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Early human handling in non-weaned piglets: Effects on behaviour and body weight

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ABSTRACT

Early handling of animals including tactile stimulation (TS) has been shown to have beneficial effects on the physical and psychological development of species where considerable maternal interaction, e.g. in the form of licking, already occurs. But little is known about the magnitude of these effects, if any, in species without this natural mechanism. Piglets from 13 litters ($N = 127$) were subjected to four treatments: AH – all piglets in a litter received TS; NH – none of the piglets in a litter received TS; 50/50H – half of a litter received TS and 50/50NH – half of a litter did not receive TS. The TS was performed by a human stroking the back of the piglet for 2 min from 5 to 35 days of age. At 4 weeks of age the piglets were tested twice in an open-field/human-approach test, with either a familiar (F) or an unfamiliar person (U). Body weight was measured at birth, 5, 9 and 12 weeks of age. In the tests, AH and 50/50H piglets allowed more physical contact, regardless of the familiarity of the person (AH: 22.5 ± 2.3 F; 24.1 ± 2.3 U, 50/50H: 18.1 ± 2.2 F; 25.3 ± 2.2 U, $P = 0.05$). Additionally, AH piglets vocalized least and were least often in the perimeter zone (PZ) of the arena (AH grunts: 6.0 ± 0.8, $P = 0.002$; 12.8 ± 0.8, $P = 0.0005$; 18.1 ± 0.8, $P = 0.08$; AH PZ: 3.9 ± 0.8, $P = 0.009$; 9.1 ± 0.8, $P = 0.003$; 12.6 ± 0.7, $P = 0.004$ in isolation, stationary and moving person phase, respectively). In contrast, NH piglets vocalized most and were most often in the PZ. 50/50NH had higher body weights at 12 weeks than 50/50H, whereas AH and NH piglets were intermediate (AH: 36.9 ± 0.9, NH: 35.6 ± 1.0, 50/50H: 34.2 ± 1.3, 50/50NH: 37.8 ± 1.3, $P = 0.03$). This study suggests that early handling changed the way piglets reacted to challenging situations in that handled piglets showed behaviour suggesting they were less fearful in a novel environment and less fearful of being handled by people in general. Interestingly though, the early handling did not result in the same beneficial effects on the physical development of the piglets, as handled piglets were not heavier. Instead, it was the 50/50NH piglets that were the heaviest, implying that the daily presence of a human moving around in the pen to handle the 50/50H piglets seemed to stimulate weight gain.

1. Introduction

Human handling at an early age, by applying tactile stimulation (TS), is considered an important element influencing the development of young rodents (Fernández-Teruel et al., 2002). It is known in rodents and several

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other species that there is a sensitive period during early postnatal development when environmental manipulations can result in permanent changes in the hypothalamic–pituitary–adrenal (HPA) axis function, behaviour and body weight (Champagne et al., 2009; Weaver et al., 2000). Biologically, it is expected that human handling in early infancy causes small doses of stress in the animals, modifying and accelerating the development of their central nervous system. More specifically, tactile stimulation has been shown to increase the glucocorticoid receptor sites in the hippocampus, changing the responses of the HPA axis of handled rats compared to non-handled animals (Levine, 2005). Such changes seemed to be linked with a greater resistance to pathogens, a higher weight gain and greater efficiency in cognitive ability tests (Levine, 1960, 1957, 1956). According to Kolb et al. (2003), this seemingly benign exposure to challenges in early life enhances motor and cognitive skills in adulthood, due to neurochemical changes in brain function and plasticity.

Lately, researchers have applied tactile stimulation in young farm animals and found that animals showed more docile behaviour and reduced fear towards humans (sheep: Boivin et al., 2000; goats: Boivin and Braastad, 1996; horses: Ligout et al., 2008; rabbits: Verwer et al., 2009), better immune status (sheep: Caroprese et al., 2006) and reduced abomasal lesions (calves: Lensink et al., 2000). However, most work done in this field has been carried out in farm animal species where tactile stimulation is an important part of maternal care. There is less work on the general effects of additional handling in early life on species where there is little, if any, maternal tactile stimulation, e.g. pigs. In relation to pigs' maternal care, for instance, sows do not help their offspring to get out of their foetal membranes, nor lick or assist piglets in their teat-seeking activity, but they do spend time building and keeping the nest in good condition (Jensen, 1988). This is in contrast to rats (Liu et al., 2000), which usually give tactile stimulation to the offspring within the nest, or to ungulates, which are involved in a high number of physical interactions with their offspring. There are clear survival benefits for these altricial and precocial offspring of this physical stimulation by the mother (Nowak et al., 2000). Pigs, on the other hand, are in the middle of these extremes since they have a developed sensorial capacity at birth but an inefficient thermoregulatory mechanism. Such differences and the lack of a natural mechanism related to maternal tactile stimulation might be important for the level of stress that pigs experience when being touched by humans compared to the species already studied.

