Altering the timing of an immunocastration vaccine (Improvac®) to reduce its impact on attributes of pig performance

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Executive Summary

Boar taint, an objectionable odour and flavour detected in the cooking of pork from entire male pigs, has become a limitation to the consumption of and demand for Australian pork. As slaughter weight increases so does the concentration of androstenone and skatole, the two major components that contribute to boar taint.

Traditionally boar taint has been controlled by physical castration within the first week of life, but compared to entire male pigs physical castrates are fatter and have poorer feed conversion efficiency. The development of the vaccine Improvac® is an effective means of controlling boar taint but has the production advantage in that the pig has all the performance attributes of an entire male up until it receives the second vaccination, recommended at four to five weeks before slaughter. However, Improvac treated boars have an increase in feed intake and weight gain following the second vaccination compared to entire males (Dunshea *et al*, 2001) and as a consequence there may also be an increase in depth of backfat (Dunshea *et al*, 2001; Oliver *et al*, 2003; Pauly *et al*, 2009) to the extent that it makes some producers question the cost effectiveness of the practice. The aim of this experiment was to measure the response of entire males and the incidence of boar taint when the second Improvac vaccination is given at different times before slaughter (0, 2, 3, 4 or 6 weeks).

Even when the second vaccination was given only two weeks pre-slaughter there was total control of boar taint, indicated by the concentrations of androstenone and skatole being below threshold levels. The sharp decline in testosterone levels indicated a cessation of testicular function, and there was a linear decrease in the weight and physical dimensions of testes in line with the time between second vaccination and slaughter. However, some individual animals that had been treated with Improvac and were free of boar taint had testes of similar size to the control animals. Since testicle width and weight are used by processors in many countries to determine which carcasses might contain boar taint, other screening methods besides testes weight along similar lines to what was examined in this experiment may be required to determine tainted carcasses.

There was a significant linear trend for depth of backfat (P2) to increase as the time between the second vaccination and slaughter increased. However, there was no significant difference in growth rate or feed conversion efficiency related to the time of the second vaccination.

The second vaccination of Improvac is currently recommended to be given four to five weeks before slaughter. The present results suggest control of boar taint can be achieved when the second vaccination is given as late as two weeks before slaughter. The latter strategy reduces the likelihood of an increase in backfat of Improvac treated pigs compared to entire males and hence any decrease in payment by processors. This gives producers greater flexibility when selecting pigs vaccinated with Improvac for slaughter provided no pigs are sold less than two weeks post-vaccination. Since the levels of skatole in this experiment were all below threshold levels, further research in a commercial piggery is required to be certain that both components of boar taint are eliminated when Improvac is given two weeks before slaughter.

Table of Contents

1. Introduction

Producers have reacted to consumer demand for leaner pork by producing entire male pigs instead of castrates. Not only do entire male pigs have less subcutaneous fat than castrates at the same slaughter weight, but they also have a better feed conversion efficiency and an overall higher percentage of valuable cuts (Campbell and Taverner, 1988; Dunshea *et al*, 1993; Pauly *et al*, 2008). At the same time there has been an increase in slaughter weights because transport and slaughter charges are calculated on a per pig basis, hence the heavier the pig the lower the costs per kg produced. Between 1980 and 2000, average slaughter weight in Australia increased from 55 to 73 kg, but has since reached a plateau (Australian Pig Annual, 2009). As slaughter weight increases so do the concentration of androstenone and skatole, the two compounds that contribute to boar taint (Dunshea *et al*, 2001; Walstra *et al*, 1999). The incidence of boar taint, an objectionable odour and flavour detected in the cooking of meat, has thus become a major limitation to the consumption of and demand for Australian pork.

As an alternative to physical castration, which is normally conducted during the first week of life, a vaccine has been developed in Australia which effectively eliminates boar taint through immunocastration while achieving most of the gains of entire male pigs. The vaccine developed by Pfizer (Improvac®) blocks the activity of gonadotrophin releasing factor (GnRF), which is the signal for the release of luteinising hormone and follicle stimulating hormones from the pituitary. As a consequence, testicular development is stopped and there is no further production of androstenone. At the same time, clearance of skatole by the liver, which is blocked by steroid hormones from the mature testes, is increased. The Improvac vaccine consists of a primary injection that is normally given at approximately 10 weeks of age, and then the second vaccination given 4-5 weeks before slaughter. The advantage of immunocastration is that the pig has all the attributes of an entire male up until it receives the second vaccination.

Boars vaccinated with Improvac have an increase in feed intake and weight gain following the second vaccination compared to entire males (Dunshea *et al*, 2001; Oliver *et al*, 2003), which is attributed to their decrease in aggression and sexual behaviour (Cronin *et al*, 2003). However, there is also an increase in backfat (Pauly *et al*., 2009), and this factor has limited the uptake of this technology in Australia because producers may be penalised on price. The recommendation that the second vaccination should be given 4 to 5 weeks before slaughter is thought to be a conservative approach, since this guarantees that the compounds causing boar taint will have been metabolised and excreted from the body. If we were still able to eliminate boar taint by giving the second vaccination closer to the time of slaughter then the possibility of the technology increasing the depth of backfat would be reduced. The aim of this experiment was to measure the effects of giving the second Improvac vaccination at different times before slaughter on performance, carcass characteristics and incidence of boar taint in entire male pigs.

Hypothesis

That reducing the time between when entire male pigs are given the second vaccination with Improvac and their slaughter will reduce the increase in backfat depth while still reducing the incidence of boar taint.

2. Methodology

The experiment was conducted at the Department of Agriculture and Food Western Australia's (DAFWA) Medina Research Centre, and the experimental protocol used in this study was approved by the DAFWA Animal Ethics Committee and the Murdoch University Animal Ethics Committee. The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2004). The experiment was conducted from February to April 2009.

