










Immunocastrated female pigs' social and feeding behaviour

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ABSTRACT - This study compared the social and feeding behaviour of immunocastrated female pigs using different immunocastration protocols. We used seventy-two gilts from industrial crossbreeding (Agroceres × Topigs) at 15 weeks of age. The pigs were distributed in a randomised design with three treatments. The second dose of the immunocastration vaccine was applied in two groups, six weeks before slaughter (I6) and four weeks before slaughter (I4). A non-immunised group (NI) was used for comparison. Due to the divergence in the time of immunisation between the two groups, an individual comparison of each immunocastrated group with the control group (NI vs. I6 and NI vs. I4) was performed. Social behaviour was analysed through the collection of images by a monitoring camera, and the analysis period was from 6:00 to 18:00 h, with behaviour observed every ten minutes. Feeding behaviour data were collected by the automated FIRE® system. Anti-GnRH immunisation and the different protocols of application did not influence most social and feeding behaviours.



Keywords: feeding, gilts, immunocastration, pre-slaughter, welfare

1. Introduction

Slaughter weight is economically significant for producers and the swine meat industry by increasing the efficiency of the production process and reducing costs (Rodrigues et al., 2018). The definition of slaughter weight for males considers fat content and feed conversion (Vieira et al., 2004; Rosa et al., 2008), but for females, puberty is also considered. Because productive performance is negatively affected by puberty, females are slaughtered with a lower weight than their production potential (Hinson et al., 2012; Rodrigues et al., 2018).

The age at which gilts reach puberty depends on diet, male presence, and lineage (Lents et al., 2020). For example, female Meishan swine manifest sexual maturity between three and four months, while Duroc, Landrace, Large White, and crossbred animals do so between four and eight months of age (Legault et al., 1983; Neal et al., 1989). During oestrus, female pigs initiate a hormonal cascade, decreasing voluntary feed intake and increasing feed conversion ratio (Hinson et al., 2012; Rodrigues et al., 2019). To suppress oestrus, immunocastration is presented as an effective and non-invasive method. The immunocastration vaccine is an alternative technique to avoid oestrus, with two doses of an anti-GnRH vaccine, inducing the synthesis of anti-GnRH antibodies and the

release of gonadotropins (Bohrer et al., 2014). This temporary involution of the reproductive system can help increase the slaughter weight in female pigs.

By observing animals' behaviour and comparing it with pre-established standards, it is possible to measure the individual state and make inferences about welfare (Broom and Fraser, 2010; Baptista et al., 2011). Few studies have examined the influence of the anti-GnRH vaccine on female swine behaviour. However, immunocastration increases weight gain (Van den Broeke et al., 2016), reduces aggressivity, and improves welfare (Di Martino et al., 2018).

The objective of this experiment was to compare the social and feeding behaviour of immunocastrated female pigs using different immunocastration protocols.

2. Material and Methods

The study was carried out in Santa Maria, Rio Grande do Sul, Brazil (latitude: 29°41'29" S, longitude: 53°48'3" W, elevation: 139 m). The procedures adopted in this experiment were in accordance with the provisions of Federal Law No. 11,794 of 8 October 2008 and Decree No. 6,899 of 15 July 2009, under Case No. 5495110619 of the local Ethics Committee of Animal Use (CEUA).

2.1. Animals

The experiment used 72 industrial crossbred female pigs (Agroceres × Topigs) with an initial body weight of 60±6.47 kg and an age of 105 days. At the end of the experiment period, the animals were 173 days old and weighed 128±11.74 kg.

2.2. Experimental design and installations

The animals were housed in six pens, each with 15 m² of free area (1.25 m² per female), in a facility with a height of 3.2 m that was curtain-sided, had a concrete floor and an aluzinc with polystyrene roof, and without a light programme. Each pen was equipped with two bite-ball drinkers and an automated Feed Intake Recording Equipment (FIRE[®]) feeding system. The females received feed and water *ad libitum*. Ambient conditions were maintained for thermal comfort; the temperature showed a minimum of 15.2 °C and a maximum of 33.9 °C, with an average of 22.6 °C. Gilts were not exposed to mature boars in the experimental period, but oestrus signs were checked twice a day (6:00 and 14:00 h) by reflex test at lumbar pressure.

