, *CYB5A* (ENSSSCG00000004875) is found on SSC1 (165,902,018-165,937,614 bp). *CYB5A* is a very important regulator of the *CYP17A1* function [Billen and Squires 2009]. In humans, it is located on chromosome 18q23 (Ensembl). The expression levels of *CYB5A* are closely correlated with androstenone levels in the fat tissue in pigs [Lin *et al.* 2005a]. This suggests that it could be possible to lower the androstenone levels by carefully selecting animals with low levels of cytochrome B5 [Gray and Squires 2013]. Lin *et al.* [2005a] identified a G>T polymorphism located at 8 bp upstream of the ATG codon (MN_001001770.1:c-8G>T) of the *CYB5A* gene associated with lower androstenone levels in fat. The frequencies of individual genotypes were *GG* 84.8%, *GT* 12.4% and *TT* 2.8%. The *TT* homozygotes have shown a significantly lower activity of *CYB5A*. These results were confirmed by Peacock *et al.* [2008] as well. On the other hand, the different effects of *CYB5A* gene have been reported by Zamaratskaia *et al.* [2008]. The *T* allele, particularly genotype *GT* was associated with low levels of androstenone in the serum and also with low levels of skatole in the fat tissue, but no relationship was observed between *CYB5A* and adrostenone levels in the fat tissue. Genotype *TT* was not included in the analysis, as only two homozygotes *TT* were detected. The effect of genotype was, however, live weight-dependent; androstenone levels were affected in lighter pigs and skatole levels in heavier pigs. However, any practical use of their data is arguable at this point, especially due to the small effect of *CYB5A* on adrostenone in fat and also due to a low frequency of the *T* allele in the observed population.

LHB gene (luteinizing hormone beta polypeptide)

LHB (ENSSSCG00000003151) in pigs is located on SSC6 (50,063,956-50,065,003 bp) (Ensembl). The gene is responsible for signalling the beginning of steroid hormone synthesis in Leydig cells at the onset of puberty [Duijvesteijn *et al.* 2010] and also affects the activity of ovaries [Dasgupta *et al.* 2012]. In humans, *LHB* is located on chromosome

19q13.32 (Ensembl). It has been reported that in humans mutations of this gene can be connected with breast cancer [Giovangrandi *et al.* 2001] or with the occurrence of ovarian cysts [Dasgupta *et al.* 2012]. Due to its location on SSC6, Duijvesteijn *et al.* [2010] considered this gene to be a possible candidate gene for androstenone levels.

Genes encoding HSD – hydroxysteroid dehydrogenases

HSD are a group of enzymes that take part in steroid hormone biosynthesis from cholesterol in mammals. Unlike the enzymes from CYP family, which are controlled by one gene, there are several isoforms for the *3HSD*s and several isozymes of the *17HSD*s, each a product of a distinct gene. The number of isoforms or isozymes varies in different species, in tissue distribution, catalytic activity (whether they function predominantly as dehydrogenases or reductases), substrate and cofactor specificity, and subcellular localization. [Payne and Hales 2004].

HSD17B7 (hydroxysteroid (17-beta) dehydrogenase 7): In pigs *HSD17B7* gene (ENSSSCG000000063378) is located on the SSC4 (95,571,648-95,594,175 bp) (Ensembl). The enzyme works as a catalyst of the last step in the androgen and estrogen synthesis [Payne and Hales 2004]. In humans, the *HSD17B7* gene is found on chromosome 1q23 (Ensembl). The study on human and mouse proved that 17βHSD7 catalyses the reduction of keto group in either 17- or 3- position of the substrate [Törn *et al.* 2003]. Chen *et al.* [2007] found a negative correlation between hepatic 17βHSD7 expression and androstenone levels in fat tissues. Testicular expression of 17βHSD7 does not seem to affect the androstenone levels in fat tissue in any way. There have also been detected strong correlations between 3βHSD and 17βHSD7 in non-castrated boars, which suggests that these two genes have similar regulatory mechanisms. This study discovered a strong negative correlation between the expression of hepatic 17βHSD7 and the expression of plasma E1S (estrogen), which suggests a very important role of 17βHSD7 in the estrogen metabolism of pigs.

Genes controlling androstenone metabolism

Genes encoding HSD – hydroxysteroid dehydrogenases

HSD3B (3 beta-hydroxysteroid dehydrogenase): In pigs, the *HSD3B* gene (ENSSSCG00000006719) is located on chromosome SSC4 (111,555,566-111,564,192 bp) (Ensembl). The enzyme coded by *HSD3B* is active during the beginning of steroid synthesis from pregnenolone [Payne and Hales 2004]. In humans, *HSD3B* is located on chromosome 1p13.1 (Ensembl). *HSD3B* expression in man has been confirmed in placenta, skin, breast tissue, ovaries, suprarenal glands, liver and testis [Simard *et al.* 2005]. Doran *et al.* [2004] reached a conclusion that the level of androstenone metabolism is determined by the level of hepatic 3βHSD expression. Pigs with high concentrations of androstenone in their fat have a low expression of 3βHSD in the liver and testis [Chen *et al.* 2007]. The expression of hepatic (non-testicular) 3βHSD shows a negative correlation with the fat tissue androstenone levels. With the help of

cloning and sequencing the gene-coding region it has been found that both hepatic and testicular 3βHSD have the same sequences, which leads to the assumption that the expression of one gene in the liver and testis is controlled by different mechanisms [Nicolau-Solano *et al.* 2006]. Kim *et al*. [2013] identified 8 polymorphisms, with the SNP5 polymorphism in the Duroc breed having a direct influence on androstenone levels. The study also found that pigs of the NM_003534677.2:g.165262G>A with genotype *GG* show significantly lower levels of androstenone, when compared with other genotypes. The different expression levels of this enzyme could be the defining factor for the rate of androstenone metabolism in the liver, which can affect the levels of hepatic CYP2E1 and thus the speed of skatole degradation in the liver. The effect of this gene is also different for different breeds, for example, the Meishan breed exhibited lower expression levels than the Large White breed [Doran *et al.* 2004].

