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PAPER

Longitudinal effects of environmental enrichment on behaviour and physiology of pigs reared on an intensive-stock farm

Arianna Manciocco,[1,2] Marco Sensi,[3] Livia Moscati,[4] Lorenzo Battistacci,[4] Giovanni Laviola,[1] Gianfranco Brambilla,[5] Augusto Vitale,[1] Enrico Alleva[1]

[1]Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, Roma, Italy
[2]Dipartimento di Progettazione Molecolare, Consiglio Nazionale delle Ricerche, Roma, Italy
[3]Laboratorio di Diagnostica di Base, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy
[4]Laboratorio di Chimica Clinica e Benessere Animale, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy
[5]Dipartimento Ambiente e Connessa Prevenzione Primaria, Istituto Superiore di Sanità, Roma, Italy

Abstract

The aim of this paper was to provide a longitudinal evaluation of the effects of physical enrichments on the behaviour and physiology of intensive stock-farming pigs. Twenty-eight crossbred pigs of both sexes, were exposed to four types of enrichments (hemp ropes, steel chains, plastic balls, rubber hoses) over a period of eleven weeks. This investigation was based on specific abnormal behaviours and physiological indicators, including hematologic parameters. For behavioural score, focal sampling was used with recording of abnormal behaviours (body-, tail- and ear-biting), belly nosing, running, and interaction with objects (for Enriched pigs). The presence of skin injuries was also recorded. In general, the frequency of abnormal behaviours was significantly reduced in the Enriched group. A time-related profile appeared in the use of the enrichments. Males showed higher occurrence of skin injuries than females. Physiological measurements, such as levels of complement system, white blood cells and neutrophils, were lower in pigs from the Enriched group. Enriched pigs, as a whole, presented much lower levels of serum DHEA-S concentration over two weeks. The findings of this study show the successful provision of appropriate enrichments to encourage behaviours which may result in satisfactory animal oral interaction with the enriching objects, preventing them biting pen-mates. In this respect, the objects proposed were strongly effective in producing changes in behaviour which could mitigate inadequate conditions, such as the relationship between animal body weight and the available space allowance.

Corresponding author: Dr. Arianna Manciocco, Reparto di Neuroscienze Comportamentali, Dipartimento di Biologia Cellulare e Neuroscienze, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Roma, Italy.
Tel. +39.06.49902107 – Fax: +39.06.4957821.
E-mail: arianna.manciocco@iss.it

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Introduction

The well-being of swine has become an important issue affecting the income of farmers and suppliers. This interest is due to the fact that the quality of life appears to be associated with food production and meat quality (Beattie *et al*., 1995; Klont *et al*., 2001; Mellor and Stafford, 2001). The rigorous selection, aimed at obtaining individuals with elevated reproductive and productive performances, has often resulted in farm animals in a poor state of health (Appleby, 2005; Deen *et al*., 2005; Stafford and Gregory, 2008). Several studies have reported chronic immuno-suppression caused by the inability to cope with stress, such as that due to high stock densities or regrouping events, in commercial lines of pigs (Zanella *et al*., 1991; Broom and Johnson, 1993).

Welfare problems can be also identified by a variety of behavioural responses and changes in these can indicate a poor state of well-being (Fraser, 1995). Furthermore, the existence and extent of behaviours that may lead to injury can indirectly indicate a status of frustration or other forms of distress (Broom, 1986).

There is consolidated evidence to show that the characteristics of the housing environment have a marked influence on the behaviour and welfare of animals, both those raised for biomedical investigation or farm animals (Beattie *et al*., 2000; Olsen *et al*., 2002; Laviola *et al*., 2004; Dong *et al*., 2007; Brenes *et al*., 2008). In particular, the provision of straw seems to represent a suitable strategy to alleviate the presence of injurious behaviours in swine (Petersen *et al*., 1995; Kelly *et al*., 2000; Guy *et al*., 2002; Van de Weerd *et al*., 2006; Studnitz *et al*., 2007). In fact, under natural or semi-natural conditions, pigs spend a large part of the daytime exploring the surrounding environment; mainly sniffing, chewing and biting edible and inedible items (Graves, 1984; Stolba and Wood-Gush, 1984). The pigs seem to be highly motivated to behave in this way (Horrel and Ness, 1995; Day *et al*., 1996) and this can, therefore, be considered a priority need for these animals (Jensen and Toates, 1993; Studnitz *et al*., 2007).

Nevertheless, standard farm rearing environments are primarily designed to maximize the production efficiency and quite often these conditions do not permit the introduction of bedding material. Therefore, in intensive breeding conditions, the motivation to explore and root can induce the animals to bite and chew protuberant areas, such as tails and ears, of pen-mates. When these activities become frequent and pervasive, this may indicate unsuccessful attempts to cope with an inadequate environment (McGlone, 1986; Lawrence and Terlouw, 1993; Olsen *et al*., 2002) and they can be considered to be abnormal behaviours (Wiepkema and Koolhaas, 1993; Broom, 1996; Kelly *et al*., 2000; Day *et al*., 2002).

