agriculture

MDPI

Article

Evaluation of Behavioral Aspects after Intradermal and Intramuscular Vaccine Application in Suckling Piglets

Manuel Göller, Nicole Kemper and Michaela Fels *

Institute for Animal Hygiene, Animal Welfare and Farm Animal Behavior, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, D-30173 Hannover, Germany; manuel.goeller@tiho-hannover.de (M.G.); nicole.kemper@tiho-hannover.de (N.K.)

* Correspondence: michaela.fels@tiho-hannover.de; Tel.: +49-511-856-8954

Received: 30 October 2020; Accepted: 11 December 2020; Published: 15 December 2020

check for updates

Abstract: The aim of this study was to analyse the behavioral aspects of suckling piglets after an intradermal vaccination method in comparison to an intramuscular vaccination applied on the seventh day of life. Possible effects on piglet welfare should be evaluated. Under field conditions, 135 suckling piglets from 12 litters were vaccinated against *Mycoplasma hyopneumoniae*—64 of those intradermally and 71 intramuscularly, from six litters each. For behavioral analyses, videos were recorded per pen, starting three days before the vaccination and ending three days after the vaccination. In the video analyses, the observation periods 6.00–10.00, 13.00–17.00, and 19.00–21.00 were analysed via scan sampling for the behaviors lying, standing, walking, suckling, and social contact. In the behavioral observations, in all piglets, the most frequent behavior was lying, followed by suckling at the sow's teats. After vaccination, less lying behavior and more suckling behavior were assessed in intradermally vaccinated piglets compared to intramuscularly vaccinated piglets, which indicates that the piglets were not impaired by stress following vaccination. The results of this study showed that intradermal needle-free vaccination has clear advantages, as it caused fewer vaccination-associated behavior changes in suckling piglets than the intramuscular vaccination method with a needle.

Keywords: farrowing farms; health management; immune response; immunization; suckling piglets; behavior

1. Introduction

In modern pig husbandry systems, preventive animal health management is of the utmost importance. In addition to biosecurity measures, advanced vaccination programmes, taking into account the respective life stages of the animals, are one cornerstone for realizing optimal animal health. In veterinary medicine, numerous vaccines for pigs have been approved and registered for intramuscular injection. However, intramuscular administration can have some disadvantages, especially in large animal populations, for which the hygienically required needle change for at least each pen is not practicable. Moreover, especially for suckling piglets in the first days of life, vaccination can be a considerable strain, but can be obligatory when the infection pressure in the herd is high. For example, *Mycoplasma hyopneumoniae*, which is the causative agent of enzootic pneumonia, is present in many pig herds and, under the protection of maternal antibodies, suckling piglets are often already infected, though without initially developing symptoms. The vaccination of suckling piglets at the end of the first week of life ensures early protection against infection immunity [1]. In the absence of immune protection, the symptoms usually only become apparent after weaning. Affected animals show chronic coughing with a low mortality and high morbidity. They are significantly more susceptible to other serious pathogens due to damage to the ciliated epithelium of the respiratory tract. The infection causes major economic losses worldwide [2–4]. Therefore, numerous vaccines against *Mycoplasma hyopneumoniae* for pigs are commercially available, and most of them are injected intramuscularly. For reasons of labor economy and hygiene, alternatives to intramuscular injection were already sought over 20 years ago [5,6]. Intradermal vaccination represents a practicable option. Here, the vaccine is applied into the skin by means of compressed air and causes a strong immune reaction there. Therefore, no needles are used for intradermal vaccination, which would also solve the problem related to the environmentally friendly disposal of used needles. This method has been known in human medicine for over eight decades [7]. In veterinary medicine, the first studies on the efficacy of intradermal vaccination compared to the intramuscular application of vaccines in the fight against Aujeszky's disease were conducted in the 1990s [6]. For over 10 years, an applicator has been on the market in Germany, with which commercially available vaccines against *Mycoplasma hyopneumoniae*, Porcine Circovirus Type 2 (PCV2), and Porcine Reproductive and Respiratory Syndrome Virus (PRRS) can be applied needle-free intradermally. Studies have shown that intradermal vaccination is equivalent to intramuscular vaccination in terms of the induction of an adequate immune response and the resulting protection at significantly lower-dose volumes [8–10]. Previous studies have dealt not only with the induced immune response of intradermal vaccination, but also with the local vaccination reactions and the penetration depth of the applied drugs (e.g., [3,10]).

In addition, the risk of the cannula breaking during vaccination [5], as well as the risk of the hematogenic transmission of pathogens from animal to animal [11,12], are reduced. Moreover, the risk of injuries to the vaccinating persons from needle sticks can be lowered. The manner in which the vaccine is administered also has an impact on animal welfare, and the fact that intradermal vaccination is clearly beneficial in this respect was first shown for pregnant sows [13].

To date, detailed investigations of the effects of intradermal vaccination on the behavior of sensitive suckling piglets are lacking. The present pilot study aims to contribute to the understanding of the effects of vaccination application methods on the behavior of suckling piglets under field conditions.

2. Materials and Methods

The study was carried out on a commercial German pig farm, where 240 sows (BHZP Viktoria and Danbreed) were kept and managed in a three-week rhythm with a suckling period of 28 days. All of the animals were housed in accordance with EU and German law. The study was approved by the Animal Welfare Officer of the University of Veterinary Medicine, Hannover, Germany.

The pregnant sows were brought over to the farrowing unit one week before the expected farrowing. Per batch (i.e., every three weeks), a group of 30 sows was housed in a farrowing compartment. During the study period, the sows and their litters were kept in farrowing pens (197 cm × 259 cm) with crates for the sows on a partially slatted floor. The creep areas (47 cm × 152 cm) for piglets were equipped with floor heating and infrared heat lamps. The sows were fed twice a day automatically. Water was available ad libitum and the drinker was integrated into the feeding trough of the sow. From three days of age until weaning, piglets were given dry feed ad libitum in the farrowing pen. Three nipple drinkers per pen were available for the piglets. The farrowing unit was illuminated 12 h per day (from 8.00 to 20.00). Male piglets were castrated before the study began and all piglets were tail docked at the same time. Piglets were weaned at their 28th day of life.

