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Published in:

Applied Animal Behaviour Science

DOI:

[10.1016/j.applanim.2018.02.004](https://doi.org/10.1016/j.applanim.2018.02.004)

First published: 14/02/2018

Document Version

Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Yang, C-H., Ko, H-L., Hofmann, LS., Llonch, L., Manteca, X., Camerlink, I., & Llonch, P. (2018). Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm. *Applied Animal Behaviour Science*, 202, 7 - 12. <https://doi.org/10.1016/j.applanim.2018.02.004>

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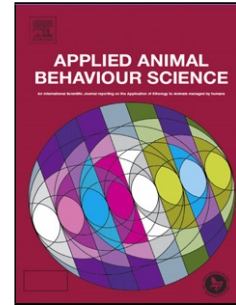
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Accepted Manuscript

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PII: S0168-1591(18)30068-6
DOI: <https://doi.org/10.1016/j.applanim.2018.02.004>
Reference: APPLAN 4599

To appear in: *APPLAN*

Received date: 27-11-2017
Revised date: 1-2-2018
Accepted date: 11-2-2018

Please cite this article as: Yang, Chung-Hsuan, Ko, Heng-Lun, Hofmann, Laura Salazar, Llonch, Lourdes, Manteca, Xavier, Camerlink, Irene, Llonch, Pol, Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm. *Applied Animal Behaviour Science* <https://doi.org/10.1016/j.applanim.2018.02.004>

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Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm

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Highlights

- Housing of neonatal piglets was enriched with substrate or hanging objects
- Environmental enrichment enhanced object play behaviour during lactation
- Neonatal enrichment had no impact on piglet growth during lactation and weaning
- Piglets which had received substrate did not show elevated cortisol at weaning

Abstract

Environmental enrichment is a legal requirement for European pig farms. The suitability of enrichment materials for neonatal pigs is understudied and has not been tested in commercial settings. This study investigates the effect of hanging objects and substrate as two enrichment strategies pre-weaning, and compares the effect of these enrichment objects on play behaviour, aggression, growth and stress

coping ability during lactation until 10 days after weaning. Farrowing crates were equipped with either six hanging objects (OB), a substrate box with wood bark (SUB), or nothing (control; CON). The behaviour of over 600 piglets (~210 piglets / treatment) was recorded weekly by instantaneous scan sampling (10 seconds / piglet, repeated 6 times per day for 6 days). Aggression was monitored through skin lesions on focal piglets on 1 day before weaning and 1 and 2 days after weaning. Piglets were weighed individually every week. Stress coping ability was assessed through salivary cortisol from a sample of six piglets per litter on 1 day before (baseline), and on days 1 and 2 after weaning. Both enrichment groups showed more object play during lactation as compared to the control group ($P < 0.001$). The amount of object play increased linearly with age ($P < 0.001$). Enrichment did not affect social play or locomotor play during lactation. Enrichment did not influence the amount of skin lesions before weaning, but heavier piglets had more skin lesions ($P < 0.01$). The enrichment strategies had no influence on weight gain at any stage. The baseline of the salivary cortisol concentration was similar amongst the treatment groups; however, the cortisol concentration of the object group and control group was significantly higher at one day after weaning than at baseline ($P < 0.001$) whereas the substrate group showed no significant increase. In conclusion, providing either hanging objects or substrate alone could encourage neonatal piglets to express more object play behaviour. Compared to the hanging objects, providing substrate in the commercial neonatal environment demonstrated to decrease piglets' stress at weaning, and therefore increase animal welfare.

Keywords: Cortisol, enrichment, neonatal environment, piglet, play behaviour

1 Introduction

The provision of environmental enrichment in commercial pig farms is closely related to the animals' welfare. Previous research demonstrated that pigs reared in enriched housing conditions express more natural behaviour (e.g. rooting), perform less agonistic behaviour and achieve better growth compared to pigs reared in barren housing conditions (Beattie et al., 2000; Martin et al., 2015). Many studies have shown that providing enrichment can reduce the occurrence of harmful redirected behaviour, such as tail biting (e.g. reviewed by D'Eath et al., 2014). Provision of extra space, objects and substrate

are the most common environmental enrichment methods adopted in the studies regarding housing conditions for pigs. Although different types of enrichment have been studied in relation to harmful behaviour such as tail biting, there has been little study on the effect of different enrichment types, and the comparison thereof, on positive behaviour such as play, in the pre-weaning stage. Moreover, increase of space availability in the farrowing pens is less likely to be implemented as an enrichment strategy in intensive farms, even if positive effects are demonstrated, because it may reduce the optimal use of space per weaned piglet.

