Effects of different enrichment devices on some welfare indicators of post-weaned undocked piglets

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Running title: Enrichment devices for weaned piglets

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Abstract

Two experimental trials were carried out in order to test the effectiveness of different environmental enrichments in improving the welfare of weaned pigs. A total of 120 undocked piglets was used. In trial one, group C1 received a metal chain and group WL a wooden log mounted on a frame. In trial two, the enrichments proposed were a hanging chain (group C2), an edible block (group ED) and a wooden briquette (group WB) mounted on a frame. The effectiveness of the enrichments was assessed in terms of animal behaviour, cortisol from bristles, hematologic and hematic profiles, cutaneous (skin and tail) lesions. Growth parameters were also recorded. Although some differences were detected in growth parameters in trial 1 (with C1 group having better productive outcomes than WL group) and some minor differences were observed in animal behaviour in both trials, the overall welfare status did not differ among the experimental groups. On the other hand, no welfare issues emerged in groups C1 and C2, receiving the enrichment device which is generally believed to be scarcely attractive, i.e. the hanging chain. We can therefore conclude that,

if no managerial errors are made (floor space availability, feed inadequacy, group stability, microclimate, illumination), under the tested experimental conditions, hanging chains can provide a sufficient environmental enrichment for undocked piglets, even when compared to more attractive enrichments (*e.g*. an edible block).

Keywords animal welfare, blood parameters, environmental enrichment, intensive husbandry, pig, weaners

1. Introduction

The term "environmental enrichment" is used widely in the literature to indicate improvements to captive animal environment. However, from a scientific point of view, it should only be applied to modifications capable of improving the biological functioning of captive animals (Newberry 1995). In the case of pigs, a successful enrichment should decrease the incidence of abnormal patterns of behaviour (stereotypies, belly nosing, ear and tail biting) and increase the frequency of species-specific behaviours such as social interactions, foraging end exploration (Petersen *et al* 1995; van de Weerd & Day 2009; Telkänranta, *et al* 2014a).

The provision of manipulable materials to pigs of all ages is mandatory in the European Union since January 2013 (Directive 2008/120/EC). However, the use of substrates listed in the directive (straw, hay, wood, sawdust, mushroom compost, peat) is not always feasible for farmers. Although straw indeed has the highest potential to be the "gold standard" enrichment material (Bracke et al., 2006), its use, especially in slatted systems, can cause difficulties for slurry management (Scott *et al* 2007; EFSA 2007). On the other hand, indestructible objects such as metal chains or tyres are considered not sufficient to provide for the exploratory needs of pigs and, according to EFSA (2007) recommendations, they may be used as a supplement to destructible and rooting materials but not as a substitute for them. The main reason for such a provision is that such enrichments, according to the literature, can apparently provide only marginal welfare benefits in terms on animal welfare, since they allow pigs to perform manipulatory behaviours, but not actual rooting behaviours (i.e., "to turn up by digging with the snout or nose" - American Heritage® Dictionary of the English Language, 2011), therefore the need for exploration may not be met by indestructible objects (EFSA, 2007). However, there is some evidence that it could be possible to design successful point-source

enrichment-objects, provided that they are able to sustain interest for a protracted period of time (van de Weerd & Day 2009) and that no competition for access to the enrichment occurs (Jensen *et al* 2010). According to Bulens et al. (2016), the provision of straw blocks reduced pen mates manipulation (e.g., tail and ear biting, belly-nosing) in finishing pigs. As it has been extensively reviewed by Bracke *et al.* (2006), various enrichment tools and materials have been proposed for piglets, including: cloth strips, rubber hoses, different amounts of straw, ropes, wood blocks, wood beams, straw racks, dog toys, mineral blocks, roughage and substrates (compost, earth, sawdust, peat). Their main conclusions were that metal objects show very few significant welfare benefits; and that rubber, rope, wood, roughage and substrates have more benefits than metal objects, but less than straw and compound objects. However, the review highlights how relatively little has been reported about mineral blocks and wood used as environmental enrichments for piglets. Trickett et al. (2009) compared the use of rope and wood as enrichments for weaned piglets and found that rope had a good attractiveness but, despite object alternation, habituation still occurred reducing the long-term attractiveness of the enrichments. Similar results were found in weaners by Blacksaw et al (1997), who observed a progressive decrease over time in interactions with the toy. However, both studies agreed that suspended or fixed objects are the most hygienic and attractive way to effect enrichment.

The aim of the present work is to gain new insights on the effectiveness in improving the welfare level of post-weaned piglets, assessed through behaviour, health, physiology, and performance traits. The investigated enrichment-objects were made with poorly investigated materials (poplar wood, sawdust briquette and edible block), and compared to metal chains which are widely used when animals are raised on slatted floors. To this aim, a wide array of haematological, biochemical and behavioural parameters was measured to assess possible differences depending on the enrichment material used. If effective (*i.e.,* able to reduce stress indicators), the proposed enrichment tool might represent a viable alternative to straw especially on slatted floors, where the use of rootable substrates is ruled out by the constraints of manure collection and handling systems (Westin et al., 2013).

2. Materials and Methods

The trials were carried out in the facilities of the Department of Veterinary Medical Sciences (DIMEVET) of the University of Bologna, Italy, in accordance with current Italian legislation implementing European

Council Directive 2008/120 on swine protection. The institutional Ethics Committee of the University of Bologna approved the experimental protocol (Authorization Prot. n. 2-IX/9 – 27.02.2012). In order to mimic farm conditions (*i.e.*, to provide environmental enrichment materials to all categories of pigs, according to the provisions set by the mentioned Directive), the experimental protocol did not include a negative control (i.e., without enrichment) group.

2.1 Animals, housing and feeding

A total of 120 crossbred (Landrace × Large White) castrated male weaners were used in two separate and independent trials (n=60 per trial). Their tails were left undocked. Animals were weaned at 25 days of age and allowed to adapt to the new environmental conditions for three days. Animals' health status was monitored in order to identify possible health problems. At 28 days of age, the experimental groups were formed on the basis of their litter and body weight (BW) and the environmental enrichments were provided. Piglets were kept in collective flat-deck cages on a slatted metal floor, with a floor space of 2 m^2 per cage. Each cage was equipped with a nipple drinker (water was available *ad libitum*) and a collective stainless steel feeder (0.2 m wide x 1 m long). Piglets were located in temperature- and humidity-controlled rooms equipped with a forced-air ventilation system (RH was kept at 65% during the whole trial; T was kept at 28° at the beginning of the trial and gradually reduced of approximately 0.5°C per week, until the temperature of 24°C was reached at the end of the trial).

Feed was provided *ad libitum*, in a pelleted form (3887 kcal DE/kg DM, CP 20.4% DM). Lighting was entirely artificial and was supplied by neon tubes (12 hours of light per day, from 7:00 to 19:00). In both trials, each replicate experienced one enrichment device for all the duration of the trial. Pictures of the enrichment devices are given in the Supplementary material (Figure S1).

