

1  
2  
3**JAST (Journal of Animal Science and Technology) TITLE PAGE**

Upload this completed form to website with submission

<b>ARTICLE INFORMATION</b>	<b>Fill in information in each box below</b>
<b>Article Type</b>	Research article
<b>Article Title (within 20 words without abbreviations)</b>	Response to environmental enrichment of weanling pigs on growth, behaviour and welfare after weaning
<b>Running Title (within 10 words)</b>	Environmental enrichment in weanling pigs
<b>Author</b>	Junhyung Lee <sup>1</sup> , Seungmin Oh <sup>2</sup> , Minju Kim <sup>3,4</sup>
<b>Affiliation</b>	1University of Guelph, Department of Animal Biosciences, Guelph, ON, Canada, N1G 2W1 2Gyeongbuk Livestock Research Institute, Yeongju, 63052, Republic of Korea 3School of Animal Life Convergence Science, Hankyong National University, Ansong 17579, Korea 4Institute of Applied Humanimal Science, Hankyong National University, Ansong 17579, Korea
<b>ORCID (for more information, please visit <a href="https://orcid.org">https://orcid.org</a>)</b>	Junhyung Lee ( <a href="https://orcid.org/0000-0002-7937-7817">https://orcid.org/0000-0002-7937-7817</a> ) Seungmin Oh ( <a href="https://orcid.org/0000-0001-8848-8028">https://orcid.org/0000-0001-8848-8028</a> ) Minju Kim ( <a href="https://orcid.org/0000-0001-6950-0458">https://orcid.org/0000-0001-6950-0458</a> )
<b>Competing interests</b>	No potential conflict of interest relevant to this article was reported.
<b>Funding sources</b> State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
<b>Acknowledgements</b>	Not applicable.
<b>Availability of data and material</b>	Upon reasonable request, the datasets of this study can be available from the corresponding author.
<b>Authors' contributions</b> Please specify the authors' role using this form.	Conceptualization: Kim MJ. Data curation: Lee JH Formal analysis: Lee JH. Methodology: Oh SM. Software: Oh SM. Validation: Kim MJ. Investigation: Lee JH. Writing - original draft: Lee JH, Oh SM. Writing - review & editing: Kim MJ.
<b>Ethics approval and consent to participate</b>	This article does not require IRB/IACUC approval because there are no human and animal participants.

4

**5 CORRESPONDING AUTHOR CONTACT INFORMATION**

<b>For the corresponding author (responsible for correspondence, proofreading, and reprints)</b>	<b>Fill in information in each box below</b>
First name, middle initial, last name	Minju Kim
Email address – this is where your proofs will be sent	minjukim@hknu.ac.kr
Secondary Email address	

Address	School of Animal Life Convergence Science, Hankyong National University, Ansong 17579, Korea
Cell phone number	+82-10-2962-7183
Office phone number	031-670-5124
Fax number	

6  
7

ACCEPTED

## 8 **Abstract**

9 The experiment was carried out to examine the growth, behaviour, and welfare response of weaning pigs  
10 to environmental enrichment from d 1 to d 28 after weaning. A total of 240 weaning pigs with average  
11 initial body weight (BW)  $6.56 \pm 0.17$  kg) were randomly allotted to one of the four treatments on the  
12 basis of initial BW. A completely randomized design was used to conduct this study. There were ten pigs  
13 per pen, with 6 replicates for each treatment. The experimental treatments were control, EE-1 (inclusion  
14 of play object until one week after weaning), EE-2 (inclusion of play object until two weeks after  
15 weaning), and EE-4 (inclusion of play object until four weeks after weaning). The pigs raised the EE-2  
16 and EE-4 treatments had greater average daily gain ( $P=0.002$ ). The average daily feed intake ( $P=0.016$ )  
17 was increased in the EE-2 treatment in phase 1. The pigs in the EE-4 treatment had greater average daily  
18 gain ( $P=0.039$ ) and average daily feed intake ( $P=0.030$ ) in phase 2 than pigs raised in the control  
19 treatment, and overall (average daily gain:  $P=0.006$ , average daily feed intake:  $P=0.014$ ). The pigs under  
20 enriched environment treatments (EE-2 and EE-4) decreased BW uniformity in phase 1 ( $P=0.006$ ) and  
21 phase 2 ( $P<0.001$ ) than pigs in the control treatment. The incidence of diarrhea was lowered ( $P<0.001$ ) in  
22 early phase 1 (d 7 after weaning) under environmental enrichment treatments (EE-2 and EE-4). Behaviour  
23 traits exhibited reduced agonistic behaviour, such as biting ( $P=0.018$ ), tail biting ( $P=0.001$ ), and ear biting  
24 ( $P=0.016$ ) under environmental enrichment treatments (EE-2 and EE-4) in phase 1. The skin lesion score  
25 was reduced ( $P=0.015$ ) in the EE-4 treatment in phase 1. Hair cortisol was reduced in the EE-4 treatment  
26 ( $P=0.032$ ) at the end of phase 2, however, there were no significant differences in salivary cortisol  
27 concentration. These findings demonstrated beneficial effects on growth, group uniformity, behaviour,  
28 incidence of diarrhea, skin lesions, and concentration of hair cortisol through exposure to environmental  
29 enrichment after weaning.

