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Indication that the presence of older conspecifics reduces agonistic behaviour in piglets at weaning

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ABSTRACT

Piglets weaned under commercial systems are greatly stressed by maternal separation, abrupt changes in the diet and mixing of litters. Intensive agonistic interactions exacerbate this challenge for piglets. We investigated effects of older conspecific presence in the nursery pen after weaning. Ninety-six 21 days old piglets were weaned and housed in eight pens, distributed equally by gender and by genetic relatedness. In four pens, a 4 months old conspecific was present. In order to test the effects of the presence of an older conspecific in the piglets' agonistic interactions, total skin lesions, agonistic behaviour and vocalizations were assessed on four consecutive days after weaning. Despite the low number of studied animals, the presence of the older animal decreased aggressive interactions, time spent in fights, number of lesions and duration of vocalizations. The presence of an older conspecific improved the welfare of piglets at weaning, possibly by acting as social reference.

1. Introduction

Piglets reared in commercial farms are weaned between 21 and 35 days of age. This is premature and is likely to be stressful to piglets since natural weaning starts when piglets are 91 days old and occurs in a gradual way (Jensen and Stangel, 1991). Weaning piglets at 21 or 28 days has negative consequences for growth rate and leads to endocrine stress responses (Colson et al., 2006b). Stressors faced by the piglets at weaning include abrupt changes in the diet from liquid to solid; environmental changes; social disturbance caused by litter-mixing and abrupt maternal separation (Lewis and Berry, 2006; Campbell et al., 2013).

Studies were performed to measure stress during weaning and to decrease the stress at this time. The consequences of premature weaning compromise piglet development (Colson et al., 2006b), causing immunological problems (Campbell et al., 2013), and losses for the producer. Special attention has been given to the aggressive behaviours caused by mixing litters (Colson et al., 2006b; Erhard et al., 1997; McGlone and Curtis, 1985; Oczak et al., 2013; Turner et al., 2006). Previous studies have characterized aggressive behaviour between weaned piglets (Jensen and Yngvesson, 1998; Souza et al., 2006; Stukenborg et al., 2011; Yuan et al., 2004). Factors such as age at weaning (Colson et al., 2006a; Pitts et al., 2000) and uniformity of piglets (Jensen and Yngvesson, 1998) contribute to the occurrence of aggression. Socialization of litters prior to weaning (D'Eath, 2005; Salazar et al., 2018) and enrichment of the pens (Melotti et al., 2011) decrease aggression. However, as far as we know, no research has investigated the importance of a more complex social environment to mitigate weaning stress.

When mixing unfamiliar piglets for the first time, a new dominance relationship is formed before social bonds become established (Rault, 2012). In horses, the presence of an adult non-relative animal in the group increases locomotion and decreases both vocalizations and cortisol concentrations of weaned foals. In the same study, aggressive and abnormal behaviour was observed only in the groups without an older horse (Rault, 2012).

In semi-natural conditions, when piglets of a litter are introduced to the other members of the group, they spend a great part of their time with other individuals of the same age and part of the time sniffing the oldest animals of the group (Newberry and Wood-Gush, 1986). This behaviour can indicate that piglets have knowledge of the other

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members of the group and they socialize with those members. When piglets are reared in a complex social environment, the oldest animals may have a position as a behaviour modulator reducing the aggressiveness of the younger pigs. Evidence of the complex influence of an older animal, such as regulating the social behaviour of younger animals, is well known. Older animals can be valuable, to promote social behaviour learning, ingestion of food and reduction of stress in challenging moments such as weaning (Bourjade et al., 2009; Bradshaw et al., 2005; De Paula Vieira et al., 2012; Henry et al., 2012).

Aggressiveness in piglets has been studied for a long time but is still a substantial problem (Camerlink et al., 2014). A new approach is needed. The hypothesis is that an older conspecific in the nursery pens might reduce the aggressive behaviour that occurs during weaning and mixing litters.

