Effects of physical enrichment and pair housing before weaning on growth, behaviour and cognitive ability of calves after weaning and regrouping

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Abstract

Housing unweaned calves individually in barren environments negatively affects their growth, cognitive ability, and adaptability to environmental changes in later life. Social housing has been shown to improve those aspects, whereas physical environmental enrichment has rarely been studied in calves. Little is known about whether the combination of both components offers further benefits. Furthermore, curiosity has been considered an intrinsic factor underpinning cognitive performance, which has yet to be determined in calves. The first objective was to compare the effects of providing physical enrichment items and pair housing calves before weaning, and their combination on the weight gain, behaviour and cognitive ability of calves once regrouped after weaning. The second objective was to investigate if calves' exploratory behaviour in a novel environment can predict their cognitive ability. Forty-eight Holstein calves were allocated to eight groups 2 days after birth. Within each group, two calves were assigned to individual pens and four to two pair pens. One individual pen and one pair pen within each group were provided with brushes, chains, teats, and nets filled with strawberry-scented hay as physical enrichment items. Remaining pens received no additional enrichment items. All calves from a group were introduced to one post-weaning pen when the youngest calf was 9 weeks of age. Calves were weighed on days 1 and 7 in post-weaning pens. They were video-recorded on days 1, 3 and 11 and behavioural data were collected. Spontaneous object recognition tests

were conducted within one week after behavioural data collection to assess cognitive ability in terms of how long after exposure calves recognised objects, indicated by differential expression of exploratory behaviour. Physical enrichment items and pair housing had interactions on average daily gain ($F_{1,33}$=5.460, p=0.026), with calves in physically enriched pair pens showing higher average daily gain than those in non-enriched pair pens and tending to show higher average daily gains than those in physically enriched individual pens. Physically enriched calves expressed more exploratory behaviour and social sniffing than non-enriched calves ($F_{1,111}$=20.691, p<0.001; $F_{1,111}$=14.433, p<0.001). Pair housed calves spent more time cross-sucking than individually housed calves ($F_{1,111}$=8.848, p=0.008). Compared with non-enriched calves, physically enriched calves were more inclined to explore the novel object than the object already presented 15-min ago (χ^2=3.282, df=1, p=0.070). There was no association between exploratory behaviour upon initial introduction to post-weaning pens and performance in object recognition tests. In conclusion, the combination of physical enrichment and pair housing improves calves' average daily gain after weaning when compared with either component alone. Physical enrichment seemed to improve calves' memory and adaptability to change, whilst pair housing did not. Calves' exploratory behaviour in novel environments may not contribute to their cognitive performance.

Keywords: pre-weaning environmental enrichment, post-weaning regrouping, average daily gain, behaviour, cognitive ability, curiosity

1. Introduction

It is a common practice on dairy farms to separate newborn calves from their dams immediately or within hours of parturition and rear them in individual pens or hutches (Mikuš et al., 2020). Thereafter, calves are weaned and moved to group pens, which must happen no later than eight weeks of age in the European Union and the United Kingdom (Council Directive 97/2/EC). At this stage, calves experience diet changes and new social and

57 physical environments (Bolt et al., 2017), all of which are considered stressors,
58 compromising their performance and welfare; signs of this include a growth check (Chua et
59 al., 2002; Vieira et al., 2010), increased undesirable social behaviours (Kerr and Wood-
60 Gush, 1987) and increased distress responses (Weary et al., 2008).
61
62 Based on the reality of periodic changes in management and environments including those
63 at regrouping after weaning, calves need to learn how to respond to and utilise complex
64 environments (Horvath, 2019). Since calves with better cognitive abilities are able to show
65 more flexible behaviour (Gaillard et al., 2014) and increased behavioural flexibility can help
66 calves better adapt to environmental changes (Horvath, 2019), calves' adaptive capacity
67 may depend on their cognitive ability. Social housing in the pre-weaning period has been
68 widely studied as a means to improve calves' capacity to adapt to environmental changes
69 since this may be a sensitive period in brain development and can impact later behavioural
70 flexibility (Meagher et al., 2015), which measures an individual's ability to adjust their
71 behaviour in response to environmental cues (Coppens et al., 2010). Calves who are
72 socially housed before weaning show higher tolerance to unfamiliar animals later in life with
73 less aggressive interactions but more non-agonistic interactions (Veissier et al., 1994). The
74 feed intake behaviours in post-weaning home pens including latency to start feeding and
75 time spent at a feeder, concentrate consumptions and weight gains are all improved by pre-
76 weaning social housing (Vieira et al., 2010).
77
78 Physical enrichment is similarly suggested to alter social skills and abilities to cope with
79 stressors (Weary et al., 2008). Studies in piglets have shown that physical enrichment
80 improved piglets' performance in a cognitive ability test (Grimberg-Henrici et al., 2016),
81 increased feed consumption in the first two days in post-weaning pens (Oostindjer et al.,
82 2010) and might reduce aggression in post-weaning pens (Kutzer et al., 2009). However, in
83 calves, the effect of pre-weaning physical enrichment on their ability to adapt to weaning and
84 regrouping has not yet been explored. Since physical enrichment may enable pre-weaning