Animals and housing

A total of 175 Large White x Landrace entire male pigs from a high-health-status commercial piggery at 16 weeks of age (59 kg live weight, LW) were used in a completely randomised block design having five treatments. The treatments involved five different second vaccination schedules before slaughter with Improvac, and consisted of a group that had no second vaccination, or the second vaccination was given at two, three, four or six weeks before slaughter. Pigs were housed in groups of seven and randomly allocated to one of five treatments with five replicates per treatment (i.e. 5 x 5 x 7 = 175, or 35 pigs per treatment). There was one treatment per pen; pigs from different treatments could not be mixed as it could influence other pig's behaviour and performance. Pigs commenced the experiment in blocks with the heaviest 35 pigs randomly allocated to the five treatments at one time.

Pigs were vaccinated according to normal commercial practice with regard to the Improvac injection, with the injection occurring high on the lateral aspect of the neck. All pigs received the initial vaccination at approximately 10 weeks of age. Pigs were weighed individually on a weekly basis until their final weighing one day prior to slaughter.

Pigs were housed in a naturally ventilated grower-finisher shed with 1/3 slatted floors and 2/3 solid concrete floor. Space allowance per pig was a minimum of 0.912 m^2. Each pen had a single-space feeder located in one corner on the solid floor, with two nipple drinkers located over the slatted area. The back wall of the pen (on the slatted area side) was mesh fencing which allowed pigs to have visual and physical contact with pigs in the adjoining pen, while the side and front walls were solid panels. Pigs were offered a single diet *ad libitum.* The diet was formulated to contain 13.2 MJ DE/kg and 0.55 g Av lysine/MJ DE (Table 1). The amount of feed consumed per pen was recorded daily through the Feedlogic system.

Slaughter protocol and lesion scores

Slaughter occurred on three separate occasions with replicates 1 and 2 slaughtered first followed by replicates 3 and 4 the next week, and replicate 5

the following week. Animals were transported to a commercial export abattoir (PPC Wholesale Food Services, Wooroloo, WA) and held in lairage overnight. Carcass weight and depth of backfat, measured at the P2 site, located 6.5 cm from the midline over the last rib, were measured on the hot carcass by abattoir staff as per normal commercial practice before carcasses were chilled. At slaughter, carcass scores were taken to measure the degree of bruising and lesions that covered the carcass to measure impact of fighting before slaughter as described by McCauley *et al*. (2001). Carcasses were scored on a 0 to 3 basis; a score of 0 was equivalent to an unmarked carcass, score 1 for one or two bruises, score 2 if there was obvious bruising and minor damage to the carcass and score 3 for severe bruising and significant amount of damage to the carcass.

Table 1 - Composition of the experimental diet (as-fed basis)

Ingredients	Content (g/kg)
Wheat	250
Barley	360
Dehulled lupins	116
Lupins	75
Mill mix	61
Reworks	50.0
Canola meal	40.5
Limesand	12.0
Dicalcium phosphate	12.0
Tallow	15
Salt	2.95
Vitamins and minerals[1]	2.50
L-Lysine HCL	2.30
Alimet	0.90
L-Threonine	0.77
Choline chloride	0.13
Calculated composition	
DE (MJ/kg)	13.20
NE (MJ/kg)	9.41
Crude protein (g/kg)	159.5
Fat (g/kg)	48.7
Available Lysine: DE (g/MJ DE)	0.55

[1] Each kilogram of vitamin and mineral premix contains 7 MIU Vitamin A, 1.4 MIU Vitamin D_3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B_1, 3 g Vitamin B_2, 1.5 g Vitamin B_6, 15 mg Vitamin B_{12} 12 g niacin, 10 mg pantothentic acid, 0.19 g folic acid, 30 mg biotin, 10.6 g Calcium pantothenatic, 60 g iron, 100 g zinc, 40 g manganese, 10 g copper, 0.2 g cobalt, 0.5 g iodine, 0.3 g selenium, and 20 g antioxidant.

Sample collection and analysis

The day before slaughter, pigs were restrained by a snout rope and two 9 mL lithium heparin vacutainers were filled with blood via vena puncture. Two aliquots of plasma were taken and frozen for later analysis.

Prior to slaughter, testes width was assessed by measuring the left testicle of each pig using a standard set of callipers. After slaughter the testes were

collected, returned to the laboratory and the epididymis was removed. Various measurements including width post-slaughter, length, volume difference and vascularity were measured on each whole individual testicle. Vascularity was measured using the Image Pro Plus software. An external vascularity photo of each individual testicle was taken with the surface area of the testicle artery and veins calculated by counting the number of pixels representative of the testicle artery and veins divided by the number of pixels representative of the whole testicle. Colour measurements were made of both sides of the cut testicle surface with no bloom time. A Chroma meter CR-400 (Minolta, Osaka, Japan) was used to measure the colour of the surface of the testicles. The testicle surface were measured in the CIE L*, a*, b* system using D65 lighting, a 2 standard observer and 8mm aperture in the measuring head standardised to a white tile. The measurement L* denotes lightness, a* relative redness and b* relative yellowness.

Subcutaneous backfat samples (approx. 50g) were taken from each individual animal after slaughter. Skin was removed from the sample and the fat was then frozen at -20°C. The fat samples were sent to Frontage laboratories (China) and analysed for androstenone and skatole levels. Androstenone was extracted from porcine fat to remove interfering lipids and was accurately determined using liquid chromatography/mass spectrometry. The calibration range for the androstenone determination was 8000 ng/g to 200 ng/g. The limit of quantitation (LOQ) for the method was 200 ng/g. Skatole was extracted from porcine fat, to remove interfering lipids, and was analysed using high performance liquid chromatography with fluorescence detection. The calibration range for the skatole determination was 700 ng/g to 20 ng/g. The LOQ for the method was 20 ng/g.