At the beginning of the experiment, the females were weighed and distributed in three treatments with 24 repetitions: NI—non-immunocastrated females (control treatment); I6—immunocastrated six weeks before slaughter; and I4—immunocastrated four weeks before slaughter. Immunocastration was carried out by the application of 2 mL Vivax[®] (Zoetis, São Paulo, SP, Brazil), following the protocol of two doses, with an interval of two weeks between doses (first dose at 105 and 119 days of age for I6 and I4, respectively; and second dose application at 119 and 133 days of age for I6 and I4, respectively). The weeks before slaughter for treatments was defined considering that the immunocastration had no effect until 10 days before the second dose vaccine application (Millet et al., 2011). To evaluate the effect of immunocastration on social and feeding behaviour, data were divided into two periods: before (i.e. at application of the first dose until the second dose), females with 105 and 119 days of age for I6 and I4, respectively; and after (i.e. two weeks after second dose application), females with 133 and 147 days of age for I6 and I4, respectively.

A completely randomised design was used to analyse the social and feeding behaviours of female pigs, as follows:

$$Y_{ij} = \mu_i + T_j + \varepsilon_{ij}$$

in which Y_{ij} = behaviour response; μ_i = overall mean value; T_j = treatment group effect; and ε_{ij} = random error. The variables had a normal distribution, and the comparison between treatments was performed using the Student's t-test with a 5% significance level.

2.3. Analyses of social behaviour

Social behaviour was recorded by monitoring cameras positioned above each pen. We used a total of 24 females, four from each pen, individually identified on the back and sides with a marker stick. The period of behaviour analysis was 12 h, from 6:00 to 18:00 h, 14 days before (before period) and 14 days after (after period) the second vaccine dose. Observations were made every 10 min using a focal sampling methodology (Lehner, 1992), totalling 72 scan samples per day for each animal. Data regarding each behaviour were determined as a percentage of daily observation scan samples (i.e. number of scan samples of behaviour \times 100/72) according to the methodology proposed by Rydhmer et al. (2010) and Machado et al. (2017). The observed behavioural patterns were as follows: eating—presence on the feeder, not necessarily eating; drinking—presence on the drinker, not necessarily drinking water; resting—right or left lateral decubitus, eyes closed or open; excretion—defecate and/or urinate; sitting—animal with the back legs bent and front legs supported on floor; rooting—exploring the environment with snout; standing—animal with four legs supported on floor; interacting—interaction without aggression, such as smelling another pig and racing; aggressivity—any agonistic interaction, such as fights, disputes, chases, and bites; and disturbed behaviour—repetitive sucking or biting behaviour on any part of the body or pen.

Given that the application of the second dose of the vaccine occurred on different dates, comparisons were made between the control group and each of the treatments individually: females immunocastrated six weeks before slaughter (I6) versus non-immunocastrated (NI vs. I6) and immunocastrated four weeks before slaughter (I4) versus non-immunocastrated (NI vs. I4). Subsequently, the data were divided to compare the effect of the immunocastration vaccine related to the application of the second vaccine dose, being grouped in before (two weeks before the second dose) or after (two weeks after the second dose).