85 calves to garner more experience dealing with external stimuli, it is expected to positively
86 impact calves' adaptive capacity in later life.
87
88 In addition, research on the combined effect of social housing and physical enrichment on
89 animals' adaptive capacity to environmental changes is growing. For example, physical
90 enrichment and social housing mitigated piglets' weaning stress and reduced their post-
91 weaning aggression (Ko et al., 2020). In calves, although combined methods have not been
92 explored, it is expected that the combination of social housing and physical enrichment may
93 further improve their cognitive ability and adaptability to environmental changes, since both
94 components may stimulate calves in different ways (Mandel et al., 2016).
95
96 The effect of different emotions on cognitive functioning is an important research area in
97 animals, which may contribute to understanding the mechanisms underlying individual
98 variation in cognitive performance (Broom, 2010). Barren environments may lead to
99 prolonged high levels of stress hormones (e.g. glucocorticoids) in livestock, which can affect
100 neurons within the hippocampus (Lupien et al., 1998). For this reason, researchers have
101 mainly focused on the impact of negative emotions induced by poor environmental
102 conditions on cognitive ability (e.g. dairy calves: Gaillard et al., 2014; broiler chickens:
103 Tahamtani et al., 2018). However, the positive emotion of curiosity is also considered an
104 intrinsic factor underpinning cognitive performance (Kidd and Hayden, 2015). In orang-utans,
105 exploratory behaviour is assessed as the indicator of curiosity to predict their problem-
106 solving abilities (Damerius et al., 2017). In young horses, exploratory behaviour towards
107 novel objects correlates with increased learning capability (Christensen et al., 2021).
108 However, in calves, whether curiosity levels are correlated with cognitive ability is still
109 unknown.
110
111 The first aim of the present study was to determine the effects of providing physical
112 enrichment items to pre-weaning calf pens, pair housing pre-weaning calves and the

combination of both components on post-weaning calves' behaviour, growth and cognitive ability. Since physical enrichment and social housing might improve calves' emotional states and cognition as well as promoting positive activities by providing different types of stimulation, it was hypothesised that 1) providing physical enrichment items and pair housing would both promote positive behaviours, such as exploratory, play, ruminating and social sniffing behaviours, and reduce undesirable behaviours, such as cross-sucking and agonistic behaviours, and also promote weight gain and performance in cognitive ability tests; 2) the combination of physical enrichment items and pair housing would have an additive effect when compared to either component alone. The second aim was to investigate associations between calves' exploratory behaviour in a novel environment and their cognitive ability. Since curiosity might underpin cognition, it was hypothesised that calves that showed more exploratory behaviour upon initial introduction to post-weaning home pens would have better performance in a cognitive ability test.

2. Materials and Methods

2.1. Animals, housing and feeding

The study was carried out at the Centre for Dairy Research, University of Reading, Reading, UK between May and November 2019 and was approved by the ethics administrator at the University and the departmental ethics coordinator. Forty-eight male registered pure Holstein calves were included from 2 days of age to 12 weeks of age. They had birth weights of 35-55 kg and were separated from dams between 24-36 hours after birth. An additional eight male registered pure Holstein calves were used for a pilot study (for details, please see 2.4.2) and were housed in standard non-enriched group pens.

For the forty-eight calves in the main study, in the first eight weeks following birth, they were reared in one of four treatments: non-enriched individual housing (n = 8 calves), physically enriched individual housing (n = 8 calves), non-enriched pair housing (n = 16 calves) and physically enriched pair housing (n = 16 calves). calves were allocated to pre-weaning

141 treatments in blocks according to their date of birth such that each housing type was
142 represented within each of eight groups (i.e. the six calves born first were assigned to the
143 first group, the next six calves born were assigned to the second group, etc). Within each
144 group, this meant that two calves were assigned into individual pens (2.4 m^2 each) and four
145 calves were assigned into two pair pens (4.8 m^2 each). One individual pen and one pair pen
146 within each group were provided with physical enrichment items: one net filled with
147 strawberry-scented ryegrass hay, one rubber teat, one plastic chain, and one stationary
148 brush for individual pens; one net filled with strawberry-scented ryegrass hay and two of all
149 other items for pair pens to reduce competition for items (see Zhang et al. 2021 for more
150 details). The aim of these items was to satisfy foraging, sucking, and grooming motivations
151 of young calves (see Mandel et al., 2016). The rest of the individual and pair pens were not
152 provided with the physical enrichment items. All pens were bedded with deep straw. All
153 calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets until
154 49 days of age. A total of 5 L/d of milk was offered to each calf until 14 days of age, followed
155 by 6 L/d from 15 to 42 days of age and 5 L/d between 43 and 49 days of age. From 50–56
156 days of age, calves received 2.5 L milk at 07:00 h only. Thereafter, calves were weaned at
157 57 days of age. In the pre-weaning and weaning period, all calves had ad libitum access to
158 concentrate (VITA concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and
159 water. Calves had auditory and visual contact with one another and limited tactile contact
160 with neighbours through the gap between the panel bars (120 mm, large enough for calves'
161 muzzles to go through the gap).
162
163 Calves within each pre-weaning group were introduced to eight post-weaning home pens
164 (six calves in each) together following the weaning of the youngest calf in the group and
165 were monitored for four weeks. The area of each post-weaning home pen is reported in
166 Table1. The lying area was bedded with deep straw and the feeding area had a concrete
167 floor. Calves had ad libitum access to total mixed ration (TMR; grass silage, maize silage
168 and concentrate) and water throughout the period.

