mRNA Expression of *CYP2E1, CYP2A19, CYP1A2, HSD3B, SULT1A1* and *SULT2A1* genes in surgically castrated, immunologically castrated, entire male and female pigs and correlation with androstenone, skatole, indole and Improvac-specific antibody levels

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Abstract: This study aimed to obtain a comprehensive look at the influence of castration on mRNA expression of the genes *CYP2E1, CYP1A2, CYP2A19, HSD3B, SULT2A1* and *SULT1A1* and their correlation with boar taint compounds (androstenone, skatole and indole) and Improvac-specific antibodies in a Czech commercial hybrid (Large White × Landrace (sow) × Duroc (boar)). Pigs were divided into groups of entire male pigs (NC), pigs castrated surgically (SC), pigs immunologically castrated and slaughtered 8 weeks (IM8) or 15 weeks (IM15) after the second dose of Improvac, and gilts (GI). Hepatic mRNA expression, measured by quantitative real-time polymerase chain reaction, differed significantly between the control group (entire male pigs) and all groups of interest for *CYP2E1, CYP1A2* and *CYP2A19*. The mRNA level of the *HSD3B* gene differed significantly between the control group and the IM8, IM15 and GI groups. *SULT1A1* gene expression was significantly different between the control group and the SC, IM8 and GI. In the case of *SULT2A1*, a significant difference was observed only between the control group and IM8 pigs. For all genes and treatment groups described above, expression was increased relative to the control. Significant differences for Improvac-specific antibodies between IM8 and IM15 groups were observed, indicating decrease of antibodies over time. Moreover, negative correlations between androstenone and mRNA levels of *CYP2A19, CYP2E1* and *SULT1A1* suggest that gene expression is suppressed.

Keywords: boar taint; immunocastration; RT-qPCR

Although entire male pigs have better feed conversion, faster growth rates, and leaner meat than do castrated male pigs (Xue et al. 1997), surgical castration of male pigs up to 7 days of age without anaesthesia is a common practice in Europe. This is to prevent boar taint, which is an unpleasant odour and flavour characteristic manifesting when pork is cooked and eaten. Boar taint is mainly caused by three compounds deposited in fat tissue: skatole, indole and androstenone. A relationship has been observed between androstenone and skatole, as a study on pig hepatocytes showed that excessive concentrations of androstenone lead to reduced skatole metabolism and its accumulation in fat (Babol et al. 1999). Surgical castration has been criticized, because it is painful for the piglets (Kluivers-Poodt et al. 2012) and creates higher risk of infection (Prunier et al. 2006). In 2011, therefore, the declaration of Brussels was signed by parties involved in the European pig sector with the aim of ending castration of male piglets in Europe in 2018 (Boars2018.com).

Immunocastration is one of the ways to reduce boar taint. This is based on the application of an analogue of gonadotropin releasing hormone (GnRH) linked to a carrier protein as included in the registered and commercially available veterinary product Improvac (Zoetis, Belgium SA). Vaccination with Improvac leads to production of GnRH-specific neutralizing antibodies and blocks the biosynthesis of testicular steroids. According to Improvac's manufacturer, two doses must be administered – the first at any time after 8 to 9 weeks of age and the second no less than 4 weeks after the first dose but 4 to 5 weeks prior to slaughter. Various studies have shown that different vaccine schedules can be used, however, such as to have a later time of slaughter after the second dose (Claus et al. 2008).

Another potential substitution for castration is genetic selection, which requires identifying candidate genes to reduce boar taint. Cytochrome P450 (CYP450) is the major family of enzymes involved in the metabolism of several xenobiotic and endogenous compounds, and it is expressed in a variety of tissues (Zamaratskaia and Rasmussen 2015). There are differences in mRNA expression among genes within the CYP450 family, especially *CYP2E1, CYP1A2* and *CYP2A19*, between female and entire male pigs (Rasmussen et al. 2011). *CYP2E1* and *CYP2A19* are directly involved in metabolism of skatole in liver. Several *in vivo* studies have shown that testicular steroids are involved in P450 regulation (Kojima et al. 2010; Brunius et al. 2012), especially by inhibiting enzyme activity of CYP2E1 (Zamaratskaia et al. 2007). The role of testicular steroids has nevertheless been shown to differ among breeds (Kojima et al. 2010).

