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Reducing the length of time between slaughter and the secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs[1]

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ABSTRACT: The objective of this study was to evaluate whether altering the timing of the secondary anti-gonadotropin-releasing factor (GnRF) immunization closer to slaughter in male finishing pigs would reduce the increase in P2 fat depth (6.5 cm from the midline over the last rib), while still limiting the incidence of boar taint. Entire male pigs are immunized against GnRF to reduce the concentration of testicular steroids that in turn limits the incidence of boar taint. Additionally, testicle measurements and color measurements were taken to examine whether they could be used to differentiate nonimmunized entire males from immunized male pigs. A total of 175 Large White × Landrace entire male pigs aged 16 wk (59 kg of BW) were used in a completely randomized design with 5 treatment groups based on the time that pigs received the secondary immunization before slaughter. Pigs were housed in groups of 7 and randomly allocated to 1 of 5 treatments with 5 replicates per treatment. The treatment groups were as follows: no secondary immunization before slaughter, and the secondary immunization given at 2, 3, 4, or 6 wk before slaughter. The P2 fat depth levels were reduced ($P = 0.054$) with the secondary immunization closer to slaughter (11.7, 11.3, 12.8, 12.6, and 13.7 mm for no secondary immunization, secondary immunization at 2, 3, 4, and 6 wk before slaughter, respectively). Androstenone concentration did not exceed the generally accepted industry sensory threshold of 1.0 μg/g of fat, and both androstenone concentration in the adipose tissue and testosterone concentrations in the blood were suppressed ($P < 0.001$) in all immunized pigs regardless of timing of the secondary immunization compared with pigs that did not receive the secondary immunization. Skatole concentration of all pigs in the experiment did not exceed the generally accepted industry sensory threshold of 0.2 μg/g. Testes weight was reduced ($P < 0.001$) with increased time between slaughter and the secondary immunization. Immunized pigs, regardless of time before slaughter, had greater L* (lightness) and b* (yellowness) color of the testicle surface ($P < 0.001$ and $P = 0.020$, respectively), and less a* (redness) color compared with entire males ($P < 0.001$). The study provides further evidence of the efficacy of the anti-GnRF immunization and indicates that the secondary immunization can be moved closer to slaughter, while still limiting the incidence of boar taint. Testicle measurements and color measurements together could provide a method of discrimination between carcasses from immunized entire males clear of boar taint and tainted carcasses.

Key words: anti-gonadotropin-releasing factor, boar taint, carcass, color measurement, pig, timing

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INTRODUCTION

Boar taint, an objectionable odor and flavor sometimes detected when pork is cooked, is caused by the production of 2 compounds, androstenone and skatole, that accumulate in adipose tissue (Babol et al., 1996). Threshold concentrations greater than 1.0 μg/g in subcutaneous fat for androstenone and greater than 0.20 μg/g for skatole have been shown to cause negative consumer reactions (Bonneau et al., 2000). Boar taint traditionally has been controlled by physical castration within the first week of life, but compared with entire male pigs, castrated males are fatter and have poorer feed conversion efficiency (Campbell and Taverner, 1988; Suster et al., 2006; Pauly et al., 2009). Immunization against gonadotropin-releasing factor (**GnRF**) is an effective means of controlling boar taint and has the production advantage in that the pig has all the performance attributes of an entire male up until it receives the secondary immunization at 4 to 5 wk before slaughter. There are limitations, however, to the current immunization schedule, such as an increase in P2 fat depth and a decrease in lean meat yield compared with entire males (Dunshea et al., 2001; Oliver et al., 2003; Pauly et al., 2009).

The current timing of the secondary immunization is based on the premise of allowing sufficient time for androstenone and skatole to be cleared from subcutaneous fat before slaughter (Dunshea et al., 2001); however, we are only aware of 1 study that investigated if a reduction in the time between the secondary anti-GnRF immunization and anticipated slaughter will affect the accumulation of these compounds in fat, while reducing the negative impacts on carcass fatness (Dunshea et al., 2008). The hypothesis tested in the present experiment was that a reduction in the time between the secondary anti-GnRF immunization and slaughter would reduce the increase in P2 fat depth, while still reducing the incidence of boar taint compounds found in adipose tissue. In addition, we investigated whether testicle or color measurements or both could be used after slaughter to differentiate between immunized pigs and entire male pigs.

MATERIALS AND METHODS

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 (1-09-06) and the Murdoch University Animal Ethics Committee (NS2247/09). 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 × Landrace entire male pigs from a high-health-status commercial piggery (Wandalup Farms Ltd., Mandurah, Western Australia, Australia) aged 16 wk (59 kg of BW) were used in a completely randomized design with 5 treatments. The treatments involved 5 different secondary immunization schedules before slaughter with an anti-GnRF immunization (Improvac, Pfizer Animal Health, Parkville), and consisted of a group that had no secondary immunization and 4 groups that were given the secondary immunization at 2, 3, 4, or 6 wk before slaughter. Pigs were housed in groups of 7. Pens were randomly allocated to 1 of 5 treatments with 5 replicates per treatment, with a total of 35 pigs per treatment. The experiment was initiated when each replicate reached the target BW.

Pigs were immunized according to normal commercial practice with the intramuscular injection on the high lateral aspect of the neck. All pigs received the initial anti-GnRF (Improvac, Pfizer Animal Health, Victoria, Australia) immunization at 10 wk of age. Pigs received 1 dose (2 mL) of the anti-GnRF immunization at both vaccination times. One dose contained 400 μg of GnRF protein conjugate. Pigs were weighed individually on a weekly basis until their final weighing 1 d before slaughter.