In the present study, pigs housed on a commercial farm either in a Standard or an Enriched pen were followed up over a period of eleven weeks. Stock density was kept according to the commercial standard requirements across the whole period of study. Our first aim was to compare the developmental expression of selected normal and abnormal behaviours by monitoring immunological and hematologic parameters. The enrichment items used were chosen on the basis of their feasibility, easy availability, low cost and *chewiness*. A second aim was to evaluate the differential use by pigs in the Enriched pen of the various objects provided according to time. Finally, we also monitored the occurrence of lesions on the skin of the animals.

Materials and methods

Animal handling to collect physiological data was carried out according to the ethical guidelines published on the website of the International Society for Applied Ethology (*http://www.applied-ethology.org/ethicalguidelines.htm*). Furthermore, animal housing and husbandry procedures followed the norms of the European Communities Council Directive 2008/120 on minimum standards for the protection of pigs (European Commission, 2008).

Animals and housing

Twenty-eight pigs [Pietrain x (Large White x Landrace)] with an initial average weight of 32.5±2 kg were used in this study. Pigs were weaned at four weeks of age. On arrival at the farm, the animals were randomly assigned to two experimental groups. Fourteen animals (7 castrated males, 7 females) were housed in a physically enriched pen (Enriched group) and 14 animals (7 castrated males, 7 females) were housed in a conventional pen (Standard group). Each pen contained a total of 50 animals. The structural characteristics of the shed limited choice of study environment to only two adjacent pens, in order to obtain homogeneous conditions of light, air quality, temperature, sound, and any other environmental factors. Therefore, the possibility that any such factors could have contributed to the observed differences between the groups can be excluded. The experimental pigs were marked with numbered ear tags, of a different color for each sex, and with a spray paint on their backs to allow the identification of each animal for behavioural observations.

The pens measured 4.0 m wide × 10.0 m long. There was a concrete floor, with narrow splits to allow drainage of feces and urine. The two adjacent pens were separated lengthwise on one side by a wall approximately 1.30 m high (Figure 1). On the short sides of each pen, there were automated sliding glass windows (1.5 m high × 3.5 m long) to provide animals with natural light. Food and water were available *ad libitum*. Two drop feeders were suspended from the ceiling, and two drinking bowls, with a push button activated by the animal's muzzle, were provided.

After six weeks on the farm, average body weight was 76.2 kg for Standard pigs and 73.3 kg for Enriched animals. At the end of the eleventh week, average body weight of all animals was 94±2 kg.

Enrichment materials

The Enriched pen contained six chains, 80 cm long × 2.5 cm in diameter, inserted in rubber hoses 50 cm long × 3.5 cm wide. These objects were hung along the dividing walls and uniformly distributed on both sides of the pen (Figure 1). Five hard, non-toxic plastic balls (diameter 25 cm, supplied by PLEXX BV, Elst, The Netherlands) with stones inside and six rubber hoses, 60 cm long x 2.5 cm wide, were placed on the floor. Finally, two hemp ropes approximately 2.0 m long were hung through 15 cm high tensile extension springs. The hemp ropes were periodically replaced following the pigs' activity.

Procedure

Behavioural data

Behavioural data were collected on the day of arrival of the animals at the facility (Time 0), 24 h later (Time 1), and two weeks (Time 2), six weeks (Time 3) and eleven weeks (Time 4) from arrival.

An observer, positioned outside the pens to minimize disturbance, recorded the pigs' behaviours before blood collection to avoid procedurally-related stress responses. The data were collected twice a day between 08.00-11.00 (morning session) and 16.30-19.30 (afternoon session). One-minute focal animal sampling was adopted, i.e. only the behaviour of one animal at a time (the focal animal) was recorded during each focal sampling (Martin and Bateson, 1993). Recording of the occurrence of behaviours such as body- , tail- and ear-biting, belly nosing, running and interaction with objects in the Enriched pen (Table 1) with 20 s inter-sample intervals (Martin and Bateson, 1993) were recorded. Biting was only recorded if it happened while the animals were not fighting. All behaviours were mutually exclusive. When recording 'interaction with the objects', the specific object used was noted. Each experimental pig was observed for a total of 5 min distributed across each single session. All 28 experimental pigs were observed across each session, and the order of intra- and inter-group observations was balanced among the sessions.