169

170 *2.2. Growth*

171 All calves were weighed on days 1 and 7 in post-weaning home pens by a wheeled scale
172 (Ritchie Agricultural, UK). Average daily gain was calculated by averaging across the 6 days.

173

174 *2.3. Behaviours in post-weaning home pens*

175 Calves' behaviours in post-weaning home pens were recorded by CCTV (Transit-PTZ,
176 Revader Security Ltd, UK) for 24 h on days 1, 3 and 11 after initial introduction to post-
177 weaning home pens (09:00 h ± 0.5 h). Behaviours, being considered as indicators of
178 adaptive capacity, defined in Table 2 (adapted from Zhang et al., 2021), were recorded using
179 instantaneous scans at 5-min intervals by watching video recordings. The behavioural
180 frequencies in the time periods of 00:00-06:00 h and 20:00-24:00 h were not recorded since
181 calves were less active during these periods.

182

183 *2.4. Cognitive ability test*

184 The spontaneous object recognition test was used to assess memory alterations by
185 measuring the difference in exploration time of novel and familiar objects (Antunes and
186 Biala, 2012). The test consisted of observing animals in the presence of two sample objects
187 (sample phase) and the observations were repeated after a certain retention time with one of
188 the sample objects replaced by a novel one (test phase). Retention time was defined as the
189 interval between the sample phase and the test phase of the object recognition test; for
190 example, 15 min retention time meant 15 min between the two phases for the calf being
191 tested. The preference for novel object in test phase indicated that re-presentation of sample
192 object existed in animal's memory since they have natural propensity to novelty (Baxter,
193 2010; Ennaceur, 2010).

194

195 2.4.1. Experimental setup

196 In this experiment, exploratory behaviour included sniffing, sucking, licking, scratching and
197 butting the objects. A square testing arena (16 m^2) was setup with the ground being covered
198 by straw. The arena was equipped with a webcam (C525, Logitech International S.A,
199 Switzerland) to record calves' behaviour. Test objects were placed on the opposite panels of
200 the entrance. The left and right objects were both 50 cm away from the left and right corner
201 and were 90 cm away from the ground. Two sets of objects were used in this experiment,
202 with the first set of three green feeders (26 cm × 26 cm × 19 cm; GN1, GN2, GN3) and one
203 blue bottle (12 cm × 12 cm × 28 cm; BE1) and the second set of three grey feeders (35 cm ×
204 12 cm × 10 cm; GY1, GY2, GY3) and one brown pipe (11 cm × 11 cm × 38 cm; BN1). The
205 testing arena and the objects were cleaned between phases to minimise olfactory traces.
206
207 2.4.2. Experimental spatial and object bias
208 A pilot study was conducted on four consecutive days before the cognitive ability test to
209 assess spatial bias in the arena and bias towards the two sets of objects. Eight male
210 registered pure Holstein calves reared in group pens with birth weight between 35 and 55 kg
211 were used when they were 10 to 12 weeks of age. All calves were individually handled to
212 habituate to the empty arena for 20-min on day 1, followed by individually exploring two
213 identical yellow brushes (11 cm × 13 cm × 32 cm) in the arena for 15-min after 5-min
214 habituation on day 2. On days 3 and 4, calves were handled to individually explore the first
215 set of objects (GN1 vs. BE1) and the second set of objects (GY1 vs. BN1) for 15-min
216 separately after 5-min habituation. The differences of the ratio of exploratory duration
217 towards the left and right yellow brushes, towards the two different objects in the first and
218 second set were tested separately by conducting pair-samples t-tests. The results showed
219 that calves did not have spatial bias ($t = -1.20$, $p = 0.270$) and bias towards the two sets of
220 objects (set one: $t = 0.39$, $p = 0.708$; set two: $t = 0.98$, $p = 0.941$).
221
222 2.4.3. Experimental procedures