2. Materials and methods

2.1. Animals, facilities and handling

The experiment was carried with approval of the Ethics Committee on Animal Use (CEUA), under the number 6240230114. Ninety-six Landrace x Large White piglets, mean weight 6.2 ± 1.5 kg, from 24 different sows were studied, together with 4 surgically castrated males of 4 months old, all purchased from the Araporanga Farm (Topgen®) – Jaguariaíva, Paraná, Brazil. The males were castrated to avoid any hormone influence. The older males and the piglets were transported to the experimental farm on the same day. They were not familiar or related to the young piglets. The sows (three primiparous and 21 between 2nd and 8th parities) were kept in commercial group-housed systems and the insemination occurred according to the farm plan. All the animals were submitted to the conventional management of the farm after birth, including teeth grinding, tail docking and ear notching. The piglets were chosen by the farm manager: 2 males and 2 females per sow. At 21 days old (D0) piglets were marked on the back for identification with permanent hair ink (Wellaton®, black color 1.0 – Wella – Procter & Gamble Co.).

After this, the piglets were weaned and put in cardboard boxes with straw, each box containing only one litter (4 piglets). The boxes were placed in a truck and the animals were taken to the University of São Paulo, Campus Fernando Costa in Pirassununga. The travel lasted 7 h (459 km) and the animals were unloaded on the next day (D1), around 0400. The animals were transported in two groups, the first in November 2014 and the second in January 2015.

At the university, the animals were housed in pens of 3 × 4 m with a full solid concrete floor and half of the pen covered with straw (sugar cane bagasse). Water and food were offered *ad libitum* in nipples and round feeders (Suin®668), respectively. The concentrate was produced in the meal factory of the Campus Fernando Costa and contained maize bran, soybean meal, premix for piglets (Uniquímica), adsorbent (Altec) and swine plasma (Despros AS.).

2.2. Data collection

The 96 piglets were housed in groups of 12 in 8 pens, 4 pens with an older conspecific (treatment) and 4 without (control). Each group of piglets was composed of three different litters with 12 piglets per pen, six males and six females (a couple per sow). The experiment was carried out in two different blocks of 48 piglets. Behavioural analysis (all the piglets from first block, n = 48) and skin lesion score (all the piglets from both blocks, n = 96) were performed to evaluate the piglets' aggressiveness, as well as analysis of vocalization to evaluate the level of welfare of the piglets (Weary and Fraser, 1995). All types of vocalisations were counted (all the piglets from the second block, n = 48), during the holding of the piglets for taken the photographs to assess skin lesions. The analyses were carried out by an experimenter who was blind to the animal treatment, since the animals were individually restrained, without any precise information about their pen mates.

Due to issues with the power system, the collected videos to analyse the aggressive behaviour from the second group were lost. Thus, here we presented the video data from 48 animals (first group). Videos were analysed during the 4 days after weaning (D1, D2, D3, and D4) from 4 different pens, 2 with the older males (+ older, treatment) and 2 with only the piglets (no older, control), totalling 48 piglets. Behaviour analysis were performed for 4 h continuously between 05h00 and 09h00. Additionally, from 09h00 to 00h00 the behaviour was analised for the first 10 min continuously for each hour. The analysis time was chosen after a pilot study, where we identified the time when the piglets were more active. The animals were not submitted to an artificial light program. A total of 440 min of videos were analysed per pen.

The images were recorded (LuxVision Infrared 720p 3.6 mm infrared LVC5125B) on an external HD until the moment of the analysis. Only one observer performed the analysis, and the frequency and duration of all agonistic interactions were recorded with the help of an ethogram (Table 1) and a chronometer (Martin and Beteson, 2007). The agonistc interactions were described as an act marked by mutual bites, head knocks or pushing between two piglets, with the duration of more than two seconds, beginning when a piglet gives another piglet a bite, head knock or push, provided that the other piglet responds. The end occurred when the animal stopped the attacks and moved away. To count a new agonistic interaction, the interval between the fights should be longer than 5 s (Moore et al., 1994). The frequency of the agonistic interactions was described as the number of agonistic interactions that started and ended during the behavioural analysis period. The duration of the agonistic interactions was described as the number of seconds between the beginning and the end of an agonistic interaction. All the piglets were individually identified, using a marker of non-toxic and non-permanent ink.

