Immunization of pigs against gonadotrophin releasing factor (GnRF) prevents boar taint and affects boar growth and behaviour

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Summary

Peri– and post–pubertal boars accumulate substances in their fatty tissue, predominantly androstenone and skatole, that are responsible for boar taint in pork. One method of inhibiting sexual development, reducing plasma gonadotrophin and testosterone and reducing the accumulation of these substances in carcass fat, is immunization against gonadotrophin releasing factor (GnRF). Recently, a vaccine (Improvac®) containing a modified form of GnRF in a low reactogenic adjuvant system has been marketed as a highly efficacious means of controlling boar taint. The vaccine decreases gonadal steroids and appears to have some additional effects on sexual, aggressive and feeding activities with resultant improvements in growth performance. It increases feed intake and growth rate of both finisher boars and gilts. While one of the consequences of increased feed intake is an increase in fat deposition, simultaneous treatment of pigs with porcine somatotrophin ameliorates this effect. Indeed, there appear to be synergies between the two technologies. Improvac or related vaccines may also be of use in other animal industries where sexual and aggressive behaviours and/or the presence of taints associated with gonadal steroids limit productive performance or product quality.

Keywords: pigs, boar taint, GnRF, immunocastration, porcine somatotrophin, growth

Introduction

During sexual development and when mature, male pigs accumulate substances in their fatty tissue, predominantly androstenone and skatole, that are regarded as the main contributors to boar taint in pork (Bonneau 1982). To avoid tainting of the meat, entire male pigs destined for fresh meat consumption in Australia and New Zealand have, until recent years, been slaughtered before sexual maturity. In other countries, including most of Asia, taint is overcome by castration of the male pig before weaning. However, castration results in significant reductions in growth performance and an excessive deposition of fat (Campbell and Taverner 1988; Dunshea *et al*. 1993a).

In recent years the average weight of pigs at slaughter in Australia has increased and continues to do so (PigStats 1998), a trend being driven by the production and processing efficiencies associated with the slaughter of heavier pigs. Pigs also appear to be maturing more rapidly and becoming sexually mature at an earlier age, perhaps due to the selection for faster growth. Since boar taint increases with sexual maturity and liveweight, the increase in slaughter weight has been associated with an increase in the risk of occurrence of the taint. One method of inhibiting sexual development, reducing plasma gonadotrophin and testosterone, and reducing the accumulation of androstenone in carcass fat, is immunization against gonadotrophin releasing factor (GnRF) (Carraty and Bonneau 1986; Dunshea *et al*. 1993b,c; Bonneau *et al*. 1994). However, most of the vaccine regimens reported to date have been inappropriate since they have required many injections, or the tissue reactions which occurred after injection of adjuvants required a long vaccination to slaughter interval, thereby negating the beneficial effects of testosterone on growth performance. It is well established that vaccines which contain mineral oil as an immuno–stimulant can cause substantial tissue damage around the site of injection (Hall *et al.* 1989; Straw *et al.* 1985, 1990).

Bonneau *et al*. (1994) reported a vaccine formulation and protocol which involved the injection of anti–GnRH/α–globulin formulation in mineral oil at 29 kg liveweight followed by the injection of an aqueous solution of the conjugate at 89 kg liveweight. While these authors reported that testes size, plasma testosterone and fat androstenone were reduced, there was a highly variable antibody titre response in the vaccinated boars and, although fat skatole concentrations were already very low, there was also no further decrease in the vaccinated pigs.

Recently, a vaccine (Improvac®) containing a modified form of GnRF in a low reactogenic adjuvant system has been developed to reduce the production and accumulation of both androstenone and skatole in the pig carcass (Hennessy *et al*. 2000; Dunshea *et al*. 2001). The vaccine formulation and protocol allow the pigs to receive the secondary immunization relatively close to slaughter. Any taint substances already present are progressively metabolised, allowing boars to be slaughtered at higher live–weights without taint, and with earlier benefit from the effects of their own testicular steroids on growth and carcass composition (Hennessy *et al*. 1997,2000; Dunshea *et al*. 2001). The decrease in testosterone appears to also have some additional effects on sexual, aggressive and feeding activities with resultant positive effects on growth performance. This paper will focus on some aspects of these improvements in growth performance.

