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# PRODUCTIVITY AND BOAR TAINT IN CASTRATES, MALE PIGS AND IMMUNOCASTRATES

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## Main conclusion

Feed conversion and lean meat percentage of immunocastrates ranked between male pigs and castrates, with no differences in daily gain between the three groups. Immunocastration reduced skatole and androstenone to very low level, but did not completely eliminate boar taint in all immunocastrates.

## Abstract

Daily gain, lean meat percentage and feed conversion ratio are significantly better in male pigs compared with castrates. This is the outcome of the study that ranks immunocastrated pigs between castrates and male pigs on all three parameters.

DLY offspring of low-androstenone boars had 50% lower androstenone levels at approx. 70 kg compared with DLY offspring of AI boars with high androstenone levels. Offspring of lowandrostenone AI boars also had lower skatole levels compared with offspring of high-androstenone AI boars – an outcome not seen in previous Danish studies. Generally, sorting AI boars based on androstenone levels will positively affect boar taint in male pigs at slaughter.

While immunocastration did lower androstenone and skatole levels, levels were still higher than seen for castrates. Immunocastration did not eliminate boar taint in all vaccinated pigs, as analyses revealed androstenone levels above 2.0 ppm in 4% of the pigs at slaughter. Nevertheless, immunocastration is an important tool in reducing androstenone and skatole in male pigs at slaughter.

Overall, immunocastration significantly reduces boar taint compared with production of male pigs and improves productivity compared with production of castrates. The drawbacks, however, include

additional costs for wages, vaccines and injection accessories and analyses for boar taint at the slaughterhouse. Furthermore, the Danish pig industry must still be able to sell products from male pigs – vaccinated and not vaccinated – in essential Asian markets.

Analyses of behaviour, penis injuries and gastric ulcers as well as cost-benefit when producing castrates, male pigs and immunocastrates will be published in two separate reports.

Permission was granted by the Danish Centre for Animal Welfare to perform biopsies and blood sampling. Permission j.no. 2016-15-0201-01080.

Permission to conduct the study was granted by the Danish Medicines Agency. Case 2018071166 concerning medicines experiments with animals.

## Background

Production of male pigs includes a range of advantages compared with production of castrates:

- 1. No surgical castration (improved animal welfare, elimination of tasks related to castration, administration of pain relief and local anaesthesia, and lower mortality rates among male piglets)
- 2. Feed conversion ratio and lean meat percentage are better in male pigs compared with castrates
- 3. Improved utilization of nutrients in the feed = lower carbon footprint.

Production of male pigs is generating increasing interest in several European countries to improve animal welfare. Several of the Denmark's main export markets still refuse to buy pork from male pigs due to, among other reasons, the risk of receiving meat with boar taint.

Boar taint is caused by skatole (produced in the gut) and androstenone (produced in the testicles), but is also affected by genetic factors, breed, age, weight and feeding. Skatole and androstenone are both hereditary [1] [2] and differ between breeds [3]. Evidence indicates that feeding affects skatole levels (but not androstenone) [4] [5] [6] [7], and that lower weight/age at slaughter reduce androstenone (but not skatole) [4] [8] [9]. It is also known that using semen from AI boars with a low level of boar taint (androstenone) leads to lower androstenone levels in the offspring. This is not seen to affect skatole levels in offspring [2] [9] [10].

Immunocastration is one of several methods for reduction of boar taint: male pigs are vaccinated with an anti-gonadotropin-releasing-hormone (GnRH) at approx. 30 kg and again 4-6 weeks before slaughter, which will trigger active immunisation against GnRH. This immunisation stops the secretion of the luteinizing hormone (LH) and the follicle stimulating hormone (FSH) from the hypophysis, which will inhibit the production of testosterone and androstenone in the testicles. Skatole is indirectly reduced as skatole decomposition in the liver is not inhibited by androstenone [11]. Antibodies against GnHR develop 4-14 days after the second vaccination. From this point, testosterone synthesis stops completely and behavioural changes are observed [12] [13].

Previous studies revealed improved feed conversion ratios and lean meat percentage in male pigs compared with castrates [10]. Danish studies also demonstrated a higher daily gain in immunocastrated pigs compared with castrates [14].

Immunocastration did reduce boar taint, but did not entirely remove boar taint [14]. This study is the first of its kind including castrates, male pigs and immunocastrates of DanBred genetics. This study combines genetics and three types of 'male pigs': castrates, male pigs and immunocastrates. Al boars were sorted according to high and low levels of androstenone, respectively, which was determined by performing a biopsy of the neck fat at approx. 100 kg.

The aim of the trial was to:

- 1. Establish the effect on boar taint (androstenone, skatole) in DLY offspring from DanBred Duroc boars with high and low androstenone levels of sorting boars on the basis of androstenone and in combination with the effect of immunocastration
- 2. Establish productivity of castrates, male pigs and immunocastrates to determine cost-benefit for 'three male genders'.

## Materials and methods

The trial comprised six groups: castrates, male pigs and immunocastrates from DanBred Duroc AI boars with high or low androstenone levels (table 1).

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Androstenone AI boars	High	Low
Castrates	120	120
Male pigs	120	120
Immunocastrates	120	120

Androstenone levels were determined in neck fat biopsies from a total of 317 DanBred Duroc boars at Bøgildgård, and of these 32 AI boars with high androstenone levels (>2.38 ppm) and 32 with low androstenone levels (<1.50) were selected for the trial.

