Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

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ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 45–56
https://doi.org/10.1080/1828051X.2018.1472531

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Enrichment devices for undocked heavy pigs: effects on animal welfare, blood parameters and production traits

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ABSTRACT

Two independent trials were carried out to test the effectiveness of different environmental enrichments in improving Italian heavy pigs' welfare. Eighty undocked Landrace × Large White barrows (body weight range: 27.1–158.4 kg) were used. In Trial 1, group C1 received a metal chain and group WL wooden logs (placed inside a specifically designed metal rack). In trial two, the enrichments proposed were a hanging chain (C2), and a vegetal edible block (EB) inside the metal rack. General health, animal behaviour, hair cortisol, blood parameters and growth performance were recorded. In Trial 1, WL pigs interacted with the enrichment at a lower extent than C1 ($p < .01$) and walked less ($p < .05$). In Trial 2, EB pigs spent less time in lateral recumbency and rooting/exploring the pen floor ($p < .05$ and $p < .01$, respectively), and interacted more with the enrichment ($p < .001$) than C2. Motivation to explore both WL and EB was maintained over time, conversely to what happened to C1 and C2. No differences were observed in hair cortisol concentration, neutrophil/lymphocyte ratio, skin lesions and growth parameters. Tail lesions were higher in WL than in C1 ($p < .05$) Minor, transient differences were found in plasma biomarkers. Overall, WL had limited effect on behaviour, whereas EB might have reduced floor over-exploration when compared to the chains. It is concluded that, although from an animal behaviour standpoint EB might be promising, regardless of the device used (C, WL or EB), all experimental groups showed similar welfare parameters levels, stimulating the necessity for wider research on enrichment design and function.

ARTICLE HISTORY
Received 22 September 2017
Revised 12 March 2018
Accepted 20 March 2018

KEYWORDS
Animal welfare; blood parameters; enrichment; intensive husbandry; heavy pigs

Introduction

The European legislation requires the permanent provision of '*manipulable materials*' to pigs of all ages (Directive 2008/120/EC). A successful enrichment material for pigs should allow the expression of species-specific behaviours (such as social interactions, foraging end exploration), maintain the pigs' interest over time and decrease the incidence of behaviours tied to boredom and frustration (e.g. stereotypies, tail biting) (van de Weerd and Day 2009; Telkänranta et al. 2014). This, in turn, should lower the incidence of tail and skin lesions (Studnitz et al. 2007).

The Commission Recommendation (EU) 2016/336 states that enrichments should be edible, chewable, investigable, manipulable, of sustainable interest, accessible, given in sufficient quantity and clean. Materials possessing all these characteristics are defined as 'optimal materials' and can be used alone, whereas 'suboptimal materials' (i.e. materials possessing most of the characteristics listed, but not all) should be used in combination with other materials. Lastly, materials providing distraction (such as chains and tyres) should not be considered as fulfilling the pigs' essential needs and therefore they should be used in combination with optimal or suboptimal materials.

Metal chains are still widespread in European intensive production, being the most common enrichment in conventional Dutch pig farms (Bracke et al. 2013), similarly to what observed by Barbieri et al. (2014) on Italian heavy pigs intended for protected designation of origin (PDO) ham production. It is out of doubt

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that, with respect to EU legislation, the use of chains is controversial; however for pigs raised on slatted the use of optimal substrates (such as straw, hay, peat) can negatively interfere with manure removal (Barbieri et al. 2014). To overcome this issue, it could be possible to design successful point-source enrichment objects. Bulens et al. (2016) showed that dispensers containing compressed straw blocks were able to attract pigs' curiosity during all finishing stages (up to 120 kg) and reduced pen mates manipulation. Similarly, Scollo et al. (2013) observed that heavy pigs showed a rather constant motivation towards long straw allocated in a metal rack.

The aim of the present study is to assess the effectiveness of point-source enrichment tools (metal racks) containing either an optimal (edible) or a sub-optimal (wooden) enrichment in heavy pigs kept for their entire production cycle on fully slatted floor. Enrichment devices were compared to the traditionally used metal chain. Effectiveness was assessed through a wide range of parameters including behaviour, physiological indicators (hair cortisol as a long-term indicator of stress exposure and blood parameters), skin and tail lesions, general health and growth traits. With respect to blood traits of heavy pigs, the paucity of information regarding the effects of subclinical stress conditions, stimulated the study of less investigated parameters which were assessed depending on their effect on the innate immune response (myeloperoxidase: Brennan et al. 2003), energy metabolism (fructosamine: Armbruster 1987), acute phase proteins and parameters linked to oxidative stress (Petersen et al. 2004; Trevisi et al. 2016).

To measure the effects of enrichments on tail biting behaviour and allow for early identification of behavioural alterations, all pigs were left undocked.

Material and methods

The trials were carried out in the facilities of the Department of Veterinary Medical Sciences (DIMEVET) of the University of Bologna, Italy. The institutional Ethics Committee of the University of Bologna approved the experimental protocol (Authorization Prot. n. 2-IX/9 – 27.02.2012). The experimental and notification procedures were carried out in compliance with Directive 86/609/EEC. In order to mimic farm conditions (i.e. to provide environmental enrichment materials to all categories of pigs, according to the rules set by the Directive 2008/120/EC on swine protection), the experimental protocol did not include a negative control (i.e. without enrichment) group.