30

## 31 **Keywords:**

32 weanling pig, body weight uniformity, behaviour, stress, welfare

33

34

## **Introduction**

35 The modern swine industry constantly addresses its interest in promoting optimal growth and  
36 increasing the market weight of pigs. In this context, it has been found that the ideal growth of weanling  
37 pigs during the weaning period is closely related to enhanced outcomes during the growth and finishing  
38 periods [1]. The weanling pigs are subjected to various stressors through environmental changes, such as  
39 separation from sows, different forms of diet, and mixing with other groups of weanling pigs [2]. In most  
40 cases, weaning stress is considered a prominent management concern related to achieving optimal growth  
41 and inducing desirable behaviors in weanling pigs. In this regard, the weaning period is a critical phase

42 for weanling pigs, which can have a significant impact and is strongly associated with reduced feed intake,  
43 higher incidence of diarrhea, and greater agonistic behavior during the early weaning period [3].

44 An enriched environment was introduced as an alternative housing system to reduce stress levels in  
45 weanling pigs induced by the weaning process, replacing the traditional barren housing system. Enriched  
46 environments enhance the welfare of livestock, which induces more instinctive behavior through different  
47 forms of housing or play object installation to enhance their optimal growth [4,5]. One relevant welfare  
48 indicator is the level of play activity, which is the natural behavior of an animal that can be a source of  
49 play and a reduction in vigilance or aggression toward the external environment [6]. Thus, supporting the  
50 play activity levels of weanling piglets could be related to reducing depression or strong negative  
51 emotions in these piglets [7]. Despite this, observing exploration or foraging behavior in weanling piglets  
52 in a conventional environment is a significant challenge. They exhibit elevated concentrations of cortisol  
53 and more reciprocal agonistic behavior, such as fighting and ear-tail biting, as an aim of social dominance  
54 and the hierarchal order with another group of weanling piglets [8,9]. As a detrimental consequence of  
55 that competitive activity, weanling piglets may also show a greater body weight (BW) variation within a  
56 group, and previous studies demonstrated that BW variation is connected to the growth performance of  
57 weanling piglets after weaning under commercial environments. Furthermore, a nonuniform growth rate  
58 can reduce the efficiency of the production cycle because of higher BW variation in weanling piglets [10-  
59 11]. This study aimed to address the impact of environmental enrichment on growth, BW uniformity,  
60 incidence of diarrhea, behavior, skin lesions, and salivary and hair cortisol levels in piglets after weaning.

61

62

## Materials and Methods

63

### Test Animals and Experimental Design

64

65

66

67

68

69

70

71

72

73

74

### Growth Performance

75 The amount of feed supplemented was measured throughout the experimental period to calculate the  
76 average daily feed intake (ADFI). The average daily gain (ADG), ADFI, and gain-to-feed ratio (G/F)  
77 were calculated at the end of each phase (phase 1: d 14, phase 2: d 28, and overall: d 0–28 after weaning).

#### 79 **Body weight uniformity**

80 Coefficient of variation (CV) of BW was calculated at the end of each phase (phase 1: d 14, phase 2: d  
81 28) as the CV by dividing the individual BW standard deviation by the mean BW.

#### 83 **Incidence of diarrhea**

84 The incidence of diarrhea was measured three times (on d 7, 14, and 28). The criteria for collecting  
85 data on the incidence of diarrhea were as follows: 1 = hard, dry pellets in a small, hard mass; 2 = hard,  
86 formed stool that remained firm and soft; 3 = soft, formed, and moist stool that retained its shape; 4 = soft,  
87 unformed stool that assumed the shape of the container; and 5 = watery, liquid stool that could be poured  
88 [12].

#### 90 **Behavior observations**

91 Piglet behavior was recorded at the end of each phase (d 14 and 28) by installing cameras (FIX  
92 Extreme Action, Camera, China) above each pen. The cameras were arranged through a cable duct  
93 located at the top of the middle of each pen to record behavior over the entire area. The video was  
94 recorded for 8 h, and the recorded video files were extracted and saved on a high-capacity USB flash  
95 drive for analysis. The observation days were at the end of each phase (phase 1: d 14, phase 2: d 28) after  
96 weaning and included an 8 h observation period (10:00–18:00). Each behavior was evaluated for 8 h  
97 using the video footage, and the number of behavioral observations was shown as the number per hour  
98 [13]. The criteria for analyzing the behavior traits are shown in Table 1.

#### 100 **Skin lesion scoring**

101 Evaluation of lesions on the body (ear, front, middle, hindquarters, and legs) or tail was conducted by  
102 inspecting the two sides of experimental weaning pigs at the end of each phase (d 14 and 28) after  
103 weaning [14].

#### 105 **Salivary and hair cortisol concentrations**

106 Saliva and hair samples were prepared and harvested at the end of phase 2 (d 28 after weaning).  
107 Medical cotton was attached to the fence of each experimental pen with string to collect saliva samples.  
108 After weaning, the pigs chewed the medical cotton for 5 to 10 min until fully wet. The cotton was  
109 retrieved using the string, and the ear tag of the pig was recorded during the chewing process. Supernatant

110 of the saliva sample (~7-8 mL) was prepared by centrifugation at  $3000 \times g$  at  $4^{\circ}\text{C}$  for 10 min and was  
111 stored at  $-20^{\circ}\text{C}$  until analysis. Salivary cortisol concentration was measured using a commercial ELISA  
112 kit (ADI-90-071, Enzo Life Sciences, Inc., US) [15]. Freshly grown hair from individual weaning pigs  
113 was collected and used for the analysis of hair cortisol concentrations. The collected hair samples were  
114 washed three times with isopropanol, followed by drying in a vacuum dryer at  $35^{\circ}\text{C}$ , and then placed in an  
115 EML plastic tube containing steel pellets and a bead beater (tacoTMPrep, 50/60 Hz 2A, GeneReach  
116 Taichung, Taiwan). Hair cortisol was extracted using methanol after crushing at Biotechnology Corp.,  
117 Taiwan. A cortisol ELISA kit (ADI-900-071; Enzo Life Sciences, Farmingdale, NY, USA) was used to  
118 determine the cortisol concentrations in the extracted sample [16].