The analysis of plasma for total testosterone concentration was conducted at Murdoch University using a commercially available kit. Total testosterone concentrations were measured with ELISA assay procedures (R & D Systems Inc, Minneapolis, MN, USA, Cat. No. KGE010) in 50µL plasma aliquots, according to the manufacturer's instructions. The manufacturer's evaluated a sensitivity of this assay of 0.030 ng/mL. All chemical analyses were performed in duplicate.

Statistical analysis

All statistical analyses were performed SAS for Windows, Version 9.13 (SAS Inst. Inc., Cary, NC). Performance traits were analysed univariately in normal linear models using the SAS MIXED procedure, with starting weight used as a covariate and block and time of second vaccination included in the model. The effects of time of second vaccination on hormone concentrations and testicle measurements were analysed according to the same normal linear model but without starting weight as a covariate. Polynomial regression was used to determine the presence of linear or quadratic treatment effects as time of second vaccination increased. Data on weekly feed intake was analysed as repeated-measures using a Gaussian model of spatial correlation in the MIXED procedure of SAS. Each pen was the experimental unit in the analyses. All means are reported as least squares means and standard error of the mean. Statistical significance was accepted at $P < 0.05$, whereas $P < 0.10$ was considered a trend.

3. Outcomes

Before the study commenced, one pig from the treatment that was assigned to the control (no second vaccination) group and one pig from the treatment that would have received the second vaccination two weeks before slaughter, were removed due to ill health. During the experiment a total of seven pigs were removed due to ill health and leg problems. The pigs removed included one pig that would not receive the second vaccination, one that would receive the vaccination two weeks before slaughter, three pigs that received the second vaccination four weeks before slaughter and two pigs that received the second vaccination six weeks before slaughter. Two more pigs were removed from statistical analysis due to one being a physical castrate and the other a female (treatment groups 6 and 0 weeks before slaughter, respectively), and feed disappearance was adjusted on the assumption that the feed intake of these two animals was the same as for the average of the pen. There were a total of 164 pigs used in the statistical analyses.

Growth performance

There was no significant difference between treatments in the LW of pigs at either the start (P=0.971) or end (P=0.223) of the experiment (Table 2). Average daily gain (ADG) and hence LW measured weekly were also not significantly different across treatments (P = 0.612) (Figure 1). Feed consumption on a per pen basis was similar at the start of the experiment, but there was a trend for this to increase for those treatment groups that received the second vaccination four or six weeks before slaughter (Figure 2). Boars that were not vaccinated had the lowest voluntary feed intake (VFI) and lowest total feed consumption, with VFI increasing as the time between second vaccination and slaughter increased (Table 2). The difference in VFI between the pigs that did not receive the second vaccination and those in the Improvac treated groups was significant (P < 0.001). There was no significant effect of treatment on feed conversion ratio (FCR).

Table 2 - Effect of time between the second Improvac® vaccination and slaughter on live weight (LW), average daily gain (ADG), voluntary food intake (VFI) and feed conversion ratio (FCR)[1] of male pigs over 42 days

	No. weeks pre-slaughter for the second vaccination						
	0	2	3	4	6	SEM	P-value
Starting LW (kg)	58.2	58.9	58.1	58.2	58.9	1.08	0.971
Final LW (kg)	105.1	104.9	104.8	107.9	107.9	1.27	0.223
ADG (g)	1114	1194	1109	1145	1246	69.6	0.612
VFI (kg/day)	2.57^{a}	2.71^{ab}	2.76^{bc}	2.90^{cd}	2.99^{d}	0.058	0.001
FCR	2.32	2.30	2.50	2.54	2.45	0.30	0.091

[1]Data calculated on pen basis; $^{a, b}$ Means in a row not having the same superscript differ significantly.

Figure 1 - Live weight for each treatment group in the six weeks pre-slaughter

Carcass characteristics

Pigs that received the second Improvac vaccination four or six weeks before slaughter had a significantly (P = 0.027) heavier carcass in comparison to those that received the second vaccination two or three weeks before slaughter (Table 3). There was a significant linear increase in carcass weight with the increase in time between slaughter and the second vaccination (P = 0.05). There was a significant trend for pigs that received the second vaccination six weeks before slaughter to have a higher backfat depth in comparison to the pigs that did not receive the second vaccination and the pigs that received the second vaccination two weeks before slaughter (P = 0.054). There was a significant linear increase in depth of backfat as the time between the second vaccination and time of slaughter increased (Figure 2, P =0.008). There was a significant trend (P=0.052) for pigs that were not vaccinated with Improvac to have the higher degree of carcass damage (Table 3). When all Improvac treatments were combined, the difference in carcass score was significantly higher for the unvaccinated Control animals (P = 0.002).

Table 3 - Effect of time between the second Improvac® vaccination and slaughter on carcass weight, P2 fat depth and carcass score[1]

Item	Number of weeks pre-slaughter of the second vaccination[2]					SEM	P-value
	Control	2	3	4	6		
Carcass weight (kg)[2]	69.2[ab]	68.3[a]	67.9[a]	71.0[b]	71.2[b]	0.78	0.027
P2 fat depth[2] (mm)	11.7[a]	11.3[a]	12.8[ab]	12.6[ab]	13.7[b]	0.56	0.054
Adjusted P2 fat depth[3] (mm)	11.7	11.4	12.8	12.5	13.7	0.61	0.1131
Carcass score[4]	1.36[a]	0.56[b]	0.34[b]	0.54[b]	0.65[b]	0.23	0.052

[1] Data calculated on pen basis; [a, b] Means in a row not having the same superscript differ. [2]Live weight at start of the experiment used as covariate in statistical analyses. [3]Carcass weight used as covariate in statistical analyses [4]Carcass score was calculated by the degree of bruising and lesions that covered the carcass.