Three batches were considered for the study. In total, data from 672 piglets from 59 sows were available and used to determine the animal health and performance, as described by Göller et al. [14]. For the present experiment, a subgroup of 135 piglets originating from 12 sows was considered. The study was based on practical preconditions. Therefore, all vaccines were applied in consultation with the farm veterinarian within the farm-specific vaccination scheme. In the experimental group, 64 piglets from six sows (two sows per batch in three batches) were vaccinated intradermally on the seventh day of life (Intra Dermal Application of Liquids (IDAL)-Vaccinator, MSD Animal Health/Intervet Deutschland GmbH, Unterschleissheim, Germany) against *Mycoplasma hyopneumoniae* (Porcilis M Hyo

ID ONCE, MSD Animal Health/Intervet Deutschland GmbH) using one-shot vaccination. For this purpose, the vaccine was administered needle-free into the skin with a dose volume of 0.2 mL at 46 bar compressed air.

In the control group, 71 piglets from six sows (two sows per batch in three batches) were vaccinated intramuscularly with the vaccine usually used on the farm (Stellamune One, Zoetis Deutschland GmbH, Berlin, Germany; one-shot vaccination). Hence, 2 mL of vaccine was injected intramuscularly with a vaccination gun (HSW ECO-MATIC® 2 mL, Henke-Sass, Wolf GmbH, Tuttlingen, Germany with a 20-gauge cannula). In both the experimental and control groups, the vaccinations were administered at the same location in the lateral neck on the left side of the body at the highest point of the base of the ear in a horizontal injection direction. Two persons carried out the vaccinations together; one person fixed the piglet and the second person vaccinated the animals.

For the present study, in each batch, piglets from two pens of the experimental and control groups, respectively, were subjected to a detailed video analysis. In total, the behavior of 12 litters, including six of the experimental group (litter size: 10.7 +/− 1.5 piglets) and six of the control group (litter size: 11.8 +/− 1.3 piglets), was analysed.

For video recordings, above each experimental and control pen, a camera (Everfocus, EQ550T, Taipeh, Taiwan) was installed and connected to a digital video recorder (Everfocus, ECOR 264-9X1, Taipeh, Taiwan), which recorded on hard drives. A total of 25 images were taken per second. Video-based behavioral analyses were carried out using the EverFocus Player Application (EFPlayer) 1.0.8.4 program (EverFocus, Taipeh, Taiwan) on a personal computer. Video recording started three days before the vaccination and ended three days after the vaccination. Therefore, videos were recorded continuously over seven days (4th–10th days of the piglets' life, Table 1). For video analysis, three observation periods were chosen per day: 6.00–10.00; 13.00–17.00; and 19.00–21.00. For all piglets, the vaccination was carried out outside the observation period on day 4 between 10:00 and 13:00. In each observation period, data were collected via scan sampling (instantaneous sampling). The video was stopped every five minutes and the number of piglets showing the following behaviors was counted: Lying; standing; walking; suckling; and social contact. Lying was defined as a recumbent position in which the piglet's body was not supported by the legs. Standing was assigned when the piglet was standing upright on its legs. Walking was defined as any form of locomotor behavior. Suckling was assigned when a piglet was in direct contact with the sow's teats, and social contact was defined as any form of body contact between different piglets, except for lying with huddling. For each observed litter and each observation moment (every five minutes), the number of piglets showing the defined behaviors was counted and converted into a percent based on the total number of littermates in the respective farrowing pen.

Table 1. Overview of observation days for video recordings, piglets' day of life, and time periods.

Day of Observation	Piglets' Day of Life	Time Period with Regard to Vaccination
1	4	3 days before vaccination
2	5	2 days before vaccination
3	6	1 day before vaccination
4	7	day of vaccination
5	8	1 day after vaccination
6	9	2 days after vaccination
7	10	3 days after vaccination

Statistical analysis was performed using IBM SPSS Statistics, Version 24. First, data (residuals) were checked for a normal distribution using histograms, Q-Q plots, and the Shapiro–Wilk test. As data were not normally distributed, and showed some outliers, non-parametric tests were applied. The Mann–Whitney U test for independent samples was performed to determine any significant differences between the experimental and control groups concerning the percentage of piglets showing different behaviors on the seven days of observation. Friedman's two-way analysis of variance for related samples was carried out to determine significant differences in the percentage of lying and suckling piglets over time (between days and daytimes) within the experimental and control groups. Pairwise comparisons were performed and *p*-values were adjusted by Bonferroni correction for multiple tests. Results were considered statistically significant if the related *p*-values were less than 0.05.

3. Results

3.1. Descriptive Results of Behavior in Suckling Piglets

Descriptive results of the behavioral analyses revealed that the most common body posture of piglets was lying. Over the entire study period, in experimental groups, an average of 53.0% of piglets within a litter were lying per observation moment, while in control groups, 58.6% of littermates were in a recumbent position per observation moment. The second most common behavior was suckling. In experimental groups, an average of 27.8% of littermates were suckling per observation moment, while in control groups, the percentage was 22.9%. The least common behavior was walking. Only 3.9% of piglets were walking per observation moment in experimental groups, while in control groups, 4.3% of piglets displayed locomotor behavior. The standing position was exhibited by an average of 8.3% of piglets per litter in experimental groups, and 7.6% in control groups, while social contact was observed in 5.2% and 5.4% of piglets per litter and observation moment, respectively.

3.2. Behavior of Piglets on Different Days of Observation

In the following, the three days before vaccination (days 1–3 of observation), the day of vaccination (day 4 of observation), and the three days after vaccination (days 5–7 of observation) are considered separately to reveal possible effects of the vaccination procedures (intradermally or intramuscularly) on the behavior of piglets. The results indicate that the piglets in the experimental and control groups showed similar levels of the studied behaviors on the three days before vaccination, i.e., at days 1, 2, and 3 of observation (Figure 1).

On the day of vaccination (day 4 of observation), significantly more piglets in the control groups were lying per observation moment compared to the experimental groups ($p < 0.0001$; Figure 1). Furthermore, in control groups, fewer piglets were observed at the sow's teats, i.e., were suckling per observation moment, than in the experimental groups ($p < 0.0001$). Concerning the other behaviors (standing, walking, and social contact), no significant differences were found between the experimental and control groups on the day of vaccination ($p > 0.05$).