Pigs were housed in a conventional grower-finisher barn that consisted of 4 rows of pens with partially slatted floors and solid concrete floor. Space allowances exceeded that for pigs of this BW according to the Australian Code of Practice. Each pen had a single feeder placed in the corner on the solid floor, with 2 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 limited physical contact with pigs in the adjoining pen. The side walls and front walls were solid panels. The diet was formulated to contain 13.2 MJ of DE/kg and 0.55 g of available lysine/MJ of DE (Table 1). The amount of feed consumed per pen was recorded through an automatic feeding system that delivered feed to each pen (Feedlogic Corp., Willmar, MN).

Sample Collection

All pigs were slaughtered at an average BW of 106 kg and average age of 22 wk. On the day before slaughter, blood was collected in two 9-mL lithium heparin Vacutainers and centrifuged at 2,000 × *g* for 15 min at room temperature. Plasma was collected and then frozen for subsequent analysis of testosterone concentration. Slaughter occurred on 3 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 mid-afternoon

Table 1. Composition of the diet (as-fed basis) offered ad libitum to all treatment groups

Item	Content
Ingredient,[1] g/kg	
Barley	378
Wheat	263
Mill run	64.1
Lupins	79
Lupin kernel	122
Canola meal	43
Tallow	16
MHA	0.95
Choline chloride	0.14
Salt	3
L-Lysine·HCl	2.40
L-Threonine	0.81
Limestone	12.5
Dicalcium phosphate	12.5
Vitamins and minerals[2]	2.60
Calculated composition	
DE, MJ/kg	13.2
NE, MJ/kg	9.4
CP, g/kg	160
Fat, g/kg	48.7
Available lysine:DE, g/MJ of DE	0.55

[1]Mill run = made up of 66% wheat bran and 33% wheat pollard. It is a by-product of the manufacture of flour and bran; MHA = methionine hydroxy analog.

[2]Supplied per kilogram of diet: 60.0 mg of Fe ($FeSO_4$); 10.0 mg of Cu ($CuSO_4$); 40.0 mg of Mn (MnO); 100.0 mg of Zn (ZnO); 0.30 mg of Se (Na_2SeO_3); 0.50 mg of I (KI); 0.20 mg of Co ($CoSO_4$); vitamin A, 7,000 IU; vitamin D_3, 1,400 IU; vitamin E, 20.0 mg; vitamin K_3, 1.0 mg; thiamine, 1.0 mg; riboflavin, 3.0 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.015 mg; pantothenic acid, 10.0 mg; folic acid, 0.2 mg; niacin, 12.0 mg; and biotin, 0.03 mg.

to a commercial export abattoir (PPC Wholesale Food Services, Wooroloo, Western Australia, Australia) with a transport length of 1 h and 10 min. Pigs were held in lairage overnight with mixing unknown pigs before slaughter the next morning. Carcass weight and depth of backfat 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. After slaughter, lesion scores on the carcass were taken to measure the impact of fighting that occurred, with a greater score indicative of an increased incidence of fighting (McCauley et al., 2001). Each carcass was given a numeric score based on the amount of damage to the entire carcass. A score of 0 was assigned to an unmarked carcass, 1 for minimal bruising to the carcass, 2 for obvious bruising present on the carcass, and a score of 3 for severe bruising and damage to the entire carcass (McCauley et al., 2001).

Before slaughter, width of testes was assessed by measuring the left testicle of each pig using a standard set of calipers. Testes were collected after slaughter, the epididymis was removed, and various measurements, including weight, width, length, and volume difference, were measured on each whole individual testicle. Volume difference was measured by placing the whole individual testicle into a known volume of water and recording the difference between the initial and final water volumes. Color measurements were made on both sides of the cut testicle surface with no bloom time (Chroma meter CR-400, Minolta, Osaka, Japan). The testicle surface was measured in the CIE L*, a*, b* system using D65 lighting, a 2 standard observer, and 8-mm aperture in the measuring head standardized to a white tile. The measurement L* denotes lightness, and a* and b* denotes relative redness and yellowness, respectively.

Chemical Analysis

Total testosterone concentrations were measured using ELISA assay procedures (KGE010, R & D Systems Inc., Minneapolis, MN) in 50-μL plasma aliquots, according to the manufacturer's instructions. The manufacturer's evaluated sensitivity of this assay was 0.030 ng/mL. All analyses were performed in duplicate.

Subcutaneous backfat samples (50 g) were taken from each individual animal after slaughter. Skin was removed from each sample, and samples were then frozen at −20°C. Androstenone (5-α-androst-16-en-3-one) was extracted from porcine fat to remove interfering lipids and was determined using liquid chromatography mass spectrometry. The calibration range for the androstenone determination was 200 to 8,000 ng/g. The limit of quantitation for the method was 200 ng/g. Skatole was extracted from porcine fat to remove interfering lipids and was analyzed using a normal phase HPLC separation with fluorescence detection. The calibration range for the skatole determination was 700 to 20 ng/g. The limit of quantitation for the method was 20 ng/g.

