



Behavior of immunocastrated pigs

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ABSTRACT - The objective of this study was to evaluate the behavior of immunocastrated male pigs compared with females and castrated males during the period before and after full immunization. A total of 30 animals were divided into three treatments, with ten animals in each (females, barrows, and immunocastrated males). The experiment was divided into three periods: 70 to 80 days of age (period 1), 81 to 110 days of age (period 2), and 111 to 140 days of age (period 3). The behavior of animals during these stages was evaluated. Immunocastrated males showed a higher rate of aggressive and sexual behavior during period 2, which decreased after the second vaccine dose. Both barrows and immunocastrated males presented high locomotion in period 1, reducing the frequency of this activity in period 3. All analyzed animals had a higher level of activities such as drinking, playing, and sexual behavior in period 1 than in the other periods, decreasing during the experiment. The remaining behavioral responses did not differ between the studied categories. Immunocastrated males had higher proportions of undesirable behaviors (aggressive and sexual) related to the surgically castrated males and females, and these were reduced after the second vaccine dose. Immunocastration is effective in the reduction of behaviors such as agonistic and sexual at the same levels observed in females and surgically castrated males. However, immunocastrated pigs are more subject to these undesirable behaviors before full immunization.

Key Words: immunocastration, sexual activity, sus scrofa, swine production, welfare

Introduction

Surgical castration has long been used as a single alternative to eliminate the taint of meat from male pigs, which is improper for consumers (Martins et al., 2013). Other indications for the castration procedure include the reduction of aggressive and sexual behavior and easeness in the management tasks during production (Thun et al., 2006). Studies have shown that surgical castration causes stress, acute and chronic pain, wound infections, and depression in weight gain (Prunier et al., 2006), making it a questionable and avoided procedure in some countries (Jaros et al., 2005). However, the animal welfare cannot be improved merely by omitting the orchietomy since male pigs have high levels of testosterone that exerts substantial behavioral effects (Rydmer et al., 2010).

The testicular function can be inhibited by the active immunization of male pigs against gonadotropin-releasing hormone - GnRH (Baumgartner et al., 2010). Therefore, the immunocastration is considered a feasible alternative as it is a practice that does not cause acute pain to animals, reducing stress (Martins et al., 2013). The immunocastration vaccine induces the formation of antibodies against GnRH, a neuropeptide that is released from the hypothalamus to stimulate the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn regulate the production of testicular steroids (Rydmer et al., 2010). These antibodies bind to the endogenous GnRH, preventing the secretion of LH and FSH from the pituitary gland, reducing the secretion of testicular steroids (Weiler et al., 2013). It has been proved that the vaccine against GnRH reduces the concentration of testicular steroids, including androstenone and skatole, lessening the size of the reproductive organs and the quantity of sperm (Jaros et al., 2005; Einarsson et al., 2009; Batorek et al., 2012).

However, few studies have focused on the behavioral consequences and welfare aspects of immunization of male pigs (Prunier et al., 2006; Baumgartner et al., 2010), since the administration of the second dose of the vaccine makes

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them behave as hogs. Thus, the objective of this study was to evaluate the behavior of immunocastrated male pigs compared to females and surgically castrated pigs during the period before and after the full immunization.

Material and Methods

This investigation was carried out according to the guidelines established by the Ethical Committee on the Animal Use in Experimentation of the Universidade Federal da Grande Dourados (Brazil), under the case No 031/2013, by the ethical principles of animal experimentation, care, and use.

The experiment took place on a commercial farm in Dourados, Mato Grosso do Sul, Brazil (latitude 22°16'30" S and longitude 54°49'00" W). The climate is considered as Cwa according to Köppen classification (humid mesothermal climate with hot summers and dry winters) with average annual temperature of 23.6 °C.

The pigs (Large White × Landrace) in the trial had their origin on a farm without breeding program, presenting medium genetic potential (Rostagno et al., 2011). A total of 30 piglets were selected from the nursery phase (70 days of age) with an average weight of 25.2±2.8 kg. The animals were distributed in a completely randomized design of three treatments (T1: female, T2: surgically castrated males, and T3: immunocastrated males), each treatment housed in a single pen. Each treatment had ten pigs, but only five animals were randomly selected for the behavioral observation and each animal was considered as an experimental unit.

