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Monitoring of behavior, sex hormones and boar taint compounds during the vaccination program for immunocastration in three sire lines

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Highlights:

- Aggressive behavior and general activity decline due to immunocastration
- Immunocastrates present with androstenone (1.9%) or skatole (3.7%) above cutoff
- Indole is unaffected by immunocastration in French Piétrain and Canadian Duroc

Keywords: immunocastration; boar taint; hormones; serum; fat; pigs

Abstract

Immunocastration (vaccination against boar taint) is an alternative method to prevent boar taint without the need for surgical castration. This study investigates the evolution of boar taint compounds in serum and fat, serum steroid compounds as well as behavior in immunocastrated pigs from 3 sire lines: 15 stress positive Belgian Piétrain (BP), 20 stress negative French Piétrain (FP), and 20 stress negative Canadian Duroc (CD). Hormone and boar taint compounds in serum were determined at 4 time points; boar taint compounds in fat were determined at 3 time points. Behavior, skin lesions, animal and pen fouling were also recorded before the first vaccination (<V1), between the first and second vaccination (<V2), and after the second vaccination (>V2). Aggressiveness, eating and drinking and general activity behavior declined from <V1 to <V2 and >V2 for all sire lines. Pigs from BP were cleaner than FP and CD pigs. Even though immunocastration was effective in general (reduced testosterone, estradiol as well as androstenone in serum) for all sire lines, some individual pigs showed either androstenone or skatole levels in fat above cutoff values. While the immunocastration mechanism works as intended for androstenone, and also for skatole for the three sire lines, the risk of carcasses with boar taint compounds above cutoff levels (respectively 1.9 and 3.7%) still remains to some extent.

Introduction

Boar taint is an unpleasant odor in fat and meat that is mainly associated with uncastrated male pigs (boars). The main compounds responsible for boar taint are androstenone (AND), skatole (SKA), and

to a lesser extent indole (IND) (Annor-Frempong et al., 1997; Claus et al., 1994; Moss et al., 1993; Rius and García-Regueiro, 2001). To avoid boar taint, most of the male piglets are surgically castrated. Immunocastration or vaccination against anti-gonadotropin releasing hormone (GnRH) is sometimes used as an alternative to eliminate boar taint without the need for surgical castration (Fuchs et al., 2009). Immunocastration is performed by giving two injections with an anti-GnRH vaccine with at least 4 weeks apart, with the second vaccination (V2) at least 4 weeks before slaughter.

Immunocastration immunizes boars against GnRH, which disrupts the hypothalamic-pituitary-gonad axis. Female pigs are similarly affected by immunocastration (Zeng et al., 2002). Immunocastration thus stops the production of male sexual steroid hormones as well as AND, and stops testes development (Oonk et al., 1998). This has also been associated with a reduction in fat SKA, in aggressive and sexual behaviors and increased passive behavior after the second vaccination (Baumgartner et al., 2010). SKA concentration is thought to be indirectly lowered by immunocastration due to the removed inhibition of SKA metabolism by AND (Zamaratskaia et al., 2008). As a consequence, AND and SKA levels in fat tissue at slaughter are reduced to the same level as barrows (Brunius et al., 2011; Dunshea et al., 2001). Although several studies confirm the reduction of male sexual steroid hormones and boar taint compound levels in fat, some studies suggest that high levels of AND and SKA in fat from immunocastrates may still occur (Batorek et al., 2012). It has been suggested that some pigs are not at all, or are less responsive to immunocastration (Batorek et al., 2012). It has also been found that certain conditions (i.e., dysentery) can lead to elevated SKA levels in fat at slaughter in all sexes, regardless of sexual maturity (Škrlep et al., 2012).

The age of reaching puberty (sexual maturity) in boars as well as the AND and SKA levels in fat at slaughter differ between breeds. It has been shown that the prevalence of Duroc boars with AND concentrations above cutoff values can reach up to 50% while this is only the case for 5 to 8% of Hampshire, Yorkshire, and Landrace boars (Zamaratskaia and Squires, 2009). Besides a difference in the age of puberty or a difference in AND synthesis for the same maturational stage, the difference in AND may also be due to differences in metabolic clearance of AND from fat tissue between breeds (Andresen, 2006). Several studies also show that SKA levels increase around puberty (Babol et al.,

2004, 1999). It has been hypothesized that these increased SKA levels around puberty are associated with increased intestinal turnover (Babol et al., 2004), also the decreased ability to metabolize SKA at that time due to AND has been shown (Squires and Lundström, 1997). Piétrain sired pigs generally have a lower growth rate than Duroc sired pigs, so slaughter age differs between fattening pigs from different sire lines (Liu et al., 2007). Lower growth rate has been associated with lower boar taint risk in some studies but the findings are not always conclusive (Bonneau, 1987; Van Den Broeke et al., 2015; Xue and Dial, 1997).

It can therefore be hypothesized that the state of pubertal development, behavior and boar taint compound levels in fat and plasma differ between fattening pigs from these sire lines at the second vaccination; a different pattern in diminution of the boar taint compounds may also be possible between these three lines. The aim of this study was therefore to compare the effect of immunocastration on hormonal blood levels, boar taint compound in serum and fat and behavior in male fattening pigs of 3 sire lines, namely Belgian Piétrain (stress positive), French Piétrain (stress negative) and Canadian Duroc (stress negative).

Materials & Methods

This experiment was approved by the ILVO Ethics Commission (Melle, Belgium) (2017/302 & 2017/295).

Animals

In 2 batches with 3 weeks difference between each round, a batch of hybrid sows (Topigs 20, n=26 in total) was inseminated. The distribution of terminal sires was 2 (2/2 in both batches) stress positive Belgian Piétrain (BP), 3 (2/3 in both, 1/3 only in 2nd batch) stress negative French Piétrain (FP), and 4 (1/4 in both, 2/4 only in the 1st batch, 1/4 only in the 2nd batch) stress negative Canadian Duroc (CD). These were used to inseminate 4 (2/4 in the 1st batch, 2/4 in the 2nd batch) sows with semen from BP boars, 5 (3/5 in the 1st batch, 2/5 in the 2nd batch) sows from FP, and 6 (3/6 in the 1st batch, 3/6 in the 2nd batch) sows from CD. Piglets of these sows were weaned at 4 weeks of age and piglets of the same sire line (but different individual boar within sire line) were grouped with 5 male piglets per pen in the

nursery (3 pens BP, 4 pens FP, 4 pens CD). At 9 weeks of age, pigs were moved to the fattening barn (keeping the same group composition) into pens (2.0 m x 2.5 m) with half slatted floors. All pigs had access to water at all times and were fed *ad libitum* with a 3-phase fattening diet, with a shift to the second phase diet at the time of the first vaccination and to the third phase diet at the second vaccination.

Pigs were immunocastrated using Improvac® (Zoetis, Zaventem, Belgium) (subcutaneous injection, 2 ml). Timing of the vaccination was determined based on average pen weight. The first vaccination was given 8 weeks before slaughter (average pen weight 50 kg) and the second vaccination (at least) 27 days before slaughter (average pen weight 80 kg). Animals were weighed weekly to determine the correct timing. Animals were slaughtered at an intended average pen weight of 115 kg (Table 1). The fasting period before slaughter was minimum 21h, transport time was 1.5h and the time in lairage was around 2h. Slaughter occurred in a commercial slaughterhouse by exsanguination after carbon dioxide stunning.