Skin injuries

Severity and location of skin injuries were recorded at Time 0, Time 2, Time 3 and at Time 4, for each experimental pig. Four categories, depending on the severity of injuries were characterized (Smulders *et al*., 2006): Category 1, pigs without any lesions; Category 2, pigs with only scratches; Category 3, pigs with lesions; Category 4, pigs with deep lesions and/or where parts of the tail or ears were missing. A scratch was defined as a superficial lesion not penetrating the skin, according to the parameters used in the SWAP program (National Pork Board of the United States, 2003). Furthermore, the animal body surface

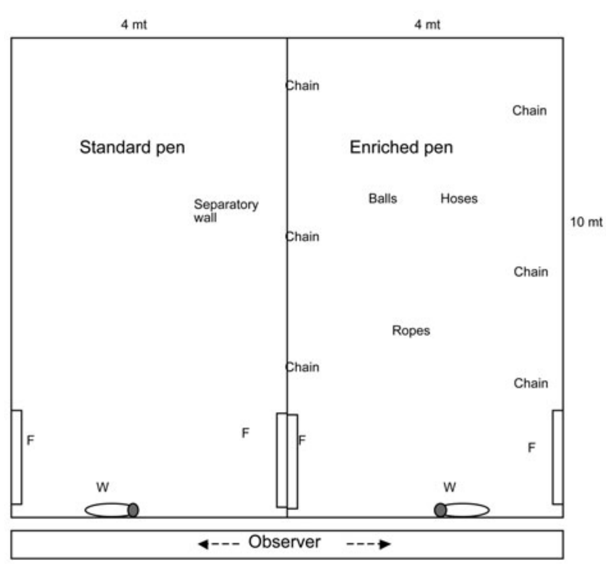

Figure 1. Experimental setting. The two experimental pens and the one-meter elevated footboard for animal observation. *Balls* and *Hoses* lay free on the floor; *Chains* and *Ropes* were hung from the wall and ceiling, respectively. F, feeder; W, water.

was divided into different areas and the location of the injuries was also taken into consideration (de Koning, 1984; Hodgkiss *et al*., 1998). A first area included head and neck (ahead), a second area comprised shoulders, back and flanks (middle), and a third area was represented by the rump, hind legs and tail (back).

Hematologic data

With the exception of Time 1, physiological data were recorded on the same days as the behavioural observations. Blood samples were collected in K3-EDTA tubes on day of the arrival at the farm (Time 0) and after two weeks (Time 2), six weeks (Time 3) and eleven weeks (Time 4). These samples were stored at -20°C. All blood samples were centrifuged for biochemical analyses (3.250 g, 15 min at 4°C).

These samples were used to determine a range of hematologic parameters using a semi-automatic electric impedance analyzer (Hemacomp 10, SEAC, Florence, Italy). The evaluated parameters were concentrations of red blood cells (RBC) and leukocytes (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), platelets and neutrophil-to-lymphocyte ratio (N:L).

For immunological parameters, the levels of serum bactericidal activity (SBA), hemolytic complement (classical pathway), lysozyme, and haptoglobin were determined. Leukogram (neutrophils, basophils, eosinophils, lymphocytes and monocytes) were determined through microscopic observation of blood smears stained by the May-Grunwald Giemsa technique.

Determination of serum lysozyme (LZM) activity was based on its lytic activity on the cell wall of *Micrococcus lysodeikticus* according to Osserman and Lawlor (1966). Results were determined after an incubation time of 18 h at +37°C. Diameter of the lysed areas was determined using a measuring viewer and compared with the lysed zones of a standard lysozyme preparation (Sigma, Milan, Italy).

The hemolytic complement assay was carried out in microtiter plates according to Barta and Barta (1993).

Oxidative stress was evaluated by measuring both pro-oxidant Reactive Oxygen Metabolites (ROMs) and anti-Oxidant species (OXY) in sera, using commercial kits supplied by Diacron International (Grosseto, Italy), according to the methodology previously described by Brambilla *et al*. (2002).

Finally, DHEAs levels in serum were evaluated. These values were dosed using commercial competitive immune-assay (DHEAs saliva) supplied by Dia Metra (Segrate, Italy) adapted to pig sera, in order to achieve a limit of quantification of 0.15 mg/mL, according to the manufacturer's instructions.

Statistical analysis

Results are reported as mean percentages ± standard errors or residual standard deviations. Each individual pig was the experimental unit.

In relation to the number of groups used in this experiment, it should be pointed out that we chose a methodology that had to take into account the logistical restrictions of field conditions (see above: Animals and housing), without detracting from the validity of the study. In particular, the independence of the observations was preserved by the fact that only the behaviour of a limited number (14) of individuals within each group (comprising a total of 50 pigs) was recorded. In a large and *not totally experimental* group, the behaviour of a randomly selected experimental animal is not necessarily affected by the behaviour of another experimental individual. In our case, it should be noted that each pen contained a number of *not experimental pigs*; nearly three times as many as the experimental animals. For further confirmation of this, a calculation of social interactions was performed by allocating scores during some randomly selected video recordings. From this analysis, it resulted that the mean percentages of interaction of experimental pigs with another experimental or *not-experimental* animal were 13.25% and 86.75% in the Standard control group, and 6.25% and 93.75% in the Enriched group, respectively. Therefore, it was much more likely that an experimental pig would have interacted with a *not experimental* subject than with another experimental pig.

With regard to statistical analysis, the day of arrival (Time 0) was considered separately since external variables (i.e. how tired the animals were after travelling) could have confounded results.

The abnormal behaviours *body-biting* (BB), *tail-biting* (TB) and *ear-biting* (EB) were analyzed both separately and together, as the *abnormal behaviours category* (ABC =

Table 1. Description of the six behaviours recorded (Jensen, 1980; Fraser and Broom, 1990).