Analyses of the biopsies taken at approx. 100 kg liveweight (table 2a) revealed androstenone levels below 1.50 ppm in 32% of the tested AI boars and above 2.38 ppm in 30% of the AI boars (figure 1). The productivity breeding index of the tested AI boars did not differ regardless of androstenone levels, nor did the productivity index differ significantly between the AI boars chosen for the trial (tables 2a and 2b). The boars chosen for the trial were used for individual sows in order to have siblings from each litter (table 2b). Skatole levels are not shown in table 2a as analyses only found quantifiable skatole levels in less than 10% (26) of the AI boars.

Androstenone level	High >2.38 ppm	Medium 1.5-2.38	Low < 1.5 ppm	All
AI boars	88	101	128	317
Androstenone, ppm	3.09	1.88	1.01	1.88
Productivity index	122	122	122	122

Table 2a. Average androstenone level and productivity index of tested DanBred Duroc boars at 100 kg

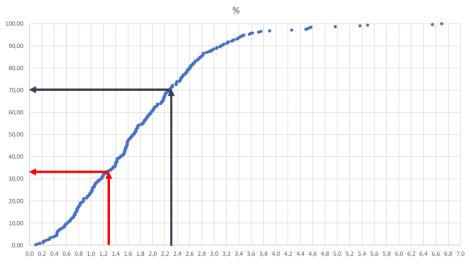


Figure 1. Androstenone levels in 317 DanBred Duroc boars tested for the trial.

Boar taint AI boars at 100 kg	High >2.38 ppm	Low < 1.40 ppm
Number of AI boars	32	32
Androstenone, ppm	3.10 (0.77)	0.87 (0.44)
Skatole, ppm	0.03 (0.02)	0.03 (0.01)
Productivity index	116 (7)	121 (7)

Table 2b. Al boars used in the trial - high and low androstenone levels, average (	(std.)
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Danish Pig Research Centre's trial station Grønhøj purchases pigs at weaning (4 weeks). For this trial, sows on the farm delivering pigs to Grønhøj were inseminated with semen from the chosen AI boars. The trial included averagely 2.5 litters per boar. At farrowing, three male pigs (siblings) from two litters per boar were randomly selected for the trial and individually marked for identification. One of them was castrated and the other two remained intact, but were assigned randomly to be either male pig or immunocastrate (table 3). At 30 kg, the pigs were individually ear-tagged and moved to finisher pens with single feeders that recorded daily feed intake for each individual pig. The pigs were assigned to groups according to gender and, where possible, according to high/low androstenone AI boars. All pigs were weighed at transfer (age: 82 days, approx. 12 weeks), before second vaccination (immunocastrates), 4-6 weeks before slaughter (age: 115 days, approx. 16 weeks), and at eight o'clock the day before slaughter.

Table 3. Offspring in trial			
Androstenone		High	Low
AI boars tested		113	114
Al boars in trial		32	32
Litters/Al-boar		2.5	2.5
Number of pigs in the trial, total		356	283
	Castrates	127	106
	Male pigs	112	91
	Immunocastrates	117	86
Number of pigs slaughtered, total		342	275
	Castrates	120	102
	Male pigs	110	88
	Immunocastrates	112	85
Dead or moved to hospital pen		14	8
ID missing at slaughterhouse		2	0
Weighed the day before slaughter, number		341	275
Slaughter data, number		340	275

## Immunocastration – vaccination with Improvac

The pigs were vaccinated with the Improvac vaccine according to the vaccination protocol supplied by Zoetis. Zoetis representatives instructed the staff at trial station Grønhøj in how to administer the vaccination. The vaccine was kept in a refrigerator: the day before use, it was placed outside the refrigerator to reach room temperature. New bottles were used for each new round of vaccinations. No pigs showed clinical signs of disease at the time of vaccination.

The first Improvac vaccination was administered the day after transfer to the finisher unit, and the second 4-6 weeks before slaughter. Representatives from Orion Pharma (Danish supplier of Zoetis products, 2019-2020) observed nearly all vaccinations. No side effects were identified. After the second vaccination, staff were asked to record significantly deviant sexual behaviour (mounting) among immunocastrates to identify potential non-responders (male pigs that fail to respond to the treatment). No observations of significantly deviant sexual behaviour were reported.

## Slaughter and sampling

All pigs were slaughtered at the economically optimum weight and were – prior to slaughter – tattooed for subsequent individual identification. Samples of neck fat taken the day after slaughter were analysed for skatole and androstenone. At the slaughter line, penis was removed for evaluation of injuries and stomachs were removed for ulcer assessment (data from these analyses are published in a separate report).

## Analyses of blood samples and fat biopsies, DLY offspring

Blood samples collected at transfer to the finisher unit, the day before the second vaccination and before slaughter were centrifuged, and plasma was separated and stored at -80 °C. Biopsy of neck fat was performed the day before the second vaccination.

By mistake, blood samples were not collected in the first of the four insertions, and consequently, the number of analysed blood samples 'at transfer' is lower than the number of samples 'before second vaccination' and 'at slaughter'.

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Plasma samples were subject to analysis for GnHR inhibitor and testosterone at the University of Hohenheim (UHOH) [15]. At vaccination, antibodies develop that inhibit the production of gonadotrophin-releasing hormone (GnHR), and to determine the efficiency of the vaccine, the GnHR inhibitor (absolute binding %) was measured, which illustrates the degree of immune response to the Improvac vaccination. In immunocastrated pigs, values below 35% indicate inadequate immunisation [13].