119

## 120 **Statistical Analyses**

121 Data generated in the present study were subjected to a statistical analysis system (SAS Institute Inc.,  
122 Cary, NC, USA) using the general linear model procedure in a completely randomized design. When  
123 significant differences were identified among the treatment means, they were separated using Tukey's  
124 Honest Significant Difference test. Statistical significance was set at  $p < 0.05$ .

125

126

127

## **Results**

### 128 **Growth performance and uniformity**

129 The effects of environmental enrichment on growth performance are summarized in Table 2. In phase 1,  
130 pigs raised in EE-2 and EE-4 had greater ADG ( $P=0.002$ ) than those raised in the control. In phase 2, pigs  
131 raised in EE-4 had greater ADG ( $P=0.039$ ) than pigs raised in the control. Overall, pigs from EE-2 and  
132 EE-4 showed a greater ADG ( $P=0.006$ ) than the control. In phase 1, the ADFI of pigs in the EE-2 was  
133 higher ( $P=0.016$ ) than the control. In phase 2, pigs treated with EE-2 showed higher ADFI ( $P=0.030$ ) than  
134 pigs reared in the control. Overall, pigs raised in the EE-4 treatment had a greater ADFI ( $P=0.014$ ) than  
135 the control. However, G/F did not exhibit any significant differences in phase 1, phase 2, or the overall  
136 period. The effects of environmental enrichment on group CV are presented in Table 3. Pigs raised in EE-  
137 2 and EE-4 had lower uniformity than the pigs raised in the control group in phases 1 ( $P=0.006$ ) and 2  
138 ( $P<0.001$ ), and overall ( $P<0.001$ ).

139

### 140 **Diarrhea incidence**

141 The effects of environmental enrichment on the incidence of diarrhea are shown in Table 4. The  
142 incidence of diarrhea was lower ( $P<0.001$ ) in pigs raised in EE-2 and EE-4 than the control treatment in  
143 early phase 1 (the first week after weaning). However, environmental enrichment did not exhibit any  
144 significant differences among the treatments at the end of phase 1 and phase 2.

145

### 146 **Behavior traits**

147 The effects of environmental enrichment on behavioral traits are shown in Table 5. Pigs raised in EE-2  
148 and EE-4 exhibited lower biting ( $P=0.018$ ), tail biting ( $P=0.001$ ), and ear biting ( $P=0.016$ ). However,  
149 there were no significant differences in behavioral traits in phase 2.

150

### 151 **Skin lesion score**

152 The effects of environmental enrichment on skin lesion scores are shown in Table 6. Pigs raised in EE-  
153 4 had lower skin lesion scores ( $P = 0.015$ ) than those raised in the control. However, there was no  
154 significant difference between the treatments in phase 2.

155

### 156 **Salivary and hair cortisol levels**

157 The effects of environmental enrichment on salivary and hair cortisol concentrations are shown in  
158 Table 7. Pigs reared in EE-4 had lower ( $P=0.032$ ) hair cortisol concentrations than pigs reared in the  
159 control. However, there was no significant difference in the cortisol levels of the saliva among the  
160 treatments.

161

162

## 163 **Discussion**

### 164 **Growth performance and uniformity**

165 Concerns regarding animals' welfare and growth have led to an increased focus on stress management.  
166 In particular, the agonistic behavior induced by the early weaning period is related to growth retardation  
167 during the growing and finishing phases of pigs [17]. Environmental enrichment as a play object  
168 installation resulted in an increase in growth performance, that is, ADG and ADFI, compared to the  
169 control group during the current study, although the enrichment effect on growth was the highest in the  
170 EE-2 group. This may result in different responses of the piglets to the object, and interest in the enriched  
171 environment appears to diminish with age during the weaning phase [18,19]. Similarly, the growth of  
172 weaning pigs raised in an enriched environment was higher than that of those raised in a commercial  
173 barren environment; however, the enriched environment did not affect growth performance until 20  
174 weeks of age [20]. As a crucial period, the weaning process is strongly connected to the growth of  
175 weaning pigs, but it is also linked to the timing of the final market weight and profitability of the producer.  
176 Accordingly, the management CV of BW just before or immediately after weaning may be used as a  
177 critical indicator to predict the final outcome of weaning pigs [21]. The observed CV of BW in the  
178 present study was approximately 10%, and the EE-4 group had the lowest CV of BW compared to the  
179 other groups, including the control group. Furthermore, enriched groups had slightly better BW evenness

180 within a group; however, various factors may contribute to this phenomenon. Therefore, further studies  
181 are warranted.