TRIAL 1:

Sixty animals were allotted to 2 experimental groups, each comprising 6 replications (*i.e.*, cages) of 5 piglets, which were subjected to the following experimental treatments

- Chain (C1) group: the environment was enriched by providing a steel chain hanging in the middle on each cage;

- Wood Log (WL) group: the environment was enriched by providing a metal frame holding in horizontal position a poplar log (10 cm in diameter, 25 cm long). The frame was attached to the cage structure approximately 10 cm above the piglets' withers, in such a way that piglets could easily access them with their snouts and rotate or bite the wood.

The average Body Weight (BW) at the beginning of the trial was 6.76 ± 0.77 kg (average ± SD).

Animals were kept under the experimental conditions for 48 days.

TRIAL 2:

Sixty animals were allotted to 3 experimental groups, each comprising 5 replications (*i.e.*, cages) of 4 piglets, which were subjected to the following experimental treatments

- Chain (C2) group: see trial 1
- Edible Block (ED) group: these cages were enriched by providing a metal frame (the same as in trial 1, installed in the same position) holding in horizontal position a cylindrical edible block (10 cm in diameter, 25 cm long). The block was specifically formulated for the experimental trial and its main ingredients were feed, alfalfa meal, sugar beet molasses, and minerals. The frame was mounted in such a way that piglets could easily access them with their snouts and rotate or bite the block;
- Wood Briquette (WB) group: in these cages, a cylinder of compressed wood shavings was mounted on the same frames described before. The briquette had the same size as the edible block.

The average Body Weight (BW) at the beginning of the trial was 6.35 ± 0.58 kg (average ± SD).

Animals were kept under the experimental conditions for 43 days.

2.2 Growth parameters

All piglets were individually weighed at the beginning, in the middle (only in trial 1) and at the end of the trial, and average daily gain (ADG) was calculated for each period. Feed intake of each replication was recorded to calculate the feed conversion ratio (FCR) for each period. The cage (5 pigs in trial 1, 4 pigs in trial 2) was taken as the experimental unit for live weight, ADG, feed consumption, FCR.

2.3 Tail and skin lesions

In each of the trials, cutaneous and tail lesions were repeatedly evaluated on all piglets according to the Welfare Quality® (2009) assessment protocol. Since the protocol does not give specific indications for the postweaning phase, the method described for growing pigs was applied as suggested by the protocol itself, and only slight modifications were made (cutaneous lesions were counted on both sides of each piglet). In particular, tail lesions were visually evaluated by a trained observer and scored as 0 (intact tail, no evidence of tail biting); 1 (superficial biting, with no evidence of fresh blood or swelling) or 2 (fresh blood, evidence of swelling or infection; or tissue missing with formation of a crust). Skin lesions were evaluated on both the sides of the body and each body region (ears, front, middle, hindquarters and legs) was scored as "a" (up to 4 lesions), "b" (5 to 10 lesions) or "c" (11 to 15 lesions). The individual piglet was then scored on a 0-to-2 scale as described in the protocol, with 0 corresponding to piglets having all body regions classified as "a" and 2 to piglets having at least two body regions or more classified as "c", or at least one body region with more than 15 lesions.

2.4 Behavioural traits

The behaviour of 20 piglets for each experimental group (4 replications for in trial 1 and 5 replications in trial 2) was videotaped over the diurnal hours (7:00 to 19:00) by means of a digital closed circuit system (Mesa, Arezzo, Italy). Cameras were mounted on a rail attached to the ceiling above the cage (approximately 3m above the ground). To allow for individual behavioural observations, 4 animal marking sticks of different colours were chosen (blue, green, red and purple – RAIDEX GmBH, Dettingenan der Erms, Germany) and assigned to 4 piglets. A spot of the corresponding colour was painted on the back of each piglet on the day before each videotaping session. The fifth piglet was left uncoloured. Piglets were videotaped over the 24 hours once or twice a week, for a total of 6 videotaping sessions in trial 1, and 12 videotaping session in trial 2. Videos were examined by a single trained observer and the behavioural patterns were assessed by scan sampling technique at 10-min intervals according to predetermined ethogram for heavy pigs (Martelli *et al*. 2014) reporting the following behaviours: standing inactive, sitting inactive (dog-sitting), sternal recumbency, lateral recumbency, walking, eating, drinking, exploring the floor, social interactions. The ethogram was adapted to the specificities in piglets' behaviour and to the trial by adding the following behaviours: tail biting, interaction with the environmental enrichment, interaction with other cage structures,

belly nosing. Results were expressed as proportion of time spent performing each behaviour. A detailed description of the behaviours observed in the ethogram is given in the Supplementary material (see Table S1). To get more insights on the use of the environmental enrichment, 3 days for each trial (one at the beginning, one in the middle and one at the end of the trial) were selected and videos for all the videotaped replicates were watched continuously (all-occurrences sampling), in order to record the number of occurrences and duration of each interaction with the environmental enrichment.

2.5 Blood and bristle sampling and analysis

For each experimental group, a sub-sample of 15 piglets was randomly selected and blood samples were collected from each piglet in concurrence with the weightings. To this aim, piglets were manually restrained on their back and 15 ml of blood were drawn from the jugular vein and collected into 2 tubes, one containing lithium heparin and the other one containing EDTA. Blood was refrigerated immediately upon collection. Blood in K-EDTA was immediately sent to the DIMEVET laboratory, where the complete blood count (CBC) was performed using the haematology analyser ADVIA 2120 (Siemens Healthcare, Milan, Italy). Blood in Li-heparin was centrifuged at 3,500×g for 15 min at 4 °C to separate plasma. Plasma was frozen (−20 °C) until analysis of biochemical and metabolic profiles. The profiles included biomarkers able to assess:

(i) energy (glucose, fructosamine, total cholesterol, triglycerides) and protein (urea, creatinine) metabolism;

(ii) liver functionality (total bilirubin, aspartate aminotransferase= GOT, γ-glutamiltransferase = GGT);

(iii) oxidative stress (total reactive oxygen metabolites = ROM, Oxygen radical absorbance capacity = ORAC);

(iv) innate immune response evaluated by myeloperoxidase (index of neutrophil activity) and by indexes of acute phase response consequent to inflammatory events (positive acute-phase proteins: serum amyloid A, haptoglobin, ceruloplasmin; parameters linked to positive acute phase proteins: globulin, zinc; negative acute phase proteins: albumin, paraoxonase = PON).

Alterations of these biomarkers during the experiment was used to assess the welfare status. In particular, an increase of positive acute phase proteins and of ROM and a reduction in negative acute phase proteins and

ORAC can to detect the presence of subclinical conditions of disease (Petersen et al., 2004; Loor et al., 2013; Jacometo et al., 2016). Moreover, the concentration of fructosamine, which reflects the glycemia concentration of the last 1-3 weeks (Armbruster 1987), can be used as indicator of under nutrition (low values), disease status, distress (high values).