*Testosterone* is a sex hormone that increases at the onset of sexual maturity in male pigs, and Improvac reduces/halts the production of testosterone. For immunocastrated pigs, values above 0.5 ng/ml indicate suboptimal effect of the vaccine [13].

Androstenone and skatole levels in fat and biopsy samples were determined using the LDTD-MS/MS method [16] (table 4).

		stenone = mg/kg)		atole = mg/Kg)
	Detection Quantification threshold threshold		Detection threshold	Quantification threshold
Biopsy	0.1	0.3	0.02	0.05
Value included in data set	0.05	0.15	0.01	0.025
Fat	0.05	0.10	0.02	0.05
Value included in data set	0.025	0.05	0.01	0.025

Table 4. Detection and quantification threshold for skatole and androstenone in biopsies and fat

## Feed: Composition of feed and analyses

In order for all pigs in the trial to have equal opportunities for maximum growth, all three genders were fed the same diet with energy and protein content above the Danish standards. The use of a highenergy/protein diet would not negatively affect growth of castrates and immunocastrates, but would allow male pigs to obtain maximum growth [17]. The diet was formulated to contain 110 feed units (+5% more than normal) and 130 g digestible protein (+8% compared with the normal content) and more amino acids. Analyses showed that the diets contained averagely 113 feed units and 16.6 g protein. Appendix 1 provides an outline of diet composition, analyses and calculations.

## Statistical analyses

#### Statistical models

The trial is designed as a split plot trial on offspring of AI boars split according to the boars' androstenone levels (low = below 1.5 ppm / high = above 2.88 ppm) as a whole-plot and three genders as sub-plot. All pigs were fed individually, and recordings were made for each single animal, but AI boars constitute a test unit.

Prior to statistical analyses, skatole and androstenone, the two primary variables, were logtransformed to reach normal distribution. Skatole, androstenone and feed conversion were subject to analysis in a generalised linear model where consideration is made to repeated recordings on boar and litter. Values presented in the tables are *back-transformed estimates LS-means*.

# Results

*Gender* defines whether the pig is a castrate, a male pig or an immunocastrate. *AI-Boar* defines selection of sires with a high or low androstenone level.

## Boar taint (skatole and androstenone) before second vaccination

Results showed no interaction between AI boars and gender for skatole and androstenone recorded before the second vaccination. Androstenone and skatole levels recorded in fat biopsies did not differ between male pigs and immunocastrates after the first vaccination. Analyses revealed that the androstenone level in male pigs of low-androstenone AI boars was 50% lower compared with male pigs of high-androstenone AI boars. Skatole levels did not differ between any of the groups.

(LS-means)							
Androstenone Al boars		High Low Significance		Low			
Offspring, gender	Male pig	Immunocastrate	Male pig	Immunocastrate	Al boars *Gender	AI boars	Gender
Number	107	109	89	86			
Weight, kg (std.)	73.4 (10.1)	70.9 (10.1)	69.9 (10.3)	70.4 (9.0)			
Age, days	115 (8)	117 (9)	113 (10)	112 (9)			
Skatole, ppm	0.10	0.10	0.10	0.09	Ns	Ns	Ns
Androstenone, ppm	0.66	0.61	0.35	0.27	Ns	P<0.01	Ns
Androstenone, ppm		0.63 <sup>a</sup>		0.31 <sup>b</sup>		P<0.01	

**Table 5**. Androstenone and skatole in biopsies taken **before** 2<sup>nd</sup> vaccination in male pigs and immunocastrates (LS-means)

a,b: Different superscripts indicate significant difference p<0.05

## Boar taint (skatole and androstenone) after slaughter

After slaughter, results showed no interaction between boar and gender (male pigs and immunocastrates) in terms of skatole. However, results did find interaction between AI boars and gender for androstenone as immunocastration led to a greater reduction in androstenone levels in offspring of high-androstenone AI boars compared with offspring of low-androstenone AI boars.

## Androstenone

Androstenone levels in male pigs of low-androstenone AI boars were 0.89 ppm lower (34% reduction) than in male pigs of high-androstenone boars.

## Skatole

Skatole levels in offspring of high-androstenone AI boars were averagely 0.02 ppm lower (20% reduction) compared with offspring of low-androstenone boars, but still higher than in samples from random castrates (tables 6 and 7).

#### Immunocastration

Vaccination with Improvac significantly reduced both skatole and androstenone levels (table 7). Androstenone levels in vaccinated male pigs of high-androstenone AI boars were 2.47 ppm lower compared with male pigs (95% reduction). Androstenone levels in vaccinated male pigs of lowandrostenone AI boars were 1.59 ppm lower compared with male pigs (93% reduction). Following immunocastration, skatole levels dropped by 0.03 ppm in offspring of both types of AI boars corresponding to a reduction of 33% and 43%, respectively, in offspring of high and low-androstenone AI boars (table 7). Skatole levels in immunocastrates at slaughter were higher than for castrates (random) (table 6).

#### Castrates

Androstenone and skatole levels in neck fat from randomly chosen castrates (59 samples) were lower than in male pigs and immunocastrates, and analyses revealed no differences between offspring of AI boars with high or low androstenone levels, respectively. Skatole levels in fat from castrates were 0.04 ppm and androstenone levels were 0.03 ppm (table 6).

Table 6. Average skatole and androstenone levels recorded in random fat samples from castrates

Androstenone AI boars	High	Low
Gender	Castrate	Castrate
Number	35	24
Skatole, ppm	0.04	0.03
Androstenone, ppm	0.03	0.03

### "Thresholds"

Immunocastration significantly reduced androstenone levels and thereby the percentage of pigs exceeding 1.00 and 2.00 ppm (table 7), which are the internationally recognized thresholds when defining boar taint. These thresholds are **not** recognized as quality criteria in a sales situation.