Pigs, housing and feeding

A total of 80 crossbred (Landrace × Large White) barrows with undocked tails were used in two independent trials ($n = 40$ in each trial). In each trial, pigs were homogeneously allotted to the experimental groups on the basis of their BW. Animals were individually identified by means of ear tags. The trials started when animals were 80 days old, at an average body weight (BW) of 27.1 kg (24.2 ± 3.2 kg and 29.9 ± 3.9 kg in Trial 1 and Trial 2, respectively) and lasted until pigs reached the average BW of 158.4 kg (159.0 ± 12.1 kg and 157.72 ± 14.0 kg, in Trials 1 and 2, respectively), for a total duration of 205 days in Trial 1 and 200 days in Trial 2. Trial 1 was carried out in Autumn–Winter, whereas Trial 2 in Spring-Summer. All pigs were daily individually monitored by a veterinarian following a classic protocol for clinical observation of pigs (Jackson and Cockcroft 2002) that included breathing rate, neurological signs, lameness, diarrhoea, pruritus and abnormal body shape and skin appearance. Pigs were vaccinated against Aujeszky disease (first dose at 80 days of age, second dose after 3 weeks and third dose at 6 months of age).

The experimental groups were formed on the basis of pigs' BW and the environmental enrichments were provided on one side of the pen. Pigs were kept in collective pens (5 pigs/pen) on a slatted floor, with a floor space of 1.3 m^2 per pig. The floor space allowance was calculated according to Petherick and Baxter (1981) on the basis of an average BW of 135 kg. Groups were maintained the same during the trial period. Each pen was equipped with a nipple drinker installed on the pen side opposite to the enrichment device (water was available *ad libitum*) and a collective stainless steel feeder. Pigs were located in temperature- and humidity-controlled rooms equipped with a forced-air ventilation system (RH was set at 65% T was set at 22 °C).

To meet the pigs' requirements, two commercial feed formulations were used (first phase up to 80 kg BW: 3640 kcal DE/kg DM, CP 17.6% DM; second phase from 80 kg BW to slaughtering: 3701 kcal DE/kg DM, CP 16.0% DM). Feed was offered twice a day (at 830 h and 1430 h) as wet (meal to water ratio = 1:3) and, according to traditional practices for Italian heavy pig production, rationed at 9% of the metabolic BW ($BW^{0.75}$), up to a maximum of 2.8 kg dry matter per pig, per day. The daily rations were adjusted every 2 weeks on the basis of the expected growth and of intermediate weighings.

Lighting was artificial and was supplied by neon tubes (12 h of light per day, from 0700 h to 1900 h).

Figure 1. Enrichment devices used in the present study. C: hanging chain; WL: wood logs placed inside a specifically-designed metal rack; ED: edible block placed inside the metal rack.

In both trials, each replicate experienced one enrichment device for all the duration of the trial. Please see Figure 1 for pictures of the environmental enrichments tested.

TRIAL 1: wooden material

Forty animals were allotted to two experimental groups, each comprising four replications (i.e. pens) of five pigs, which were subjected to the following experimental treatments.

- Chain (C1) group: the environment was enriched by providing a steel chain hanging from a side of the pen;
- Wood Logs (WL) group: the environment was enriched by providing a metal rack holding in horizontal position three poplar logs (10 cm in diameter, 1 m long). The rack was attached to the pen wall approximately 10 cm above the ground to avoid contamination with manure, and its overall height was of approximately 70 cm. Pigs could easily access the poplar logs and sniff, rotate or chew on them between the rack metal bars.

TRIAL 2: edible material

Forty animals were allotted to two experimental groups, each comprising four replications (i.e. pens) of five pigs, which were subjected to the following experimental treatments

- Chain (C2) group: see Trial 1
- Edible Block (EB) group: these pens were enriched by providing a metal rack (the same as in Trial 1, installed in the same position) holding in horizontal position an EB (approximately 10 cm thick, one metre long and 30 cm wide). The block was specifically formulated for the experimental trial and its main ingredients were cereals, forages and vegetable by-products. The frame was mounted in such a way that pigs could easily access them with their snouts and move or bite the block. Each block weighed approximately 12 kg, and it was replaced when completely consumed. The number of blocks consumed during the trial was recorded.

Growth performance and enrichment disappearance

All pigs were individually weighed at the beginning, in the middle 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 pen (5 pigs) was taken as the experimental unit for BW, ADG, feed consumption and FCR. Recording sheets were used to keep track of every time a rack was refilled with a new wood log or edible block.

Behavioural traits

The behaviour of 20 pigs for each experimental group (four replications for each group, for each trial) was videotaped over the diurnal hours (700 h to 1900 h) by means of a digital closed circuit system (Mesa, Arezzo, Italy). Cameras were mounted on a rail attached to the ceiling (approximately three metres above the ground). To allow for individual behavioural observations, four animal marking sticks of different colours were chosen (blue, green, red and purple—RAIDEX GmBH, Dettingenan der Erms, Germany) and assigned to four pigs. A spot of the assigned colour was painted on the back of each pig on the day before each videotaping session. The fifth pig was left uncoloured. Pigs were videotaped over the diurnal hours once every 2 weeks, for a total of 12 videotaping sessions in each trial. All videos were examined by a single trained observer and the behavioural patterns (daily behaviour) 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, rooting/exploring the floor and social interactions. The ethogram was adapted to the specificities in the present trials by adding the following behaviours: tail biting, interaction with the enrichment device, interaction with other pen structures. Results were expressed as proportion of time spent performing each behaviour. To get more insights on the use of the environmental enrichment, three videotaping 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 and duration of each interaction with the environmental enrichment. To avoid the confounding effect of non-explorative interactions with the environmental enrichment, only interactions with the enrichment lasting at least 10 seconds were recorded by the observer.

