

Gonadotropin-releasing hormone immunization and castration in male pigs: effects on growth, hormonal levels, antibody titer response, testicular function, back fat, and consumers' sensory perceptions

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Abstract: This study investigated the effects of gonadotropin-releasing hormone (GnRH) vaccine on pig growth, carcass quality, and sensory perceptions of pork meat by Thai consumers. Male crossbred pigs (n = 30) were separated into three groups of 10 pigs each: boars, immunocastrated pigs, and surgically castrated pigs. The immunocastrated group was immunized at 13 and 19 weeks of age with GnRH vaccine (400 µg/dose). All pigs were slaughtered at 24 weeks of age. Blood samples were collected and testes size determined. Testes weight and back fat thickness were recorded at the time of slaughter, and meat samples were collected for sensory assessment by Thai consumers. Testosterone and progesterone concentration levels decreased significantly two weeks after the second dose of GnRH ($p < 0.05$), and the GnRH antibody titer of the immunocastrated pigs was significantly high two weeks after the second dose of GnRH ($p < 0.05$). GnRH vaccine significantly reduced the weight and size of testes. Thai consumers reported no significant differences in odor or flavor among meat samples from the three groups. In conclusion, immunocastration improved growth performance, removed the need for castration surgery, and avoided “boar taint” in the meat. It has also no distinct trace of the boar taint same as pork that is currently produced from physically castrated pig which is making it acceptable to consumers.

Keywords: back fat, gonadotropin-releasing hormone vaccine, growth performance, sensory assessment, testes size

Introduction

The swine industry in many countries such as Australia and especially England, is concerned about animal welfare. In these countries, piglets are not routinely castrated, but are slaughtered after some method which used to decrease boar taint. Removal of the testes of piglets has an impact on the quality of meat in terms of odor, flavor and taste. Those three characteristics together are known as “boar taint” [13]. Consumers are more likely to refuse to accept meat with boar taint. The boar taint in pig meat and fat are caused by skatole (3-methylindole) which results from the disintegration of tryptophan amino acid and androstenone (5 α -androst-16-ene-3-one) which is produced by the testes [4]. A number of studies of consumer acceptance of the meat of castrated piglets have been conducted [1, 13]

An alternative way of diminishing the odor of male pigs is immunization against gonadotropin-releasing hormone (GnRH) using a GnRH vaccine such as Improvac (Zoetis, Thailand)

[6]. GnRH vaccine contains gonadotropin-releasing factor analogue-protein conjugate, which is similar to the GnRH hormone and is foreign to pigs. When it enters the body of the pig, it immunizes and produces an antibody which destroys GnRH [2]. The inhibition of GnRH serves to inhibit luteinizing hormone and to decrease follicle stimulating hormone. That then leads to decreased testis growth and testosterone production, two factors which affect the odor and taste of meat [17]. Progesterone, which is released from the adrenal gland, has been reported to be related to testosterone but it has not been reported to affect immunocastrated pigs. The relation between progesterone and testosterone levels has not been reported previously. Surgical castration of male pigs is regularly performed in Thailand to prevent boar taint in the carcass. However, avoiding castration may be beneficial in terms of improved growth performance and a leaner carcass. Also, this study is interested in the development of optimum immunocastrated pigs in different swine industries.

The last element in the pig chain is the consumer. Some

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studies have reported no differences in the acceptability of meat from boar pigs and castrates or female pigs, but other studies have found considerable differences [8, 11]. Sensory evaluation of immunocastrated meat by the Japanese, Filipino and European consumers has been reported [8], but none have been performed in Thailand. The objective of this study was to evaluate the effectiveness of the GnRH vaccine on growth performance, testes size and back fat in male pigs, and to conduct a sensory assessment of pork by Thai consumers.

