Effects of stocking density and environmental enrichment on behavior and fecal corticosteroid levels of pigs under commercial farm conditions

DOI:10.1016/j.jveb.2015.05.002

(Article begins on next page)

Effects of stocking density and environmental enrichment on behavior and fecal corticosteroids levels of pigs under commercial farm conditions

Paolo Cornale, Elisabetta Macchi, Silvia Miretti, Manuela Renna, Carola Lussiana, Giovanni Perona, Antonio Mimosi

PII: S1558-7878(15)00061-1

DOI: 10.1016/j.jveb.2015.05.002

Reference: JVEB 889

To appear in: *Journal of Veterinary Behavior*

Received Date: 7 July 2014

Revised Date: 14 May 2015

Accepted Date: 16 May 2015

Effects of stocking density and environmental enrichment on behavior and fecal corticosteroids levels of pigs under commercial farm conditions

Paolo Cornale[a], Elisabetta Macchi[b], Silvia Miretti[b], Manuela Renna[a], Carola Lussiana[a], Giovanni Perona[b], Antonio Mimosi[a]

Affiliations: [a]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italy; [b]Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Grugliasco (TO), Italy

E-mail addresses: paolo.cornale@unito.it, elisabetta.macchi@unito.it, silvia.miretti@unito.it, manuela.renna@unito.it, lussiana.carola@unito.it, giovanni.perona@unito.it, antonio.mimosi@unito.it

Corresponding author: Paolo Cornale, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Largo Paolo Braccini, 2 - Grugliasco (Torino) 10095, Italy, +39 011 6708576, +39 011 6708563, paolo.cornale@unito.it

ABSTRACT

In intensive pig farming of Western and Southern Europe, welfare concerns are still often related to barren environments and crowded conditions. Pig producers need to balance the requirements to improve welfare conditions at farm with practical considerations. The aim of this study was to determine the extent to which the reduction of stocking density and the provision of suspended pieces of hard wood as environmental enrichment have an influence on both behavior and fecal corticosteroids concentration in commercially housed growing-finishing pigs. A total of 640 growing pigs were arranged in a 2 × 2 factorial design with stocking density (high, 1.0 m^2/pig and low, 1.5 m^2/pig) and environmental enrichment (yes or no) as factors. Ten replicate pens were allocated to each treatment. Observations of behavior (instantaneous scan sampling) were made in each pen at 2-week intervals, when pigs were from 15 to 31 weeks of age. Fecal samples were collected to determine corticosteroids concentration in the 40 selected pens. The pigs housed in low density showed a higher (although not significant) level of exploration of pen furniture than crowded pigs (10.11% vs. 8.53%, respectively; P = 0.09). Similarly, social interactions were observed more frequently (P ≤ 0.001) among the pigs in the enriched (10.27%) than in barren (6.69%) pens. The pigs housed in barren pens had greater incidences of tail biting (barren: 1.35%, enriched: 0.42%; P ≤ 0.01) and aggression (barren: 1.30%, enriched 0.61%; P ≤ 0.05). Crowded pig in barren pens spent less time moving (2.26%) compared to other treatments. Uncrowded pig in enriched pens spent less time lying inactive (43.97%) and feeding (14.48%) compared to other treatments. Fecal corticosteroids doubled their concentration from the first (56.74 ng/g) to the last (108.10 ng/g) sampling date (P ≤ 0.001). The crowded pigs showed higher (P ≤ 0.001) concentration than the pigs housed in low stocking density (85.09 ng/g and 76.08 ng/g, respectively). No differences were found in corticosteroids concentration between the pigs housed in barren and enriched pens. To conclude, the reduction of stocking density modified the pigs behaviors and reduced the fecal corticosteroids levels, highlighting an improvement of welfare conditions. The provision of suspended pieces of hard wood modified the pigs behaviors, but did not exert relevant effects on fecal corticosteroid levels.

Key Words: stocking density, environmental enrichment, fecal cortisol, behavior, welfare, pig

INTRODUCTION

In the last decades, application of technological innovations in agricultural sectors and in animal production has led to more and more specialized techniques. However, the derived beneficial aspects in terms of productivity have often been obtained at the expense of behavioral needs and welfare of kept animals. The majority of growing-finishing pigs reared in Southern and Western Europe are housed according to intensive farming conditions and predominantly held in a barren environment. These environmental conditions limit the expression of their species-specific behaviors (De Jonge et al., 1996; Edwards, 2010). Two of the most important welfare concerns are related to high stocking densities and restriction of social and locomotory activities. A reduction of space allowance has been associated with a decline of production, a worsening of health status, and an increase in stressful and uncomfortable conditions for the animals (Barnett, 2007). In growing and finishing pigs, a reduction of space allowance is usually responsible for a decline of feed efficiency and a worsening of weight gain (EFSA, 2005). Moreover, increasing level of aggression, reducing exploratory activities and abnormal behaviors (e.g., tail and ear biting) can be observed while increasing stocking density (Hörning, 2007).

Similarly, increases in behaviors like nosing and chewing penmates as well as in the level of aggression have been shown in growing pigs reared in a barren environment (Beattie at al., 2000). Commercially farming systems usually provide minimal stimulation to the animals. Different types of environmental enrichments have consequently been proposed to ameliorate the welfare conditions of intensive kept animals (Newberry, 1995). Concerning pigs, the European Union (EU) legislation recognizes their needs to explore and manipulate, and requires the use of materials that allow them to perform these activities (EU Directive 2008/120/EC). Among the proposed enrichments, straw seems to be very effective in providing new stimuli for pigs allowing the containment of harmful social behaviors such as tail and ear biting (Bracke et al., 2006). However, the proposed solutions or even law’s requirements are not always easily applicable in commercial production systems. For example, straw and other substrates (e.g., woodshavings, mushrooms compost, peat, etc.) suggested as environmental enrichments are not often compatible with most of the commercial pig farms in Southern and Western Europe, where the use of slatted or partly slatted floor is still prevalent, since their use would necessarily require substantial structural and operational changes to manure handling systems. For this reason, in commercial housing systems the use of alternative point-sources enrichments (e.g., chains, plastic balls, rubber tyres, etc.) has been tested (van de Weerd and Day, 2009). Nowadays it's recognized that a successful enrichment should be ingestible, destructible, deformable, chewable, and 'non-routable'.