2.4. Analyses of feeding behaviour

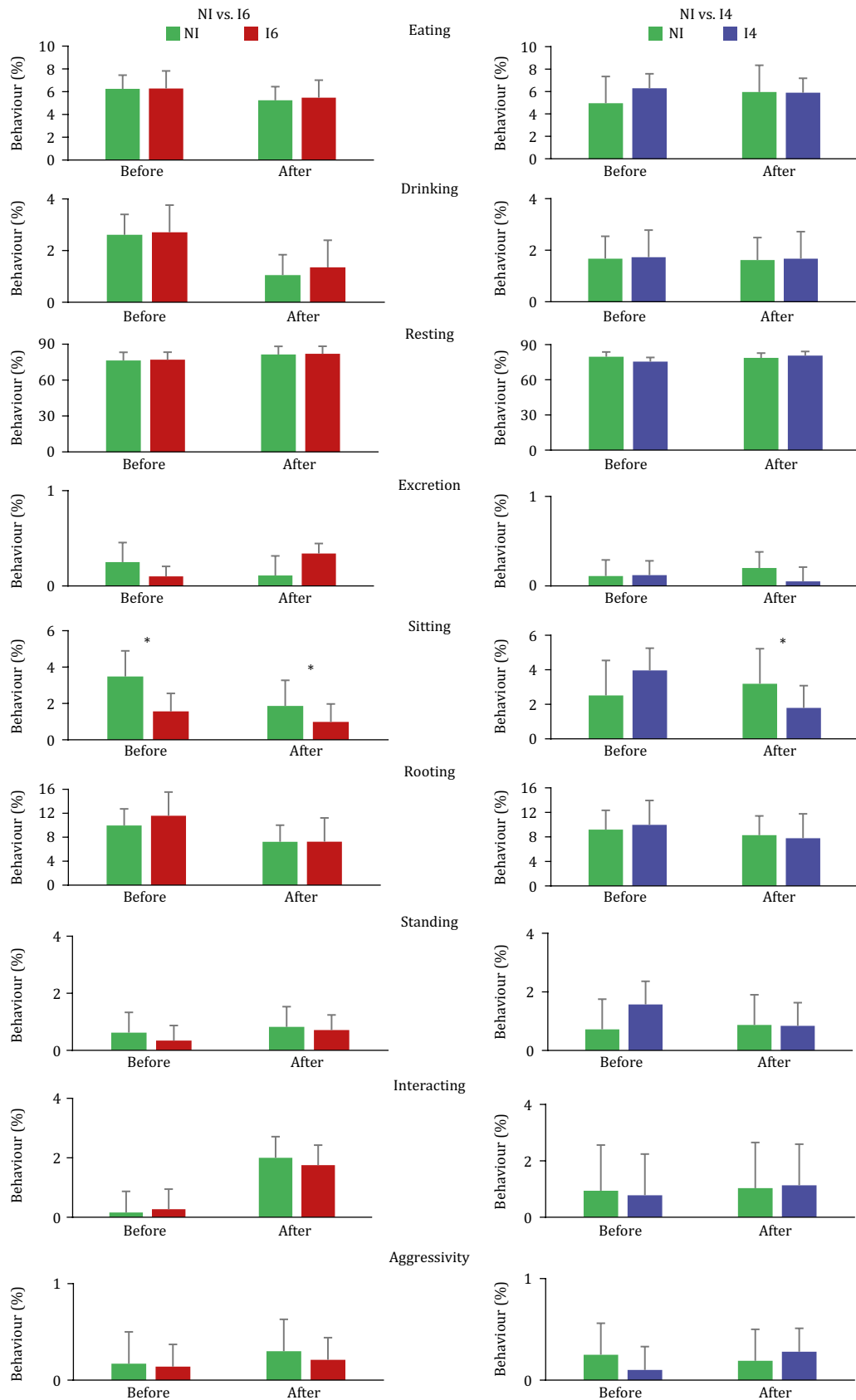
Feeding behaviour data were collected for 24 h by the FIRE[®] system, which identifies animals by reading a chip on the ear. We used 72 females, 12 from each pen. The system stored the time of entering and exiting the feeder, the weight of the animal upon entering and exiting the feeder, and the feed consumed at each visit, allowing us to calculate the feed intake per meal (kg), the number of feeder visits, and the time at the feeder per meal (minutes). The data were revised, eliminating those concerning when an animal spent a lot of time in the feeder without consuming feed, or when it entered and exited in less than five seconds.

Data regarding feeding behaviour were also separated into two periods (before and after the second dose of immunocastration vaccine) and grouped for comparisons in NI vs. I6 and NI vs. I4.

3. Results

3.1. Social behaviour

In the results of the variables determining the social behaviour of NI vs. I6 and NI vs. I4 (Figure 1), the only difference was in sitting behaviour, influenced by the second dose immunocastration vaccine in NI vs. I6 and NI vs. I4 ($P < 0.05$). Disturbed behaviours and oestrus signs were not registered in the experimental period.