Photos for lesion analysis were taken (Sony Cyber shot® DSC-HX1) of all piglets (N = 96) on days D1, D2, D3 and D4. All photos were taken in the same way as well as in the same sequence: back, right side, outer face of the right ear, inner face of the right ear and right cheek, left side, outer face of the left ear, inner face of the left ear and left cheek. All photos were taken around 18h30 on each day. Each animal was individually restrained, video recorded, photographed inside the pen, and released.

Two evaluators counted the lesions on all animals, independently, considering lesions characterized as a cut or scratch partially healed and recent injuries, characterized by red-pink color (Guy et al., 2009). To

Table 1
Ethogram with the description of behaviours assessed.

Behaviour assessed	Behaviour description
Agonistic interaction	Act marked by the exchange of bites, butting or pushing between two piglets lasting more than two seconds. The beginning is indicated when a piglet heads, bites or pushes another piglet which in turn responds with one of the actions mentioned. It ends when at least one of the animals moves away and stops fighting the bites and headbutting showing disinterest in the piglet that keeps attacking, this action must have a duration of at least five seconds to be considered a new fight.
Duration of agonistic interaction	Interval of seconds between the beginning and the end of an agonistic interaction
Frequency of agonistic interaction	How many fights occurred during the observation period
Piglet starts an agonistic interaction	Piglet that gave bite, push or headbutt to another pig, resulting in an agonistic interaction
Piglet lost an agonistic interaction	Piglet that moves away in the middle of the onslaught of another piglet or after prolonged pairing, or even, piglet that lies or sits and stops fighting back, generating disinterest and withdrawal from the other piglet
Agonistic interaction without winner	When both piglets move away at the same time or stop biting, pushing and butting for more than five seconds and then move away

verify the reliability of the counted lesions number among the two evaluators, we performed a numerical dispersion, which the data from each oberserver did not diverge more than 8.5 %. We used the mean from both observers to perform the statistics tests.

While restraining the animals of the second block (n = 48) for the photographs, we also assessed the frequency (number of vocalization events, for which, after a two second pause, it was considered as a new vocalization) and duration (total duration of each event) of the vocalizations of piglets.

Agonistic behaviour (frequency of interactions, frequency of interactions by gender, percentage of time spent in agonistic behavior, percentage of time by gender) and lesion score (number of skin lesion) data were analysed using Statistical Analysis System (SAS Inst., Inc., Cary, NC) in a multifactorial design 4 × 2 × 2 (time, treatment and gender). The unit considered was the individual, although there was one group per pen. Initially, the data were analysed for the presence of discrepant information (outliers) and to verify residual normality we used the Shapiro-Wilkes test. When the normality assumption was not found, the transformation by logarithm, square root or arcsine were used. The variable total duration of fights and mean duration of fights were transformed by logarithm. Data were submitted to analysis of variance and the model included the effect of treatment as a fixed effect and the block as a random effect.

The correlation testes were performed to assess if there was correlation between skin lesions and agonistic behavior (frequency and duration). The Pearson correlation coefficient test was applied in the data that showed residual normality, ant the correnpondent non-parametric was Spearman's rank correlation coefficient. For all analyses (mean ± SD), 5% significance was adopted.

3. Results

The frequency of agonistic interactions was lower in the presence of the older pig than in the control (Fig. 1) on: D 1, p = < 0.01; D 2, p = 0.02; and D 4, p = <0.01. There was triple interaction, between time, treatment and gender, with p = 0.04. Piglets of the older animal treatment had fewer agonistic interactions, indicating that the presence of the older conspecific was enough to reduce agonistic interactions between piglets. The frequency of agonistic interactions declined over time in both, control and treatment.

The males of the control group had a greater motivation for agonistic interactions than females, especially on the first day after mixing and on day 4 (Fig. 2; D1, p = 0.01; D4, p = <0.01).