Reduced fear towards humans is a benefit of early handling, since fear of humans is strongly related to decreases in productivity and poor welfare in all domestic species (Rushen et al., 1999). Some authors argue that the decreased avoidance of humans and increased motivation to interact with people as a result of touching and stroking may also facilitate handling procedures. Some research has been carried out with pigs, evaluating the effects of handling weanling pigs on their fear of humans (Gonyou et al., 1986; Hemsworth et al., 1986; Tanida et al., 1994). Generally, in previous research (Hemsworth and Barnett, 1992; Hemsworth et al., 1986; Tanida et al., 1995), handlers adopt a stationary squat posture and the contact has been at the initiative of the piglets themselves, with a view to making the interaction as positive as possible. However, no studies in pigs have investigated the effects of the type of handling carried out in laboratory animals, where the repeated tactile stimulation is forced and so could probably be considered a mild stressor.

There are several well-established tests to assess exploratory behaviour and fear in pigs and most of these are adapted from laboratory animal studies, e.g. the open field-test. Although not all assumptions of the test within rodent research (i.e. thigmotaxis) can be directly transferred into pig assessments (Murphy et al., 2014), this test has been widely used. According to Forkman et al. (2007), it is not clear if the results really indicate fear due to the lack of correlation with other tests. Nevertheless, Donald et al. (2011) proposed that the open-field test can be used to assess emotional states in pigs when combined with other behavioural measurements, e.g. vocalization. The human-approach test assesses fear of humans, offering also the possibility to measure the social relationship with humans, management quality, as well as identifying individual differences (Waiblinger et al., 2006).

The present study examined whether early human handling using TS would improve piglets' growth and alter their reactions in a standardized fear test. The hypothesis was that enforced and regular early human handling would result in heavier and less fearful piglets than non-handling treatments.

2. Materials and methods

2.1. Animals and management

This study was approved by the Ethical Committee of Animal Experimentation in Uppsala, Sweden, under protocol C117/11.

The experiment was conducted in 2011 at the Swedish Livestock Research Centre, Lövsta, at the Swedish University of Agricultural Sciences, Sweden. A total of 127 crossbred Hampshire × Yorkshire piglets from 13 litters were used, with an average litter size of 9.7 ± 2.5 piglets (mean ± SD). Animals were born in farrowing pens (3.84 m × 2.2 m) with partly slatted concrete floors, provided with straw and a heated creep area (1.35 m × 1.65 m). At birth, piglets were weighed and received an ear tattoo for identification. Litters born at the same time were adjusted by moving piglets between sows to make the sex ratio within each litter as even as possible. Before 4 days of age, they received an iron injection, their teeth were ground and males were castrated. Weaning was carried out at 5 weeks of age by removing the sow from the pen. Water was always available and at the age of 21 days the piglets got ad libitum access to commercial pelleted food. All piglets experienced the same daily routines performed by the facility staff.

2.2. Treatments

Piglet litters were randomly assigned to four treatments, balanced for pen location in the stable and day of birth, as follows: (1) all-handled (AH) – all piglets in a litter received tactile stimulation (TS), as explained in Section 2.3 (four litters, 42 piglets in total); (2) non-handled (NH) – none of the piglets in a litter received TS according to the standardized procedure (four litters, 40 piglets). In order to control for the sow's effect on the offspring two more treatments were included in the trial (five litters): (3) 50/50H – the half of the litter that received TS, in the same way as in handled litters (22 piglets) and (4) 50/50NH – the half of the litter that did not receive TS, but experienced the daily presence of a human in the pen to perform TS on their litter mates (23 piglets). In these two treatments the piglets were randomly assigned within the litters to be stimulated (50/50H) or not (50/50NH) within the constraint of balancing as much as possible for sex and bodyweight.

