POTENTIAL STRATEGIES TO REDUCE BOAR TAINT

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WHY CASTRATE?

Young male piglets are castrated to prevent off-odours and off-flavours (boar taint) in the meat at slaughter weight. Castration prevents boar taint, but intact males (boars) have improved feed efficiency, nitrogen retention and lean gain compared to castrates, which could result in significant economic gains to producers. The advantages of boars over castrates can be summarised as follows (EFSA, 2004):

- Superior growth rate of boars up to 13%
- Intact males may eat up to 9% less feed
- Feed conversion (to live-weight) up to 14% more efficient
- Generally leaner than castrates by up to 20%

The use of intact male pigs for pork production will improve pork production efficiency, but this also brings with it concerns about pork with boar taint and the management of entire male pigs, which exhibit more aggressive and more sexual behaviour than castrates (Lundström *et al*., 2009). A major driving force for using intact males is the growing animal welfare concerns against castration. Several EU countries will ban surgical castration in the next few years (even with anaesthetic) and some major grocery stores in the Netherlands have now decided not to sell pork from castrates. Controlling boar taint without surgical castration would, therefore, have dramatic benefits for production and consumer acceptance of pork products.

WHAT CAUSES BOAR TAINT?

Boar taint is caused by the accumulation of two compounds, androstenone and skatole, in the fat. Androstenone is a steroid produced in the testis as the boar nears puberty, and it acts as a sex pheromone to regulate reproductive development in gilts and induce a mating stance in sows. Skatole is produced as a bacterial breakdown product of the amino acid, tryptophan, in the gut. It is produced in equivalent amounts in the gut of both males and female pigs, but it is poorly metabolized and eliminated by males, so it accumulates in fat.

POTENTIAL STRATEGIES FOR CONTROL OF TAINT

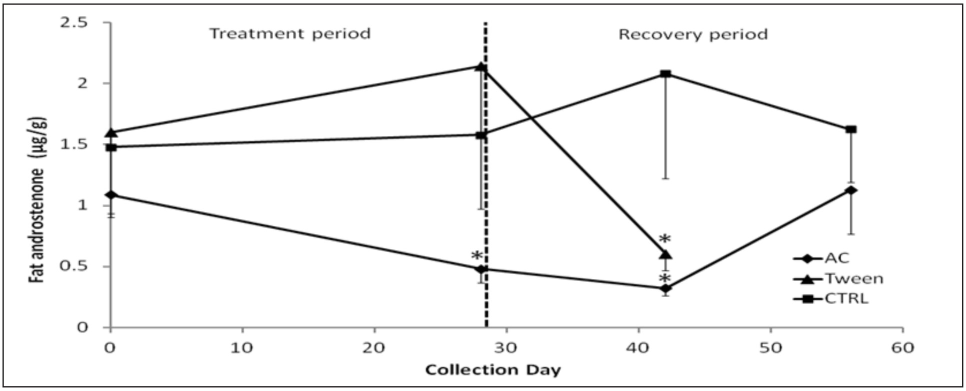
There are several promising alternatives to castration for dealing with boar taint. The amount of boar taint can be reduced by using a number of dietary additives and with good management practises. Boar taint can also be eliminated using an immunocastration vaccine (Dunshea *et al.*, 2001; Rikard-Bell *et al.*, 2009; Pauly *et al.*, 2009) and genetic markers are being developed to select pigs that have reduced levels of boar taint (Squires and Schenkel, 2008; Zamaratskaia and Squires, 2009).

NUTRITIONAL MANIPULATIONS TO REDUCE TAINT

Boar taint from skatole is affected by diet and environment (management) as well as the composition of the gut microflora. One of the main sources of tryptophan for skatole production comes from the turnover of cells lining the gut. Skatole levels can be reduced by including sources of fermentable carbohydrates (chicory inulin, raw potato starch or high amylase barley) in the diet and by using various antibiotics to alter the gut microflora. Skatole can also be absorbed from the manure, so dirty pigs of any sex can have high skatole levels in fat.

Androstenone production is controlled by the sexual maturity of the boar, so diet does not have much of an effect on the production of androstenone. Androstenone levels could be decreased by slaughter of the pigs at lighter weights before androstenone synthesis increases at puberty, but this is not economical. We have recently discovered that including binding agents (activated carbon or Tween) as dietary additives can reduce the accumulation of androstenone in fat (Fig1). However, this technology has not yet been tested under commercial production conditions.