Provision of enrichment may facilitate play behaviour. Play is a developed positive welfare indicator (Boissy et al., 2007) in young animals. For instance, Martin et al. (2015) showed that piglets reared in enriched neonatal housing conditions expressed more locomotor and social play behaviour than piglets reared in a barren environment. In addition, that study also showed less agonistic behaviour after weaning in piglets from the enriched compared to barren neonatal environment. A theoretical explanation supporting the 'play effect' is that locomotor play could prepare the animal for unexpected situations while social play enhances the necessary social skills such as conflict resolution (Bekoff, 1984; Spinka et al., 2001). Piglets reared in an enriched environment may cope better with a novel environment and avoid the undue confrontation later in their life (Spinka et al., 2001). Chaloupková et al. (2007) suggested that early play experience induced by environmental enrichment can have a profound influence on reducing agonistic behaviour even three to six months after weaning. At weaning, piglets are separated from the mother, moved to new facilities, fed with new feed and mixed with unfamiliar piglets, which altogether results in a great stress response. Cortisol is a key regulator of the stress response and its concentration in blood and saliva has been used as a stress biomarker (Colson et al., 2012)

This study aims to investigate the effect of different enrichment materials on the expression of play behaviour, growth, aggression and stress coping ability in a commercial neonatal environment of pigs. Experimental research facilities often provide more space and enrichment to the experimental animals, in line with legislation for research studies. This may already increase the amount of play as compared to commercial conditions. Testing the enrichment materials at a commercial farm provides direct applicability of these materials for on-farm use if proven to benefit welfare. Two types of enrichment

materials (substrate vs. hanging objects) were compared with each other and against no provision of enrichment (control).

2 Material and methods

This trial was conducted between May and June 2016 at a commercial pig farm in Puiggros, Lleida, Spain. The farm had a breeding stock of 1,130 sows on a routine program of 50 – 60 sows farrowing per week on average. All experimental procedures were approved by the ethical committee of the Universitat Autònoma de Barcelona (UAB). Management procedures, such as tail docking and castration, were conducted routinely by the staff of the farm.

2.1 Animals and housing

A total of 48 primiparous and multiparous Danbred sows (distribution of parities in treatments is shown in subsection 2.2 ‘Experimental design’) and their litters were studied. Sows were moved to farrowing pens 3 to 5 days before the expected farrowing date. Each sow was housed in a crate (2.00 × 0.60 m) into a farrowing pen of 2.46 × 1.66 m (**Fig. 1**). The pen had a slatted PVC floor with a 1.20 × 0.60 m heating area for the piglets. The temperature of the room of the farrowing pens was automatically set at a maximum of 30±2° C, and temperatures above activated a cooling system. If during farrowing the interval between two piglets being born exceeded 50 min, than the sow was treated with 1ml of oxytocin (Facilpart, SYVA laboratories, León, Spain), according to farm routine procedures. At 24 hours after birth, the litter size was homogenized at 13-14 piglets by fostering among litters from the same treatment. At maximum two piglets were added to or removed from a litter and the remaining piglets were allocated to artificial lactation according to the farm’s procedures. After fostering, piglets were identified with numbered ear tags with different colours among treatments. Piglets were iron supplemented by intramuscular injection one day after birth and male piglets were castrated three days after farrowing. Commercial solid feed (creep-feeding) was available for piglets two weeks before weaning by scattering it on the feeding plate and water was provided *ad libitum* by automatic drinkers. Piglets were weaned on average at 25 days of age. At weaning, sows and their litters were removed together from the farrowing pen. Piglets were separated from the sows,

loaded onto a truck and transported to the weaning units, which were located 400 m away. Piglets were housed over 16 pens with 40 piglets per pen on average, grouped by similar body weight irrespective of the treatment. Pens measured 3.1×2.2 m and had a partly slatted PVC floor (2×2.2 m) and partly solid floor (1.1×2.2 m). Air temperature was automatically set at 19°C ($18 - 20^{\circ}\text{C}$). Artificial light was on from 8:00 – 18:00 h but windows allowed natural daylight. Piglets had *ad libitum* access to water and feed (three feeders and four drinkers per pen).