The vaccine

The anti–GnRF vaccine used in the studies discussed here was manufactured and formulated by CSL Ltd, Melbourne, Australia, and is marketed as Improvac®. The proprietary adjuvant system that has been developed for the vaccine does not contain oil and causes very little irritation to pig tissues. While site reactions may not be a great problem in pigs slaughtered many months after vaccination (Oishi *et al.* 1997), it is an important consideration when pigs are being slaughtered a short time afterwards. The protocol for Improvac is to leave primary vaccination as late as possible (8–9 weeks before slaughter) and give the secondary vaccination 4 weeks later. This leaves only 4–5 weeks for the resolution of any reactions at the sites of injections, but does maximise the opportunity for benefits from boar growth characteristics.

Effect of the vaccine on testes function and boar taint

In a study involving 200 boars, the primary dose of the vaccine (2 ml) or placebo was injected subcutaneously at either 15 or 18 weeks of age and the secondary, booster, dose was administered 4 weeks later. The pigs were slaughtered 4 weeks after the booster dose. The subcutaneous administration was generally well tolerated with the majority of boars showing no reaction to either vaccine or placebo. Measurements of growth performance, plasma metabolites and behaviour were made over the final 4 to 5 weeks prior to slaughter.

At the time of the second immunization, though the boars were 19 or 21 weeks old, the serum testosterone concentrations were >2 nM in 85% of the pigs whether they had been treated 4 weeks earlier with Improvac (immunocastrates) or with placebo (Table 1; Dunshea *et al.* 2001). Even in some individual pigs as light as 55 kg the testosterone values indicated active steroidogenesis. A concentration >2 nM is considered to be biologically significant. Pigs with the capacity to produce high concentrations of testosterone also have the potential to produce androstenone and hence to have detectable levels of taint in the carcass. Within two weeks of the second dose of Improvac there was a highly significant reduction in testosterone ($P<0.001$) in the treated boars such that only 6% of animals had concentrations above 2 nM. This effect was maintained until at least four weeks after the second vaccination when only 8% of the immunocastrates had concentrations above 2 nM (Table 1). The suppression in testicular function was also evident in the 50% reduction in testes weight in the immunocastrates.

Placebo–treated boars had concentrations of androstenone in their fat that were almost eight times higher ($P<0.001$) than those vaccinated (Table 1) which had concentrations not significantly different from those in barrows (data not shown). When pooled across age groups, 24% of control boars had fat androstenone concentrations between 0.5 and 1.0 µg/g, and in a further 49% they were greater than 1.0 µg/g. In contrast, only 3% of the Improvac treated boars had concentrations of between 0.5 and 1.0 µg/g; in the remainder they were well below 0.5 µg/g, most being below the detection limit. All barrows had <0.5 µg/g. Xue and Dial (1997) and the European Union (Bonneau and Cook 1997)

Table 1 Effect of age at vaccination with Improvac® on plasma testosterone in boars, and testes weight, fat androstenone and fat skatole at slaughter (Dunshea *et al.* 2001).

	Early[a]			Late[b]		
	Boar[c]	Improvac	*P* value	Boar[c]	Improvac	*P* value
Plasma testosterone (nM)						
Secondary dose	13.7	12.7	NS	6.61	8.27	NS
Secondary dose + 2 weeks	8.52	0.51	<0.001	7.03	0.54	<0.001
Secondary dose + 4 weeks	10.5	1.16	<0.001	8.26	0.62	<0.001
Testes weight (g)	421.6	182.6	<0.001	509.6	254.4	<0.001
Fat androstenone (µg/g)	1.21	0.160	<0.001	1.05	0.126	<0.001
Fat skatole (µg/g)	0.133	0.068	<0.001	0.095	0.056	<0.001

[a] Primary and secondary vaccinations at 15 and 19 weeks of age; slaughtered at 23 weeks of age
[b] Primary and secondary vaccinations at 18 and 22 weeks of age; slaughtered at 26 weeks of age
[c] Not vaccinated

suggest that 1.0 µg/g is the threshold concentration at which androstenone becomes detectable as taint.

Skatole concentrations in the fat of boars treated with placebo were almost twice as high (P<0.001) as in the immunocastrates (Table 1) which, in turn, were not significantly different from barrows. None of the boars treated with Improvac, and none of the barrows, had concentrations that exceeded 0.25 µg/g, and most values were much lower. Xue and Dial (1997) suggest 0.25 µg/g and the European Union (Bonneau and Cook 1997) suggest 0.22 µg/g as the threshold concentration above which skatole becomes detectable as taint. When pooled across the age groups, 11% of the control boars had concentrations above those thresholds.

With the suggested threshold values as criteria, Improvac was 99 and 100% effective in suppressing skatole and androstenone, respectively.