All animals (15 BP, and 20 FP and CD) were included in observations, blood sampling, blood analysis, fat sampling, and boar taint analysis discussed further.

Observations

Observations were performed from 10 weeks of age until slaughter and were always performed in the afternoon. The observation protocol was based on protocols used in previous studies (Bekaert et al., 2012a; Van Den Broeke et al., 2015). Animal behavior was evaluated at pen level. Animal behavior was scored every two weeks by single scan sampling after a 10-min period to habituate the pigs to the presence of the observer. The duration of observation was 10×1 min per pen, with a 3-min pause between each round of the pens. The number of pigs per pen displaying the various types of behavior (Table 2) was recorded and average percentage of time spent per behavior was calculated per pen during the time period before V1 (2 or 3 series of observation), the period between V1 and V2 (2 or 3 series of observation), and the period after V2 (2 to 3 series of observation). As frequency of observation was low for some categories, these observations were merged: interaction + playing, and

manipulation + ear & tail biting. Skin lesions, animal soiling and pen soiling were scored on a scale from 0 to 4 or 5 by recording the number of pigs in each category per pen (Table 3).

Blood sampling and analysis

Blood sampling was performed via venipuncture of the jugular vein. Blood was collected in 10mL tubes with a silicone-coated interior (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Blood samples were taken from all selected pigs on 4 occasions: at the first vaccination (V1 = 8 weeks before slaughter), at the second vaccination (V2 = 4 weeks before slaughter), 2 weeks after the second vaccination (V2+2), and 1 day before slaughter (before fasting period started) (S) (Fig. 1). Serum was obtained by centrifugation at 3000rpm for 10min at 4°C. Serum samples were kept frozen at -80°C until analysis of hormonal and boar taint compound concentrations. From the serum samples, testosterone (TES), estradiol (EST), and progesterone (PRO) were determined using the commercial Cobas Testosterone II, Cobas Estradiol III, Cobas Progesterone II, and Cobas Ureal kits (Roche, Basel, Switzerland). Serum concentrations of androstenone (AND), skatole (SKA), and indole (IND) were determined in serum using U-HPLC-HR-Orbitrap-MS analysis with a limit of detection (LOD) of 1.0, 0.5, and 0.5 µg/L, a maximum repeatability of 4.7, 6.6, and 7.5% , and a maximum within-laboratory reproducibility of 6.4, 6.1, and 10.4% for AND, SKA, and IND respectively (Bekaert et al., 2012b).

Fat sampling and boar taint analysis

Fat biopsy samples were taken from neck fat at V2 and V2+2 based on the methodology described by Baes et al., (2012). In short, a modified rabbit stunning device (Klaus- Gritsteinwerk GmbH & Co., Bünde, Germany) with a reusable biopsy needle was used to obtain a fat sample of approximately 200 mg. Each sample was immediately placed on ice and kept frozen at -20°C in an Eppendorf until analysis for AND and SKA concentrations (expressed in liquid fat). Chemical analyses were performed at ELFI Analytik GbR (Neufahrn, Germany) using SIDA-HS-SPME-GC/MS with a LOD of 35, 0.1, and 0.5 ng/g, a maximum inter-essay coefficient of variation (CV) of 4.1, 2.1, and 5.0% , and a maximum intra-essay CV of 5.3, 5.0, and 1.0% for AND, SKA, and IND respectively (Fischer et

al., 2011). Some biopsy samples did not provide the required amount of fat for analysis. At V1 no biopsy samples were taken as the fat layer in these young boars was assumed to be too thin (Fig. 1).

At the slaughter line, neck fat samples were collected from all animals (5 cm x 5 cm) and stored frozen at -20°C in vacuum packed plastic bags until olfactory analysis and chemical analysis using the same analysis method as for the biopsy samples (relating to the same amount of liquid fat). Olfactory analysis of all neck fat samples collected at slaughter was performed by a trained olfactory panel of 3 experts. The panel scored each sample on a 5-point scale using the hot iron method (0 = no aberrant odor, 1 = light aberrant odor, 2 = some aberrant odor, 3 = strong aberrant odor, 4 = very strong aberrant odor) (Aluwé et al., 2017). The mean score was taken as the final score. When this exceeded 0.5, the sample was considered positive for boar taint (Heyrman et al., 2018).

Statistical analysis

Separate mixed linear models with the (natural) log of each compound as dependent variable were set up with sire line (BP, FP, and CD), time point (V1, V2, V2+2, and S) and their interaction as fixed effects. The individual animal identifier was included as random effect to correct for repeated measures.

A similar mixed linear model was set up with age as dependent variable, with the same fixed and random effects. For the 3 time periods between the 4 time points (V1 – V2, V2 – V2+2, and V2+2 – S) the daily gain was calculated. A mixed linear model was set up with daily gain as dependent variable, with sire line, time period, and their interaction as fixed and the individual animal identifier as random effect. A similar mixed linear model was also set up with all behavior categories as dependent variable and time point (before V1 (<V1), before V2 (<V2), and after V2 (>V2)) categories as fixed effect and pen as random effect. When interaction terms were non-significant ($P < 0.05$) they were removed from the final models. A post-hoc test with a Tukey correction for multiple comparisons was performed for each analysis. Normal distribution of the residuals of all models was evaluated graphically. For all analyses when $P < 0.05$ the difference was considered significant.

For the olfactory boar taint scores, no model was constructed because none of the evaluated samples exceeded the cutoff for olfactory boar taint. Spearman correlations were calculated for all pairs of compound concentrations at each time point and evaluated with a chi square test.

For V2+2 and S a new variable (1/0) was created to differentiate between animals with AND in fat > 4.00 µg/kg at S. Separate mixed linear models were set up with TES, EST, and AND in serum at these time points as dependent variable, with this new variable as fixed effect and the individual animal identifier as random effect.

A power analysis was performed for each analysis to justify the mention of trends and numerical differences. When the perceived effect size was possibly relevant and the power of the corresponding analysis was below 0.80 (due to the number of observations) we made mention of it but noted that it concerns only a trend or numerical difference for the reader to keep in mind.

All data analysis was done in R 3.2.3 (R Core Team, 2013).

Results

Timing of the vaccinations and slaughter (and corresponding sample times) were determined at pen level based on average pen weight (Table 1), resulting in comparable weights for the three groups at V1, V2, and V2+2. Only at slaughter, BP was 10 kg lighter than FP and CD. BP, FP and CD received the second vaccination at 36, 32 and 28 days before slaughter, respectively. It is notable that BP pigs are consistently around 10 days older than FP and CD pigs (except at S, where FP pigs were also 4 days older than CD pigs) ($P_{\text{time}} \leq 0.001$, $P_{\text{sire}} \leq 0.001$, $P_{\text{time*sire}} \leq 0.001$). Furthermore BP grew significantly slower from V2+2 to S compared to FP and CD, while from V1 to V2 and V2 to V2+2 BP grew significantly slower than CD with FP being intermediate ($P_{\text{time}} \leq 0.001$, $P_{\text{sire}} = 0.054$, $P_{\text{time*sire}} \leq 0.001$).