Other genes possibly involved in boar taint are those of 3β-hydroxysteroid dehydrogenases (*HSD3B, 3β-HSD*). The enzyme 3β-HSD is one of the main enzymes involved in the biosynthesis of active steroid hormones and it participates in catalysing androstenone reduction in liver (Doran et al. 2004). A relationship has been observed between high concentrations of androstenone in fat and low gene expression of *3β-HSD* in liver (Chen et al. 2007).

SULT1A1 (*SULT1A3*) and *SULT2A1* genes belong to the sulfotransferase family, where SULT2A1 is a key enzyme in the metabolism of androstenone in liver and testis (Moe et al. 2007). *SULT2A1* is located within the QTL region of SSC6 related to the androstenone level in boars (Duijvesteijn et al. 2010). A breed dependence on the expression of this gene has been reported (Moe et al. 2007). Lower levels of SULT1A1 have been connected to a high level of skatole (Lanthier et al. 2007) and also to a high level of testosterone (Kojima and Degawa 2013). The effect of polymorphisms of this gene and skatole level has been studied, revealing substitution of nucleotides causing decrease in its sulfation activity (Lin et al. 2004).

The aim of this study was to investigate the effects of castration on expression levels of important candidate genes involved in metabolism of skatole and androstenone (*CYP2E1, CYP1A2, CYP2A19, HSD3B, SULT2A1* and *SULT1A1*) in Czech commercial hybrid pigs and to evaluate correlations between expression of these genes and levels of androstenone, skatole and indole in backfat and Improvac-specific antibodies in serum. Our main hypothesis was that castration contributed to a decrease in androstenone level that is associated with an increase in expression of genes involved in degradation of skatole.

MATERIAL AND METHODS

Animals. Ninety pigs of a commercial hybrid (Large White × Landrace (sow) × Duroc (boar))

were used in this study. Piglets were weaned at 10.5 kg and kept in identical boxes (5 animals per box) in the accredited experimental barns of the Veterinary Research Institute, Brno, Czech Republic. Pigs were fed twice daily with a standard commercial diet according to the weight category. Animal care conformed to the Institute's good care practices protocol. All experimental procedures were approved by the Central Commission for Animal Welfare of the Czech Republic. Pigs were slaughtered at 174 days of age on average and mean slaughter weight was 110 kg. There were 90 pigs in total divided into five groups. In the first group, piglets were surgically castrated (SC, $n = 20$) without anaesthesia before 1 week of age. In the second and third groups, pigs were vaccinated with Improvac (Zoetis) according to the manufacturer's recommendations, then slaughtered 8 weeks (IM8, $n = 12$) or 15 weeks (IM15, $n = 8$) after the second dose. The fourth group consisted of gilts (GI, $n = 10$). Pigs in the fifth group (NC, $n = 40$) were males kept intact throughout the study.

Tissue sample collection. Samples for expression analysis were collected from the liver at slaughter, immediately submerged in RNAlater (Qiagen, Germany), and held at –70°C until further analysis. Samples for analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS) of androstenone, indole and skatole were collected from pig backfat. Blood samples for detecting Improvac-specific antibodies were taken from animals in the fifth week after the second dose of vaccine and at the time of slaughtering.

Total RNA extraction and reverse transcription reaction. Total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen, Germany) according to the manufacturer's recommendation and was quantified using a spectrophotometer (NanoDrop, Thermo Fisher Scientific, USA). RNA integrity was checked by 1.5% agarose gel electrophoresis. One microgram of total RNA was reverse-transcribed at 42°C using the Quantitec reverse transcription kit (Qiagen) with elimination of genomic DNA according to the manufacturer's manual. The reverse transcription products (cDNA) were stored at –20°C before relative quantitative real-time PCR.

Relative quantitative real-time polymerase chain reaction (qPCR). Primers for the *CYP2E1, CYP1A2, HSD3B* and *SULT1A1* genes were designed using OLIGO 4.0, where at least one of the primers