Tail and skin lesions

The presence of skin and tail biting lesions was assessed according to the Welfare Quality® protocol for growing and finishing pigs (Welfare Quality® 2009), with some slight modifications. Lesions were scored approximately every month, for a total of six assessments. The first assessment was made when animals arrived at the experimental farm, immediately before the formation of the experimental groups. Skin and tail lesions were assessed according to the Welfare Quality® (2009) protocol for growing and finishing pigs. However, due to the limited number of animals involved in the study, this protocol was slightly modified, in terms of assessment of both sides of each body region, to increase the number of assessed skin lesions. The average number of lesions between the two sides was calculated for each body region (ears, front, middle, hindquarters, and legs) and used for classification according to the Welfare Quality® protocol (2009): each region was scored as 'a' (up to 4 lesions), 'b' (5–10 lesions) or 'c' (11–15 lesions). Each pig was then scored using a 0-to-2 scale, where 0 corresponded to a pig having the full body classified as 'a', 1 to a pig having any body region with and individual score 'b' and/or a maximum of one region scored as 'c'; and 2 to a pig having at least two body regions or more classified as 'c', or at least one body region with more than 15 lesions.

Tail biting was assessed according to the following scale: 0 (intact tail, no evidence of tail biting), 1 (superficial biting but no evidence of fresh blood or swelling) and 2 (fresh blood, evidence of swelling or infection, or tissue missing with the formation of a crust).

Blood and bristle collection and analysis

For each experimental group, a sub-sample of 15 pigs was randomly selected and blood samples were collected from each pig in concurrence with the weighings. To this aim, pigs were restrained with a snare and 15 mL of blood were drawn from the jugular vein and collected into two tubes, one containing lithium heparin and the other one containing ethylenediaminetetraacetic acid (EDTA). Blood was refrigerated immediately upon collection. Blood in K-EDTA was immediately sent to the DIMEVET laboratory (Ozzano Emilia, Italy), 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 $3500 \times g$ 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:

- energy (glucose, fructosamine, total cholesterol, triglycerides) and protein (urea, creatinine) metabolism;
- liver functionality (total bilirubin, aspartate aminotransferase = GOT, γ-glutamyltransferase = GGT);
- oxidative stress (total reactive oxygen metabolites = ROM, Oxygen radical absorbance capacity = ORAC);
- 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).

For a detailed description of the methods used for the analysis of blood parameters and the related references, please see the supplemental material (SM – Section 'SM Material and Methods').

Bristles were collected at the beginning, in the middle and at the end of the trial by shaving the rump region of all pigs. Samples were handled and analysed as described by Bacci et al. (2014). In brief, bristles were washed with water and then twice with isopropanol to remove any organic residue from the surface. Once fully dried, samples were finely pulverised and pooled (a pool of bristles for each pen, i.e. four pools per treatment in each trial) then 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. Intra-assay and inter-assay coefficients of variation were 3.4% and 8.50%, respectively. The assay sensitivity was 5.4 pg/tube and was defined as the dose of hormone at 90% binding.

Statistical analysis

Data of each trial was separately analysed using the software SAS Inst. Inc. (Cary, NC; release 8.0, 2014).

The normal distribution was checked by using the UNIVARIATE procedure of SAS, by NORMAL option. Parameters that were not normally distributed (some haematic parameters) 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.).

All parameters were subjected to ANOVA using the MIXED procedure of SAS. The pen (five pigs) was taken as the experimental unit for BW, ADG, feed consumption, FCR and hair cortisol levels. Individual data were taken to be the experimental unit for behavioural observations, blood parameters, and skin and tail lesions. Environmental enrichment was used as the main effect for BW, ADG, feed consumption and FCR. Enrichment and time were used as fixed effects and pen as random effect for the following variables: interactions with the enrichments (in-continuous observations), hair cortisol levels, skin and tail lesions. For blood parameters, the statistical model applied included the fixed effect of day from the introduction of the environmental enrichment, and type of environmental enrichment. The day from the introduction of the environmental enrichment within the subject were considered as a repeated measure. Several covariance structures were tested, and the selection of compound symmetric as covariance structures was done based on the Bayesian information criterion. The pairwise comparison has been done using least significant difference (LSD) test.

Chi-squared test was used to evaluate the distribution of skin lesions and tail lesions into the three severity classes.

The significance level was set at $p < .05$ for all tests.

Results

No occurrence of disease was recorded during the trials. No pig had to be removed from the experimental protocol.

Table 1 shows the productive traits observed during the two trials. No significant differences were observed in BW, ADG, feed consumption or FCR.

The diurnal behaviour of pigs is shown in Table 2. In Trial 1, WL pigs interacted with the enrichment less than C1 pigs ($p < .01$) and walked less ($p < .05$). In Trial 2, EB pigs spent less time in lateral recumbency and rooting/exploring the pen floor ($p < .05$ and $p < .001$), and they interacted more with the enrichment ($p < .001$) and with other pen components ($p < .05$), if compared to C2 group. Figure 2 and 3 show the results of the in-continuous video analysis. In Trial 1 (Figure 2), number of interactions significantly differed between the experimental groups ($p < .01$), with WL

Table 1. Live weight and average daily gain (ADG) of heavy pigs receiving different environmental enrichment materials (C1 and C2 = hanging chains; WL = wood log; EB = edible block) in two different trials.