Genes encoding sulfotransferases

SULT2A1 (sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1): In pigs, *SULT2A1* (ENSSSCG00000003130) is located on SSC6 (49,108,567-49,119,941 bp) (Ensembl). SULT2A1 is a key enzyme in the testicular and hepatic metabolism of 5α-androstenone [Moe *et al.* 2007b]. In humans, this gene is found on chromosome 19q13.3 (Ensembl). Its function is in regulating the metabolism of xenobiotics and endogenous hormones [Huang *et al.* 2014]. A study performed by Sinclair *et al.* [2006] showed that the expression of SULT2A1 enzyme can affect the accumulation of 5α-androstenone in the fat tissue of pigs. Animals with high concentrations of 5α-androstenone and low activity of SULT2A1 also exhibit low levels of the SULT2A1 protein, when compared with animals showing low levels of 5α-androstenone. The effect of *SULT2A1* was also confirmed by the conclusions of Leung *et al*. [2010]. They found negative correlations between *SULT2A1* and boar taint in boars of the Duroc, Norwegian Landrace and Yorkshire breeds. Negative correlations between the fat tissue androstenone levels and activity of testicular SULT2A1 ($r = -0.57$; $P<0.01$) were also reported by Sinclair *et al.* [2006]. On the other hand, Moe *et al.* [2007b] found a high mRNA expression of the *SULT2A1* gene in the testis of Duroc and Landrace boars, which also showed high levels of androstenone.

SULT2B1 (sulfotransferase family, cytosolic, 2B, member 1): The *SULT2B1* gene (*ENSSSCG00000023280*) is found on SSC6 (49,700,287-49,710,764 bp) (Ensembl). In the mouse, the protein is found in two isoforms (Sult2b1a and Sult2b1b), which are controlled by the *Sult2b1* gene [Kurogi *et al.* 2010]. Human SULT2B1b catalyzes the sulfonation of 3-hydroxysteroid hormones and cholesterol, whereas SULT2B1a preferentially catalyzes pregnenolone sulfonation [Ji *et al.* 2007]. In mice, a number of interactions between the Sult2b1b and cytoskeletal proteins have been confirmed, as well as the role of Sult2b1b on keeping up the epidermal barrier [Kurogi *et al.* 2010]. In the human genome this gene is found on chromosome 19q13.3 (Ensembl). SULT2B1b has also been associated with breast cancer. Higher expression of

SULT2B1b was reported in the female populations in Africa, where there is a lower incidence of breast cancer when compared with Caucasian populations [Dumas *et al.* 2008]. The expression of this gene in pigs can depend on the breed. In the study published by Moe *et al.* [2007a] there was no difference in the expression of hepatic *SULT2B1* in connection with the androstenone levels in the Landrace breed. However, for the Duroc breed the expression of *SULT2B1* in animals with high levels of androstenone was 35% lower than in the animals with low levels of androstenone. Concerning the expression of testicular *SULT2B1*, both of the breeds showed lower expression levels, which was more pronounced in the Duroc breed. As suggested by Panella-Riera *et al.* [2008], there were speculations that the expression of *SULT2B1* is influenced by testosterone. Nevertheless, this was disproved by Zamaratskaia *et al.* [2012], who could not find any influence of testosterone nor estron sulfate on the *SULT2B1* expression.

TEAD3 (TEF-5) gene (*TEA domain family member 3*): *TEAD3* (ENSSSCG00000001544) is located on SSC7 (36,085,701-36,100,686 bp) in pigs. In humans, this gene is found on chromosome 6p21.2 (Ensembl). The human version of this gene is capable of regulating the *HSD3B* transcription, which significantly influences the degradation of androstenone in liver [Robic *et al.* 2012]. The manifestation of transcription factors was also studied in the skeletal muscles, cardiac muscle and placenta, which so far shows the highest levels of expression. Nonetheless, even here the effect of *TEAD3* is not direct and it works through the activation of other genes, which influence embryonal development [Jacquemin *et al.* 1999]. According to Robic *et al.* [2011a], *TEAD3* was considered to be one of the possible target genes for androstenone due to its location on chromosome SSC7; however, the study proved no direct effect of this gene. Several pig populations were tested for SNP at 726 bp in the 5´ UTR of the first exon (GenBank, Accession Number NM_001037966). The study found high correlations between *TEAD3* and *HSD3B* in pigs, but no difference was observed between expression levels measured in different genotypes of the SNP studied [Robic *et al.* 2012].

Genes for skatole

The results of current studies suggest that *CYP2A6* and *CYP2E1* are the two main genes of the CYP family which have the most significant effect on the phase I of skatole metabolism in the liver [Diaz and Squires 2000]. According to Duijvesteijn *et al.* [2010] and Chen *et al.* [2008], the gene known in the human genome as *CYP2A6* is identical with the pig *CYP2A19* gene, although in a number of studies concerning boar taint this gene is being referred to as *CYP2A6*. Wiercinska *et al*. [2012] stated that other potentially important metabolism regulators apart from *CYP2E1* are also *CYP2A19* and *CYP2C49*. Skinner *et al*. [2006] in their work also tested the effects of *CYP2C18* in a hybrid Danish population (Landrace - Yorkshire - Duroc), but the study proved no significant effect of this gene. Matal *et al.* [2009] also confirmed the influence

of *CYP2E1* and recommended further investigations of the effects of *CYP2A19* and *CYP1A2* genes on the skatole metabolism and the origin of its metabolites. A major part in phase II of skatole metabolism is played by *SULT1A1* [Babol *et al.* 1998a]. Lanthier *et al.* [2007] studied prepubescent boars and in addition to studies of the effects of *CYP2E1* and *SULT1A1* on the metabolism of skatole, their work was also focused on the influence of aldehyde oxidase (*AO*) on skatole metabolism.