Before: two weeks before second immunocastration dose, females with 105 and 119 days of age for I6 and I4, respectively.
 After: two weeks after second immunocastration dose, females with 133 and 147 days of age for I6 and I4, respectively.
 * Difference by Student's t test between the treatments.

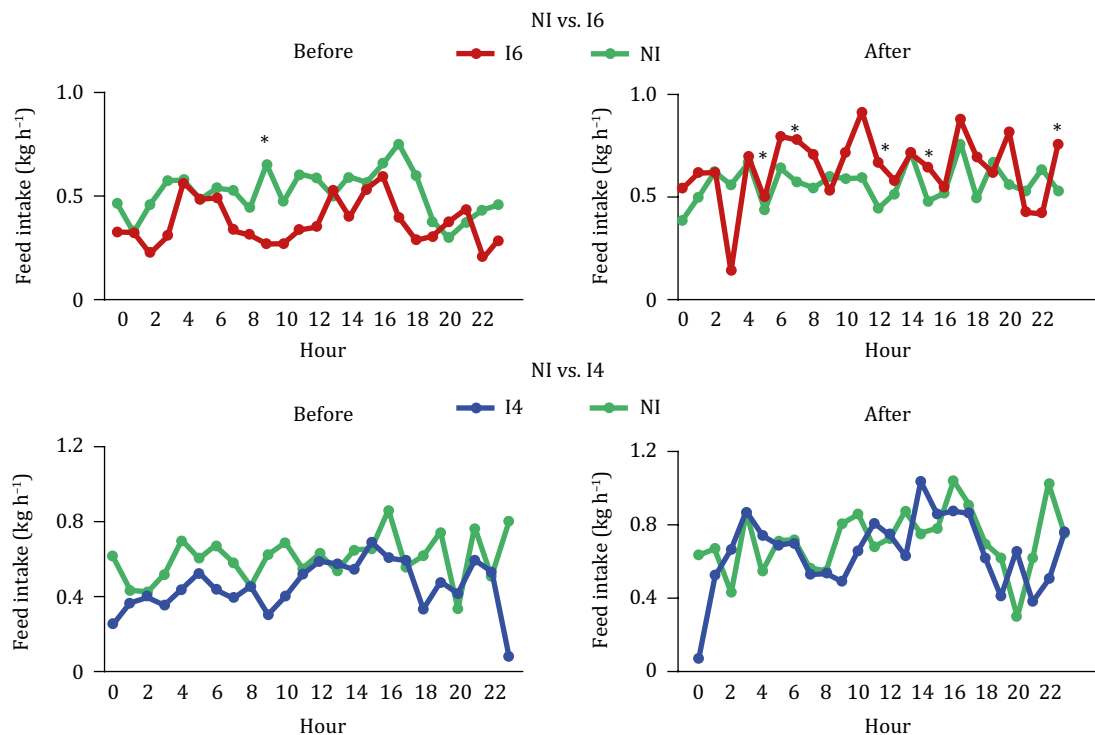
Figure 1 - Means and standard deviation of behaviour variables of female pigs non-immunocastrated (NI) vs. immunocastrated six weeks before slaughter (I6; NI vs. I6), and NI vs. immunocastrated four weeks before slaughter (I4; NI vs. I4), before and after second dose of immunocastration vaccine.

3.2. Feeding behaviour

The daily feed intake per meal of I6 vs. NI showed some differences ($P < 0.05$) after the second dose of immunocastration between 5:00 and 7:00 h, and at 12:00 and 16:00 h, with I6 consuming more feed (Figure 2). In contrast, NI vs. I4 presented no differences during the day.

The number of daily visits to the feeder in NI vs. I6 showed some differences ($P < 0.05$) before and after the application of the second dose of the immunocastration vaccine (Figure 3). Before, I6 presented fewer visits at 13:00 h and the highest number at 16:00 h. For the period after the second dose, the number of visits increased at 13:00 h, decreased at 15:00 h, and increased at 16:00 h. The greatest number of visits to the feeder occurred between 4:00 and 18:00 h for NI vs. I6 and NI vs. I4 (Figure 3), with no influence of immunocastration in this behaviour.