There was a triple interaction between time, treatment and gender

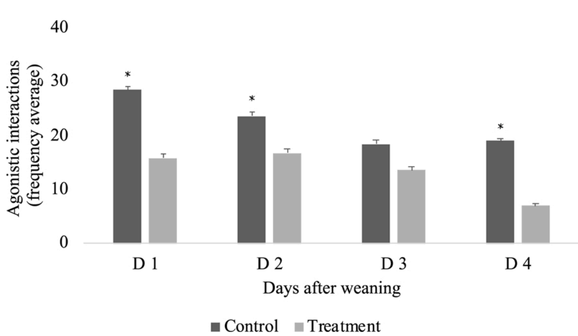

Fig. 1. Frequency of agonistic interactions. Frequency of agonistic interactions observed in piglets weaned at 21 days old (D0) and mixed at D1 in the presence (treatment, n = 24) or absence (control, n = 24) of an older conspecific. Data obtained by indirect observation. Mean value (± SD) of 24 animals for each independent treatment. * Indicates difference between treatments on the same day (factorial analysis, triple interaction time x treatment x gender p = 0.04 and SEM = 0.40 and the difference between control and treatment for D 1 p = <0.01 and SEM = 0.76 for D p 2 = 0.02 and SEM = 0.72 and p = <0.01 D 4 and SEM = 0.51).

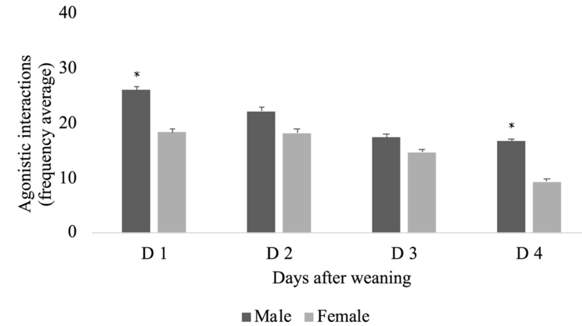

Fig. 2. Frequency of agonistic interactions according to gender. Frequency of agonistic interactions between genders in piglets weaned at 21 days old (D0) and mixed in D1. Data obtained by indirect observation. Mean value (± SD) of 24 animals for each gender. * Indicates difference between genders in the same day (factorial analysis, triple interaction time x treatment x gender p = 0.04 and SEM = 0.40 and difference between males and females for D1 p = 0.01 and SEM = 0.76 for D4 p = <0.01 and SEM = 0.51).

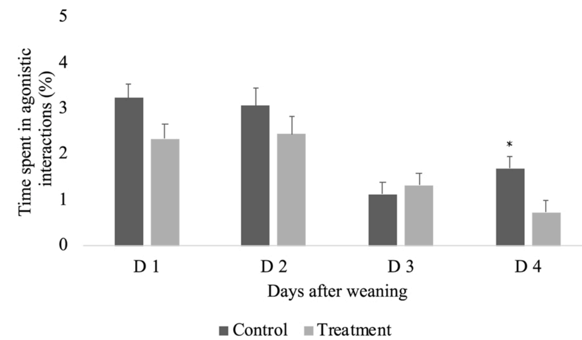

Fig. 3. Percentage of time spent on agonistic interactions. Percentage of time spent in agonistic interactions in piglets weaned at 21 days old (D0) and mixed at D1 in the presence (treatment n = 24) or absence (control n = 24) of an older conspecific. Mean value (± SD) of 24 animals for each independent treatment. * Indicates difference between treatments on the same day (factorial analysis, triple interaction time x treatment x gender p = 0.03 and SEM = 0.27 and the difference between control and treatment for D 4 p = <0.01 and SEM = 0.51).

(Fig. 3; p = 0.03) and interaction between treatment and time (Fig. 3; p = 0.02). The control group spent more time performing agonistic interactions than the piglets grouped with the older conspecific (Fig. 3; D4 p = < 0.01). Males spent more time performing agonistic interactions than females (Fig. 4; p = 0.05).

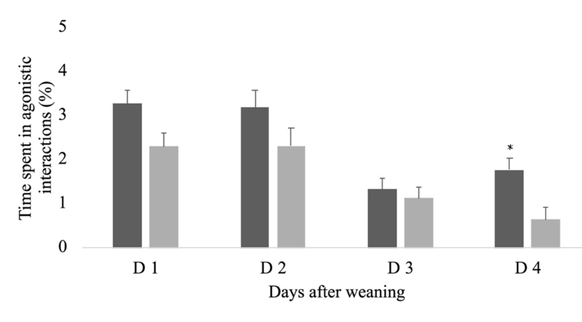

Fig. 4. Percentage of time spent in agonistic interactions according to gender. Percentage of time spent in agonistic interactions according to gender in piglets weaned at 21 days old (D0) and mixed in D1. Data obtained by indirect observation. Mean value (± SD) of 24 animals for each gender. * Indicates difference between treatments on the same day (factorial analysis, triple interaction time x treatment x gender p = 0.03 and SEM = 0.27 and the difference between male and female to D 4 p = <0.01 and SEM = 0.27).