	Trial 1		
	C1	WL	RMSE
Body weight, kg			
Initial weight (0 day)	31.36	29.74	2.21
Weight at 124 days	109.86	107.76	9.00
Final weight (205 days)	158.91	159.13	12.78
ADG (kg/day)			
0–124 days	0.633	0.629	0.07
125–205 days	0.606	0.634	0.08
1–205 days	0.622	0.631	0.06
Feed consumption (kg/days)			
1–124 days	1.891	1.891	–
125–205 days	2.788	2.788	–
1–205 days	2.247	2.247	–
FCR			
1–124 days	3.09	3.05	0.15
125–205 days	4.71	4.48	0.20
1–205 days	3.64	3.53	0.42
	Trial 2		
	C2	EB	RMSE
Body weight, kg			
Initial weight (0 day)	25.04	24.91	3.20
Weight at 127 days	107.17	109.11	11.92
Final weight (200 days)	155.35	158.09	14.13
ADG, kg/days			
1–127 days	0.652	0.668	0.08
127–200 days	0.651	0.662	0.07
1–200 days	0.652	0.666	0.06
Feed consumption, kg/day			
1–127 days	1.749	1.749	0.10
127–200 days	2.943	2.943	0.02
1–200 days	2.190	2.190	0.07
FCR			
1–127 days	2.68	2.62	0.11
127–200 days	4.52	4.45	0.10
1–200 days	3.36	3.29	0.10

No statistically significant difference was detected between the experimental groups ($p > .05$).
ADG: average daily gain; FCR: feed conversion ratio; RMSE: root mean square error.

Table 2. Diurnal behaviour (700 h–1900 h) of pigs that received different environmental enrichment materials in two different trials.

	Trial 1				Trial 2			
	C1	WL	*p* Value	RMSE	C2	EB	*p* Value	RMSE
Standing inactive	2.18	1.96	n.s.	2.74	2.43	2.95	+	2.84
Sitting inactive (dog sitting)	1.17	1.34	n.s.	2.44	2.76	2.66	n.s.	3.89
Lateral recumbency	25.85	25.57	n.s.	14.36	42.87	39.06	*	17.24
Sternal recumbency	37.01	37.32	n.s.	12.74	19.29	21.45	+	12.42
Total recumbency	62.86	62.89	n.s.	12.12	62.16	60.51	n.s.	12.33
Walking	2.78	2.14	*	2.49	1.64	1.97	+	3.14
Rooting/exploring the floor	17.82	18.79	n.s.	10.32	15.90	13.39	***	7.07
Positive interaction	1.66	1.54	n.s.	2.71	4.00	4.34	n.s.	3.46
Aggressive interaction	0.52	0.32	n.s.	1.24	0.62	0.60	n.s.	1.07
Tail biting	0.12	0.06	n.s.	0.55	0.04	0.06	n.s.	0.24
Total social interactions	2.31	1.92	n.s.	3.17	4.66	5.00	n.s.	3.58
Interaction with the enrichment	1.84	0.64	**	3.06	1.12	2.85	***	3.20
Manipulation of pen components	1.51	1.17	n.s.	3.83	0.79	1.27	*	2.09
Total interactions with pen	3.36	1.81	**	5.04	1.91	4.12	***	3.76

C1 and C2: hanging chains; WL: wood log; EB: edible block; n.s.: not significant. Pigs were videotaped once every two weeks, for a total of 12 videotaping sessions in each trial. Videos were then examined by scan sampling technique at 10-min intervals. Data are expressed as percentage of total observed behaviours.
$p > .05$; $+p < 0.1$; $*p < .05$; $**p < .01$ and $***p < .001$ within the same trial.