Statistical Analysis

All statistical analyses were performed using SAS (SAS Inst. Inc., Cary, NC). Performance traits were analyzed univariately in normal linear models using the SAS MIXED procedure, with starting BW used as the covariate and replication and time of secondary immunization included in the model. The effects of time of secondary immunization on hormone and testicle measurement (androstenone, skatole, and testosterone and testicle measurements) were analyzed according to the same normal linear model but without starting BW as the covariate. Polynomial regression was used to determine the presence of linear or quadratic treatment effects as time of the secondary immunization increased. Data on weekly ADFI was analyzed as repeated measures using a Gaussian model of spatial correlation in the MIXED procedure of SAS:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \delta x_{ijk} + \varepsilon_{ijk},$$

where Y_{ijk} is the observed response; μ is the overall mean; α_i is replication; β_j is the effect of time of secondary immunization; γ_k is the effect of week; $(\beta\gamma)_{jk}$ is the

Table 2. Effect of time between the secondary anti-gonadotropin-releasing factor immunization and slaughter on HCW, P2 fat depth (6.5 cm from the midline over the last rib), and carcass score[1]

		Secondary immunization before slaughter, wk					*P*-value		
Item	Control[2]	2	3	4	6	SEM	Overall	Linear	Quadratic
HCW,[3] kg	69.21[ab]	68.29[a]	67.93[a]	70.90[b]	71.16[b]	0.80	0.027	0.046	0.133
Dressing %	65.82	65.13	64.82	65.86	65.94	0.73	0.743	0.731	0.319
P2 fat depth,[3] mm	11.70[a]	11.30[a]	12.80[ab]	12.60[ab]	13.70[b]	0.60	0.054	0.008	0.442
Score[4]	1.36[a]	0.56[b]	0.34[b]	0.54[b]	0.65[b]	0.23	0.052	0.069	0.015

[a,b]Means in a row not having the same superscript differ ($P < 0.05$).

[1]Data were calculated on a pen basis; 5 replicates/treatment.

[2]Control did not receive the secondary immunization.

[3]Body weight at the start of the experiment was used as a covariate in statistical analyses.

[4]Carcass score was calculated by the degree of bruising and lesions that covered the carcass. Carcasses were scored on a 0 to 3 basis. Score 0 was equivalent to an unmarked carcass, score 1 was carcasses with 1 or 2 bruises, score 2 was carcasses with obvious bruising and minor damage, and score 3 was carcasses with severe bruising and a substantial amount of damage to the carcass.

interaction between time of secondary immunization and week; δ is a regression on starting BW (x); and ε_{ijk} is the residual error. Each pen was the experimental unit in the analyses. All means are reported as least squares means and pooled SEM. Statistical significance was accepted at $P \leq 0.05$, whereas $P < 0.10$ was considered a trend.

RESULTS

Before and during the experiment, a total of 9 pigs were removed due to ill health. Two pigs were removed from the experiment before it started, and 7 pigs were removed during the experiment for a variety of health- and leg-related problems. Pigs removed were split equally across experimental treatments. Two more pigs were removed from the statistical analysis because one was a castrated male and the other a female. Therefore, a total of 164 pigs were used in the final statistical analyses.

Carcass Characteristics

The impact of the immunization against GnRF on P2 fat depth resulted in a strong trend for P2 fat depth to be reduced when time between slaughter and the secondary immunization was decreased ($P = 0.054$; Table 2). On average, pigs that received the secondary immunization 6 wk before slaughter had a 2-mm increase in P2 fat depth compared with the pigs that did not receive the secondary immunization and those that received the secondary immunization 2 wk before slaughter. There was a linear increase ($P = 0.008$) in P2 fat depth with time between the secondary immunization and time of slaughter. Pigs that received the secondary immunization 4 or 6 wk before slaughter had heavier ($P < 0.05$) carcasses compared with those that received the secondary immunization 2 or 3 wk before slaughter. There was also a linear increase ($P = 0.046$) in carcass weight with time between slaughter and the secondary immunization. However, dressing percentage was unaffected by the timing of the secondary immunization ($P = 0.743$).

Carcass Lesions

There was a strong trend for the pigs that did not receive the secondary immunization to have a greater carcass lesion score compared with pigs that received the secondary immunization ($P < 0.05$). When immunization treatments were pooled, the pigs that did not receive the secondary immunization had carcass lesion scores 2.5 times greater ($P = 0.002$).

Growth Performance

There were no significant differences in BW between immunization groups at the start or at the end of the experiment. Altering the timing of the secondary immunization did not influence ADG or G:F across treatments (Table 3). However, pigs that did not receive the secondary immunization had less ($P < 0.05$) ADFI than pigs that received the secondary immunization 3, 4, and 6 wk before slaughter. There was no difference between the pigs that did not receive the secondary immunization and pigs that received the secondary immunization 2 wk before slaughter. There was a linear increase ($P < 0.001$) in ADFI with time between the secondary immunization and slaughter. The increase in ADFI became apparent 2 wk after immunization regardless of the timing of the immunization (Figure 1).

Indicators of Boar Taint

Pigs that did not receive the secondary immunization had fat androstenone concentrations at least 7 times greater ($P < 0.05$) than all of the immunized pigs regardless of the timing of the immunization (Table 4). The androstenone content of fat from all groups of pigs that received the secondary immunization was not dif-

Table 3. Effect of time between the secondary anti-gonadotropin-releasing factor immunization and slaughter on initial BW, final BW, ADG, ADFI, and G:F[1]

		Secondary immunization before slaughter, wk					*P*-value		
Item	Control[2]	2	3	4	6	SEM	Overall	Linear	Quadratic
Initial BW, kg	58.2	58.9	58.1	58.3	58.9	1.1	0.971	—	—
Final BW, kg	105.1	104.9	104.8	107.9	107.9	1.3	0.223	0.062	0.521
ADG, g/d	1,114	1,194	1,109	1,145	1,246	70	0.612	0.266	0.586
ADFI, kg/d	2.57[a]	2.71[ab]	2.76[bc]	2.90[cd]	2.99[de]	0.06	0.001	<0.001	0.923
G:F	0.432	0.439	0.402	0.395	0.413	0.020	0.388	0.224	0.495

[a–e]Means in a row not having the same superscript differ ($P < 0.05$).