Males from the surgically castrated treatment were subjected to orchiectomy on the seventh day of age. The procedure was done without anesthetic, having both testicles removed after local disinfection, with one transversal incision of the scrotum with a scalpel, and cutting the spermatic cords. After this procedure, repellent and healing cream was applied to the incision wound.

The chosen piglets for immunocastration were vaccinated using two dosages of Vivax®, which contains a modified form of GnRH protein conjugate in an aqueous adjuvant system. The first dosage (2 mL/pig injected subcutaneously just behind and below the base of the ear) was applied 60 days before slaughter (80 days of age) and the second dosage, 30 days before slaughter (110 days of age) (Figure 1).

All piglets were reared under the same experimental conditions, in a conventional swine housing (20 m × 8.0 m), ceilings of 4.0 m, east-west orientation, masonry walls, and clay tiles. The house consisted of concrete floor pens on the front area and a shallow pool in the rear area. The open

sidewalls were 1.55 m high and had polypropylene curtains for environmental control. The pens had a herd density of 1.0 m²/pig and semi-automatic feeders and drinkers were available.

The study lasted 70 days including the phases of growing (70 to 110 days) and finishing (110 to 140 days). All pigs were fed the same diet (Table 1), using the nutritional recommendations by Rostagno et al. (2011) for hogs during growth and females during the finishing phase. Diets and water were given *ad libitum* during all the experimental period.

The study was subdivided into three periods: period 1, 70 to 80 days of age (before the application of the first dose of GnRH protein conjugate on T3); period 2, 81 to 110 days of age (between the application of the first and the second dose of GnRH protein conjugate on T3); and

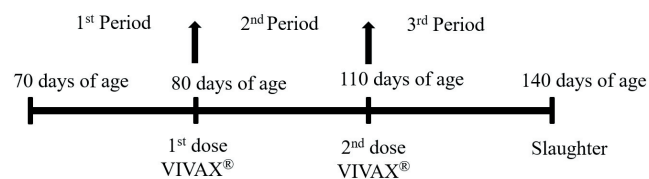


Figure 1 - Schema of the immunocastration protocol.

Table 1 - Centesimal diet composition and nutritional characteristics for growing and finishing pigs

	Growing* (%)	Finishing** (%)
Ingredient		
Soybean meal	27.60	22.00
Corn	68.50	75.30
Soybean oil	0.50	0.13
Salt	0.40	0.35
Dicalcium phosphate	1.40	1.00
Limestone	0.71	0.60
L-lysine	0.40	0.25
DL-methionine	0.10	0.05
L-threonine	0.14	0.07
Vitamin/mineral mix	0.25	0.25
Calculated nutritional values		
Metabolizable energy (kcal/kg)	3229.10	3236.59
Crude protein (%)	18.4	16.2
Ether extract (%)	3.46	3.25
Crude fiber (%)	2.65	2.47
Calcium (%)	0.70	0.55
Available phosphorus (%)	0.36	0.28
Sodium (%)	0.18	0.16
Chlorine (%)	0.29	0.26
Lysine (%)	1.44	0.89
Methionine (%)	0.35	0.28
Methionine + cystine (%)	0.62	0.52
Threonine (%)	0.74	0.61
Tryptophan (%)	0.19	0.16

* Recommendation for boars.

** Recommendation for females, according to Rostagno et al. (2011).

period 3, 111 to 140 days of age (after the application of the second dose of GnRH protein conjugate on T3).

The pigs were identified by numbers in the lumbar area, made with a marker with ink stick. The behavioral assessment was carried out by direct observation, always made by the same observers. The number of animals and the activities performed during the three periods were noted on a worksheet. Each period consisted of three days of observation, distributed at the beginning, middle, and end of the period. The behavior was rated in 5-min intervals along 8 h every day (7.30 to 15.30 h), according to Martin and Bateson (2007). Trained observers were placed inside the house in a way not to interfere with the behavior of pigs. The observers were rotated between treatments to avoid the effect of the observer. A table was built with the observations, characterizing the proportions of time spent in each behavior. Adjustments from previous research were made to describe the behavior of pigs (Campos et al., 2010; O'Connell et al., 2004; Pandorfi et al., 2006) (Table 2).