2.3. Tactile stimulation procedures

Piglets from the AH and 50/50H treatments were subjected to TS from 5 days of age until weaning (35 days of age). The procedure was performed once a day until 21 days of age and then once every second day until weaning. It took place between 9:00 and 15:00 h with the exact time varying systematically between days and treatments. The procedure was carried out by two women, always wearing blue long sleeved cloth overalls and green boots.

In every handling session, one experimenter entered the pen and calmly removed the sow from the pen to another area in the same stable. When the sow was removed from the pen, one person caught a piglet and handed it to the other person who stood inside the creep area, which was closed off from the rest of the pen during handling. This person released the piglet on the floor in the creep area while the other person caught another piglet. When the two piglets were in the creep area both people sat down on the floor of the creep area and, taking a piglet into their lap, started to stroke it gently from head to back for 2 min, at a rate of 1 stroke/s.

During the first min of handling the piglet was always kept in the person's lap and stroked, but it was released to the floor after this time, if it was resisting or vocalizing, and the TS continued with the piglet on the floor. From the age of 21 days and onwards, piglets only received the TS while they were on the floor. This was due to the practicalities of restraining struggling or bigger piglets. The procedure resulted in all piglets receiving TS for the full 2 min since the creep area was so small that piglets were always within arm's reach. What makes this procedure different from one aimed at 'taming' the piglets was that the early handle was enforced, as was done in the rat and horse studies referred to earlier (Levine, 1960; Ligout et al., 2008). Every day, before starting the new handling session, we checked the order in which the piglets were stimulated in the previous session and re-arranged it to avoid bias in the order of TS.

The sows in the non-handled litters were separated from the piglets for the same amount of time as the handled litters.

2.4. Open-field/human-approach test

Four piglets from each litter were tested individually in a novel environment at 4 weeks of age. They were tested twice, once with a familiar and once with an unfamiliar person, in a balanced design (half of the piglets were first tested with the familiar person and then with the unfamiliar person, and the other half had the opposite order) with an interval of 2 days between the tests. Two familiar and two unfamiliar women were involved in the tests. The familiar people (who had performed the TS) wore the same blue long sleeved cloth overalls and green boots they had used when handling the piglets. Even the non-handled piglets were expected to have habituated to the presence of the familiar handlers due to their regular presence in the stable. The two unfamiliar people wore white overalls and had never been in the stable prior to testing.

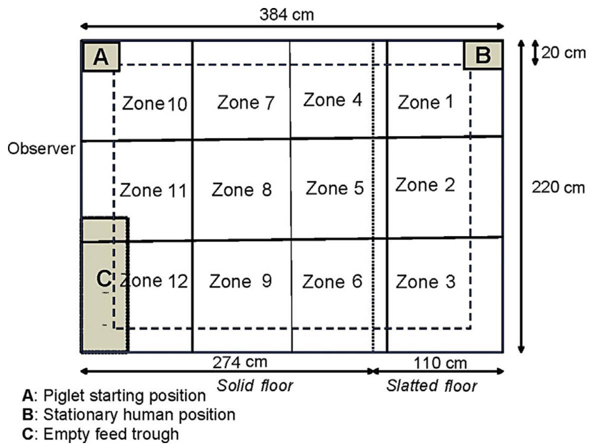

Fig. 1. Diagrammatic representation of the open-field/human-approach test arena. The test arena was divided in 12 different zones indicated by the solid lines and the dashed line represents the perimeter zone. The piglet was released in the corner ('A') and the stationary person was situated by 'B'. 'C' was an empty feed trough.

The test arena was an empty and disinfected pen (3.84 m × 2.2 m), identical to their home pen, but with no straw on the floor and no creep area (Fig. 1). It was located in a neighbouring stable within the same building. The arena was divided into twelve 0.7 m^2 zones and a perimeter zone within 20 cm of the pen wall. The boundaries to the zones were painted on the floor according to Fig. 1.

In total, 68 piglets were tested (36 males and 32 females; NH = 16; AH = 16, 50/50H = 18; 50/50NH = 18). Each group of four randomly selected piglets (two pairs, balanced for sex and weight) in each litter was kept in the creep area of the home pen during the time it took to test all the animals from their litter. In the 50/50 treatments, four 50/50NH and four 50/50H piglets from each litter were tested, with the exception of one litter which contained fewer than eight piglets. For this litter, we tested three piglets from each 50/50 treatment.