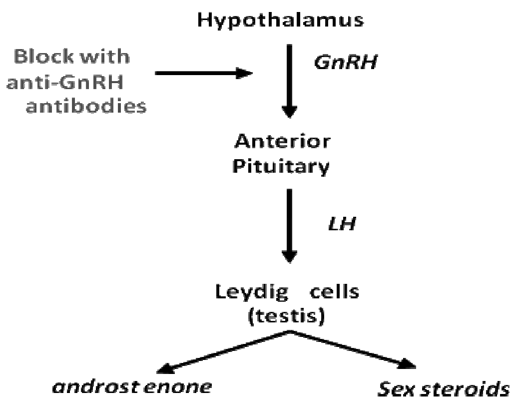
Figure 1. Profile of androstenone concentrations in fat of boars treated with activated carbon (AC,◆; n=13), Tween (▲ ; n=13) and boars fed a control diet (CTRL, ■; n=11). Values are plotted as means ± SE, * indicates values significantly different (P<0.05) from values at day 0.



IMMUNOCASTRATION

A promising method for controlling boar taint is by immunocastration, instead of surgical castration. Immunocastration works by injecting a vaccine (Improvest® or Improvac® from Pfizer Animal Health), which stimulates the production of antibodies against gonadotropin releasing hormone (GnRH). GnRH is produced by the hypothalamus in the brain to drive the release of luteinizing hormone (LH) by the pituitary gland, which stimulates the development of the testis. The antibodies inactivate GnRH to shut down testicular development to the same extent as surgical castration (Fig 2) and eliminate the production of androstenone and the sex steroids. Levels of skatole are also low in immunocastrated pigs, and this is most likely due to enhanced metabolic clearance by the liver after steroid production is suppressed, as occurs in surgically castrated pigs. Immunocastration

Figure 2. Regulation of testis function by GnRH and LH



also reduces the aggressive and sexual behaviour of boars.
To immunize pigs, two doses of Improvac (2 ml of 200 ug/ml GnRH conjugate) are given at an interval of at least 4 weeks, with the second dose given 4 to 6 weeks before slaughter (Dunshea *et al*., 2001). This allows the pig to grow as a normal, intact male until just before slaughter, when it is immunologically castrated. Immunocastration eliminates the production of the anabolic sex steroids, but since the vaccine is given to pigs near slaughter, they produce sex steroids to grow as normal boars for most of their life and retain the performance advantages of intact boars. The effects of immunocastration on performance from a number of different studies are summarized in Table 1.

Compared with intact males, immunocastrated pigs grow faster, have a similar or slightly decreased feed efficiency and have higher fat content in their carcass (Table 1). This is attributable to increased feed intake, which is primarily due to reduced aggression and mounting behaviours following immunization. However, compared with surgically castrated pigs, immunocastrated pigs may grow faster or

Table 1 Effect of immunocastration (Improvac vaccine) of male pigs on performance, hormone levels and sexual development. Larger scale studies

Results are expressed as % of the control intact males and (% of surgical castrates); - = not determined (modified from Prunier *et al.*, 2006).

#animals per treatment	Growth rate	Feed efficiency Gain/feed)	fat	Reference
100	121 (119)	103 (115)	114 (85)	Dunshea et al., 2001
60	110 (103)	-	-	Cronin et al., 2003
28	109	96	116	Oliver et al., 2003
270	(101)	-	(98)	Jaros et al., 2005
47	113 (115)	95 (105)	103 (98)	Zamaratskaia et al., 2008
23	(99)	(108)	(92)	Hennessey et al., 2006a
24	(110)	(110)	(92)	Hennessey et al., 2006b
48	113	95	115	Rikard-Bell et al., 2009

grow at a similar rate, but consistently have improved feed efficiency and decreased fat content. This is attributable to the positive effects of androgens secreted from the testes prior to immunization.