After weaning, the number of skin lesions was higher for piglets in the control group on the first and second days (Fig. 5; D1 and D2 $p = <0.01$). This indicates that the older conspecific was effective in reducing aggressive behaviour, and hence, decreasing the number of lesions.

The duration of vocalization in piglets with the older pig present was lower than in the controls (Fig. 6; D1, $p = 0.01$; D2, $p = 0.03$; D4, $p = 0.02$).

Regarding the correlation between total skin lesions and frequency of agonistic interactions, only in day 4 there was a correlation (Fig. 7; $R^2 = 0.11$; $r = 0.33$, $p = 0.02$). At the day 3, the correlation showed p value $= 0.09$, while $R^2 = 0.06$, $r = 0.24$ (Fig. 7)

Only on the day 3, there was a correlation between duration of agonistic interactions and total skin leions (Fig. 8, $r = 0.33$, $p = 0.02$).

4. Discussion

Here we showed that the presence of an older conspecific improved the social context of piglets after weaning. The older conspecific presence decreases the skin lesion, and frequency of agonistic interactions in recently weaned piglets. The increased frequency of agonistic interactions on D4 for males from the control group may be due to uncertainty in the new dominance relationship in the early days. Moreover, this uncertainty position in the group hierarchy can indicate stress in these individuals (DeVries et al., 2003). However, aggressiveness behaviour can lead to establishing of dominance hierarchies, so it is possible that increased aggression on day 4, might induce to a more stable dominance relationships in the long-therm. The fatigue caused by the long trip or memory impairments due to the large number of stressful factors at weaning (Berry and Lewis, 2001; Lewis and Berry, 2006; Weary et al., 2008) may have contributed to the delay in establishing a concrete hierarchy, which could take several days. These data are not in agreement with those that affirm that hierarchy formation occurs immediately within 48 h (Meese and Ewbank, 1973; Souza et al., 2006; Yuan et al., 2004). Moreover, a recent study reported that the behaviour data from 48 h after weaning are enough to characterize agonistic behaviour (Büttner et al. 2019). To our knowledge, this is the first evidence for a modulator role of an older individual reducing aggressivesness in piglets.

It is very important to highlight that aggressive behaviour in pigs is an enormous issue. A recent review analysed research since 1970 to reduce aggressive behaviour in pigs, an important topic in animal research (Peden et al., 2018). In addition to the agonistic interactions, the number of skin lesions on piglets was lower in the first 48 h when an older conspecific was present. These data agree with previous reports,

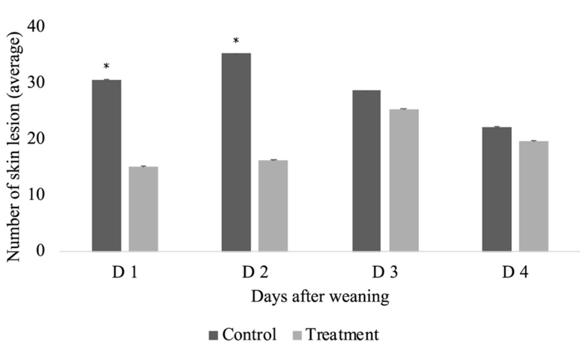

Fig. 5. Skin lesion score. Skin lesion score in piglets weaned at 21 days old (D0) and mixed atD1 in the presence (treatment $n = 48$) or absence (control $n = 48$) of an older conspecific. Data obtained by indirect observation. Mean values (± SD) of 48 animals in each independent treatment. * Indicates difference between treatments on the same day (factorial analysis, dual interaction time x treatment $p = <0.01$ and SEM $= 0.88$ for D1 $p = <0.01$ and SEM $= 0.06$ and D2 $p = <0.01$ and SEM $= 0.07$).