Among male pigs of high-androstenone AI boars, 96.1% were identified with androstenone levels above 1.0 ppm and among immunocastrates this number was 14.0%. Among male pigs of low-androstenone AI boars, 71.3% were identified with androstenone levels above 1.0 ppm and among immunocastrates this number was 10.3% (table 7).

Of all male pigs, androstenone levels above 2.00 ppm were found in 45.9% of the pigs and in 3.75% of the immunocastrated pigs.

The percentage of male pigs with skatole levels above 0.25 ppm (current rejection limit for boar taint in Denmark) was 7.7% and for immunocastrates 0.9% for offspring of high-androstenone AI boars. Among offspring of low-androstenone AI boars, 2.3% male pigs were rejected and 0% immunocastrates were rejected.

## Boar taint after slaughter

 Table 7. Skatole and androstenone in fat samples collected from male pigs and immunocastrates after slaughter.

Androstenone Al boars (Boars)		High		Low	Sign	ificance	
Gender	Male pig	Immunocast.	Male pig	Immunocast.	Boars*Gender	Boars	Gender
Number	103	107	87	85			
Liveweight, kg	120.9	120.7	120.5	120.2			
Skatole, ppm	0.09	0.06	0.07	0.04	Ns	P=0.04	<0.01
% > 0.25 ppm	7.7	0.9	2.3	0.0			
Androstenone, ppm	2.60	0.12	1.71	0.12	<0.01	<0.01	<0.01
% > 1.00 ppm	96.1	14.0	71.3	10.6			
% > 2.00 ppm	57.3	2.8	34.5	4.7			

## Correlations - skatole and androstenone

Analyses showed a significant correlation between androstenone levels in neck fat before the second vaccination and at slaughter, as was also the case for skatole. For immunocastrates results showed a very low, but significant correlation between skatole at slaughter and skatole/androstenone at second vaccination (table 8a).

**Table 8a.** Correlation between skatole and androstenone in biopsies in DLY offspring. Correlation coefficient with

 P value in parenthesis.

		After sla	After slaughter		
		Androstenone	Skatole		
Biopsy before 2 <sup>nd</sup> vaccination,	Androstenone	0.55 (p<0.01)	0.15 (ns)		
male pigs	Skatole	0.07 (ns)	0.38 (p<0.01)		
Biopsy before 2 <sup>nd</sup> vaccination,	Androstenone	0.13 (ns)	0.14 (0.04)		
immunocastrates	Skatole	-0.03 (ns)	0.14 (0.05)		

For male pigs, results revealed a low, but significant correlation between androstenone levels of Al boars and their offspring. There was no correlation between androstenone levels of Al boars and skatole levels in offspring. As skatole levels of many of the Al boars were below the detection limit (table 8b), it was not possible to establish a correlation between skatole levels of Al boars and their offspring.

 Table 8b.
 Correlation for skatole and androstenone between Duroc AI boars and DLY male pigs.
 Correlation

 coefficient with P value in parenthesis.
 Coefficient with P value in parenthesis.
 Coefficient with P value in parenthesis.

		D boars - all		
DLY offspring		Androstenone	Skatole	
Male pigs	Androstenone	0.19 (p<0.01)	0-0.05 (ns)	
Immunocastrates	Androstenone	-0.04 (ns)	0.07 (ns)	

## Testosterone and GnRH inhibitor in blood - male pigs and immunocastrates

Overall, results showed no interaction between AI boars and gender in testosterone and GnHR inhibitors at any of the blood samples. Results showed no differences between male pigs and immunocastrates in testosterone and GnHR inhibitors at transfer.

### Testosterone

Testosterone levels at slaughter were significantly lower in immunocastrates compared with male pigs, but analyses revealed identical levels at transfer (age: 82 days, approx. 12 weeks) and before the second vaccination (age: 115 days, 16 weeks). Testosterone levels in blood were significantly higher for male pigs of high-androstenone AI boars at the second vaccination and at slaughter (age: 150 days, approx. 21 weeks).

*GnRH inhibitor* (absolute binding %) in blood showed no interaction between AI boars and gender. There were no differences in immune response at transfer (table 9).

Blood samples collected before the second vaccination and the day before slaughter showed a significantly higher immune response in immunocastrates than in male pigs: 21% of all immunocastrates exhibited an immune response below 35%, which indicates a suboptimal response to the vaccination [13]. Testosterone levels in immunocastrates with an immune response below 35% were nearly five times higher than the levels found in immunocastrates that were effectively immunised.

Analyses identified a correlation of -0.48 (p<0.001) between GnHR and testosterone at slaughter for immunocastrates, but this correlation was not identified for male pigs (data not shown in table).