Materials and Methods

Animal handling

All procedures conducted with the animals were approved by the Animal Ethics Committee (S12-2555), and followed the guidelines for handling of laboratory animals established by the Animal Ethics Committee of Thailand. The study included a total of 30 male crossbred pigs (Large White \times Landrace \times Duroc) pigs, of which 20 were boars and 10 were surgically castrated. The animals were divided into three groups of ten pigs each. Group I was surgically castrated pigs; group II was uncastrated pigs; and group III was uncastrated male pigs which had been injected with a 400 μ g dose of GnRH vaccine at 13 and 19 weeks of age. All pigs were slaughtered at 24 weeks of age. Groups I and II were treated with normal saline and served as negative and positive controls, respectively. The pigs were weighed at 13 weeks of age; the weights of the groups were found to be not significantly different ($p < 0.05$). They were then separated for sex to pens of 10 pigs each which was provided with an evaporative cooling system. The pigs were fed commercial, unmedicated, corn-based grower and finisher diets. At 24 weeks of age, the pigs were weighed and the amount of feed consumed calculated to evaluate growth performance (body weight and average daily gain [ADG]).

Blood samples and testes size measurements (for groups II and III) were performed at 13, 19, 21 and 23 weeks of age. Blood serum was separated for measurement of testosterone and progesterone concentrations by enzyme-linked immunosorbent assay (ELISA) [3] and for GnRH antibody using Indirect Non-competitive ELISA [20]. Testes size (length) was measured using vernier calipers [16].

At slaughter, the testes were removed from the carcass then weight and the length measurements were made. Following that, the testes were fixed with 10% formalin. Immediately after this fixative step, a sample was prepared for light microscopy investigation. Back fat thickness was recorded as the mean of the fat thickness on the first rib, last rib and lumbo-sacral bone (6.5 cm from the midline over the spinal cord).

Sensory evaluation

After slaughter and chilling, the longissimus thoracis muscle was collected and samples of the meat were prepared in

1.5 \times 1.5 \times 1.5 cm³ cube, packaged in aluminum foil and baked in an oven at 180°C for 15 min. The samples were labeled using random 3-digit codes [21]. The cooked meat samples were provided to 122 Thai consumers, 51 men and 71 women, ages 19 to 45, for sensory evaluation. Each consumer tasted three meat samples. To decrease potential bias from having already eaten one sample of meat, consumers were given unsalted crackers and water to eat between samples. After tasting all three samples, the consumers completed a questionnaire which included questions on odor, flavor and taste. All parameters were scored using a five point Likert scale: 1, dislike a lot; 2, dislike; 3, can't differentiate; 4, like; and 5, like a lot [21].

Statistical analysis

Testosterone and progesterone concentrations and the GnRH antibody titer were analyzed using the general linear model (two-way repeated measurement in ANOVA), and compared between the groups using the one-way ANOVA. Testes size and back fat thickness were analyzed using the paired-samples t-test. The Chi-squared test was used to analyze the sensory evaluation. The significance level for all the parameters was $p < 0.05$.

Results

During the experiment, pig No. 1 in the boar group was removed from the experiment because of a leg problem, and pig No. 9 in the immunocastrated group was removed from the experiment because the vaccine could not be expected to have the desired effect, so, the boar group and the immunocastrated group had only 9 pigs each.

Antibody response

The level of the GnRH antibody titer⁻¹ was used to monitor the effect of the GnRH vaccine. There were no significant differences in the levels of the GnRH antibody titer⁻¹ between the first and the second serum samples or between levels at the time of the initial dose and the time of the second dose for any of the groups. However, the immunocastrated group had significantly higher levels than both the other two groups at two and at four weeks following the second dose ($p < 0.001$) (Table 1). The GnRH antibody titer⁻¹ of the serum sample from the immunocastrated group taken two weeks after the second dose was 25,379 units, while the GnRH antibody titer⁻¹ of the non-vaccinated groups (the boars and the surgically castrated) at the same time was less than 3,000 units.