Genes affecting phase I of skatole metabolism

Genes of the CYP (cytochrome P450) family

Gene CYP2A6 (CYP2A19) **(**cytochrome P450, family 2, subfamily A, polypeptide 6). This gene (ENSSSCG00000022808) is located on SSC6 [Diaz and Squires, 2000; Lin *et al.* 2004a] (44,853,498-44,876,802 bp)(Ensembl). In the human genome, the gene is found on chromosome 19q13.2 [Ensembl). The *CYP2A6* gene is associated with the metabolism of some of the tobacco carcinogens, mainly nicotine [Yamazaki *et al.* 1999]. Liu *et al.* [2013] studied the effect of gene polymorphism *CYP2A6*4* on the incidence of lung cancer in smokers. Although the study did not find any significant relationship in general population, it did confirm a significant effect on the Asian population of smokers. CYP2A6 is considered to be one of the most important enzymes regulating the skatole metabolism in pigs. Diaz and Squires [2000] and Lin *et al.* [2004a] also confirmed its significant role in skatole metabolism and assumed that a close monitoring of CYP2A6 levels and activity could help to regulate the levels of skatole in pigs. Pigs with a high activity of CYP2A6 also show very low levels of skatole [Lin *et al.* 2004a]. Conversely low CYP2A6 activity is connected with an excessive acccumulation of skatole in the fat tissue of pigs [Chen *et al.* 2008]. Lin *et al.* [2004a] detected a deletion mutation, in which a guanine base is deleted at the nucleotide position 421 (GenBank, Accession Number AY091516.1), which leads to the shift of reading frame and changes its length from 1485 to 612 bp. The results of this study suggest that this deletion leads to inactivation of *CYP2A6* and a loss of enzymatic activity, which results in excessively high levels of skatole in pigs.

CYP2E1 (cytochrome P450, family 2, subfamily E, polypeptide 1). Another gene with a significant role in the skatole hepatic metabolism is *CYP2E1* (ENSSSCG00000010780). It is found on chromosome SSC14 in location 153,477,961-153,490,404 bp. In the human genome this gene is located on chromosome 10q26.3 (Ensembl). The *CYP2E1* gene expression is a subject of intensive studies in humans as well, mainly due to its role in the metabolism of xenobiotics and carcinogens [Gonzalez 2005]. Its effect on carcinogens metabolism was studied not only in humans, but also in mice [Konstandi *et al.* 2013], rats and rabbits [Koop and Casazza 1985]. Konstandi *et al.* [2013] studied the effects of female hormones on regulation of CYP2E1 and reached the conclusion that CYP2E1 is indeed influenced by female hormones, which could have a negative effect on the metabolism of many toxic and carcinogenic substances. It is also being studied in connection with alcohol (ethanol) metabolism and oxidative stress in

hepatocytes, in relation to liver damage in alcoholics [Lu and Cederbaum 2008]. In addition, it also takes part in the metabolism of ketone bodies (which are released during periods of fasting) and thus influences gluconeogenesis [Koop and Casazza 1985]. The activity of CYP2E1 in the liver significantly determines the total concentration of skatole and its metabolites in the fat tissue of pigs [Babol *et al.* 1998b]. *CYP2E1* expression is in a negative correlation with skatole accumulation in pigs. *CYP2E1* expression and the level of skatole in fat tissue are in inverse proportion to each other [Lin *et al*. 2006]. Skinner *et al*. [2005] discovered a SNP in the promoter region of *CYP2E1*, which could be related to skatole fat deposition in Danish pig production populations. However, no QTL for skatole were found in this area and that is why it seems highly improbable that this mutation could be connected with the formation of boar taint [Robic *et al.* 2008]. Lin *et al.* [2006] identified a substitution mutation G>A in nucleotide 1423 in the coding region of *CYP2E1* gene (GenBank Accession Number NM_214421.1), which replaces the original amino acid alanine - Ala^{475} with Thr^{475}. This mutation causes a significant decrease in *CYP2E1* expression. The evaluated data suggest that the mutation could be at least partially responsible for high levels of skatole in pigs. *CYP2E1* is a gene which affects not only the concentrations of skatole, but also the metabolism of indole. Mörlein *et al.* [2012] studied the effect of SNP AJ697882.1:g.2412 C>T (in the promoter region) in two production populations of crossbred Duroc pigs. The frequencies of individual genotypes were *CC* (25%), *CT* (52%) and *TT* (23%), with the genotype *CC* showing significantly higher levels of skatole and indole than the other genotypes. The activity of *CYP2E1* promoter is affected by two main factors; COUP-TF1 and HNF-1α. Androstenone is capable of reducing the promoter's activity by binding with HNF-1α and blocking its binding with the promoter. This phenomenon could explain the inhibition of CYP2E1 activity in isolated hepatocytes and also the low CYP2E1 expression in animals with high levels of androstenone *in vivo*. [Tambyrajah *et al.* 2004].

CYB5A. The *CYB5A* gene has already been described above as one of the genes regulating androstenone synthesis. Wiercinska *et al.* [2012] studied the effect of CYB5A on skatole metabolism and believe, that CYB5A can affect not only CYP17A1, but also some other isoforms of cytochrome P450, in an inhibitory or stimulatory way and can thus influence the metabolism of skatole. The study found that the *CYB5A* effect is inconclusive and very specific for individual genes, however by influencing *CYP2A19, CYP2E1* and *CYP2C49* genes it could be possible to use it in order to regulate skatole levels.