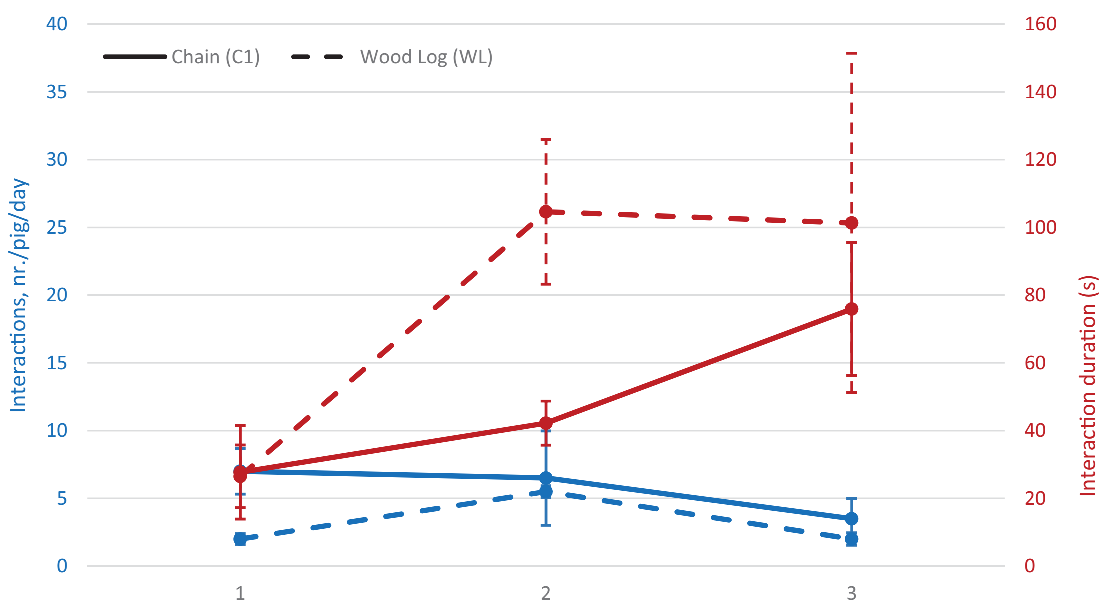

Figure 2. Median values (all-occurrences sampling) of the interactions with the environmental enrichment tools, metal chain (—) or wooden log (- - -), observed during Trial 1: median of the number of occurrences (blue lines) and average duration (red lines). Interactions were observed over 3 days, one at the beginning, one in the middle and one at the end of the experimental trial (points 1, 2 and 3 of the horizontal axis, respectively). The number of interactions significantly differed between the experimental groups ($p < .01$). The effect of time was also significant ($p < .01$ for interactions number, $p < .001$ for interaction duration).

pigs interacting a lower number of times with the enrichment if compared to C1 ($p < .1$). The effect of time was also significant ($p < .01$ for interactions number, $p < .001$ for interaction duration, $p < .001$ for total daily duration, see Figure 2). Regardless of the experimental group, interaction duration increased as the trial progressed, whereas both interaction number and total duration peaked at the second observation day and decreased towards the end of the trial. In Trial 2 (Figure 3), both time and environmental enrichment had a significant effect on interaction duration ($p < .05$ for both). EB pigs carried out longer interactions with the enrichment if compared to C2. Average interaction duration increased over time in EB group. Total daily duration was affected both by treatment and time ($p < .05$ and $p < .01$, respectively, see Figure 3).

During Trial 1, the replacement of consumed wood logs occurred rarely (on three occasions during the entire trial). EBs disappearance during Trial 2 showed high variability: the four replications consumed 3, 4, 5

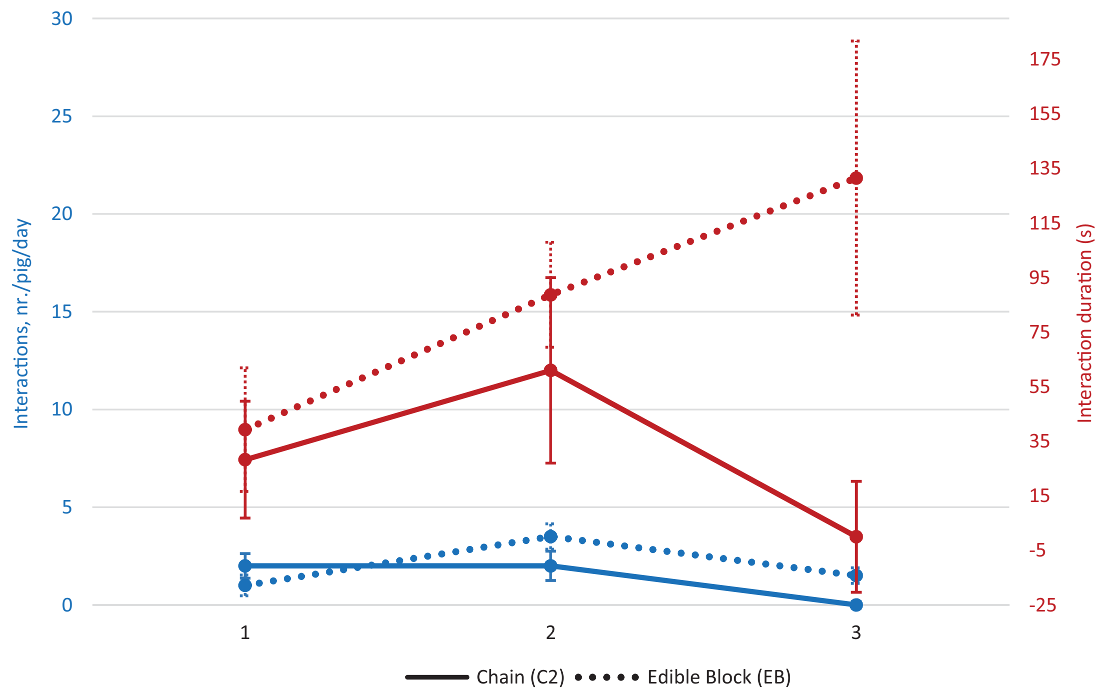

Figure 3. Median values (all-occurrences sampling) of the interactions with the environmental enrichment tools, metal chain (—) or edible block (●●●●), observed during Trial 2: number of occurrences (blue lines) and average duration (red lines). Interactions were observed over 3 days, one at the beginning, one in the middle and one at the end of the experimental trial (points 1, 2 and 3 of the horizontal axis, respectively). The duration of interactions significantly differed between the experimental groups ($p < .05$). The effect of time was also significant ($p < .01$ for number of interactions, $p < .05$ for interaction duration).

and 11 edible blocks. On average, the rack of every pen was refilled with a new block once a month, with an average disappearance of 70 g per pig, per day. However, due to the slatted floors on which animals were raised, no recording of refusals (and, therefore, of actual ingestion) could be made.