The behaviors standing still, lying awake, and sitting were considered the same at the time of statistical analysis and were identified as idleness. The sexual behavior, rooting other swine, and the agonistic behavior were seen as undesirable behaviors.

Statistical analysis was conducted using a nonparametric test for data repeated in time in a factorial model schema. The R software (R Core Team, 2015) was applied using the nparLD package with LD-F2-F2 function (Noguchi et al., 2012). In this study, 15 animals were stratified into three

periods: period 1, period 2, and period 3 (Factor T1). In each stratification, the animals were assigned to treatments as follows: A1 = females, A2 = castrated males, and A3 = immunocastrated males (Factor A).

The scores of the observed data for each animal were recorded at 97-time points per day (Factor T2) to evaluate the effect of the time, treatment, and their interaction. Independent random vectors can describe the statistical model of this test. The null hypothesis of the main effect A, the main time T1 effect, and the interaction between A and T1 (AT1) are expressed regarding the marginal distribution functions:

$$H_0^F(A): \bar{F}_1 = \dots = \bar{F}_a.$$

$$H_0^F(T1): \bar{F}_1 = \dots = \bar{F}_t.$$

$$H_0^F(AT1): F_{is} = \bar{F}_i - \bar{F}_s + F_{.s}, i = 1, \dots, a; s = 1, \dots, t,$$

$\bar{F}_i = \frac{1}{t} \sum_{s=1}^t F_{is}$ denotes an average distribution over time by group treatment i ; $i = 1, \dots, a$, $\bar{F}_s = \frac{1}{a} \sum_{i=1}^a F_{is}$ shows the average distribution in the treatment groups for the time point s ; and $s = 1, \dots, t$, $e \bar{F}_e = \frac{1}{at} \sum_{i=1}^a \sum_{s=1}^t F_{is}$ means the overall average and distribution.

To analyze the frequency of the behaviors, the average of the three days (291 points) was considered for all animals used in the treatment and each animal was regarded as an experimental unit. For this, the ANOVA was applied to compare the frequency of behaviors between treatments and periods.

Results and Discussion

There were differences between the treatments, periods, and their interactions (Table 3). Castrated males differed from females and immunocastrated males. By contrast, immunocastrated males and females did not differ. For the periods, there was a difference between periods 1 and 2, periods 1 and 3, but not between periods 2 and 3.

There were differences between treatments for the following behaviors: rooting other swine (RO), agonistic behavior (AB), and sexual behavior (SB). Concerning the period, there were differences between sleeping (S), commuting up (C), rooting the pen (RP), and sexual behavior (SB) (Tables 4 and 5).

Regarding the behavioral frequency, the pigs, regardless of their condition in all periods, remained most of the time sleeping and the highest frequency of this behavior appeared in period 2 and 3. In period 1, the pigs spent less time sleeping than in the other periods, probably due to

Table 2 - Observed behaviors of castrated and immunocastrated males and females during growing and finishing

Behavior	Description
Sleeping	Pig is lying, resting or sleeping on its lateral or belly.
Idleness	Pig is not doing activities. Pig is standing or lying still.
Commuting up	Slow walking movement.
Defecating/urinating	Pig is defecating or urinating.
Rooting the pen	Rooting the pen floor, lateral walls and around the feeder.
Rooting other swine	One pig rooting (any body part) or munching (tail and ear) another pig.
Agonistic behavior	Fighting, biting, or stretching other with the teeth. Giving or receiving head-knocks or bites. Head beating.
Sexual behavior	Mounting or being mounted.
Eating	Pig is with the head in feeder or waiting for food beside the feeder.
Drinking	Pig is with the mouth on the drinker ingesting water.
Playing	Pig galloping, sometimes in circular motion, moving around the body axis.

the initial exploratory behavior. For confined animals, it is expected that they spend most of their time resting or sleeping (Broom and Fraser, 2010). A range of dynamic behaviors, mainly rooting, is considered as a good indicator of motivation and welfare, since in the wildlife, boars usually forage to find and examine sources of food.