		An	drostenone	Al boars (B	oars)	Significance			
		High		L	ow	Boars* Gender	Boars	Gender	
Gender		Male pig	Immuno- cast.	Male pig	Immuno- cast.				
Testosterone,	Number	59	69	62	61				
ng/ml	Pre-1 <sup>st</sup> vacc	1.79	1.68	1.79	1.59	ns	Ns	ns	
	Number	105	112	86	85				
	Pre-2 <sup>nd</sup> vacc	2.64	2.49	2.23	2.10	ns	p=0.03	ns	
	Number	116	112	90	66				
	At slaughter	4.15ª	1.22 <sup>b</sup>	3.45°	0.95 <sup>b</sup>	ns	p=0.04	p<0.01	
GnRH	Number	61	69	62	61				
inhibitor, Absolute	Pre-1 <sup>st</sup> vacc	5.56	5.56	5.68	5.71	ns	Ns	ns	
binding %	Number	105	113	86	86				
	Pre-2 <sup>nd</sup> vacc	5.21 <sup>a</sup>	14.34 <sup>b</sup>	5.40ª	15.50 <sup>b</sup>	ns	Ns	p<0.01	
	Number	116	112	90	85				
	At slaughter	5.25	37.03	5.36	39.10	ns	Ns	p<0.01	

## Table 9. Testosterone (ng/ml plasma) and GnRH inhibitor binding (%) (LS-means)

a,b,c: Different superscripts indicate significant difference p<0.05

For male pigs, results showed a significant correlation at slaughter between testosterone, androstenone and skatole (table 10). In other words: testosterone levels before slaughter may indicate whether pigs will have high or low levels of skatole and androstenone. Analyses revealed a weak (but significant) correlation between GnHR inhibitors and androstenone level. Among immunocastrates,

results demonstrated a significant correlation at slaughter between testosterone, GnHR inhibitors and androstenone, but no correlation with skatole (table 10).

				After slaughter			
			No.	Androstenone	Skatole		
Male pigs	Slaughter	Testosterone	199	0.55 (<0.01)	0.31 (<0.01)		
		GnRH	199	0.15 (0.04)	0.07 (ns)		
Immunocastrates	Slaughter	Testosterone	133	0.68 (<0.01)	0.11 (ns)		
		GnRH	191	0.46 (<0.01)	-0.03 (ns)		

**Table 10.** Correlation between testosterone and GnRH inhibitor at transfer, 2<sup>nd</sup> vaccination and slaughter in male pigs and immunocastrates. Correlation coefficient incl. P value in parenthesis.

## Weight and age

No interaction was observed at slaughter for gender and boar and age and weight. Weight at transfer differed between genders as castrates were *heavier* than male pigs and immunocastrates. Liveweight at slaughter also differed as castrates weighed *less* than male pigs and immunocastrates. Carcass weight, however, was identical, which is attributed to a greater dressing loss in male pigs and immunocastrates (table 12). Age at transfer (first vaccination), second vaccination and slaughter was identical for all groups. Age at slaughter varied from 149-153 days with a variation of 6-7 days (table 11). Variations in start and end weight were greater than normal as regard was made to gender (draw at the time of birth), litter mates and a fairly even number of pigs in each pen.

Androstenone Al boars		High		Low				
Gender	Castrate	Male pig	Immuno- castrate	Castrate	Male pig	Immuno- castrate		
Number	127	pig 112	117	106	91	86		
Weight at transfer, kg	39.5	37.7	38.1	37.2	36.6	36.5		
	(8.0)	(7.3)	(6.6)	(4.6)	(5.6)	(4.6)		
Age at transfer, days	83.1 (7.6)	83.5	84.6	81.4	81.4	80.9		
		(7.7)	(8.4)	(6.7)	(8.1)	(7.1)		
Weight at 2 <sup>nd</sup>	74.6	74.3	70.9	73.9	69.9	70.4		
vaccination, kg	(10.4)	(10.1)	(10.1)	(11.8)	(10.3)	(9.0)		
Age at 2 <sup>nd</sup>	115	115	117	114	113	112		
vaccination, days	(8)	(8)	(9)	(9)	(10)	(9)		
Age at slaughter,	149.6	150.1	152.6	149.5	148.7	149.3		
days	(6.9)	(6.5)	(6.3)	(6.9)	(7.0)	(6.8)		
Weight day before	119.5	120.9	120.7	118.8	120.5	120.2		
slaughter, kg	(4.8)	(6.5)	(6.8)	(5.0)	(6.2)	(6.3)		

**Table 11.** Weight and age in the growth period, average with standard deviation.

## Productivity

Results revealed no interaction between boar and gender or effect of boar on daily gain, feed intake, feed conversion ratio and dressing loss (appendix 2). Results did reveal an effect of gender on daily gain, feed intake, feed conversion ratio and lean meat percentage. For lean meat percentage, no interaction was observed between gender and boar, but results did find an effect of boar, which is surprising as index did not differ between AI boars with high and low androstenone levels (appendix 2).

### Daily gain

Daily gain differed significantly between genders in the entire period. Immunocastrates grew slower than castrates and male pigs. In the period from transfer to the second vaccination, castrates had a higher daily gain than immunocastrates, with male pigs ranked in-between. In the period from the second vaccination to slaughter, male pigs had a higher daily gain than castrates and immunocastrates that both had identical gain (table 12).

#### Feed intake

In the period from transfer (first vaccination) to the second vaccination, feed intake was identical for male pigs and immunocastrates both eating less than the castrates. In the period from the second vaccination until slaughter, no differences were detected between castrates and immunocastrates that both had a higher feed intake than the male pigs (table 12) (figure 2).