The average time spent per feeder visit (Figure 4) indicated longer meals in the morning period after the second dose of anti-GnRH vaccine for I6 and I4 when compared with the control treatment.



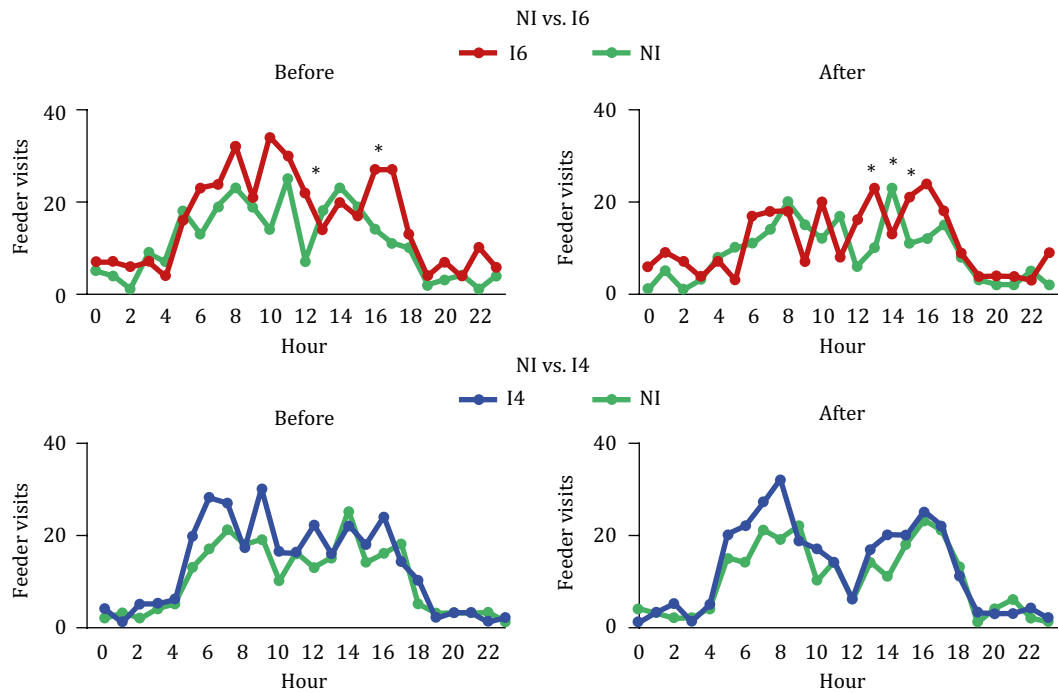
Before: two weeks before second immunocastration dose, females with 105 and 119 days of age for I6 and I4, respectively.
After: two weeks after second immunocastration dose, females with 133 and 147 days of age for I6 and I4, respectively.
* Difference by Student's t test between treatments.

Figure 2 - Feed intake per meal (kg h^{-1}) per hour of female pigs non-immunocastrated (NI) and immunocastrated six (I6; NI vs. I6) and four (I4; NI vs. I4) weeks before slaughter, before and after the second dose of immunocastration vaccine.

4. Discussion

4.1. Social behaviour

Analysing the social behaviour between NI vs. I6 and NI vs. I4, the most frequent activities were, in decreasing order, resting, rooting, and eating, representing about 94% of the total activities observed