was designed to span exon–exon junction for eliminating amplification of genomic DNA. All others had been described previously by other authors (Sinclair et al. 2006; Chen et al. 2007; Nygard et al. 2007; Svobodova et al. 2008; Svobodova 2011; Rasmussen et al. 2011 – Supplementary Table S1 in Supplementary Online Material (SOM)). The specificity of the primer set for each gene was verified by 3% agarose gel electrophoresis and by direct Sanger sequencing of amplicons using an ABI PRISM 3500 genetic analyser (Thermo Fisher Scientific). The resultant sequences were analysed for homologous counterparts in the GenBank database using the BLAST network service (http//:www.ncbi.nlm.nih.gov/BLAST). Reaction for RT-qPCR was prepared using Power Sybr® Green Master Mix (Thermo Fisher Scientific) in triplicates for each sample and for non-template negative control. The reaction mixture consisted of 1 µl of cDNA, 10 pmol/µl of each primer, 10 µl of Power Sybr® Green Master Mix, 0.2 µl of AmpErase® Uracil N-glycosylase (UNG) (Thermo Fisher Scientific), and 8 µl of RNase-free water. The final reaction volume was 20 µl. The RT-qPCR was performed on a Rotor-Gene Q device (Qiagen), with cycling conditions consisting of hold at 50°C/2 min, denaturation at 95°C/10 min, and 40 cycles of 95°C/15 s and 60°C/1 min. The absence of non-specific products and specificity of each product were confirmed by analysis of the melting curves. We tested five reference genes selected from the literature (Supplementary Table S1 in SOM) – *PPIA, TBP1, TOP2B, GAPDH* and *HPRT* – using the geNorm algorithm (Vandesompele et al. 2002) and determined the expression stability values (M) to be 0.387, 0.389, 0.399, 0.640 and 0.737, respectively. Based on these results, we chose the most stable of these – *PPIA, TOP2B* and *TBP1* – as reference genes for subsequent expression analysis. The standard curve was measured for each primer pair individually using Rotor-Gene Q software, Version 2.1.0 to determine the reaction efficiency, which was between 0.9 and 1.06 and had R^2 value between 0.98 and 0.99.

Detection of Improvac-specific antibodies (hereinafter Improvac_Ab). A complex of GnRH with diphtheria toxin was used as antigen in enzyme-linked immunosorbent assay for detecting Improvac-specific antibodies in blood serum. Details of the method used are in Supplementary Material S1 in SOM.

Determination of the boar taint compounds. Indole, skatole and androstenone levels were detected in backfat using LC-MS/MS with high resolution. Details of the method used are in Supplementary Material S2 in SOM.

Statistical analysis. Relative mRNA expression was calculated from the Ct values obtained and normalized against mRNA expression of *PPIA, TOP2B* and *TBP1*. The mean expression in the group of entire male pigs was arbitrarily set to 1. The effect of treatment on mRNA expression of genes between groups was evaluated using an unpaired *t*-test. The *P*-values were regarded as significant when $P \leq 0.05$. One way ANOVA (GLM) in SAS, Version 9.4 (SAS Institute Inc., USA) was used for evaluating the different levels of Improvac-specific antibodies between the groups.

Multivariate statistical analysis of the data was performed using STATISTICA, Version 13.3 (TIBCO Software Inc., USA). Experimental data were modified into a data set X ($n \times m$), where $n = 11$ variables and $m = 80$ cases (male pigs 40 NC, 20 SC and 20 IM). The first step was to carry out exploratory data analysis (EDA) for each variable that included assessment and finding outlier objects to determine whether linearity of the relationships could be assumed and to verify the data provided in terms of normality, homogeneity, homoscedasticity and point interval estimates. The androstenone, indole and skatole variables showed difference from normal distribution, and so the data were

further modified by Box–Cox transformation. In the next step, the multivariate statistical methods (principal component analysis (PCA) and factor analysis (FA)) were used to evaluate the relationships between experimental variables. The entire data set was modified by scaling for the use of multivariate statistical modelling. The principal components PC1 and PC2 in the PCA were generated based on the variance and correlation of individual variables. Latent variables F1 and F2 in FA were extracted based on linear combinations followed by Varimax rotation of the first two factors. Within the multivariate statistical modelling, the Pearson correlation coefficient matrix was calculated in PCA.

RESULTS

Real-time PCR detection. Electrophoresis results showed a single band, and no non-specific products or primer dimers were generated during the PCR amplification cycle. Specificity of the PCR products was confirmed by sequencing the PCR amplicons and performing a BLAST database search against the GenBank sequences. All sequences for all genes were found to be identical with the given genes in the GenBank database.