Glucose, total protein, albumin, total cholesterol, triglycerides, total bilirubin, creatinine, urea, GOT, GGT were detected at 37°C by a clinical auto-analyzer (ILAB 650, Instrumentation Laboratory, Werfen, Bedford, MA) using commercial kits purchased by Instrumentation Laboratory, Werfen (IL Test).

Ceruloplasmin, haptoglobin, PON, and MPO were determined with dedicated methods adapted to ILAB 650 conditions. Ceruloplasmin was determined following minor modification of the method proposed by Sunderman & Nomoto (1970); haptoglobin (HP) was determined using the method proposed by Skinner et al. (1991); PON activity was assessed by adapting the method of Ferré et al. (2002), as previously described by Bionaz et al. (2007) and MPO activity was determined using the colorimetric method of Bradley et al. (1982), in which MPO reacts with hydrogenperoxide, producing H2O and O− and O− reacts with the O− dianisidinedihydrochloride, an electron donor, releasing H2O and a coloured compound.

Zn was determined by a commercial kit (Wako Chemicals GmbH, Neuss, Germany). ROM were measured using a method patented by Diacron International S.r.l. (Grosseto, Italy) and expressed as mg of hydrogen peroxide per 100 mL of plasma. Serum amyloid A (SAA) concentration was assessed with a commercial ELISA immunoassay kit (Tridelta Development Ltd., Manynooth, Co. Kildare, Ireland). Total antioxidants were assessed through the oxygen radical absorbance capacity (ORAC) assay. This method measures a fluorescent signal from a probe (fluorescein) that decreases in the presence of radical damage (Cao & Prior, 1999). The analysis of ORAC was performed with a multidetection microplate reader equipped with a dual reagent injector (BioTek Synergy2, Winooski, VT). Lastly, globulins were calculated as the difference between total protein and albumin.

Bristles were collected at the beginning and at the end of the trial by shaving the rump region of all piglets. Samples were handled and analysed as previously described by Bacci *et al.* (2014). In brief, bristles were washed with water and then twice with isopropanol in order to remove any organic residue from the surface. Once fully dried, samples were finely pulverized and incubated overnight with methanol for steroid

extraction. After centrifugation, methanol was collected and air-dried, and the dry extracts were analysed using a validated radioimmunoassay. Data were reported as pg of cortisol/ mg of bristle.

Since it has been possible to collect only a little amount of hair from each piglet, the analysis has been carried out on a pool of bristles for each cage (i.e., 6 pools per treatment in trial 1 and 5 pools per treatment in trial 2).

2.6 Statistical analysis

Data of each trial was separately analysed using the STATISTICA 10 package (StatSoft, 2011) or SAS Inst. Inc. (Cary, NC, USA; release 8.0, 2014).

For growth parameters, normality of data was assessed by the Kolmogorov–Smirnov test and the data obtained were submitted to analysis of variance using environmental enrichment as the main effect. The cage (5 pigs in trial 1, 4 pigs in trial 2) was taken as the experimental unit for live weight, ADG, feed consumption, FCR, behavioural observations and cortisol from bristles; individual data were taken to be the experimental unit for cutaneous and skin lesions and blood parameters.

For hematic parameters, the normal distribution was checked by using Proc UNIVARIATE (SAS Inst. Inc., Cary, NC, USA; release 8.0) by NORMAL option. Parameters that were not normally distributed received a log transformation to satisfy normality and homogeneity of variance assumptions underlying linear models. Through the text, the data are presented in the original scale (mean and s.e.m.).Transformed data were subjected to ANOVA using the MIXED procedure of SAS. The statistical model applied included the fixed effect of day from the introduction of the environmental enrichment, type of environmental enrichments and their interaction. The subject within the type of environmental enrichment was considered as a repeated measure. The pairwise comparison has been done using least significant difference (LSD) test.

For nonparametric data (behavioural traits, blood parameters, lesion and tail score), the Mann-Whitney test (trial A) or the Kruskall-Wallis test (trial B) were used. The chi-squared test was used to evaluate the distribution of skin and tail lesions in the severity classes. The significance level for all statistical tests was set at $P < 0.05$.

3. Results

Growth parameters of both the experimental trials are shown in table 1. In trial 2, no significant differences were observed between the experimental groups. Conversely, in trial 1 piglets of the C1 group showed significantly higher intermediate and final body weights ($P = 0.01$) when compared to the WL group. Consequently, group C1 had a significantly higher ADG during the first period (P = 0.001) and considering the whole trial (P = 0.01). Feed consumption of WL piglets was significantly lower than in group C1 both during the second period and during the whole trial (P = 0.001). Significant differences were also observed in FCR, with significantly lower FCR in C1 group during the first period and in WL group during the second period (P = 0.001). Overall FCR tended to be lower in the WL group (P = 0.07).

As concerns cutaneous lesions (skin and tail lesion scores, see Supplementary material, table S3), no significant differences were detected between the experimental groups during the trials. However, it should be highlighted that, in both trials, tail score distribution indicated a numerically lower degree of lesion severity in the "enriched" than in the control (i.e., "chain") groups. In trial 2, tail score distribution showed tendentially (P<0.1) less severe tail wounds in ED when compared with C2 group.

Table 2 shows the behavioural patterns recorded during the two trials. Some statistical differences were detected between the ethograms of the experimental groups: in trial 1, piglets in the WL group spent more time standing inactive and rooting/exploring the floor, but less time manipulating cage components than piglets in the C1 group ($P = 0.03$, $P = 0.02$ and $P = 0.001$, respectively). Overall, the WL group showed a lower level of activity than the C1 group ($P = 0.03$). In trial 2, the lowest level of activity was recorded in ED and the highest in WB group, with C2 being intermediate ($P = 0.001$). As concerns the individual behaviours, WB piglets spent significantly more time eating ($P = 0.001$) and tended to interact more with the environmental enrichment ($P = 0.07$) than the other two experimental groups, whereas ED piglets spent more time resting in sternal recumbency ($P = 0.03$) when compared to the other two experimental groups. Lastly, piglets in the C2 group spent more time drinking ($P = 0.02$) and having positive social interactions ($P = 0.001$) than the other two experimental groups.