### 182 **Incidence of diarrhea**

183 As a common condition, post-weaning diarrhea is a well-known disease that is induced by weaning  
184 stress in weaned piglets worldwide. Since the start of the weaning process, weaned pigs quickly adapt to  
185 various environmental changes, such as different forms of diet and mixing with other pigs. Furthermore,  
186 as a result of these changes, weaned pigs exhibit abnormal symptoms, such as lower feed intake and a  
187 higher incidence of diarrhea, which are closely associated with the retarded growth of pigs [22-24]. In the  
188 present study, pigs raised in the EE-4 group showed the lowest incidence of diarrhea in the early post-  
189 weaning period (d 7 post-weaning), although they had a higher incidence of diarrhea than those in the rest  
190 of the weaning period. Few studies have examined the incidence of diarrhea in weaned pigs in  
191 environments enriched with play objects to demonstrate the precise relationship between environmental  
192 enrichment and the incidence of diarrhea. However, few studies have reported that feed intake and CV of  
193 BW can be used as indicators to measure the proper growth of weaned pigs [25]. Weaning stress, which is  
194 strongly associated with an increase in the inflammatory response in the gastrointestinal tract, is one of  
195 the various stressors contributing to the incidence of diarrhea in weaned pigs [26]. In this regard, the  
196 results of the present study, which are closely related to previous results showing improved ADG, ADFI,  
197 and CV of BW conditions in an enriched environment, may contribute to another positive effect of  
198 enriched environments in weaning pigs.

### 199 **Behavioral traits**

200 The behavior of animals is considered an essential factor in their growth and welfare, and animals must  
201 be exposed to objects that are sufficient to maintain their behavior [27]. It is well known that agonistic  
202 behavior during the early post-weaning period, which is common in commercial barren environments, is  
203 directly related to poor feed intake and growth in weaning pigs. In the present study, pigs exhibited  
204 reduced agonistic behavior, including torso, leg, ear, and tail biting, during the early post-weaning period  
205 (d 14). The results of the present study are consistent with previous studies showing that an enriched  
206 environment led to elevated play behaviors [28,29] or more proactive and explorative behavior in  
207 weaning pigs [28,30,31]. Enriched housed pigs have been shown to spend more time playing before  
208 weaning and up to 7 weeks after weaning, and these behaviors are strongly related to less agonistic  
209 behavior [28,29]. Moreover, these results were associated with increased weight gain and adaptability to  
210 the new environment of weaning pigs, and exposure to enriched housing could impact later life behavior  
211 and welfare [32]. Therefore, it can be inferred from these results that positive behavioral changes in  
212 weaning pigs in an enriched environment are a better outcome in terms of the growth and welfare of  
213 weaning pigs.

### 214 **Skin lesion score**



215 The consequences of the weaning process, which is the phased formation of reciprocal dominance  
216 relationships through agonistic behavior, are well-known phenomena [33]. Most weaned pigs show this  
217 aggressive tendency by fighting or biting other pigs from different groups. This behavior manifests as an  
218 accumulation of skin lesions throughout the body, legs, ears, and tail [34,35]. The results of the present  
219 study showed that pigs raised under exposure to the enriched environment had reduced skin lesion scores  
220 compared to the control group, and there was a trend towards a decrease in the number of skin lesions as  
221 exposure time to the enriched environment increased. These results are linked to those of previous studies  
222 that observed that over 20% lowered oral activities related to skin lesions in pigs reared in enriched  
223 environments [36], and the response to enriched environments is correlated with the age of the pigs [37].  
224 These facts led us to conclude that access to an enriched environment contributed to a reduced skin lesion  
225 score, which is consistent with the diminished reciprocal agonistic behavior observed in the present study.

### 226 **Salivary and hair cortisol levels**

227 When animals encounter stressful conditions, such as a shortage of environmental enrichment, they can  
228 activate the hypothalamic-pituitary-adrenal axis, which is associated with the secretion of glucocorticoid  
229 cortisol, an indicator of stress levels measured in blood, urine, saliva, and hair [38]. In the present study,  
230 the EE-4 group had lower hair cortisol concentrations during the weaning phase; however, the enriched  
231 environment did not affect salivary cortisol concentrations. These results are consistent with previous  
232 studies that have shown that barren environments adversely affect the welfare of weaned pigs and that it  
233 is connected to the increment of hair cortisol levels in pigs raised in a barren environment [20,39].  
234 Cortisol concentration in hair is a response to chronic stress that is distinct from salivary cortisol levels,  
235 which are related to acute stress response in weaning pigs [40]. These responses indicate that they were  
236 subjected to sustained stress over weeks while growing in a non-enriched environment, similar to the  
237 present study. However, in contrast to the present study, previous studies have demonstrated increased  
238 salivary cortisol concentrations in pigs raised in barren environments [41,42]. In this context, the weaning  
239 process, including relocation or social mixing, is a stressor that can be characterized by elevated salivary  
240 cortisol levels. However, salivary cortisol levels recovered to normal ranges within 8 h, indicating that it  
241 is most likely an acute stress response [43,44]. There was a 1.47-fold increase in salivary cortisol  
242 concentration after weaning; however, this increase was reduced by 1.26-fold greater two days after  
243 weaning. This can be explained as a transient response that progressively decreases concentration [45].  
244 Therefore, environmental enrichment conditions may be consistently more favorable for reducing stress  
245 on the growth and welfare of weaning pigs; however, further research is required to refine the appropriate  
246 assay that may influence the results.