One piglet at a time was calmly transferred to the test arena using a trolley and placed in a corner of the arena. As soon as the piglet was released, the test began. Behaviour and location of the piglet were scored live by an observer standing outside the test arena and the familiar/unfamiliar person recorded latencies as described in the next paragraph. The location of the piglet (zone) was registered considering where the head and at least one front leg were located and it was recorded instantaneously every 5 s. This data was used later on to calculate the movement between zones within each phase of the test. One-zero registration of vocalizations (high pitched squeals and grunts) was also made in this same time interval.

The test lasted for 5 min. In the first part of the test, the piglet was left alone in the test arena for 1 min (isolation phase: 0–1 min). During this phase, the familiar/unfamiliar person stood outside the arena. After 1 min, the person quietly entered, walked to the corner and sat down, staying passive for 2 min (stationary person: 1–3 min). The latency for the piglet to approach the person (defined as a front leg and the head in the zone where the person was sitting) was recorded as well as the latency to first physical contact (initiated by the piglet towards the passive person). In the next part of the test (moving person: 3–5 min) the person stood up, waited for 5 s, then started walking slowly towards the piglet at a rate of 1 step/s with the aim of touching it. When within one arm's length of the piglet (and if the piglet was standing still), the person slowly bent down to touch it. The total number of attempts and the total number of accepted strokes was recorded.

2.5. Piglet performance

To monitor the body development, each piglet was weighed at birth and at 5, 9 and 12 weeks of age.

2.6. Statistical analyses

The statistical analyses were performed using the SAS software (version SAS 9.2, SASTM Inst., Cary, NC, USA) and the R environment (R Development Core team, 2011). All variables were tested to verify normality (Shapiro–Wilk test) and homogeneity of variance.

When the residuals of the dataset followed a normal distribution (UNIVARIATE procedure), analysis of variance (ANOVA) was used within the MIXED procedure and when they did not, the GLIMMIX procedure was utilized, taking into account the Poisson distributed data.

To analyze body weight, we considered the effects of treatment, sex, age (weeks) and the interactions between them as fixed effects and piglet nested within litter as a random effect. Birth weight was included as a covariate. Regarding the behavioural variables, the frequencies of changing zone (as a measure of activity, based on changes between location), location in the perimeter zone, vocalizations (high pitched squeals and grunts), stroke attempts, accepted strokes and mean number of strokes per attempt were determined in the three study phases (isolation, stationary person and moving person). The following independent variables were considered: treatment, sex and familiarity (familiar/unfamiliar person) and their interactions as fixed effects and piglet nested within litter as a random effect. When developing the models for the variables, fixed effects that had no significant influence in the model were removed and the data set re-analyzed.

The significance of effects was tested with the Tukey's post hoc adjustment test. For the two variables, latency to approach and latency to physical contact with the person, the analyses were conducted using the survival methodology in R Software (R Development Core team, 2011). We treated such observations as censored and performed the analysis with the Cox proportional hazards model including the effects of treatment, sex and familiarity as fixed effects, and piglet nested within litter as random effect. Results are presented as means and standard errors.

3. Results

As high pitched squeals represented less than 10% of piglet's vocalizations and there were no differences among treatments ($\chi^2 = 4.27$, $P = 0.24$, df = 66), only the results for grunting are presented.

3.1. Open-field/human-approach test

3.1.1. Isolation phase

When alone in the test arena, the frequency of changing zones was affected by treatment ($F = 3.72$, $P = 0.01$, df = 66) and by the familiarity of the person who was going to enter the pen 1 min later, which was not expected ($F = 10.25$, $P = 0.002$, df = 66). AH and NH piglets were recorded as having moved more often between zones than the piglets from the mixed treatment litters, 50/50H and 50/50NH (Fig. 2A). In addition, piglets from NH treatment were more frequently seen in the perimeter zone, whereas AH piglets were there least often compared to those from the other treatments ($F = 4.14$, $P = 0.009$; Fig. 2A). Regardless of treatment, in the isolation phase piglets moved more often between zones when it was a familiar person who was about to enter the pen compared to when an unfamiliar person stood outside the arena ($F = 10.25$, $P = 0.002$, df = 66, familiar: 12.81 ± 0.27 and unfamiliar: 11.0 ± 0.27).