Concentration of TES and EST in serum (Fig 2 (a) and (b)) declined after the second vaccination in all sire lines. In CD pigs, PRO in serum declined consistently from V2 to V2+2 and S, while for BP and

FP no significant difference between time points was observed. At V1 and V2 PRO in serum was significantly higher for CD than BP, with FP being intermediate (Fig 2 (c)).

AND concentration in serum (Fig. 2 (d)) in CD showed a significant increase from V1 to V2, followed by significantly lower concentrations after V2 until S. In BP, AND in serum showed significantly lower concentrations after V2. This was not the case for FP, in which the AND concentration in serum remained low at all time points. Concentration of SKA in serum (Fig. 2 (e)) significantly rose in FP from V1 to V2 after which it dropped to a significantly lower concentration at V2+2 and S. For BP, the same significant decline from V2 to V2+2 and S is seen, while this is only significant at S for CD. At V1, SKA in serum for BP is significantly higher than CD and FP. Serum IND concentration (Fig. 2 (f)) increased significantly after V2 for CD and from V2+2 to S compared to V1 for FP pigs respectively, while IND concentration in BP pigs remained low at all time points after V2 and was significantly highest at V1.

In fat, AND concentration showed an interaction between sire line and time point (Fig 2 (g)). Two weeks after the second vaccination, AND concentration in fat was significantly lower than V2 in all sire lines. None of the samples exceeded the cutoff of 2000 $\mu\text{g/kg}$ (Heyrman et al., 2017) at V2 and V2+2. At slaughter, levels were higher compared to V2+2 for all sire lines, and even significantly higher than at V2 for BP and FP. At slaughter, 1 out of 54 samples (1/20 FP) exceeded the cutoff level.

Skatole concentration in fat significantly differed between time points as well as sire lines but no significant interaction was found. BP showed lower SKA concentrations than FP and CD (Fig 2 (h)). A gradual reduction in skatole fat concentration was observed after the second vaccination. SKA at S was significantly lower than at V2 and V2+2 in all sire lines. At V2, SKA levels above the cutoff of 250 $\mu\text{g/kg}$ were found in 6 of 45 samples (Heyrman et al., 2017) (4/16 CD and 2/16 FP). At V2+2, 2 of 48 samples exceeded cutoff level (1/16 CD and 1/17 FP) and at S, 2 of 54 samples exceed cutoff level (2/20 FP). Based on the score of the olfactory panel, 0 of the 54 samples collected at slaughter scored were determined positive for boar taint.

The relation between boar taint compounds in serum and fat, and between testosterone in serum and AND in serum and fat, at V2 and S are given in Fig 3 (a), (b), (c), and (d). Correlation between testosterone and androstenone in serum is not significant at either time point, while correlation for testosterone and androstenone in fat showed a significant correlation at V2, but not at S. Correlation between AND in serum and AND in fat was significant at V2 (Fig. 3 (e)), but not at S (Fig. 3 (f)). No significant correlation between SKA in serum and in fat was observed at either V2 (Fig. 3 (g)) or S (Fig. 3 (h)).

Comparing pigs with AND in fat $> 4.00 \mu\text{g/kg}$ at S and those with lower concentrations we see a trend ($P = 0.059$) for higher TES in serum for pigs at V2+2 and S with higher AND in fat at S. There was also a trend ($P = 0.078$) for higher EST in serum for pigs at V2+2 and S with higher AND in fat at S. There was no significant difference ($P = 0.246$) for AND in serum for pigs at V2+2 and S with higher or lower AND in fat at S.

BP pigs were less active from $<V2$ to $>V2$ compared to $<V1$, CD and FP pigs were less active from $>V2$ compared to $V1$ (Fig. 4(a)). Manipulation and ear- & tail biting behavior significantly decline up to $>V2$ compared to $<V1$ and $<V2$ for all sire lines (Fig. 4 (b)). Eating and drinking and aggressive behavior was significantly lower at $>V2$ than at $>V1$ with $<V2$ being intermediate (only for eating and drinking). These behaviors did not differ significantly between sire lines (Fig.4 (c), and (e)).

Interaction and playing behavior did not differ significantly between time points or sire lines (Fig. 4 (d)). Mounting behavior was not significantly different between sire lines nor time points (Fig. 4 (f)). Ano genital sniffing behavior was significantly higher for CD at $<V2$ (Fig. 4 (g)). Skin lesion score was significantly higher at $>V2$ compared to $<V1$ and intermediate at $<V2$, with no difference between sire lines (Fig. 4 (h)). Animal fouling was lower for BP than FP and CD, with no difference between time points (Fig. 4 (i)). Pen fouling was significantly lower for BP compared to FP with CD having intermediate values (Fig.4(j)).

Discussion

Biopsy sampling of fat for boar taint compound analysis has been introduced in a number of studies in order to evaluate the effectiveness of intervention strategies (Wesoly et al., 2016) or to test boar taint in live breeding candidates (Baes et al., 2012). One other study evaluated AND, SKA, and IND in fat in immunocastrates compared to entire male pigs at 70, 100, and 120kg. This was however not done using biopsies but by slaughtering 4 animals per sex at 70, and 100 kg while slaughtering 12 animals per sex at 120 kg. None of the immunocastrates in this study showed boar taint compound concentrations above cutoff at any weight (Font-i-Furnols et al., 2016).

The testosterone profile did not differ between sire lines, nor did the occurrence of sexual and aggressive behavior or the skin lesion score. Generally, peak levels of TES, and EST reached at the onset of puberty differ between studies. This can be due to the effect of nutritional status, stress, social rank, and individual differences between animals on reaching puberty (Lanthier et al., 2006). The serum levels of puberty hormones can therefore increase during a long timeframe (Lanthier et al., 2006; Sinclair et al., 2001; Van den Broeke et al., 2016; Zamaratskaia et al., 2004). The order of magnitude of TES concentrations seen at V1 (though we have no measurements from before this time point) and the decline after V2 in the present study may be indicative of the onset of puberty.

In serum, an increase in AND at the time of the second vaccination for CD is consistent with the onset of puberty (Sinclair et al., 2001; Zamaratskaia et al., 2004). For the FP animals the AND concentration remained low at V2 and was not significantly different for BP, although a numerical increase was observed. However, one must keep in mind that there is a wide age range when animals reach puberty and that the increase in AND during puberty can come later or not at all. One study found no difference in luteinizing hormone (LH) or TES profile between pigs with high or low AND serum levels at puberty (Bonneau et al., 1987). In the present study, FP and CD grew faster and were thus younger than BP when they reached the target weight for vaccination. A possible hypothesis therefore, is that FP show less pubertal development at this age and thus show lower serum AND levels, while CD are closer to sexual maturity at the same age. Further support for this hypothesis is the significantly higher EST level at V1 and numerically higher at V2 for CD compared to BP and FP, as

well as the significantly higher PRO concentration at V1 and V2 of CD compared to BP, with FP showing intermediate values.