Testicular structure and function

In four series samples, all of the surgically castrated groups consistently had levels of testosterone concentration of less than 0.5 ng/mL. In contrast, most of the boars and most of the immunocastrated group had more than 2 ng/mL at the time of the second dose. Two weeks after the second dose,

Table 1. Effect of GnRH vaccine on plasma GnRH antibody titer⁻¹ levels

Time	Boar (n = 9)	Immunocastrated (n = 9)	Surgically castrated (n = 10)	<i>p</i> value
Initial dose	2,827 ± 239	3,060 ± 226	3,405 ± 214	0.097
Second dose	2,349 ± 422	2,928 ± 398	2,994 ± 377	0.523
Second dose + 2 weeks	2,818 ^a ± 3,322	25,379 ^b ± 3,132	2,815 ^a ± 2,971	< 0.001
Second dose + 4 weeks	2,533 ^a ± 913	12,337 ^b ± 861	3,794 ^a ± 817	< 0.001

^{a,b}Difference significant at $p < 0.05$. Data are presented as mean ± SEM.

Table 2. Effect of GnRH vaccine on testosterone and progesterone concentrations

Time	Boar (n = 9)	Immunocastrated (n = 9)	Surgically castrated (n = 10)	<i>p</i> value
Testosterone (ng/mL)				
Initial dose	2.080 ^a ± 0.364	1.993 ^a ± 0.344	0.182 ^b ± 0.326	< 0.05
Second dose	2.626 ^a ± 0.407	2.480 ^a ± 0.384	0.108 ^b ± 0.364	< 0.001
Second dose + 2 weeks	3.489 ^a ± 0.432	0.373 ^b ± 0.407	0.210 ^b ± 0.386	< 0.001
Second dose + 4 weeks	3.725 ^a ± 0.537	0.397 ^b ± 0.506	0.304 ^b ± 0.480	< 0.001
Progesterone (ng/mL)				
Initial dose	0.287 ^a ± 0.041	0.372 ^a ± 0.038	0.072 ^b ± 0.036	< 0.001
Second dose	0.454 ^a ± 0.063	0.426 ^a ± 0.060	0.129 ^b ± 0.057	< 0.05
Second dose + 2 weeks	0.426 ^a ± 0.053	0.182 ^b ± 0.050	0.137 ^b ± 0.047	< 0.05
Second dose + 4 weeks	0.474 ^a ± 0.042	0.286 ^b ± 0.040	0.153 ^b ± 0.038	< 0.001

^{a,b} Difference significant at $p < 0.05$. Data are presented as mean ± SEM.

Table 3. Effect of GnRH vaccine on testes length and weight

Time	Boar (n = 9)	Immunocastrated (n = 9)	<i>p</i> value
During the experimental period			
Testes length (cm)	–	–	–
Initial dose	13.4 ^a ± 0.4	11.9 ^b ± 0.4	< 0.05
Second dose	16.5 ± 0.6	16.2 ± 0.6	0.737
Second dose + 2 weeks	16.6 ^a ± 0.7	12.3 ^b ± 0.7	< 0.001
Second dose + 4 weeks	16.8 ^a ± 0.5	12.1 ^b ± 0.5	< 0.001
At slaughter			
Testes size (cm)	16.2 ^a ± 0.4	11.6 ^b ± 0.3	< 0.001
Testes weight (g)	478 ^a ± 26	129 ^b ± 8	< 0.001

^{a,b}Difference significant at $p < 0.05$. Data are presented as mean ± SEM.

the immunocastrated group had a significantly lower serum testosterone concentration (< 0.5 ng/mL) than the boar group ($p < 0.001$) (Table 2). Although the level of progesterone in the boar and the immunocastrated groups was similar to the level of testosterone two weeks after the second dose, the immunocastrated group had a serum progesterone concentration of < 0.3 ng/mL which is significantly lower than the boar group ($p < 0.001$) (Table 2).