Figure S4 and S5 (given in the Supplementary material) show the number of occurrences and the duration of the interaction with the environmental enrichment material. In trial 1 (see figure S4), no statistically significant difference was found in the number or in the duration of the interactions. However, interactions lasted tendentially more (P<0.1) in WL than in C1 (on average 27.3 vs. 17.28 s). In trial 2, no significant

278 difference was observed in interaction number or duration, but the number of interactions tended to increase
279 as time passed (P<0.1, see figure S5). The increase in interaction duration is more evident for ED and WB
280 groups and is due to the presence of some individuals that continued to be interested in the environmental
281 enrichment during the entire trial, without showing any decreasing trend (data not shown).
282 No significant differences were detected between the experimental groups in cortisol from bristles (table 3),
283 in the complete blood cell count and in the neutrophil-to-lymphocyte ratio (N/L, see table 4).
284 In the first trial, the presence of different environmental enrichments determined some differences in the
285 metabolic profile (table 5). Twenty-one days after the introduction of WL, the concentration of glucose,
286 albumin and PON in plasma were lower (P<0.01) in comparison with C1. These variations were transient
287 and disappeared at third assessment. The concentrations of GOT and GGT increased during the experiment
288 in both the environment enrichments, but the increase was smaller in WL in comparison with C1 group
289 (P<0.001 and P<0.001 respectively), until the third assessment. Moreover, concentrations of triglycerides
290 were higher (P<0.01) and concentrations of SAA tended to be lower (P<0.10) at the third assessment in WL
291 in comparison with C1.
292 In the second trial (table 6) the comparison among the environmental enrichments has been limited at two
293 assessments, separated by 43 days. The differences in comparison to the control group (C2) at the end of the
294 trial were smaller and limited to triglycerides lower in ED (P<0.01) and WB (P<0.05), and total protein
295 higher in ED (P<0.1). At the beginning of the trial, glucose was higher in ED than in WB (P<0.05), total
296 antioxidants (ORAC) were tendentially higher in ED than in C2 (P<0.1) and GGT was tendentially lower
297 (P<0.1) in WB than in C2. Such small differences however disappeared at the second assessment.
298 In both trials, the prevalence of piglets with positive acute phase protein (eg. SAA and HP) concentrations
299 over the threshold of severe inflammations (>0.1 and >1.5 g/L for SAA and HP, respectively) was quite low:
300 2.2% of piglets in trial 1 and about 13% of piglets in trial 2.
301
302 ***4. Discussion***
303 The aim of the present work was to study the consequences of the use of three point-source, destructible
304 enrichment-objects, which might represent a viable enrichment on slatted floors, on post-weaned piglets'
305 welfare. The point- source enrichment objects tested (poplar wood, sawdust briquette and edible block) were

306 compared to an indestructible object (*i.e.*, the widely used metal chain). Their effectiveness was assessed
307 using a wide range of behavioural, health, physiology, and performance parameters.
308
309 Growth parameters
310 Overall, growth parameters recorded in the two trials were less favourable (similar or lower ADG, increased
311 feed intake and FCR) than the data available in literature on piglets of similar age (*e.g.*, Trickett et al. 2009;
312 Leliveld et al. 2013). This difference was expected and in agreement with the fact that these pigs are
313 intended for the production of Parma Ham, an Italian PDO (protected designation of origin) dry-cured ham
314 whose production rules require the use of raw tights from pigs of at least nine months of age and weighing
315 on average 160kg at slaughter (Consortium for Parma Ham, 1992). Therefore, such production requires the
316 use of genotypes that reach high BW in relatively longer times, *i.e.*, less efficient if compared with other
317 meat types.
318 Growth parameters differed between the experimental groups in trial 1, but not in trial 2. Overall, in trial 1
319 the WL group showed worse production parameters than the C1 group (lower body weight and ADG,
320 reduced feed intake). FCR was higher in the first period, but lower in the second when compared to C1
321 group. The improved feed conversion in WL group during the second period may indicate how, in spite of
322 their relatively low daily gain and feed intake, these animals' body size has increased, resulting (because of
323 the low feed consumption) in better FCR in comparison to C1 group. However, the worsening of productive
324 parameters in WL group cannot be ascribed to wood chewing or ingestion, since the animals have barely
325 notched it. It has been observed that pig-specific enrichment objects usually do not influence performance
326 parameters negatively, and that negative effects are mainly found when the enrichment provided does not
327 fulfil all the pigs' requirements (Van de Weerd et al. 2009). Within this context, it cannot be ruled out that
328 WL may have represented a worse environmental enrichment than hanging chains, being less manipulable
329 or, at least, less easily chewable and movable. In fact, in WL piglets some transient negative changes were
330 observed at the blood profile. WL showed a marked reduction of albumin and PON at 2nd assessment in
331 comparison to control, which suggests a slight reduction in liver functionality, likely as consequence of
332 previous inflammatory events (Gruys et al., 1998; Bertoni and Trevisi, 2013). It should however be
333 highlighted that no behavioural or physiological signs of impaired welfare have been detected in the WL

group at the end of the experimental period. For example, the plasma changes were transient and other plasma indices were more favourable in comparison to piglets of the control group (e.g. the lower concentrations of liver transaminase and the lower concentration of SAA at the third assessment). Thus, the overall metabolic and inflammatory conditions did not differ among groups tested in trial 1.

Skin and tail lesions

As concerns skin and tail lesions, the absence of differences in their level (*i.e.*, lesion score) and severity (*i.e.*, score distribution) indicates how the environmental enrichment materials proposed have determined no substantial modifications in animal aggressive behaviours. However, it should be highlighted that the level of skin and tail lesions recorded is very low in all the experimental groups if compared to the results obtained by Temple et al. (2011), who applied the Welfare Quality® protocol to intensively reared growing pigs. To our knowledge, no literature is available on the application of the Welfare Quality® skin and tail lesion score to post-weaned piglets. Tail lesion distribution across the severity classes was similar among the experimental groups, with the majority of piglets (especially in trial 2) having intact tails, and only a minority showing severe lesions. Such a distribution indicates a considerably lower level and severity of tail biting in all the experimental groups if compared to what has been observed in undocked weaners in other studies (Tellkantra et al. 2014b). Overall, the low number of lesions observed is of further interest if we consider that the post-weaning period is critical for the development of oral behaviour redirection (massaging, tail biting), especially when piglets are reared in barren environments (van de Weerd et al., 2005; Tellkantra et al. 2014b). Besides, in both trials lesion frequency and severity were reduced in the "enriched" groups. Therefore it cannot be ruled out that the alternative enrichment devices might have, although not significantly, reduced the piglets' exploratory behaviour directed towards the tail of the pen-mates. The low number of piglets with severe lesions is also confirmed by the low frequency of piglets with severe inflammatory conditions, diagnosed in accordance with the low concentrations of positive acute phase proteins (e.g. SAA and HP). Despite the thresholds of these proteins which identify clinical cases are not well defined, their high concentrations represent a systemic response after a severe psychological stress, injuries or infections (Chen et al., 2003; Jacobson et al., 2004; Hansson et al., 2011; Pomorska-Mol et al., 2013). In the present experiment, the number of piglets with clear inflammation has been defined utilizing the threshold of 0.1 mg/L for SAA and 1.5 mg/L for HP. In trial 1 less than 3% of piglets showed severe