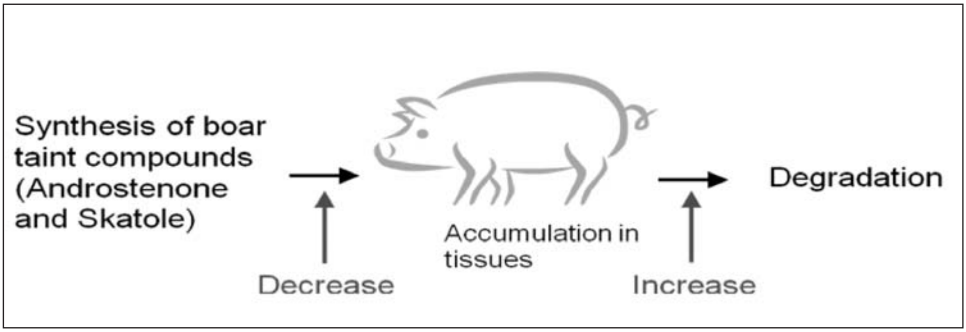
Improvac is presently licensed in 53 countries around the world, the most recent launch being in the European Union, but is not yet available in North America. Studies in many of these countries with male pigs immunized with Improvac have consistently found that immunocastration is an effective method of reducing testicular function and preventing boar taint while preventing some of the lost production efficiency and carcass quality that occurs with surgical castration. However, the main concern about immunocastration is uncertainty about consumer acceptance of immunocastrated pork, and the adoption of Improvac has been slow in several EU countries.

GENETIC SELECTION

The use of genetic markers to produce lines of pigs that are free of boar taint but otherwise grow as normal boars is a long term solution to raising entire male pigs for pork production. Genetics can affect both the production and metabolism of boar taint compounds, and these effects can be found both within breeds and among different breeds. For example, levels of androstenone are much higher in Durocs than in the white pig breeds. There is also a wide variability in the amount of boar taint that individual pigs have within a breed. The heritability of both androstenone and skatole is moderate to high, but previous attempts to select for pigs with low boar taint have resulted in reproductive problems (reviewed in Zamaratskaia and Squires, 2009). The development of specific genetic markers for boar taint would minimize these negative effects on reproduction.

The accumulation of boar taint compounds is controlled by both their rates of synthesis and degradation (Fig 3). A number of studies have shown differences in expression of candidate genes encoding enzymes involved in the metabolism of boar taint compounds, but only a few studies have reported SNPs in these genes that are correlated with levels of boar taint. A recent report from Norway (Moe *et al.*, 2009) compared a large number of single nucleotide polymorphisms (SNPs) to boar taint in Duroc and Norwegian Landrace breeds. They found significant marker effects for fat androstenone in Duroc, but not in Landrace, and significant marker effects for fat skatole in both breeds. Individual markers explained from 2.5-16.3% of the total variation in the traits.

Figure 3. Strategy for development of genetic markers



We have developed genetic markers for low boar taint based on SNPs in candidate genes that were selected based on functional studies carried out at University of Guelph over the past 20 years, which characterized the metabolites and enzymes of the metabolic pathways involved in the secretion and degradation of the boar taint compounds (Squires and Schenkel 2008).

We have a database of about 1300 animals representing 8 different lines, comprising 6 breeds (Duroc, Hampshire, Landrace, Large-white, Pietrain and Yorkshire, n=76 to 219), that we have used for the discovery and validation of SNP markers in the DNA. For SNP discovery, we compared the sequences of candidate genes from pools of DNA obtained from animals from the extremes of the boar taint phenotypes in each line. We then genotyped all the animals in our database for each SNP and conducted association analysis for each SNP with the amount of boar taint from androstenone and skatole in the carcass. The ultimate goal is to identify the causative mutations in the handful of most important genes, and then use these markers in breeding programs to develop lines of pigs that are free of boar taint but otherwise grow as normal boars.

So far, we have about 80 effective SNPs in 28 candidate genes for boar taint. The number of significant SNPs across lines varied from 5 to 17 and from 3 to 16 for skatole and androstenone, respectively (Table 2 and 3). In addition, 65% of the 80

effective SNPs were associated with both skatole and androstenone, which corroborates with the reported moderate positive genetic correlation between these two boar taint compounds (e.g., Tajet *et al*. 2006). Application of the markers to produce pigs homozygous for the favourable alleles would decrease fat skatole levels by 20-53% and fat androstenone by 26-61%, depending on the line.
We also determined that none of these markers were associated with negative effects on production traits (Back fat, Loin Depth, Front leg score, Rear leg score, Subjective Live muscle/conformation score, Daily Gain).