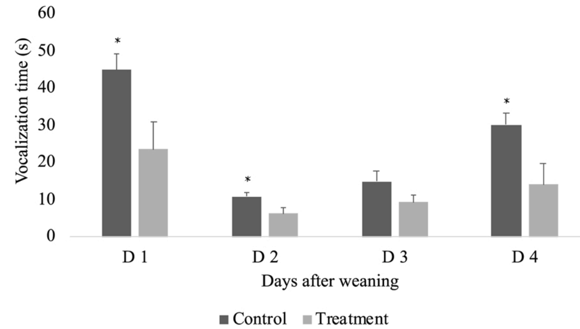

Fig. 6. Duration of vocalization. Duration of vocalization time of piglets during restraint. Piglets weaned at 21 days old (D0) and mixed at D1 in the presence (treatment $n = 24$) or absence (control $n = 24$) of an older conspecific. Data obtained by direct observation. Mean values (± SD) of 24 animals in each independent treatment. * Indicates difference between treatments on the same day (no interactions, for D1 $p = 0.01$ and SEM $= 5.72$, D2 $p = 0.03$ and SEM $= 1.37$, D4 $p = 0.02$ and SEM $= 4.41$).

where the lesion score was used to evaluate the degree of injury caused by assault and agonistic behaviour in piglets (Parratt et al., 2006). It showed that there is a decrease in the number of lesions over days. The use of a skin lesion score has been identified as a method for assessing aggressiveness in piglets, one way of estimating the aggressiveness of individual animals (Turner et al., 2006).

In other species of gregarious animals, the importance of social context in the maternal separation period has been demonstrated. An example is for elephants that live in a matriarchal hierarchy but whose offspring often become orphans due to the illegal ivory trade. It was demonstrated that such orphans present serious socialization and aggressiveness problems, since they do not have all the complexity of a social environment with animals of different ages and status in the group. In a reserve, young elephants attacked young rhinos and killed many of them. This behaviour has ceased after the placement of older males together with the younger group in an enclosure (Bradshaw et al., 2005). The presence of an older unrelated horse in weaning paddocks caused decreased vocalization, increased locomotion and reduced cortisol concentration in weanling foals (Henry et al., 2012). Foals without the presence of the older horse showed aggression and other abnormal behaviours. In swine it was shown that the physical presence of a boar in a pen with sows that have been mixed reduced the amount of aggressive behaviour, lesions, and salivary cortisol when compared with sows without the presense of a boar or sows that have only the visual information of a boar (Séguin et al., 2006).

Regarding the behavior of the older pig toward the piglets, we observed different responses to piglets' aggressive behavior. During the agonistic interactions among the piglets, the older pig intervened, in order to cease the interaction. In one pen, the older pig intervened in 18 out of 53 intense interactions observerd. However, in the other pen, the old pig intervened in 11 out of the 50 intense aggressive interactions. In the first pen abovementioned, these interventions done by the older pig took place by simple approaching the piglets involved, moving toward them (11 events), or separating the piglets with his snout, pushing them out of the interaction (7 events). In the other pen, the older pig interrupted the agonistic interations in 8 situations by pushing them out of the agonistic interaction, and 3 times moving towards the piglets. The observations of the existing active character in the intervention of the older pig in the agonistic interacions were made during the study. However, the experimental design did not allow us to perform a systematic data collection on this fascinating observation, which requires further studies.

In pigs a study conducted in semi-natural conditions, showed that piglets of a litter are introduced by the mother to other members of the group (Newberry and Wood-Gush, 1986). This behaviour suggests that piglets acquire knowledge of the other members of the group and then