Tail and skin lesions are shown in Table 3. No difference between the experimental groups was observed when the groups were formed (time 0, i.e. pre-trial assessment). The table shows the average value of the subsequent five assessments which were carried out during each trial. Figures SM4 and SM5, reported in the Supplemental Materials, show the evolution of tail and skin lesions across Trials 1 and 2, respectively.

In both trials, skin lesion score presented no statistically significant differences between the experimental groups ($p > .05$). As concerns tail lesions, in Trial 1 they were significantly more severe in WL than in C1 group ($p < .05$), due to a higher proportion of tails classified as 'moderate' in the WL group. In Trial 2, no differences were found between the experimental groups for tail lesions. An effect of time on skin lesions was observed, both in Trial 1, with an increase in the last two assessments ($p < .01$, see Figure SM4), and in Trial 2, with higher skin score at the second assessment in both experimental groups ($p < .01$, see Supplementary Figure SM5).

Hair cortisol concentration and selected blood parameters are shown in Table 4 (for the complete blood parameters, please see the Supplemental Material, Tables SM6, SM7 and SM8). No statistically significant difference in hair cortisol or neutrophil/lymphocyte (N/L) ratio was observed in both trials. As concerns biochemical parameters, some significant differences between the experimental groups were observed. In particular, at the beginning (1st assessment) of Trial 1 WL pigs had lower GGT values ($p < .05$) and showed higher triglycerides concentration ($p < .01$) if compared to the C1 group. These differences at the beginning of the trial disappeared at later assessments. At the second assessment, WL pigs showed higher haptoglobin ($p < .05$) than C1. At the end of the trial, WL pigs had higher glucose ($p < .001$) and lower zinc ($p < .05$) concentration if compared to C1.

In Trial 2, at the first assessment EB pigs had lower triglycerides and higher zinc concentration than C2 ($p < .001$ and $p < .05$, respectively). At the second assessment, lower bilirubin concentration was observed in EB pigs ($p < .05$). At the third assessment, the only difference observed concerned AST/GOT values, which were higher in EB pigs ($p < .05$).

Table 3. Skin and tail lesions (assessed according to the Welfare Quality® protocol, 2009) of pigs receiving different environmental enrichment materials.

	Trial 1			Trial 2		
	C1	WL	RMSE	C2	EB	RMSE
Pigs	20	20	–	20	20	–
Average skin score[1], pt.	0.49	0.50	0.25	0.56	0.69	0.29
Average tail score[2], pt.	0.11	0.24**	0.20	0.26	0.31	0.27
Skin lesions severity classes, %						
None or slight lesion (0)	52.0	52.0	–	46.0	35.0	–
Moderate lesion (1)	47.0	46.0		52.0	61.0	
Severe lesion (2)	1.0	2.0		2.0	4.0	
Tail lesions severity classes, %						
Intact tail (0)	89.0	77.0*	–	77.0	73.0	–
Moderate tail lesion (1)	11.0	22.0*		21.0	23.0	
Severe tail damage (2)	0	1.0		2.0	4.0	

C1 and C2: hanging chains; WL: wood log; EB: edible block. Average scores and class distributions are calculated on the 5 assessments carried out on all pigs during each trial.
A significant statistical difference in a trial is shown by a superscript on the WL (Trial 1) or on the EB (Trial 2) value (*$p < .05$; **$p < .01$).
A time effect was detected on skin lesions in both trials ($p < .01$), as shown in the Supplementary materials (graphs SM4 and SM5).

Table 4. Selected blood parameters and cortisol from bristles of heavy pigs (mean and Standard Error = SE) receiving different environmental enrichment materials.

Trial 1							
Time (days)	1st assessment (day 1)		2nd assessment (day 124)		3rd assessment (day 205)		
Group	C1	WL	C1	WL	C1	WL	SE
Hair cortisol, pg/mg[a]	5.11	9.32	7.13	6.46	11.02	13.21	0.617
N/L ratio[1]	0.53	0.51	0.68	0.63	0.90	1.0	0.050
Glucose[2], mmol/L	6.10	6.28	4.98	4.94	6.99	9.58***	0.383
Triglycerides[2], mmol/L	0.363	0.487**	0.271	0.297	0.423	0.479	0.041
GGT[3], U/L	121.25	71.0*	109.7	101.4	52.2	54.7	19.46
Haptoglobin[4], g/L	1.02	0.98	1.09	1.39*	1.62	1.79	0.133
Zinc[5], µmol/L	19.77	19.63	15.14	16.48	19.88	16.92*	1.193

Trial 2							
Time, days	1st assessment (day 1)		2nd assessment (day 127)		3rd assessment (day 200)		
Group	C2	EB	C2	EB	C2	EB	SE
Hair cortisol, pg/mg[a]	5.24	4.63	4.76	4.79	5.64	6.96	0.259
N/L ratio[1]	0.44	0.48	0.57	0.53	0.53	0.50	0.020
Triglycerides[2], mmol/L	0.61	0.47***	0.35	0.36	0.30	0.31	0.039
Total bilirubin[3,4], µmol/L	2.04	2.50	1.31	0.98*	1.11	1.10	0.431
AST/GOT[3], U/L	52.45	51.13	43.10	40.86	47.00	56.87*	4.390
Zinc[5], µmol/L	13.65	15.41*	15.56	17.05+	17.96	19.31	0.852

C1 and C2: hanging chains; WL: wood log; EB: edible block. A significant statistical difference at the same assessment is shown by a superscript on the WL (Trial 1) or on the EB (Trial 2) value (+$p < .1$; *$p < .05$; **$p < .01$; ***$p < .001$).
GGT: Gamma-glutamyltransferase; GOT/AST: Glutamyl oxaloacetic transaminase; N/L: Neutrophil/Lymphocyte ratio.
[a]Assessed on a pool of bristles for each pen (i.e. four pools per treatment in each trial).
Type of indicators: [1]innate immune response (leukocyte indexes); [2]energy metabolism; [3]liver functionality; [4]positive acute phase proteins; [5]parameters linked to positive acute phase proteins.