223 Each calf was tested for 15-min and 60-min retention times on two consecutive testing days.
224 Calves attended the test within one week after the recording of post-weaning home
225 pen behaviours and took turns to be tested on same testing days.
226
227 Each calf was individually handled to habituate to the empty testing arena for 20-min one
228 day before testing. On the first testing day, the following pattern was used: a testing calf
229 habituated to the empty testing arena for 5-min. Then, in the sample phase, the calf was
230 removed and two identical objects (GN1 and GN2 or GY1 and GY2; used for alternate
231 calves at alternate retention time) were placed on the panels, followed by letting the calf
232 back to the testing arena for 15-min to explore both objects. Thereafter, the calf was
233 transported back to its post-weaning home pen. The test phase was initiated after 15-min
234 retention time. Following habituation to the empty testing arena for 5-min (these 5-min were
235 included in the total retention time) a novel object and a familiar object in the same set used
236 in the sample phase (BE1 and GN3 or BN1 and GY3) was placed in the testing arena and
237 the calf was allowed 15-min to explore both objects. For each set of objects, the novel object
238 and familiar object were separately placed on the left and right side of the panel in one test
239 and changed side in the next test. After the test phase, the calf was transported back to its
240 post-weaning home pen. The procedure was repeated on the second testing day with 60-
241 min retention time.
242
243 Eight calves' videos in the sample phase were randomly chosen and watched to measure
244 the difference of the ratio of exploratory duration towards the left and right objects to ensure
245 there was no spatial bias to the testing arena by conducting pair-samples t-tests. The results
246 showed that calves did not have spatial bias ($t = -0.924$, $p = 0.386$). In the test phase, we
247 determined discrimination index [$= (T_N - T_F) / (T_N + T_F)$] as the difference between the
248 exploratory duration of the novel object (T_N) and the familiar object (T_F) divided by the sum of
249 exploratory duration of the novel and familiar objects (Ennaceur and Delacour, 1988). A
250 greater value of discrimination index indicated a better object recognition memory ability.

2.5. Associations between exploratory behaviour and cognitive performance

Calves' behavioural videos (recorded as described in Section 2.3.) were observed from initial introduction to post-weaning home pens until 20:00 h on that day and frequencies of exploratory behaviour (defined in Table 2) were recorded using instantaneous scans at 5-min intervals. The ratio of exploratory behaviour for every calf was expressed as the frequency of a calf's exploratory behaviour from initial introduction to post-weaning home pens until 20:00 h divided by the frequency of all behaviours observed during this period. Thereafter, the associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens, and discrimination indices for 15-min and 60-min retention times in cognitive ability test were measured.

2.6. Statistical analyses

Data were analysed using SPSS Statistics (version 27.0.1.0, IBM) with individual calf as statistical unit. Significant differences were declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.10$.

Calves' average daily gains were analysed by a univariate general linear model, incorporating the fixed factors of physical enrichment items, pair housing and the interaction between these two factors and the covariates of calves' birth weight and days in pre-weaning pens after weaning. A post hoc test (LSD) was carried out thereafter to identify differences among treatment means.

Calves' behavioural variables in post-weaning home pens were collected by one observer and were expressed as proportions of total scans. All variables except play were analysed by generalized linear mixed models respectively. Play was not analysed because it was rarely expressed. For the data structure, the subjects were post-weaning home pen number and calves' ID number; the repeated measure was days in post-weaning home pens. The

fixed effects were physical enrichment items, pair housing and the interaction between the two factors, post-weaning home pen number, days in post-weaning home pens, calves' birth weight and average temperature of the barn during the testing day. The random effects were calves' ID number and area of post-weaning home pens. LSD was used to undertake pairwise comparisons. Thereafter, to reduce the risk of chance significant results due to multiple testing, adjusted p-values were calculated to control the false discovery rate (Jafari and Ansari-Pour, 2019).

Calves' behavioural variables in cognitive ability test were collected by one observer. In the test phase, the variables of discrimination indices for 15-min and 60-min retention times were analysed by generalized linear models, incorporating the factors of physical enrichment items, pair housing, the interaction between these two factors, object set and location of the testing arena, and calves' birth weight and average temperature of the barn during the testing day were used as covariates in the model. Before conducting generalized linear models, calves that did not show exploratory behaviour towards novel and familiar objects in the test phase (seven calves for 15-min retention time and nine calves for 60-min retention time) were discarded from the analysis because those calves might not have learnt how to recognise the objects or they might not be motivated to explore the objects.

Associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens and discrimination indices for 15-min and 60-min retention times in the object recognition test were analysed by linear regressions, incorporating a dependent variable of discrimination indices for 15-min or 60-min retention time and independent variables of physical enrichment items, pair housing, the interaction between these two factors and the ratio of exploratory behaviour upon initial introduction to post-weaning home pens. Calves that were discarded from the analysis of cognitive ability test data were also discarded from this analysis.

To determine inter-observer reliability, another observer watched the post-weaning home pen behaviour videos of three pens by randomly choosing one pen from days 1, 3 and 11 separately. For the cognitive ability test, eight calves' test phase videos for 15-min retention time and eight calves' test phase videos for 60-min retention time were randomly selected and watched by another observer who was blind to the pre-weaning treatments. Pearson correlations were used to compare the reliability between the two observers. The results showed strong positive relationships between both observers for post-weaning home pen behaviour ($r = 0.994$, $p < 0.001$) and cognitive ability test ($r = 0.996$, $p < 0.001$).