Genes affecting phase II of skatole metabolism

Sulfotransferases

SULT1A1 (sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1): In pigs, the *SULT1A1* gene (ENSSSCG00000021557) is located on SSC3 [Lin *et al.* 2004b] in (Scaffold GL892962.1: 15,128-17,110) (Ensembl). Genes for phenol

sulfotransferases and their functions have been previously described for a human population [Babol *et al.* 1998a]. In human genome *SULT1A1* is located on chromosome 16p12.1 (Ensembl). In human medicine, this gene is intensively studied mainly due to its involvement in carcinogenesis. Several *SULT1A1* mutations have been connected with certain cancer types [Lin *et al.* 2004b], such as cancers of lung, colon, stomach, kidney, uterine cervix or urinary bladder, caused by a G>A polymorphism, which changes arginine (Arg^{213}) to histidine (His^{213}) [Zheng *et al.* 2003], oesophagus cancer in men [Wu *et al.* 2003] or breast cancer in women [Tengström *et al.* 2012]. The main role of *SULT1A1* in pigs lies in the phase II of skatole metabolism [Babol *et al.* 1998b]. Lin *et al* [2004b] studied *SULT1A1* polymorphism in 69 boars of European breeding populations (Yorkshire, Duroc, Landrace, Pietrain and crossbreds Landrace x Duroc; Large White x Duroc and Large White x Pietrain) and identified a substitution mutation (A>G) in the 546 nucleotide, located in the coding region of the gene (AY193893.1: c439A>G). This mutation causes the transformation of lysine - Lys^{147} to glutamine - Glu^{147} and results in reduced enzymatic activity of SULT1A1. This mutation could be at least partially responsible for the low catalytic activity of SULT1A1 and subsequently high concentrations of skatole in fat tissue. Whereas Lin *et al.* [2004b] detected *SULT1A1* on chromosome SSC3, Varona *et al.* [2005] were unable to detect the QTL in this area in a studied Landrace pig population. It is therefore necessary to conduct further studies and to take into account the breed or hybrid combination of the pigs studied. Skinner *et al.* [2006] attempted to confirm these conclusions in Large White and Meishan hybrids, as well as in the Danish production populations, but they were unable to find the previously mentioned mutation.

Relationship between the genes for androstenone and skatole

During the period of puberty there is an increase of skatole levels in the fat tissue of boars, which then correlates with the levels of androstenone [Babol *et al.* 1999]. However, no possible effect of certain genes on the androstenone and skatole level at the same time was discussed in the above mentioned publications of Wiercinska *et al.* [2012], Doran *et al.* [2004] and Tambyrajah *et al.* [2004].

Conclusion

Based on the information discussed in this review it is clear that careful breeding selection using selected candidate genes could present a suitable solution to problem of boar taint in the pork. However, this approach also has its risks, which need to be taken into consideration. Several potential candidate genes have been selected in order to regulate androstenone and skatole levels in fat tissue in pigs. In the case of some of these genes, their effect on androstenone and skatole levels has already been clearly demonstrated and researchers identified mutations causing either increase or decrease in the expression of these genes. Nonetheless, the effects of these mutations have not

been unequivocally confirmed. Several studies even describe the reciprocal effects of certain genes on each other – such as the effect of *CYB5A* on *CYP17A1* expression [Billen and Squires 2009]. Based on that, it is obvious that boar taint is the result of a larger number of genes, which mutually affect each other. Considering the fact that the genes affecting boar taint are located in the same chromosomal areas as genes coding enzymes necessary for sex hormones synthesis we also need to take into account any possible negative effects on fertility of the pigs. The above mentioned studies show possible negative effects of these genes on the fertility of boars and sows [Tajet *et al.* 2006; Strathe *et al.* 2013]. The potential negative effects of these genes could also be found in the area of meat quality traits. Due to low levels of androstenone there could be potential decrease in production parameters and carcass composition. Therefore it is appropriate to focus on such genes and SNPs which would be able to lower androstenone and skatole levels without affecting performance parameters [Moe *et al.* 2009].

REFERENCES

1. ANDRESEN O., 2006 – Boar taint related compounds: Androstenone/skatole/other substances. ***Acta Veterinaria Scandinavica*** 48 (Suppl 1), S5.
2. BABOL J., SQUIRES E.J., LUNDSTRÖM K., 1998a – Relationship between oxidation and conjugation metabolism of skatole in pig liver and concentrations of skatole in fat. ***Journal of Animal Science*** 76, 829-838.
3. BABOL J., SQUIRES J., LUNDSTRÖM K., 1998b – Hepatic metabolism of skatole in pigs by cytochrome P4502E1. ***Journal of Animal Science*** 76, 822-828.
4. BABOL J., SQUIRES E.J., LUNDSTRÖM K., 1999 – Relationship between metabolism of androstenone and skatole in intact male pigs. ***Journal of Animal Science*** 77, 84-92.
5. BILLEN M.J., SQUIRES E.J., 2009 – The role of porcine cytochrome b5A and cytochrome b5B in the regulation of cytochrome P45017A1 activities. ***Journal of Steroid Biochemistry and Molecular Biology*** 113, 98-104.
6. BONNEAU M., WALSTRA P., CLAUDI-MAGNUSSEN C., KEMPSTER A.J., TORNBERG E., FISCHER K., DIESTRE A., SIRET F., CHEVILLON P., CLAUS R., DIJKSTERHUIS G., PUNTER P., MATTHEWS K.R., AGERHEM H., BEAGUE M.P., OLIVER M.A., GISPERT M., WEILER U., VON SETH G., LEASK H., FURNOLS M.F.I., HOMER D.B., COOK G.L., 2000 – An international study on the importance of androstenone and skatole for boar taint: IV. Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting carcasses on the slaughter line, main conclusions and recommendations. ***Meat Science*** 54, 285-295.
7. DE CAMPOS C. F., LOPES M. S., E SILVA F. F., VERONEZE R., KNOL E. F., LOPES P. S., GUIMARÄES S. E. F., 2015 – Genomic selection for boar taint compounds and carcass traits in a commercial pig population. ***Livestock Science*** 174, 10-17.
8. CLAUS R., HOFFMANN B., KARG H. 1971 – Determination of 5α-androst-16-en-3-one, a boar taint steroid in pigs, with reference to relationships to testosterone. ***Journal of Animal Science*** 33, 1293-1297.
9. CHEN G., BOURNEUF E., MARKLUND S., ZAMARATSKAIA G., MADEJ A., LUNDSTRÖM K. 2007 – Gene expression of 3b-hydroxysteroid dehydrogenase and 17b-hydroxysteroid dehydrogenase in relation to androstenone, testosterone, and estrone sulphate in gonadally intact male and castrated pigs. ***Journal of Animal Science*** 85. 2457-2463.