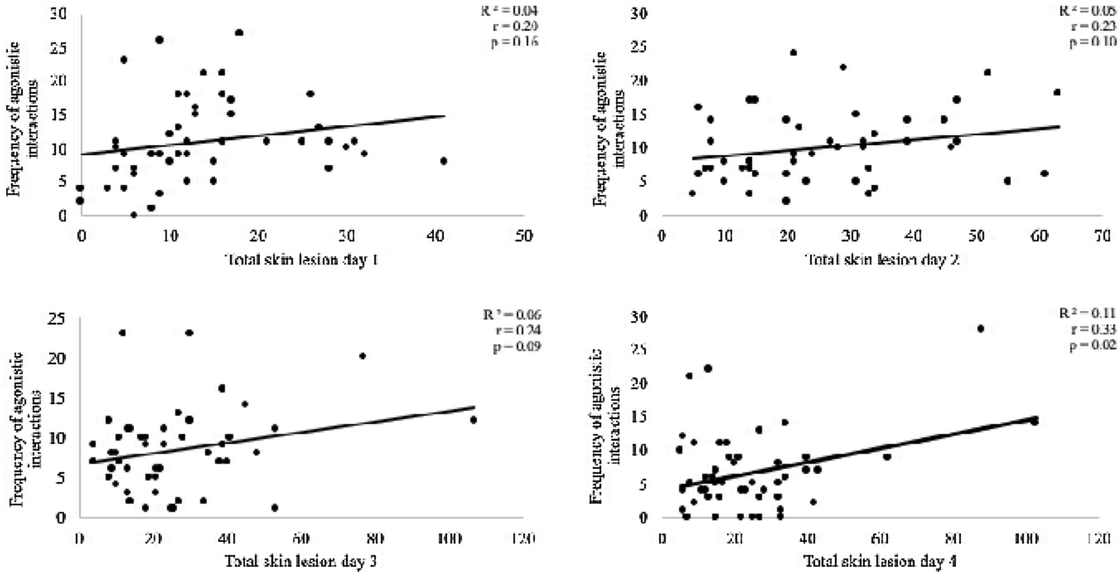

Fig. 7. Correlation between total skin lesion and frequency of agonistic interations, per day of analysis. The analysis was performed with Pearson correlation test, once the data showed normality. The statistical results and p-value are described in each graph.

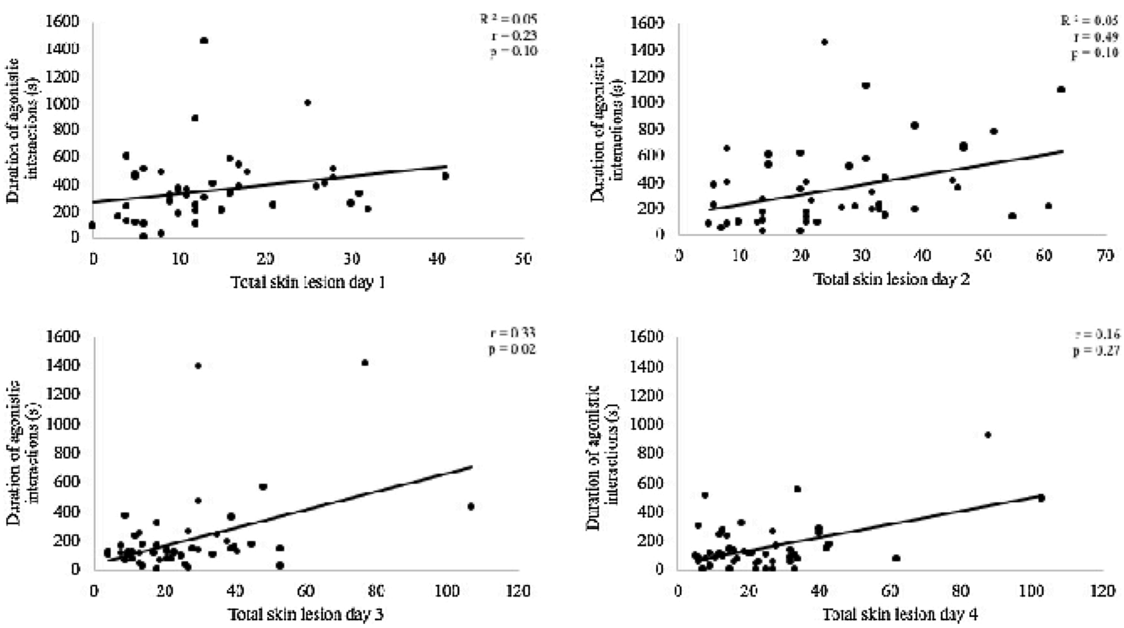

Fig. 8. Correlation between total skin lesion and duration of agonistic interations, per day of analysis. The analysis of the days 1 and 2, were performed with Pearson correlation test, once the data showed normality. The analysis of the days 3 and 4 were performed with Spearman correlation test, since the data was not showing normality. The statistical results and p-value are described in each graph.