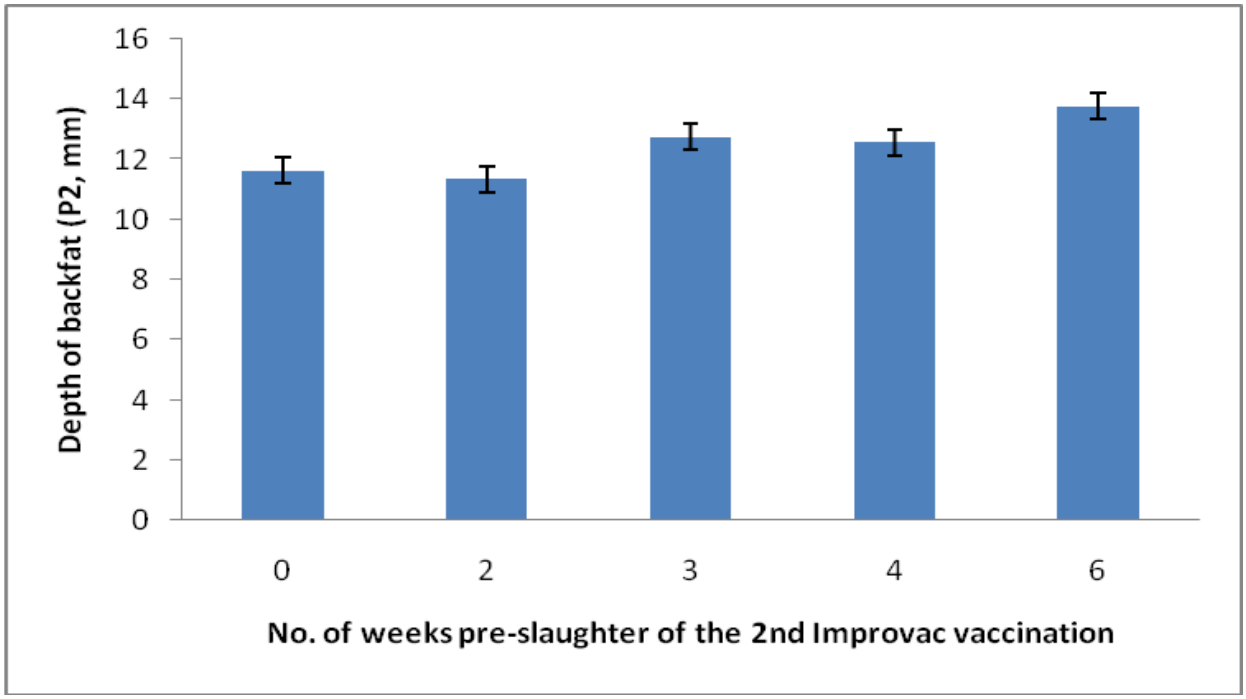

Figure 2 - Depth of backfat for pigs receiving the second vaccination of Improvac at different times pre-slaughter

Boar taint

Concentration of androstenone and skatole in adipose tissue (subcutaneous fat) and testosterone concentration in blood plasma are shown in Table 4. Pigs that did not receive the second vaccination had androstenone levels nine times

greater (P < 0.001) than all of the Improvac treated pigs regardless of vaccination time before slaughter. The androstenone levels of the Improvac treated pigs with alternate times before slaughter were not significantly different (P > 0.05) from one another. A high proportion (56%) of pigs that did not receive the second vaccination exceeded the sensory threshold value of 1.0 µg/g for androstenone in the adipose tissue (Figure 3). No pigs in the Improvac treatments exceeded the threshold value. Testosterone concentration in plasma, which is an indicator of testes function, followed a similar pattern to androstenone levels in the adipose tissue. The pigs that did not receive the second vaccination had testosterone levels at least two times greater than those of the Improvac treated pigs (P < 0.001). Skatole levels in fat were not different between all treatment groups (P = 0.52). Two pigs that did not receive the second vaccination had values of 0.16 µg/g and 0.18 µg/g, however across all treatment groups no pigs exceeded the threshold value of 0.20 µg/g for skatole in adipose tissue.

Table 4 - Effect of time between the second Improvac® vaccination and slaughter on the concentrations of androstenone and skatole in adipose tissue and testosterone in blood plasma.[1]

	No. of weeks pre-slaughter of the second vaccination						
	0	2	3	4	6	SEM	P-value
Androstenone (µg/g)	0.91[a]	0.11[b]	0.11[b]	0.10[b]	0.13[b]	0.053	< 0.001
Skatole (µg/g)	0.05	0.04	0.03	0.04	0.04	0.010	0.52
Testosterone (ng/g)	5.24[a]	1.11[b]	1.31[bc]	1.57[bc]	1.77[c]	0.205	<0.001

[1]Data calculated on pen basis; [a, b] Means in a row not having the same superscript differ.