Together with behavioral analysis and other animal-based parameters (e.g., body conditions, injuries, etc.), physiological measurements (i.e., hormonal) are also of particular value in welfare assessment (Möstl and Palme, 2002). The hypothalamus-pituitary-adrenal (HPA) axis is activated when animals react to stressful events (Moberg, 2000). The activation leads to, among other things, an increased synthesis and release into the circulation of glucocorticosteroids (Woodman, 1997). Quantification of glucocorticosteroids in blood unfortunately requires capture, restraint and blood sampling. These tasks on animals result in a rapid release of corticosteroids into the circulation making hormone blood levels of little use in chronic stress studies. Consequently, during the past decade there have been increased efforts to develop non-invasive sampling methods for corticosteroids and their metabolites quantification in secreted or excreted material. Hormonal consequences of stressful conditions have been studied in pigs (Mormède et al., 2007) and van de Weerd and Day (2009) reported some studies concerning the effect of environmental enrichments on cortisol level in pigs. However, analyses of the fecal concentration of corticosteroids and their metabolites as a mean to non-invasively assess animal welfare have been poorly studied in this species (Palme, 2012).

Pig producers must balance the requirements to provide appropriate welfare improvements with practical considerations: applicability in commercial practice, cost implications, impact on performance and product quality, etc. The aim of the present study was therefore to determine if a reduction of stocking density and the introduction of suspended pieces of hard wood as environmental enrichment may affect behavior and fecal corticosteroids concentration in growing-finishing pigs under commercial farm conditions.

MATERIALS AND METHODS

All procedures and treatments were in compliance with the ethical guidelines of the International Society for Applied Ethology (ISAE, 2002) and with the European Directives (2001/88/EC and 2001/93/EC) on the minimum standards for the protection of pigs.

Animals and housing

The study was held at a commercial pig unit for growers and finishers located in N-W Italy (latitude: 44° 43' 28" N; longitude: 7° 48' 34" E; altitude: 545 m a.s.l.) from May to October 2012. A total of 968 [(Landrace × Yorkshire) × Duroc] hybrid pigs of both sexes (females and castrated males) were initially enrolled in the experiment. Due to the large number, the pigs were acquired in two batches from the same supplier. The pigs were previously reared under the same conditions. Briefly, all pigs were teeth clipped and partially tail docked at approximately 3d of age; afterwards, they were weaned at 3 weeks of age. Prior to being enrolled in the experiment, the pigs were exposed to the same transport. At farm entry, pigs were 13 weeks of age with an average weight of 25±1.2 kg.

Animals were housed in two adjacent buildings. Each building consisted of one single room containing 27 (+1 hospital pen) and 35 (+1 hospital pen) pens, respectively, equally distributed at each side of a central corridor. The pens measured 2.98 m × 6.63 m and they were equipped with concrete slatted floors, except for the feeding area, which was equipped with solid concrete floor. Pens partitions, made of concrete blocks, were fenced to allow visual contact among pigs in adjacent pens. The two buildings were equipped with an automatically controlled natural ventilation system: adjusting the inlet and outlet vents regulated the natural airflow through the buildings. Natural lighting was sufficient during the whole experimental procedures. Artificial light was mainly used only during husbandry tasks and to provide at least a light period of 12 h per day.

Pigs in both houses received the same dry pelleted diets (from 13 to 17 weeks of age: 19.0% crude protein, 5.2% crude fiber, 1.1% lysine, 13.3 MJ of digestible energy (DE)/kg; from 17 weeks of age until slaughter: 17.8% crude protein, 4.6% crude fiber, 1.1% lysine, 13.4 MJ DE/kg). Diets were automatically provided *ad libitum* every day at morning (approximately at 7 am) in a multiple space dry feeder. Water was freely available from two nipple drinkers per pen.

All pigs were vaccinated against Aujeszky's disease according to laws prescription (Italian Ministry of Health, 1997).

Experimental treatments

When growing pigs arrived at farm, they were randomly divided into the two buildings. The pigs of the first batch were housed in the first building. This building had space for 513 pigs, consisting of 27 pens housing 19 animals each. The resulting stocking density was equal to 1.0 m^2/pig (high stocking density, HD). Currently, it represents the EU minimum space allowance for pigs over 110 kg live weight (EU Directive 2008/120/EC). The pigs of the second batch arrived at farm one week after the first batch and they were housed in the second building. This building had spaces for the remaining 455 pigs, consisting of 35 pens housing 13 animals each. In this case, the stocking density was equal to 1.5 m^2/pig (low stocking density, LD). This value is usually indicated in organic pig production as the maximum stocking density in indoor housing (IFOAM, 2005).

Whilst half of the pens in both buildings were kept in their original configuration (barren pens, BP), the other half was equipped with an environmental enrichment (enriched pens, EP). The enrichments were realized on-farm and consisted of a cylindrical piece of hard wood (*Robinia pseudoacacia* L.) suspended from its center on a chain at pig head level. The wooden pieces were 35 cm in length and had a diameter of 6 to 10 cm. The wooden pieces were replaced once during the experimental period depending on their wear. Each pen was equipped with two environmental enrichments, placed on each side of the pen partitions.

External pens (in the corners of the buildings) as well as hospital pens were excluded from the selection procedure. Of the remaining 54 pens, 40 were randomly selected and followed during the experimental period (20 weeks). The percentage of males to females was similar in each pen and did not vary across treatment. The selected pens were arranged in a 2 × 2 factorial design with 10 replications (pens) each: high density – barren pen (HD-BP), high density – enriched pen (HD-EP), low density – barren pen (LD-BP), and low density – enriched pen (LD-EP). Therefore, a total of 640 pigs were involved in the experimental measurements.

Data collection

During the first two weeks after entry, the pigs were allowed to overcome the transport's stress, and to habituate in the new surrounding and groups formation. Since pigs housed in LD building arrived at farm with one-week interval than the pigs housed in HD building, data collection in the two buildings was carried out on alternate weeks to ensure that pigs were at the same age when data were collected. The same observers assessed all the pens. When a pig was removed from one of the selected pens due to healthy problems or severe injuries, no replacements were made to avoid disruption of the social structure within the groups. However, a pig's removal from a pen determined a variation in the experimental density. Therefore, at each sampling date, only pens with the initial stocking density (19 animals in HD and 13 animals in LD) were considered in the subsequent statistical analysis.