The decline in TES, and EST concentrations in serum after V2 is consistent with literature and the mode of action of immunocastration (Brunius et al., 2011; Van den Broeke et al., 2016; Zamaratskaia et al., 2008). In accordance with this decline in sex hormones, activity and aggressive behavior also declined after V2 as reported in previous studies (Baumgartner et al., 2010; Cronin et al., 2003; Zamaratskaia et al., 2008). It is notable that skin lesion score increased up to >V2 even though aggressive behavior declined. Considering that there is no significant difference in skin lesion score between <V2 and >V2 this result is less unusual than it appears, aggressive behavior at <V2 is not significantly different from <V1. It also has to be kept in mind that the skin lesion score is in a sense a cumulative measure since previous scars and wound only disappear as they heal and might more easily increase in number than decline. In all sire lines, eating and drinking behavior was significantly depressed after the second vaccination and was intermediate after the first vaccination. Although the pigs' feed intake increases after the second vaccination, they spend less time feeding; in other words, their feeding rate increases (Aluwé et al., 2016).

The serum AND concentration after the second vaccination showed a significant decline in the BP and CD sire line. This is consistent with results from literature (Zamaratskaia et al., 2009). The same decline was seen for the level of AND in fat from V2 to V2+2 in all sire lines. The subsequent increase in AND concentration in fat at slaughter (fat tissue collected after slaughter) for all sire lines was not expected and was not in accordance with the low levels of androstenone in serum collected 1 day before slaughter. It should be noted, however, that barrows and even gilts can show elevated levels of boar taint compounds (specifically $AND \geq 1000 \mu\text{g/kg}$) and that some barrows do receive high scores for sensory boar taint at slaughter (Prusa et al., 2011). This means that boar taint may occur to some extent in pig production in any case but does not necessarily translate to problematic boar taint prevalence, although the underlying mechanism in the Prusa's study might include unremoved internal testes or registration problem of the carcass as well. One FP animal in the present study did show elevated AND concentrations in fat (above the cutoff of $2000 \mu\text{g/kg}$) after slaughter; nonetheless, the

olfactory panel determined this sample to be negative for boar taint. TES levels and AND levels in serum at slaughter were not elevated for this animal (Fig. 3 (f)). A recent study (Wesoly et al., 2014) showed that aggressive and stressful conditions during transport may increase AND concentrations in fat of boars based on a comparison of pigs slaughtered after different transport and pre-unloading times. For barrows or immunocastrates this has not yet been studied. It could be hypothesized that stress during transport also resulted in an increase of AND level from V2+2 towards slaughter in this trial. Another hypothesis is that for some pigs the vaccination was less effective in reducing testicular function (so-called non-responders) (Batorek et al., 2012). For those pigs in Fig 3 (d) that showed higher TES in serum the day before slaughter as well as higher AND in fat after slaughter, the immunocastration might have been less effective. Moreover, the correlation between TES in serum and AND in fat at this time point is non-significant. Also, when comparing pigs at S with AND in fat higher or lower than 4.00 µg/kg we see a trend for higher TES and EST at V2+2 and S for the former group of pigs, but a non-significant difference for AND in serum at V2+2 and S. This gives some argument to the possibility of non-response in some pigs. Note that pigs exhibiting elevated TES concentrations in serum at slaughter do not necessarily exhibit elevated AND concentration in either serum or fat at slaughter (Fig. 3 (f)). This can also be said for most entire male pigs, given the low prevalence of boar taint in the general population (Aluwé et al., 2014; van Wagenberg et al., 2013). For those pigs with low serum TES levels on the day before slaughter but with elevated AND in fat after slaughter, the proposed effect of stress during transport could be at play (both processes may possibly be connected). Stress can possibly also affect SKA in fat after transport, due to its effect on colonic function or SKA metabolism (Wesoly et al., 2014). The overall evolution of SKA in fat was largely as expected, and SKA did not show the same increase after slaughter as AND (Fig. 2 (h)). But as Fig. 3 (h) illustrates, 2 samples had SKA levels in fat above the 250 µg/kg cutoff (these were both samples with fat AND levels well below cutoff). A possible experiment to further clarify the observed boar taint compound patterns close to slaughter would be to include sample collections of serum and fat before transport, after transport and after slaughter to evaluate the evolution of the boar taint compounds within this time span. Also a large scale screening for boar taint compounds of immunocastrate, barrow, and even gilt carcasses in a practical setting would clarify the relevance of

this remaining risk for boar taint. Especially considering that all 3/54 samples in this study above AND and SKA cutoffs at slaughter were not confirmed as positive for olfactory boar taint by the trained panel (false negatives). Considering individual differences in sensitivity for panelists reported in literature it is not uncommon to have inconsistencies between chemical and olfactory evaluation (Trautmann et al., 2016). Since there were 0 samples positive for olfactory boar taint we could not calculate sensitivity on the low number of samples in the current study. We know however from evaluation of our trained panel (data not shown) that sensitivity is 0.72. A larger scale evaluation could give more relevant insights into the overall prevalence of olfactory boar taint in these populations.

Before the second vaccination, CD boars showed numerically higher AND concentrations in fat, which is in accordance with previously established higher AND concentrations in fat of CD boars (Xue et al., 1996). The numerically lower AND concentrations in fat at V2 for BP and FP are also in accordance with previous findings (Wesoly and Weiler, 2012). It has been hypothesized that lean breeds such as Piétrain have lower AND and SKA concentrations in fat as a result of the lower proportions of saturated fatty acids and faster turnover in fat tissue (Wesoly and Weiler, 2012).

Notably, at the time of the second vaccination, fat AND levels and serum TES, EST and PRO were not significantly different between BP and FP (CD was also only significantly different from BP in PRO concentration). At that same time point, serum concentration of AND was significantly higher in BP in comparison to FP. Most Piétrain sires in Belgian are homogenous carriers of RYR1 (stress positive), resulting in higher lean meat percentages in the fattening pigs, which are heterogeneous carriers of RYR1. These type of Piétrain sires are very common in Flanders and therefore represent commercial practice. In a previous study Piétrain entire male pigs (stress positive) were compared with Large White (LW) and Belgian Landrace stress negative (BN) entire male pigs. Stress positive Piétrain entire male pigs had the lowest skatole level compared to LW and BN. At 110 kg of slaughter weight, P also had a lower androstenone level compared to LW (Aluwé et al., 2011). This does confirm the lower boar taint levels for lean meat type of pigs. In another study the effect of a polymorphism of the MC4R gene in Piétrain boars (known to result in higher lean meat percentage)

was also linked to lower boar taint compounds (Van Den Broeke et al., 2015). These associations with RYR1 and MC4R were however not included within the scope of this study.