Regarding vocalization (grunts), significant effects of treatment ($F = 3.59$, $P = 0.002$, df = 66), and familiarity of the person about to enter the pen ($F = 5.38$, $P = 0.02$, df = 66) were found. NH piglets vocalized most, AH piglets least, with both 50/50 treatments being intermediate ($F = 3.59$, $P = 0.002$, df = 66). Piglets from all treatments grunted more in the isolation phase when they would be tested with an unfamiliar person compared to the familiar person (familiar: 3.5 ± 0.3 and unfamiliar: 14.9 ± 0.5, $F = 5.38$, $P = 0.02$).

3.1.2. Reactions to a stationary person

In keeping with the results during the isolation phase, treatment ($F = 4.54$, $P = 0.006$, df = 63) and familiarity ($F = 4.60$, $P = 0.036$, df = 63) affected the reactions of piglets when the stationary person was in the arena. NH and 50/50H piglets moved more between zones than AH and 50/50NH ($F = 4.54$, $P = 0.006$, df = 63) and NH piglets were more frequently located in the perimeter zone than piglets from the other treatments ($F = 5.23$, $P = 0.003$, df = 63; Fig. 2B).

With regard to grunting, the results were also similar to those found in the isolation phase. NH piglets grunted more than AH piglets, whereas 50/50H and 50/50NH piglets were intermediate between them ($F = 6.07$, $P = 0.0005$, df = 66). No differences were found between treatments regarding the latencies to approach the person ($\chi^2 = 0.13$, $P = 0.45$, df = 0.11) or to physical contact ($\chi^2 = 15.91$, $P = 0.22$, df = 12.5).

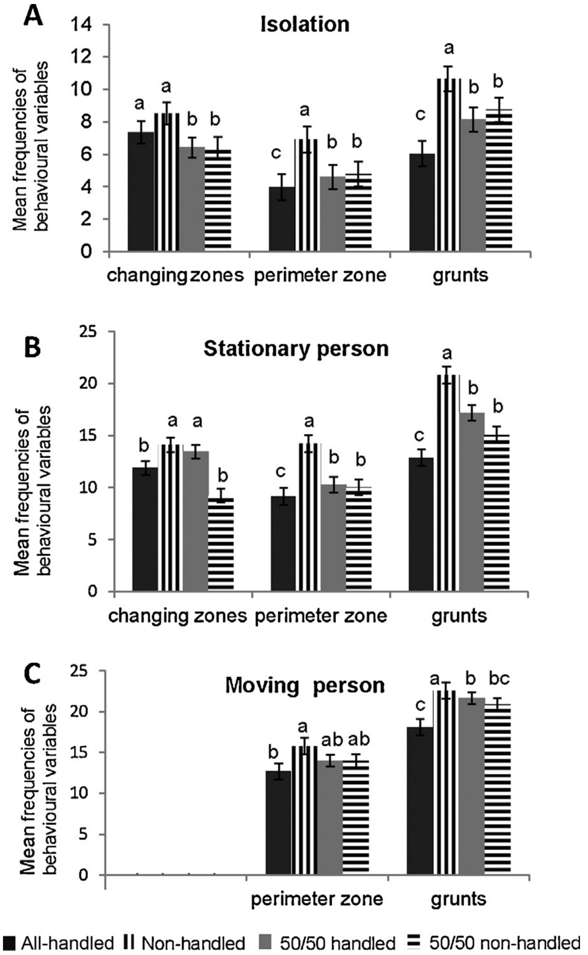

Fig. 2. Behaviour results in the open-field/human approach test. Mean of the frequencies (±SE) of changing zones, location in the perimeter zone and grunts in all treatments during the isolation phase of the test (A), with the stationary person (B) and with the moving person (C) according to the treatments. Significant differences at the 0.05 level are presented.

All piglets grunted more together with the unfamiliar person compared to when they were with the familiar person ($F = 5.42$, $P = 0.02$, df = 63; familiar: 15.9 ± 0.56 and unfamiliar: 16.8 ± 0.56). However, all piglets moved between more zones when with the familiar person ($F = 4.60$, $P = 0.03$, df = 63; familiar: 13.10 ± 0.77 and unfamiliar: 11.04 ± 0.77).