We estimated mRNA expression for *CYP2E1, CYP2A19, CYP1A2, HSD3B, SULT1A1* and *SULT1A2* genes (Figure 1). In comparison with entire male
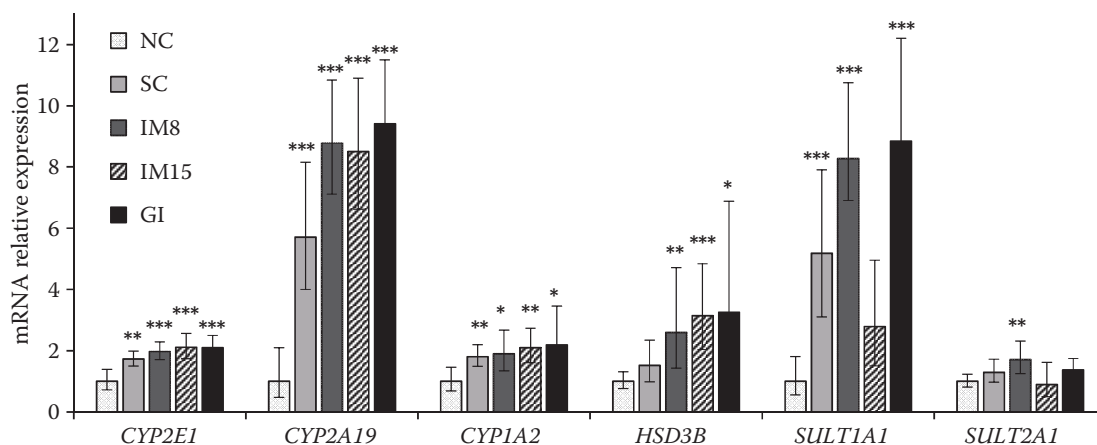

Figure 1. mRNA Expression of *CYP2E1, CYP1A2, CYP2A19, HSD3B, SULT1A1* and *SULT2A1* in liver from male pigs non-castrated (NC), surgically castrated (SC), immunocastrated at 8 weeks (IM8) and at 15 weeks (IM15) prior to slaughter and from gilts (GI). Values are means ± 95% confidence intervals expressed relative to non-castrated male pigs, the mean for which was arbitrarily set to 1

significant difference at *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ between each of the treated groups (SC, IM8, IM15, GI) and control group (NC), respectively

Table 1. Pearson's correlation coefficients between the boar taint compounds in backfat, expression of genes, and Improvac antibodies. Boldface text indicates a statistically significant correlation ($P < 0.05$)

Compound	Impro-vac_Ab	Indole	Skatole	Andro-stenone	*CYP1A2*	*CYP2A19*	*CYP2E1*	*HSD3B*	*SULT1A1*	*SULT2A1*
Androstenone	−0.1922	**0.4981**	**0.3302**	1.0000	**−0.2312**	**−0.3782**	**−0.4378**	−0.1821	**−0.2437**	−0.1921
Skatole	−0.0128	−0.1517	1.0000	**0.3302**	−0.1035	**−0.2700**	**−0.2598**	−0.1309	−0.1765	−0.0530
Indole	−0.1517	1.0000	**0.8101**	**0.4981**	−0.1378	**−0.3327**	**−0.3320**	−0.2020	−0.1849	−0.1075
Improvac_Ab	1.0000	−0.1517	−0.0128	−0.1922	0.2303	**0.5017**	**0.3867**	**0.4580**	0.1532	0.1244

pigs, mRNA expression was higher in the surgically castrated group for *CYP2E1*, *CYP2A19*, *CYP1A2* and *SULT1A1*. The IM8 group showed higher expression for all studied genes, IM15 similarly but with the exception of *SULT* genes. Expression in gilts was higher for all genes except *SULT2A1*. The greatest differences in expression were found for the *CYP2A19* gene (570–940%), on the contrary, the lowest for the *SULT2A1* gene, where the difference in expression was only demonstrated in the IM8 group.

We found statistically significant differences ($P = 0.0007$) in the mean levels of Improvac-specific antibodies between the groups IM8 and IM15 (LSM 1.893, 1.157, respectively). When compared to the level of antibodies at 5 weeks after the second dose, the 8 weeks group (IM8) had mean decrease of 13% while the 15 weeks group (IM15) had mean decrease of 48%.

Multivariate analysis of boar taint compounds, gene expressions and Improvac-specific antibodies. Exploratory data analysis did not identify those variables tested. No significant extreme outlier was identified, only significant differences in the skewness from normality in variables indole, skatole and androstenone. These skewed variables were modified by Box–Cox transformation. Selected statistical parameters estimates are given in Supplementary Table S2 in SOM.