362 inflammations; in trial 2, the percentage increased to 13%. In both trials, the introduction of the
363 environmental enrichments has not affected the frequency of the severe inflammation in the population,
364 which seems largely dependent to other environmental factors, not easily detectable. Interestingly in the trial
365 2, the WB showed better results of ED in term of inflammatory conditions. In fact, the higher concentration
366 of Zinc (which is sequestered in the liver during inflammatory events - Bertoni and Trevisi, 2013-) and the
367 lower concentration of SAA suggests a lower inflammatory events or a less severe inflammation in WB than
368 in ED (Jacobson et al., 2004; Hansson et al., 2011).
369 Behavioural observations
370 The differences observed between the experimental groups in trial 1 were mainly due to an overall reduced
371 activity (i.e., higher degree of calmness) of group WL (increase in the percentage of behaviours such as
372 standing inactive and rooting/exploring the floor; decrease in cage components exploration). In trial 2, the
373 higher degree of calmness was observed in ED group (increased sternal recumbency, reduction in positive
374 social interactions) and the lowest in WB (reduced sternal recumbency, increase in time spent eating and
375 interacting with the enrichment), with C2 group being intermediate. The time spent interacting with the
376 environmental enrichment was similar between the experimental groups in trial 1, whereas in trial 2 the
377 enrichment that tended to involve the piglets more was the WB. Overall, in the 2 trials the time spent
378 manipulating the environmental enrichment by all experimental groups was higher if compared to the results
379 described by Trickett et al (2009). Although such a percentage of time is very low if compared with the
380 occupational level provided by straw (Kelly et al., 2000), it has been demonstrated that in rats the
381 behavioural changes observed in the enriched environment were due to the presence of the enrichments
382 themselves in the cages (indirect effects) and not due merely to rats interacting with the enrichment (Abou-
383 Ismail et al., 2010). In the case of rats, environmental enrichment promoted longer bouts of sleep and
384 diminished aggressive behaviour, improving welfare. Similarly, in pigs, it cannot be ruled out that the
385 presence of enrichment could have improved welfare even when animals spent little time in direct contact
386 with it, *i.e.*, that the frequency of object use alone may not be indicative of improved/impaired welfare
387 (Tellkantra et al. 2014b). This observation would be in agreement with the higher calmness levels that were
388 observed in groups WL (trial 1) and WB (trial 2). Unexpectedly, piglets did not show an increased interest
389 towards the edible material when compared to the hanging chain. However, such a result can be at least

390 partially explained by the fact that animals were fed *ad libitum*. The greater use on the wooden briquette by
391 piglets when compared to the edible block might be due to the fact that the wooden briquette was more
392 friable (i.e., more destructible and manipulable) than the edible block (Studniz et al., 2007). No alterations
393 were detected in the harmful social behaviours (aggressive interactions, tail biting, massaging).
394 The observation of videos in continuous showed that in trial 1 piglets tended to carry out longer interactions
395 with the wood log than with the chain, probably due to the fact that the wood log was more manipulable and
396 smelling and might have captured the interest of piglets for longer times if compared with the metal chain. In
397 trial 2, over time piglets tended to increase the amount of time they spent interacting with the enrichment (in
398 particular with the wood briquette and the edible block). However, the increase was not homogeneously due
399 to all piglets, but to the presence of some subjects, which continued to interact with the enrichments for the
400 entire duration of the trial, without showing the decreasing trend that is typically observed when habituation
401 occurs (Trickett et al., 2009). This finding shows that not all piglets find equally attractive the same
402 enrichment, but also confirms that the proposed enrichments may be more capable of capturing the piglets'
403 attention. However, it would be interesting to analyze if such an interest is maintained as the piglets grow up.
404 Hair cortisol and haematologic parameters
405 As concerns hair cortisol levels, no significant differences were detected between the groups at the same
406 sampling time. This shows that the materials used for environmental enrichment did not activate the
407 hypothalamic-pituitary-adrenocortical response in terms of chronic stress. When comparing cortisol values
408 of the same group at the 2 different experimental times, it is noticeable that the first ones are slightly higher.
409 This might be related to the last few days of intrauterine life and lactation since maternal cortisol blood
410 concentration rises before and during delivery, and returns at normal values at weaning (Whitely *et al.* 1984).
411 As concerns the haematological parameters, the absence of differences in CBC or in N/L ratio between the
412 experimental groups indicates that none of the experimental groups was subjected to sub-chronic stressors. In
413 fact, under environmental stressors the N/L ratio tends to increase in pigs (as extensively reviewed by Kick
414 et al. 2011). Overall, parameters fell within the reference intervals for the swine specie (Thorn 2000). From
415 the comparison between trial 1 and trial 2, discrepancies can be observed between the two trials in the
416 differential leukocyte count. In trial 2, total leukocytes at the beginning of the trial were higher than in trial 1,
417 and the difference is due to a higher number of neutrophils that considerably diminished in the second

418 assessment. Although we did not carry out any specific analysis, the presence of a subclinical viral infection
419 (probably caused by PCV2 – Porcine Circovirus type 2) in these piglets cannot be ruled out. The presence of
420 a circovirus infection could explain both the neutrophilia observed at the beginning of trial 2 and the reduced
421 growth rate of these piglets if compared to the results obtained in trial 1, although no overt clinical signs
422 were observed. Moreover, neutrophilia (together with lymphopenia) is commonly observed in PCV2
423 infections (Gauger et al 2011).
424 The higher number of total leukocytes in the trial 2 in comparison with the trial 1, also agrees with the
425 different inflammatory profile. In fact, in the trial 2 the incidence of piglets with positive acute phase protein
426 (eg. SAA and HP) concentrations over the threshold of severe inflammations was higher in comparison with
427 trial 1 (about 13% vs 2.2% of the piglets).
428
429 ***5. Conclusion***
430 The results obtained from the present research trials did not allow to identify among the materials tested an
431 environmental enrichment material being particularly effective in improving piglet welfare if compared with
432 the metal chain. This observation can be drawn considering the fact that no peculiar difference has been
433 detected in behavioural, physiological or growth parameters of piglets receiving the innovative
434 environmental enrichment materials when compared to piglets receiving the traditionally used hanging
435 chains. Unexpectedly, piglets did not show an increased interest even towards the edible material. Although
436 our data refer to animals kept in small groups (4 or 5 piglets/cage), the overall results indicate that under our
437 experimental conditions piglets receiving the metal chain attained a satisfactory welfare level. In fact, in spite
438 of their theoretically low enrichment level and of the intact tails, no tail-biting outbreak occurred and no
439 behavioural or biochemical alteration were observed. Therefore, without devaluing the importance of
440 adequate enrichment tools, under practical farming conditions attention should be paid not to allow the use of
441 enrichments as a mean to compensate for poor environmental conditions or to overlook underlying welfare
442 issues.
443 Overall, the results of the present study highlight a basic issue related to the inner nature and meaning of
444 environmental enrichment itself. The fact that several enrichment devices (differing in materials and/or
445 design) had similar effects, urges a reflection on what is an effective enrichment tool, and what only attracts