Behavioral measurements

Behavioral observations were carried out when pigs were from 15 to 31 weeks of age. Instantaneous scan sampling of each pen was performed to determine the number of pigs performing each activity provided in the predetermined ethogram (Table 1) adapted from Guy et al. (2002a) and van de Weerd et al. (2006). The observer recorded the pigs' activities from outside the pen. During the experimental period, behaviors were recorded at 2-week intervals for 9 times, one day per each selected week. Pigs were observed during 3 periods (at 9 am, 11 am, and 1 pm) each observation day. Scan samples were repeated three times in the each period with a 10-minute interval. All considered behavioral activities were mutually exclusive.

Measurements of fecal corticosteroids concentration

Feces collection was carried out at 2-week intervals and was always scheduled the day before the behavioral measurements to avoid that other experimental tasks could affect corticosteroids concentrations. For the determination of baseline fecal corticosteroids concentration (FCC) of each pen, fecal samples were collected twice when pigs were 14 weeks of age.

FCC in pigs as an index of circulating cortisol has a 48-hour time lag to extraction (Möstl et al., 1999). The distribution of corticosteroids concentration in pig's feces is not homogeneous and thus the whole sample has to be collected and subsequently homogenized prior to assay (Carlsson et al., 2007). After defecation, feces were sampled from the bedding and immediately refrigerated to be transported to the laboratory, where samples were thawed at –20°C until analysis.

To extract steroids from nonliquid matrices (such as dried solids) feces were subjected to an organic phase extraction using ethanol; the use of ethanol is recommended as a mean to completely solubilize the dried steroid because certain steroids have limited aqueous solubility (Cook, 2012).

Extraction and determination of corticosteroids in the feces were carried out as previously reported by Prola et al. (2013). Briefly, fecal samples were kiln dried at 55°C for 24 h, thoroughly crushed, and five aliquots of pulverized feces (0.20 g each) were put into extraction tubes, which were then sealed with a Teflon cap. Next, 1 mL of ethanol (Sigma Aldrich, St. Louis, MO, USA) for every 0.1 g of solid was added to each tube, and the mixture was shaken vigorously for 30 min. Samples were centrifuged at 3,300 × g for 15 min, and the supernatant recovered in a clean tube for evaporation to dryness in a SpeedVac (ThermoFisher Scientific, Waltham, MA, USA). Extracts were stored at –80°C. Extracted samples were dissolved into 100 µL ethanol followed by at least 400 µL of kit Assay Buffer (Arbor Assays, Ann Arbor, MI, USA), then they were vortexed and rested for 5 min twice to ensure complete steroid solubility. FCCs were determined using a pan-specific cortisol enzyme immunoassay kit (K003; Arbor Assays, Ann Arbor, MI, USA) validated for dried fecal extracts. All analyses were repeated twice. It is uncertain to which extent native molecules and immunoreactive metabolites of cortisol were quantified in the kit used. Consequently we have used the terminology fecal corticosteroid concentration (FCC). Inter- and intra-assay coefficients of variation were less than 10%. The test's sensitivity was determined by measuring the least amount of hormone standard consistently distinguishable from the zero concentration standard and was calculated to be 17.3 pg/mL.

According to the manufacturer, the cortisol kit presents the following cross reactivity: 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisolone, 1.2% with corticosterone and 1.2% with cortisone. Serial dilutions (1:4, 1:8, 1:16, and 1:32) of fecal samples were assayed to test for parallelism against the standard curve ($P < 0.05$ for all assays). The mean recovery rate of cortisol added to dried feces was 96.7%.

Statistical analyses

For all the data analyses, the pen was the experimental unit. The pen was treated as a random effect and nested within treatment. Data were analyzed as repeated measures mixed models (REML) in SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA) with stocking density (D), environmental enrichment (E), and their interaction (D×E) as fixed effects. While analyzing FCC, age of animals was also considered as fixed effect. Concerning behavior measurements, data were first collated and percentage of each behavioral activity of the ethogram was expressed as ratio of the total number of observations for the three observation moments of the day. Normality of residuals was checked with graphical methods and Kolmogorov-Smirnov test. Data, with the exception of 'Lying' behavior and FCC, were subjected to LOGIT transformation to meet the assumptions of REML (homogeneity of variance, normality of error and linearity), and then reanalyzed. Significance was declared at $P \leq 0.05$, and statistical trend are considered as $P < 0.10$. Results of statistical analysis are reported as estimate least-squares means. Results are always presented as untransformed data.

RESULTS

Behavioral activities

Table 2 presents the frequencies of the considered behavioral activities. The mounting behavior was not analyzed because it was seen very rarely. The pigs spent the majority (>50%) of the observation time lying on the floor pens. The second most observed behavior was feeding activity, followed by exploration of pen furniture and social interactions. The incidence of the other considered behaviors was under the 5% of the observation time for scan samples.

The overall effect of stocking density showed a tendency just on exploration of pen furniture. Difference in the percentages of exploring pen between the two stocking density treatments approached significance (HD: 8.53%, LD: 10.11%; $P = 0.09$).

The presence of environmental enrichment significantly affected most of the behavioral activities. Pigs in the enriched pens spent more time performing social positive interactions than pigs in barren pens (EP: 10.27%, BP: 6.69%; $P \leq 0.001$). On the other hand, the incidences of tail-biting (BP: 1.35%, EP: 0.42%; $P \leq 0.01$) and aggressive behavior (BP: 1.30%, EP: 0.61%; $P \leq 0.05$) were significantly greater in the pigs housed in the barren pens.

The percentage of time spent moving through the pen was significantly lower in the pigs housed in high density and barren pens (HD-BP) compared to LD treatments, with HD-EP pigs showing an intermediate value between LD treatments and pigs housed in HD-BP. For low density and enriched pens (LD-EP), scan samples of behavior showed that the pigs spent a larger percentage of observation time feeding if compared to all other treatments and lower percentage of observation time lying, although it was not statistically different from the value detected in the enriched pens of high density treatment.

HD and LD pigs spent similar percentages of observed time exploring the environmental enrichment (4.23% and 4.35%, respectively). Stocking density and environmental enrichment did not affect drinking and excreting (plus urinating) activities.

Fecal corticosteroids concentration

Unreliable results were obtained from the samples collected when the pigs were at 21 weeks of age and consequently they were not considered in the statistical analysis.

FCC baseline values were not different among treatments (HD-BP: 50.63 ng/g, HD-EP: 47.78 ng/g, LD-BP: 48.27 ng/g, LD-EP: 47.95 ng/g). Furthermore, the baseline values were not different with the concentration detected at the first sampling date.