On the three days after vaccination (days 5–7 of observation), there were still significant differences between the behaviors of piglets in the experimental and control groups. During the three days, fewer lying piglets and more suckling piglets were counted per observation moment in the experimental groups compared to the control groups ($p < 0.05$, Figure 2). No significant differences were found for the other behaviors between the experimental and control groups on the three days after vaccination ($p > 0.05$).

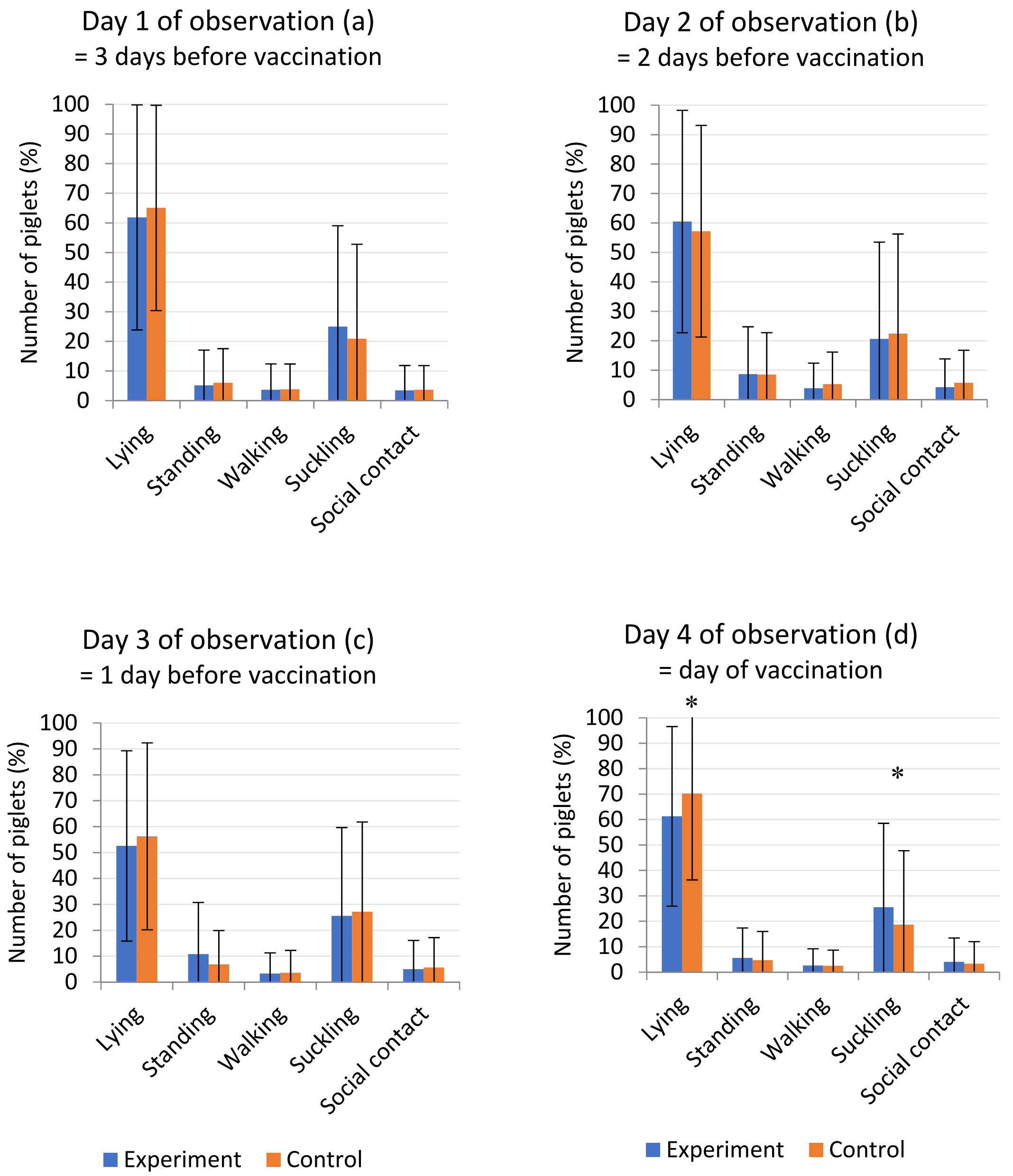

Figure 1. Percentage of piglets per litter (means and standard deviations) showing different behaviors in experimental and control groups on the three days before vaccination (**a**–**c**), as well as on the day of vaccination (**d**). * $p < 0.0001$.