[1]Data were calculated on a pen basis; 5 replicates/treatment.

[2]Control did not receive the secondary immunization.

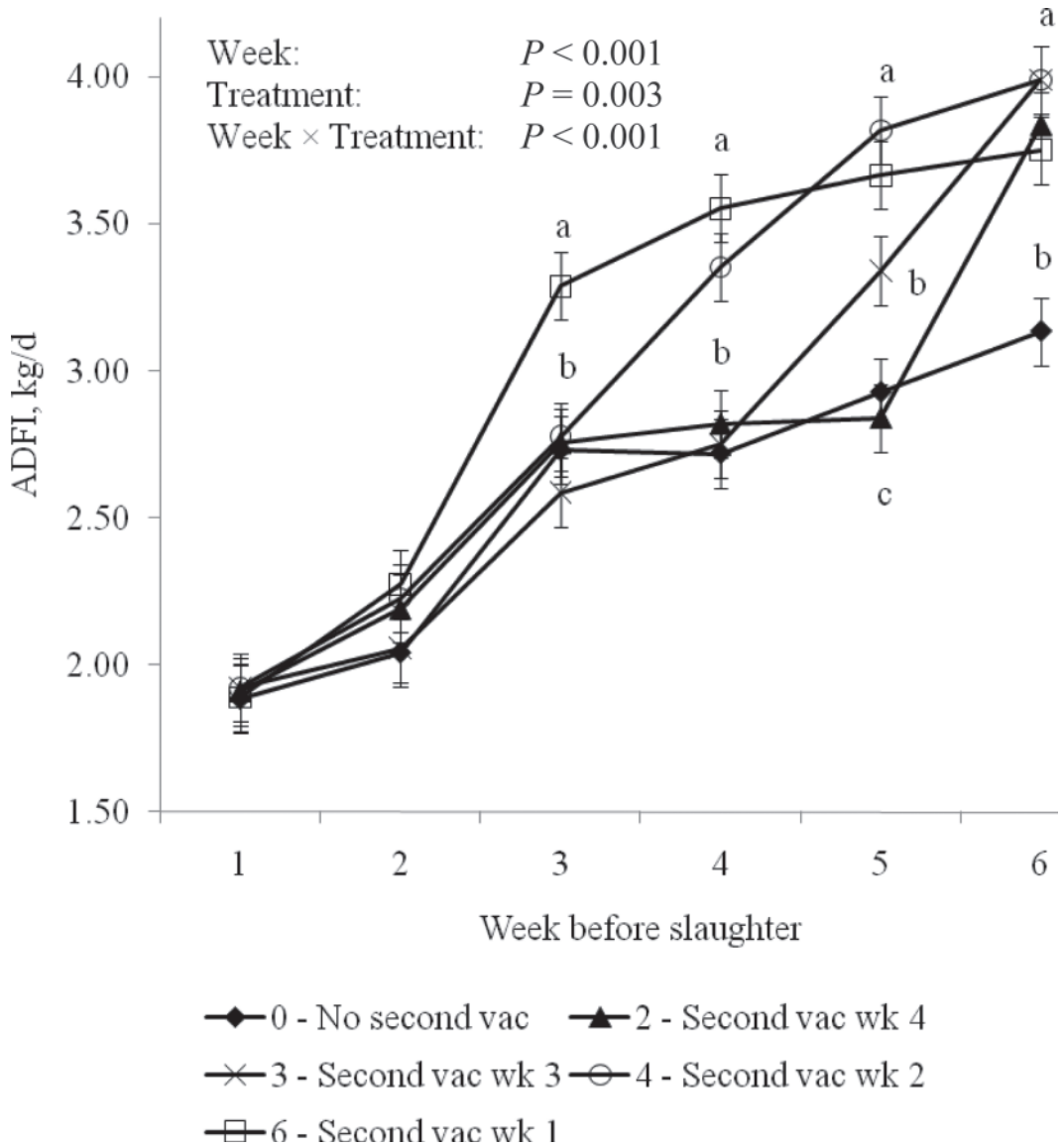

Figure 1. Average daily feed intake for each treatment group during the 6-wk period before slaughter. Pigs that received the secondary anti-gonadotropin-releasing factor immunization (vaccination, vac) 2 wk before slaughter were injected at wk 5; pigs that received the second immunization 3 wk before slaughter were injected at wk 4; pigs that received the second immunization 4 wk before slaughter were injected at wk 3; and pigs that received the second immunization 6 wk before slaughter were injected at wk 1. [a–c]Means within a week not having the same letter differ ($P < 0.001$).