Positive or negative correlations are shown in Table 1. Androstenone was positively correlated with skatole and indole and at the same time negatively correlated with mRNA expression of *CYP1A2, CYP2A19, CYP2E1* and *SULT1A1*. Skatole and indole correlated negatively only with *CYP2A19* and *CYP2E1*. Improvac-specific antibodies were positively correlated with mRNA expression levels of *CYP2A19*, *CYP2E1* and *HSD3B*. These findings are supported by the two different multivariate mathematically independent models – PCA and FA (Figures 2, 3). In the PCA model, the variables *CYP2A19*, *CYP2E1*, *SULT1A1* and Improvac_Ab were correlated positively with one another and the variable androstenone correlated negatively with the previous set of variables (the variable was on the opposite side from the origin and their
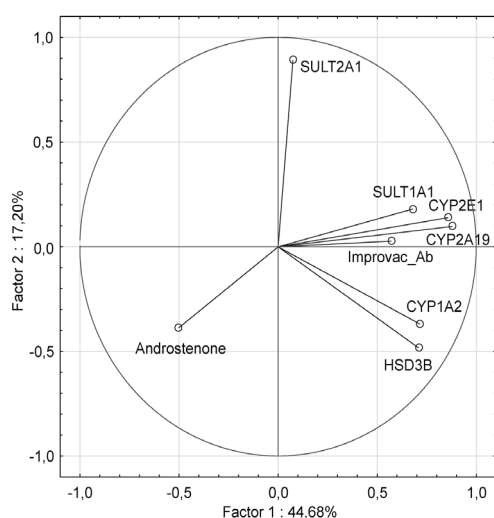

Figure 2. Loadings plot from principal component analysis (PCA) model for selected variables
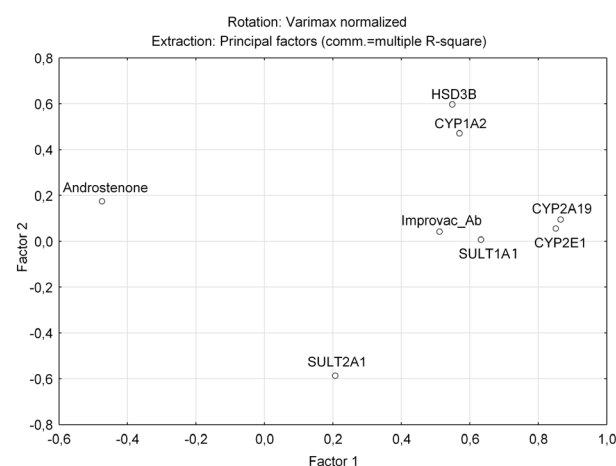

Figure 3. Factor loadings plot from factor analysis (FA) model for selected variables

geometric angles were close to 180°). In the FA model, the variables *CYP2A19*, *CYP2E1*, *SULT1A1* and Improvac_Ab again correlated positively with one another and the variable androstenone again correlated negatively with the previous set of variables (the variable was on the opposite side from the point F1 = 0.0 and F2 = 0.0, similarly to the PCA model).

DISCUSSION

In our study, we investigated mRNA levels of selected genes involved in metabolism of boar taint compounds between entire male pigs (NC) and surgically castrated pigs (SC), immunocastrated pigs slaughtered at different ages (IM8, IM15) and gilts (GI) of a commercial hybrid. It has been shown that castrated and immunocastrated pigs have higher activities for several CYP450 isoforms (Zamaratskaia et al. 2009). CYP2E1, CYP2A19 and CYP1A2 are considered mainly responsible for skatole metabolism in liver of pigs (Matal et al. 2009). We found significant differences in mRNA levels of *CYP2E1*, *CYP2A19* and *CYP1A2* between the control group (entire male pigs) and SC, IM8, IM15 and GI pigs, with all of these groups having higher expression of those genes compared to the control. This finding is in agreement with Brunius et al. (2012), who had found higher mRNA expression of *CYP2E1* and *CYP1A2* in surgically and immunologically castrated Yorkshire × Landrace pigs. In Bama miniature pig, mRNA levels of *CYP2E1* and *CYP2A19* decline after 6 months of age in entire male pigs due to the onset of sexual maturity (Wang et al. 2015), thus suggesting an effect of sex hormones on gene expression. Female pigs show higher expression of *CYP1A2* and *CYP2E1* than do entire male pigs, suggesting gender-related difference in P450 activity (Rasmussen et al. 2011).