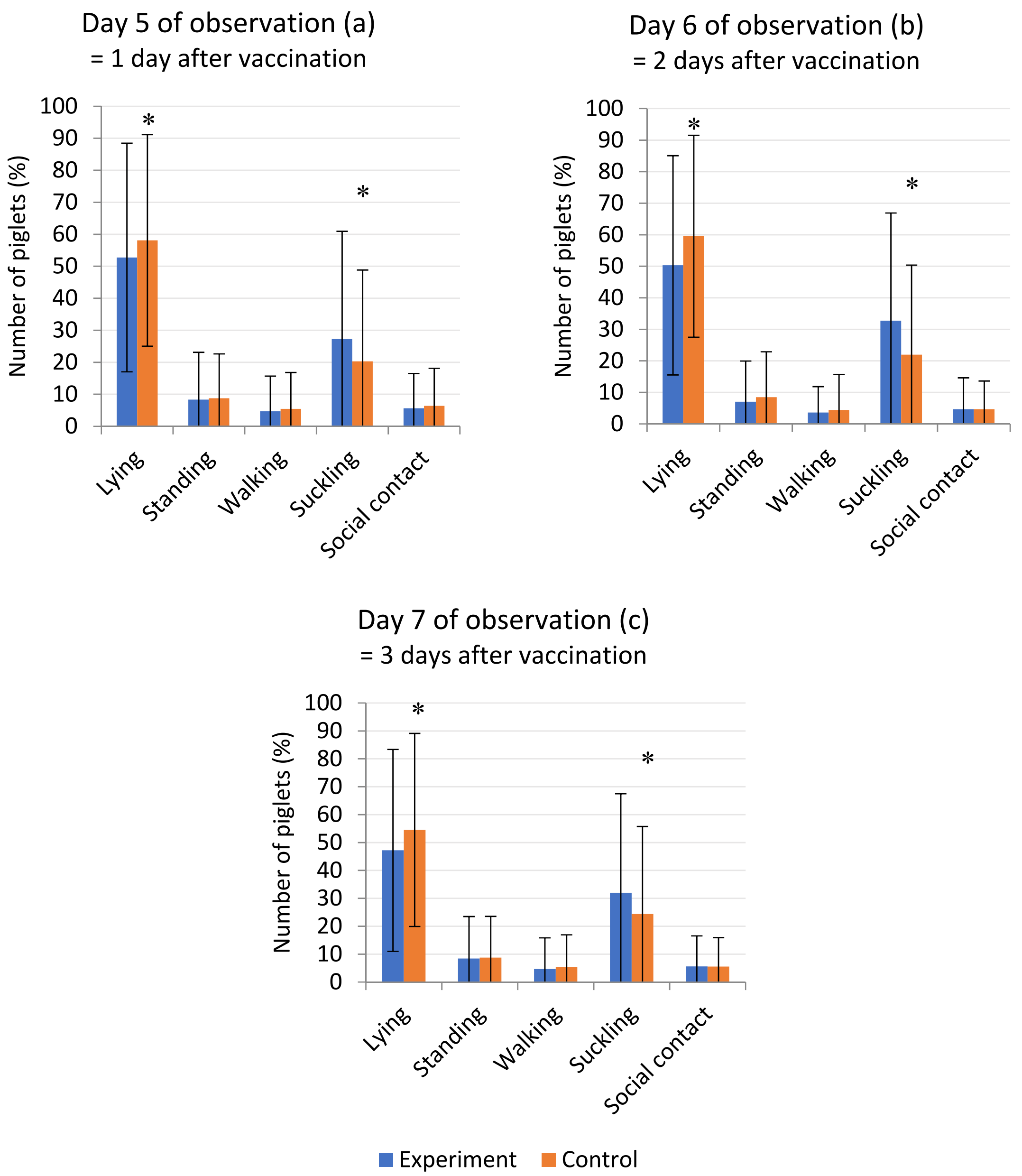

Figure 2. Percentage of piglets per litter (means and standard deviations) showing different behaviors in the experimental and control groups on the three days after vaccination (**a**–**c**). * $p < 0.01$.

3.3. Behavior of Piglets over Time

Considering the behaviors of lying and suckling in detail over time, it was shown that in both groups (experimental and control groups), there was a significant decrease in the number of lying piglets (%) from the first to third day of observation, i.e., during the three days before vaccination ($p = 0.003$ for the experimental groups and $p < 0.0001$ for the control groups; Figure 3). On the day of vaccination (day 4 of observation), the number of lying piglets increased in both groups compared to the day before vaccination ($p < 0.0001$); however, this increase was higher for the control groups than for the experimental groups ($p < 0.001$). On the day after vaccination, the number of lying piglets decreased again in both groups ($p < 0.0001$). However, during the three days after vaccination, there were still more lying piglets in the control groups than in the experimental groups ($p < 0.05$). No significant difference in the number of lying piglets was found between the first day after vaccination (day 5 of observation) and the third day after vaccination (day 7 of observation) in either the experimental groups or the control groups ($p = 1.000$; Figure 3).

Concerning the percentage of suckling piglets, a similar level was found on the three days before vaccination for the experimental and control groups (Figure 3). There was no significant difference between day 1 and day 3 of observation (i.e., before vaccination) in either the experimental groups ($p = 1.000$) or the control groups ($p = 0.319$). While the number of suckling piglets remained constant on the day of vaccination in the experimental groups ($p = 1.000$), it decreased significantly in the control groups ($p = 0.003$). On the three days after vaccination, the number of suckling piglets increased in both groups; however, there was still a higher percentage of suckling piglets in the experimental groups compared to the control groups ($p < 0.05$). While the number of suckling piglets increased slightly from day 4 to day 5 of observation ($p > 0.05$), it rose significantly in the experimental groups ($p = 0.012$), as well as in the control groups ($p < 0.0001$), from day 4 to day 7 of observation. Between days 5 and 7 and between days 6 and 7 of observation, there were no significant differences in either the experimental or control groups ($p > 0.05$).

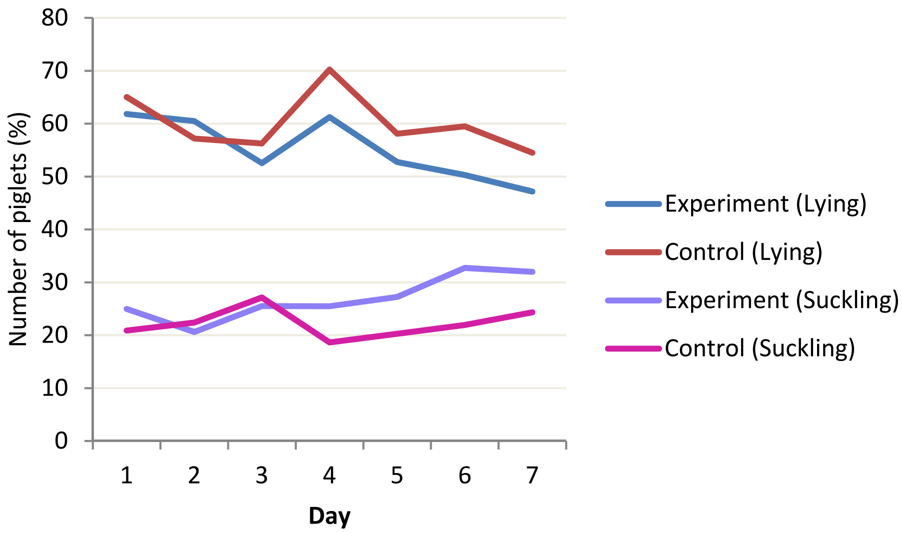

Figure 3. Mean percentage of lying and suckling piglets per litter in the experimental and control groups over seven days (days 1–3 = before vaccination, day 4 = day of vaccination, and days 5–7 = after vaccination).