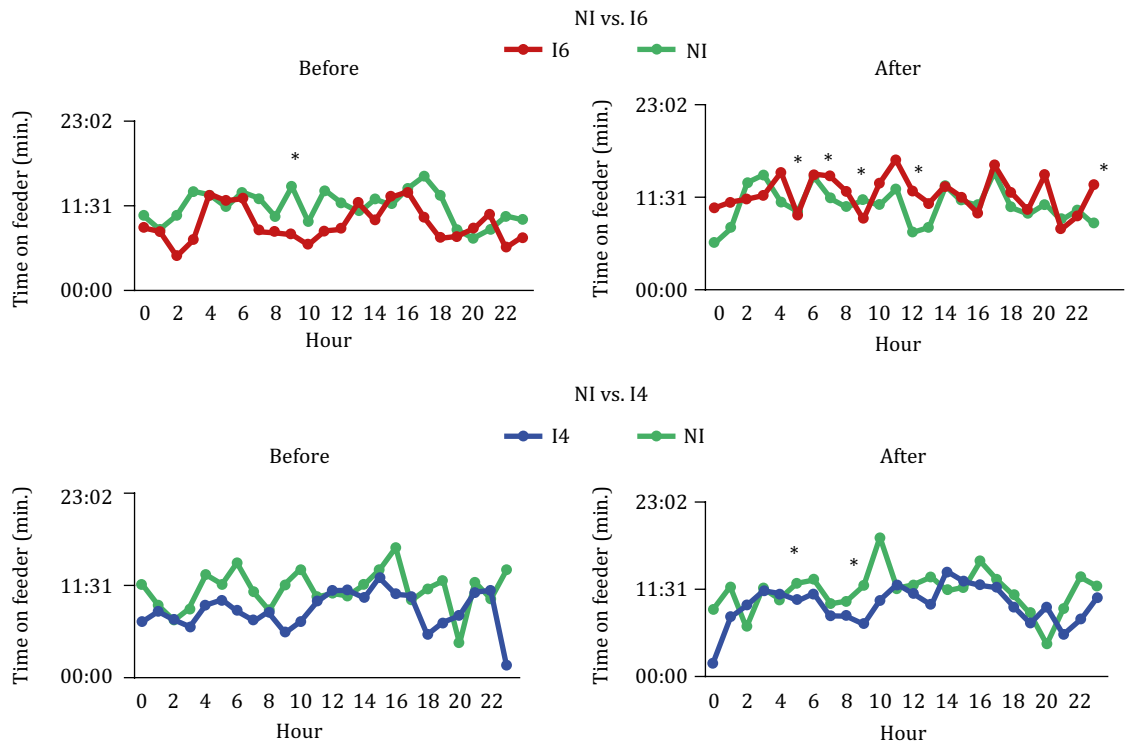


Before: two weeks before second immunocastration dose, females with 105 and 119 days of age for I6 and I4, respectively.

After: two weeks after second immunocastration dose, females with 133 and 147 days of age for I6 and I4, respectively.

* Difference by Student's t test between treatments.

Figure 3 - Total of daily feeder visits per hour of female pigs non-immunocastrated (NI) and immunocastrated six (I6; NI vs. I6) and four (I4; NI vs. I4) weeks before slaughter, before and after the second dose of immunocastration vaccine.



Before: two weeks before second immunocastration dose, females with 105 and 119 days of age for I6 and I4, respectively.

After: two weeks after second immunocastration dose, females with 133 and 147 days of age for I6 and I4, respectively.

* Difference by Student's t test between treatments.

Figure 4 - Time on feeder per meal (minutes) per day of female pigs non-immunocastrated (NI) and immunocastrated six (I6; NI vs. I6) and four (I4; NI vs. I4) weeks before slaughter, before and after the second dose of immunocastration vaccine.

in the period. Growing pigs showed a daytime rhythm of activity, composed of social interactions and exploratory activities, spending approximately 70% of their time inactive (Maselyne et al., 2014). The active period represented about 30% of the time and included movement, standing, and lying periods, food and water intake, and social and aggressive behaviours (Maselyne et al., 2014).

The act of rooting is an innate behaviour of pigs, but it was not affected by immunocastration in I6 and I4. Swine are intensely domesticated, and exploratory behaviour keeps them aware of the availability of resources essential for their survival when in natural conditions (Machado et al., 2017). Exploring habits depend on the environment; finishing pigs housed in enriched environments have a considerably increased activity frequency (Zwicker et al., 2012).

Immunocastration decreased the frequency that females spent sitting in NI vs. I4 after the second vaccine dose of vaccine ($P < 0.05$); this coincided with an increase in resting time (75.5% after and 80.6% before; $P > 0.05$) and aggressivity (0.1% after and 0.3% before; $P > 0.05$). In NI vs. I6, this behaviour was different before and after the application of the second vaccine dose, being higher for the NI group. In a study by Rodrigues et al. (2019), sitting behaviour was not affected by female immunocastration; however, they observed a decrease in standing time in immunocastrated females.