10. CHEN G., CUE R.A., LUNDSTRÖM K., WOOD J.D., DORAN O., 2008 – Regulation of CYP2A6 protein expression by skatole, indole, and testicular steroids in primary cultured pig hepatocytes. ***Drug Metabolism and Disposition*** 36, 56-60.
11. DASGUPTA S., SIRISH P.V.S., NEELAVENI K., ANURADHA K., SUDHAKAR G., MOHAN REDDY B. 2012 – Role of luteinizing hormone β-subunit gene variants among South Indian women with polycystic ovary syndrome. ***GENE*** 494, 51-56.
12. DAVIS S.M., SQUIRES E.J., 1999 – Association of cytochrome b(5) with 16-androstene steroid synthesis and the testis and accumulation in the fat of male pigs. ***Journal of Animal Science*** 77, 1230-1235.
13. DESLANDES B., GARIEPY C., HOUDE A., 2001 – Review of microbiological and biochemical effects of skatole on animal production. ***Livestock Production Science*** 71, 193-200.
14. DIAZ G.J., SKORDOS K.W., YOST G.S., SQUIRES E.J., 1999 – Identification of phase I metabolites of 3-methylindole produced by pig liver microsomes. ***Drug Metabolism and Disposition*** 27, 1150-1156.
15. DIAZ G.J., SQUIRES E.J., 2000 – Metabolism of 3-methylindole by porcine liver microsomes: Responsible cytochrome P450 enzymes. ***Toxicological Sciences*** 55, 284-292.
16. DIAZ G.J., SQUIRES E.J., 2003 – Phase II *in vitro* metabolism of 3-methylindole metabolites in porcine liver. ***Xenobiotica*** 33, 485-498.
17. DORAN E., WHITTINGTON F.A., WOOD J.D., MCGIVAN J.D., 2004 – Characterisation of androstenone metabolism in pig liver microsomes. ***Chemico-Biological Interactions*** 147, 141-149.
18. DUIJVESTEIJN N., KNOL E.F., MERKS J.W.M., CROOIJMANS R., GROENEN M.A.M., BOVENHUIS H., HARLIZIUS B., 2010 – A genome-wide association study on androstenone levels in pigs reveals a cluster of candidate genes on chromosome 6. ***BMC Genetics*** 11, 42.
19. DUMAS N.A., HE D., FROST A.R., FALANY C.N., 2008 – Sulfotransferase 2B1b in human breast: Differences in subcellular localization in African American and Caucasian women. ***Journal of Steroid Biochemistry and Molecular Biology*** 111, 171-177.
20. ENGELSMA K. A., BERGSMA R., HARLIZIUS B., DUCRO-STEVERINK D., KNOL E., 2007 – Genetic parameters for components of boar taint and their relation with carcass quality and female fertility. EAAP 58th, Dublin, Ireland, August 26-29. Session 10, Poster 27.
21. ERNST C. W., STEIBEL J. P., 2013 – Molecular advances in QTL discovery and application in pig breeding. ***Trends in Genetics***, 29 (4), 215-224.
22. GIOVANGRANDI Y., PARFAIT B., ASHEUER M., OLIVI M., LIDEREAU R., VIDAUD M., BIECHE I., 2001 – Analysis of the human CGB/LHB gene cluster in breast tumors by real-time quantitative RT-PCR assays. ***CANCER Letters*** 168, 93-100.
23. GONZALEZ F.J., 2005 – Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. ***Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*** 569, 101-110.
24. GRAY M.A., SQUIRES E.J., 2013 – Effects of nuclear receptor transactivation on steroid hormone synthesis and gene expression in porcine Leydig cells. ***Journal of Steroid Biochemistry and Molecular Biology*** 133, 93-100.
25. GREGERSEN V.R., CONLEY L.N., SORENSEN K.K., GULDBRANDTSEN B., VELANDER I.H., BENDIXEN C., 2012 – Genome-wide association scan and phased haplotype construction for quantitative trait loci affecting boar taint in three pig breeds. ***BMC Genomics*** 13, 22.
26. GRINDFLEK E., BERGET I., MOE M., OETH P., LIEN S., 2010 – Transcript profiling of candidate genes in testis of pigs exhibiting large differences in androstenone levels. ***BMC Genetics*** 11, 4.
27. GRINDFLEK E., LIEN S., HAMLAND H., HANSEN M.H.S., KENT M., VAN SON M., MEUWISSEN T.H.E., 2011a – Large scale genome-wide association and LDLA mapping study identifies QTLs for boar taint and related sex steroids. ***BMC Genomics*** 12, 362.