The behaviors eating, idleness behavior, defecating and urinating, drinking, and playing did not differ in the present study (Table 5). On the other hand, immunocastrated animals, regardless of the evaluation period, had a higher frequency ($P < 0.05$) of undesirable behavior (agonistic behavior, sexual behavior, and rooting other swine) than in the other treatments. Mounting, as a sexual characteristic, is part of the standard behavior of pigs. Hintze et al. (2013) found that a higher frequency of mounting (51.6%) was related to sexual behavior, and it lasted between 1 and 10 s. The authors suggest that sexual mounting causes more screaming by the mounted animal, indicating some distress and lack of welfare in the pigs. However, there was a gradual decrease in the incidence of sexual behavior during

the experimental periods, which can be justified by the fact that in period 1, the immunocastrated condition had not received the first dose of vaccine, thus behaving as boars. After the second dose, the animals were fully immunized, with a decrease in testosterone production, which reduced the frequency of sexual behaviors. Although boars are more aggressive than castrated males, the expression of the undesirable behaviors, in the present study, can be related to the lack of environmental stimuli in the pen.

According to Cronin et al. (2003) and Rydhmer et al. (2006), boars express more sexual behavior than castrated pigs, regardless of the method of castration. The influence of the immunocastration on the testosterone concentration in male pigs was studied by Albrecht et al. (2012). The authors observed that immunocastrated males showed a similar level of testosterone as in boars. After the second dose of the vaccine, this level decreased, bringing a significant reduction in the agonistic and sexual behaviors, comparable to those levels of castrated pigs.

According to Andersen et al. (2000), the agonistic interactions are a means of defining the social hierarchy in the collective stall after mixing the groups. The observations made in this study corroborate Baumgartner et al. (2010), who observed an increased incidence of agonistic behaviors in immunocastrated pigs before the first vaccination, compared with the pigs castrated surgically, and decreased after receiving the second dose.

The more aggressiveness present in boars, as well as a higher incidence of sexual behavior, may suggest an insufficiency on the use of immunocastration, since it caused a decrease in the welfare status, and it may impair the meat and carcass quality. However, over the experimental period, there was a decline in the incidence of these behaviors, showing that the vaccine, besides preventing the occurrence of boar taint, is effective in reducing the unwanted behaviors. Thus, the most critical period to the

Table 3 - Results of statistical analysis for the categories, periods, and their interactions by nonparametric factorial conducted by nparLD package: F2-LD-F2 function

Situation	DF	P-value
Treatments	2	0.428542e-13
Periods	2	0.999067e-14
Treatment × Period	4	0.887495e-02
Female × Castrated	1	0.386758e-13
Female × Immunocastrated	1	0.492207e-01
Castrated × Immunocastrated	1	0.456405e-09
P1 × P2	1	0.225524922
P1 × P3	1	0.823273e-02
P2 × P3	1	0.454838e-01

DF - degrees of freedom.

P1 - Period 1 - prior to application of the 1st dose of the immunocastration vaccine (70-80 days old); P2 - Period 2 - between the 1st and 2nd application of the vaccine dose (80-110 days old); P3 - Period 3 - after application of the 2nd dose of immunocastration vaccine (110-140 days old).

Table 4 - Frequency (%) of the observed behaviors for treatments and periods

Category	Behavior frequency (%)										
	S	I	C	D/U	RP	RS	AB	SB	E	D	P
T1	54.60	18.75	3.00	0.44	9.30	1.40b	0.61ab	0.10b	10.10	1.43	0.27
T2	54.01	18.43	2.90	0.45	10.36	1.70b	0.35b	0.30ab	10.10	1.20	0.20
T3	55.98	17.94	2.97	0.45	9.25	3.80a	1.47a	0.82a	10.40	0.42	0.30
Period											
1	47.20b	19.59	3.92a	0.39	12.6a	1.89	0.84	0.77a	11.38	1.12	0.30
2	54.80a	16.81	2.68b	0.59	7.90b	3.25	0.98	0.30ab	11.17	1.48	0.04
3	55.90a	18.28	2.33b	0.42	9.10b	1.80	0.61	0.16b	10.70	0.59	0.11

S - sleeping; I - idleness; C - commuting up; D/U - defecating/urinating; RP - rooting the pen; RS - rooting other swine; AB - agonistic behavior; SB - sexual behavior; E - eating; D - drinking; P - playing.