3.1.3. Reactions to a moving person

There were no main effects of treatment or familiarity of the person on the movement between different zones in this phase of the test, but a tendency for a significant interaction ($F = 2.33$, $P = 0.08$, df = 63). Closer examination of this interaction revealed that piglets from the NH treatment moved more often between zones than AH piglets regardless the familiarity of the person. However, AH piglets moved more between zones when a familiar person was present in the arena compared to when it was an unfamiliar one (Table 1).

Piglets from the AH treatment were least often in the perimeter zone whereas NH piglets were most often in this area ($F = 4.85$, $P = 0.004$, df = 63; Fig. 2C). Piglets from the NH treatment tended to vocalize more than piglets from the other treatments ($F = 5.16$, $P = 0.08$).

50/50H piglets allowed the unfamiliar person to approach to within one arm's length, resulting in a greater number of stroke attempts ($F = 3.09$, $P = 0.03$, df = 63) compared to piglets in the other treatment groups (Fig. 3). There was no difference in the behaviour of the pigs towards the familiar person attempting to stroke them. Ultimately though, more strokes were successfully given to AH and 50/50H piglets than to NH and 50/50NH piglets irrespective of whether the person was familiar or unfamiliar to them ($F = 2.78$, $P = 0.05$, df = 63; Fig. 3). There was a tendency for a sex effect in respect to stroke attempts, in which female piglets tended ($F = 3.01$, $P = 0.08$, df = 63) to allow more stroke attempts (6.13 ± 0.32) than males (5.32 ± 0.31).

As expected, AH and 50/50H piglets had a higher mean number of strokes per attempt, which means that they accepted more strokes with fewer trials ($F = 2.98$, $P = 0.04$, df = 63) compared to NH and 50/50NH piglets, in response to both the familiar and unfamiliar person.

Table 1
Means of the frequencies (±SE) of changing zones in the presence of a familiar and an unfamiliar moving person in the open field/human-approach test according to the treatments.

Group	Familiar	Unfamiliar
All-handled	16.63 ± 0.96^{Ab}	14.50 ± 0.96^{Bb}
Non-handled	19.94 ± 0.96^{a}	18.31 ± 0.96^{a}
50/50 handled	16.17 ± 0.91^{b}	16.28 ± 0.91^{ab}
50/50 non-handled	18.82 ± 0.93^{ab}	18.29 ± 0.93^{a}

Statistical differences in the columns (a, b) and in the rows (A, B). Significant differences are presented at the 0.05 level.

3.2. Performance

There was a significant interaction between treatment and age ($F = 3.21$, $P = 0.03$, df = 120) as differences in body weight between treatments were found only when the piglets were 12 weeks old (Table 2). The 50/50H piglets were lighter than piglets from the 50/50NH treatment and from groups where all piglets were handled (AH treatment).

4. Discussion

Our results showed that regular and forced early human handling using tactile stimulation altered piglets' behaviour in a standardized fear test. Besides the predicted reduction of fear in the presence of humans, their choice of location in the arena differed when they were alone in the arena. Although both NH and AH piglets were more active in the test arena compared to piglets from the 50/50 treatments, piglets that were not handled (NH) spent more time close to the walls, while handled piglets (AH) were more often in the centre areas of the open-field arena. This supports the findings in other species (Denenberg et al., 1973; Hughes, 1971). On the other hand, there was no evidence for the prediction, based on the results from other species (McClelland, 1956; Napolitano et al., 2005) that handling (by tactile stimulation) stimulated growth. Instead, this

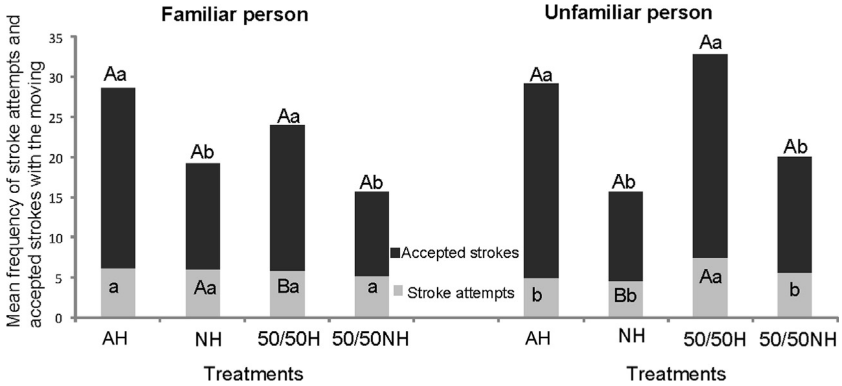

Fig. 3. Interactions with the moving person. Means of the frequencies (±SE) of stroke attempts and accepted strokes by piglets with familiar and unfamiliar persons according to the treatments. Significance was declared at 0.05 level (a, b = statistical differences between treatment groups; A, B = statistical differences between familiar and unfamiliar moving persons in the human-approach test).