### 248 **Conclusion**

249 The use of play objects for 2 or 4 weeks improved ADG and ADFI, lowered CV of BW, diarrhea  
250 incidence, agonistic behavior, skin lesion score, and hair cortisol concentration. The results showed that

251 the use of play object for one week was not adequate. Further research is required to refine more specific  
252 periods related to providing enriched environments through additional parameters, such as the installation  
253 of different types of play objects and how this environment might also impact the growing and finishing  
254 phases.

255

256

## 257 **Acknowledgments**

258

259

260

ACCEPTED

## 261 **References**

- 262 1. Hosseindoust AR, Lee, SH., Kim JS., Choi YH., Kwon IK, Chae BJ. Productive performance of  
263 weanling piglets was improved by administration of a mixture of bacteriophages, targeted to control  
264 Coliforms and Clostridium spp. shedding in a challenging environment. *J Anim Physiol Anim Nutr.*  
265 2017; 101(5):98-107. <https://doi.org/10.1111/jpn.12567>
- 266 2. Lee SH, Hosseindoust AR, Kim JS, Choi YH, Lee JH, Kwon IK, Chae BJ. Bacteriophages as a  
267 promising anti-pathogenic option in creep-feed for suckling piglets: Targeted to control Clostridium  
268 spp. and coliforms faecal shedding. *Livest Sci.* 2016;191:161-4.  
269 <https://doi.org/10.1016/j.livsci.2016.08.003>
- 270 3. Hosseindoust AR, Lee SH, Kim JS, Choi YH, Noh HS, Lee JH, Jha PK, Kwon IK, Chae BJ. Dietary  
271 bacteriophages as an alternative for zinc oxide or organic acids to control diarrhoea and improve the  
272 performance of weanling piglets. *Vet Med.* 2017 Feb 13;62(2):53-61.  
273 <https://doi.org/10.17221/7/2016-VETMED>
- 274 4. Poole TB. The nature and evolution of behavioural needs in mammals. *Anim Welf.* 1992;1(3):203-  
275 220. <https://doi.org/10.1017/S0962728600015013>
- 276 5. Kim KH, Hosseindoust A, Ingale SL, Lee SH, Noh HS, Choi YH, Jeon SM, Kim YH, Chae BJ.  
277 Effects of gestational housing on reproductive performance and behavior of sows with different  
278 backfat thickness. *Asian-australas. J Anim Sci.* 2016;29(1):142. <https://doi:10.5713/ajas.14.0973>
- 279 6. Newberry RC, Wood-Gush DGM, Hall JW. Playful behaviour of piglets. *Behav Process.*  
280 1988;17(3):205-216. [https://doi.org/10.1016/0376-6357\(88\)90004-6](https://doi.org/10.1016/0376-6357(88)90004-6)
- 281 7. Spinka M, Newberry RC, Bekoff M. Mammalian play: training for the unexpected. *Q Rev Biol.*  
282 2001;76(2):141-168. <https://doi.org/10.1086/393866>
- 283 8. Fraser D, Phillips PA, Thompson BK, Tennessen T. Effect of straw on the behaviour of growing  
284 pigs. *Appl Anim Behav Sci.* 1991;30(3-4):307-318. [https://doi.org/10.1016/0168-1591\(91\)90135-K](https://doi.org/10.1016/0168-1591(91)90135-K)
- 285 9. Stricklin WR, Mench JA. Social organization. *Vet Clin North Am Food Anim Pract.* 1987;3(2):307-  
286 22. [https://doi.org/10.1016/s0749-0720\(15\)31154-3](https://doi.org/10.1016/s0749-0720(15)31154-3)
- 287 10. Choi YH, Hosseindoust A, Kim MJ, Kim KY, Lee JH, Kim YH, Kim JS, Chae BJ. Additional  
288 feeding during late gestation improves initial litter weight of lactating sows exposed to high ambient  
289 temperature. *Rev Bras Zootec.* 2019; 28:48.
- 290 11. López-Vergé S, Gasa J, Farré M, Coma J, Bonet J, Solà-Oriol D. Potential risk factors related to pig  
291 body weight variability from birth to slaughter in commercial conditions. *Transl Anim Sci.*  
292 2018;2(4):383-395. <https://doi.org/10.1093/tas/txy082>