446 stereotyped behaviours. Besides, there would be possibilities that enrichments considered similar by humans
447 could have different effects on behavior and performance of animals. For these reasons, there is a clear need
448 for further studies on what components of environmental enrichment do actually influence the animal as a
449 whole (e.g., behaviour, physiology etc.) or only in part (lesions, etc.) and how it happens.
450
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455
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577 **Table 1**: Live weight and average daily gain (ADG) of piglets receiving different environmental enrichment
578 materials (C1 and C2 = hanging chains; WL = Wood Log; ED = Edible block; WB = Wood Briquette)

		Trial 1			
		C1	**WL**	**RMSE**	**p-value**
Replications (cage)	n	6	6		
Body Weight					
Initial weight (0 d)	kg	6.77	6.74	0.79	0.95
Weight at 21 d	kg	14.15	12.07	1.20	0.01
Final weight (48 d)	kg	31.99	28.67	1.41	0.01
Average Daily Gain (ADG)					
ADG 0-21 d	g/day	351	275	2.81	0.001
ADG 21-48 d	g/day	660	624	3.63	0.12
ADG 0-48 d	g/day	526	474	2.02	0.01
Feed Consumption					
1-21 d	g/day	762	762	-	-
22-48 d	g/day	1203	943	8.20	0.001
1-48 d	g/day	1010	864	4.62	0.001
Feed Conversion Ratio (FCR)					
FCR 1-21d		2.19	2.78	0.19	0.001
FCR 22-48 d		1.83	1.51	0.11	0.001
FCR 1-48 d		1.92	1.82	0.089	0.07

		Trial 2				
		C2	**ED**	**WB**	**RMSE**	**p-value**
Replications (cage)	n	5	5	5		
Body Weight						
Initial weight (0 d)	kg	6.44	6.46	6.36	0.48	0.94
Final weight (43 d)	kg	24.49	24.86	26.19	2.40	0.62
ADG:						
ADG 0-43 d	g/d	429	428	461	4.68	0.47
Feed Consumption						
0-43 d	g/d	837	837	941	4.06	0.37
FCR						
FCR 0-43 d		1.94	1.95	2.05	0.22	0.72

579

580

Table 2: Diurnal behaviour (7:00 to 19:00) of piglets receiving different environmental enrichments (data are expressed as a percentage of total observed behaviours). (C1 and C2 = hanging chains; WL = Wood Log; ED = Edible block; WB = Wood Briquette)

	Trial 1			Trial 2			
	C1	**WL**	**P-value**	**C2**	**ED**	**WB**	**P-value**
Standing inactive[1]	3.54	4.42	0.03	3.24	2.69	2.69	n.s.
Sitting inactive (dog sitting) [1]	1.22	1.31	n.s.	1.30	1.36	1.29	n.s.
Sternal recumbency[1]	28.50	27.01	n.s.	36.20	38.01	35.32	0.03
Lateral recumbency[1]	36.46	38.92	n.s.	38.04	37.84	38.37	n.s.
Eating[2]	14.11	13.93	n.s.	9.94	9.95	11.94	0.001
Drinking[2]	2.49	2.20	n.s.	1.99	1.40	1.47	0.02
Walking[2]	2.74	3.14	n.s.	1.95	1.54	1.61	n.s.
Rooting/Exploring the floor[2]	1.89	2.24	0.02	0.71	0.95	0.86	n.s
Positive interaction[2]	3.65	3.33	n.s.	2.01	1.53	1.61	0.001
Aggressive Interaction[2]	1.77	1.45	n.s.	0.08	0.09	0.08	n.s.
Tail biting[2]	0.69	0.54	n.s.	0.05	0.05	0.05	n.s.
Massaging[2]	0.73	1.23	n.s.	2.66	2.61	2.47	n.s.
Interaction with the enrichmen[2] t	0.66	0.57	n.s.	0.54	0.50	0.79	n.s
Manipulation of cage components[2]	1.64	0.74	0.001	0.49	0.62	0.56	n.s.
Total inactive[1]	69.63	71.66	0.03	78.78	79.85	77.66	0.001
Total active	30.37	28.34	0.03	21.22	20.15	22.34	0.001

[1] Inactive behaviours
[2] Active behaviours

588 **Table 3:** Cortisol from bristles of piglets receiving different environmental enrichments (C1 and C2 =
589 hanging chains; WL = Wood Log; ED = Edible block; WB = Wood Briquette)

	Trial 1				
	C1		**WL**	**RMSE**	**P-value**
Replication	6		6		
1st assessment	8.39		9.72	1.72	0.52
2nd assessment	5.11		9.32	2.34	0.25
	Trial 2				
	C2	**ED**	**WB**	**RMSE**	**P-value**
Replication	5	5	5		
1st assessment	7.81	7.27	7.52	1.62	0.89
2nd assessment	5.24	4.63	5.81	1.92	0.33

590

591

592 **Table 4:** Complete blood count and N/L ratio of piglets receiving different environmental enrichments (C1
593 and C2 = hanging chains; WL = Wood Log; ED = Edible block; WB = Wood Briquette)

Trial 1							
	1st assessment		**2nd assessment**		**3rd assessment**		
	C1	**WL**	**C2**	**WL**	**C1**	**WL**	**SE**
Haematocrit (%)	41.1	39.2	35.8	33.5	39.4	39.8	0.45
Haemoglobin (g/dL)	12.6	12.0	10.5	9.8	11.6	11.5	0.14
Erythrocytes ($x10^6$/µL)	7.196	6.956	6.642	6.354	7.264	7.305	0.07
Leukocytes (/µL)	10730	10668	17732	15901	16425	15327	554.24
Neutrophil (/µL)	3747	3645	5885	5544	5013	4965	261.72
Lymphocyte(/µL)	6209	5045	9931	8946	10149	9361	324.73
N/L ratio	0.60	0.66	0.61	0.64	0.53	0.51	0.03
Trial 2							
	1st assessment			**2nd assessment**			
	C2	**ED**	**WB**	**C2**	**ED**	**WB**	**SE**
Haematocrit (%)	39.7	39.8	41.1	32.1	31.6	34.2	0.51
Haemoglobin (g/dL)	12.0	11.3	11.5	9.1	9.0	9.8	0.19
Erythrocytes ($x10^6$/µL)	6.623	6.930	6.919	7.573	7.689	7.801	0.07
Leukocytes (/µL)	14371	13874	14671	15385	16652	17379	521.56
Neutrophil (/µL)	6275	6262	6192	3977	4424	4709	277.37
Lymphocyte (/µL)	6815	6635	7566	9767	10637	10495	336.57
N/L ratio	0.96	0.98	0.84	0.44	0.42	0.45	0.04

594 No significant difference was detected at the statistical analysis (P>0.05).

595

596 **Table 5** –Plasma biomarkers of piglets (mean and Standard Error = SE) receiving different environmental
597 enrichments (C1 = hanging chains; WL = Wood Log) during the trial 1.