3.4. Behavior of Piglets for Different Daytimes

Considering the different daytimes of observation (Figure 4), some differences between the experimental and control groups became evident. While the numbers of lying piglets in the mornings of the day before vaccination (day 3), the day of vaccination (day 4), and the day after vaccination (day 5) were similar for the experimental groups ($p > 0.05$), there was a significant difference for the control groups between the mornings of days 3 and 5, with fewer lying piglets on day 5 ($p = 0.022$). More noticeable differences became apparent in the afternoons and evenings of the days of observation. On the day of vaccination (day 4), the percentage of lying piglets increased, especially in the afternoon, compared to the afternoon of the day before vaccination (day 3). This increase in lying behavior in the afternoon was found in the experimental groups ($p = 0.004$), as well as in the control groups ($p < 0.0001$). However, there were more lying piglets in the afternoon of day 4 in the control groups than in the experimental groups ($p < 0.0001$). In the afternoon of day 5 (i.e., the day after vaccination), the number of lying piglets decreased again compared to the afternoon of day 4 (experimental groups: $p = 0.001$; control groups: $p < 0.0001$) and there was no significant difference between day 5 and day 3 in the experimental groups ($p = 1.000$), in contrast to the control groups, in which still more piglets were lying ($p = 0.036$).

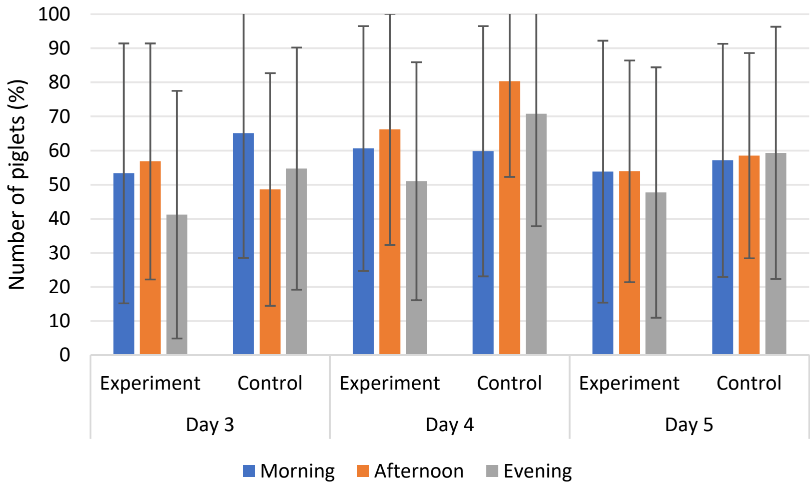

Figure 4. Mean percentage of lying piglets per litter in the experimental and control groups in the morning (6.00–10.00), in the afternoon (13.00–17.00), and in the evening (19.00–21.00). Day 4 = day of vaccination.

In the control groups, the increase in lying behavior was also evident during the evening of day 4. When comparing the number of lying piglets in the control groups between the evenings of days 3 and 4, a significant difference became evident, with more lying piglets in the evening of day 4 ($p = 0.004$). Furthermore, there was still a significant difference between the experimental and control groups, with more lying piglets in the control groups than in the experimental groups ($p < 0.0001$). From the evening of day 4 to the evening of day 5, the number of lying piglets in the control groups decreased, but not significantly ($p = 0.158$). In the experimental groups, no significant differences were found for the number of lying piglets between the evenings of days 3, 4, and 5 of observation ($p > 0.05$).

The suckling behavior was also affected by the daytime (Figure 5). While there were no significant differences between the mornings of days 3, 4, and 5 ($p > 0.05$), some differences became evident for the afternoon and evening.

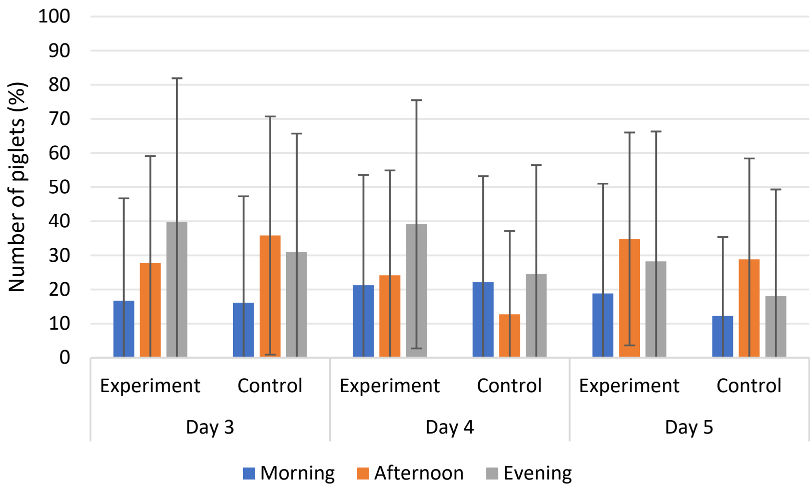

Figure 5. Mean percentage of suckling piglets per litter in the experimental and control groups in the morning (6.00–10.00), in the afternoon (13.00–17.00), and in the evening (19.00–21.00). Day 4 = day of vaccination.

Looking at the situation on the day of vaccination (day 4), it was shown that the percentage of suckling piglets decreased in the afternoon compared to the afternoon of day 3 in both the experimental and control groups; however, this decrease was higher in the control groups than in the experimental groups. Furthermore, this decrease in suckling behavior was only identified as statistically significant for the control groups ($p < 0.0001$), whereas for the experimental groups, no significant decrease was found from day 3 to day 4 ($p = 0.797$). In the control groups, the number of suckling piglets increased significantly from the afternoon of day 4 to the afternoon of day 5 ($p < 0.0001$), without showing any significant difference compared to the afternoon of day 3 ($p = 0.720$). Additionally, in the experimental groups, the number of suckling piglets increased from the afternoon of day 4 to the afternoon of day 5 ($p < 0.0001$). For the evening, no significant differences in suckling behavior were found between the three days in the experimental groups, while the number of suckling piglets decreased significantly from the evening of day 3 to the evening of day 5 in the control groups ($p = 0.02$).

4. Discussion

The aim of this pilot study was to evaluate two methods of vaccinating suckling piglets based on behavioral reactions with an impact on animal welfare. Therefore, behavioral analyses were carried out on the three days before vaccination, on the day of vaccination, and on the three days after vaccination.