At the time of the initial dose, the immunocastrated group and the boar group differed significantly in testes length and weight ($p < 0.05$), but at the time of the second dose there was very little difference. However, by two weeks after the

second dose, the difference was again significant ($p < 0.001$) (Table 3). That difference was also present at the time of slaughter ($p < 0.001$) (Table 3). However, by 2 weeks after the second dose, the testes size of immunocastrated pigs had decreased substantially, by approximately 4 cm, indicating that the vaccine can not only suppress testicular growth, but can actually decrease testicle size. These histopathology results clearly demonstrate the effect on the testes of the boar group and immunocastrated group. The diameters of the ductus epididymides and the seminiferous tubules of the immunocastrated pigs were smaller than those of the boar group. In addition, Leydig cells and spermatocytes were rare in the

seminiferous tubules of the immunocastrated pigs compared to the boar group (Fig. 1).

Growth performance and carcass quality

This experiment commenced when the pigs were 13 weeks

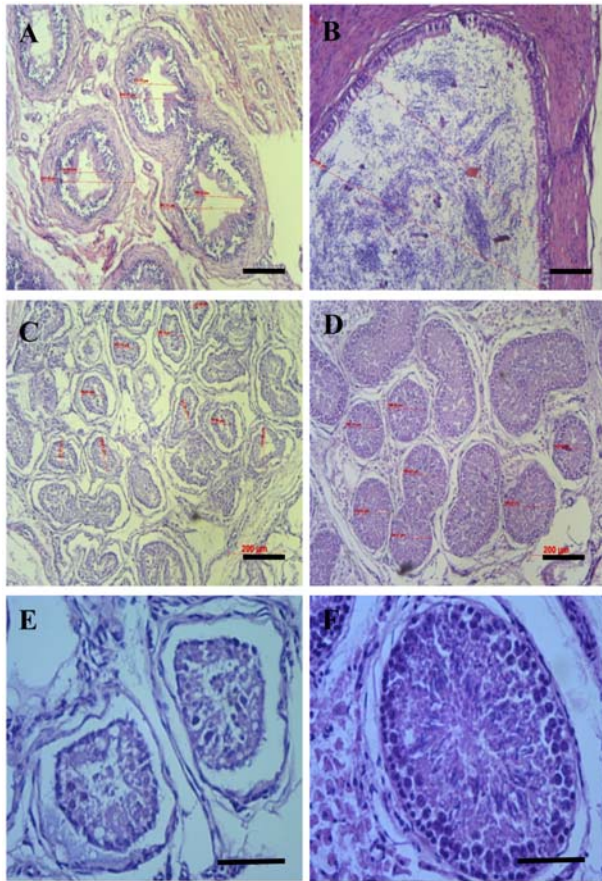


Fig. 1. Histology of testes showing ductus epididymides and seminiferous tubules. (A) Diameter of ductus epididymides of the immunocastrated group. (B) Diameter of ductus epididymides of the boar (non-castrated) group. (C) Diameter of seminiferous tubules of the immunocastrated group. (D) Diameter of seminiferous tubules of the boar (non-castrated) group. (E) Leydig cells and spermatocyte in seminiferous tubules of the immunocastrated group. (F) Leydig cells and spermatocytes in seminiferous tubules of the boar group. H&E strain. 50 \times (A-D), 200 \times (E and F).

of age and ended when they were 24 weeks, a period of 11 weeks. There was no significant difference in growth performance among the groups ($p > 0.05$) (Table 4). Body weight gain and ADG of the immunocastrated group, however, showed a trend similar to that of the boar group. As for carcass quality, the back fat thickness of all the groups did not show any significant difference ($p > 0.05$), with an average for all groups of 2.5 to 2.6 cm.

Sensory evaluation

Sensory evaluation related to the use of GnRH vaccine has not previously been reported in the literature. In this study, the consumers consisted of a veterinary student and personnel of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand. Consumer assessment of odor and flavor of meat from the different groups was not significantly different ($p > 0.05$), but more than twice as many consumers rated the taste of the boar group as “dislike a lot” compared to the other two groups (21% for the boar group vs. 10.1% and 9.5% for the immunocastrated and surgically castrated groups, respectively ($p < 0.05$) (Table 5).