Trial 1							
Time (days)	**1st assessment (day 0)**		**2nd assessment (day 21)**		**3rd assessment (day 48)**		**SE**
Group	**C1**	**WL**	**C1**	**WL**	**C1**	**WL**	
Glucose (mmol/l)	6.64	6.84	7.37	6.53**	6.10	6.28	0.212
Cholesterol (mmol/l)	2.24	2.33	2.29	2.56	2.15	2.27	0.127
Urea (mmol/l)	4.41	4.64	2.91	3.05	4.99	5.17	0.241
Zinc (µmol/l)	16.39	16.78	30.28	28.68	19.77	19.63	0.079
Ceruloplasmin (µmol/l)	13.45	12.21	11.55	11.57	13.26	12.26	0.697
Total Protein (g/l)	53.10	52.58	54.79	52.84+	60.19	60.60	0.778
Albumins (g/l)	32.93	32.96	32.98	29.72**	38.27	38.15	0.773
Globulin (g/l)	20.16	19.63	21.81	23.12	21.92	22.45	0.032
AST/GOT (U/l)	52.44	54.31	92.70	63.04***	64.46	46.14**	0.079
GGT (U/l)	41.93	40.25	120.2	68.21**	121.3	70.96*	0.118
Total bilirubin (µmol/l)	1.60	1.62	1.23	1.79	0.80	0.68	0.132
Haptoglobin (g/l)	0.94	0.97	1.07	1.19	1.02	0.98	0.104
Paraoxonase (U/ml)	35.95	34.34	33.84	23.62**	45.42	48.71	2.21
Triglycerides (mmol/l)	0.389	0.414	0.666	0.700	0.363	0.487**	0.061
Creatinin (µmol/l)	99.2	102.4	69.0	67.4	82.8	86.1	2.16
ROMt (mg H_2O_2/ 100 ml)	33.26	28.72+	22.26	19.12	26.05	22.08	0.056
Myeloperoxidase (U/l)	352	381	635	683	621	625	26.0
Fructosamine (µmol/l)	52.35	51.39	39.58	35.80	42.24	40.85	2.074
ORAC (µmol/l)	8201	8449	8634	9086	9631	9987	314.9
Serum amyloid A (µg/ml)	7.06	14.95	7.97	23.04	46.43	5.87+	14.13

598 A significant statistical difference at the same assessment is shown by a superscript on the WL value (+
599 P<0.10; * P<0.05; ** P<0.01).

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602 **Table 6:** Plasma biomarkers of piglets (mean and Standard Error = SE) receiving different environmental
603 enrichments (C2 = hanging chains; ED = Edible block; WB = Wood Briquette) during the trial 2.

Trial 2							
Time (days)	**1st assessment (day 0)**			**2nd assessment (day 43)**			
Group	**C2**	**ED**	**WB**	**C2**	**ED**	**WB**	**SE**
Glucose (mmol/l)	6.82	7.38	6.59(1)	5.68	5.54	5.38	0.37
Cholesterol (mmol/l)	2.03	2.00	2.04	2.71	2.55	2.55	0.17
Urea (mmol/l)	4.18	3.84	4.29	4.52	4.59	4.63	0.34
Zinc (µmol/l)	11.47	12.61	12.55	13.76	15.41	15.07	1.16
Ceruloplasmin (µmol/l)	19.28	19.50	18.89	16.17	14.78	16.57	1.75
Total Protein (g/l)	54.35	54.12	53.82	60.14	62.90(2)	62.32	1.61
Albumins (g/l)	34.13	33.66	33.28	35.96	36.73	36.99	0.96
Globulin (g/l)	20.21	20.45	20.53	24.18	26.17	25.33	1.51
AST/GOT (U/l)	70.11	77.60	72.19	51.21	51.13	52.00	7.88
GGT (U/l)	96.32	83.57	58.01(3)	48.77	43.61	45.60	0.21
Total bilirubin (µmol/l)	1.90	2.00	1.86	2.00	2.50	1.79	0.20
Haptoglobin (g/l)	0.93	1.05	1.12	1.22	1.21	1.32	0.17
Paraoxonase (U/ml)	33.95	34.63	33.29	30.54	29.97	33.56	3.35
Triglycerides (mmol/l)	0.509	0.473	0.413	0.612	0.474 **	0.488 *	0.06
Creatinin (µmol/l)	100.3	101.5	97.2	93.6	95.6	94.2	3.76
ROMt (mg H_2O_2/ 100 ml)	30.22	31.83	31.11	28.84	26.89	27.89	1.70
Myeloperoxidase (U/l)	370	701	410	408	469	438	0.19
Fructosamine (µmol/l)	44.36	46.55	42.12	28.50	28.95	29.86	4.36
ORAC (µmol/l)	9105	10344(2)	9923	11173	11198	10751	622.51
Serum amyloid A (µg/ml)	110.70	34.69	49.94	21.92	65.19	61.03	0.47

604 Significant statistical difference with C2 at the same time point is shown by a superscript on the ED and WB
605 values (* P<0.05; ** P<0.01); (1) P<0.05 between ED and WB group; (2) P<0.1 between C2 and Ed group;
606 (3) P<0.1 between C2 and WB group.

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Figure S1: pictures of the environmental enrichment devices used during the experimental trials. C=metal chain; WL=Wood log; WB=Wood briquette; EB=Edible Block.

613 **Table S2:** Description of the behaviours observed (Martelli *et al*. 2014, modified)

Behaviour	Description
Standing inactive	Standing, neither moving forward or backward
Sitting inactive (dog-sitting)	Sitting on its tail with its foreleg stretched under the body
Sternal recumbency	Lying down on its belly
Lateral recumbency	Lying down on one side
Walking	Walking through the cage
Eating	Eating from the feeder or chewing food
Drinking	Mouth in contact with the drinker and water being ingested
Rooting/Exploring the floor	Aimless rooting, sniffing, touching the cage floor
Positive Interaction	Social, explorative, interactions performed by one pig and directed towards one or more pigs (e.g. nose-to nose, nose-to-body)
Aggressive Interaction	Interactions (bites or head butts) ending with the victim piglet fleeing the performer
Interaction with the environmental enrichment	Any explorative interaction (sniffing, rooting, nosing or chewing) performed towards the enrichment.
Manipulation of cage components	Nosing, rooting, or chewing equipment (feeder or metal bars).
Tail biting	The performer holds the tail of the victim in its mouth
Massaging	Repetitive rooting movement with the snout on another piglet (belly-nosing or nosing any other body part).
Others	Other unlisted activities (e.g. running, playing)