The interacting behaviour is related to young animals' pleasure and welfare (Massari et al., 2015). In the present study, there was no variation in the groups. Similarly, aggressivity was not influenced by immunocastration, perhaps because the slaughter age at 173 days in older females (more than 196 days) reduces aggressivity (Di Martino et al., 2018).

4.2. Feeding behaviour

The role of hormonal factors correlated with changes in feeding behaviour is relevant, as GnRH is responsible for triggering a series of events, including decreased serum progesterone and increased feed intake (Van den Broeke et al., 2016). When comparing the periods before and after immunocastration, I6 and I4 females increased feed intake two weeks after the second dose compared to NI because of the effect of immunocastration.

The highest frequency of feeder visits had a defined daytime distribution, from 4:00 to 18:00 h, similar to that reported by Gonyou et al. (1992) for housed pigs (between 8:00 and 16:00 h). After the second dose of the immunocastration vaccine, compared with NI, I6 pigs spent more time on the feeder than in the period before; this could be due to the increase in feed intake per meal without increasing the number of feeder visits for that period. The number of feeder visits and the time on the feeder were also influenced by body weight; feeder visits decreased, increasing feed intake per meal, resulting in a decrease in the time on the feeder (Hyun et al., 1997).

It is widely accepted that after the second dose of immunisation, male pigs reduce their aggression and sexual behaviour, increasing feeder visits and feed intake (Zamaratskaia et al., 2008; Weiler et al., 2013). In the same way, females are expected to redirect oestrus-related behaviours to other activities because immunocastration leads to the total suppression of reproductive cyclicity and reduced aggression (Dalmau et al., 2015; Di Martino et al., 2018). This suppression, including the levels of serum progesterone, and the involution of the ovaries and uterus increases the growth rate and feed intake after the second dose (Dunshea and McCauley, 2001; Gómez-Fernández et al., 2013; Dalmau et al., 2015; Van den Broeke et al., 2016; Batista Samaniego and Saavedra Arcia, 2017; Rodrigues et al., 2018).

The social and feeding behaviours in this study were not greatly affected by immunocastration; a possible explanation for this is that these pigs did not start their reproductive cycle within 133 days (I6) or 147 days (I4). Puberty in female swine usually occurs between 180 and 240 days of age (Lea and England, 2019), or between 131 and 201 days of age (Fontes and Rodrigues, 2014). The age at puberty is influenced by the lineage of animals (Lents et al., 2020). Considering the industrial crossbreeding used in this study, it is possible that at 133 and 147 days of age, oestrus did not manifest, which would explain the absence of differences in the results.

5. Conclusions

Immunocastration protocols with the application of a second dose six or four weeks before slaughter had no significant influence on behaviour variables in females slaughtered at 128 kg and 173 days old.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.R. Klein, D.C. Batista, A.D. Verardi and V. Oliveira. Data curation: D.R. Klein, D.C. Batista, J.S. Kunzler, J.M. Medeiros, R.S. Spagnol and H.C.M. Muniz. Formal analysis: D.R. Klein, A.D. Verardi and V. Oliveira. Funding acquisition: A.R.B. Quadros and V. Oliveira. Investigation: D.R. Klein, D.C. Batista, J.S. Kunzler, J.M. Medeiros, R.S. Spagnol and H.C.M. Muniz. Methodology: D.R. Klein, D.C. Batista, A.D. Verardi and V. Oliveira. Project administration: D.C. Batista, J.S. Kunzler and J.M. Medeiros. Resources: D.R. Klein, J.S. Kunzler, J.M. Medeiros, A.R.B. Quadros and V. Oliveira. Software: D.R. Klein and V. Oliveira. Supervision: D.R. Klein, D.C. Batista, J.S. Kunzler, J.M. Medeiros and V. Oliveira. Validation: D.R. Klein, D.C. Batista and V. Oliveira. Visualization: D.R. Klein and V. Oliveira. Writing-original draft: D.R. Klein, D.C. Batista, A.D. Verardi and V. Oliveira. Writing-review & editing: D.R. Klein and V. Oliveira.

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