Growth responses

It is generally accepted that barrows exhibit inferior feed conversion efficiency and are fatter than boars (Campbell and Taverner 1988; Dunshea *et al.* 1993a) and it might be anticipated that GnRF vaccinated boars would also perform more poorly than entire boars. However, the growth rates of boars treated with Improvac is greater than that of boars treated with placebo while feed conversion efficiencies are generally unchanged. For example, in the study outlined above the younger and older treated boars grew 10% and 30% faster, respectively, than the control boars over the four weeks following the second dose of the vaccine (Table 2). The increased growth rate appears to be due to an increase in feed intake rather than to any change in feed conversion ratio. For example, the feed intake of the immunocastrates was approximately 15% higher than that of the controls (Table 2) and for the older pigs, feed intakes of barrows were 10% greater and of treated boars 16% greater than the control boars (P=0.097). In both age groups the FCR of control and treated boars were similar. However, there was an increase in P2 backfat in the older boars treated with Improvac.

The better growth performance following the secondary vaccination may be related to a decrease in aggressive and sexual behaviours as a consequence of suppression of testicular function. At around 19–22 weeks of age, when the secondary vaccination was given, pigs are well advanced in puberty and normally display an increasing amount of sexual behaviours and related aggression. It is well established that castrated pigs consume more feed than entire males (Campbell and Taverner 1988; Dunshea *et al*. 1993a) and that this is probably related to the low concentrations of testosterone in the barrows. The wild boar, which exhibits wide seasonal fluctuations in testosterone, stops eating when testosterone concentrations are at their peak (Weiler *et al*. 1996). In the domestic boar, which exhibits less marked seasonal variation in both plasma testosterone feed intake, there is still a strong negative correlation (P<0.001) between testosterone and feed intake (Weiler *et al*. 1996). Therefore, the reduction in testosterone may account for the increased feed intake that occurs after immunization against GnRH, but whether the reduced intake is a direct effect of testosterone on satiety is unknown. It may be that the increase in feed intake occurs because the vaccinated boars are no longer involved in sexual or aggressive activities that detract from time spent eating. It is also possible that the lower level of aggression and sexual behaviour allows energy to be directed towards carcass growth rather than into non–productive activities. While there is little change in feed conversion efficiency, it may be that the energy saved by not fighting is negated by the small inefficiency associated with the slight increase in fat deposition in Improvac treated boars. Bulls vaccinated with a similar GnRH vaccine displayed less sexual activity and lower aggression compared with control bulls when observed at pasture (Finnerty *et al*. 1996; Jago *et al.* 1997). Thus, it seems reasonable to conclude that the improved growth performance following vaccination is, at least in part, a result of reduced sexual and aggressive activities over the last weeks preceding slaughter.

Recent on–farm observations have suggested that entire male pigs do not grow as well in groups as would be anticipated from performance data generated in individual pens. It is possible that the reduced performance under group housing conditions may be due to sexual and aggressive activities. Since Improvac

Table 2 Effect of age at vaccination with Improvac® on growth performance of boars (Dunshea *et al.* 2000, 2001).

	Early[a]			Late[b]		
	Boar[c]	**Improvac**	***P* value**	**Boar[c]**	**Improvac**	***P* value**
Daily gain (g)	786	868	0.051	858	1119	<0.001
Feed intake (g/d)	2.44	2.81	<0.01	2.79	3.40	0.097
Feed conversion ratio	3.03	3.05	NS	3.30	3.10	NS
P2 back fat (mm)	11.1	11.9	NS	12.6	15.1	<0.01

[a] Primary and secondary vaccinations at 15 and 19 weeks of age; slaughtered at 23 weeks of age
[b] Primary and secondary vaccinations at 18 and 22 weeks of age; slaughtered at 26 weeks of age
[c] Not vaccinated

can reduce this 'maleness', McCauley *et al.* (2000a) conducted a study to determine whether it could be used to reduce such activities and minimise their effects upon growth performance of boars housed under commercial conditions. A total of 120 entire boars, 120 immunocastrates, and 60 barrows were used to study the interactions between sex and housing (group vs individual pens). There were 4 pens of entire boars, of Improvac–treated boars, and of barrows, with 15 animals per pen, and 4 blocks of individually housed entire boars and of immunocastrates with 15 animals of each type per block. Improvac (2 ml) was administered at 14 and 18 weeks of age and pigs were fed *ad libitum*; studies of behaviour, including video recordings, were made between 18 and 23 weeks.