In serum, SKA concentration of BP showed a significant decline between V1 and V2+2 up to the day before slaughter, FP showed a significant decline between V2 and V2+2 up to S, while CD showed a significant decline between V2+2 up to S. The decline of SKA is more gradual than that of the other compounds. This might be due to the fact that the decline in SKA happens only once testicular steroid levels have lowered and no longer have an inhibiting effect on SKA metabolism in the liver (Zamaratskaia et al., 2009). Based on this relationship, the lowest serum SKA level could have been expected for FP based on the lower AND level for FP, this was indeed the case at V1, but the same can be said for CD. Also keeping in mind the short half-life of SKA in fat compared to AND (hours versus days) (Prunier et al., 2013), this hypothesis might not hold true. Other factors could be at play that explain the high values of SKA in fat of pigs after second vaccination. It has been hypothesized that pre-pubertal metabolism of SKA is regulated by different enzymes (Lanthier et al., 2007), and that EST can be responsible for the inhibition of SKA catabolism (Zamaratskaia et al., 2007). As EST did show elevated levels at V2 for FP, other testicular steroids may have inhibited SKA metabolism in these pigs, resulting in comparable SKA levels at V2 for all sire lines even though AND in serum at V2 is low for FP. The high values of SKA in fat of pigs after V2+2 remain unexplained, however; besides metabolism of SKA, production in the gut might also be part of the explanation. SKA in fat is in general significantly lower in BP as compared to FP and CD. This could be due to the differences in lean meat percentage mentioned previously or the slower growth/lower feed intake leading to less degradation of the intestinal cell wall and hence lower SKA production (Zamaratskaia and Squires, 2009). Insulin like growth factor (IGF-1) has been linked with SKA since it is influenced by nutrition and plays a role in intestinal growth and apoptosis (Claus and Raab, 1999). It is clear from Table 1 that BP pigs grow significantly slower than the FP and CD, especially in the period between V2+2 and S. Generally Piétrain sires are selected for more lean meat growth with less optimal growth rates than Duroc sires which alternatively show a higher fatness (Morales et al., 2013). This difference in feed intake and accompanying smaller amount of manure produced (Rigolot et al., 2010) is also confirmed

by the difference that was observed in animal fouling, with BP being significantly cleaner than FP and CD. It has further been proposed in literature that heavily fouled pigs might show higher SKA concentration by absorbing SKA through their skin and lungs, this has however not been established (Wesoly et al., 2016).

It has been hypothesized that the reduction of IND in immunocastrated pigs is also due to the effect of the suppressed steroid synthesis on its metabolism, analogous to SKA (Zamaratskaia et al., 2008).

Several studies also indicate that immunocastration is effective in reducing IND in fat as well as serum (Font-i-Furnols et al., 2012; Zamaratskaia et al., 2008). The increase in serum IND concentrations for FP and CD even after the second vaccination is therefore not in accordance with expectations.

Nevertheless, corresponding results can be found in literature. In the study of Zamaratskaia et al. (2008) immunocastrated pigs had lower IND concentrations compared to entire males, but also showed a slight increase in IND concentration in serum between the first vaccination and slaughter. It is known that there are likely many candidate genes having small effects on boar taint and that there can be differences in the expression of enzymes responsible for SKA and IND metabolism (Zamaratskaia and Squires, 2009). Hence, breed differences can be expected. In FP and CD this may play a role in IND metabolism, which might not be affected by suppression of steroid synthesis. The metabolism of IND has not yet been thoroughly studied, however, and the importance of IND is not sufficiently clear. One other possible hypothesis is that feeding behavior plays a role in these elevated IND concentrations in serum for FP and CD, as it has been found that immunocastrates showing higher feed intake and growth ratio also show high IND concentrations in fat at slaughter, leading to IND concentrations higher than entire male pigs (Weiler et al., 2012). Further, SKA values in fat were significantly lower and SKA values in serum were numerically lower for BP. The lower growth rate and supposed lower manure production of BP contribute to this hypothesis. It would be interesting to perform a similar experiment including analysis of IND concentrations in fat biopsies before and after slaughter to see if these elevated IND levels in serums translate to increased IND fat levels. Taking fecal levels of SKA and IND into account would further help to clarify the physiological cause of these results.

Conclusion

Immunocastration is confirmed to be effective in reducing aggressive behavior in Belgian Piétrain, French Piétrain, and Canadian Duroc. IND in serum seemed unaffected by immunocastration in FP and CD, while it declined in BP, possibly due to different metabolic processes. Even though immunocastration was generally effective in reducing boar taint compound levels for all sire lines, 3 pigs did show either elevated levels of AND (1.9% of total pigs) or SKA (3.7% of total pigs) in fat at slaughter. Still, no pigs were considered positive for boar taint in fat by a trained olfactory panel. Further research, using biopsy sampling to follow up boar taint compound level in fat, could clarify whether processes related to stress during transport and slaughter are at play and to evaluate if and why boar taint possibly occurs in immunocastrates in commercial settings.

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References

- Aluwé, M., Degezelle, I., Depuydt, L., Fremaut, D., Van Den Broeke, A., Millet, S., 2016. Immunocastrated male pigs: Effect of 4 v. 6 weeks time post second injection on performance, carcass quality and meat quality. *Animal* 10, 1466–1473.
<https://doi.org/10.1017/S1751731116000434>
- Aluwé, M., Heyrman, E., Theis, S., Sieland, C., Thurman, K., Millet, S., 2017. Chicory fructans in pig diet reduce skatole in back fat of entire male pigs. *Res. Vet. Sci.* 115, 340–344.

<https://doi.org/10.1016/j.rvsc.2017.06.016>

- Aluwé, M., Millet, S., Bekaert, K.M., Tuytens, F.A.M., Vanhaecke, L., De Smet, S., De Brabander, D.L., 2011. Influence of breed and slaughter weight on boar taint prevalence in entire male pigs. *Animal* 5, 1283–1289. <https://doi.org/10.1017/S1751731111000164>
- Aluwé, M., Tuytens, F., Millet, S., 2014. Field experience with surgical castration with anaesthesia, analgesia, immunocastration and production of entire male pigs: performance, carcass traits and boar taint prevalence. *Animal* 9, 500–508. <https://doi.org/10.1017/S1751731114002894>
- Andresen, Ø., 2006. Boar taint related compounds : Androstenone / skatole / other substances. *Acta Vet. Scand.* 48, 1–4. <https://doi.org/10.1186/1751-0147-48-S1-S5>
- Annor-Frempong, I., Nute, G.R., Whittington, F.W., Wood, J.D., 1997. The problem of taint in pork—II. The influence of skatole, androstenone and indole, Presented individually and in combination in a model lipid base, on odour perception. *Meat Sci* 47, 49–61. [https://doi.org/http://dx.doi.org/10.1016/S0309-1740\(97\)00039-9](https://doi.org/http://dx.doi.org/10.1016/S0309-1740(97)00039-9)
- Babol, J., Squires, E.J., Lundström, K., 1999. Relationship between metabolism of androstenone and skatole in intact male pigs. *J. Anim. Sci.* 77, 84–92. <https://doi.org/1999.77184x>
- Babol, J., Zamaratskaia, G., Juneja, R.K., Lundström, K., 2004. The effect of age on distribution of skatole and indole levels in entire male pigs in four breeds: Yorkshire, Landrace, Hampshire and Duroc. *Meat Sci.* 67, 351–358. <https://doi.org/10.1016/j.meatsci.2003.11.008>
- Baes, C., Mattei, S., Luther, H., Ampuero, S., Sidler, X., Bee, G., Spring, P., Hofer, a., 2012. A performance test for boar taint compounds in live boars. *Animal* 7, 714–720. <https://doi.org/10.1017/S1751731112002273>
- Batorek, N., Čandek-Potokar, M., Bonneau, M., Van Milgen, J., 2012. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. *Animal* 6, 1330–1338. <https://doi.org/10.1017/S1751731112000146>
- Baumgartner, J., Laister, S., Koller, M., Pfützner, A., Grodzyski, M., Andrews, S., Schmoll, F., 2010.