Similarly, the *HSD3B* gene is involved in androstenone metabolism, where the 3β-HSD enzyme catalyses the transformation of androstenone to β-androstenol (Doran et al. 2004). Low 3β-HSD activity is associated with high accumulation of androstenone in fat (Nicolau-Solano et al. 2006). The expression of *HSD3B* in our study was significantly higher in IM8, IM15 and GI pigs. Chen et al. (2007) had found higher expression of *HSD3B* in liver of immunocastrated and surgically castrated Landrace × Yorkshire pigs compared to entire

male pigs. Also in Yorkshire × Landrace pigs, the expression of *HSD3B* in liver has been observed to be elevated in pigs surgically castrated and castrated by Improvac than in entire male pigs (Rasmussen et al. 2012).

The *SULT1A1* gene was significantly upregulated in the SC, IM8 and GI groups (but not in IM15) compared to the entire male group. Similar to our results, Kojima and Degawa (2013) found increase in mRNA levels of *SULT1A1* and *SULT2A1* in surgically castrated Meishan pigs. In the Landrace breed, however, they found no differences, which fact might suggest breed dependence. The *SULT2A1* mRNA expression was significantly higher only in the IM8 group. Contrary to our findings, Rasmussen et al. (2012) found no difference in expression of *SULT2A1* between castrated and entire males in Yorkshire × Landrace pigs. Haplotypes have been found in the QTL region on SSC6 related to low and high levels of androstenone, and *SULT2A1* is located within this region (Duijvesteijn et al. 2010). This might suggest an effect of single nucleotide polymorphisms on the different gene expression of *SULT2A1*, but further investigation should be undertaken of mechanisms controlling mRNA expression of *SULT2A1*.

We tested prolonged times until slaughter after the second Improvac vaccination dose (8 and 15 weeks) and their effect on expression of genes involved in metabolizing skatole and androstenone. Improvac's manufacturer recommends slaughter of pigs at 4 to 5 weeks after the second dose. Claus et al. (2008) had determined, however, that the onset of testosterone synthesis ranged from 10 to 24 weeks, varying among individuals, whereas the effect of immunocastration lasted for at least 10 weeks. While in IM8 pigs we observed significantly higher expression of *CYP2E1*, *CYP1A2*, *CYP2A19*, *HSD3B*, *SULT2A1* and *SULT1A1*, in IM15 pigs expression was significantly higher only in the cases of *CYP2E1*, *CYP1A2*, *CYP2A19* and *HSD3B* when compared to the control group (entire male pigs). Moreover, the decrease of Improvac-specific antibodies in the group of pigs with the longest time until slaughter (15 weeks) compared to the group of pigs slaughtered after 8 weeks demonstrates a reduction in efficiency of the Improvac vaccine. Together with this finding, gradual decrease of antibody levels through increasing time (5, 8 and 15 weeks) since the second vaccination was demonstrated. This finding is in agreement with

the manufacturer's recommendation to slaughter 4–5 weeks after the second vaccination, and indicates that after 8 weeks GnRH antibodies may decline below effective levels (Improvac.com). All the findings described above correspond with the results of our previous study (Sladek et al. 2018), showing that the IM15 pigs had in their testis structure more numerous interstitial endocrine cells and thicker epithelium in *tubuli seminiferi*, thus suggesting onset of redeveloping spermatogenesis. Although we would hypothetically have expected a decrease in mRNA expression of genes connected to degradation of boar taint compounds, no mRNA levels of genes in groups IM8 and IM15 differed with statistical significance. Our results (Figure 1) do, however, suggest tendencies for decreased expression of several genes connected with decrease of Improvac-specific antibodies. This indicates that morphological changes probably have not been yet fully reflected in the decrease of gene expression at this age.