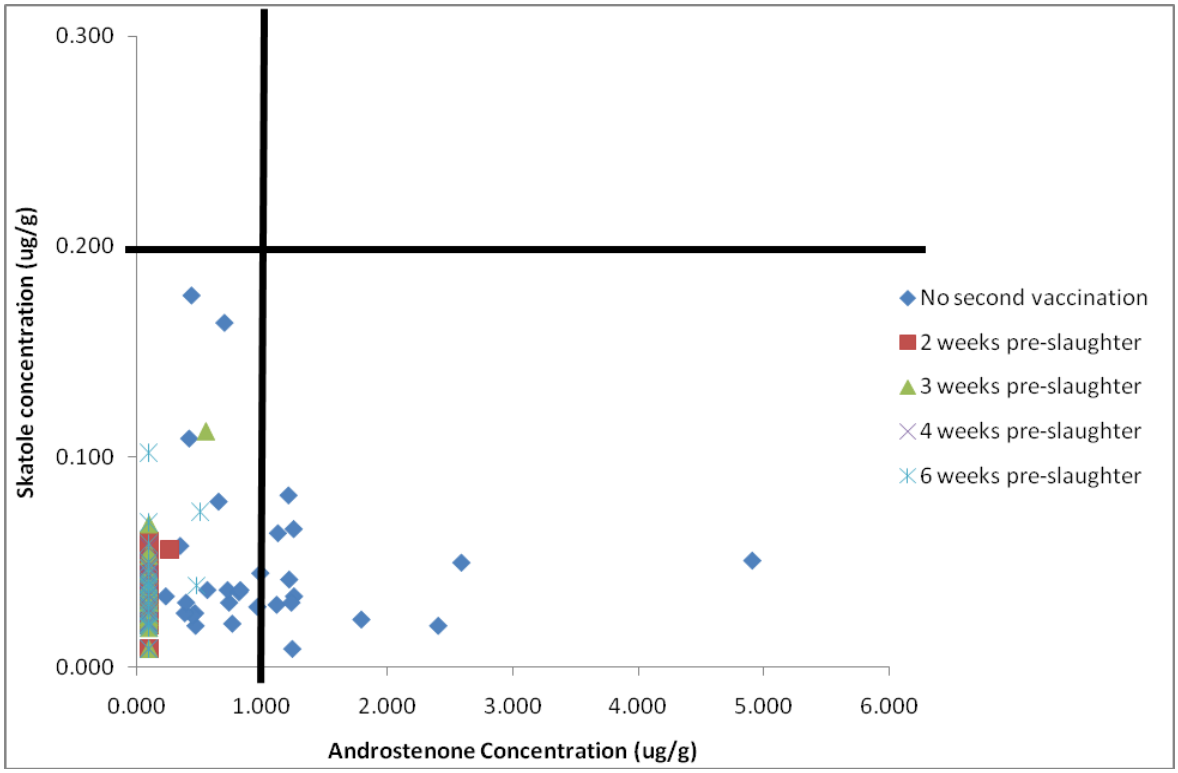

Figure 3 - Relationship between fat concentration of skatole and androstenone for control boars and Improvac treated boars given the second vaccination at alternate time before slaughter. Threshold values for androstenone and skatole concentration are indicated

Testicular function

The impact that varying the time of the second vaccination had on a number of physical measures of the testes is presented in Table 5. Testes weight (Figure 4) and volume were significantly reduced as the period between second vaccination and slaughter was increased (P<0.001). Testes width measured before and after slaughter, and testes length measured after slaughter, were also significantly altered with the timing of the second vaccination (P < 0.001). Testicle width and length increased with decreasing time between the second vaccination and slaughter.

Table 5 - Effect of time between the second Improvac® vaccination and slaughter on testicle weight, volume difference, testicle width pre-slaughter, testicle width post-slaughter and testicle length[1]

	No. weeks pre-slaughter of the second vaccination[2]						
	0	2	3	4	6	SEM	P-value
Testicle weight (g)	209^{a}	162^{b}	134^{b}	98^{c}	64^{d}	10.24	< 0.001
Testicle volume (mL)	215^{a}	164^{b}	137^{b}	99^{c}	69^{d}	9.95	< 0.001
Testicle width pre-slaughter (mm)	69^{a}	62^{b}	57^{bc}	55^{c}	49^{d}	1.84	< 0.001
Testicle width post-slaughter (mm)	59^{a}	54^{b}	50^{bc}	45^{c}	39^{d}	1.77	< 0.001
Testicle length (mm)	102^{a}	95^{ab}	87^{b}	79^{c}	65^{d}	2.66	< 0.001

[1]Data calculated on pen basis; $^{a, b, c, d}$ Means in a row not having the same superscript differ.