Stocking density significantly ($P \leq 0.001$) affected the average level of corticosteroids measured during the whole experimental period. In fact, while there were no differences in FCC between the pigs housed in barren and enriched pens (82.03 ng/g and 79.14 ng/g, respectively), the pigs housed with high stocking density showed higher FCC (85.09 ng/g) if compared to the pigs housed in low stocking density pens (76.08 ng/g).

Figure 1 shows the FCC variations during the experimental period. FCC significantly ($P \leq 0.001$) increased with the increasing of age and live weight of pigs: FCC at the last sampling date shows almost double value (108.10 ng/g) if compared to the first sampling date (56.74 ng/g). Furthermore, at the end of the experimental period, stocking density shows a significant effect analyzing FCCs at each sampling date. At the second-last sampling date, FCC levels detected in pigs housed in high stocking density (HD-BP: 110.03 ng/g and HD-EP: 112.57 ng/g) were higher ($P \leq 0.01$) if compared to pigs housed in low stocking density (LD-BP: 90.40 ng/g and LD-EP: 85.45 ng/g). Similarly, higher ($P \leq 0.05$) FCCs were detected at the last sampling date in pigs housed in high density pens (HD-BP: 123.59 ng/g and HD-EP: 111.15 ng/g) than in low density pens (LD-BP: 102.06 ng/g and LD-EP: 94.76 ng/g).

DISCUSSION

Behavioral activities

In the present study, since the pen size was constant among treatments, stocking density decreased with increasing group size. Therefore, stocking density and group size effects were confounded, and group size could have affected the obtained results. However, it is worth to point out that several studies (EFSA, 2005; Schmolke et al., 2002; Street et al., 2008; Turner et al., 2003) suggested that the influence of stocking density on pigs productivity and behaviors (e.g., lying, tail biting, social interaction, etc.) seems to be predominant on group size effect. Moreover, the same studies showed that no effects or negligible effects were detected while comparing different group sizes (at the same stocking density), especially if an adequate space allowance is provided to pigs.

In the current study, the pigs housed in HD pens ate less frequently than those housed in LD-BP and LD-EP pens, the latter spending the highest detected level in feeding activity. Similar results were reported by Street and Gonyou (2008). These authors hypothesized that crowded conditions may be responsible for hindering feeder access. The same authors did not ascribe the reduced feeding frequency to higher level of aggression: in fact, an increase in competition at the feeder did not occur in crowded pigs and they observed a lack of difference in injuries prevalence, which are indexes of aggressive behaviors. Furthermore, higher level of aggression would be expected with restricted feeding: Baxter (1985) suggested that pigs in stable groups could be aggressive when there is a feed competition because the resource is limited. Similar considerations reported by Street and Gonyou (2008) might be partially supported by the reduction of moving activity observed in HD-BP pigs of our study. Concerning pig productive performance, no effects of stocking density or environmental enrichment were observed: live weights of pigs at the end of the experimental period were comparable (HD-BP: 158.6 kg; LD-BP: 161.9 kg; HD-EP: 165.2 kg; LD-EP: 165.6 kg). This suggests that the pigs housed in HD pens probably compensated the reduced feeding frequency through longer meals. The same feeding strategy was already reported by Wolter et al. (2000): they suggested that crowded pigs ate fewer but longer meals than uncrowded pigs. More recently, Jensen et al. (2012) expressed similar considerations, concluding that there is no evidence that productivity can be improved by increasing space allowance of finishing pigs.

Concerning the effect of stocking density, we detected a tendency on exploration of pen furniture, with the pigs housed in low density showing a higher explorative level than crowded pigs. Our results did not support previous results indicating that an increased space *per se* without enrichment causes a reduction in locomotory and exploratory activities (Whittaker et al., 2012). However, our study confirms the conclusion of the same authors: enrichment plays a greater role in modifying behavior that space allocation did.

As suggested by Newberry (1995), an environmental enrichment represents any modification of a barren environment aiming at improving biological functioning of captive animals. A variety of studies exist on the effect of environmental enrichments in pigs' behavior and welfare (see the review of van de Weerd and Day, 2009). As already discussed above, although straw bedding has the highest potential to meet the criteria that define a successful enrichment, it does not apply to the majority of pig farms due to the incompatibility with current liquid-slurry handling systems. For this specific reason, marginal or point-source enrichments have been tested. In the present study, the pigs housed in the enriched pens showed more active behaviors (e.g., exploring, interacting, moving, etc.) than the pigs housed in barren environment. However, only explorative behaviors towards penmates were statistically different between barren and enriched housed pigs. Similar results were reported by Guy et al. (2002b); in the same study, the pigs with an enrichment object in their pen also exhibited more positive social interactions. Furthermore, some recent studies (Tönepöhl et al., 2012; Telkänrantaa et al., 2014) highlighted that the provision of point-source objects as minimal environmental enrichments in pigs could increase the level of overall activity if compared to pigs housed in barren conditions. The results of our trial and those of the above mentioned studies seem to contrast with the hypothesis that pigs reared in barren environments have elevated level of motivation to explore and interact in comparison to pigs reared in enriched pens (Stolba and Wood-Gush, 1980). However, an explanation to these different results might be provided by the diversity and the amount of enrichment used. As reported by van de Weerd et al. (2006), one of the main consequences of providing objects as environmental enrichments is that pigs can easily lose interest on them. In this case, the level of exploratory motivation decreases as pigs become familiar and they can redirect inappropriate stimuli towards penmates. Our results suggest that the provided enrichment is effective, since 'negative' behaviors (i.e., aggressive behavior and tail biting) were less performed by the pigs housed in the enriched pens.