Positive or negative correlations between mRNA expression, boar taint compounds (androstenone, skatole and indole) in backfat, and Improvac-specific antibodies in serum were studied using multivariate statistical methods. Skatole and indole are reported to be strongly correlated in fat (0.70), and moderately positive correlations have been found between skatole and androstenone (0.31) and between indole and androstenone (0.38) levels in fat (Bidanel et al. 2006). In the crossbred population Czech Large White × (Czech Large White × Czech Landrace) these correlations were 0.38, 0.54 and 0.25, respectively (Zadinova et al. 2017). We observed positive correlations between skatole and indole, and similarly between androstenone and skatole and indole. Our study also found moderate negative correlations between mRNA expression of *CYP2E1* and *CYP2A19* genes and skatole, indole and androstenone. In the case of *CYP1A2*, negative correlation was found only with androstenone. To the best of our knowledge, no previous studies have analysed correlations between boar taint compounds and *CYP2E1*, *CYP1A2* and *CYP2A19* mRNA levels, but it has been reported by Zamaratskaia et al. (2009) that enzyme activities of several cytochrome P450 isoforms, including CYP2E1, are lower in entire male pigs compared to surgically and immunocastrated pigs. It is likely that the higher CYP450 activity (for at least some isoforms) is caused by the absence or low levels of testicular steroids, which otherwise inhibit CYP450 expression at the level of transcription (Gillberg et al. 2006) and thus could be negatively correlated with these genes. Similarly, Brunius et al. (2012) reported *CYP2E1*, *CYP1A2* and *CYP1A19* mRNA levels to be negatively correlated with testosterone, which is a testicular steroid together with androstenone and might have an effect on skatole metabolizing enzymes in the liver (Doran et al. 2002; Zamaratskaia et al. 2007). Lanthier et al. (2007) studied the enzyme SULT1A1 in the liver of prepubescent Yorkshire pigs and found negative correlation between the enzyme and skatole levels in plasma and backfat, thus suggesting that SULT1A1 is important in the metabolism of skatole. We found no correlation between *SULT1A1* and skatole, but we did observe a weak negative correlation with androstenone. No significant correlations between boar taint compounds and *HSD3B* and *SULT2A1* mRNA were found. Rasmussen et al. (2012) also found no correlations between *SULT2A1* mRNA and androstenone, but they did find a moderate negative correlation between *HSD3B* mRNA and androstenone level. Similarly, Chen et al. (2007) reported negative correlation between *HSD3B* mRNA and fat androstenone, but we found no significant correlations between this gene and boar taint compounds. The two independent and mathematically different statistical methods PCA and FA confirm negative correlation between the tested variables androstenone on the one hand and *CYP2A19*, *CYP2E1*, *SULT1A1* and Improvac_Ab on the other. From a biological point of view, this means that when the levels of androstenone in backfat rise, the levels of *CYP2A19, CYP2E1, SULT1A1* and Improvac_Ab decrease.

We also observed positive and statistically significant correlations between Improvac-specific antibodies level and *CYP2A19, CYP2E1* and *HSD3B* genes. Although negative correlation between Improvac-specific antibodies and androstenone levels was not statistically significant, the multivariate analyses did imply a negative relationship between androstenone and Improvac-specific antibodies, as had been assumed based on knowledge of the mechanism connecting androstenone and Improvac-specific antibodies. The strength of mutual relationships between these variables might be caused by individual variability among animals and other factors that cannot be included

into the analysis but that will influence the level of antibodies.

Considering the complex nature of the genetic determination of metabolite levels in connection with boar taint, it is not easy to determine a clear relationship between steroid hormones and genes known to metabolize skatole, indole and androstenone in the liver. Sequential changes, along with the influence of environmental factors, are of great importance and can explain individual differences. Moreover, breed differences in expression of genes connected to the metabolism of boar taint compounds have been described (Moe et al. 2007; Kojima et al. 2010). Therefore, our findings are specific to our set of Czech commercial hybrid pigs.

CONCLUSION

Our study provides a more comprehensive look at selected genes involved in the metabolism of either skatole or androstenone in the liver of a commercial Czech breed and contributes to understanding how castration relates to mRNA expression of those genes and their relationship with boar taint compounds and Improvac-specific antibodies. Furthermore, the significant differences observed between the control and treatment groups suggest that *CYP2E1, CYP2A19, CYP1A2, HSD3B, SULT1A1* and *SULT2A1* genes are upregulated in surgically and immunologically castrated pigs and provide useful information about possible candidate genes for further studies. These differences show that hepatic expression of these genes is regulated differently between boars and barrows at the level of transcription. Moreover, the use of multivariate statistical methods for modelling the relationship between boar taint compounds, immunological parameters, and gene expression seems to be a promising approach. Further study of these genes could be useful especially for detecting genetic polymorphisms in relation to differential expression of these genes, and these might be used for genomic selection.

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