28. GRINDFLEK E., MEUWISSEN T.H.E., AASMUNDSTAD T., HAMLAND H., HANSEN M.H.S., NOME T., KENT M., TORJESEN P., LIEN S., 2011b – Revealing genetic relationships between compounds affecting boar taint and reproduction in pigs. ***Journal of Animal Science*** 89, 680-692.
29. GROßE-BRINKHAUS CH., STORCK L. C., FRIEDEN L., NEUHOFF CH., SCHELLANDER K., LOOFT CH., THOLEN E., 2015 – Genome-wide association analyses for boar taint components and testicular traits revealed regions having pleiotropic effects. ***BMC Genetics*** 16, 36.
30. GUNAWAN A., SAHADEVAN S., NEUHOFF CH., GROßE-BRINKHAUS CH., GAD A., FRIEDEN L., TESFAYE D., THOLEN E., LOOFT CH., UDDIN M. J., SCHELLANDER K., CINAR M. L., 2013 – RNA Deep sequencing reveals novel candidate genes and polymorphisms in boar testis and liver tissues with divergent androstenone levels. ***PLoS ONE*** 8, e63259**.**
31. HUANG C., ZHOU T., CHEN Y., SUN T., ZHANG S., CHEN G., 2014 – estrogen-related receptor err alpha regulation of human hydroxysteroid sulfotransferase (sult2a1) gene expression in human caco-2 cells. ***Journal of Biochemical and Molecular Toxicology*** 28, 32-38.
32. JACQUEMIN P., CHEN Z., MARTIAL J.A., DAVIDSON I., 1999 – Genomic structure and chromosomal mapping of the mouse transcription factor TEF-5 (*Tead3*) gene. ***Mammalian Genome*** 10, 632-634.
33. JI, Y., MOON I., ZLATKOVIC J., SALAVAGGIONE O.E., THOMAE B.A., ECKLOFF B.W., WIEBEN E.D., SCHAID D.J., WEINSHILBOUM R.M., 2007 – Human hydroxysteriod sulfotransferase SULT2B1 pharmacogenomics: Gene sequence variation and functional genomics. ***Journal of Pharmacology and Experimental Therapeutics*** 322, 529-540.
34. KIM J.M., AHN J.H., LIM K.S., LEE E.A., CHUN T., HONG K.C., 2013 – Effects of *hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1* polymorphisms on fat androstenone level and gene expression in Duroc pigs. ***Animal Genetics*** 44, 592-595.
35. KONSTANDI M., CHENG J., GONZALEZ F.J., 2013 – Sex steroid hormones regulate constitutive expression of *Cyp2e1* in female mouse liver. ***American Journal of Physiology-Endocrinology and Metabolism*** 304, E1118-E1128.
36. KOOP D.R., CASAZZA J.P., 1985 – Identification of ethanol – inducible P-450 isozyme 3A as the acetone and acetol monooxygenase of rabbit microsomes. ***Journal of Biological Chemistry*** 260, 3607-3612.
37. KUROGI K., SAKAKIBARA Y., KAMEMOTO Y., TAKAHASHI S., YASUDA S., LIU M.-C., SUIKO M., 2010 – Mouse cytosolic sulfotransferase SULT2B1b interacts with cytoskeletal proteins via a proline/serine-rich C-terminus. ***FEBS Journal*** 277, 3804-3811.
38. LANTHIER F., LOU Y., SQUIRES E.J., 2007 – Skatole metabolism in the intact pre-pubescent male pig: The relationship between hepatic enzyme activity and skatole concentrations in plasma and fat. ***Livestock Science*** 106, 145-153.
39. LEE, G.J., ARCHIBALD,A.L., LAW A.S., LLOYD S., WOOD J., HALEY C.S., 2005 – Detection of quantitative trait loci for androstenone, skatole and boar taint in a cross between Large White and Meishan pigs. ***Animal Genetics*** 36, 14-22.
40. LEUNG M.C.K., BOWLEY K.L., SQUIRES E.J., 2010 – Examination of testicular gene expression patterns in yorkshire pigs with high and low levels of boar taint. ***Animal Biotechnology*** 21, 77-87.
41. LIN Z., LOU Y., SQUIRES E.J., 2004a – Molecular cloning, expression and functional characterization of the *cytochrome P450 2A6* gene in pig liver. ***Animal Genetics*** 35, 314-316.
42. LIN Z.H., LOU Y.P., SQUIRES E.J., 2004b – Molecular cloning and functional analysis of porcine *SULT1A1* gene and its variant: a single mutation *SULT1A1* causes a significant decrease in sulfation activity. ***Mammalian Genome*** 15, 218-226.
43. LIN Z.H., LOU Y.P., PEACOCK J., SQUIRES E.J., 2005a – A novel polymorphism in the 5 ‘ untranslated region of the porcine cytochrome b5 (*CYB5*) gene is associated with decreased fat androstenone level. ***Mammalian Genome*** 16, 367-373.