Table 2
Means body weight (±SE) of piglets at birth, 5, 9 and 12 weeks of age according to the treatments.

Groups	Birth weight	5 weeks old	9 weeks old	12 weeks old
All-handled	1.71 ± 0.58	10.09 ± 0.34	25.8 ± 0.74	36.94 ± 0.97[a]
Non-handled	1.62 ± 0.57	10.40 ± 0.36	25.41 ± 0.76	35.56 ± 1.0[ab]
50/50 handled	1.58 ± 0.80	9.79 ± 0.49	24.52 ± 1.02	34.23 ± 1.3[b]
50/50 non-handled	1.60 ± 0.78	10.06 ± 0.48	25.52 ± 1.00	37. 80 ± 1.3[a]

Statistical differences in the columns (a, b). Significance was declared at 0.05 level.

study shows that in the 50/50 litters it was the seemingly milder stressor of been disturbed but not handled, that had maximal beneficial effects for growth performance. There was no difference in body weight between the all handled and non-handled litters. Thus, in a species where there is usually only limited maternal tactile stimulation of the young, the effects of early human handling and stimulation on the psychological level were supported, but the effects on physical development seemed sensitive to the level of stimulation stress.

Although compared to other species such as mice, rats and cattle, it is rare to see sows physically stimulating their offspring by licking or massaging them, the tactile stimulation applied in the AH treatment had significant effects on how piglets responded to the challenges they were exposed to during the test in this study. The usual interpretation from open field tests in rats is that remaining in the perimeter zone (an anti-predatory strategy) is indicative of a higher level of anxiety and fear (Donald et al., 2011), and increased vocalization has been interpreted as an indicator of a negative emotional state (Düpjan et al., 2008; Von Borell and Ladewig, 1992). When alone in the first part of the open-field/human-approach test, AH piglets were less frequently located in the perimeter zone and vocalized less than the piglets from the other treatments. Animals from mixed litters (50/50H and 50/50NH piglets) responded in an intermediate way, while NH piglets vocalized most and were more frequently in the perimeter zone. These results from the open-field test should be interpreted cautiously, since there is no confirmation of thigmotaxis (wall-hugging behaviour) in pigs, even under drug manipulation (Andersen et al., 2000). So it seems that the variable perimeter zone cannot easily be interpreted as a measure of anxiety and fear, as would be done with rats. On the other hand, Weaver et al. (2000) found similar results to ours in that handled pigs were in the outer squares (perimeter zone) less often. Perhaps NH pigs spend more time in the perimeter looking for a way to escape whereas AH piglets use the full potential of the whole arena. A larger ethogram recording the actual behaviours performed in the different locations by the different categories of pigs during the test would be necessary to investigate these possible explanations.

During the ‘moving person phase’, there was a tendency for AH piglets to be more active when a familiar person was present in the arena compared to when an unfamiliar person was there. Since these piglets also showed fewer signals of distress, e.g. less vocalization, it might be that the familiar person was acting as a sort of ‘social support’ for piglets to move more in the arena, suggesting reduced fear. This result supports previous work showing that pigs were more active in the arena when together with a familiar person (Tanida and Nagano, 1998; Tanida et al., 1995; Terlow and Porcher, 2005). A similar, although an unexpected effect of familiarity was found regarding vocalization also in the ‘isolation phase’, most probably because piglets recognized the person that was just about to enter the arena. According to Hemsworth et al. (1994), this human recognition can happen in situations of intensive handling, although in our case piglets could also have recognized the colour of the overall worn by the person (Koba and Tanida, 1999).