Levels of aggression available in the literature are highly variable. There are several factors that can affect the level of aggression in pigs. Pigs are social animals and their social groups are based upon dominance hierarchy. It is commonly agreed that, when unfamiliar pigs are brought together, the formation of a hierarchy order is establish within 24 or 48 hours (Deen, 2010). Samarakone and Gonyou (2007) tested difference in productivity and aggression between group sizes of 18 and 108 pigs per pen. 'Social negative' behaviors (including aggression and tail biting) did not differ between the two groups, but they progressively decreased over the following 48 hours after group formation: the percentage of time spent fighting varied from 3.5-4.5%, detected at group formation, to 1.0-1.5% after two days. Although finishing pigs are usually in stable social groups, there are still several factors that can affect their aggression level: breed, sex, amount and quality of available space, amount of feed and feeding distribution, etc. (Deen, 2010). In an extensive study concerning the application of the Welfare Quality® in growing pigs housed in intensive conditions (Temple et al., 2011), the authors detected an averaged level of "negative social interaction" equal to 3.6% of all pigs behaviors. Mattiello et al. (2003) observed behaviors of heavy pigs reared at different space allowances in three housing systems. They found an overall level of 'social negative' interaction around 2-3% of total observed behaviors. On the other hand, the levels of aggression detected in our study are higher than results previously published in other comparable studies. For example, Bolhuis et al. (2006) studied the effects of rearing and housing environment on behavior of finishing pigs. Examining the results of the "barren" pens only, the aggression levels ranged from 0.05 to 0.38% of the observed behaviors. More recently, Camerlink et al. (2012) detected mean level of aggression equal to 0.18% of observation time in finishing pigs. As the authors suggested, the stable situation and to avoid mixing unfamiliar pigs helped that aggression hardly occurred in their study. On the basis of the above-mentioned data, our results are in line with other studies. The experimental conditions might have contributed to maintain this level of aggression among pigs.

The activity of enrichment exploration was not influenced by stocking density; similar results were obtained in a previous trial where pen size was not found to influence toy use (Apple and Craig, 1992).

Recently, Tönepöhl et al. (2012) firstly used a piece of wood on a chain as environmental enrichment for pigs. Pigs housed in the enriched pens were allowed to manipulate either a plastic star on a chain or a piece of wood on a chain. These authors reported that pigs in enriched pens were less inactive and even only point-source enrichments may exert positive effects on animal welfare. Similar conclusions have been more recently reported by Telkänranta et al. (2014) that also supported that suspended pieces of wood may be promising environmental enrichments for pigs.

Fecal corticosteroids concentration

Limited available spaces as well as barren environments were widely shown to adversely affect adrenocortical hormones, with consequent well-being reduction (SVC, 1997; Möstl et al., 1999). The concentration of cortisol in blood depends on the species: pigs showed baseline levels ten times higher than cows, and more than twice higher in response to a stressor (Mormède et al., 2007). Furthermore, the same authors outlined that it is sufficient to expose a pig to a novel environment to significantly increase blood cortisol. Whittaker at al. (2012) reviewed the effect of space on pig's welfare. They reported that gilts housed in group with low space allowance (1 m^2) showed increased plasma corticosteroids concentration compared to groups with higher space allowance (2 and 3 m^2), with consequent negative effects on reproductive performance. van de Weerd and Day (2009) reported that, while higher levels of plasma cortisol were shown in pigs housed in crowded pens compared with uncrowned ones, there was no difference in plasma cortisol concentrations between enriched and barren pens. This is in agreement with the results obtained in the present study on fecal corticosteroids.

By contrast, unchanged levels of basal free cortisol concentration were reported in fattening pigs housed in pens with different space allowance, and even lower levels were detected in gilts with reduced space compared to control group (Mormède et al., 2007). More recently, Marco-Ramell et al. (2011) compared physiological parameters of pigs housed at different stocking densities. Differently from what we detected, these authors observed that serum cortisol was not altered in higher density (0.25 m^2/pig vs. 0.50 m^2/pig) but it is worth mentioning that pigs were involved in a quite short trial (i.e., 26 days).

However, available results on the effects of enrichment objects in pigs are still unclear (van de Weerd and Day, 2009).

Assessments of corticosteroids, their metabolites, and other stress sensitive molecules in feces are increasingly used to monitor the stress of animals (Cook, 2012). Besides the added advantage of allowing non-invasive and easy sampling, the analysis of these compounds in feces can be a particularly useful indicator of chronic, long-term stress since they provide an estimation of cortisol secreted during a time period rather than a point value detected in blood samples (Millspaugh and Washburn, 2004). As reported by Palme (2012), in the last decade an increasing literature has been carried out on fecal cortisol/corticosterone metabolites measurement in farmed animals; however, very few studies investigated it on pigs.

Cortisol metabolites in cattle feces were shown to increase after transport and after adrenocorticotropic hormone administration (Palme et al., 1999). Similarly, Lexen et al. (2008) concluded that the measurement of fecal cortisol metabolites could be used as a parameter to monitor adrenocortical activity in sheep during shearing and transport. The use of fecal cortisol to assess stress levels over long-term conditions in horses was also suggested by Hughes et al. (2010). A reduced level of fecal corticoid metabolites in mink observed during nine months was detected in the presence of increased environmental complexity (occupational materials) (Hansen et al., 2007).

Royo et al. (2005) published one of the few papers on fecal cortisol in pigs, studying the effect of repeated housing in metabolic cages on fecal excretion of cortisol. Cortisol level increased in feces at the first stay in metabolic cage, but not in the following visits. The authors suggested that fecal cortisol could be used as a measure of acute stress.

To the best of our knowledge, it is the first time that the assessment of fecal corticosteroids levels has been used to evaluate long-term stress in pigs under commercial farm conditions. Our results on fecal corticosteroids confirm those previously reported on plasma cortisol, which appeared to be unaffected by enrichment objects (van De Weerd and Day, 2009).

The results of the presents study seem to suggest that an increasing stocking density strongly affects fecal corticosteroids concentration and modified some behavioral activities of growing-finishing pigs. On the other side, the provision of point-source enrichment-objects seems to affect pig's behaviors but not the corticosteroids concentrations in feces. A possible answer to such difference may be found in the extremely complex mechanisms that regulate the overall response to stress at the physiological, hormonal, and behavioral level.

Any change, event, or modification in the rearing environment represents external stimuli for animal. The organism responds to the homeostasis's perturbation (i.e., stress) to return system to equilibrium. According to intensity and duration of stimuli, the stress response can be both beneficial and detrimental to the organism. From a hormonal point of view, stress elicits the activation of the HPA axis causing the release of corticosteroids in blood (Mormède et al., 2007). For this reason, corticosteroids plasma levels are used as index of stress. Environmental enrichment induced a rise in plasma corticosteroids concentration in rats (Moncek et al., 2004) and horses (Fureix et al., 2013). However, there is a lack of agreement and knowledge about the effects of environmental enrichment on plasma corticosteroids and there are contrasting results in the available literature. Young (2003) reported a reduction of plasma cortisol among physiological evidences to support that an environmental enrichment works properly. On the contrary, as we already mentioned, van de Weerd and Day (2009) detected no effects of environmental enrichments on plasma cortisol of pigs housed in barren and enriched pens. Therefore, it's difficult to hypothesize a significance and which results we would have obtained in our study by analyzing plasma cortisol. For this reason, as already suggested by many authors (see for example Fureix at al., 2013), we used fecal samples rather than plasma in order to avoid bias caused by sampling procedures and to assess chronic stress.