On all days of analysis, the most frequent behavior—or, rather, body posture—of suckling piglets was lying, followed by suckling at the sow's teats. Locomotor activity or social contacts accounted for only a small proportion of behavior throughout the day. The same was reported by other studies showing that young piglets mainly spent the day lying down and suckling [15–17]. It was precisely in these behaviors that the present study revealed differences between the two methods of vaccination. On the day of vaccination, as well as on the three days after vaccination, on average, more piglets within a litter were lying in groups that had been vaccinated intramuscularly (control groups) than in groups that had been vaccinated intradermally (experimental groups). Furthermore, on the same days, fewer piglets were observed at the sow's teats in the control groups compared to the experimental groups. Therefore, piglets vaccinated intradermally were more active and expressed more suckling behavior on the day of vaccination and thereafter. It was previously shown that piglets suffering from stress were less active, showed less activity on the teats, and had reduced social behavior [18]. Therefore, the differences in those behaviors found in the present study may indicate that piglets vaccinated intradermally experienced less stress than piglets vaccinated intramuscularly. Video observations offer indirect conclusions about stress or pain and are therefore a valuable tool for studies on animal welfare. Although the measurement of direct stress parameters such as cortisol also allows for an assessment of animal welfare, this method has several disadvantages [18]. It may be influenced by animal handling procedures for blood or saliva sampling and it is difficult to realize in practice, especially for suckling piglets. Therefore, cortisol measurement was not used in this study.

The time required per piglet for intradermal vaccination is shorter than that for intramuscular vaccination [14]. Therefore, the animals are exposed to the stressful vaccination procedure for a shorter time—probably positively affecting animal welfare. A recent study [19] found a lower frequency of aversive behavioral responses (attempts at flight and vocalization) both during and immediately after vaccination in intradermally vaccinated three-week-old piglets compared to an intramuscularly vaccinated group, indicating reduced stress when piglets are vaccinated intradermally. Other authors postulated that needle-free intradermal vaccination reduced vaccination-related pain in growing pigs [20]. They studied the behavior of weaned piglets and reported that a greater percentage of piglets vaccinated intramuscularly displayed high-pitch vocalizations and retreat attempts at the time of injection. Furthermore, piglets vaccinated intramuscularly explored a rope less frequently after the vaccination than piglets with needle-free vaccination. In total, a significant decrease in active behavior was found in the group with intramuscular vaccination compared to the group with intradermal vaccination, which is very similar to the results obtained for suckling piglets in the present study.

However, in the present study, we found a significant increase in lying behavior in both the experimental intradermally vaccinated group and the control group with intramuscular injection on the day of vaccination compared to the day before vaccination. This indicates a stressful vaccination procedure in both groups. According to Cook et al. [21], who compared groups of weaners that were vaccinated intramuscularly with groups that were not vaccinated at all, vaccination in general is followed by an increase in inactivity, also affecting clustering behavior in piglets, particularly up to 7.5 h after vaccination. The same authors compared groups of weaned piglets vaccinated intramuscularly with groups given an intramuscular injection of 0.9% saline and control groups without any treatment [22]. In the period between three and eight hours after vaccination, there was an increase in huddling behavior in the vaccinated animals relative to both other groups and relative to the day before vaccination [22]. According to the authors, the increase in lying with huddling indicated that a febrile response after vaccination disrupted normal activity in piglets [22]. Therefore, there is a clear relationship between vaccination and behavior changes in piglets, whereas other injections did not cause similar behavioral reactions [22]. This was also supported by McGlone et al. [17], who did not find any differences in lying behavior between male suckling piglets with no injection and intramuscular or subcutaneous injection with saline.

However, it is also possible that pain plays a role in determining behavioral reactions after vaccination. Pain is also associated with behavior changes in piglets, as reported by Herskin et al. [23] for tail docking and by Prunier et al. [24] for castration. However, pain-related behavior is often associated with increased activity [18,25], whereas in the present study, active behavior decreased in both the experimental and control groups. Therefore, pain does not seem to be a major problem on the days following vaccination, even if pain is reported at the moment of vaccination in growing pigs and sows [13,20]. In sows, it was observed that intramuscularly vaccinated animals that did not show any sign of fear before the vaccination exhibited a total withdrawal from the observer during a fear-to-human test the day after vaccination [13]. This could indicate that the sows were remembering a previous painful experience. Furthermore, sows that were vaccinated with intramuscular injection showed decreased activity the day after vaccination compared to needle-free vaccinated sows [13]. This corresponds to the results of the present study for suckling piglets.

It was surprising that the differences in lying and suckling behavior between the experimental and control groups of the present study were still evident three days after vaccination. To the best of our knowledge, no other study has analysed a similarly long observation period after vaccination; thus, these results are interesting. However, it must be emphasized that on the first day after vaccination, there was a decrease in lying behavior in both the experimental and control groups, indicating some kind of recovery in both groups. The recovery phase for suckling pigs may take longer than that for older pigs, as suckling pigs have a greater need for rest than older pigs. Nevertheless, there were still more lying piglets in the control groups than in the experimental groups on the days after vaccination, indicating an even longer recovery period in control groups. However, the reasons for this remain speculative. Differences in immune response could also play a part. In this regard, it would have been interesting to investigate a few more days after the vaccination to find out at what time this difference in behavior between experimental and control groups disappears.

In contrast to lying behavior, suckling behavior was only affected by intramuscular vaccination in control groups and did not show any significant differences in intradermally vaccinated groups. There was a significant decrease in the number of suckling piglets in control groups on the day of vaccination compared to the day before vaccination. A lower number of suckling piglets in control groups than in experimental groups was still observed on the three days after vaccination, even if suckling activity was increasing again on those days in both groups. Reduced suckling behavior can be an expression of stress or pain, as it was also found after the castration of male piglets [18,26,27]. Hence, in considering suckling as a welfare indicator, a decrease in this behavior may indicate reduced animal welfare in piglets vaccinated with intramuscular injection compared to piglets vaccinated intradermally. Collecting more data on various farms would help confirm this assumption. However, the short-term change in suckling behavior did not lead to a difference in daily weight gain between experimental and control groups over a period of seven days, as shown in our previous study [14]. Therefore, long-term effects of the vaccination method on the growth performance are not expected. In this respect, it must also be considered that we did not record the suckling itself, but rather the time spent on the sow's teats. Perhaps the piglets in the experimental groups, which were more active, were also more motivated to suckle the sow's teats. As a result, they may have stayed at the teats more often, without always taking milk. Udder massage is also performed outside suckling [28], and this might have been more obvious in the more active piglets of the experimental groups in the present study. A connection between the level of activity and suckling bouts initiated by piglets has already been reported by other authors [29]. It was also previously shown that the number of piglets massaging the udder when no suckling occurred increased during the first 10 days of the piglets' life [28], which could explain the increasing number of piglets at the sow's teats from day 4 to day 7 of observation in both groups of the present study (i.e., from the 7th to the 10th days of the piglets' lives). It was also reported that the average proportion of a litter taking part in a suckling event increased especially from day 8 to day 10 of the piglets' life, which corresponds to our findings [28].