2.2 Experimental design

To study the effect of different enrichment materials for piglets on their behaviour and performance, sows ($n = 48$) and their litters were randomly assigned to one of the three different treatments; control (CON, $n = 16$), substrate (SUB; $n = 16$), or hanging objects (OB; $n = 16$). The kind of substrate and the hanging objects were selected from a variety of them after a preliminary study. Those receiving higher attention from piglets were chosen. There was one primiparous sow in the CON group and four in the SUB and OB group. Piglets in the control treatment had a barren environment without any added enrichment material. In the substrate treatment, the same farrowing pen as in the control treatment was now provided with a white plastic box ($60 \times 40 \times 10$ cm) with wood bark (**Fig. 1**) that was replenished daily. In the object treatment, the farrowing pen was attributed with six hanging objects; two KONG® Durable (Salisbury, United Kingdom), two wired thick ropes that were hang by a string into the roof, and two plastic balls with ropes (Nayeco, Barcelona, Spain) which were attached to the ceiling with metal chains. The three kinds of objects were kept at piglet eye level throughout the trial and were placed out of the sow's reach (**Fig. 1**). Both the substrate and the objects were provided from one day after farrowing until weaning. The experimental trial lasted from one day after farrowing until ten days post-weaning.

2.3 Behavioural observations

Behaviour of piglets during lactation was recorded live on six observation days by three observers who received training until achieving acceptable repeatability ($r = 0.82$). Observation days were at 4, 5, 11, 12, 19 and 23 days after birth and included a 6-hour observation period, distributed over two

assessment periods; one in the morning (from 10:00 h to 13:00 h) and one in the afternoon (from 15:00 h to 18:00 h). Behaviour was assessed using 10-sec instantaneous scan sampling (i.e. every 10 seconds for a different piglet). Each observer performed two assessments per day (morning and afternoon). The ethogram focused on locomotor, social and object play behaviour, with other general behaviours such as aggression, suckling, resting and 'out of sight' (**Table 1 and Supplementary files**). The ethogram was based on Martin et al. (2015) and was tested using three 30-min pilot sessions at the commercial farm. Although some studies might use the term exploration to describe pig-object interaction (e.g. see Beattie et al., 1995), the previously described definitions of exploratory behaviour hardly differed from definitions for object play (Wesler and McCall, 1976; Einon, 1983). Thus, according to recommendations from studies above, once the piglet interacted with the object (e.g. biting, chewing or rooting) this was in the current study recorded as object play rather than exploration. For individual observation, piglets were identified by a number on their back every morning before observations (from 07:00 h to 09:00 h) using a permanent marker pen, which was applied on a first-caught-first-marked basis without individual recognition.

2.4 *Skin lesion scoring*

Aggressive interactions were estimated based on the number of fresh skin lesions. To facilitate counting, the body was divided into three regions: front (head, neck, shoulders and front legs), middle (flanks and back) and rear (rump, hind legs and tail). The number of lesions was recorded but the length or diameter of the lesions was not considered. Freshness was judged subjectively by lesion colour and the estimated age of scabbing. According to Turner et al. (2006), the number of fresh skin lesions reflects the number of bites a pig has received in the previous 24 to 48 hours. For each treatment group, 66 focal piglets (six piglets / litter of the 11 largest litters) were selected for skin lesion scoring. From each litter, the lightest and heaviest male and female were selected as well as a male and female with a weight around the median pen weight. Lesions were scored 1 day pre-weaning (-1), 1 day post-weaning (+1) and 2 days post-weaning (+2).

2.5 *Performance data*

Piglets were individually weighed weekly over a 6 week period (day 1, 7, 14, 21, 28, and 35 after birth). Weighing always took place in the morning and was done by randomly picking up a piglet and placing it in a plastic box (60 × 40 × 20 cm) on a weigh scale (Balanza Cobos CW-PB4040-60). Average Daily Gain (ADG) was calculated per week by [weight age B – weight age A / number of days between A and B].

2.6 *Saliva cortisol concentration*

Saliva samples were collected to assess the cortisol concentration of the piglets. The samples were collected during the morning (from 09:00 to 11:30) at 1 day before weaning (Basal), 1 day (+1) and 2 days (+2) after weaning. The same piglets that were selected for skin lesion counts were also sampled for saliva. The piglets were held by a person who offered a sponge (Salivette®) in the mouth to be chewed during at least 30 seconds. Subsequently, the Salivette® was immediately inserted into a 10 ml tube and kept at 4°C temperature for later centrifugation. The tubes were centrifuged for 10 minutes at 3000 rpm to collect the saliva. Samples that had less than 100 µl were discarded. The saliva obtained was stored at -20°C until analysed. In total, 539 saliva samples were assessed to detect cortisol concentrations by using cortisol ELISA detection kits (Neogen Europe, Ayr, UK). The precision within tests was assessed by calculating intra-assay coefficients of variation from all duplicated samples analysed. The inter-assay coefficients of variation were calculated from 10 pool samples with markedly different concentrations and analysed per duplicate in each ELISA kit. The linearity of dilution was determined by using 1:1; 1:2; 1:5 and 1:10 dilutions of pools with EIA buffer.