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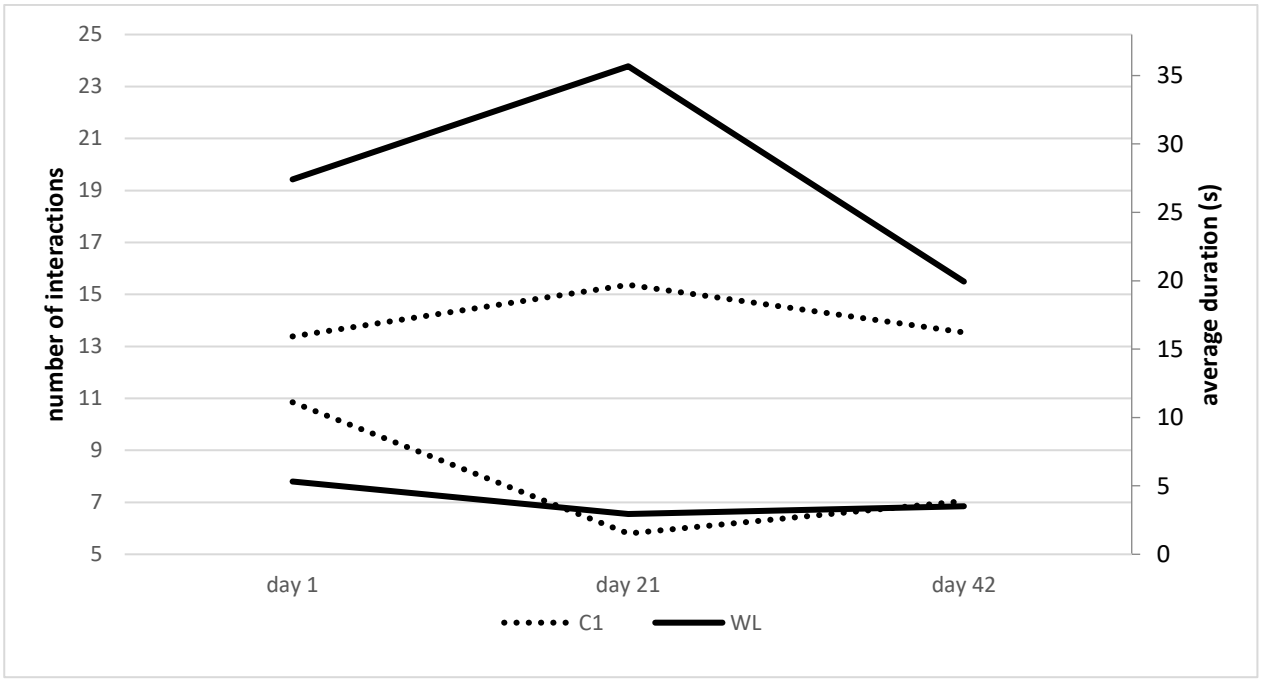
Table S3: Skin and tail lesions of piglets receiving different environmental enrichment materials. (C1 and C2 = hanging chains; WL = Wood Log; ED = Edible block; WB = Wood Briquette)

Trial 1			
		C1	**WL**
Animals	n°	30	30
1st assessment (d 14)			
Skin lesions	pt	0.27	0.23
Tail lesions	pt	0.20	0.03
2nd assessment (d 36)			
Skin lesions	pt	0.00	0.07
Tail lesions	pt	0.48	0.67
3rd assessment (d 48)			
Skin lesions	pt	0.38	0.43
Tail lesions	pt	1.00	0.76
Overall skin score distribution			
Intact skin	%	78,5	75.6
Moderate wounds	%	21.5	24.4
Severe wounds	%	0	0
Overall tail score distribution			
Intact tail	%	56.6	68.9
Moderate tail lesion	%	30.08	14.4
Severe tail damage	%	12.6	16.7

Trial 2				
		C2	**ED**	**WB**
Animals	n°	20	20	20
1st assessment (d 15)				
Skin lesions	pt	0.15	0.20	0.25
Tail lesions	pt	0.11	0.11	0
2nd assessment (d 29)				
Skin lesions	pt	0.50	0.40	0.53
Tail lesions	pt	0.45	0.25	0.26
3rd Assessment (d 42)				
Skin lesions	pt	0.65	0.32	0.43
Tail lesions	pt	0.32	0.05	0.26
Overall skin score distribution				
Intact skin	%	58.3	68.3	68.3
Moderate wounds	%	40.0	31.7	31.7
Severe wounds	%	1.7	0	0
Overall tail score distribution				
Intact tail	%	76.7	86.7	81.7
Moderate tail lesion	%	18.3	13.3	18.3
Severe tail damage	%	5.0	0	0

No significant difference was detected at the statistical analysis (P>0.05).

Figure S4 – Interactions with the environmental enrichments observed during trial 1: number of occurrences and average duration



628 **Figure S5 –** Interactions with the environmental enrichments observed during trial 2: number of occurrences
629 and average duration

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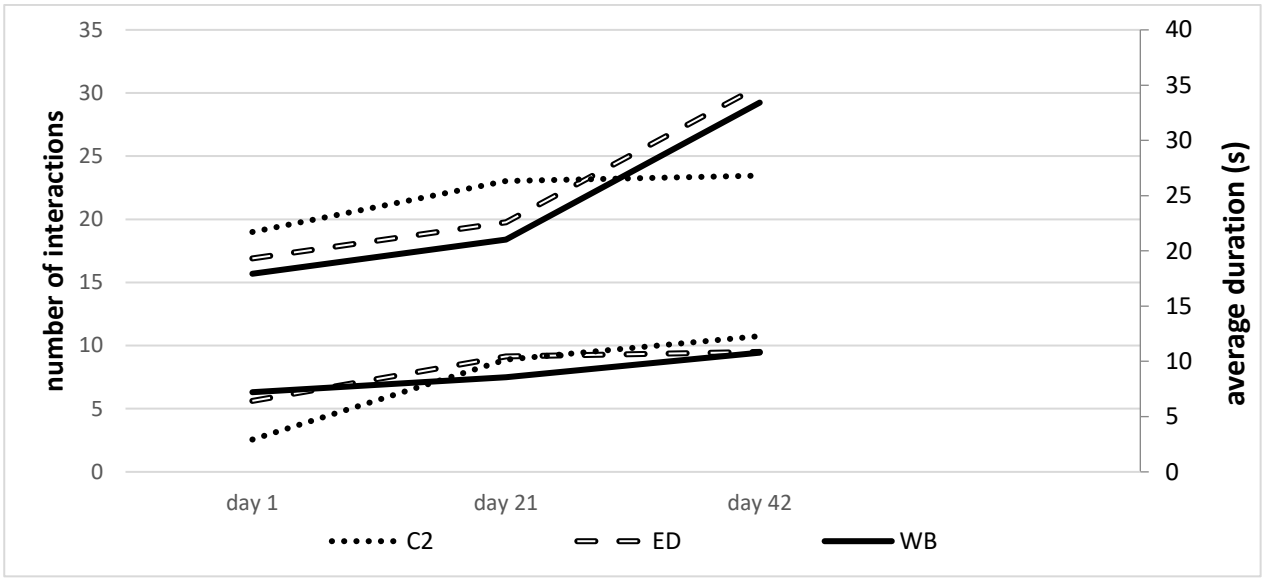

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