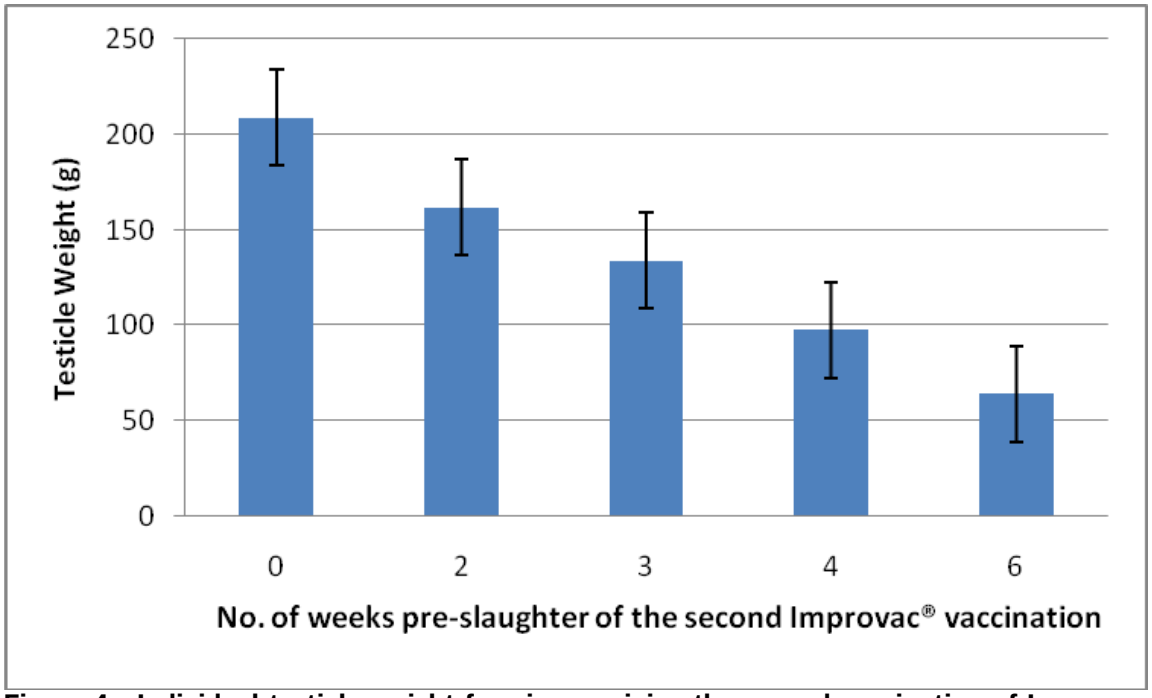

Figure 4 - Individual testicle weight for pigs receiving the second vaccination of Improvac at different times pre-slaughter

The effect of altering the timing of the second Improvac vaccination on Chroma metre results and vascularity percentage is presented in Table 6. There was a significant difference in the L* measurement ($P < 0.001$), a* measurement ($P < 0.001$) and the b* measurement ($P = 0.017$). The measurement of lightness (L*) and redness (a*) were both significant as a quadratic response ($P = 0.001$ and $P < 0.001$, respectively) while there was a significant linear response in the measurement of yellowness (b*, $P = 0.001$). However, there was high variation amongst the data and the L* measurement had the most consistent relationship with time of second vaccination. Vascularity percentage between the pigs that did not receive the second vaccination and the Improvac treatments regardless of times before slaughter was not significant ($P = 0.099$).

Table 6 - Effect of time between the second Improvac® vaccination and slaughter on Chroma meter colour measurements L*, a* and b*, and on vascularity percentage

	No. of weeks pre-slaughter of the second vaccination						
Item	0	2	3	4	6	SEM	P-value
L*[2]	49.2[a]	55.2[b]	53.9[b]	54.9[b]	53.2[b]	0.81	<0.001
a*[3]	20.8[a]	17.2[b]	18.1[bc]	17.9[bc]	19.2[c]	0.42	<0.001
b*[4]	6.4[a]	6.6[ab]	7.2[bc]	7.0[ac]	7.6[c]	0.22	0.017
Vascularity %[5]	80.0	72.6	66.2	64.5	58.9	5.83	0.099

[1]Data calculated on pen basis; [a, b, c, d] Means in a row not having the same superscript differ. [2]L* = lightness. [3]a*= redness. [4]b* = yellowness. [5]Vascularity percentage calculated based on the pixel amount of the surface testicle arteries and veins over the pixel amount of the entire testicle.

4. Discussion

There are production advantages to raising entire male pigs (boars) in comparison to barrows and Improvac treated boars. Boars have reduced feed intake and better FCR than barrows (Babol and Squires, 1995; Andersson *et al*., 1997; Xue *et al* 1997; Bonneau, 1998) and Improvac treated boars (Pauly *et al*., 2009). However, in comparison to boars and barrows, Improvac treated pigs had an increase in ADG (Dunshea *et al*., 2001). Dunshea *et al*. (2001) and Cronin *et al*. (2003) stated that this increase in ADG was a result of the decrease in sexual and aggressive behaviour as a consequence of the Improvac vaccination. Moore *et al*. (2009) also reported that as a result of the Improvac vaccination, ADG and VFI increased. In the present study in which pigs were housed in groups of seven, there was no significant effect on ADG with the alternate timing of the second vaccination (P = 0.149). However, under commercial conditions with a larger group size and increased interaction likely between individual pigs, then we could expect entire male pigs to have had a reduced ADG in comparison to Improvac treated pigs regardless of vaccination time before slaughter.

Oliver *et al*. (2003) reported that pigs treated with Improvac had higher carcass weights in comparison to pigs that had not received the Improvac vaccination (P value = 0.003). In the current experiment there was a significant linear increase in carcass weight as the time between second vaccination and slaughter increased. However, Dunshea *et al*. (2001), McCauley *et al*. (2003) and Zamaratskaia *et al*. (2008a) found no significant difference between carcass weights of Improvac treated pigs and entire male pigs, possibly due to them giving the second vaccination at four weeks before slaughter rather than six weeks as in this experiment.

One of the aims of this experiment was to reduce the effect that the current Improvac vaccination schedule has on increasing the depth of backfat (P2). In this experiment there was a significant linear increase in P2 as the time between second vaccination and slaughter increased. As producers are paid according to carcass weight and P2, treating pigs with Improvac too long before slaughter could have a negative impact on price. Pigs vaccinated two weeks before slaughter had an average P2 fat depth of 11.5 mm, whereas those that received the second vaccination six weeks before slaughter had an average P2 of 13.8 mm. The more than 2 mm difference in P2 would have important ramifications for any producers who are paid on a weight and grade system such as that in Australia where the prime grade is often for pigs with a P2 of 12mm or less. Based on our results, producers would have received a similar price per kg for pigs vaccinated two weeks before slaughter as they would have for entire males (Control) because there was no significant difference in either carcass weight or P2. Therefore the impact of the Improvac vaccination on P2 fat depth, and hence potential returns to pork producers, is reduced when the second vaccination is given closer to slaughter.