2.7 *Statistical analysis*

Data were analyzed using SAS (Statistical Analysis System, version V.8; SAS Institute, Cary, NC, USA) version 9.3 and a P-value <0.05 was considered significant. Data was reported as means ± standard error.

2.7.1 *Behaviour*

During lactation, behaviour was recorded at a pen level and the frequency of observed behaviours per

pen was divided by the number of piglets per observation pen to obtain a proportion per pig (to account for the varying number of piglets per pen). Observations were averaged per day by litter. Play behaviours were grouped due to their low occurrence into the major categories of locomotor play, social play and object play. All other behaviours were grouped together as 'other' as the main interest was in play behaviour (note that aggressive behaviour was assessed by skin lesions rather than behavioural observations). The category 'other behaviour' showed a normal distribution of the residuals but all play behaviours did not. Accordingly, the proportion of behaviour spent on object play and social play were arcsine square root transformed, which resulted in a normal distribution. The proportion spent on locomotor play remained not normally distributed (even after transformation) due to the large amount of zeroes and was therefore converted into a binary trait, either being absent at pen level (0) or present (1). Normally distributed data were analysed in linear mixed models (MIXED Procedure) with the behaviour as response variable and the treatment and observation day as fixed variable. The litter was included as random factor to account for multiple observations per litter. Locomotor play was analysed with a Generalized Linear Mixed Model (GLIMMIX Procedure) with a binary distribution and logit link function. The binary variable 'locomotor play' was the response variable and treatment and observation date were the fixed variables. Sow was included as random factor.

2.7.2 Skin lesions

For the analysis, the pre-mixing, 1 day post-mixing, and 2 day post-mixing scores were used. The count of skin lesions was strongly skewed and residuals remained not normally distributed even after square root transformation. Instead, untransformed data was analysed in a Generalized Linear Mixed Model (GLIMMIX Procedure) with a Poisson distribution and log link function. The number of skin lesions was the response variable with as fixed variables the treatment, sex, and body weight at weaning. Litter of origin and weaning pen were included as random factors to account for maternal effects and pen effects.

2.7.3 Growth performance

Body weight and ADG showed a normal distribution of the residuals. Data on body weight and ADG

were analysed using linear mixed models (MIXED Procedure), with body weight and ADG as response variables. The fixed effects were treatment, sex and parity (biological sow). The random effects were the litter of origin (nursing sow) and the weaning pen.

2.7.4 Salivary cortisol concentration

Salivary cortisol concentration followed a non-parametric distribution and data was Log transformed to reach normality. To assess differences in salivary cortisol a linear mixed model (MIXED Procedure) was used, considering treatment, sample (basal, +1 and +2), and the interaction treatment×sample as fixed effects, and sampling time as a covariable. When the ANOVA showed significant differences ($P < 0.05$), a least square means comparison test (LSMEANS) adjusted to multiple comparison test of Tukey was carried out. In order to assess the differences in cortisol variation between treatments, a linear mixed model (MIXED Procedure) was used again but considering the same variables as previous analysis but including basal cortisol as a covariable. In this analysis only cortisol of post weaning samples “+1” and “+2” were assessed.

3 Results

3.1 Behaviours

The SUB and OB groups showed 1.8 times more object play behaviour than piglets from the control group (**Fig. 2**; both $P < 0.001$). The SUB and OB group did not differ from each other for object play ($P = 0.51$). The amount of object play linearly increased with the age of the piglets, showing an increase each week of observation ($P < 0.001$). The amount of object play behaviour did not differ between treatments among each observation day ($P > 0.10$). The OB group showed more social play than the SUB group (**Fig. 2**; $P = 0.04$) but both of them did not differ from the control ($P = 0.64$). In this case, the proportion of observations spent on social play tended to increase with age ($P = 0.06$), with the highest proportions of social play in the 2nd week after birth (day 11 and 12). Locomotor play did not differ between treatment groups ($P = 0.86$) and was unaffected by age ($P = 0.21$). The proportion on other behaviour was higher for CON as compared to SUB ($P < 0.001$) and OB groups ($P < 0.001$) due to the lower proportion of observations on object play in the control group.