One calf's data for all measures were discarded due to an abscess on its tongue. Another calf's data for average daily gain and behaviours in its post-weaning home pen were discarded due to diarrhoea. Owing to technical problems, the data of eight calves' post-weaning home pen behaviours on days 1 and 3 were discarded.

3. Results

3.1. Growth

Physical enrichment items and pair housing had interactions on average daily gain ($F_{1,33} = 5.433$, $p = 0.026$; Figure 1), with calves from physically enriched pair pens showing increased average daily gain compared to those from non-enriched pair pens ($p = 0.009$) and tending to show increased average daily gain than those from physically enriched individual pens ($p = 0.093$).

3.2. Behaviours in post-weaning home pens

Physically enriched calves expressed more exploratory behaviour and social sniffing than non-enriched calves ($F_{1,111} = 20.691$, adjusted $p < 0.001$; $F_{1,111} = 14.433$, adjusted $p < 0.001$; Table 3). Pair housed calves showed increased time spent lying next to familiar calves than individually housed calves ($F_{1,111} = 8.812$, adjusted $p = 0.032$). Cross-sucking behaviour was more frequent in pair housed calves than in individually housed calves ($F_{1,111} = 8.848$,

adjusted p = 0.008). There were no interactions between physical enrichment items and pair housing with respect to the incidence of natural and undesirable behaviours.

3.3. Cognitive ability test

For the 15-min retention time, physically enriched calves tended to show higher discrimination index compared to non-enriched calves (χ^2 = 3.282, df = 1, p = 0.070, Table 4). Individually and pair housed calves showed similar discrimination indices (χ^2 = 0.060, df = 1, p = 0.806). There were no interactions between physical enrichment items and pair housing with respect to discrimination index (χ^2 = 0.837, df = 1, p = 0.360).

For the 60-min retention time, non-physically and physically enriched calves showed similar discrimination indices (χ^2 = 1.242, df = 1, p = 0.265). Individually and pair housed calves showed similar discrimination indices (χ^2 = 1.130, df = 1, p = 0.288). There were no interactions between physical enrichment items and pair housing with respect to discrimination index (χ^2 = 0.706, df = 1, p = 0.401).

3.4. Associations between exploratory behaviour and cognitive performance

There were no associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens, and discrimination index for 15-min retention time in cognitive ability test ($\mathcal{B}$ coefficient ± SE: 0.092 ± 3.875; t = 0.237, p = 0.814; n = 40) and 60-min retention time in cognitive ability test ($\mathcal{B}$ coefficient ± SE: -2.894 ± 3.615; t = -0.801, p = 0.430; n = 38).

4. Discussion

Although this study could be considered as comparing enrichment types, we have not used the term ‘enrichment’ here for social housing. Environmental enrichment is a vague term in the way it is often applied in the field of applied ethology. The term implies improvements of the initial environment. In calves, environmental enrichment is intended to improve their

363 biological functioning, fulfil behavioural requirements, help cope with stressors, reduce
364 frustration, and promote positive emotions (Mandel et al., 2016). Although the initial
365 environment varies between studies, since individual housing is widely used in the dairy
366 industry, many researchers treat this as the baseline and suggest that social housing should
367 be considered a type of environmental enrichment (e.g. Bloomsmith et al., 1991; Mandel et
368 al., 2016). In contrast, others do not categorize social housing as a form of environmental
369 enrichment (e.g. Costa et al., 2016) since calves are gregarious animals, housing them in
370 groups is practice that satisfies their basic needs. In addition, the benefits of group housing
371 have been gradually accepted by an increasing number of farmers, and housing pre-
372 weaning calves in groups is becoming more popular in a number of countries. Therefore, as
373 the dairy industry develops, we would argue that social housing should not be considered a
374 type of environmental enrichment but rather a minimum standard of calves' early
375 environment.
376
377 *4.1. Growth*
378 The combination of physical enrichment items and pair housing improved or tended to
379 improve calves' average daily gain after weaning and regrouping when compared with either
380 component alone. Although in the present study frequencies of TMR intake and rumination
381 did not show statistical differences between treatments, the mean values for calves from
382 physically enriched pair pens were numerically higher than those from the other treatments.
383 Therefore, the combination of physical enrichment items and pair housing may reduce stress
384 responses towards mixing in novel environments (Kutzer et al., 2009), thus increasing feed
385 consumption and rumination resulting in improved weight gain. In contrast, neither providing
386 physical enrichment items nor pair housing to pre-weaning calves affected their weight gain
387 after weaning and regrouping. This is in agreement with studies in pigs (Oostindjer et al.,
388 2010) and in calves (Duve and Jensen, 2012). These results may be attributed to the
389 tremendous amount of stress that animals are subjected to during environmental change.
390 The sudden transformation to novel environments with new feeds and mixing with unfamiliar