Voluntary feed intake was significantly lower in entire male pigs in comparison to the Improvac treated animals. Improvac vaccination has a positive effect upon VFI (Moore *et al*., 2009) compared to entire males possibly due to the decrease

in aggressive and sexual behaviour (Dunshea *et al*., 2001 and Cronin *et al*., 2003). Dunshea *et al*. (2001) suggested that there is a strong negative correlation between testosterone and VFI. Zamaratskaia *et al*. (2008a) also reported a direct negative effect between oestrogen levels and feed intake. Entire male pigs, in comparison to immunised boars, produce large quantities of testosterone and oestrogen especially during puberty (Zamaratskaia *et al*., 2008a). Therefore the reduction in testosterone and oestrogen levels due to immunocastration may account for the increased feed intake in immunised pigs. Aggressive and sexual behaviour do decrease as a direct result of the lower levels of testosterone and oestrogen.

Previous studies have confirmed the efficacy of the Improvac vaccination in preventing boar taint, by reducing both androstenone and skatole below threshold levels (Dunshea *et al*., 2001; McCauley *et al*., 2003; Pauly *et al*., 2009 and Zamaratskaia *et al*., 2008a). The current recommendation for the second Improvac vaccination is 4 to 5 weeks before slaughter to ensure taint substances present in the adipose tissue are cleared. However there are limitations to the current schedule such as an increase in backfat and decrease in lean meat in comparison to entire male pigs (Zamaratskaia *et al*., 2008a and Pauly *et al*., 2009). In the current experiment the pigs that did not receive the second vaccination had fat androstenone levels that exceeded ($P < 0.001$) the Improvac treated boars regardless of vaccination time before slaughter. Fifty six per cent of the carcasses from the pigs that did not receive the second vaccination exceeded the androstenone threshold of 1.0 µg/g. Therefore we have shown that Improvac is an effective means of reducing the concentration of androstenone to below threshold levels when given at least two weeks before slaughter.

Walstra *et al.* (1999) found that more than 60% of the carcasses had androstenone levels above 0.5 µg/g and 30% exceeded the threshold of 1.0 µg/g. In the current study, all pigs that received the second vaccination two weeks or more before slaughter exhibited reduced androstenone concentrations and all were below the threshold level. Bonneau *et al*. (1994), using an alternate immunocastration vaccine with a schedule of three separate vaccination times, also found that androstenone and testosterone levels were suppressed when the last vaccination was given two weeks before slaughter.

Fat skatole levels were found to be similar ($P = 0.52$) between all treatments in the current experiment although there was a trend for the values to be highest for the Control treatment. No pigs exceeded the threshold value of 0.20 µg/g skatole. Other researchers reported that entire male pigs had higher skatole levels than Improvac treated pigs (Dunshea *et al*., 2001; McCauley *et al*., 2003 and Pauly *et al*., 2009) possibly related to the increased testosterone levels in entire males. Increased testosterone levels result in a decrease in metabolic clearance of skatole, thus increased concentration of skatole in the adipose tissue. However, Zamaratskaia (2008a) reported that the effect of immunocastration on skatole varies in different studies due to variability in methods used to measure skatole. Walstra *et al*. (1999) found that only 11% of entire male pigs that had androstenone levels above 0.5 µg/g had skatole levels above 0.2 µg/g. The pigs in this experiment were comparatively clean and

lightly stocked in comparison to commercial practices which may account for the low skatole concentrations across treatments. Improvac treatment results in the suppression of testosterone concentration, therefore an increase in metabolic clearance of skatole from the adipose tissue. As testosterone levels were suppressed regardless of the time of the second vaccination before slaughter, using the Improvac vaccination would ensure skatole, an indicator of boar taint, to also be reduced under most circumstances.

The results from the present experiment confirm the finding of Dunshea *et al*. (2001), with testosterone levels being suppressed when the second Improvac vaccination was given two weeks before slaughter. Zamaratskaia *et al*. (2008a) found that animals immunised against GnRF had undetectable levels of testosterone in plasma. Previously Dunshea *et al*. (2001) reported that two weeks following the second Improvac vaccination, secretion of testosterone had been suppressed. Claus *et al*. (2007) reported that GnRF antibodies peaked four to six days after vaccination, and significantly reduced ($P < 0.001$) concentrations of testosterone five to ten days after vaccination. As a result, the luteinising hormone concentrations were significantly decreased ($P < 0.001$) and androstenone levels lowered ($P < 0.001$) in the four to eight day period after vaccination. Based on our results and that of others, it would appear that a period of two weeks between second vaccination and slaughter is adequate to enable the immunological changes required to take effect and for the clearance of androstenone present in the adipose tissue to occur.

The fighting and aggression between pigs that did not receive the second vaccination in this experiment, as measured by the degree of carcass lesions, was higher in comparison to the pigs treated with Improvac regardless of when the second vaccination was given. Dunshea *et al*. (2001), Cronin *et al*. (2002) and Zamaratskaia *et al*. (2008b) all reported that entire male pigs were more prone to aggressive behaviour and mounting in comparison to immunocastrates. While the current experiment was not done under commercial conditions, where pigs would be housed in larger groups with the opportunity for greater social interaction, it still showed how Improvac can reduce damage to carcasses when given even just two weeks before slaughter presumably by reducing aggressive behaviour between pigs.

To determine whether the Improvac treatment has been effective in controlling boar taint, screening methods such as testes weight and visual internal appearance of an individual testicle after slaughter have been used in various countries. Dunshea *et al*. (2001) and Einarsson (2006) both suggest screening is essential to determine if the vaccination has been successfully administered. In pigs aged 23 or 26 weeks of age at slaughter that had received the second vaccination four weeks before slaughter, Dunshea *et al*. (2001) recommended that a paired trimmed testes weight exceeding 350g and 400g, respectively, could be considered suspect by processors and therefore not clear of boar taint. Suspect carcasses would be removed from the slaughter line before entering the consumer market.