socialize with those members. When piglets are reared in a complex social context, other individuals may have a position as a behaviour modulator reducing the aggressiveness of the younger pigs. Moreover, the affective experience of socialization with the older conspecific during a sensitive period like waning may increase the social skills of pigs, which in turn can improve their welfare by adjusted aggressive behaviour. Evidence of the complex influence of an older animal, regulating the social behaviour of the younger, are well known. As in other species, (Bradshaw et al., 2005; De Paula Vieira et al., 2012; Henry et al., 2012), older animals can promote learning of social behaviour (Bourjade et al., 2009).

In our study, the frequency of agonistic interactions decreased over the period of observation for piglets, both those with and without the presence of the older pig. The reduction over time is expected, given that the hierarchy is established in the first days after the mixing of litters (Yuan et al., 2004). The agonistic interactions occur mainly and with greater intensity in the first 48 h after the mixing. However, they may continue for longer, until a new hierarchy is established (Meese and Ewbank, 1973; Souza et al., 2006; Yuan et al., 2004). On day four there was a difference from day three in agonistic interactions of males and females without the older conspecific. This result agrees with previous reports, in which males tend to be involved in more severe fights and initiated more agonistic interactions than females (Colson et al., 2006a). It is plausible that the same did not occur with males in the presence of the older conspecific as it reduced the stress and the agonistc interactions of piglets.

The vocalizations may indicate states, or moods, or emotions that can lead to specific behaviours (Manteuffel et al., 2004). Therefore, the vocalization is considered a useful indicato to infer about the welfare or stress of an individual (Weary and Fraser, 1995). There is evidence that in pigs a very loud tonal sound is used as a signal associated with fear of contexts, while low-frequency and rough sounds are assigned to more aggressive emotions (Seyfarth and Cheney, 2003). In a speculative way, the shorter duration of vocalizations for animals weaned with the older conspecific may indicate a better preparation to deal with the situation. However, the piglets could be overall less aroused, and vocalized less. This in turn may indicate that the more complex social environment in weaning animals made them more prepared to deal with certain situations.

When restraining the pigs for photos for the lesion score, we noticed in the first group that the piglets which were in the presence of the older conspecific appeared to have a lower duration of vocalizations when compared with the control group. It appeared to the observers that the duration of vocalization was longer in the control piglets than in the older conspecific treatment during restraining. This difference in the duration of vocalization suggests that piglets with the older pig have better welfare than the controls (arousal maybe was lower in this group). Thus, providing support for the importance of a complex social environment at weaning. Similarly, Hötzel et al. (2011) reported that piglets with a combination of various stressors after weaning showed a higher frequency of vocalizations. The authors also attributed this response due to their fear, since they were isolated from the sow. We agre that more studies with detailed analysis of vocalizations (intensity, duration, frequency, among others) in a more complex piglet social environment would be very important to understand deeply this valuable indicator.

Additionally, we did not find a strong correlation between those variable because could due to the intensity of the interaction, instead of just frequency and duration. In other words, how is the enagement in the aggressive behavior seems to be more determinant to cause skin lesions, instead of simply unitis of time. Moreover, since there is a difference regarding the gender, this could have affected the outcomes of the correlation tests.

We would like to addres that one of the limitations of this study is the number of the experimental groups. Although we studied the animals as individual for all analysis, we evaluated only 96 piglets. Moreover, an evaluation over the time could provide valuable information, such as performance, feed convertion rate, among other, since the piglets could start to eat solid food faster, by learning from the older conspecific.

5. Conclusion

Despite the low number of studied animals, we showed that an older pig reduces aggressiveness of piglets at weaning. This reduction differs with gender and time. The frequency of agonistic interactions was lower in the presence of the older conspecific when compared with the control group. This result indicates possible management strategies to improve the welfare of piglets during weaning using an older animal, more experienced in the social organization of these gregarious animals. From an evolutionary point of view, in gregarious animals it would be adaptive for an older individual to facilitate social organization.

Declaration of Competing Interest

The authors report no declarations of interest.

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