- The behaviour of male fattening pigs following either surgical castration or vaccination with a GnRF vaccine. *Appl. Anim. Behav. Sci.* 124, 28–34.
<https://doi.org/10.1016/j.applanim.2010.01.004>
- Bekaert, K.M., Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander, D.L., Verheyden, K., De Brabander, H.F., Vanhaecke, L., Tuytens, F.A.M., 2012a. Predicting the likelihood of developing boar taint: Early physical indicators in entire male pigs. *Meat Sci.* 92, 382–385. <https://doi.org/10.1016/j.meatsci.2012.04.005>
- Bekaert, K.M., Vanden Bussche, J., François, S., Tuytens, F.A.M., De Brabander, H.F., Vandendriessche, F., Vanhaecke, L., 2012b. A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds. *J. Chromatogr. A* 1239, 49–55.
<https://doi.org/10.1016/j.chroma.2012.03.060>
- Bonneau, M., Carrié-Lemoine, J., Prunier, A., Garnier, D.H., Terqui, M., 1987. Age-related changes in plasma LH and testosterone concentration profiles and fat 5 α -androstenone content in the young boar. *Anim. Reprod. Sci.* 15, 241–258. [https://doi.org/10.1016/0378-4320\(87\)90046-7](https://doi.org/10.1016/0378-4320(87)90046-7)
- Bonneau, M., 1987. Effects of age and live weight on fat 5 α -androstenone levels in young boars fed two planes of nutrition. *Reprod. Nutr. Dev.* 27, 413–42.
- Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A., Lundström, K., 2011. Early immunocastration of male pigs with Improvac® - Effect on boar taint, hormones and reproductive organs. *Vaccine* 29, 9514–9520. <https://doi.org/10.1016/j.vaccine.2011.10.014>
- Claus, R., Lacorn, M., Danowski, K., Pearce, M.C., Bauer, A., 2007. Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. *Vaccine* 25, 4689–4696. <https://doi.org/10.1016/j.vaccine.2007.04.009>
- Claus, R., Raab, S., 1999. Influences on Skatole Formation from Tryptophan in the Pig Colon. Springer, Boston, MA, pp. 679–684. https://doi.org/10.1007/978-1-4615-4709-9_87

- Claus, R., Weiler, U., Herzog, A., 1994. Physiological aspects of androstenone and skatole formation in the boar-A review with experimental data. *Meat Sci.* 38, 289–305.
[https://doi.org/10.1016/0309-1740\(94\)90118-X](https://doi.org/10.1016/0309-1740(94)90118-X)
- Cronin, G.M., Dunshea, F.R., Butler, K.L., McCauley, I., Barnett, J.L., Hemsworth, P.H., 2003. The effects of immuno- and surgical-castration on the behaviour and consequently growth of group-housed, male finisher pigs. *Appl. Anim. Behav. Sci.* 81, 111–126. [https://doi.org/10.1016/S0168-1591\(02\)00256-3](https://doi.org/10.1016/S0168-1591(02)00256-3)
- Dunshea, F., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K.A., Lopaticki, S., Nugent, E.A., Simons, J.A., Walker, J., Hennessy, D.P., 2001. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Anim. Sci.* 79, 2524–2535.
- Fischer, J., Elsinghorst, P.W., Bücking, M., Tholen, E., Petersen, B., Wüst, M., 2011. Development of a candidate reference method for the simultaneous quantitation of the boar taint compounds androstenone, 3 α -androstenol, 3 β -androstenol, skatole, and indole in pig fat by means of stable isotope dilution analysis-headspace solid-phase micro. *Anal. Chem.* 83, 6785–6791.
<https://doi.org/10.1021/ac201465q>
- Font-i-Furnols, M., Carabús, A., Muñoz, I., Čandek-Potokar, M., Gispert, M., 2016. Evolution of testes characteristics in entire and immunocastrated male pigs from 30 to 120kg live weight as assessed by computed tomography with perspective on boar taint. *Meat Sci.* 116, 8–15.
<https://doi.org/10.1016/j.meatsci.2016.01.008>
- Font-i-Furnols, M., Gispert, M., Soler, J., Diaz, M., García Regueiro, J.A., Diaz, I., Pearce, M.C., 2012. Effect of vaccination against gonadotrophin-releasing factor on growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured ham production. *Meat Sci.* 91, 148–154. <https://doi.org/10.1016/j.meatsci.2012.01.008>
- Fuchs, T., Thun, R., Parvizi, N., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Klein, G., Sudhaus, N., Beilage, E. grosse, 2009. Effect of a gonadotropin-releasing factor vaccine on follicle-stimulating hormone and luteinizing hormone concentrations and on the development of

testicles and the expression of boar taint in male pigs. *Theriogenology* 72, 672–680.

<https://doi.org/10.1016/j.theriogenology.2009.04.025>

Heyrman, E., Millet, S., Tuytens, F.A.M., Ampe, B., Janssens, S., Buys, N., Wauters, J., Vanhaecke, L., Aluwé, M., 2018. On farm intervention studies on reduction of boar taint prevalence: Feeding strategies, presence of gilts and time in lairage. *Res. Vet. Sci.* 118, 508–516.

<https://doi.org/10.1016/j.rvsc.2018.05.008>

Heyrman, E., Millet, S., Tuytens, F.A.M., Ampe, B., Janssens, S., Buys, N., Wauters, J., Vanhaecke, L., Aluwé, M., 2017. Olfactory evaluation of boar taint: Effect of factors measured at slaughter and link with boar taint compounds. *Animal* 11, 2084–2093.

<https://doi.org/10.1017/S1751731117000994>

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. *Livest. Sci.* 106, 145–153. <https://doi.org/10.1016/j.livsci.2006.07.009>

Lanthier, F., Lou, Y., Turner, M.A., Squires, E.J., 2006. Characterizing developmental changes in plasma and tissue skatole concentrations in the prepubescent intact male pig. *J. Anim. Sci.* 84, 1699–1708. <https://doi.org/10.2527/jas.2005-633>

Liu, G., Jennen, D.G.J., Tholen, E., Juengst, H., Kleinwächter, T., Hölker, M., Tesfaye, D., Ün, G., Schreinemachers, H.J., Murani, E., Ponsuksili, S., Kim, J.J., Schellander, K., Wimmers, K., 2007. A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Anim. Genet.* 38, 241–252. <https://doi.org/10.1111/j.1365-2052.2007.01592.x>

Morales, J.I., Serrano, M.P., Cámara, L., Berrocoso, J.D., López, J.P., Mateos, G.G., 2013. Growth performance and carcass quality of immunocastrated and surgically castrated pigs from crossbreds from Duroc and Pietrain sires. *J. Anim. Sci.* 91, 3955–3964. <https://doi.org/10.2527/jas2012-6068>