As expected, differences in behavioral activities were detected between the pigs housed in barren and enriched pens. Therefore, our results confirm what previously reported in literature. Moreover, our results support the hypothesis that the provision of a suspended piece of hard wood is an effective environmental enrichment for growing-finishing pigs.

On the other side, density showed a strong effect on corticosteroids levels of pigs. This is not an unexpected result since the assessment of corticosteroids in pig's feces allows the evaluation of chronic stress (Cook, 2012). In fact, the corticosteroids difference between the pigs housed in the two stocking density increase during the experiment, and it became significant at the end of the productive cycle. Some, but non-negligible, effects of stocking density were also observed on behaviors. The statistical analysis showed an effect of density on exploration of pen furniture. As we already reported in the manuscript, we probably did not detect a significant effect on aggression level due to the experimental conditions (e.g., mixing unfamiliar pigs was avoided).

CONCLUSION

Stocking density and environmental enrichments constitute two aspects that can be modified by pig producers at farm level. In this study, a reduction of stocking density determined modifications in pigs behaviors and a significant reduction in fecal corticosteroids levels, highlighting an improvement of animal welfare conditions. When considering marginal environmental enrichments, the biggest challenge for point-source enrichment objects is to ensure that the enrichments are practical and effective. Suspended pieces of hard wood in the growing-finishing pigs modified their behaviors, but did not exert relevant effects on fecal corticosteroid levels.

Finally, we can conclude that, when considering enrichment and density effects on pig welfare at farm level, it is advantageous to detect simultaneously behavioral and physiological parameters because they may provide different information of the same complex mechanism, and, therefore they may both contribute in the assessment of pig welfare at farm level.

ACKNOWLEDGEMENT

The authors wish to gratefully thank Dr. Ugo Benedetto for his willingness to take part in the project. Maria Mola and Mauro Vissio are also acknowledged for technical assistance and care of animals. All authors contributed to conceive the idea for the paper, design the experiment, perform the study, and collect all the data. Data analysis and manuscript drafting were mainly carried out by the first author, with the help and inputs from all coauthors. The study includes only noninvasive procedures (i.e., behavioral observations of the pigs and feces collection), without any alteration in the regular husbandry and management practices. There was no external funding for this project. We are grateful to anonymous referee and the editor for comments that considerably improved the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Apple, J.K., Craig, J.V., 1992. The influence of pen size on toy preference of growing pigs. Appl. Anim. Behav. Sci. 35, 149-55.

Barnett, J.L., 2007. Effects of confinement and research needs to underpin welfare standards. J. Vet. Behav.: Clin. Appl. Res. 2, 213-218.

Baxter, M.,1985. Social space requirements of pigs. In: Zayan, R. (Ed.), Social Space for Domestic Animals. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp. 116-127.

Beattie, V.E., O'Connel, N.E., Kilpatrick, D.J., Moss, B.W., 2000. Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. Anim. Sci. 70, 443-450.

Bolhuis, J.E., Schouten, W.G.P., Schrama, J.W., Wiegant, V.M., 2006. Effects of rearing and housing environment on behaviour and performance of pigs with different coping characteristics. Appl. Anim. Behav. Sci. 101, 68-85.

Bracke, M.B.M., Zonderland, J.J., Lenskens, P., Schouten, W.G.P., Vermeer, H., Spoolder, H.A.M., Hendriks, H.J.M., Hopster, H., 2006. Formalised review of environmental enrichment for pigs in relation to political decision making. Appl. Anim. Behav. Sci. 98, 165-182.

Camerlink, I., Bijma, P., Kemp, B., Bolhuis, J.E., 2012. Relationship between growth rate and oral manipulation, social nosing, and aggression in finishing pigs. Appl. Anim. Behav. Sci. 142, 11-17.

Carlsson, H.-E., Lyberg, K., Royo, F., Hau, J., 2007. Quantification of stress sensitive markers in single fecal samples do not accurately predict excretion of these in the pig. Res. Vet. Sci. 82, 423-428.

Cook, N.J., 2012. Review: Minimally invasive sampling media and the measurement of corticosteroids as biomarkers of stress in animals. Can. J. Anim. Sci. 92, 227-259.

De Jonge, F.H., Bokkers, E.A.M., Schouten, W.G.P., Helmond, F.A., 1996. Rearing pigs in a poor environment: developmental aspects of social stress in pigs. Physiol. Behav. 68, 571-578.

Deen, J., 2010. Pigs: Behavior and welfare assessment. In: Breed, M.D., Moore, J. (Eds.), Encyclopedia of Animal Behavior. Academic Press, Oxford, pp.731-739.

EFSA (European Food Safety Authority), 2005. The welfare of weaners and rearing pigs: effects of different space allowances and floor types. Scientific Report of European Food Safety Authority – AHAW panel. Available at: http://www.efsa.eu.int/science/ahaw/ahaw_opinions/1203/ahaw_op_ej268_pigwelfare_report_en3.pdf. Accessed October 04, 2011.

Fureix, C., Benhajali, H., Henry, S., Bruchet, A., Prunier, A., Ezzaouia, M., Coste, C., Hausberger, M., Palme, R., Jego, P., 2013. Plasma cortisol and faecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: do stereotypic horses cope better with poor environmental conditions? BMC Vet. Res. 9 (3), http://dx.doi.org/10.1186/1746-6148-9-3.

Guy, J.H., Rowlinson, P., Chadwick, J.P., Ellis, M., 2002a. Behaviour of two genotypes of growing–finishing pig in three different housing systems. Appl. Anim. Behav. Sci. 75, 193-206.

Guy, J.H., Whitehead, N., Cussins, R.A., 2002b. The Pig Mobile: a simple environmental enrichment device for growing pigs in barren pens. In: Abstract 36th International Congress of the ISAE, August 2002. Egmond aan Zee, The Netherlands, (abstract), p. 142.