In the current experiment three methods were used to determine the effectiveness of the Improvac vaccination. Pigs that did not receive the second vaccination overall had the heaviest weight per individual testicle and greatest

length and width in comparison to the Improvac treated boars. With increasing time between slaughter and the second Improvac vaccination, there was a concurrent decrease in weight, width and length of the treated animals' testes. In this experiment, the paired testes weight of 350g used by Dunshea *et al*. (2001) for pigs slaughtered at 23 weeks of age, to determine the efficacy of the vaccine and its ability to clear boar taint compounds, could not be used as an indication that the carcasses were clear of boar taint. There were a total of 18 animals in the different treatments that exceeded the testes weight cut off assigned by Dunshea *et al*. (2001) but did not have androstenone or skatole levels that exceeded the threshold values. Three animals overall that did not exceed the testes weight cut off, however, did exceed the threshold values. Using testes weight as the only method of screening suspect carcasses for boar taint is not one hundred percent reliable, especially if the second vaccination is given up to two weeks before slaughter. Genotype, age and management differences may have a further effect upon testes weight thus decreasing the effectiveness of using testes weight as a screening process.

Chroma meter colour measurements were taken to determine whether the colour measurements could be used as a method to confirm the efficacy of the vaccine on an individual pig testicle. The chroma meter L* measurement, which denotes lightness, the a* measurement relative redness and the chroma meter b* measurement which denotes yellowness, were significantly different ($P < 0.001$, $P < 0.001$, $P = 0.017$, respectively) between the pigs that did not receive the second vaccination and the pigs that received the second Improvac vaccination at alternate times. There, was, however a large amount of variation in the samples thus no cut off value was suitable to determine the efficacy of the vaccination based on the chroma meter measurements. It is certainly recommended that these measures are included in any subsequent experiments with Improvac, especially if larger numbers of animals are to be used.

5. Application of Research

The results of this experiment indicate that the indicators of boar taint, androstenone and skatole, can be eliminated when the second vaccination with Improvac is given two weeks before slaughter. While it is still recommended that the second vaccination is given four weeks before slaughter, the results of the current experiment mean that if producers find that a proportion of animals have reached the target slaughter weight only two or three weeks after vaccination, then they can be safely sold as being free of boar taint. This of course relies on pigs having correctly received the first and second vaccinations, and as it is expensive to measure boar taint some measure of testes size is recommended since there is such a strong correlation between the weight, volume and dimensions of testes and the time between the second vaccination and slaughter. Other measures of testes function, such as colour, may be used to improve the accuracy of determining which animals have been successfully vaccinated against boar taint.

The results from this experiment also clearly showed that as the time between second vaccination and slaughter increases, so to does the increase in P2. The increase in P2 means some producers and processors might question the use of

Improvac regardless of the evidence for how it can control boar taint. By showing that boar taint could be controlled without any increase in P2 when pigs receive the second vaccination just two weeks before slaughter, this greatly improves the attraction of using Improvac by pork producers. However, producers contemplating the use of Improvac in this way should first discuss this strategy with their consultant veterinarian and ensure that no pigs are sent to slaughter at less than two weeks post-vaccination.

6. Conclusion

The major finding from this experiment was that the second Improvac vaccination can be moved closer to slaughter and still prevent boar taint by reducing androstenone and skatole levels below threshold levels. If the increase in P2 with using Improvac is a problem, then producers should certainly consult their veterinarian about reducing the time between second vaccination and slaughter because of the linear increase in P2 with increased time between second vaccination and slaughter. Carcass lesions were also lower in the animals that were vaccinated with Improvac, regardless of when the second vaccination was given, indicating the very rapid effect it has on reducing aggressive behaviour. Based on the results of this research, pork producers have greater flexibility in how they might use Improvac while still being assured that they have reduced the compounds responsible for boar taint.

7. Limitations/Risks

The official recommendation for the use of Improvac is that the second vaccination be given four to five weeks before slaughter. The results of this experiment indicate that the second vaccination will effectively control boar taint when given only two weeks before slaughter. Any decision to slaughter pigs on a routine basis with an average interval of 2-3 weeks post second vaccination should first be discussed with the clients' veterinarian. Pigs should certainly not be sold any sooner than two weeks after the second vaccination as there is no data to support freedom from boar taint. The major risk is that producers who start to sell some pigs two weeks after receiving the second vaccination may think that only one week will be satisfactory, but there is no guarantee from the results of this and other experiments that boar taint will be controlled under these circumstances. The other potential limitation is that processors will not be prepared to accept pigs with testes that are larger than those they may currently be used to seeing (i.e. vaccination at four to five weeks before slaughter) because they are not confident that boar taint has been sufficiently controlled.

8. Recommendations

The findings in this research project will greatly increase the flexibility and appeal for producers to use Improvac. Given the degree to which other countries are accepting Improvac, the Pork CRC and Pfizer should use these results as the basis for a campaign to promote the use of this excellent product by the Australian industry. At the same time, processors need to be convinced that it has many advantages over physical castration and that the current decrease in carcass weight that is happening in many states is not sufficient to guarantee optimal eating quality (i.e. no boar taint).

Under most circumstances pigs treated with Improvac would be assumed to have similar nutrient requirements to those of females of the same genotype. Recent results from a Pork CRC supported experiment of Moore *et al* (unpublished), in which the lysine requirements of entire male and female finisher pigs have been determined, would suggest that this assumption may not be valid and that some research needs to be conducted in this area.

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