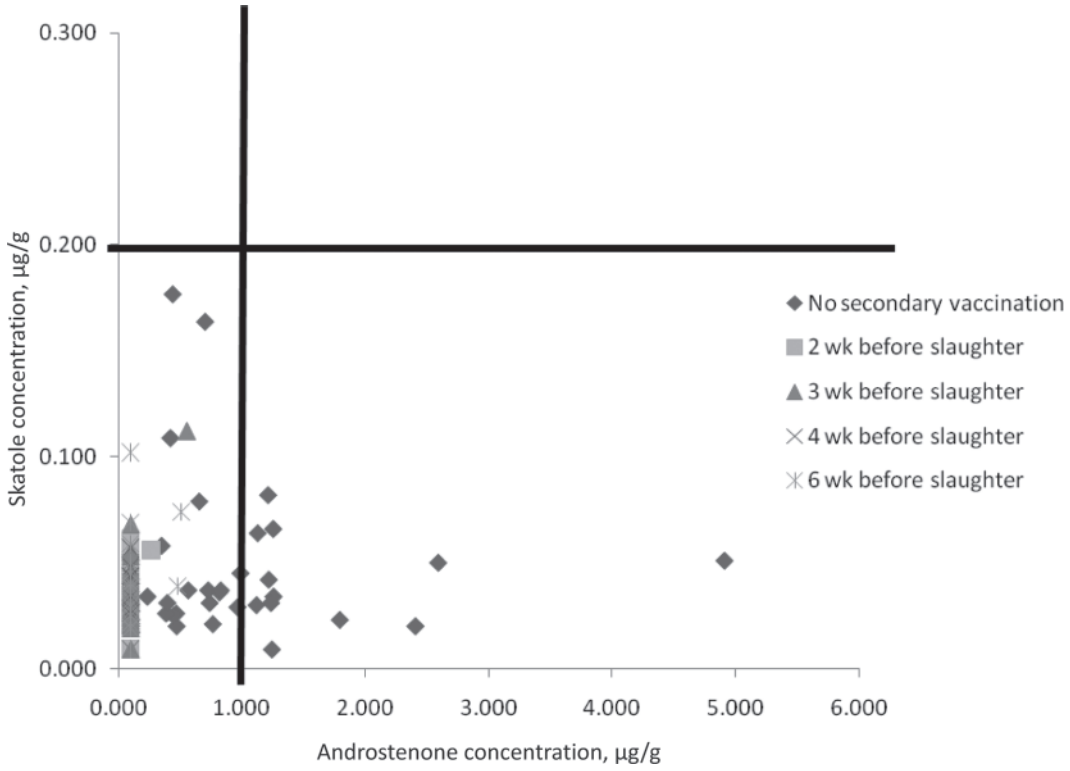

Figure 2. Relationship between skatole and androstenone concentrations of fat in pigs that did not receive the secondary immunization (vaccination) and pigs given the secondary immunization against gonadotropin-releasing factor at an alternative time before slaughter. The horizontal line cutting from the y-axis represents the skatole concentration threshold value, and the vertical line cutting from the x-axis represents the androstenone concentration threshold value. Threshold values for androstenone and skatole concentrations are 1.0 and 0.20 µg/g, respectively.

ferent (Figure 2). Pigs that did not receive the secondary immunization had plasma testosterone concentrations approximately 3 times greater ($P < 0.05$) than pigs that received the secondary immunization (Table 4). However, the pigs that received the secondary immunization 6 wk before slaughter had greater plasma testosterone compared with the pigs that received the secondary immunization 2 wk before slaughter ($P < 0.05$). Fat skatole concentrations were not different among treatment groups.

Testicle and Color Measurements

Individual testicle weight decreased with time between secondary immunization and slaughter (linear and quadratic, $P < 0.001$; Table 5). Several pigs that received the secondary immunization, in particular those that were immunized 2 wk before slaughter, had heavy individual testicle weights. The heavy testicle weights, however, were not associated with increased concentrations of the indicators of boar taint, androstenone and skatole (Figures 3 and 4, respectively). Testicle volume decreased (linear and quadratic, $P < 0.001$) with time between secondary immunization and slaughter.

Testicle width before and after slaughter increased with decreasing time between the secondary immunization and slaughter (linear and quadratic, $P < 0.001$). The testicle width of the pigs that received the secondary immunization 2, 3, 4, and 6 wk before slaughter was 10, 16, 20, and 28% less, respectively, than the pigs

Table 4. Effect of time between the secondary anti-gonadotropin-releasing factor immunization and slaughter on the concentrations of androstenone and skatole in adipose tissue and testosterone in blood plasma[1]

		Secondary immunization before slaughter, wk					*P*-value		
Item	Control[2]	2	3	4	6	SEM	Overall	Linear	Quadratic
Androstenone, µg/g	0.91[a]	0.11[b]	0.11[b]	0.10[b]	0.13[b]	0.05	<0.001	<0.001	<0.001
Skatole, µg/g	0.05	0.04	0.03	0.04	0.04	0.01	0.52	0.23	0.19
Testosterone, ng/g	5.24[a]	1.11[b]	1.31[bc]	1.57[bc]	1.77[c]	0.21	<0.001	0.002	<0.001

[a–c]Means in a row not having the same superscript differ ($P < 0.05$).

[1]Data were calculated on a pen basis; 5 replicates/treatment.

[2]Control did not receive the secondary immunization.

Table 5. Effect of time between the secondary anti-gonadotropin-releasing factor immunization and slaughter on testicle weight, volume, width before slaughter, width postslaughter, and length[1]

		Secondary immunization before slaughter, wk					P-value	
Item	Control[2]	2	3	4	6	SEM	Overall	Linear
Testicle weight, g	209[a]	162[b]	134[bc]	98[d]	64[e]	10	<0.001	<0.001
Testicle volume, mL	215[a]	164[b]	137[bc]	99[d]	69[e]	10	<0.001	<0.001
Testicle width before slaughter, mm	69[a]	62[b]	57[bc]	55[cd]	49[d]	2	<0.001	<0.001
Testicle width postslaughter, mm	60[a]	54[b]	50[c]	45[c]	39[d]	2	<0.001	<0.001
Testicle length, mm	102[a]	95[ab]	87[b]	79[c]	65[d]	3	<0.001	<0.001

[a–e]Means in a row not having the same superscript differ ($P < 0.05$).