- 293 12. Kim, TG, Kim MJ, Lee JH, Moturi J, Ha SH, Tajudeen H, Mun JY, Hosseindoust A, Chae BJ.  
294 Supplementation of nano-zinc in lower doses as an alternative to pharmacological doses of ZnO in  
295 weanling pigs. *J Anim Sci Technol.* 2002;64(1):70. <https://doi.org/10.5187/jast.2022.e2>
- 296 13. Oh S, Hosseindoust A, Ha S, Moturi J, Mun J, Tajudeen H, Kim J. Metabolic responses of dietary  
297 fiber during heat stress: effects on reproductive performance and stress level of gestating sows.  
298 *Metabolites.* 2022;12(4):280. <https://doi.org/10.3390/metabo12040280>
- 299 14. Fu L, Li H, Liang T, Zhou B, Chu Q, Schinckel AP, Yang X, Zhao R, Huang R. Stocking density  
300 affects welfare indicators of growing pigs of different group sizes after regrouping. *Appl Anim  
301 Behav Sci.* 2016;174:42-50. <https://doi.org/10.1016/j.applanim.2015.10.002>
- 302 15. Nejad JG, Ataallahi M, Park KH. Methodological validation of measuring Hanwoo hair cortisol  
303 concentration using bead beater and surgical scissors. *J Anim Sci Technol.* 2019;61(1):41.  
304 <https://doi.org/10.5187/jast.2019.61.1.41>
- 305 16. Moturi J, Hosseindoust A, Tajudeen H, Mun JY, Ha SH, Kim JS. Influence of dietary fiber intake  
306 and soluble to insoluble fiber ratio on reproductive performance of sows during late gestation under  
307 hot climatic conditions. *Sci Rep.* 2022; 17;12(1):19749. <https://doi.org/10.1038/s41598-022-23811-8>
- 308 17. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. *J Anim Sci  
309 Biotechnol.* 2013;4(1):19. <https://doi.org/10.1186/2049-1891-4-19>
- 310 18. van de Weerd HA, Day JE. A review of environmental enrichment for pigs housed in intensive  
311 housing systems. *Appl Anim Behav Sci.* 2009;116(1):1-20.  
312 <https://doi.org/10.1016/j.applanim.2008.08.001>
- 313 19. Docking CM, Van de Weerd HA, Day JEL, Edwards SA. The influence of age on the use of  
314 potential enrichment objects and synchronisation of behaviour of pigs. *Appl Anim Behav Sci.*  
315 2008;110(3-4):244-257. <https://doi.org/10.1016/j.applanim.2007.05.004>
- 316 20. Beattie VE, Walker N, Sneddon IA. Effects of environmental enrichment on behaviour and  
317 productivity of growing pigs. *Anim Welf.* 1995;4(3):207-220.  
318 <https://doi.org/10.1017/S0962728600017802>
- 319 21. Francis DA, Christison GI, Cymbaluk NF. Uniform or heterogeneous weight groups as factors in  
320 mixing weanling pigs. *Can J Anim Sci.* 1996;76(2):171-176. <https://doi.org/10.4141/cjas96-026>
- 321 22. Lee JJ, Kyoung H, Cho JH, Choe J, Kim Y, Liu Y, Kang J, Lee H, Kim H, Song M. Dietary yeast  
322 cell wall improves growth performance and prevents of diarrhea of weaned pigs by enhancing gut  
323 health and anti-inflammatory immune responses. *Animals.* 2021;11(8):2269.  
324 <https://doi.org/10.3390/ani.11082269>

- 325 23. Choi Y, Hosseindoust A, Ha SH, Kim J, Min Y, Jeong Y, Mun J, Sa S, Kim J. Effects of dietary  
326 supplementation of bacteriophage cocktail on health status of weanling pigs in a non-sanitary  
327 environment. *J Animal Sci Biotechnol.* 2023;14(1):1-7. <https://doi.org/10.1186/s40104-023-00869-6>
- 328 24. Lee J, Hosseindoust A, Kim M, Kim K, Choi Y, Moturi J, Song C, Lee S, Cho H, Chae B. Effects of  
329 hot melt extrusion processed nano-iron on growth performance, blood composition, and iron  
330 bioavailability in weanling pigs. *J Anim Sci Technol.* 2019;61(4):216.  
331 <https://doi:10.5187/jast.2019.61.4.216>
- 332 25. Wensley MR, Tokach MD, Woodworth JC, Goodband RD, Gebhardt JT, DeRouchey JM,  
333 McKilligan D. Maintaining continuity of nutrient intake after weaning. II. Review of post-weaning  
334 strategies. *Transl Anim Sci.* 2021;5(1):txab022. <https://doi.org/10.1093/tas/txab022>
- 335 26. McCracken BA, Spurlock ME, Roos MA, Zuckermann FA, Gaskins HR. Weaning anorexia may  
336 contribute to local inflammation in the piglet small intestine. *J Nutr.* 1999;129(3):613-619.  
337 <https://doi.org/10.1093/jn/129.3.613>
- 338 27. Tajudeen H, Moturi J, Hosseindoust A, Ha S, Mun J, Choi Y, Sa S, Kim J. Effects of various cooling  
339 methods and drinking water temperatures on reproductive performance and behavior in heat stressed  
340 sows. *J Anim Sci Technol.* 2022;64(4):782. <https://doi:10.5187/jast.2022.e33>
- 341 28. Bolhuis JE, Schouten WG, Schrama JW, Wiegant VM. Behavioural development of pigs with  
342 different coping characteristics in barren and substrate-enriched housing conditions. *Appl Anim  
343 Behav Sci.* 2005;93(3-4):213-228. <https://doi.org/10.1016/j.applanim.2005.01.006>
- 344 29. Bolhuis JE, Schouten WG, Schrama JW, Wiegant VM. Effects of rearing and housing environment  
345 on behaviour and performance of pigs with different coping characteristics. *Appl Anim Behav Sci.*  
346 2006;101(1-2):68-85. <https://doi.org/10.1016/j.applanim.2006.01.001>
- 347 30. Beattie VE, O'connell NE, Moss BW. Influence of environmental enrichment on the behaviour,  
348 performance and meat quality of domestic pigs. *Livest Prod Sci.* 2000;65(1-2):71-79.  
349 [https://doi.org/10.1016/S0301-6226\(99\)00179-7](https://doi.org/10.1016/S0301-6226(99)00179-7)
- 350 31. Averós X, Brossard L, Dourmad JY, de Greef KH, Edge HL, Edwards SA, Meunier-Salaün MC. A  
351 meta-analysis of the combined effect of housing and environmental enrichment characteristics on the  
352 behaviour and performance of pigs. *Appl Anim Behav Sci.* 2010;127(3-4):73-85.  
353 <https://doi.org/10.1016/j.applanim.2010.09.010>
- 354 32. Luo L, Reimert I, Middelkoop A, Kemp B, Bolhuis JE. Effects of early and current environmental  
355 enrichment on behavior and growth in pigs. *Front vet sci.* 2020;7:268.  
356 <https://doi.org/10.3389/fvets.2020.00268>
- 357 33. Mendl MT. The social behaviour of non-lactating cows and its implications for managing sow  
358 aggression. *Pig Vet J.* 1994;34:9-20.