391 peers may result in considerable stress for calves (Hulbert and Moisá, 2016), especially for
392 those without experiencing both social and external stimuli before and thus, this
393 overshadows the differences arising in their pre-weaning period.
394
395 *4.2. Behaviours in post-weaning home pens*
396
397 Exploration is a process of information gathering for animals (Rojas-Ferrer et al., 2020),
398 which may help animals to better control or predict new environments (Wood-Gush and
399 Vestergaard, 1993). In the present study, the expression of exploratory behaviour in post-
400 weaning home pens was promoted by pre-weaning physical enrichment. Since in pre-
401 weaning pens, the provision of additional items may attract calves' attention to explore them
402 (Zobel et al., 2017), the high exploratory motivation of physically enriched pre-weaning
403 calves may persist in post-weaning group pens. The increased expression of exploratory
404 behaviour towards peers (social sniffing) in the same post-weaning home pens for calves
405 with pre-weaning physical enrichment may also corroborate this view. By contrast, pre-
406 weaning pair housing had no effect on calves' exploratory behaviour towards post-weaning
407 home pens and peers in the same pens. This is in contrast with the finding of Jensen et al.
408 (1997), which indicated the lack of social housing early in life could delay exploratory
409 behaviour. The difference may be because in the present study individually housed pre-
410 weaning calves could have olfactory, visual and limited tactile interactions with neighbours in
411 pair pens through the bars of panels and thus, they might have acquired some social
412 experience like that of pair-housed calves.
413
414 Social lying reflects the focal animal's choice of having a social partner during rest (Duve
415 and Jensen, 2012), which may indicate high social tolerance to peers in the same pens
416 (Estevez et al., 2007). The timing of recording calves' behaviours in their post-weaning home
417 pens in the present study was selected to end on 11 after initial introduction to post-weaning
418 home pens because new social relationships can be well established and other activities

419 normally return to basic levels after this time (Bøe and Færevik, 2003; Færevik et al., 2007).
420 Although pre-weaning pair housing increased the expression of social lying in post-weaning
421 home pens towards the previously familiar calves throughout this period in the present study,
422 this does not mean they have better tolerance. Since calves reared in the same pre-weaning
423 social pens can establish strong bonds with each other (Raussi et al., 2010), the higher
424 expression of social lying in post-weaning home pens towards previously familiar calves may
425 indicate they are maintaining their strong relationships and increasing safety in novel
426 environments (Grignard et al., 2000). Færevik et al. (2007) also found that introducing calves
427 with their companions to a new group, they rested more with familiar companions in the first
428 three days after grouping. Since in the present study pre-weaning pair housing and pre-
429 weaning physical enrichment items did not affect social lying with unfamiliar calves in post-
430 weaning home pens, both methods may not have effects on calves' social capacity after
431 weaning and regrouping.
432
433 Cross-sucking, as an undesirable behaviour in calves, may result in hair loss, inflammation
434 and disease in receivers (Jensen, 2003). Since the present study illustrated that pre-weaning
435 pair housing increased the expression of cross-sucking in post-weaning home pens, the
436 higher expression of the undesirable behaviour may reflect frustration in the performing calf
437 (Costa et al, 2016), and thus may indicate poor adaptability of calves with pre-weaning social
438 experience to the weaning situation. In contrast, pre-weaning physical enrichment had no
439 effect on the expression of cross-sucking in post-weaning home pens. Although providing
440 physical enrichment items such as dry teats may redirect calves' cross-sucking motivation to
441 the items and reduce their expression of the non-nutritive oral behaviour in the pre-weaning
442 period (e.g. Newberry, 1995), yet the items obviously do not have a long-term impact on
443 reducing cross-sucking. Appropriate physical enrichment items may be needed for post-
444 weaning calves to redirect their cross-sucking motivation.
445

446 Agonistic behaviour usually refers to the negative side of social interactions in animals
447 (Chaloupková et al., 2007), which may result in a tremendous cost to economic efficiency
448 and animal welfare owing to stress and injury (Fraser and Rushen, 1987). In the present
449 study, both pre-weaning pair housing and physical enrichment had no effect on the agonistic
450 behaviour of post-weaning calves. Since calves from the different pre-weaning treatments
451 rarely expressed agonistic behaviour in post-weaning home pens, it may indicate that cattle
452 at a young age have limited motivation for resource monopolisation (Davies and Houston,
453 1984) and aggression (Veissier et al., 2001).
454
455 *4.3. Cognitive ability test*
456 In the present study, physically enriched calves tended to be better at discriminating familiar
457 and novel objects than non-enriched calves for the 15-min retention time suggesting that
458 physical enrichment might improve calves' object recognition memory ability. Pair housing
459 had no effect on the discrimination indices for the 15-min and 60-min retention times
460 indicating that pair housing might not improve calves' object recognition memory. The results
461 of effect of physical enrichment on calves' memory ability in the current study agree with
462 Martin et al. (2015) in piglets. However, the findings of pair housing not affecting calves'
463 memory ability in the current study are in contrast to those reported by Gaillard et al. (2014).
464 They demonstrated that pair housed calves showed reduced exploration in repeated object
465 recognition test, but individually housed calves did not, which indicated that only pair housed
466 calves learned to recognize the recurring object. The differences in the results of these
467 studies may be attributed to the different housing designs. In the present study, individually
468 housed pre-weaning calves had limited physical contact with neighbours, whilst in the
469 Gaillard et al. (2014) individually housed calves did not have any physical contact with
470 neighbours. Since limited physical contact between calves stimulates the expression of
471 social behaviours and reduces their fear of novel situations (Jensen and Larsen, 2014), it is
472 reasonable to deduce that individually housed calves with limited physical contact with
473 neighbours may have acquired some social experience and improved their cognitive ability.