Discussion

Earlier studies on the effects of environmental enrichments on growth traits have yielded conflicting results. Beattie et al. (2000) observed increased growth rates and carcase weight in pigs that were allowed extra space and a rack containing peat and straw. Our results failed to find such improvements in growth parameters, in agreement with the studies carried out by Klont et al. (2001) and Bulens et al. (2016), who also observed no differences in productive parameters when pigs were raised in straw-enriched vs. barren environments. However, given the limited number of replications used in the present trials, productive data should be considered as only indicative pending further and extensive on-farm testing.

A few studies investigated the behavioural traits of heavy pigs. The results from the present trials are in substantial agreement with previous finding from our research group (Scipioni et al. 2009, Martelli et al. 2015) confirming that heavy pigs tend to spend most of the daily hours (at least 60%) lying in resting position (either lateral or sternal recumbency). In both trials behaviour significantly differed between the two experimental groups. In Trial 1, WL pigs spent significantly less time interacting with the environmental enrichment if compared to C1. This could indicate that animals found the wood logs to be less attractive than the hanging metal chain. It cannot be ruled out that the wood log, similarly to what has been already observed for piglets (Nannoni et al. 2016), may have represented a less effective environmental enrichment than hanging chains, being less manipulable or, at least, more difficultly accessible. The all-occurrences observation of the interactions with the enrichment tools confirmed that pigs carried out significantly less (but longer) interactions with the wood log than with the metal chain. However, the number of interactions towards both enrichments increased in the middle of the trial but underwent a sharp decline as time progressed, probably due to habituation (Van De Weerd et al. 2005; Trickett et al. 2009). It should also be considered that, as pigs grow older, they show overall decreasing levels of activity, becoming progressively more inactive (Van De Weerd et al. 2005). This could, in part, explain the reduced number of interactions with the enrichments at the end of the trial in both treatments.

In Trial 2, pigs receiving the edible enrichment tool were more active and directed their explorative activities towards the edible block, reducing the time they spent rooting/exploring the pen floor if compared to C2. This result could indicate that the edible block had a good attractivity for pigs, which were motivated to explore it even between the metal bars of the rack. The block might also have helped to reduce the exploratory behaviour directed towards the floor. This reduction should be regarded as positive since the over-exploration of the floor, especially in barren environments such as slatted floors, can be considered as a stereotyped behaviour (Scipioni et al. 2009).

Similarly, Jensen et al. (2010) observed that the provision of maize silage decreased oral behaviour directed towards pen mates and pen components. As concerns rooting materials for heavy pigs, the results from Scollo et al. (2013) and Di Martino et al. (2015) are comparable to those shown in the present study: pigs were motivated to explore straw in the feeding rack and decreased their overall explorative behaviour directed towards other pen components.

The analysis of the interactions with the enrichment tools showed that, although the number of interactions with the metal chain and with the edible block was similar, pigs carried out significantly longer interactions with the edible block, especially at the end of the trial. Similarly to what has been observed in Trial 1, the number of interactions towards both enrichments increased in the middle of the trial but then declined as time progressed, therefore habituation, together with decreasing overall activity, cannot be ruled out.

In both trials, the most interesting differences in enrichments use are observable at the end of the production cycle, when WL and EB pigs interacted for longer periods with the enrichment if compared to C1 and C2. Therefore, the motivation to explore the enrichments placed inside the metal rack was maintained over time, conversely to what happened with the metal chains. This observation is in agreement to what has been previously observed by Scollo et al. (2013) and Bulens et al. (2016), who also used racks or dispensers to hold the rooting materials. However, since pigs in these trials were restricted-fed, the high motivation at the end of the trial might be, at least in part, due to foraging behaviours directed towards the enrichment materials. Overall, the occupational level of both chains and wood logs was low and comparable to what observed by Scott et al. (2007) for hanging plastic toys (below 2% of observations) whereas the edible bock showed a significantly higher occupational value (ca. 3%).

A very low occurrence of aggressive interactions and tail biting behaviour was observed in all the experimental groups. These behavioural observations are confirmed by the low prevalence of severe skin lesions during the whole rearing period in all the experimental groups. As concerns tail lesions, they were increased only in the WL group, due to the higher number of lesions classified as moderate in that group. It may suggest that wood logs placed in the rack were not enough rootable and destructible by the pigs, contrarily to the edible block. According to Moinard et al. (2003), providing animals with an enrichment which is not sufficiently manipulable may increase the incidence of tail biting. Overall, the presence of severe damages on the tail was low in all the groups. This finding is relevant considering that the tails were left undocked. However, under our experimental conditions, other factors may have helped preventing tail lesions, such as high space allowance, low number of pigs per pen, presence of environmental enrichments and stability of the groups. For example, Di Martino et al. (2015) observed increased tail lesions when undocked pigs were kept under challenging conditions (high stocking density on fully slatted floors) even if the pens were enriched with straw racks suggesting a possible major role played by space allowance, or at least by a complex combination of these two factors (Larsen et al. 2017).