Behaviour	Description
Body-biting	Taking any part of the body of a pen-mate, except tail or ears, into the mouth
Tail-biting	Taking the tail of a pen-mate into the mouth
Ear-biting	Taking an ear of a pen-mate into the mouth
Belly nosing	Repeatedly nudging with snout into the belly of a lying pen-mate
Running	Running or scampering in the pen
Interaction with objects	Taking into the mouth, pushing, pulling, biting, chewing, licking an enrichment

Table 2. Number of scans (%) with Standard (S) and Enriched (E) pigs engaged in the different behaviours over eleven weeks.

Behaviour	Standard/Enriched status								Significance				
	Time 1		Time 2		Time 3		Time 4		Standard/ Enriched status	Sex	Time	Time x Standard/ Enriched status	RSD
	S	E	S	E	S	E	S	E					
ABC	26.98	5.55[a]	17.06	4.76[a]	8.72	5.95	22.22	9.52[a]	***	***	***	**	1.94
BB	6.35	0.79[a]	7.93	1.18[a]	5.16	3.57	13.49	4.76[a]	***	ns	***	**	1.23
TB	0.39[b]	5.16	0[b]	0.79	0.39	0.79	1.18	***	ns	**	***	0.56	
EB	16.66	4.36	3.96	3.57	2.77	1.98	7.93	3.57	***	ns	***	**	1.12
Belly nosing	5.16	2.38	9.44	3.17[a]	8.72	6.72	0	0.79	**	ns	***	ns	0.95

ABC, body-biting + tail-biting + ear-biting; BB, body-biting; TB, tail-biting; EB, ear-biting; S, Standard status; E, Enriched status; ***P≤0.01; **P≤0.05; ns, not significant (P>0.05); RSD, residual standard deviation; [a]S *vs* E P<0.05 within Standard/Enriched status x Time; [b]S *vs* E P<0.01 within Standard/Enriched status x Time.

BB+TB+EB). For all variables, analyses of variance (ANOVA) were performed to detect the effect of Standard/Enriched status, sex and their interaction. Furthermore, ANOVA for repeated measurements (adding the subject effect) was performed to detect changes over time of these factors and their interactions. In the Enriched condition, the factor *type of object* was included in the ANOVA model to detect the use of different objects and its interaction with sex and time. When significant main effects were found, the Tukey test was used for multiple comparisons (Edwards, 1985).

Skin injuries were analyzed using the logistic regression analysis method to evaluate the effect of condition, sex and time on the injuries incurred and on their location on the body of the pig. Significance level was P≤0.05. All statistical analyses were performed using Statview 5.0 (SAS, 2001).

Results

Behavioural measures

Enriched pigs spent significantly less time with abnormal behaviours than Standard pigs. A significant interaction between Standard/Enriched status and time was also found (Table 2). Post-hoc comparisons revealed that Standard pigs showed a significantly higher level of abnormal behaviours than Enriched pigs at Time 1, Time 2 and Time 4 (Table 2). A main effect of condition was found at Time 0 (Standard/Enriched status, P<0.01, *data not shown*).

When considering each behaviour separately, levels of the redirected oral activities significantly changed through time (Behaviour x Time, P<0.01). In particular, body-biting significantly increased during the experimental period (Table 2), but at much lower levels in the Enriched group. As shown in Table 2, Enriched animals showed significantly less body-biting than the Standard group at Time 1, Time 2 and Time 4.

Tail-biting also changed significantly over time, showing an overall reduction. Enriched pigs were significantly less involved in this activity than Standard pigs. Post hoc comparisons show that Enriched animals were significantly less involved in tail-biting at Time 1, Time 2 (Table 2).

Finally, ear-biting was observed at peak levels at Time 1 and Time 4, and was observed much less on the other observation days (Table 2). This reduced profile was also significantly more marked in Enriched pigs than in Standard pigs (Table 2).

With respect to belly nosing, a main effect of time was found (Table 2), with a significant decrease between Time 3 and Time 4. At Time 2, Enriched pigs were markedly less involved in belly nosing than Standard pigs.

Very low levels of running behaviour (running away) were shown by both groups of animals across the whole experimental period (mean percentage 0.2% and 0.5% of scans in Standard and Enriched groups, respectively; *data not shown*). No main effects or significant interactions were found.

Type of enrichment

A main effect of type of enrichment (Enrichment, P<0.05) and of time (Time, P<0.05) were found. Post hoc comparisons showed that significantly more time was spent in interaction with the steel chains than with the plastic balls (Figure 2). Furthermore, the pigs approached the objects much more frequently at Time 2 than at Time 3 (Figure 3).

Results showing the use of single object types introduced across time are reported in Table 3. In particular, a main effect of time in the interaction with hemp ropes was found. At Time 2, pigs were observed being involved in interaction with ropes in approximately 15% of scans, whereas at Time 3 no interaction with ropes was observed (Table 3). With regard to the interaction with steel chains, a difference between Time 2 and Time 3 seemed to appear, although this was not significant. Furthermore, the interest in the plastic balls was high at Time 3, decreasing at Time 4. Finally, the interaction with rubber hoses showed a declining profile up to Time 3.