44. LIN Z.H., LOU Y.P., SQUIRES E.J., 2005b – Identification of a single nucleotide polymorphism in porcine testis cytochrome P450-c17 (CYP17) and its effect on steroidogenesis. ***Biochemical Genetics*** 43, 531-542.
45. LIN Z.H., LOU Y.P., SQUIRES E.J., 2006 – Functional polymorphism in porcine CYP2E1 gene: Its association with skatole levels. ***Journal of Steroid Biochemistry and Molecular Biology*** 99, 231-237.
46. LIU Y., XU Y., LI F., CHEN H., GUO S., 2013 – CYP2A6 deletion polymorphism is associated with decreased susceptibility of lung cancer in Asian smokers: a meta-analysis. ***Tumor Biology*** 34, 2651-2657.
47. LU Y., CEDERBAUM A.I., 2008 – CYP2E1 and oxidative liver injury by alcohol. ***Free Radical Biology and Medicine*** 44, 723-738.
48. LUKIĆ B., PONG-WONG R., ROWE S. J., DE KONING D. J., VELANDER I., HALEY C. S., ARCHIBALD A. L., WOOLLIAMS J. A., 2015 – Efficiency of genomic prediction for boar taint reduction in Danish Landrace pigs. ***Animal Genetics*** 46, 607-616.
49. LUNDSTRÖM K., ZAMARATSKAIA G., 2006 – Moving towards taint-free pork – alternatives to surgical castration. ***Acta Veterinaria Scandinavica***. 48(Suppl 1). S1.
50. MATAL J., MATUSKOVA Z., TUNKOVA A., ANZENBACHEROVA E., ANZENBACHER P., 2009 – Porcine CYP2A19, CYP2E1 and CYP1A2 forms are responsible for skatole biotransformation in the reconstituted system. ***Neuroendocrinology Letters*** 30, 36-40.
51. MOE M., GRINDFLEK E., DORAN O., 2007a – Expression of 3 beta-hydroxysteroid dehydrogenase, cytochrome P450-c17, and sulfotransferase 2B1 proteins in liver and testis of pigs of two breeds: Relationship with adipose tissue androstenone concentration. ***Journal of Animal Science*** 85, 2924-2931.
52. MOE M., MEUWISSEN T., LIEN S., BENDIXEN C., WANG X.F., CONLEY L.N., BERGET I., TAJET H., GRINDFLEK E., 2007b – Gene expression profiles in testis of pigs with extreme high and low levels of androstenone. ***BMC Genomics*** 8, 405.
53. MOE M., LIEN S., BENDIXEN C., HEDEGAARD J., HORNSHOJ H., BERGET I., MEUWISSEN T.H.E., GRINDFLEK E., 2008 – Gene expression profiles in liver of pigs with extreme high and low levels of androstenone. ***BMC Veterinary Research*** 4,29.
54. MOE M., LIEN S., AASMUNDSTAD T., MEUWISSEN T.H.E., HANSEN M.H.S., BENDIXEN C., GRINDFLEK E., 2009 – Association between SNPs within candidate genes and compounds related to boar taint and reproduction. ***BMC Genetics*** 10, 32.
55. MÖRLEIN D., LUNGERSHAUSEN M., STEINKE K., SHARIFI A.R., KNORR C., 2012 – A single nucleotide polymorphism in the CYP2E1 gene promoter affects skatole content in backfat of boars of two commercial Duroc-sired crossbred populations. ***Meat Science*** 92, 739-744.
56. NAKAMURA Y., XING Y., HUI X.G., KUROTAKI Y., ONO K., COHEN T., SASANO H., RAINEY W.E., 2011 – Human adrenal cells that express both 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) and cytochrome b5 (CYB5A) contribute to adrenal androstenedione production. ***Journal of Steroid Biochemistry and Molecular Biology*** 123, 122–126.
57. NICOLAU-SOLANO S.I., MCGIVAN J.D., WHITTINGTON F.M., NIEUWHOF G.J., WOOD J.D., DORAN O., 2006 – Relationship between the expression of hepatic but not testicular 3 beta-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissued. ***Journal of Animal Science*** 84, 2809-2817.
58. OSKAM I.C., LERVIK S., TAJET H., DAHL E., ROPSTAD E., ANDRESEN O., 2010 – Differences in testosterone, androstenone, and skatole levels in plasma and fat between pubertal purebred Duroc and Landrace boars in response to human chorionic gonadotrophin stimulation. ***Theriogenology*** 74, 1088-1098.

59. PANELLA-RIERA N., MOE M., GRINDFLEK E., OLIVER M.A., WOOD J.D., DORAN O., 2008 – Effect of sex steroids on expression of sulfotransferase 2B1 immunoreactive protein in primary cultured porcine hepatocytes. ***Livestock Science*** 118, 223-230.
60. PAYNE A.H., HALES D.B., 2004 – Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. ***Endocrine Reviews*** 25, 947-970.
61. PEACOCK J., LOU Y., LUNDSTRÖM K., SQUIRES E.J., 2008 – The effect of a c.-8G > T polymorphism on the expression of *cytochrome b5A* and boar taint in pigs. ***Animal Genetics*** 39, 15-21.
62. QUINTANILLA R., DEMEURE O., BIDANEL J.P., MILAN D., IANNUCCELLI N., AMIGUES Y., GRUAND J., RENARD C., CHEVALET C., BONNEAU M., 2003 – Detection of quantitative trait loci for fat androstenone levels in pigs. ***Journal of Animal Science*** 81, 385-394.
63. RAMOS A.M., DUIJVESTEIJN N., KNOL E.F., MERKS J.W.M., BOVENHUIS H., CROOIJMANS R., GROENEN M.A.M., HARLIZIUS B., 2011 – The distal end of porcine chromosome 6p is involved in the regulation of skatole levels in boars. ***BMC Genetics*** 12, 35.
64. ROBIC A., LARZUL C., BONNEAU M., 2008 – Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: A review. ***Genetics Selection Evolution*** 40, 129-143.
65. ROBIC A., FEVE K., LARZUL C., BILLON Y., VAN SON M., LIAUBET L., SARRY J., MILAN D., GRINDFLEK, E., BIDANEL, J.P., RIQUET J., 2011a – Expression levels of 25 genes in liver and testis located in a QTL region for androstenone on SSC7q1.2. ***Animal Genetics*** 42, 662-665.
66. ROBIC A., LE MIGNON G., FEVE K., LARZUL C., RIQUET J., 2011b – New investigations around *CYP11A1* and its possible involvement in an androstenone QTL characterised in Large White pigs. ***Genetics Selection Evolution*** 43,15.
67. ROBIC A., LARZUL C., GRINDFLEK E., CHEVILLON P., HOFER A., FEVE K., IANNUCCELLI N., MILAN D., PRUNIER A., RIQUET J., 2012 – Molecular characterization of the porcine *TEAD3 (TEF-5)* gene: examination of a promoter mutation as the causal mutation of a quantitative trait loci affecting the androstenone level in boar fat. ***Journal of Animal Breeding and Genetics*** 129, 325-335.
68. ROWE S. J., KARACAÖREN B., DE KONING D. J., LUKIĆ B., HASTINGS-CLARK N., VELANDER I., HALEY C. S., ARCHIBALD A. L., 2014 – Analysis of the genetics of boar taint reveals both single SNPs and regional effects. ***BMC Genomics***, 15, 424.
69. SAHADEVAN S., THOLEN E., GROßE-BRINKHAUS C., SCHELLANDER K., TESFAYE D., HOFMANN-APITIUS M., CINAR M. U., GUNAWAN A., HÖLKER M., NEUHOFF C., 2015 – Identification of gene co-expression clusters in liver tissues from multiple porcine populations with high and low backfat androstenone phenotype. ***BMC Genetics***, 16, 21.
70. SIMARD J., RICKETTS ML., GINGRAS S., SOUCY P., FELTUS F.A., MELNER M.H. 2005 – Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5–4 isomerase gene family. ***Endocrine Reviews*** 26, 525–82.
71. SINCLAIR P.A., HANCOCK S., GILMORE W.J., SQUIRES E.J., 2005 – Metabolism of the 16-androstene steroids in primary cultured porcine hepatocytes. Journal of Steroid ***Biochemistry and Molecular Biology*** 96, 79-87.
72. SINCLAIR P.A., GILMORE W.J., LIN Z., LOU Y., SQUIRES E.J., 2006 – Molecular cloning and regulation of porcine *SULT2A1*: relationship between SULT2A1 expression and sulfoconjugation of androstenone. ***Journal of Molecular Endocrinology*** 36, 301-311.
73. SKINNER T.M., DORAN E., MCGIVAN J.D., HALE C.S., ARCHIBALD A.L., 2005 – Cloning and mapping of the porcine *cytochrome-p450 2E1* gene and its association with skatole levels in the domestic pig. ***Animal Genetics*** 36, 417-422.
74. SKINNER T.M., ANDERSON J.A., HALEY C.S., ARCHIBALD A.L., 2006 – Assessment of *SULT1A1*, *CYP2A6* and *CYP2C18* as candidate genes for elevated backfat skatole levels in