- Moss, B.W., Hawe, S.M., Walker, N., 1993. Sensory thresholds for skatole and indole, in: Colloques de l'INRA.
- Oonk, H.B., Turkstra, J.A., Schaaper, W.M.M., Erkens, J.H.F., Schuitemaker-de Weerd, M.H., Van Nes, A., Verheijden, J.H.M., Meloen, R.H., 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. *Vaccine* 16, 1074–1082. [https://doi.org/10.1016/S0264-410X\(98\)80101-1](https://doi.org/10.1016/S0264-410X(98)80101-1)
- Prunier, A., Brillouët, A., Merlot, E., Meunier-Salaün, M.C., Tallet, C., 2013. Influence of housing and season on pubertal development, boar taint compounds and skin lesions of male pigs. *Animal* 7, 2035–43. <https://doi.org/10.1017/S1751731113001596>
- Prusa, K., Nederveld, H., Runnels, P.L., Li, R., King, V.L., Crane, J.P., 2011. Prevalence and relationships of sensory taint, 5 α -androstenone and skatole in fat and lean tissue from the loin (Longissimus dorsi) of barrows, gilts, sows, and boars from selected abattoirs in the United States. *Meat Sci.* 88, 96–101. <https://doi.org/10.1016/j.meatsci.2010.12.008>
- R Core Team, R: A Language and Environment for Statistical Computing, 2013.
- Rigolot, C., Espagnol, S., Pomar, C., Dourmad, J.Y., 2010. Modelling of manure production by pigs and NH₃, N₂O and CH₄ emissions. Part I: Animal excretion and enteric CH₄, effect of feeding and performance. *Animal* 4, 1401–1412. <https://doi.org/10.1017/S1751731110000492>
- Rius, M.A., García-Regueiro, J.A., 2001. Skatole and indole concentrations in Longissimus dorsi and fat samples of pigs. *Meat Sci.* 59, 285–291. [https://doi.org/10.1016/S0309-1740\(01\)00081-X](https://doi.org/10.1016/S0309-1740(01)00081-X)
- Sinclair, P.A., Squires, E.J., Raeside, J.I., Britt, J.H., Hedgpeth, V.G., 2001. The effect of early postnatal treatment with a gonadotropin-releasing hormone agonist on the developmental profiles of testicular steroid hormones in the intact male pig. *J. Anim. Sci.* 79, 1003–1010.
- Škrlep, M., Batorek, N., Bonneau, M., Fazarinc, G., Šegula, B., Čandek-Potokar, M., 2012. Elevated fat skatole levels in immunocastrated, surgically castrated and entire male pigs with acute dysentery. *Vet. J.* 194, 417–419. <https://doi.org/10.1016/j.tvjl.2012.04.013>

- Squires, E. J., & Lundström, K., 1997. Relationship between cytochrome P450IIE1 in liver and levels of skatole and its metabolites in intact male pigs. *Journal of animal science*, 75(9), 2506-2511.
- Trautmann, J., Meier-Dinkel, L., Gertheiss, J., Mörlein, D., 2016. Boar taint detection: A comparison of three sensory protocols. *Meat Sci.* 111, 92–100. <https://doi.org/10.1016/j.meatsci.2015.08.011>
- Van Den Broeke, A., Aluwé, M., Janssens, S., Wauters, J., Vanhaecke, L., Buys, N., Millet, S., Tuytens, F.A.M., 2015. The effect of the MC4R gene on boar taint compounds, sexual maturity and behaviour in growing-finishing boars and gilts. *Animal* 9, 1688–1697. <https://doi.org/10.1017/S1751731115001135>
- Van den Broeke, A., Leen, F., Aluwé, M., Ampe, B., Van Meensel, J., Millet, S., 2016. The effect of GnRH vaccination on performance, carcass, and meat quality and hormonal regulation in boars, barrows, and gilts. *J. Anim. Sci.* 94, 2811–2820. <https://doi.org/10.2527/jas2015-0173>
- van Wagenberg, C.P.A., Snoek, H.M., van der Fels, J.B., van der Peet-Schwering, C.M.C., Vermeer, H.M., Heres, L., 2013. Farm and management characteristics associated with boar taint. *Animal* 7, 1841–8. <https://doi.org/10.1017/S1751731113001328>
- Weiler, U., Götz, M., Schmidt, A., Otto, M., Müller, S., 2012. Influence of sex and immunocastration on feed intake behavior, skatole and indole concentrations in adipose tissue of pigs. *Animal* 1–9. <https://doi.org/10.1017/S175173111200167X>
- Wesoly, R., Jungbluth, I., Stefanski, V., Weiler, U., 2014. Pre-slaughter conditions influence skatole and androstenone in adipose tissue of boars. *Meat Sci.* 99, 60–67. <https://doi.org/10.1016/j.meatsci.2014.08.015>
- Wesoly, R., Stefanski, V., Weiler, U., 2016. Influence of sampling procedure, sampling location and skin contamination on skatole and indole concentrations in adipose tissue of pigs. *Meat Sci.* 111, 85–91. <https://doi.org/10.1016/j.meatsci.2015.08.015>
- Wesoly, R., Weiler, U., 2012. Nutritional influences on skatole formation and skatole metabolism in the pig. *Animals* 2, 221–242. <https://doi.org/10.3390/ani2020221>

- Xue, J., Dial, G.D., Holton, E.E., Vickers, Z., Squires, E.J., Lou, Y., Godbout, D., Morel, N., 1996. Breed Differences in Boar Taint: Relationship between Tissue Levels of Boar Taint Compounds and Sensory Analysis of Taint. *J. Anim. Sci.* 74, 2170–2177. <https://doi.org/10.2527/1996.7492170x>
- Xue, J., Dial, G.D., 1997. Raising intact male pigs for meat: Detecting and preventing boar taint. *J. Swine Heal. Prod.* 5, 151–158.
- Zamaratskaia, G., Andersson, H.K., Chen, G., Andersson, K., Madej, A., Lundström, K., 2008. Effect of a gonadotropin-releasing hormone vaccine (ImprovacTM) on steroid hormones, boar taint compounds and performance in entire male pigs. *Reprod. Domest. Anim.* 43, 351–359. <https://doi.org/10.1111/j.1439-0531.2007.00914.x>
- Zamaratskaia, G., Babol, J., Madej, A., Squires, E.J., Lundström, K., 2004. Age-related variation of plasma concentrations of skatole, androstenone, testosterone, oestradiol-17 β , oestrone sulphate, dehydroepiandrosterone sulphate, triiodothyronine and IGF-1 in six entire male pigs. *Reprod. Domest. Anim.* 39, 168–172. <https://doi.org/10.1111/j.1439-0531.2004.00496.x>
- Zamaratskaia, G., Chen, G., Lundström, K., 2006. Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochromes P450E1 and P450A6 in pigs. *Meat Sci.* 72, 331–338. <https://doi.org/10.1016/j.meatsci.2005.07.020>
- Zamaratskaia, G., Gilmore, W.J., Lundström, K., Squires, E.J., 2007. Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. *Food Chem. Toxicol.* 45, 676–681. <https://doi.org/10.1016/j.fct.2006.10.023>
- Zamaratskaia, G., Rydhmer, L., Andersson, H.K., Chen, G., Lowagie, S., Andersson, K., Lundström, K., 2008. Long-term effect of vaccination against gonadotropin-releasing hormone, using ImprovacTM, on hormonal profile and behaviour of male pigs. *Anim. Reprod. Sci.* 108, 37–48. <https://doi.org/10.1016/j.anireprosci.2007.07.001>
- Zamaratskaia, G., Squires, E.J., 2009. Biochemical, nutritional and genetic effects on boar taint in

entire male pigs. *Animal* 3, 1508–1521. <https://doi.org/10.1017/S1751731108003674>