[1]Data calculated on pen basis; 5 replicates/treatment.

[2]Control did not receive the secondary immunization.

that did not receive the secondary immunization. Testes length followed a similar pattern as testicle width, and increased as time between slaughter and the secondary immunization decreased (linear and quadratic, $P < 0.001$). However, there was no difference in testicle length between the pigs that did not receive the secondary immunization and pigs that received the secondary immunization 2 wk before slaughter.

Pigs that received the secondary immunization regardless of time before slaughter had a greater ($P < 0.05$) lightness measurement (L*) and smaller ($P < 0.05$) redness measurement (a*) than the pigs that did not receive the secondary immunization (Table 6). The lightness and redness responses over time were quadratic ($P < 0.001$) in nature. Pigs that did not receive the secondary immunization also had a decreased ($P < 0.05$) yellowness (b*) measurement compared with the pigs that received the secondary immunization 3 or 6 wk before slaughter. There was also a linear increase ($P = 0.001$) in the yellowness measurement with time between the secondary immunization and slaughter.

DISCUSSION

The hypothesis that a decrease in the time between the secondary anti-GnRF immunization and slaughter of male finishing pigs would reduce the extent of the increase in fat depth, while still reducing the incidence of boar taint compounds, was supported by the findings of the present study. The data clearly demonstrated that fat depth was reduced when the secondary immunization was given closer to slaughter and that androstenone concentration in the adipose tissue and testosterone concentration in the blood were suppressed in all immunized animals regardless of the timing of the secondary immunization. Pigs that received the secondary immunization 2 wk before slaughter had an average P2 fat depth of 11.4 mm, whereas those that received the secondary immunization 6 wk before slaughter had an average P2 of 13.6 mm. The more than 2 mm difference in P2 could have important ramifications for financial returns to pork producers in markets where fat depth is important.

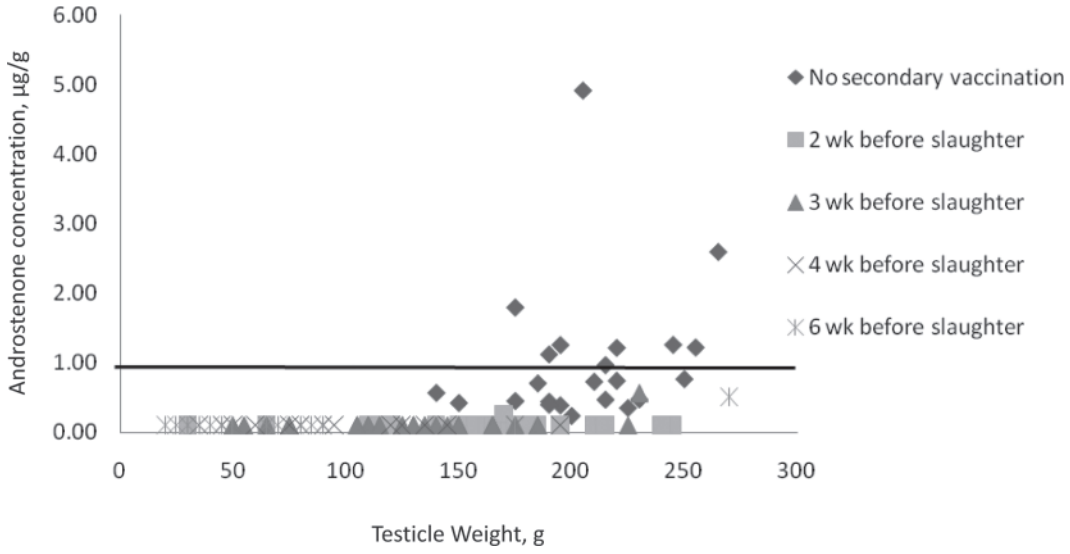

Figure 3. Relationship between individual testicle weight and androstenone concentration of fat in pigs that did not receive the secondary immunization (vaccination) and pigs given the secondary immunization against gonadotropin-releasing factor at a different time before slaughter. The horizontal line represents the androstenone concentration threshold value, which is 1.0 µg/g.

Table 6. Effect of time between the secondary anti-GnRF immunization and slaughter on color measurements, L*, a*, and b*, of testes[1]

		Secondary immunization before slaughter, wk					*P*-value		
Item	Control[2]	2	3	4	6	SEM	Overall	Linear	Quadratic
L*[3]	49.2[a]	55.2[b]	53.9[b]	54.9[b]	53.2[b]	0.8	0.002	0.060	<0.001
a*[4]	20.8[a]	17.2[b]	18.1[bc]	17.9[bc]	19.2[c]	0.4	<0.001	0.270	<0.001
b*[5]	6.4[a]	6.6[abc]	7.2[bc]	7.0[abc]	7.6[c]	0.2	0.02	0.001	0.872

[a–c]Means in a row not having the same superscript differ ($P < 0.05$).

[1]Measured with Chroma meter (CR-400, Minolta, Osaka, Japan). Data were calculated on a pen basis; 5 replicates/treatment.

[2]Control did not receive the secondary immunization.

[3]L* = lightness.

[4]a* = redness.

[5]b* = yellowness.