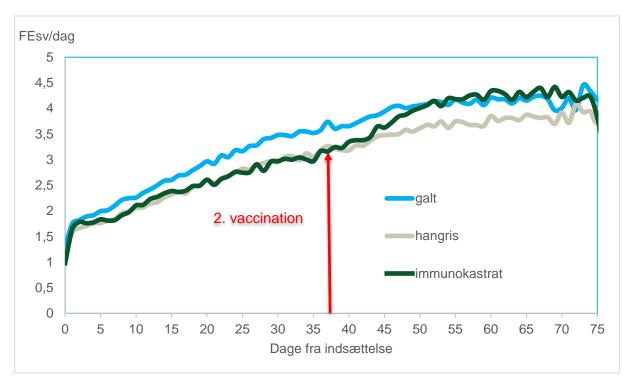


Figure 2. Feed intake from transfer to finisher unit until slaughter, castrates (blue), male pigs (light brown) and immunocastrates (green).

#### Feed conversion ratio

In the period from transfer to the second vaccination, feed conversion ratio was equal for immunocastrates and male pigs and better than that of castrates. From the second vaccination and until slaughter and for the entire period, male pigs had the best feed conversion, castrates the poorest and immunocastrates in-between (table 12).

#### Lean meat %

The lowest lean meat percentage was found in castrates and the highest in male pigs, with immunocastrates in-between (table 12). Offspring of low-androstenone AI boars had a significantly higher lean meat percentage compared with offspring of high-androstenone boars (62.4% vs 61.6%). There are no obvious explanations for this.

## Dressing loss

The lowest dressing loss of 24.8% was observed for castrates (table 12), while for male pigs and immunocastrates dressing loss was 26.5%. Dressing loss in castrates was 1.7% lower for castrates as unlike male pigs and immunocastrates they did not have testicles to be removed.

Boars			All		Sem	
Gender		Castrate	Male	Immuno-		Significance
			pig	castrate		gender
Pigs		235	203	203	0.5	0.42
Daily gain, g	Period 1	1,132ª	1,104 <sup>ab</sup>	1,090 <sup>b</sup>	0.01	0.03
	Period 2	1,229 <sup>a</sup>	1,301 <sup>b</sup>	1,252ª	0.01	<0.01
	Entire period	1,196 <sup>ab</sup>	1,212 <sup>a</sup>	1,180 <sup>b</sup>	0.01	<0.01
Feed conversion,	Period 1	2.31ª	2.11 <sup>b</sup>	2.15 <sup>b</sup>	0.02	<0.01
FUgp/day	Period 2	3.19 <sup>a</sup>	2.70 <sup>b</sup>	3.01°	0.02	<0.01
	Entire period	2.76 <sup>a</sup>	2.41 <sup>b</sup>	2.61°	0.01	<0.01
Feed conversion,	Period	2.33ª	2.06 <sup>b</sup>	2.06 <sup>b</sup>	0.03	<0.01
FUgp/kg	Period 2	3.45 <sup>a</sup>	3.08 <sup>b</sup>	3.30 <sup>c</sup>	0.03	<0.01
	Entire period	2.96 <sup>a</sup>	2.60 <sup>b</sup>	2.73 <sup>℃</sup>	0.02	<0.01
Lean meat %		60.1ª	63.5 <sup>b</sup>	62.6 <sup>c</sup>	0.2	<0.01
Slaughter weight, kg		89.6	89.2	88.9	0.4	ns
Slaughter factor		1.33ª	1.36 <sup>b</sup>	1.36 <sup>b</sup>	0.01	<0.01

Table 12. Daily gain, feed intake, feed conversion ratio, slaughter weight and lean meat %.

a, b, c different superscripts within a row indicate significant difference p<0.05.

Period 1: Transfer to before 2<sup>nd</sup> vaccination.

Period 2: Before 2<sup>nd</sup> vaccination to slaughter.

Total period: From transfer to slaughter.

# Discussion

## Boars

In this study, sorting AI boars according to androstenone levels generated a difference in androstenone in male pigs of 0.9 ppm and a difference in skatole of 0.02 ppm. In two previous studies, androstenone levels in offspring varied from 0.5 to 1.1 ppm. No study previously found an effect on skatole depending on androstenone levels in fat biopsies of AI boars (Duroc boars) [2] [9]. The correlation between AI boars' androstenone levels and offspring androstenone levels in this trial was 0.19 (p=0.007), which was also found in a previous trial [9].

## Boar odorants

Analyses of neck fact biopsies taken before the second vaccination (at 16 weeks) revealed significantly higher androstenone levels (0.63 ppm) in DLY offspring of high-androstenone AI boars compared with offspring of low-androstenone AI boars (0.31 ppm). For male pigs analyses revealed a significant correlation (0.19) in androstenone levels between Duroc AI-boars and DLY offspring, which confirms the hereditary trait of androstenone [1].

Skatole and androstenone were determined in neck fat biopsies taken before the second vaccination (at 16 weeks) and of fat samples taken at slaughter. For male pigs, recordings made at 16 weeks (70 kg) and at slaughter (approx. 21 weeks, 115 kg) found a significant correlation for skatole (0.38) and androstenone (0.55), i.e. recordings made at an early point in the pigs' lives may predict the level of boar taint at slaughter. In a previous trial, biopsies were performed multiple times on the same male pig at increasing weight and results here revealed a correlation for androstenone between the first recording at 60 kg and at 110 and 120 kg of 0.78 and 0.72, respectively, [4], which is higher than the correlation found in this trial.

Before the second vaccination, results showed no significant difference in androstenone and skatole between male pigs and immunocastrates. At slaughter, immunocastrates had significantly lower

skatole and androstenone levels than male pigs regardless of the androstenone levels of the AI boars. This confirms the assumption that the first vaccination functions as a primer and that active immunisation is not obtained until the second vaccination. This is also confirmed by the GnHR values that reveal some degree of immune response before the second vaccination and a significantly greater response at slaughter. Immunisation inhibits testosterone production and thereby testicular function, which inhibits the production of androstenone. This also lowers the indirect deposit of skatole in fat tissue through increased capacity for skatole decomposition in the liver. Immunocastration was generally efficient in reducing skatole and androstenone levels, but not to the low levels obtained in castrates, which was also the outcome in previous trials [13],[14]. Results revealed androstenone levels higher than 1.00 and 2.00 ppm in immunocastrates.