- 359 34. McGlone JJ. A quantitative ethogram of aggressive and submissive behaviors in recently regrouped  
360 pigs. *J Anim Sci.* 1985;61(3):556-566. <https://doi.org/10.2527/jas1985.613556x>
- 361 35. O'Connell NE, Beattie VE. Influence of environmental enrichment on aggressive behaviour and  
362 dominance relationships in growing pigs. *Anim Welf.* 1999;8(3):269-279.  
363 <https://doi.org/10.1017/S0962728600021758>
- 364 36. Manciooco A, Sensi M, Moscati L, Battistacci L, Laviola G, Brambilla G, Vitale A, Alleva E.  
365 Longitudinal effects of environmental enrichment on behaviour and physiology of pigs reared on an  
366 intensive-stock farm. *Ital J Anim Sci.* 2011;10(4):e52. <https://doi.org/10.4081/ijas.2011.e52>
- 367 37. Hill JD, McGlone JJ, Fullwood SD, Miller MF. Environmental enrichment influences on pig  
368 behavior, performance and meat quality. *Appl Anim Behav Sci.* 1998;57(1-2):51-68.  
369 [https://doi.org/10.1016/S0168-1591\(97\)00116-0](https://doi.org/10.1016/S0168-1591(97)00116-0)
- 370 38. Ghassemi Nejad J, Ghaffari MH, Ataallahi M, Jo JH, Lee HG. Stress concepts and applications in  
371 various matrices with a focus on hair cortisol and analytical methods. *Animals.* 2022;12(22):3096.  
372 <https://doi.org/10.3390/ani12223096>
- 373 39. van der Staay FJ, van Zutphen JA, de Ridder MM, Nordquist RE. Effects of environmental  
374 enrichment on decision-making behavior in pigs. *Appl Anim Behav Sci.* 2017;194:14-23.  
375 <https://doi.org/10.1016/j.applanim.2017.05.006>
- 376 40. Short SJ, Stalder T, Marceau K, Entringer S, Moog NK, Shirtcliff EA, Wadhwa PD, Buss C.  
377 Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and  
378 weekly urinary cortisol measures. *Psychoneuroendocrinology.* 2016;71:12-18.  
379 <https://doi.org/10.1016/j.psyneuen.2016.05.007>
- 380 41. Grimberg-Henrici CG, Vermaak P, Elizabeth Bolhuis J, Nordquist RE, van der Staay FJ. Effects of  
381 environmental enrichment on cognitive performance of pigs in a spatial holeboard discrimination  
382 task. *Anim Cogn.* 2016;19:271-283. <https://doi.org/10.1007/s10071-015-0932-7>
- 383 42. de Jong IC, Prelle IT, van de Burgwal JA, Lambooij E, Korte SM, Blokhuis HJ, Koolhaas JM.  
384 Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and  
385 the circadian rhythm in cortisol in growing pigs. *Physiol Behav.* 2000;68(4):571-578.  
386 [https://doi.org/10.1016/S0031-9384\(99\)00212-7](https://doi.org/10.1016/S0031-9384(99)00212-7)
- 387 43. Blecha F, Pollmann DS, Nichols DA. Immunologic reactions of pigs regrouped at or near weaning.  
388 *Am J Vet Res.* 1985;46(9):1934-1937. PMID: 4051298
- 389 44. Nejad JG, Ghaseminezhad M, Sung K, Hoseinzadeh F, Cabibi J, Lee J. A cortisol study; facial hair  
390 and nails. *J Steroids Horm Sci.* 2016;7(2):177. <https://doi.org/10.4172/2157-7536.1000177>

391 45. Escribano D, Ko HL, Chong Q, Llonch L, Manteca X, Llonch P. Salivary biomarkers to monitor  
392 stress due to aggression after weaning in piglets. *Res Vet Sci.* 2019;123:178-183.  
393 <https://doi.org/10.1016/j.rvsc.2019.01.014>

394  
395

ACCEPTED

## Tables and Figures

Table 1. Ethogram used for the behavioral observations

Behavior	Definition
Nosing	Nosing another part of the body of a penmate
Biting	Biting on substrates in pens
Mounting	Standing on hind legs while having front legs on another pig`s back (not the sows)
Tail biting	A pig chews, sucks or plays with another`s ears
Ear biting	A pig chews, sucks or plays with another`s tails.
Aggression	Horizontal or vertical knocking with the head or forward thrusting with the snout toward a penmate; intense mutual/individual ramming or pushing a penmate; biting a penmate, except ear or tail