3.2 Skin Lesions

The number of fresh skin lesions was low at all three sampling days, with on average 0.2 ± 0.1 lesions pre-weaning; 2.5 ± 0.3 lesions at 1 day post-weaning and 0.7 ± 0.1 lesions at 2 days post-weaning. The treatment groups did not differ in the number of fresh skin lesions prior to weaning ($P = 0.24$), at 1 day post-weaning ($P = 0.74$) or at 2 days post-weaning ($P = 0.31$). Before weaning, heavier piglets had more fresh skin lesions than lighter piglets ($b = 0.4 \pm 0.1$ lesions / kg; $P < 0.01$), but at weaning body weight had no influence on the number of fresh skin lesions (1 day post-weaning $P = 0.27$; 2 days post-weaning $P = 0.68$). Males tended to accumulate more fresh lesions at weaning than females ($P = 0.07$), but did not differ in skin lesion count pre-weaning ($P = 0.71$) or at 2 days post-weaning ($P = 0.67$).

3.3 Body weight and average daily gain

Body weight and average daily gain (ADG) did not differ between the treatment groups throughout the duration of the trial (**Fig. 3**) (at all weighing moments $P > 0.10$). The three treatment groups showed the same pattern, with a peak in ADG between day 14 and 21, followed by a drop in the week of weaning (Fig. 3). Piglets of primiparous sows were, throughout the trial, lighter than piglets born at multiparous sows, with piglets of primiparous sows being 0.25 ± 0.13 kg lighter at birth ($P = 0.05$) and being 0.60 ± 0.21 kg lighter at the end of the trial ($P < 0.01$). ADG was lower in piglets of primiparous sows as compared to piglets of multiparous sows before weaning (at all weeks $P < 0.001$) but not after weaning (ADG day 21 – 28 $P = 0.81$; day 28 – 35 $P = 0.60$). Male and female piglets did not differ in weight or ADG at any of the weighing moments (each weighing moment $P > 0.10$).

3.4 Salivary cortisol concentration

The average salivary cortisol per sampling day according to treatment is given in Table 2. The concentration of cortisol before weaning (Day 0) did not differ between treatments ($P = 0.58$). In the CON group, the concentration of cortisol was significantly increased from Day 0 to Day 1 (Day 0 - 1;

Mean 4.02 ± 0.708 , $P < 0.001$) and dropped again from Day 1 to Day 2 (Day 1 - 2; Mean -2.91 ± 0.530 , $P = 0.02$) while the data from OB group showed an increase followed by a decrease (Day 0 - 1: Mean 2.12 ± 0.647 , $P < 0.001$; Day 1 - 2: -3.33 ± 0.512 , $P < 0.001$). However, in the SUB group the cortisol concentration showed no significant change at weaning (Day 0 - 1; $P = 0.278$) and only tendency to drop in Day 1 - 2 ($P = 0.06$). Furthermore, the cortisol concentration was significantly higher in the CON group in Day 1 than in the SUB group ($P = 0.045$). No difference was found in the cortisol concentration among treatment groups in Day 2 ($P > 0.05$).

4 Discussion

Environmental enrichment may contribute to the reduction of abnormal behaviours in pigs and has as such been included in EU legislation as a requirement at farms. Here we investigated the suitability of two types of enrichment objects (hanging objects and wood bark substrate) and their effect on play behaviour, aggression, growth and stress coping ability in neonatal piglets.

It was hypothesised that piglets in the treatment groups would show more play behaviour, would express less agonistic behaviour, have a greater growth performance and cope better with weaning compared to housing conditions without additional enrichment. Results confirmed a higher dedication to playing during lactation in both enriched treatments (SUB and OB) and an improved ability to cope with weaning stress in SUB compared to OB and CON. Implications of neonatal enrichment for performance during lactation and weaning could not be confirmed in this study.

4.1 Behaviour

Piglets in the OB and SUB groups performed more object directed behaviour than the CON group. The present result confirms the previous findings that piglets reared in enriched housing conditions express more play behaviour than piglets in barren environments (Bolhuis et al., 2005; Chaloupková et al., 2007; Martin et al., 2015). Studies suggested that explorative behaviour (which in this study has been taken as object play) such as rooting, chewing and sniffing the enrichment materials has a high priority in pig's behaviour. It has been demonstrated that pigs living in semi-natural environments

spend more than half of the time exploring the surrounding (Stolba and Wood-Gush, 1989; Jensen and Stangel, 1991). Pigs explore their surroundings for both extrinsic and intrinsic purposes (Wood-Gush and Vestrgaard, 1989). Extrinsic exploration is related to foraging and finding a favourable place to lie down while the purpose of intrinsic exploration is gathering the information from their current environments. Thus, explorative behaviour is not only motivated by an immediate goal (e.g. foraging) but also curiosity (Wood-Gush and Vestrgaard, 1989). Both enrichments in the present study contained no nutritional value. Hence, this result suggests that the provision of these two kinds of enrichment materials might be related to the intrinsic exploration and, as a result, to some extent fulfil the psychological need (exploring the environment motivated by curiosity) of piglets. This inevitably avoids pigs from being bored, and thus improves their welfare (Dawkins, 1990).