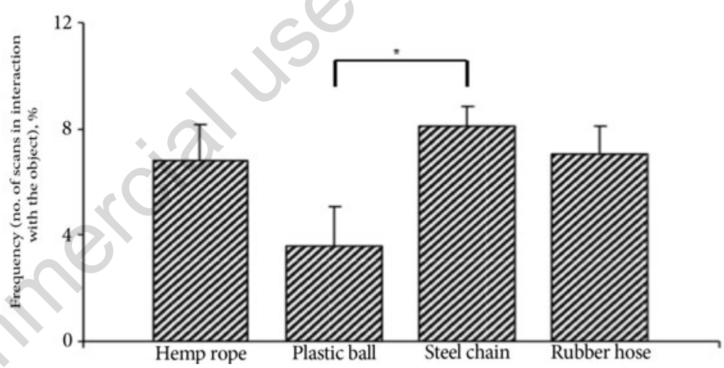

Figure 2. Frequency of interaction with the four enrichment items (mean percentage of scans ± SE) (N=14). Interaction with hemp ropes, plastic balls, steel chains and rubber hoses in the whole experimental period *P<0.05. The percentages were computed as the number of scans in which the pigs were involved in the interaction with the object on total number of scans carried out.

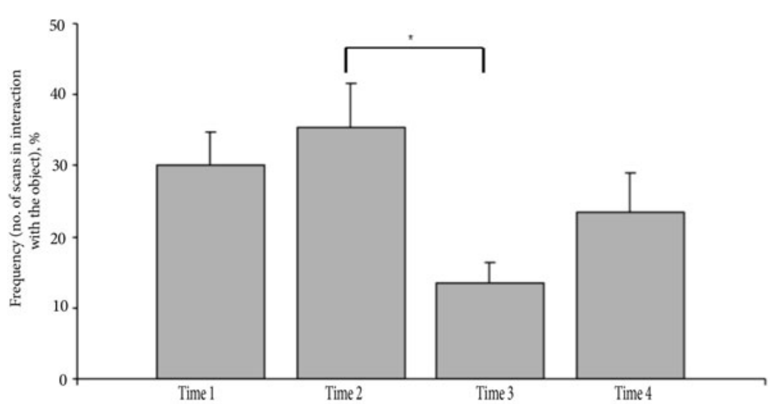

Figure 3. Frequency of interaction with the enrichments across eleven weeks (mean percentage of scans ± SE) (N=14). Interaction with objects after 24 h (Time 1), two (Time 2), six (Time 3) and eleven (Time 4) weeks * P<0.05. The percentages were computed as the number of scans in which the pigs were involved in the interaction with the objects on total number of scans carried out.

Skin injuries

Upon arrival, a total of 7 experimental pigs, which were randomly assigned to the Enriched and Standard pens, presented injuries (3 in Enriched and 4 in Standard pens, respectively). From Time 2 to Time 4, a total of 21 scratches (category 2) and 10 lesions (category 3) were scored. In general, no animal was classified within the most serious category 4. The results are shown in Table 4.

Logistic regression analysis did not provide any evidence that the presence of injuries (scratches + lesions) was a function of the Standard/Enriched status. However, a significant sex difference was found (Table 4). Males had a four times higher probability of presenting injuries than females. A tendency towards higher probability of injury appeared during the interval from Time 0 to Time 2 (P=0.08).

When only the presence of lesions was considered, significant effects of Standard/Enriched status and sex were found. The pigs housed in the absence of enrichments showed a six times higher probability to have lesions on the skin than those of the Enriched group, and males more than females. The presence of lesions significantly increased from Time 0 to Time 3 (Table 4). Furthermore, during Time 3 the Standard pigs showed a higher number of pigs with lesions than Enriched animals (a total of 5 *vs* 2 experimental pigs, respectively). No significant effects on the presence of scratches were found.

With regard to the distribution of the injuries to the body areas (data not shown), no effects of Standard/Enriched status were found. A sex effect was found for the presence of injuries to the head area, with males showing a three times higher probability to present injuries to head and/or neck than females (P<0.05). Furthermore, the presence of injuries in this area significantly increased from Time 0 to Time 2 (five times higher) (P<0.05) and from Time 2 to Time 3 (six times higher) (P<0.05). An increase in injuries in the middle area was found from Time 0 to Time 2 (P<0.05).

Physiological measurements

With respect to serum bactericidal activity, i.e. haptoglobin, lysozyme and basophil levels, no significant effects on Standard/Enriched status were found (Table 5). Levels of hemolytic complement were significantly lower in Enriched pigs than in Standard pigs.