T1 - females; T2 - surgically castrated males; T3 - immunocastrated males.

Period 1 - 70-80 days old; Period 2 - 80-110 days old; Period 3 - 110-140 days old; 100% = 8 h. Means followed by different letters in the column differ statistically by Tukey's test ($P < 0.05$).

Table 5 - Average frequency (min±SD) of the observed behaviors over a day of observation for all treatments

Behavior (min±SD)	Treatment		
	T1 (n = 10)	T2 (n = 10)	T3 (n = 10)
Sleeping	248.55±3.09	260.34±47.97	261.56±31.61
Idleness	91.45±10.01	90.56±7.24	77.12±12.30
Commuting up	14.56±2.01	14.39±7.58	14.35±7.31
Defecating/urinating	2.23±0.76	2.56±0.18	2.34±1.15
Rooting the pen	52.23±4.62	46.47±23.78	44.71±6.20
Rooting other swine	9.34b±0.88	6.78b±1.01	16.33a±6.96
Agonistic behavior	3.01ab±1.66	1.43b±0.19	7.12a±3.03
Sexual behavior	0.23b±0.38	1.45ab±2.21	4.01a±3.05
Eating	50.22±10.59	48.01±10.39	50.56±8.06
Drinking	6.95±5.00	7.01±2.18	1.56±1.50
Playing	1.23±1.34	1.00±1.20	0.34±0.33

T1 - females; T2 - surgically castrated males; T3 - immunocastrated males.
SD - standard deviation.

Means followed by different letters in the rows differ statistically by Tukey's test (P<0.05).

animal welfare would be the stage at which pigs begin to express their sexual characteristics when they received the second dose of vaccine, about 30 days before slaughter.

Velarde et al. (2008), Rydhmer et al. (2010), Baumgartner et al. (2010), and Andersson et al. (2012) assessed the effect of the immunocastration vaccine on the sexual and aggressive expression in the behavior of male pigs. The authors found that the behavior is modified after the second dose of the hormone vaccine with a reduction in sexual behavior compared with boars, at levels similar to surgically castrated pigs, and that these changes remained until slaughter. According to Claus et al. (2007), until the second dosage, the hypothalamic-pituitary-gonadal axis of vaccinated animals remains intact, even providing behavior of boars.

Fabrega et al. (2010) compared the performance and behavior of immunocastrated pigs and surgically castrated males and found a reduction of the activities of immunocastrated males at three days after the first dosage of the vaccine. The authors suggest that vaccination against GnRH might be associated with improvements in well-being during sexual maturity, as it reduces sexual activity and aggressive behavior, along with the productive benefits such as better growth rate and feed conversion.

The higher frequency of rooting other animals by immunocastrated animals suggests some association with sexual behavior. Behaviors such as sniffing or tweaking the genital area, riding accompanied by pelvic movements, and accepting to be mounted have been observed in pigs before puberty. Sex play may be related to the sexualization process and is more frequent among males than in females (Berry and Signoret, 1984).

All animal categories spent more time commuting up in period 1, reducing the frequency of the activity in

subsequent periods. The occurrence of exploratory behaviors early on can be justified by the need to recognize the place, decreasing during the day due to housing habituation. Also, the fact that the animals were getting heavier over the periods may have contributed to their remaining more time in leisure. The frequency of locomotor activity has been identified as healthy and is used by young animals to establish social dominance (Donaldson et al., 2002).

Amongst the active behaviors, it was observed that the animals dedicated significant time to the exploration activity of the pen components (approximately 9.64%), similar to the time spend on food. This fact indicates that even pigs reared on a pen with a nutritionally balanced diet do not lack the motivation to explore and search for food (Beattie and O'Connell, 2002; Studnitz et al., 2007).

Conclusions

Immunocastration is effective in the reduction of behaviors such as agonistic and sexual at the same levels observed in females and surgically castrated males. However, immunocastrated pigs are more subject to these undesirable behaviors before full immunization.

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