It is worth noting that the amount of object play in the substrate group might be underestimated. During the study, two different types of usage of substrate were observed. The box of substrate in some pens was wet when replenished because of presence of faeces and urine. In contrast, the substrate in other pens was always dry. We could not determine any environmental reason, such as climate or facility defaults, why some litters soiled the substrate box but others did not. However, the different usage of substrate influenced the object play in the substrate groups since piglets never chewed or explored the box of wet substrate evidencing that piglets lost interest rapidly towards enrichment once they were soiled with faeces. Thus, the actual amount of object play in the substrate group might be higher if the substrate was dry.

Chaloupková et al. (2007) and Martin et al. (2015) suggested that the enriched environment would increase locomotor play and social play in pre-weaning piglets but this could not be confirmed according to results from our study. This inconsistency could be associated with pen design in the studies. The former studies provided both enlarged space and substrate at the same time while our trial had no confounding between space and enrichment, as no adjustment of space was made. Regarding the effect of space allowance, Apple and Craig (1992) found that pigs in larger pens tend to perform less object play than pigs in smaller pens but no effect was found on the duration of play. Jarvis et al. (2002) also compared the effect of increased space allowance and provision of substrate and showed that extra space, or allowance of locomotion, had a greater effect on gilt behaviour than provision of

enrichment.

The results evidence that the amount of object play increased along with the age of piglets. This is consistent with other studies (Newberry et al., 1988; Blackshaw et al., 1997; Bolhuis et al., 2005) which suggest that the development of cognitive abilities during the neonatal period facilitate playing. On the other hand, in our study, the amount of locomotor play was found not to be age related. The finding is in contradiction with Newberry et al. (1988), who demonstrated that locomotor play was age related, peaking between 2 to 6 weeks after birth. The conflicted result could be explained again by the difference of housing condition. Piglets in the Newberry et al. (1988) were reared in the 2.3 ha Pig Park while in our study limited space was provided. Hutton (1983) demonstrated that piglets reared in a confined space perform less locomotor play than unrestricted space. Thus, the space limitation during lactation may restrict the natural behaviour of piglets with likely implications for their welfare.

4.2 *Skin lesions*

The different treatments had no significant effect on the number of skin lesions at all three sampling days. The result is in line with previous studies (O'Connell et al., 2004; Chaloupková et al., 2007; Martin et al., 2015). These studies support that the early play experience does not affect the amount of aggressive events during the routine weaning procedure. This might be because fighting is inevitable when forming a hierarchy between newly mixed pigs (Meese and Ewbank, 1973) irrespective of play behaviour during the neonatal period. In this study, weaned piglets were mixed with regard to their body weight, following the routine management of the commercial farm, irrespective of the treatment group, which might have confounded the treatment effects. However, the fact that results are in line with previous research suggests that these may not be deviated because of the experimental design.

Despite the null effect on aggression after mixing, the effect of early play experience on aggressive behaviour in later stages of pig's life seems to be positive. Martin et al. (2015) showed that aggression remains high in frequency in both enriched and barren housing groups in the first three days after mixing but drops sharply in the enriched groups after seven days. Supporting this, Chaloupková et al. (2007) and Munsterhjelm et al. (2009) also suggested that pigs with early experience of substrate

showed less aggressive behaviour than pigs without the experience in later life. However, as aggressive behaviour was only assessed until two days after weaning, the long-term effect could not be confirmed in this study.

4.3 *Growth*

The data shows that enrichment, either hanging objects or substrate, had no effect on overall ADG, which is in line with previous research. Beattie et al. (1995) showed that neonatal enrichment had no effect on growth performance from farrowing until twenty weeks after birth. Conversely, according to Schaefer et al. (1990), when enrichment is provided to newly weaned piglets, it has positive effects for ADG (Schaefer et al., 1990) but also decreases agonistic behaviour during this critical period. The aforementioned authors suggested that providing objects might not only repartition the energy from fighting to growth, but decrease the anxiety level, and thus improve the growth rate. Comparing the studies from Beattie et al. (1995) and Schaefer et al. (1990), in addition to the age difference (lactating vs. newly weaned piglets, respectively) there is a fundamental difference in the enriched pen design. Schaefer et al. (1990) adopted a teeter-totter device as enrichment, while Beattie et al., (1995) provided extra space and designed several areas that provided different kinds of substrates. As discussed in previous paragraphs, different environmental enrichment methods may lead to variation in the impact for welfare and productivity.