ACCEPTED



Table 2. Effects of environmental enrichment on growth performance in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Phase 1 (d 0-14)						
ADG, kg	288.10 <sup>b</sup>	302.02 <sup>ab</sup>	323.69 <sup>a</sup>	316.31 <sup>a</sup>	8.58	0.002
ADFI, kg	417.13 <sup>b</sup>	429.92 <sup>ab</sup>	460.26 <sup>a</sup>	447.75 <sup>ab</sup>	12.84	0.016
G/F,	0.69	0.70	0.70	0.71	0.01	0.168
Phase 2 (d 15-28)						
ADG, kg	406.79 <sup>b</sup>	409.88 <sup>ab</sup>	414.40 <sup>ab</sup>	433.21 <sup>a</sup>	9.14	0.039
ADFI, kg	617.08 <sup>b</sup>	620.33 <sup>ab</sup>	626.43 <sup>ab</sup>	654.04 <sup>a</sup>	12.43	0.030
G/F,	0.66	0.66	0.66	0.66	0.01	0.892
Overall (d 0-28)						
ADG, kg	347.44 <sup>b</sup>	355.95 <sup>ab</sup>	369.05 <sup>a</sup>	374.76 <sup>a</sup>	7.36	0.006
ADFI, kg	517.10 <sup>b</sup>	525.13 <sup>ab</sup>	543.34 <sup>ab</sup>	550.89 <sup>a</sup>	10.36	0.014
G/F,	0.67	0.68	0.68	0.68	0.01	0.212

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain to feed ratio.

<sup>a-b</sup>means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Table 3. Effects of environmental enrichment on uniformity in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Phase 1 (d 14)						
CV	12.43 <sup>a</sup>	11.77 <sup>ab</sup>	10.86 <sup>b</sup>	10.45 <sup>b</sup>	0.53	0.006
Phase 2 (d 28)						
CV	13.49 <sup>a</sup>	12.41 <sup>ab</sup>	11.37 <sup>bc</sup>	10.50 <sup>c</sup>	0.58	<0.001
Overall (d 28)						
CV	13.49 <sup>a</sup>	12.41 <sup>ab</sup>	11.37 <sup>bc</sup>	10.50 <sup>c</sup>	0.58	<0.001

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning; CV, coefficient of variation.

<sup>a-c</sup>means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Table 4. Effects of environmental enrichment on uniformity in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Phase 1 (d 7)						
Diarrhea incidence	3.77 <sup>a</sup>	3.28 <sup>ab</sup>	2.83 <sup>b</sup>	2.80 <sup>b</sup>	0.21	<0.001
Phase 1 (d 14)						
Diarrhea incidence	2.27	2.30	2.50	2.37	0.50	0.968
Phase 2 (d 28)						
Diarrhea incidence	1.70	1.67	1.82	1.80	0.44	0.981

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning. <sup>a-b</sup> means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Table 5. Effects of environmental enrichment on behaviour in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Phase 1 (d 14)						
Nosing	9.56	10.19	10.00	9.27	0.46	0.221
Biting	6.48 <sup>a</sup>	6.08 <sup>ab</sup>	5.38 <sup>b</sup>	5.31 <sup>b</sup>	0.39	0.018
Mounting	0.63	0.56	0.58	0.54	0.22	0.984
Tail biting	4.17 <sup>a</sup>	3.63 <sup>ab</sup>	3.29 <sup>b</sup>	3.02 <sup>b</sup>	0.24	0.001
Ear biting	2.88 <sup>a</sup>	2.54 <sup>ab</sup>	2.02 <sup>b</sup>	2.04 <sup>b</sup>	0.28	0.016
Aggressive	2.52	2.23	1.77	1.83	0.29	0.056
Phase 2 (d 28)						
Nosing	5.04	4.96	5.23	5.27	0.45	0.880
Biting	1.31	1.10	1.17	1.25	0.33	0.924
Mounting	0.67	0.71	0.69	0.73	0.19	0.990
Tail biting	0.63	0.71	0.60	0.56	0.25	0.945
Ear biting	0.69	0.75	0.71	0.67	0.20	0.978
Aggressive	0.71	0.63	0.63	0.75	0.24	0.936

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning. <sup>a-b</sup>means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Table 6. Effects of environmental enrichment on skin lesion score in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Phase 1 (d 14)						
Skin lesion score	45.40 <sup>a</sup>	43.80 <sup>ab</sup>	40.74 <sup>ab</sup>	37.23 <sup>b</sup>	2.41	0.015
Phase 2 (d 28)						
Skin lesion score	78.76	75.50	75.90	78.09	3.02	0.644

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning. <sup>a-b</sup>means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

ACCEPTED

Table 7. Effects of environmental enrichment on skin lesion score in weanling pigs

Item	Control	EE-1	EE-2	EE-4	SEM	P-value
Saliva						
Cortisol	4.79	4.45	4.30	4.07	0.51	0.564
Hair						
Cortisol	76.07 <sup>a</sup>	75.10 <sup>ab</sup>	72.05 <sup>ab</sup>	68.97 <sup>b</sup>	2.40	0.032

EE-1, inclusion of play object until one week after weaning; EE-2, inclusion of play object until two weeks after weaning; EE-4, inclusion of play object until four weeks after weaning; <sup>a-b</sup>means with different superscripts in the same row differ significantly( $p < 0.05$ ).

ACCEPTED