In the present study, there was no effect on ADG or the G:F with the alternative timing of the secondary immunization. The ADFI was greater in pigs that received the secondary immunization 6 wk before slaughter compared with the pigs that did not receive the secondary immunization and the pigs that received the secondary immunization 2 or 3 wk before slaughter. Immunization against GnRF increases ADFI (Moore et al., 2009) possibly because of the decrease in aggressive and sexual behavior (Dunshea et al., 2001; Cronin et al., 2003). The pigs that received the secondary immunization, regardless of time before slaughter, had an overall decreased proportion of greater carcass lesion scores compared with the pigs that did not receive the secondary immunization. Thus, the increased ADFI may be associated with decreased aggression in pigs that received the secondary immunization at alternative times before slaughter. However, differences in feed intake have been reported between individually housed intact males and immunized males (McCauley et al., 2000), and Weiler et al. (1996) stated that there is a strong negative correlation between testosterone concentrations and ADFI. It has also been reported that there is a direct negative effect between estrogen concentrations and feed intake (Bonavera et al., 1994). Entire male pigs, in comparison with immunized boars, produce large quantities of testosterone and estrogen, especially during puberty (Zamaratskaia et al., 2008). In the present study, the pigs that did not receive the secondary anti-GnRF immunization had testosterone concentrations approximately 3 times greater than the immunized pigs, regardless of time before slaughter. The pigs that did not receive the secondary immunization, which are basically intact, untreated males, also

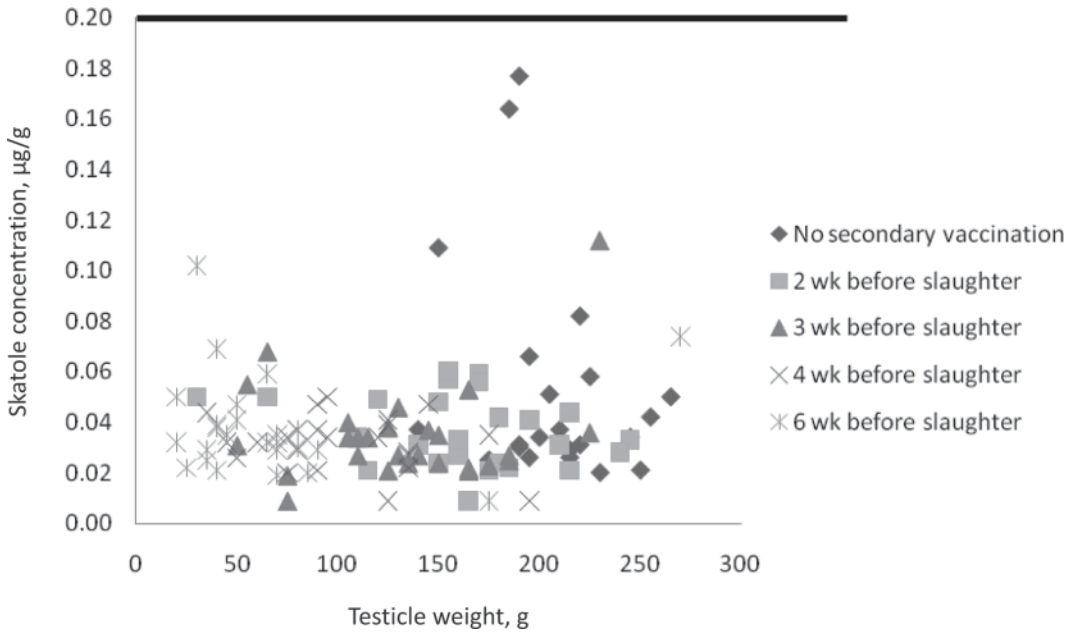

Figure 4. Relationship between individual testicle weight and skatole concentration of fat in pigs that did not receive the secondary immunization (vaccination) and pigs given the secondary immunization against gonadotropin-releasing factor at different time before slaughter. The horizontal line represents the skatole concentration threshold value.

had the least overall ADFI, which supports the notion that feed intake is related to testosterone and estrogen concentrations. The decrease in testosterone concentrations also results in a decrease in aggressive behavior and an increase in ADFI (Cronin et al., 2003).

Timing of the secondary immunization is critical to ensure castration and allow the maximum expression of the effect of anabolic hormones on growth and body composition. In the current experiment, pigs that did not receive the secondary immunization had fat androstenone concentrations that exceeded all animals that received the secondary immunization regardless of immunization time before slaughter. A total of 81% of the carcasses from the pigs that did not receive the secondary immunization exceeded 0.5 μg of androstenone/g, and 56% of those exceeded 1.0 μg of androstenone/g. Therefore, a total of 45% of the carcasses from the pigs that did not receive the secondary immunization exceeded the androstenone threshold of 1.0 μg/g. All pigs that received the secondary immunization 2 wk before slaughter had androstenone concentrations below the current threshold level of 1.0 μg/g. All of animals that received the secondary immunization 3, 4, or 6 wk before slaughter also exhibited androstenone concentrations less than this threshold value. The results confirm the efficacy of immunizing against GnRF and show that the secondary anti-GnRF immunization can be moved closer to slaughter and still allow enough time for androstenone, an indicator of boar taint, to be suppressed and metabolically cleared from the adipose tissue.

Dunshea et al. (2001) previously reported that 2 wk after the secondary immunization, secretion of testosterone had been suppressed and the results from the present experiment confirm these findings. Similarly, Claus et al. (2007) reported that plasma testosterone concentrations were suppressed within 5 to 10 d after the secondary anti-GnRF immunization. The present results provide definitive data on the timing of the secondary immunization on both testosterone and androstenone concentrations. The results show that reducing the timing of the secondary anti-GnRF immunization to as little as 2 wk does not adversely affect the efficacy of the technology and still enables sufficient time to clear androstenone present in the adipose tissue.