Moreover, the effect of forced human handling extended the acceptance of physical contact, since tactile stimulated (AH and 50/50H) piglets accepted a higher number of strokes compared to piglets from the other treatments. This is in line with other previous studies of non-forced handling, which showed that piglets exposed to human contact early in life are easier to approach and handle later in life (Hemsworth and Barnett, 1992; Hemsworth et al., 1986; Tanida et al., 1995). Furthermore, it seems that handled piglets generalized their acceptance of physical contact (accepted strokes), since there was no effect of familiarity, as has also been shown previously (Hemsworth et al., 1994). What is new in our study, besides that the pigs here were younger when they were tested, is that the handler stroked all piglets and that the human handling was enforced, whereas in the previous studies piglets were touched only if they approached the handler. According to Ligout et al. (2008), who studied two different handling methods (forced and non-forced human-contact) in horses (a species in which the dam shows early maternal tactile stimulation), only forced handling reduced the later fear reactions of foals towards humans. That reduced fear of piglets to humans based on handling procedures in which pats and strokes were given whenever the piglet approached (Hemsworth and Barnett, 1992), may suggest that piglets respond to both forced and non-forced social interactions and regardless of whether, from the piglets' perspective, the stimuli are experienced positively or negatively, which is in keeping with Levine (1960).

In order to better interpret the findings from this study, some practical aspects of the methodology when implementing the treatments should be addressed. For instance, because of the need to select only specific individuals in the litter, 50/50H piglets were probably exposed to a slightly longer catching procedure and so perhaps to slightly higher stress levels than piglets collected in the AH treatments. Similarly, the experience with humans for 50/50NH piglets was probably not the same as that of the NH piglets, since the former were exposed to the attempts by a person in the pen to catch the 50/50H piglets in their litter. Thus, although the inclusion of the split litter treatments allows us to control for sow/litter effects in a better way, we should be cautious in equating the handling of piglets from the 50/50 treatments (50/50H and 50/50NH) with those in AH and NH treatments, respectively.

In fact, these potential differences in how the piglets' experienced the procedure around the handling may explain some contradictory findings, such as the behavioural similarities (movement between zones) between 50/50NH and AH piglets during the "stationary person phase" of the open-field/human-approach test. It seems that being close to humans in the home pen, but not necessarily being caught and stroked by them, was enough to stimulate 50/50NH piglets to develop similar behaviour to AH piglets, i.e. possible habituation to the closeness or handling by people. Likewise, the similarities between 50/50H and NH regarding the measurement of movement between zones in this phase of the open-field/human-approach test can be treated following the same logic, i.e. possible sensitization or unfamiliarity to handling or to the closeness of people. These results might have practical implications at the farm level, since it seems that regular exposure early in life to the mere presence of a human entering the pen shows similar beneficial effects to the whole time consuming procedure involving tactile stimulation. Although we do not know how much of this effect is due to exposure to the person or to the stress and interaction between the piglets that their entrance may generate.

The differences in experiencing humans might also explain why 50/50H piglets seemed to avoid the familiar person in the "moving person phase". Fewer successful attempts to stroke the piglets could be made by the familiar person compared to the unfamiliar one (a stroke attempt was only performed when piglets were one arm's length away and standing) even though 50/50H were less active than NH piglets (less movement between zones).

Regarding body weight, the prediction based on studies with rodents, was that the handled animals would be heaviest (Levine, 1960, 1957, 1956; McClelland, 1956). This effect was not significantly different between the AH and NH treatments and we found an unexpected effect at 12 weeks of age in that 50/50NH piglets were significantly heavier than 50/50H. This led to the suggestion that the more moderate stressor, of being exposed to humans but not handled was optimal for physical development, whereas the combination of a potentially longer catching period and forced handling was too great a stressor, as seen with 50/50H piglets. It has been shown previously that when experienced as too stressful, human handling affected pig's weight gain negatively (Gonyou et al., 1986; Hemsworth and Barnett, 1991; Hemsworth et al., 1981). Given this apparent sensitivity to the level of stress caused directly or indirectly by the early handling, it would have been interesting to have followed the piglets for longer than 12 weeks to investigate any long term body weight consequences of the different treatments.

In conclusion, our results showed that forced early human handling reduced piglets' fear of humans and resulted in active piglets with low vocalization in a novel environment, perhaps suggesting less fear even when isolated. The expected benefits of human handling by tactile stimulation on body weight however could not be supported, seeming rather sensitive to the level of stimulation stress. Nevertheless from a practical point of view there were beneficial effects of the human tactile stimulation treatments that may be useful to explore further. Tactile stimulation may help prepare offspring to maximize their potential in a novel environment as well as potentially making them easier to handle, whereas merely entering the pen may stimulate weight gain.

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