commercial and experimental pig populations. ***Animal Genetics*** 37, 521-522.

75. STRATHE A.B., VELANDER I.H., MARK T., KADARMIDEEN H.N., 2013 – Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace. ***Journal of Animal Science*** 91, 2587-2595.
76. TAJET H., ANDRESEN, O., MEUWISSEN T., 2006 – Estimation of genetic parameters of boar taint; skatole and androstenone and their correlations with sexual maturation. ***Acta Veterinaria Scandinavica***. 48(Suppl 1). S9.
77. TAMBYRAJAH W.S., DORAN E., WOOD J.D., MCGIVAN J.D., 2004 – The pig CYP2E1 promoter is activated by COUP-TF1 and HNF-1 and is inhibited by androstenone. ***Archives of Biochemistry and Biophysics*** 431, 252-260.
78. TENGSTRÖM M., MANNERMAA A., KOSMA V.-M., HIRVONEN A., KATAJA V., 2012 – *SULT1A1* rs9282861 polymorphism-a potential modifier of efficacy of the systemic adjuvant therapy in breast cancer? ***BMC Cancer*** 12, 257.
79. TÖRN S., NOKELAINEN P., KURKELA R., PULKKA A., MENJIVAR M., GHOSH S., COCA-PRADOS M., PELTOKETO H., ISOMAA V., VIHKO P. 2003 – Production, purification, and functional analysis of recombinant human and mouse 17β-hydroxysteroid dehydrogenase type 7. ***Biochemical and Biophysical Research Communications*** 305, 37-45.
80. VARONA L., VIDAL O., QUINTANILLA R., GIL M., SANCHEZ A., FOLCH J.M., HORTOS M., RIUS M.A., AMILLS M., NOGUERA J.L., 2005 – Bayesian analysis of quantitative trait loci for boar taint in a Landrace outbred population. ***Journal of Animal Science*** 83, 301-307.
81. WIERCINSKA P., LOU Y., SQUIRES E.J., 2012 – The roles of different porcine cytochrome P450 enzymes and cytochrome b5A in skatole metabolism. ***Animal*** 6, 834-845.
82. WU M.T., WANG Y.T., HO C.K., WU D.C., LEE Y.C., HSU H.K., KAO E.L., LEE J.M., 2003 – *SULT1A1* polymorphism and esophageal cancer in males. ***International Journal of Cancer*** 103, 101-104.
83. XUE J.L., DIAL G.D., 1997 – Raising intact male pigs for meat: Detecting and preventing boar taint. ***Swine Health and Production*** 5, 151-158.
84. YAMAZAKI H., INOUE K., HASHIMOTO M., SHIMADA T., 1999 – Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. ***Archives of Toxicology*** 73, 65-70.
85. YOKOYAMA M. T., CARLSON J. R., 1979 – Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. ***American Journal of Clinical Nutrition*** 32, 173-178.
86. ZAMARATSKAIA G., LOU Y., PEACOCK J., RYDHMER L., ANDERSSON H.K., JUNEJA R.K., CHEN G., LUNDSTRÖM K., SQUIRES E.J., 2008 – Effect of polymorphism in the porcine cytochrome b5 (*CYB5A*) gene on androstenone and skatole concentrations and sexual development in Swedish pig populations. ***Animal*** 2, 190-196.
87. ZAMARATSKAIA G., SQUIRES E.J., 2009 – Biochemical, nutritional and genetic effects on boar taint in entire male pigs. ***Animal*** 3, 1508-1521.
88. ZAMARATSKAIA G., STEFANOVIC S., LUNDSTRÖM K., DORAN O., 2012 – Expression of the hepatic skatole- and androstenone-metabolising enzymes in entire male pigs of two live weights. ***Livestock Science*** 145, 124-130.
89. ZHENG L.Z., WANG Y.F., SCHABATH M.B., GROSSMAN H.B., WU X.F., 2003 – Sulfotransferase 1A1 (SULT1A1) polymorphism and bladder cancer risk: a case-control study. ***CANCER Letters*** 202, 61-69.