Group–housed pigs grew more slowly (–170 g/d), ate less (–370 g/d), and had a slightly poorer feed:gain (0.10 g/g) than individually–housed pigs (Table 3). The immunocastrates ate more (550 g/d) and grew faster (150 g/d) than entire boars. With the exception of the period between 18 and 20 weeks of age (the two weeks after the second vaccination) there were no interactions between sex and housing. In the two weeks after the final vaccination, Improvac increased growth rate in the group housed pigs but not in the individually housed pigs. However, from 20 weeks of age Improvac increased feed intake and growth rate to a similar extent regardless of housing, but there was no effect on FCR; backfat at the P2 site was increased (+2 mm). Barrows grew at a similar rate to entire males but ate more and had a higher feed:gain than boars, particularly over the latter stages of the study. Surgical castrates were also fatter than boars at the P2 site (+4 mm).

Video recordings taken at 21 weeks of age showed that, within the group–housed pigs, the entire boars exhibited the greatest number of bouts of agonistic (aggressive) behaviour, 29.5 per pig per day compared with 9.5 for both immunocastrates and barrows (SED ±4.66). Vaccination also decreased sexual activity (mounting), and the amount of time spent eating was increased (G. Cronin, unpublished data). It appears that at least part of the improved growth performance observed in treated boars that are housed in groups is associated with decreased sexual and aggressive behaviours.

Synergistic effects of immunocastration and porcine somatotrophin

In an attempt to reduce the effect on backfat of the increase in feed intake after immunocastration, a study was made with 48 finisher pigs fed *ad libitum* in individual pens of the interactions between Improvac, porcine somatotrophin (Reporcin®, pST), and sex (McCauley *et al.* 2000b). Porcine somatotrophin, in contrast to Improvac, generally reduces P2 backfat and feed intake.

Improvac was administered at 14 and 18 weeks of age, and pST (5 mg/d) was administered daily from 18 weeks of age. Pigs were weighed weekly and feed disappearance was measured from 18 weeks of age until slaughter at 23 weeks of age. The effect of pST on daily gain and feed intake each week was similar over the experiment, whereas Improvac was most effective in the latter part of the study. Over the entire treatment period, boars grew more quickly than gilts and pigs treated with pST grew faster than their control counterparts (Table 4). Improvac alone had little effect on daily gain in individually penned pigs but there appeared to be a synergy with pST in both sexes as indicated by the interaction between the treatments. Pigs treated with both Improvac and pST grew 210 g/d faster than control pigs which is much more than would be predicted from responses to pST (+60 g/d) or Improvac (–40 g/d) alone.

Improvac successfully negated in boars, but not in gilts, the reduction in feed intake induced by pST. Backfat was decreased by pST but not altered by Improvac nor different between sexes (Table 4). Thus, pST and the vaccine could be used in conjunction to negate the increase in backfat thickness. The effect of Improvac in gilts is probably due to inhibition of ovarian activity since GnRF is involved in the secretion of

Table 3 Effect of sex, Improvac® vaccine and housing on growth performance of finisher pigs (McCauley *et al.* 2000a)[a].

	Group pen			Individual pen			
	Boar[b]	**Improvac**	**Barrow**	**Boar[b]**	**Improvac**	**SED[d]**	***P* value[c]**
Daily gain (g/d)	908	1079	944	1098	1225	37.4	<0.001
Feed intake (kg/d)	2518	3050	2871	2881	3463	118.9	<0.001
Feed conversion ratio	2.80	2.88	3.05	2.64	2.83	0.256	0.047
P2 back fat (mm)	13.5	15.3	17.4	13.5	16.0	1.64	<0.001

[a] Primary and secondary vaccinations at 14 and 18 weeks of age; slaughtered at 23 weeks of age
[b] Not vaccinated
[c] The main effect of H was deliberately confounded with block effects in the design and thus cannot be legitimately reported
[d] *P* value and standard error of the differences relate only to comparing sex (including Improvac as a separate sex) within housing

pituitary LH and FSH which are essential for ovarian function. Cycling gilts exhibit oestrus activity and reduce their feed intake around the time of ovulation and it is likely that Improvac treatment minimises the impact of oestrus on growth performance of gilts.

The effect of immunocastration on growth rate and feed intake appears to be most pronounced in group–housed boars and Oliver *et al.* (2001) studied interactions between Improvac administered at 13 and 17 weeks of age, pST (5 mg/d) administered daily from 17 weeks of age, and sex in 224 finisher pigs (112 boars, 112 gilts) housed in 32 pens of 7 pigs. Pigs were weighed and group feed disappearance was measured from 17 weeks of age until slaughter at 21 weeks. Growth rate in both sexes, compared with untreated pigs, was increased by Improvac (means 1276 vs 1155 g/d, P<0.001) and by pST (1254 vs 1177 g/d, P<0.001). Boars treated with both agents gained at a faster rate (1404 ± 33 g/d) than those treated with only pST and than the control pigs (P<0.001); they tended to grow at a faster rate than pigs receiving Improvac alone (P<0.08), which increased daily gain in gilts from 1000 to 1166 g/d (P < 0.001) and increased feed intake by 18% (P<0.01). Treatment with both Improvac and pST resulted in feed intakes similar to pigs receiving neither treatment (P>0.5), and in higher lean tissue accretion rates than in control pigs (P<0.09) but similar fat accretion rates (P>0.4). These data confirm that the effects of Improvac and pST are at least additive and in some cases synergistic. The combined treatment offers an opportunity for Australian pig producers to maximise growth performance while improving the quality of the pork.