Fat skatole concentrations were uniformly decreased and similar between all treatments in the current experiment with no pig exceeding the threshold value of 0.20 μg/g for skatole in the adipose tissue. Others have reported greater skatole concentrations in entire male pigs compared with immunized pigs (Dunshea et al., 2001; McCauley et al., 2003; Pauly et al., 2009). The difference is possibly related to the increased androstenone concentrations in entire males because increased androstenone concentrations might decrease the rate of skatole clearance (Zamaratskaia and Squires, 2008). Walstra et al. (1999) found that only 11% of entire male pigs that had androstenone concentrations greater than 0.5 μg/g fat had skatole concentrations greater than 0.20 μg/g of fat. In the present study, only 1 animal (0.16 μg/g) was found to have a skatole concentration that approached the threshold of 0.20 μg/g and an increased androstenone concentration (0.71 μg/g).

To determine whether anti-GnRF immunization was effective in eliminating indicators of boar taint, screening methods such as testes weight and visual internal appearance of an individual testicle after slaughter have been used commercially in Brazil (E. Poleze, Pfizer Animal Health, São Paulo, Brazil, personal communication). Dunshea et al. (2001) and Einarsson (2006) both agree screening postslaughter is essential to determine if the immunization has been fully effective. The present results showed that testicle weight per se may not be an appropriate indicator of the effectiveness of the anti-GnRF immunization in eliminating boar taint. This is because several the immunized pigs had individual testicle weights and widths that exceeded the pigs that did not receive the secondary immunization but the immunized pigs were clear of boar taint compounds. Dunshea et al. (2001) used paired trimmed testes weights for the cut-off as to whether immunization had been effective. Pigs aged 23 or 26 wk of age at slaughter, of the particular Large White × Landrace genotype used and raised under group conditions, that had received the secondary immunization 4 wk before slaughter and had paired trimmed testes weight exceeding 350 and 400 g, respectively, could be considered suspect for boar taint by processors and removed from the consumer market (Dunshea et al., 2001).

In the current study, individual testicle weight, testicle width and length, and color measurements were used as potential screening methods to determine the effectiveness of the anti-GnRF immunization after slaughter. In the present study, the paired testes weight of 350 g, as suggested by Dunshea et al. (2001) for pigs slaughtered at 23 wk of age to determine the efficacy of immunization against GnRF and its ability to clear boar taint compounds, could not be used as an indication that the carcasses were clear of boar taint. A total of 18 animals in the different treatments exceeded the testes weight cut-off assigned by Dunshea et al. (2001) but did not have androstenone or skatole concentrations that exceeded the threshold values. Three animals overall that did not exceed the testes weight cut-off, however, did exceed the threshold values. Therefore, and based on data obtained in this study, using testes weight as the only method of screening suspect carcasses for boar taint is not completely reliable.

Color measurements were taken to determine whether they could be used as a potential screening method to confirm the efficacy of the immunization against GnRF on an individual pig testicle. Immunized pigs had greater L* measurements and thus an overall lighter testicle surface compared with entire males. A greater L* value has been found to be associated with less in vivo hemoglobin concentration (Klont et al., 1999). The immunized pigs, regardless of timing of the secondary immunization, had decreased a* color measurements and thus decreased intensity of redness

of the testicle surface compared with pigs that did not receive the secondary immunization, which seems to confirm the association between L* measurement and hemoglobin concentration. A greater a* color measurement was found to be associated with increased heme pigments, resulting in greater mitochondrial activity (Dunne et al., 2005). Therefore, because the pigs that did not receive the secondary immunization had greater a* measurements, it could be argued that their testes were more active than the immunized pigs, which could be related to overall greater testosterone concentration present in the pigs that did not receive the secondary immunization. The immunized pigs also had greater b* color measurements compared with the pigs that did not receive the secondary immunization.

The L* measurement had the most consistent relationship with timing of the secondary immunization. Based on our results, an individual testicle weight that exceeds 170 g and has an L* measurement less than 53.0 could indicate the carcass is tainted or at least suspect of being tainted. In this study, only 1 pig with an individual testicle weight less than the threshold (140 g) and a L* measurement less than the cut-off (52.4) exceeded the 0.5-μg intermediate androstenone value. Based on the data from this experiment, a combination of a physical testicle measurement and color measurement could provide a means to distinguish tainted carcasses from clear carcasses, and the associations need to be investigated further.

In conclusion, our hypothesis that a decrease in the time between the secondary anti-GnRF immunization and slaughter of male finishing pigs would reduce the extent of the increase in P2 fat depth while still reducing the incidence of boar taint compounds, androstenone and skatole, was confirmed. Immunization against GnRF was associated with marked reductions in testosterone concentrations. Carcass lesions were reduced in pigs that received the secondary immunization regardless of time before slaughter in comparison with pigs that did not receive the secondary immunization, indicating that anti-GnRF immunization reduces aggressive behavior. Testicle width and weight was increased with a decrease in the time between secondary immunization and slaughter, and therefore it alone cannot be used as a method of discrimination between immunized and nonimmunized pigs. A combination of a series of physical testicle measurements in association with color measurements indicates a possible discrimination method. The results of this experiment increase the flexibility as to how immunization against GnRF could be used commercially.

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