Zamaratskaia, G., Zlabek, V., Chen, G., Madej, A., 2009. Modulation of porcine cytochrome P450 enzyme activities by surgical castration and immunocastration. *Animal* 3, 1124–1132. <https://doi.org/10.1017/S1751731109004510>

Zeng, X.Y., Turkstra, J.A., Tsigos, A., Meloen, R.H., Liu, X.Y., Chen, F.Q., Schaaper, W.M.M., Ria Oonk, H.B., Guo, D.Z., van de Wiel, D.F., 2002. Effects of active immunization against GnRH on serum LH, inhibin A, sexual development and growth rate in Chinese female pigs. *Theriogenology* 58, 1315–1326. [https://doi.org/10.1016/S0093-691X\(02\)01036-1](https://doi.org/10.1016/S0093-691X(02)01036-1)

Table 1 Age and weight of immunocastrates of 3 sire lines, Belgian Piétrain (BP), French Piétrain (FP), and Canadian Duroc (CD) sire lines at 4 time points: at first vaccination (V1 – 8 weeks before slaughter), at second vaccination (V2 – 4 weeks before slaughter), 2 weeks after second vaccination (V2+2), and 1 day before slaughter (S). Daily gain is also calculated for the 3 periods between these 4 time points. Values are mean $\pm$ standard deviation. Significant differences for age and daily gain between sire lines within time points and periods are indicated by a, b.

	V1	V2	V2+2	S
Weight (kg)				
BP	52.5 $\pm$ 8.9	78.3 $\pm$ 9.7	95.7 $\pm$ 11.0	110.5 $\pm$ 15.5
FP	49.7 $\pm$ 5.0	80.6 $\pm$ 8.3	100.2 $\pm$ 10.6	121.0 $\pm$ 13.6
CD	53.5 $\pm$ 3.4	81.8 $\pm$ 5.5	99.7 $\pm$ 8.5	119.6 $\pm$ 8.8
Age (days)				
BP	102 ^b $\pm$ 1	130 ^b $\pm$ 1	144 ^b $\pm$ 1	166 ^c $\pm$ 1
FP	92 ^a $\pm$ 3	121 ^a $\pm$ 1	135 ^a $\pm$ 1	153 ^b $\pm$ 7
CD	92 ^a $\pm$ 6	116 ^a $\pm$ 3	129 ^a $\pm$ 2	144 ^a $\pm$ 2
	V1 – V2	V2 – V2+2	V2+2 – S	
Daily gain (g/day)				
BP	953 ^a $\pm$ 89	1110 ^a $\pm$ 129	670 ^a $\pm$ 523	
FP	1022 ^{ab} $\pm$ 118	1297 ^{ab} $\pm$ 310	1332 ^b $\pm$ 311	
CD	1152 ^b $\pm$ 109	1336 ^b $\pm$ 270	1394 ^b $\pm$ 124	

Table 2 Ethogram of recorded behaviors

Behavior	Definition
Inactive	Pig is sleeping (eyes closed) or lying down
Eating/drinking	Pig is eating or drinking at feeding tray or drink nipple
Moving	Pig is walking around without exhibiting any other behavior
Interaction ¹	Pig is sniffing other pig without showing aggressive behavior
Playing ¹	Pig is playing with another pig without showing aggressive behavior
Manipulation ²	Pig is manipulating/biting distraction material, feeding trough, pen wall,..
Ear or tail biting ²	Pig is biting the ear or tail of another pig
Aggression	Thrusting by head-knocking or biting in the air, pushing another pig away, biting or lifting another pig
Mounting	Pig (attempts) to place front legs over front or back end of other pig and (attempts) to copulate
Anogenital sniffing	Nose of pig is in 5cm proximity to anogenital zone of other pig

¹Clustered as interaction + playing

²Clustered as manipulation + ear & tail biting

Table 3 Definitions for the category scales used in the animal observations

Category	Skin lesions ^{1,2}	Animal soiling ¹	Pen soiling ¹
0	None: No scratches or other wounds	Clean: all pigs are clean	Clean: the pen surface is clean
1	Very light: 1-3 scratches <5cm, 1-2 scratches of 5-15cm, no other wounds	Very light: up to 25% of the pig is soiled	Very light: up to 25% of the pen surface is soiled
2	Light: 4-6 scratches <5cm, 3-4 of 5-15cm or 1-2 >15cm, no other wounds	Light: 25-50% of the pig is soiled	Light: 25-50% of the pen surface is soiled
3	Some: >6 scratches <5cm, >4 scratches 5-15cm, >3 scratches >15cm or other wounds	Some: 50-75% of the pig is soiled	Some: 50-75% of the pen surface is soiled
4	Severe: Multiple severe wounds on part of the body	Heavy: more than 75% of the pig is soiled	Heavy: more than 75% of the pen surface is soiled
5	Very severe: Multiple severe wounds on entire body		

¹The protocol for these observations used photographs for each category to help the observer. The pen was not entered for these observations, the observer stood in front of the pen right up to the side.

²Both sides of the pig was considered as a whole

Figure 1 Schematic representation of the timing of measurement

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Figure 2 Testosterone (a), estradiol (b), progesterone (c), androstenone (d), skatole (e), indole (f) in serum and androstenone (g) and skatole (h) in fat for immunocastrates of 3 sire lines (Belgian Piétrain (BP), French Piétrain (FP), and Canadian Duroc (CD)) at 4 time points: first vaccination (V1 = 8 weeks before slaughter), second vaccination (V2 = 4 weeks before slaughter), 2 weeks after second vaccination (V2+2), and 1 day before slaughter (S). Values are mean $\pm$ standard error. Ps indicates p-value for differences between sire lines, Pt indicates p-value for differences between time points, and Ps*t indicates p-value for the interaction. For all analyses a, b, c indicates significant differences between time points within sire lines, and x, y indicates significant differences between sire lines within time points. All compounds are shown on logarithmic scale.

Figure 3 Scatter plot of testosterone in serum vs. androstenone in serum at second vaccination (V2) (a) and at slaughter (S) (b), testosterone in serum vs. androstenone in fat at V2 (c) and S (d), androstenone in serum vs. fat at V2 (e) and S (f), and of skatole in serum vs. fat at V2 (g) and S (h). Correlations (r) and p-value (P) are given for all samples together and for Belgian Piétrain (BP), French Piétrain (FP), and Canadian Duroc (CD) separately.

Figure 4 Recorded behavior before the first vaccination ($<V1$), before the second vaccination ($<V2$), and after the second vaccination ($>V2$) for immunocastrates from 3 sire lines (Belgian Piétrain (BP), Canadian duroc (CD), and French Piétrain (FP)). P_s indicates p-value for differences between sire lines, P_t and P_{s*t} indicates p-value for differences between time points. For all analyses a, b indicates significant differences between time points within sire lines, and x, y indicates significant differences between sire lines within time points.

Highlights:

- Aggressive behavior and general activity decline due to immunocastration
- Immunocastrates present with androstenone (1.9%) or skatole (3.7%) above cutoff
- Indole is unaffected by immunocastration in French Piétrain and Canadian Duroc

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Figure 1

Figure 2

Figure 3

Figure 4