Hansen, S.W., Malmkvist, J., Palme, R., Damgaard, B.M., 2007. Do double cages and access to occupational materials improve the welfare of farmed mink? Anim. Welf. 16, 63-76.

Hörning, B., 2007. Space allowance. In: A. Verlade and R. Geers (Ed.) On-farm monitoring of pig welfare. Wageningen Academic Publishers, Wageningen, Netherlands, pp. 121–126.

Hughes, T., Creighton, E., Coleman, R., 2010. Salivary and fecal cortisol as measures of stress in horses. J. Vet. Behav.: Clin. Appl. Res. 5, 59-60.

IFOAM (International Federation of the Organic Agriculture Movements), 2005. Basic Standards for Organic Agriculture and Food Processing, 2005 version. Bonn, Germany. pp. 126.

ISAE (International Society of Applied Ethology) Ethics Committee, 2002. Guidelines for Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research. Accessed Jan. 18, 2012. Available at: http://www.applied-ethology.org/ethical guidelines.html. Accessed October 04, 2011.

Italian Ministry of Health, 1997. Decreto 01 aprile 1997, Piano nazionale di controllo della malattia di Aujeszky nella specie suina. - G.U. Serie Generale, n. 103 del 06 maggio 1997.

Jensen, T., Nielsen, C.K., Vinther, J., D'Eath, R.B., 2012. The effect of space allowance for finishing pigs on productivity and pen hygiene. Livest. Sci. 149, 33-40.

Lexen, E., El-Bahr, S.M., Palme, R., Möstl, E., 2008. Monitoring disturbances in sheep by measuring glucocorticoid metabolites in faeces. Wien Tierärztl. Monat. – Vet. Med. Austria 95, 64-71.

Marco-Ramell, A., Pato, R., Peña, R., Saco, Y., Manteca, X., Ruiz de la Torre, J.L., Bassols, A., 2011. Identification of serum stress biomarkers in pigs housed at different stocking densities. Vet. J. 190, 66-71.

Mattiello, S., Tosi, M.V., Hinzl, E., Carenzi, C., 2003. Il benessere del suino pesante: effetto della densità e del sistema di stabulazione. Rivista di Suinicoltura 10, 157-161.

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol. 138, 189-199.

Moberg, G.P., 2000. Biological response to stress: implications for animal welfare. In: Moberg, G.P., Mench J.A. (Ed), The biology of animal stress: basic principles and implications for animal welfare, CABI Publishing, Wallingford, UK, pp. 1-22.

Moncek, F., Duncko, R., Johansson, B.B., Jezova, D. 2004. Effect of environmental enrichment on stress related systems in rats. J Neuroendocrinol 16: 423–431.

Morméde, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C.G., Richard, S., Veissier, I., 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92, 317-339.

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Dom. Anim. Endocrinol. 23, 67-74.

Möstl, E., Messmann, S., Bagu, E., Robia, C., Palme, R., 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J. Vet. Med. A 46, 621-32.

Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44, 229-243.

Palme, R., 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim. Welf. 21, 331-337.

Palme, R., Robia, C., Messmann, S., Hofer, J., Möst, E., 1999. Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. Wien. Tierärztl. Mschr. 86, 237-241.

Prola, L., Cornale, P., Renna, M., Macchi, E., Perona, G., Mimosi, A., 2013. Effect of breed, cage type, and reproductive phase on fecal corticosterone levels in doe rabbits. J. Appl. Anim. Welf. Sci. 16(2), 140-149.

Royo, F., Lyberg, K., Abelson, K.S.P., Carlsson, H.E., Hau, J., 2005. Effect of repeated confined single housing of young pigs on fecal excretion of cortisol and IgA Scand. J. Lab. Anim. Sci. 32, 33-37.

Samarakone, T.S., Gonyou, H.W., 2008. Productivity and aggression at grouping of grower-finisher pigs in large groups. Can. J. Anim. Sci. 88, 9-17.

Schmolke, S.A., Li, Y.Z., Gonyou, H.W., 2003. Effect of group size on performance of growing-finishing pigs. J. Anim. Sci. 81, 874-878.

Stolba, A., Wood-Gush, D.G.M., 1980. Arousal and exploration in growing pigs in different environments. Appl. Anim. Ethol. 6, 382-383.

Street, B.R. Gonyou, H.W., 2008. Effects of housing finishing pigs in two group sizes and at two floor space allocations on production, health, behavior and physiological variables. J. Anim. Sci. 86, 982-991.

SVC (Scientific Veterinary Committee), 1997. Report on the welfare of intensively kept pigs. Available from http://ec.europa.eu/food/fs/sc/oldcomm4/out17_en.pdf Accessed February 21, 2011.

Telkänranta, H., Bracke, M.B.M., Valros, A., 2014. Fresh wood reduces tail and ear biting and increases exploratory behaviour in finishing pigs. Appl. Anim. Behav. Sci. 161, 51-59.

Temple, D., Dalmau, A., Ruíz de la Torre, J.L., Manteca, X., Velarde, A., 2011. Application of the Welfare Quality® protocol on intensively kept growing pigs in Spain. J. Vet. Behav. 6, 138-149.

Tönepöhl, B., Appel, A.K., Welp, S., Voß, B., von Borstel, U.K., Gauly, M., 2012. Effect of marginal environmental and social enrichment during rearing on pigs' reactions to novelty, conspecifics and handling. Appl. Anim. Behav. Sci. 140, 137-145.

Turner, S.P., Allcroft, D.J., Edwards, S.A., 2003. Housing pigs in large social groups: A review of implications for performance and other economic traits. Livest. Prod. Sci. 82,39-51.

van de Weerd, H.A., Day, J.E.L., 2009. A review of environmental enrichment for pigs housed in intensive housing systems. Appl. Anim. Behav. Sci. 116, 1-20.

van de Weerd, H.A., Docking, C.M., Day, J.E.L., Breuer, K., Edwards, S.A., 2006. Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. Appl. Anim. Behav. Sci. 99, 230-247.

Whittaker, A.L., Van Wettere, W.H., Hughes, P.E., 2012. Space requirements to optimize welfare and performance in group housed pigs-a review. Am. J. Anim. Vet. Sci. 7, 48-54.

Wolter, B.F., Ellis, M., Curtis, S.E., Parr, E.N., Webel, D.M., 2000. Group size and floor-space allowance can affect weanling-pig performance. J. Anim. Sci. 78, 2062-2067.