4.4 *Salivary cortisol*

Colson et al. (2012) showed that social mixing and environmental change had an additive stressful effect on piglets, based to a significant increase of cortisol concentration in saliva after weaning. In our study, salivary cortisol after weaning increased in OB and CON groups but this did not happen in the SUB group. According to this, environmental enrichment with substrate during the neonatal period might improve the ability of piglets to adapt to weaning stress in commercial farming situation. The reason for this stress mitigation at weaning could be due to a rise in object play. Spínka et al. (2001) states that early play experience could help piglets to cope better with the novel environment. However, the different effect on the coping ability among the enrichment methods mentioned above implies that

both enrichment materials may stimulate play differently. However, we could not find any reference in the literature that can explain a biological reason for such a difference between both enrichment materials. Still, the results of this study provide significant evidence to consider neonatal environmental enrichment as a strategy to reduce stress at weaning.

5 Conclusion

This study identifies the effect of hanging objects and wood bark substrate as enrichment material for piglets reared under commercial farming conditions. The results show that the provision of hanging objects or substrate alone encourages piglets to play more than in a barren environment. Enrichment did not affect aggressive behaviour at weaning, estimated by the number of skin lesions. Similarly, the effect of neonatal enrichment was insignificant on growth during the neonatal phase up to weaning. However, neonatal enrichment using substrate may have positive effects for the welfare of piglets as it reduces the stress response during the weaning challenge. These findings suggest that neonatal enrichment provides the opportunity to promote positive behaviour in neonatal piglets and reduce the level of stress in a critical period such as weaning without having a negative impact on piglet growth. Future research should investigate whether the reported positive effects on behaviour and welfare remain over time and whether neonatal enrichment has an impact on later stages of pig's life.

6 Acknowledgement

Thanks are due to Ramaderies Miqueló S.L. for offering their facilities to conduct this study. We also acknowledge the technical support from the staff at the Animal and Feed Science Department of UAB for their technical help during the experiment. PL received support from a Marie Curie Cofund Fellowship within the 7th European Community Framework Program (TECSPR15-1-0040). This project was funded by "Proyectos I+D+I Convocatoria RETOS 2015" (AGL2015-68373-C2-1-R) from the Ministry of Economy, Industry and Competitiveness of the Spanish Government. IC was funded by the Biotechnology and Biological Sciences Research Council (BBSRC). SRUC receives financial support from the Scottish Government.

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Figures

Figure 1 Treatment design with farrowing pens for the control group (A), substrate enriched pens (B), and pens with hanging objects (C).