Other opportunities

There are also opportunities to use Improvac or other anti–GnRF vaccines to control behaviour and increase growth in other species. For example, Vaxstrate® has been commercially available to control sexual behaviour and reduce pregnancies in extensively grazed female cattle in Northern Australia (Hoskinson *et al.* 1990). Although its efficacy was clearly demonstrated, the product was released at a time when beef prices were low and, consequently, so was the uptake of the technology. With higher beef prices, as they are currently, the use of an immunocastration vaccine for this class of cattle may be more attractive and a similar product may have applications in more intensive systems of cattle production. For example, Cook *et al.* (2000) recently reported that when bull calves were actively immunised against GnRF, with a resultant suppression of testicular function, there were improvements in meat tenderness, and their growth and carcass characteristics were similar to those observed in steers. Huxsoll *et al.* (1998) found that the immunisation decreased plasma testosterone and aggressive behaviour in beef bulls and improved carcass quality; growth performance was similar to that of control bulls. Aggressive and sexual behaviour of grazing bulls was reduced for up to 5 months after they were immunised (Jago *et al.* 1997). Further work to better define injection protocols may help to develop a product for the grazing dairy bull calf industry.

Another attractive prospect is the use of anti–GnRF vaccines to control rutting in deer. One of the problems with commercial deer production is that feed intake and growth performance of stags is depressed during the breeding season (rut). It has been found in New Zealand that active immunisation can partially reduce the effects of rutting on growth performance (Freudenberger *et al.* 1991), although this effect is not always sustained (Ataja *et al.* 1992). Work in our laboratory showed that the immunisation delayed rather than prevented rutting and modifications of, for example, injection protocols may be necessary for effective short–term responses (Simons 1998).

Most meat goats are farmed and marketed when sexually mature, and there is anecdotal evidence that meat taints are present in male goats. Production gains are not likely to be significant in an extensively farmed system, but there may be behavioural benefits from use of an anti–GnRF vaccine.

Chickens are quite different in the nature of the sexual effects on productivity. However, the endocrine changes that occur in layer hens at the time of moulting may be amenable to manipulation by techniques similar

Table 4 Effect of sex, porcine somatotrophin (Reporcin®) and Improvac® vaccine on growth performance of finisher pigs (McCauley *et al.* 2000b)[a].

Reporcin® (P)	**0 mg/d**				**5 mg/d**				
Improvac® (I)	**0 mL**		**2 mL**		**0 mL**		**2 mL**		
Sex (S)	**Boar**	**Gilt**	**Boar**	**Gilt**	**Boar**	**Gilt**	**Boar**	**Gilt**	**Significance[b]**
Daily gain, g/d	1379	1127	1240	1185	1405	1220	1599	1332	S***, P***, PxI*
Feed intake, g/d	3578	3052	3667	3639	2975	2948	3408	2891	S***, P***, I**, SxPxI**
P2 backfat, mm	19.7	15.6	19.5	18.2	14.7	16.6	16.1	14.7	P*

[a] Primary and secondary vaccinations at 14 and 18 weeks of age; slaughtered at 22 weeks of age. The somatotrophin was administered daily between 18 and 22 weeks

[b] *P<0.05, **P<0.01, ***P<0.001

to those used in pigs and would provide another management tool for that industry.

Conclusion

These studies show that it is practicable to make significant changes to the gonadotrophic axis of the pig and produce useful alterations to the physiology and behaviour of the animal. The reduction in sex hormone levels, particularly in males, is reproducible and results in the elimination of boar taint, a decrease in sexual behaviours, and an increase in feed intake. Our experience with the use of pST and its mode of action enables the design of treatments which take advantage of its complementary action with immunocastration to produce a combined effect that can be greater than with either treatment alone, particularly in gilts. In a more general sense, these studies indicate that it is possible to use agents that consistently alter major components of the endocrine makeup of animals to provide productivity benefits over a period of a few weeks.

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