Table 2. Summary of Marker Effects for Skatole

Breed	# of Effective markers	R^2	Favourable Allele Frequency	Current geometric mean	Mean with favourable allele	% Change
Duroc	13	0.59	.05 - .95	40.9	24.0	-41.2
Hampshire	17	0.42	.28 -.85	97.5	54.4	-44.2
LW-Duroc	5	0.09	.06 - .87	96.5	77.6	-19.6
Landrace	13	0.42	.02 - .76	59.2	35.0	-40.9
Large White	10	0.18	.15 -.74	72.2	48.2	-33.3
Pietrain	12	0.45	.10 - .88	63.4	29.6	-53.3
Sire Line	9	0.33	.03 - .92	42.1	22.2	-47.2
Yorkshire	10	0.19	.30 - .91	24.8	15.9	-35.7

Table 3. Summary of Marker Effects for Androstenone

Breed	# of Effective markers	R^2	Favourable Allele Frequency	Current geometric mean	Mean with favourable allele	% Change
Duroc	11	0.41	.03 - .96	1.38	0.67	-51.6
Hampshire	3	0.13	.21 - .81	0.79	0.31	-60.9
LW-Duroc	16	0.35	.06 - .78	2.14	1.23	-42.7
Landrace	14	0.32	.05 - .79	0.52	0.29	-44.1
Large White	5	0.08	.07 - .96	0.55	0.38	-31.3
Pietrain	12	0.51	.10 - .94	0.35	0.18	-47.3
Sire Line	10	0.27	.09 - .74	0.88	0.54	-38.6
Yorkshire	7	0.16	.17 - .92	0.55	0.41	-26.0

We next validated our 80 SNP marker set in 6 unrelated lines of pigs from JSR Genetics. The number of significant SNPs across lines varied from 5-13 and from 2-10 for skatole and androstenone, respectively (Table 4), with 12 SNPs associated with both skatole and androstenone. Across all lines, the SNPs explained an average of 51% (range 33% to 73% based on R^2 values) of the skatole variance and an average of 50% (range 24% to 74%) of the androstenone variance. This confirmed that our markers are effective in unrelated lines of pigs and demonstrated their potential for wide applicability in pig breeding. These findings represent significant progress towards a genetic solution for boar taint. Work is continuing to characterize additional SNPs for boar taint.

Table 4. Summary of significant markers for fat skatole and androstenone in JSR lines

	Line	n	Number of significant markers	R^2	% above the cutoff threshold[1]
Skatole	1	49	9	0.51	20.0
	2	51	5	0.33	7.7
	3	55	13	0.65	3.5
	4	32	12	0.73	2.6
	5	41	5	0.35	6.5
	6	42	5	0.52	0
	Overall	***45***	***24***	***0.51***	***7.5***
Androstenone	1	49	10	0.67	32.0
	2	51	3	0.23	100.0
	3	55	6	0.49	29.8
	4	33	7	0.74	13.2
	5	41	2	0.24	29.8
	6	42	10	0.61	4.5
	Overall	***45***	***27***	***0.50***	***37.5***

[1] % of individuals above the cutoff threshold for detectable presence of boar taint for skatole (200 ng/g) and androstenone (1.0 ug/g).

We are now working with Ontario Swine Improvement, the Canadian Centre for Swine Improvement and other provincial swine improvement organizations to validate these markers in commercial swine breeds in Canada. The specific objectives of the project are to:

- Investigate the association between genetic markers and boar taint levels in major Canadian swine breeds;
- Study the relationship between economically important performance traits and genetic markers for boar taint;
- Develop methods for the potential selection against boar taint using genetic markers;
- Provide recommendations and guidelines to breeders interested in selecting against boar taint; and
- Disseminate results to the larger industry.

The control of boar taint by marker-assisted selection will eliminate the need for castration. This will significantly improve the profitability of pork production and address animal welfare concerns about castration that are now a hot topic in several EU countries.

CONCLUSIONS

Castration to prevent boar taint limits productivity and increases animal welfare concerns of commercial pork production, so alternative strategies for controlling taint are needed. Immunocastration effectively controls boar taint, but the development of low boar taint lines of pigs by marker assisted selection would provide a long term solution to the problem. Various dietary manipulations can also be used to decrease taint, so that genetic selection might not need to completely eliminate taint. Eliminating the need for castration of male piglets will improve pork quality and consistency, profitability, environmental impact and animal welfare of pork production. In terms of production efficiencies it is anticipated that the use of entire male pigs will improve profits per pig by more than $5, which is based on analyses that were conducted previously by de Lange and Squires (1995) and adjusted to 2010 economic conditions. Intact males also produce less manure and thus excrete less nitrogen and phosphorous in the manure than castrates, thereby decreasing the environmental impact of pig production.

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