Discussion

The main objective of this study was to evaluate the effect of immunocastration of pigs with GnRH vaccine on the hormones progesterone and testosterone which indirectly affect boar taint and on testes function (physical characteristics and histology of testes). Unlike many other studies, this research directly assessed the effect on consumer acceptance of the meat through sensory evaluation (odor, flavor and taste of the meat).

The high GnRH antibody titer⁻¹ in immunocastrated pigs compared to the boar group and the surgically castrated group, especially after the second dose, is in accordance with several previous studies [6, 20, 21].

This study found that immunocastration successfully reduced hormones to levels similar to those observed in surgically castrated pigs. This finding is in accordance with studies by Dunshea *et al.* [6] and Zeng *et al.* [21] which used a similar protocol and GnRH dosage (about 400 μ g/dose). However, Zeng *et al.* [21] reported that some pigs in that experiment were unresponsive to the vaccine. The present study had one unresponsive immunocastrated pig (No. 9)

Table 4. The effect of GnRH vaccine on growth performance and back fat

Index (growth performance)	Boar (n = 9)	Immunocastrated (n = 9)	Surgically castrated (n = 10)	p value
Initial weight (kg)	63.77 \pm 1.95	60.77 \pm 1.76	62.20 \pm 1.83	0.539
Final weight (kg)	114.77 \pm 3.2	108.33 \pm 14.33	105.20 \pm 11.08	0.222
Body weight (kg)	51.00 \pm 2.96	47.55 \pm 4.69	43.00 \pm 2.26	0.258
Average dairy gain (g/d)	653.84 \pm 38.04	651.44 \pm 64.55	537.50 \pm 28.25	0.126
Back fat thickness (cm)	2.5 \pm 0.1	2.5 \pm 0.2	2.6 \pm 0.1	0.788

Difference significant at $p < 0.05$. Data are presented as mean \pm SEM.

Table 5. Effect of GnRH vaccine on sensory evaluation (percent)

	Boar (n = 122)	Immunocastrated (n = 122)	Surgically castrated (n = 122)	p value
Odor				
Dislike a lot	12.0	17.1	12.3	0.480
Dislike	43.0	46.7	37.7	0.419
Like	36.0	32.4	44.3	0.185
Like a lot	9.0	3.8	5.7	0.292
Flavor				
Dislike a lot	14.9	10.9	9.3	0.445
Dislike	34.7	40.0	30.8	0.365
Like	41.6	40.9	49.5	0.369
Like a lot	8.9	8.2	10.3	0.862
Taste				
Dislike a lot	21.0 ^a	10.1 ^b	9.5 ^b	< 0.05
Dislike	28.6	39.4	24.8	0.065
Like	41.9	37.4	48.6	0.265
Like a lot	8.5	13.1	17.1	0.180

^{a,b}Difference significant at $p < 0.05$. Value evaluation by percentage frequency of score.

which had high testosterone and progesterone concentrations and normal testes growth. The initial dose of Improvac vaccine had no apparent physiological effect on testes function which were normal both in size and testosterone concentration. However, 2 weeks after the second dose, testes size and testosterone concentration in pig No. 9 decreased markedly. The reduction of progesterone (produced by the adrenal gland) was similar to the reduction of testosterone concentration. Improvac vaccine inhibited GnRH which directly affected the release of progesterone, the precursor of testosterone, by the adrenal gland.

Testes size and weight were significantly reduced in the immunocastrated pigs in our study as was the case in other studies [6, 20, 21]. Histological examination of testes tissue showed that the testes of the immunocastrated pigs had undergone a decrease in diameter of the ductus epididymides and seminiferous tubules. Additionally, there was an absence of Leydig cells and spermatocytes within the seminiferous tubules. These results clearly indicate a significant effect on reduction of spermatogenesis. Turkstra *et al.* [18] reported that the use of the GnRH vaccine in ponies induces atrophy of the testes, leading to a failure of spermatogenesis and to sterility. In the present study, pigs immunized with GnRH vaccine also exhibited a decrease in aggression compared to the boar pigs.