Another potential explanation is that post-weaning group housing may reverse the deficits of brain development caused by pre-weaning individual housing. This stems from Bredy et al. (2003) who determined that the negative effects of low maternal care on rats' cognitive ability could be reversed by post-weaning environmental enrichment. Since calves can establish new social bonds with unfamiliar calves within 2 weeks after regrouping (Færevik et al., 2007) and may improve their learning ability within the short period (Lensink et al., 2006), calves may have become familiar with each other and may have improved cognitive ability before attending the cognitive ability test in the third or fourth week after regrouping.

4.4. Associations between exploratory behaviour and cognitive performance

In the present study, calves' exploratory behaviour after the initial introduction to post-weaning home pens might be considered as an indicator of curiosity. Curiosity refers to the motivation of information-seeking and is reflected in approaching and exploring novel stimuli (Damerius et al., 2017). The behaviour was only recorded from initial introduction to post-weaning home pens until 20:00 h that day because although animals have curiosity to novel information, it can be diminished when satiation occurs by continuing exposure (Kidd and Hayden, 2015). In the present study, there was no linear association between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens and discrimination indices for 15-min and 60-min retention times in the cognitive ability test. Conversely, in horses, positive associations between exploratory behaviour towards novel objects and learning performance on the tasks of visual discrimination and pressure-release have, however, been reported (Christensen et al., 2021). One of the potential reasons for the different results is the different measures of curiosity. In Christensen et al. (2021), the testing duration of exploratory behaviour towards novelty for each animal was several minutes whereas in the present study the testing duration for each calf spanned several hours. Since exploratory motivation decreases over time as focal animals progressively habituate to the novelty (e.g. Van de Weerd and Day, 2009), the measure of curiosity in the present study may be less sensitive and not reflect calves' initial curiosity levels towards the novel

environments. Moreover, the present study investigated the association between animal's exploratory behaviour and their object recognition memory, but Christensen et al. (2021) investigated the correlation between animal's exploratory behaviour and cognitive flexibility. Since cognitive flexibility is expressed as the ability to change behaviours according to the changes of environmental conditions (Nilsson et al., 2015), its fundamental process may include two executive functions, memory and inhibition, which enable individuals to adaptively control their thought and action (Buttelmann and Karbach, 2017). Therefore, it is reasonable to infer that compared with simple memory, flexibility in rule learning is a more complex form of learning. To understand the mechanisms underlying calves' cognitive performance, how exploratory behaviour affects their performance of tasks requiring cognitive flexibility needs to be further studied.

5. Conclusion

Pre-weaning physical enrichment may improve calves' memory and stimulate their exploration of new environments after weaning and regrouping. Pre-weaning pair housing, meanwhile, increased calves' sucking behaviour towards peers after weaning and regrouping. The combination of physical enrichment and social housing during pre-weaning period improved calves' growth after weaning and regrouping compared to either of these alone. Calves' exploratory behaviour in novel environments may not contribute to their cognitive performance, but this needs further confirmation by studies of associations between exploratory behaviour and more complex cognitive tasks.

Declaration of Competing Interest

None.

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532 weaning home pen behaviour and cognitive ability test to help us determine inter-observer
533 reliability.
534

535 **References**

536 Antunes, M., Biala, G., 2012. The novel object recognition memory: neurobiology, test
537 procedure, and its modifications. Cogn. Process. 13, 93-110. https://doi.org/10.1007/s10339-
538 011-0430-z.
539 Baxter M.G., 2010. "I've seen it all before": explaining age-related impairments in object
540 recognition. Theoretical comment on Burke et al. (2010). Behav. Neurosci. 124, 706-709.
541 https://doi.org/10.1037/a0021029.
542 Bloomsmith, M.A., Brent, L.Y., Schapiro, S.J., 1991. Guidelines for developing and
543 managing an environmental enrichment program for nonhuman primates. Lab. Anim. Sci.
544 41, 372-377. PMID: 1658487.
545 Bøe, K.E., Færevik, G., 2003. Grouping and social preferences in calves, heifers and cows.
546 Appl. Anim. Behav. Sci. 80, 175-190. https://doi.org/10.1016/S0168-1591(02)00217-4.
547 Bolt, S.L., Boyland, N.K., Mlynski, D.T., James, R., Croft, D.P., 2017. Pair housing of dairy
548 calves and age at pairing: effects on weaning stress, health, production and social networks.
549 PLoS One 12, e0166926. https://doi.org/10.1371/journal.pone.0166926.
550 Bredy, T., Humpartzoomian, R., Cain, D., Meaney, M., 2003. Partial reversal of the effect of
551 maternal care on cognitive function through environmental enrichment. Neuroscience 118,
552 571-576. https://doi.org/10.1016/S0306-4522(02)00918-1.
553 Broom, D.M., 2010. Cognitive ability and awareness in domestic animals and decisions
554 about obligations to animals. Appl. Anim. Behav. Sci. 126, 1-11.
555 https://doi.org/10.1016/j.applanim.2010.05.001.
556 Buttelmann, F., Karbach, J., 2017. Development and plasticity of cognitive flexibility in early
557 and middle childhood. Front. Psychol. 8, 1040. https://doi.org/10.3389/fpsyg.2017.01040.