Blood samples from the Enriched group showed lower levels of white blood cells (WBC) than Standard pigs (Table 5). Enriched pigs showed a significantly higher level of neutrophils than Standard pigs, whereas the percentages of circulating eosinophils in the blood samples of the Enriched pigs were significantly lower compared with swine housed in the Standard pen. Interestingly, the Enriched pigs showed a progressive reduction in these white blood cells from Time 2 to Time 4, whereas swine housed in the Standard pen showed an increment between Time 2 and 3. Values of circulating lymphocytes and eosinophils consistently changed across time only in Standard pigs.

All hematologic parameters considered showed significantly lower levels in the samples from the Enriched group than in those from the Standard group, except the concentration of red blood cells (RBC) which showed no difference between groups. In this study, a significant difference in neutrophil-to-

Table 3. Number of scans (± SE) (%) with pigs in interaction with enrichments.

Enrichment	Time 1	Time 2	Time 3	Time 4
Hemp ropes	5.95±2.03	15.07±3.95	0.00	6.35±2.68
Steel chains	7.93±2.13	11.90±3.62	2.77±1.41	9.92±4.10
Plastic balls	3.96±1.48	4.58±1.29	8.10±2.06	2.38±1.12
Rubber hoses	12.33±2.28	5.95±1.70	4.36±1.05	5.55±1.14

Table 4. Logistic model coefficients for skin injuries and lesions.

	Exp (Coef)		95% lower		95% upper	
	Injuries	Lesions	Injuries	Lesions	Injuries	Lesions
Standard	1.460	6.391	0.620	1.082	3.437	37.769
Sex: m	4.277**	6.387*	1.755	1.054	10.426	37.522
Time 2 *vs* 0: yes	2.931	2.718	0.877	2.047	9.794	8.687
Time 3 *vs* 0: yes	1.477	1.688b	0.432	1.088	5.051	2.574
Time 4 *vs* 0: yes	1.000	4.553	0.281	4.483	3.556	4.909

**P<0.01;*P<0.05.

Table 5. Means and significance of the main effect condition and its interaction with time for the immunological, hematologic, oxidative and hormonal parameters.

Item	Standard/ Enriched status S	Standard/ Enriched status E	Standard/ Enriched status	Significance Standard/ Enriched status x time	RSD
Immunological parameters					
Serum bactericidal activity, %	26.68	27.39	ns	ns	9.88
Hemolytic complement, CH 50/100 μL	90.18	78.68	**	ns	22.87
Lysozyme, μg/mL	3.83	3.73	ns	ns	0.80
Haptoglobin, mg/mL	0.86	0.82	ns	ns	0.61
Hematologic parameters					
WBC, x10^3/L	19.98	17.83	**	ns	4.12
Neutrophils, %	36.30	42.42	***	ns	9.50
Lymphocytes, %	57.35	52.82	ns	**	8.77
Eosinophils, %	3.41	2.42	ns	**	1.89
Monocytes, %	2.57	1.94	ns	ns	2.03
Basophils, %	0.35	0.37	ns	ns	0.80
HCT, %	33.31	31.03	**	ns	3.80
RBC, x10^6/L	6.92	6.73	ns	ns	0.70
MCV, fl	48.44	45.57	**	ns	5.66
HGB, g/ld	10.06	9.42	**	ns	1.17
MCH, pg	14.62	13.82	ns	ns	1.76
MCHC, %	30.19	29.80	ns	ns	2.64
Platelets, x10^3/L	470.23	421.66	ns	ns	93.72
Oxidative stress parameters					
N:L ratio	0.683	0.892	**	***	0.140
ROMs, mM	39.89	41.11	ns	ns	5.52
PAO, μM	215.10	211.73	ns	ns	23.40
DHEA-S, μg/mL	0.248	0.238	ns	**	0.12

S, Standard pigs; E, Enriched pigs; ***P≤0.01; **P≤0.05; ns, not significant (P>0.05); RSD, residual standard deviation.

lymphocyte ratio (N:L) was seen between the two groups. Furthermore, there was a significant change in this ratio in the Enriched and Standard pigs through time. Nevertheless, post hoc comparisons showed a significant difference only at Time 1.

There was no significant change in ROM levels of Enriched and Standard groups through the experimental period. An effect of Standard/Enriched status across time was found with regard to DHEA-S values. In general, the DHEA-S values from Enriched pigs were characterized by greater variability across time than those obtained from Standard pigs. Post hoc comparisons found that Enriched pigs showed significantly lower levels of DHEA-S during Time 2 than Time 1.

Discussion

The data from this study give a clear indication that the presence of manipulative objects provides welfare advantages to rearing pigs. Mixing unfamiliar pigs is a stressful event for the animals, which leads to an increase in fighting, abnormal behaviours, and plasma cortisol concentrations (Blecha *et al*., 1985; Moore *et al*., 1994) and a potential reduction in production gains (Tan *et al*., 1991; Stookey and Gonyou, 1994). In this study, after 24 h from the regrouping, the frequency of oral activities redirected towards pen-mates was 5% for Enriched pigs, whereas it reached 27% among pigs housed in the absence of objects. Therefore, the presence of physical objects can represent a valid tool to manage a critical time window for the social dynamic of pigs, routinely presented in the commercial farm.