At all the sampling points (beginning, middle and end of each trial), cortisol concentration in bristles did not differ between the experimental groups indicating the absence of chronic stressors activating the hypothalamic–pituitary–adrenal axis. Casal et al. (2016)

reported a significant decrease in hair cortisol concentration of pigs supplied with enrichment materials for two months, if compared to pigs raised in barren environments. However, in the cited study a negative control group (i.e. without enrichment) was used, which could explain the differences observed between the experimental groups. Conversely, in the present study, all groups received an environmental enrichment tool, and they had similar effects on animal welfare, resulting in no differences between the experimental groups. It is also worth noting that overall, the values observed by Casal et al. (2016) are in good agreement with the present study, and with findings from Trevisan et al. (2017) on Tanzanian pigs. Other studies (for example Valent et al. 2017) found considerably higher hair cortisol levels in pigs subjected to either positive or negative handling.

Although the two trials were independent and statistical analysis was carried out separately, it can be seen that hair cortisol concentration at the last assessment was numerically higher in Trial 1 than in Trial 2. The difference is probably due to the fact that the last assessment was carried out during winter in Trial 1 and during summer in Trial 2. In fact, despite the ventilation system, temperature in the rooms tended to be higher during the summer months. Similarly to what we observed in a previous study (Bacci et al. 2014), the prolongation of adverse situations (e.g. hot season) may result in a reduction of circulating cortisol and, therefore, in a reduced cortisol accumulation in hair.

The absence of significant differences in the N/L ratio, further confirms the fact that none of the experimental groups experienced chronic stress. In other words, under our experimental conditions, also the pigs receiving the lowest enrichment level (i.e. the metal chain) showed N/L values comparable to the more enriched groups. The analysis of plasma biomarkers detected only minor, transient differences in triglycerides and zinc concentration (Trials 1 and 2), GGT and haptoglobin values (Trial 1), bilirubin and AST/GOT values (Trial 2).

Overall, the inflammo-metabolic profile of pigs enrolled in our trials confirmed that they were in a satisfactory health condition (Kaneko et al. 1997; Chen et al. 2003; Hellwing et al., 2007). In particular, the haptoglobin, which is a marker of acute inflammatory events, has been within the range of clinically normal pigs (1.42 ± 0.02 as standard error mg/mL; Chen et al. 2003). Only at the end of Trial 1, in both groups average haptoglobin concentrations was slightly higher than the upper range of healthy pigs (Chen et al. 2003), suggesting the presence of a subclinical inflammatory condition. Another marker of inflammatory events, rarely monitored previously in pig, is ceruloplasmin (Martínez-Subiela et al. 2007; Trevisi et al. 2016). High concentrations of this protein suggest the presence of prolonged inflammatory events. Interestingly, the concentration of the ceruloplasmin is progressively increased with the age of the pig and reached the highest levels (more than 20 μmol/L) at the same time as haptoglobin (3rd assessment of trial 1). A progressive increase over time has been observed for many other markers (ROM, total antioxidant (ORAC), paroxonase, globulin, creatinine) and in all treatments. This suggests that pigs, in this physiological period (quick growth and with a high accumulation of adipose tissue) could have undergone a mild inflammation and oxidative stress, which are independent of the presence of environmental enrichments and likely related to the physiological status. The gradual raise of these biomarkers with the age suggests the need of more studies to better define the physiological range of these parameter in fattening pigs.

As concerns the differences between treatments, in the Trial 1 at the 2nd assessment the mild differences in haptoglobin and ceruloplasmin do not highlight the presence of generalised inflammatory events in the animals (Chen et al. 2003), but they simply signal the sporadic presence of few subjects with subclinical and transitory problems in both the groups. At the third assessment, the metabolic profile is slightly better in the C1 group (lower and more physiological level of the blood glucose, suggests the presence of more calm subjects; Martínez-Miró et al. 2016) but the inflammatory profile does not show differences between the experimental groups.

In Trial 2, at the second assessment C2 pigs appear to be in a slightly worse metabolic state than EB, having a tendency ($p < .1$) towards lower zinc levels (probably as a consequence of the difference observed at the beginning of the trial) and higher bilirubin levels (indicating a mild difficulty for the liver in its excretion; Assenat et al. 2004). In both cases, differences are mild, transient and not indicative of a pathologic state. At the end of the trial, EB pigs had higher AST/GOT values in comparison with C2, presumably due to a higher muscular activity (Pettersson et al. 2008) rather than to a liver damage, given the basic similarity in other plasma parameters.

Conclusions

The results obtained from the present research trials allowed the identification of point-source enrichment materials capable of attracting and maintaining pigs' interest over time. Contrarily to wood logs, which had a limited effect, the use of the EB reduced the over-exploratory behaviours without altering the main physiological, behavioural and productive (growth traits) parameters in comparison with the use of hanging chains. The overall results, however, indicate that all the groups attained a similar welfare level. Under our experimental conditions (small groups, space allowance above the minimum legislation requirement), no tail biting outbreak occurred despite the undocked tails, and the occupational level provided by the enrichments was low, even when edible blocks were provided. On the whole, these findings seem to indicate that other factors such as space allowance and farm management might be, for heavy pigs, even more important than environmental enrichments in order to attain a satisfactory welfare level of animals.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Progetto AGER [grant number 2011-0280].

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