Woodman, D.D., 1997. The adrenal glands. In: Whiley J. (Ed), Laboratory animal endocrinology. Hormonal action, control mechanisms and interactions with drug. Whiley & Sons Ltd. Chichester, England, pp. 253-286.

Young, R.J. 2003. Environmental Enrichment for Captive Animals. Blackwell Science, Oxford. pp. 240.

Figure captions

Figure 1. Effects of stocking density and environmental enrichment on corticosteroids concentration (ng/g) in pigs feces (** $P \leq 0.01$; * $P \leq 0.05$). Different letters (a, b) represent significant differences among treatments for each sampling date ($P \leq 0.05$). HD-BP, high density-barren pen; HD-EP, high density-enriched pen; LD-BP, low density-barren pen; LD-EP, low density-enriched pen.

Table 1. Definitions of behavior for scan animal samples adapted from Guy et al. (2002) and van de Weerd et al. (2006)

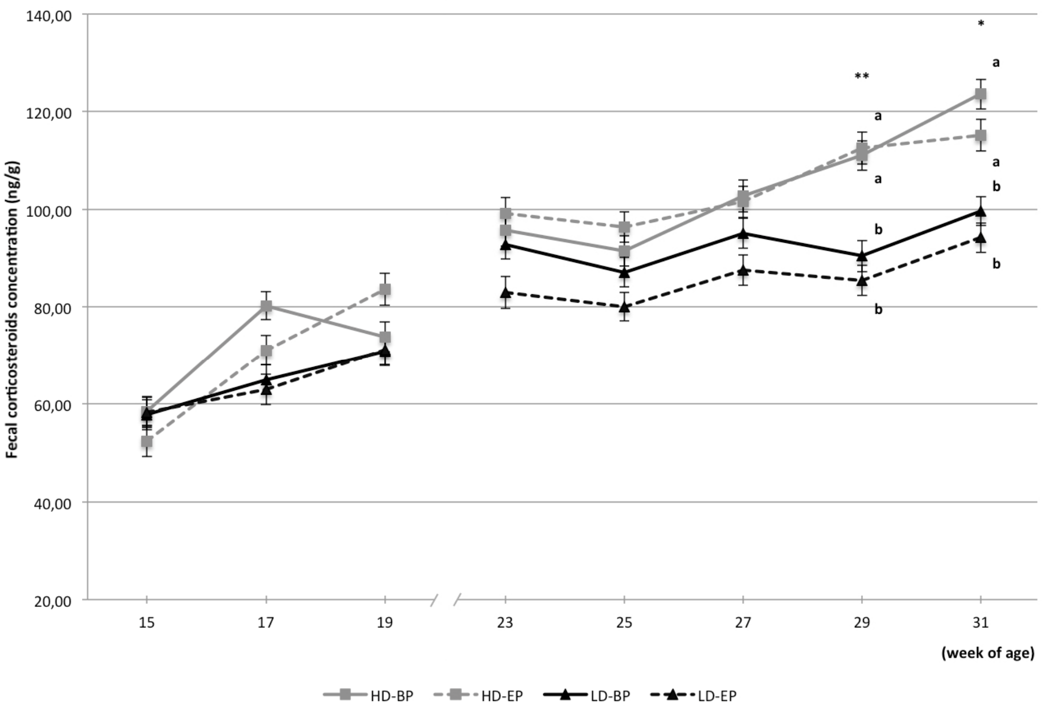
Behavior	Description
Feeding	Pig stands in front of feeder with head lowered in feed hopper
Drinking	Pig stands, either with mouth touching or holding nipple drinker, or with snout in water bowl
Excreting or urinating	Pig stands in process of excreting or urinating
Exploring pen furniture	Pig stands and actively sniffs, noses, bites or chews floor and any part of the pen furniture
Examining enrichment	Pig stands and actively sniffs, noses, bites or chews the environmental enrichment
Social activity	Pig stands or lies and noses, lick or nibbles any part of a pen-mate’s body
Aggressive behavior	Pig violently bites or knocks another group member with his head
Tail-biting	Pig holds a penmate’s tail in its mouth and bites it
Mounting	Pig stands or attempts to stand, with front legs on back of another group member
Moving	Pig walks, trots or runs around the pen
Lying	Pig lies motionless on side or sternum with eyes closed

Table 2. Effects of stocking density and environmental enrichment on frequency of pigs behaviors (% of total observation time)

Behaviors	High density		Low density		Effects		
	Barren pen	Enriched pen	Barren pen	Enriched pen	D	E	D×E
	HD-BP	HD-EP	LD-BP	LD-EP			
Feeding	10.89^{c}	11.25^{c}	12.71^{b}	14.48^{a}	*	*	*
Drinking	4.81	5.17	4.38	5.92	ns	ns	ns
Excreting or urinating	2.13	2.15	1.98	2.61	ns	ns	ns
Exploring pen furniture	7.92	9.31	9.17	10.97	0.09	ns	ns
Examining enrichment	-	4.23	-	4.35	ns	-	-
Social activity	7.43	9.74	5.98	10.81	ns	***	ns
Aggressive behavior	1.33	0.64	1.28	0.59	ns	*	ns
Tail-biting	1.41	0.56	1.29	0.29	ns	**	ns
Moving	2.26^{b}	2.53^{ab}	2.98^{a}	3.16^{a}	*	ns	0.07
Lying	59.67^{a}	52.44^{ab}	59.33^{a}	43.97^{b}	**	***	0.06

[1] Significance of effects of stocking density (D), environmental enrichment (E), and their interaction (D×E) is indicated; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; tendency $P < 0.10$; ns, not significant.

[2] a, b, c: different letters at the same row means significant difference within treatments ($P \leq 0.05$).



140,00
120,00
100,00
80,00
60,00
40,00
20,00
Fecal corticosteroids concentration (ng/g)
15
17
19
23
25
27
29
31
(week of age)
**
*
a
a
a
a
b
b
b
b
HD-BP
HD-EP
LD-BP
LD-EP

Highlights

- We evaluated how to ameliorate pigs' welfare under commercial farm conditions
- We considered behavior and fecal corticosteroid concentration as welfare indicators
- Reducing stocking density modified behavior and reduced fecal corticosteroids level
- The provision of suspended pieces of wood in pens box modified pigs behavior
- The same piece of wood did not exert relevant effect on fecal corticosteroids level