Androstenone levels in male pigs averaged 2.6 ppm (high-androstenone AI boars) and 1.7 ppm (lowandrostenone AI boars), respectively. German studies found an average androstenone level of 0.41 ppm in male pigs. For immunocastrates, androstenone levels found in this trial (0.11 ppm) corresponded with the levels found in the German studies (0.12 ppm). However, the German studies reported significantly higher skatole levels in male pigs (0.13) and immunocastrates (0.07) compared with this trial where skatole levels in male pigs were 0.07-0.09 (boars low/high) and in immunocastrates 0.04-0.06 (boars low/high). This corresponds to the differences between DLY and PLY hybrids found in a Danish study [3].

The difference in androstenone levels between offspring of AI boars with high and low androstenone levels was 0.87, which corresponds to previous findings of differences ranging from 0.41 to 1.1 ppm [2] [9]. No trials have previously identified a significant difference in skatole levels between offspring of high-androstenone AI boars and low-androstenone AI boars like the difference found in this trial where skatole was reduced by 20% (0.02 ppm) by using low-androstenone AI boars to sire DLY offspring.

## Testosterone and GnRH

Plasma samples taken from male pigs and immunocastrates at transfer (approx. 12 weeks of age), before the second vaccination (approx. 16 weeks of age) and before slaughter (approx. 21 weeks of age) were analysed for GnHR inhibitors and testosterone levels. Analyses showed no differences at transfer; before the second vaccination, however, no differences in testosterone were observed, but immunocastrates did show increasing GnHR immune response which indicates incipient immune response. At slaughter immunocastrates had a significantly higher immune response and lower testosterone levels, as expected, compared with male pigs. This corresponds with findings in other trials [13][15]. A German study identified significantly higher testosterone levels (39 ng/ml) [13] than in this trial where average testosterone levels were approx. one tenth lower (3.8 ng/ml). This corresponds with results of another German study [15].

One fifth of all immunocastrates displayed an unsatisfactory immune response (GnHR inhibitor) to the vaccine as values were below 35% [13]. Immunocastrates with low immune responses had high testosterone levels in blood. It is unclear why such a large percentage of the immunocastrates did not respond optimally to the vaccination. However, other studies have also found suboptimal response to the vaccine among immunocastrates [18] [19]. Inadequate immune response is generally attributed to stress, disease or inaccurate handling of the vaccine. However, no pigs in this trial showed any sign of stress or disease during the vaccination which was administered in controlled environments.

Among male pigs, results showed high testosterone levels in offspring of high-androstenone AI boars, which was not previously found. This corresponds with the correlations observed between testosterone levels and androstenone levels [4].

## Productivity

#### Daily gain

Results showed significant effect of gender on daily gain. From transfer to the second vaccination, castrates had a significantly higher daily gain than the immunocastrates, with male pigs in between. From the second vaccination until slaughter, male pigs had a significantly higher daily gain than the castrates and immunocastrates. In the entire period, male pigs had a numerically higher daily gain than the castrates and a significantly higher gain than the immunocastrates. Previous studies also found a high daily gain at the beginning of the growth period and the lowest in the end in castrates, and these studies also found a higher daily gain after the second vaccination in immunocastrates compared with castrates and male pigs [20] [21]. The lower daily gain in male pigs may be attributed to the protein content of the feed that was inadequate for the pigs to reach maximum productivity [20]. All three genders in this study were fed the same diet, with identical energy and protein content above the standard, specifically aimed at benefitting the growth potential of male pigs [17].

#### Feed intake

From transfer to the second vaccination, castrates had a significantly higher feed intake compared with immunocastrates and male pigs, which also explains the high daily gain. From the second vaccination, immunocastrates' feed intake increased – and was significantly higher than that of male pigs, but still significantly lower than castrates, which corresponds with previous findings [21]. This increase for the immunocastrates was attributed to GnHR inhibition. There are indications that decreasing testosterone levels positively affected appetite and reduced aggressive behaviour. Further research is required to determine whether and why testosterone affects appetite.

#### Feed conversion ratio

Not surprisingly, castrates had a poorer feed conversion over the entire period compared with the male pigs, with immunocastrates in-between. From transfer to the second vaccination, male pigs and immunocastrates had a significantly better feed conversion than castrates. After the second vaccination, when active immunisation sets in, feed conversion of the immunocastrates deteriorated and was ranked overall between castrates and male pigs. This is explained by the fact that the immunocastrates' feed intake increased after the second vaccination without their daily gain correspondingly exceeding that of the male pigs [21].

The improved feed conversion of male pigs is attributed to multiple factors, such as gender hormones that accelerate the excretion of growth hormone (GH). GH is excreted from the hypophysis through IGF-1 stimuli from the liver that is triggered by insuline and gender hormones: the higher the gender hormone level, the better the feed conversion. Consequently, a reduction in gender hormones following immunocastration will lead to a lower GH and poorer feed conversion. Previous studies found castrates to have the lowest IGF-1 measured in plasma the day before slaughter compared with male pigs and immunocastrates in-between [22].