In our study, both the immunocastrated and the boar pigs weighed more at slaughter than the surgically castrated pigs. Similar results were found by Dunshea *et al.* [6], Cronin *et al.* [5] and Jaros *et al.* [12], all of whom reported that there was no effect on the overall growth performance between immunocastrated pigs and surgically castrated pigs. However, Improvac vaccine has the added effect of improving growth performance during the growing-finishing period, *i.e.*,

after the second injection. This is probably due to higher feed consumption during the period and thus the reduced aggression from the mounting events combined with a reduction in aggressive mounting events [7]. Other research has reported that immunocastrated boars have better ADG than surgically castrated boars as a result of the anabolic effects being mediated by testicular steroids [9]. In our study, the amount of back fat of the immunocastrated pigs, the boar pigs and the surgically castrated pigs did not show a significant difference, unlike the findings of other studies, most of which reported that the back fat thickness of immunized pigs was lower than that of boars and surgically castrated pigs [6]. In fact, the decrease in the testosterone levels strongly affected was correlated with a significant increase in the feed intake of barrows [19]. The surgically castrated pigs consumed more feed than the boars. It has additionally been demonstrated that testosterone can promote muscle growth via the somatotropin axis and that it has a clear negative correlation to body-fat mass [17]. Moreover, back fat thickness is controlled by the endocrine system which is related to metabolic change (lipid metabolism) [15]. However, there are many other factors which affect carcass quality, *e.g.*, genetics, nutrients and management of feed, which may have had an effect on back fat in addition to differences in back fat measurement methods.

Sensory evaluation was conducted in three areas, odor, flavor and taste, unlike some other studies which investigated only odor and flavor [8]. The researchers felt that acceptability to the Thai consumer would depend on taste as well as odor and flavor. In this study, no significant differences were found in consumer ratings for odor or flavor among immunocastrated meat (IM), boar meat (BM) and surgically castrated meat (SM). In the case of taste, however, ratings differed sig-

nificantly, with BM rated lower than both IM and SM ($p < 0.05$). These results still fail to clearly indicate the difference between the BM and the other meat. These results differ from Font i Furnols *et al.* [8] which reported that the odor and flavor of BM was less acceptable to consumers than SM and IM meat as was female meat (FM). Other studies have reported results similar to our findings, *i.e.*, no difference in the acceptability of BM, SM and FM meat. Many factors affect consumer acceptance of the odor and flavor of BM meat such as the level of boar taint, androstenone or skatole [10]. In addition, the quantity of fat in the meat and the cooking process used likely also affect the opinion of consumers [14]. Both androstenone and skatole levels, which have maximum fat, impacted the strength of boar taint and, thus, acceptability. Font i Furnols *et al.* [8] reported that boar taint affected consumer acceptance, stating that if the level of androstenone and skatole from the meat were more than 1 $\mu\text{g/g}$ and 0.1 $\mu\text{g/g}$, respectively, the meat would be considered unacceptable. In our study, the samples of BM probably had low levels of androstenone and skatole or a low level of the subcutaneous fat. Furthermore, the acceptability of pig meat also depends on factors like the gender, age and culture of consumers in different countries. The results of this study do not provide an unequivocal sensory evaluation for the odor and flavor of the meat. The sensory evaluations in this study may be specific to Thailand and the Thai culture.

This study supports the idea that immunocastration can be used in piglet management as an alternative “castration” process for swine management in Thailand. Immunization against GnRH can decrease levels of the hormones progesterone and testosterone, which are the precursors of androstenone and skatole which affect levels of boar taint. Moreover, immunocastration reduces the size of the testes and the aggressiveness of the male pig, making it similar to a surgically castrated pig or a female pig, and this is detrimental to animal welfare. Those effects indicate a reduction in the reproductive function. Although the results regarding growth performance and carcass quality still remain inconclusive, the Thai consumer is probably able to differentiate, based on taste, between boar meat and immunocastrated meat or surgically castrated meat. Further studies need to be conducted employing consumers who have been specifically trained in sensory evaluation to more fully understand consumer attitudes towards immunocastrated meat.

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