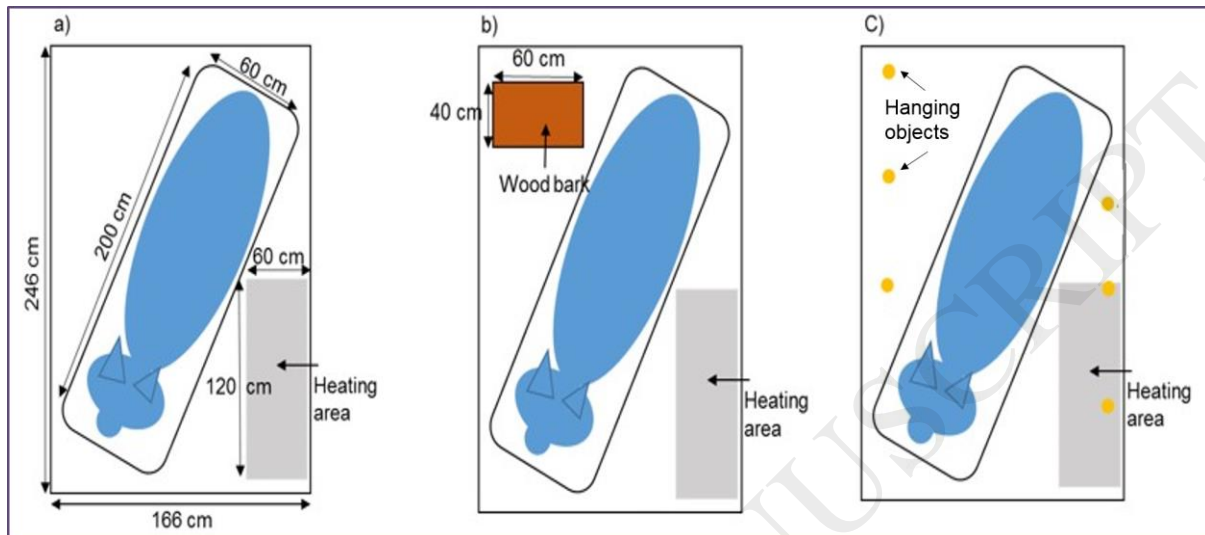


Figure 2. Proportion of observations spent on social play and object play in the control group (CON), substrate group (SUB) and object group (OB) in LSmeans with SE for the arcsine square root transformed values.

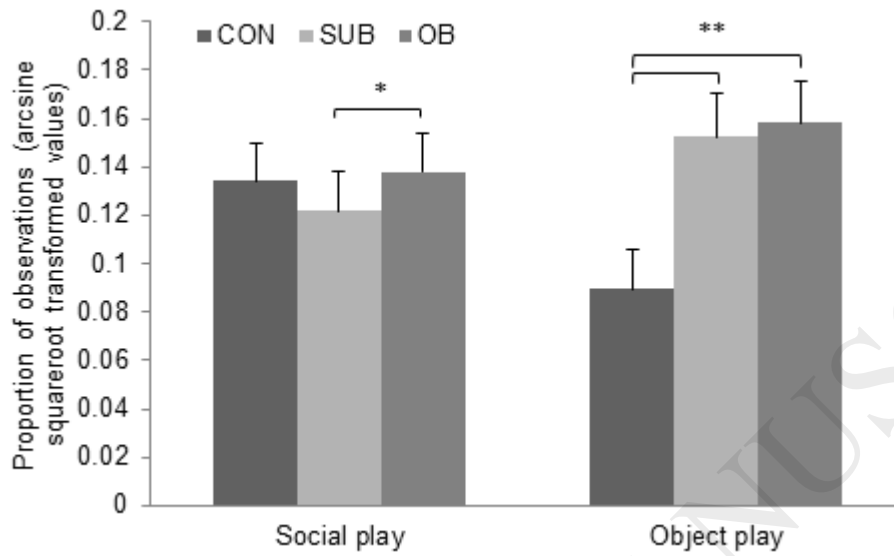


Figure 3. Average daily gain (B) by week for the control group (CON), the substrate group (SUB), and the group with objects (OB) in LSmeans with SE.

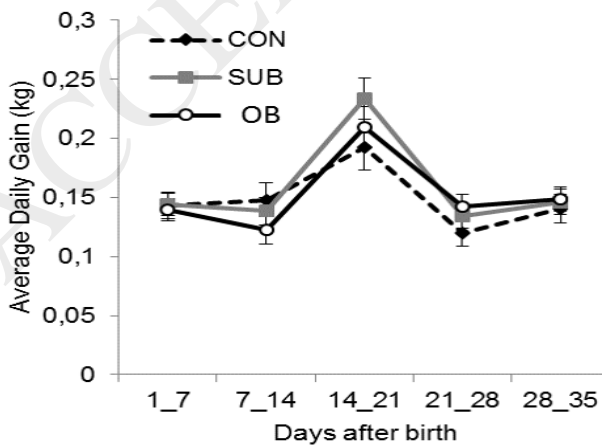


Table 1. Ethogram of clustered behaviours as used in the analyses. For the full ethogram see the Supplementary files.

Behavioural category	Description
Locomotor play	Piglet is either scampering, running, pivoting, head tossing, flopping, or hopping individually
Social play	Piglet pushes, nudges, chases, or climbs another piglet
Object play	Piglet is manipulating an enrichment object (hanging objects or substrate) or pen features
Other	Aggression, suckling, resting, all other behaviour and being out of sight

Table 2. Average concentration (ng/ml) of piglets' salivary cortisol according to treatment group (CON: control; OB: object; SUB: substrate) and sampling day.

	CON	OB	SUB
Basal	3.94±0.454 ^b	5.93±0.473 ^b	5.53±0.452 ^{a,b}
Day 1	7.98±0.543 ^a	8.50±0.516 ^a	7.57±0.590 ^a
Day 2	5.13±0.279 ^b	5.22±0.306 ^b	5.07±0.422 ^b

Different subscripts correspond to significant differences by $P < 0.05$ between sampling days within each treatment.