These results are in apparent contrast with the study by Scott *et al*. (2006), which reported that the presence of straw and a hanging toy in the pens over 12 weeks was not able to affect the levels of behaviour directed at pen-mates. The availability of an increased variety (four types of different objects) and quantity (a total of 19 items in the pen) of enrichment objects may explain the apparent discrepancy. The presence of 4 objects which differed in shape, consistency and pen location, may account for a stimulation of rooting and chewing activities by the animals. Furthermore, the presence of numerous objects may have reduced the competition between the pigs, making the individual interaction with these items easier and safer, therefore, encouraging use.

With regard to levels of interaction with each of the enrichment items, the objects were used by the pigs from 35% after two weeks, to 13% during the sixth week of their daily time budget. These percentages are only partly in accordance with the available literature, reporting lower levels in the use of enrichments. Van de Weerd *et al*. (2003) investigated the use of 74 different objects by weaner and grower pigs, reporting a daily interaction level of about 5% after five days. With a more popular enrichment, such as straw, 20% of the day spent with the item was observed (de Jong *et al*., 1998).

Furthermore, we found that pigs switched their interest to the different objects throughout the study, showing an initial preference for rubber hoses lying on the ground, and then for hemp ropes hanging from the ceiling, while animals maintained a constant interest in hanging steel chains inserted in rubber hoses. The general preference for bitable and changeable material, such as rubber hose and hemp rope, appears to agree with other studies (Hill *et al*., 1998; Van de Weerd *et al*., 2003; Studnitz *et al*., 2007). Instead, the interest in interacting with steel chains inserted in rubber hoses may be explained by the heterogeneity (steel and rubber together) and complexity of the enrichment as a whole. Also, the possibility of a sensory feedback component, such as a *fresh* sensation obtained from biting steel, cannot be excluded and may have a particularly relevant role in the hot summer season. Another important factor in enrichment preference could be the age of animals; indeed the motivation to interact with specific enrichments has been found to be related to the age of the pigs (Grandin and Curtis, 1984; Hill *et al*., 1998). In this study, it is possible that the shift in the enrichment-item preference can be associated with the fact that animals gain the ability to chew and destroy the objects with age. In fact, the rubber hoses laid on the floor are easily available for the pigs from around three months of age, while the ropes hung from the ceiling can likely be accessible only when the animals have grown. Furthermore, the progressive increase in the ratio of body size to space allowance may make hung objects more suitable than objects lying on the floor.

Interestingly, high levels of interaction (up to 15%) were seen with the hemp ropes after two weeks. In general terms, this was the time at which interaction with enrichments reached its peak (up to 35% of observations). This result should be considered along with the absence of tail-biting events in the Enriched pen. In contrast, incidences of tail-biting were highest in the Standard pen. The destructibility of the ropes, associated with the elasticity provided by the extension springs located on the top, may have solicited a strong interest from the pigs.

However, it should be noted that interest towards the objects decreased with time. The need to find novel objects to stimulate the animals' interest or the failure to use the enrichments, because of weight gain and associated animal density in the pen, are two possible explanations. These two factors could have also affected the general increase in the occurrence of bites on the body of pen-mates across time. In particular, the increase in body size and the correlated reduction of available space per individual may have played a role in making the head and back of pen-mates more easily accessible than other body areas.

Many authors consider belly nosing to be mainly due to a lack of maternal contact caused by early weaning (e.g. between 7 and 21 days of age) (Gonyou *et al*., 1998; Worobec and Duncan, 1999) and this can explain the fact that incidences of this behaviour decreased over time. Nevertheless, in this study the pigs were weaned at four weeks. Therefore, incidences of this behaviour could also be due to other factors. Belly nosing is considered an element of the fighting behaviour of the wild pig (Beuerle, 1975). Moreover, a dominant-subordinate dynamic represented by abdominal nudging or belly rooting has been described as *normal* behaviour (LAREF, 2008). In this study, the term *belly nosing* is taken to mean a repetitive nudging of the abdomen of a lying sleeping pig from one standing or lying pig. Most of the time, the receiver remains passive. Sometimes, when the nudging become a little more insistent and the belly gets lifted, the receiver lifts up the head and shams a bite. The absence of bites and the lying position of the receiver would exclude the interpretation of the belly nosing as a fighting behaviour. The increase in belly nosing over six weeks seems to be in contrast with the hypothesis of a dominant-subordinate relationship, which is the social relationship commonly established in groups of swine within approximately 48 h of mixing (McGlone, 1986). Furthermore, the Enriched pigs showed very low levels of this behaviour after two weeks, corresponding to the peak of interaction with the environmental enrichments. These findings seem to suggest that the animals, once objects were available, appeared less interested in nudging pen-mates' abdomens. From the sixth week of observation, a reduction in pushes was observed; it should be noted that the progressive gain in body weight limited the space allowance, and therefore also limited the possibility for the animals to behave in this way.