In the present study, the connection between the change in behavior and the vaccination procedure became particularly obvious due to the fact that an increase in lying behavior was observed in both groups during the afternoon of day 4 (i.e., after the vaccination was carried out in the morning). However, in the control groups, more pigs were lying at that time than in the experimental groups with intradermal vaccination, indicating a stronger reaction in the groups with intramuscular vaccination. This was also confirmed by the decrease in lying behavior on the afternoon of day 5 to the level of the day before vaccination in experimental groups, which was not found for control groups. Piglets of control groups also changed their lying behavior in the evenings after vaccination, in contrast to the experimental groups. Therefore, considering the daytimes makes the effect of vaccination on the behavior of piglets even more apparent.

Suckling behavior also changed in response to the vaccination. After the vaccination on the morning of day 4, the number of suckling piglets decreased in the afternoon of day 4 in both groups; however, this decrease was higher for the control groups than for the experimental groups. On the day after vaccination, suckling behavior was observed more often again in both groups, indicating a recovery from the vaccination procedure.

Even if it was previously reported that novelty and handling itself can be stressful and lead to behavior changes in pigs [17,25], in the present study and in some earlier studies [17,21,22], different behavioral reactions were shown following various management procedures, such as vaccination methods or a simple injection of saline. Therefore, except for handling itself, the procedure thereafter can cause different levels of stress or pain with an impact on animal welfare. In this study, the intradermal vaccination procedure triggered less welfare-associated behavior changes than the intramuscular vaccination. It has to be considered that in the present study, different vaccines were used for intradermal and intramuscular vaccination due to practical reasons on the commercial farm. The aim of the study was to compare the intradermal vaccination method with the method (including the vaccine) usually applied on this farm. Of course, an effect of the vaccine itself on the piglets' behavior cannot be completely ruled out. However, since other studies on weaners and sows, each using the same vaccine for intradermal and intramuscular administration, also found positive effects of the intradermal method on the pigs' behavior [13,20], we assume that the effect of the method itself was also strong in the present study. Nevertheless, it has to be emphasized that this is a pilot study and further research on a larger number of farms using the same vaccine for intradermal and intramuscular vaccination should be carried out to confirm these promising results for the intradermal vaccination of suckling piglets.

5. Conclusions

In this study, it was shown that the intradermal needle-free vaccination caused fewer vaccination-associated behavior changes in suckling piglets than the intramuscular vaccination method with a needle. From an animal welfare point of view, less lying behavior and more suckling behavior in intradermally vaccinated piglets compared to intramuscularly vaccinated piglets indicate clear advantages of the intradermal vaccination method. The needle-free vaccination is fast and gentle, and seems to be less stressful for the animals. Taking into account the hygienic advantages, intradermal vaccination seems to be very promising for application in suckling piglets.

Author Contributions: Conceptualization, N.K. and M.F.; methodology, N.K. and M.F.; validation, M.F.; formal analysis, M.G. and M.F.; investigation, M.G.; resources, N.K.; data curation, M.G. and M.F.; writing—original draft preparation, M.F., N.K., and M.G.; writing—review and editing, N.K. and M.F.; visualization, M.F.; supervision, N.K. and M.F.; project administration, N.K.; funding acquisition, N.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by MSD Animal Health/Intervet Deutschland GmbH, Unterschleissheim, Germany.

Acknowledgments: We gratefully acknowledge the support provided by the farmer and his willingness to contribute to this study. Moreover, we thank the helpers from the institute for their support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Nathues, H. Epidemiology, Diagnostics and Prevention of Enzootic Pneumonia in Pig Herds (in German: Epidemiologie, Diagnostik und Bekämpfung der Enzootischen Pneumonie in Schweinebeständen). Habilitation Thesis, University of Veterinary Medicine, Hannover, Germany, 12 September 2013.
2. Selbitz, H.J. Enzootische Pneumonie (in German: Enzootic Pneumony). In *Tierärztliche Impfpraxis*; Selbitz, H.J., Moos, M., Eds.; Enke Verlag: Stuttgart, Germany, 2006; p. 47.
3. Tassis, P.D.; Papatsiros, V.G.; Nell, T.; Maes, D.; Alexopoulos, C.; Kyriakis, S.C.; Tzika, E.D. Clinical evaluation of intradermal vaccination against porcine enzootic pneumonia (Mycoplasma hyopneumoniae). *Vet. Rec.* **2012**, *170*, 261. [CrossRef] [PubMed]
4. Zimmermann, W.; Plonait, H. Mycoplasmen- oder Enzootische Pneumonie, EP (Enzootic Pneumonia). In *Lehrbuch der Schweinekrankheiten*; Waldmann, K.H., Wendt, M., Eds.; Parey Verlag: Stuttgart, Germany, 2004; pp. 135–141.
5. Chase, C.C.; Daniels, C.S.; Garcia, R.; Milward, F.; Nation, T. Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. *J. Swine Health Prod.* **2008**, *16*, 254–261.
6. Vannier, P.; Cariolet, R. Vaccination of pigs against Aujeszky's disease by the intradermal route using live attenuated and inactivated virus vaccines. *Vet. Microbiol.* **1991**, *26*, 11–23. [CrossRef]
7. Baxter, J.; Mitragotri, S. Needle-free liquid jet injections: Mechanisms and applications. *Expert. Rev. Med. Devices* **2006**, *3*, 565–574. [CrossRef]
8. Beffort, L.; Weiß, C.; Fiebig, K.; Jolie, R.; Ritzmann, M.; Eddicks, M. Field study on the safety and efficacy of intradermal versus intramuscular vaccination against Mycoplasma hyopneumoniae. *Vet. Rec.* **2017**, *181*, 348. [CrossRef]
9. Eblé, P.L.; Weerdmeester, K.; Hemert-Kluitenberg, F.; Dekker, A. Intradermal vaccination of pigs against FMD with 1/10 dose results in comparable vaccine efficacy as intramuscular vaccination with a full dose. *Vaccine* **2009**, *27*, 1272–1278. [CrossRef]
10. Ferrari, L.; Borghetti, P.; Gozio, S.; De Angelis, E.; Ballotta, L.; Smeets, J.; Blanchaert, A.; Martelli, P. Evaluation of the immune response induced by intradermal vaccination by using a needle-less system in comparison with the intramuscular route in conventional pigs. *Res. Vet. Sci.* **2011**, *90*, 64–71. [CrossRef]
11. Baker, S.R.; Mondaca, E.; Polson, D. Evaluation of a needle-free injection device to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus. *J. Swine Health Prod.* **2012**, *20*, 123–128.