#### Lean meat %

Castrates had the lowest lean meat percentage and male pigs the highest, with immunocastrates placed in-between. Male pigs had a lean meat percentage of 63.5, which was 3.4 percentage points higher than in castrates. This difference is approx. 1 percentage point higher than found in previous trials [10], and is likely attributed to a high protein (and energy) content of the diet which has allowed the male pigs to exploit their full potential for lean meat growth.

#### Dressing loss

As testicles are removed from male pigs and immunocastrates, dressing loss is 1.7% higher and the slaughter factor is higher than that of castrates. Results showed no differences between male pigs and

immunocastrates in dressing loss. The increased dressing loss must be taken into account when selecting pigs for slaughter.

# Conclusion

Male pigs had the highest daily gain, lean meat percentage and the best feed conversion; castrates had the lowest gain, lean meat percentage and poorest feed conversion. Immunocastrates ranked between male pigs and castrates on all three parameters.

Assessing AI boars according to androstenone levels (high/low) generated a difference in androstenone of 50% at approx. 70 kg (offspring of high-AI boars: 0.63 ppm; offspring of low-AI boars: 0.31 ppm androstenone). At slaughter, the difference had dropped to 34% between offspring of high-AI boars (2.61 ppm) and of low-AI boars (1.70 ppm) androstenone. Immunocastration reduced androstenone and skatole levels to very low levels (0.13 ppm androstenone and 0.06 ppm skatole). Immunocastration did not eliminate boar taint in all vaccinated pigs, as analyses revealed androstenone levels above 2.0 ppm in 4% of the pigs at slaughter.

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Trial. 1553 NAV nr.: 150-1202

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# Appendix 1

	Unit	Calculated nutrient content	Analysis result, avg.
FUgp*/100 kg	FUgp/100 kg ingredient	110	113
Crude protein	% of ingredient	16.66	16.56
Fat	% of ingredient	3.64	3.82
Ash	% of ingredient	5.31	4.53
Water	% of ingredient	13.4	12.2
Calcium	g/kg ingredient	7.7	7.3
Phosphorus	g/kg	4.9	4.9
Lysine	g/kg	11.0	10.5
Methionine	g/kg	3.6	3.4
Cysteine + Cystine	g/kg		2.8
Phytase activity	FTU/Kg	1,000	1,405

#### Analysed nutrient content (average of four trial rounds)

Calculated nutrient content compared with standard

	Guarantees avg. 4 rounds	Standard 30-110 (2018)	Compared with standard
FUgp*/kg	1.10	-	
Crude protein g dig./FUgp	130	120	108%
Fat, %	3.51	-	
Ash, %	5.02	-	
Calcium	7.00	6.20	113%
Phosphorus dig. 200% phytase	2.74	2.50	110%
Lysine std. dig./FUgp	9.23	7.70	120%
Methionine std. dig./FUgp	2.95	2.30	128%
Meth + cystine std. dig./FU	5.20	4.50	116%
Threonine std. dig./FUgp	5.90	5.10	116%
Tryptophan e. BCR std. dig./FUgp	1.80	1.54	117%
Valine std. dig./FUgp	5.80	5.20	112%

\*) Energy content per kg diet for finishers, 30-100 kg 1.07 FUgp = 13.4 MJ ME = 9.6 MJ NE = 7.9 MJ physiological energy.

# Appendix 2

Daily gain, feed intake, feed conversion ratio, slaughter weight and lean meat % (LS-means) Basis slaughter weight \* 1.31

Androstenone AI boars		High		Low			SEM	Significance		
Gender	Castrate	Intact	Immuno- castrate	Castrate	Intact	Immuno- castrate		Boars* Gender	Boars	Gender
Number	127	112	117	106	91	86				
Period 1 Daily gain, g	1,130	1,110	1,080	1,140	1,100	1,110	0.02	0.53	0.9	0.47
Period 2 Daily gain, g	1,250	1,300	1,260	1,210	1,300	1,240	0.02	0.36	<0.01	0.22
Total period Daily gain, g	1,200	1,210	1,180	1,190	1,210	1,190	0.01	0.39	0.008	0.83
Period 1 Feed intake, FUgp/day	2.67	2.30	2.30	2.69	2.36	2.36	0.04	ns	ns	p<0.01
Period 2 Feed intake, FUgp/day	3.98	3.50	3.78	3.79	3.51	3.77	0.04	ns	ns	p<0.01
Total period Feed intake, FUgp/day	3.27	2.85	3.00	3.23	2.88	3.01	0.03	ns	ns	p<0.01
Period 1 Feed conversion ratio, FUgp/kg	2.07	1.86	1.92	2.07	1.90	1.88	0.03	0.38	<0.0001	0.99
Period 2 Feed conversion ratio, FUgp/kg	2.81	2.14	2.67	2.88	2.39	2.69	0.03	0.63	<0.0001	0.64
Total period Feed conversion ratio, FUgp/kg	2.46	2.14	2.33	2.46	2.15	2.31	0.02	0.52	<0.0001	0.93
Carcass weight, kg	90.0	89.0	88.9	89.1	89.4	88.8	0.5	ns	ns	ns
Slaughter factor	1.33	1.35	1.36	1.34	1.36	1.36		ns	p<0.01	ns
Lean meat %	59.7	63.2	62.0	60.5	63.7	63.1	0.3	0.31	=0.01	<0.01

Period 1: Transfer to before 2<sup>nd</sup> vaccination.

Period 2: Before 2<sup>nd</sup> vaccination to slaughter.

Total period: From transfer to slaughter.



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