Regarding skin injuries, the absence of deep lesions may suggest a low level of aggressiveness in this genetic line of pigs. A redirected behaviour of biting the objects introduced in the pen may explain the strongly reduced probability of lesions among the Enriched pigs. Furthermore, the numerous scratches may be partly due to the animals rubbing on the metallic door and feeders in the pens.

WBC count revealed an evident leukocitosis status (>20,000 cell/mm^3×10^9/L) in about 40% of the pigs on arrival, basically irrespective of group allocation. Within WBC parameters, the leukocyte formula revealed also a reduction of the granulocytes (<35%) (Nemi, 1993), accompanied by a correspondent rise in lymphocyte count (>65%); this rise was very apparent on arrival and gradually re-balanced by the sixth week. There was a clear change in the leukocyte formula over the eleven weeks; the increase in the presence of lesions at Time 3 could explain this event. Indeed, it should be noted that changes in this immunological parameter were less relevant in the Enriched pigs which showed a consistently lower number of lesions than Standard pigs.

Therefore, such findings may be explained as a consequence of ongoing bacterial infections, such as enzootic subclinical pneumonia typical of intensive pig farming systems, as well as of skin injuries due to the unfavorable ratio of body mass to space allowances (Pallares *et al*., 2008).

Some other significant differences between the two groups of animals were seen in the other blood and immunological parameters. A significantly lower value of the Complement activity in the Enriched group (78 *vs* 90) would suggest the presence of sub-clinical inflammatory processes. For eosinophils (2 *vs* 3), the differences noted may be reasonably ascribed to a different parasitic status on an individual basis (Evans, 2006).

Higher levels of haptoglobin found in Standard pigs with respect to Enriched animals at Time 2 would seem to agree with high levels of abnormal behaviours shown by the Standard group after two weeks of mixing. In fact, both these parameters are commonly considered physiological and behavioural reactions to stressful conditions, respectively (Smulders *et al*., 2006; Candiani *et al*., 2008). Therefore, a particularly high presence of acute phase proteins can indicate unsuccessful attempts by Standard pigs to cope with discomfort. Conversely, Enriched pigs could have dealt with stress situations more efficiently. There was only a slight difference in RBC parameters among the two groups, with Enriched pigs again showing a significantly lower value of HTC (31 *vs* 33), MCV (45 *vs* 48) and HGB (9 *vs* 10) with respect to Standard pigs. However, the levels recorded all fall within the range reported in intensively farmed pigs in the literature (Nemi, 1993) and may be interpreted as a small disturbance induced by the ongoing sub-clinical inflammatory processes reported above.

With regard to oxidative stress parameters, we can infer that genetic selection for lean pigs has caused the appearance of some undesirable traits which are likely to worsen the process of adaptation to modern husbandry techniques. The percent weight of the heart muscle has decreased from 0.38% in wild boars to 0.21% in modern Landrace pigs (Brambilla *et al*., 2002). The resulting tissue hypoxia induces conditions of persistent oxidative stress response which paves the way to serious clinical conditions like Mulberry Heart Disease (Brambilla *et al*., 2002). In fact, lean muscle pigs show abnormally high serum concentrations of reactive oxygen metabolites (ROMs) compared to rural swine (Brambilla *et al*., 2002). In our research, the highest ROM levels were noted especially on arrival in both groups, at the same time as a rise in a coping anti-oxidant response (Ballerini *et al*., 2003).

On a time basis, a general trend in the rise of DHEA level has been noted in females, irrespective of enrichment. In contrast, the presence of the physical enrichments could seem to have an effect on DHEA levels in males across the first two weeks. Nevertheless, the large number of sera with threshold levels did not allow for a sound correlation of DHEA levels with the effects of the enrichment. A more rigorous evaluation of DHEA level in pig sera should allow an appropriate correlation with cortisol levels, as far as an inverse correlation of these two parameters is well consolidated in the scientific literature as a consequence of chronic stress situations. In our case, we did not consider cortisol determination in sera because it probably interfered with acute stress situations determined by animal handling.

Conclusions

As suggested by the British Department for Environment, Food and Rural Affairs, in situations in which a substrate material cannot be provided, enrichment objects should be offered instead (Defra Code of Recommendations for the Welfare of Livestock: Pigs, 2003). The findings of this study show the successful provision of appropriate enrichments encourage behaviours which may entice the animal to carry out satisfactory oral activities on these objects, preventing them biting pen-mates. In this respect, the objects proposed were strongly effective in producing changes in behaviour which could mitigate inadequate conditions, such as the relationship between animal body weight and the available space allowance. Within this frame, the outcomes from our study support the reliability of the minimal space requirements set by the EU Directive 2001/93 to prevent stressful and associated abnormal behavioural conditions in intensive pig farming.

Furthermore, adequate conditions for farm animals can be guaranteed by making available a suitable routine procedure to farmers to assess their animals' welfare levels. Monitoring of selected behavioural parameters (i.e. biting of tails and ears), together with skin lesion checks, may be a user-friendly management strategy on farms with elevated animal density and limited numbers of workers. Further studies are ongoing to better understand the relationship between behaviour, and hormonal and genetic traits of the commercial line of pigs considered in this study.

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