12. Martelli, P.; Saleri, R.; Cavalli, V.; De Angelis, E.; Ferrari, L.; Benetti, M.; Borghetti, P. Systemic and local immune response in pigs intradermally and intramuscularly injected with inactivated Mycoplasma hyopneumoniae vaccines. *Vet. Microbiol.* **2014**, *168*, 357–364. [CrossRef]
13. Temple, D.; Escribano, D.; Jiménez, M.; Mainau, E.; Cerón, J.J.; Manteca, X. Effect of the needle-free "intra dermal application of liquids" vaccination on the welfare of pregnant sows. *Porc. Health Manag.* **2017**, *3*, 9. [CrossRef]
14. Goeller, M.; Fels, M.; Gerdts, W.R.; Kemper, N. Intradermal versus intramuscular vaccine application in suckling piglets—Comparison with regard to dermal reactions, performance and procedural aspects. *Tierärztl. Praxis. Ausg. G Grosstiere/Nutztiere* **2018**, *46*, 317–322.
15. Johnson, A.K.; Morrow-Tesch, J.L.; McGlone, J.J. Behavior and performance of lactating sows and piglets reared indoors oroutdoors. *J. Anim. Sci.* **2001**, *79*, 2571–2579. [CrossRef] [PubMed]
16. Fels, M.; Giersberg, M.; Bill, J.; Gillandt, K.; Kemper, N. Auswirkungen eines einheitlichen Beschäftigungsangebotes während der Säugezeit und nach dem Absetzen auf das Verhalten und den Integumentzustand von Ferkeln. *Züchtungskunde* **2016**, *88*, 241–253.
17. McGlone, J.; Guay, K.; Garcia, A. Comparison of intramuscular or subcutaneous injections vs. castration in pigs—Impacts on behavior and welfare. *Animals* **2016**, *6*, 52. [CrossRef] [PubMed]
18. Hay, M.; Vulin, A.; Genin, S.; Sales, P.; Prunier, A. Assessment of pain induced by castration in piglets: Behavioral and physiological responses over the subsequent 5 days. *Appl. Anim. Behav. Sci.* **2003**, *82*, 201–218. [CrossRef]
19. Scollo, A.; Minervini, S.; Galli, M.C.; Cevidalli, A.; Bortoletto, G.; Romano, G.; Gottardo, F. Evaluation of pain and stress in three-week old piglets in relation to route of vaccine administration. *Livest. Sci.* **2020**, *233*. [CrossRef]
20. Temple, D.; Jiménez, M.; Escribano, D.; Martin-Valls, G.; Diaz, I.; Manteca, X. Welfare benefits of intradermal vaccination of piglets. *Animals* **2020**, *10*, 1898. [CrossRef]
21. Cook, N.J.; Chabot, B.; Lui, T.; Bench, C.J.; Schaefer, A.L. Infrared thermography detects febrile and behavioral responses to vaccination of weaned piglets. *Animal* **2015**, *9*, 339–346. [CrossRef]
22. Cook, N.J.; Bench, C.J.; Liu, T.; Chabot, B.; Schaefer, A.L. The automated analysis of clustering behavior of piglets from thermal images in response to immune challenge by vaccination. *Animal* **2018**, *12*, 122–133. [CrossRef]
23. Herskin, M.S.; Di Giminiani, P.; Thodberg, K. Effects of administration of a local anaesthetic and/or an NSAID and of docking length on the behavior of piglets during 5 h after tail docking. *Res. Vet. Sci.* **2016**, *108*, 60–67. [CrossRef]
24. Prunier, A.; Bonneau, M.; von Borell, E.H.; Cinotti, S.; Gunn, M.; Fredriksen, B.; Giersing, M.; Morton, D.B.; Tuyttens, F.A.M.; Velarde, A. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Anim. Welf.* **2006**, *15*, 277–289.
25. Taylor, A.A.; Weary, D.M.; Lessard, M.; Braithwaite, L. Behavioral responses of piglets to castration: The effect of piglet age. *Appl. Anim. Behav. Sci.* **2001**, *73*, 35–43. [CrossRef]
26. McGlone, J.J.; Hellman, J.M. Local and general anesthetic effects on behavior and performance of two and seven-week-old castrated and uncastrated piglets. *J. Anim. Sci.* **1988**, *66*, 3049–3058. [CrossRef] [PubMed]
27. McGlone, J.J.; Nicholson, R.I.; Hellman, J.M.; Herzog, D.N. The development of pain in young pigs associated with castration and attempts to prevent castration-induced behavioral changes. *J. Anim. Sci.* **1993**, *71*, 1441–1446. [CrossRef]
28. Jensen, P.; Stangel, G.; Algers, B. Nursing and suckling behavior of semi-naturally kept pigs during the first 10 days postpartum. *Appl. Anim. Behav. Sci.* **1991**, *31*, 195–209. [CrossRef]
29. Stangel, G.; Jensen, P. Behavior of semi-naturally kept sows and piglets (except suckling) during 10 days postpartum. *Appl. Anim. Behav. Sci.* **1991**, *31*, 211–227. [CrossRef]