558 Chaloupková, H., Illmann, G., Bartoš, L., Špinka, M., 2007. The effect of pre-weaning
559 housing on the play and agonistic behaviour of domestic pigs. Appl. Anim. Behav. Sci. 103,
560 25-34. https://doi.org/10.1016/j.applanim.2006.04.020.
561 Christensen, J.W., Ahrendt, L.P., Malmkvist, J., Nicol, C., 2021. Exploratory behaviour
562 towards novel objects is associated with enhanced learning in young horses. Sci. Rep. 11, 1-
563 10. https://doi.org/10.1038/s41598-020-80833-w.
564 Chua, B., Coenen, E., Van Delen, J., Weary, D., 2002. Effects of pair versus individual
565 housing on the behavior and performance of dairy calves. J. Dairy Sci. 85, 360-364.
566 https://doi.org/10.3168/jds.S0022-0302(02)74082-4.
567 Coppens, C.M., De Boer, S.F., Koolhaas, J.M., 2010. Coping styles and behavioural
568 flexibility: towards underlying mechanisms. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 365,
569 4021-4028. https://doi.org/10.1098/rstb.2010.0217.
570 Costa, J., Von Keyserlingk, M., Weary, D., 2016. Invited review: effects of group housing of
571 dairy calves on behavior, cognition, performance, and health. J. Dairy Sci. 99, 2453-2467.
572 https://doi.org/10.3168/jds.2015-10144.
573 Damerius, L.A., Graber, S.M., Willems, E.P., Van Schaik, C.P., 2017. Curiosity boosts
574 orang-utan problem-solving ability. Anim. Behav. 134, 57-70.
575 https://doi.org/10.1016/j.anbehav.2017.10.005.
576 Davies, N., Houston, A., 1984. Territory economics, in Krebs, J.R., Davies, N.B. (Eds.),
577 Behavioural Ecology: an Evolutionary Approach. Blackwell, Oxford, pp. 148–169.
578 Duve, L., Jensen, M., 2012. Social behavior of young dairy calves housed with limited or full
579 social contact with a peer. J. Dairy Sci. 95, 5936-5945. https://doi.org/10.3168/jds.2012-
580 5428.
581 Ennaceur, A., 2010. One-trial object recognition in rats and mice: methodological and
582 theoretical issues. Behav. Brain Res. 215, 244-
583 254.https://doi.org/10.1016/j.bbr.2009.12.036.

584 Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory
585 in rats. 1: Behavioral data. Behav. Brain Res. 31, 47-59. https://doi.org/10.1016/0166-
586 4328(88)90157-X.
587 Estevez, I., Andersen, I.L., Nævdal, E., 2007. Group size, density and social dynamics in
588 farm animals. Appl. Anim. Behav. Sci. 103, 185-204.
589 https://doi.org/10.1016/j.applanim.2006.05.025.
590 Færevik, G., Andersen, I.L., Jensen, M.B., Bøe, K.E., 2007. Increased group size reduces
591 conflicts and strengthens the preference for familiar group mates after regrouping of weaned
592 dairy calves (Bos taurus). Appl. Anim. Behav. Sci. 108, 215-228.
593 https://doi.org/10.1016/j.applanim.2007.01.010.
594 Fraser, D., Rushen, J., 1987. Aggressive behavior. Vet. Clin. North Am. Food Anim. Pract. 3,
595 285-305. https://doi.org/10.1016/S0749-0720(15)31153-1.
596 Gaillard, C., Meagher, R.K., Von Keyserlingk, M.A., Weary, D.M., 2014. Social housing
597 improves dairy calves' performance in two cognitive tests. PLoS One 9, e90205.
598 https://doi.org/10.1371/journal.pone.0090205.
599 Grignard, L., Boissy, A., Boivin, X., Garel, J., Le Neindre, P., 2000. The social environment
600 influences the behavioural responses of beef cattle to handling. Appl. Anim. Behav. Sci. 68,
601 1-11. https://doi.org/10.1016/S0168-1591(00)00085-X.
602 Grimberg-Henrici, C.G., Vermaak, P., Bolhuis, J.E., Nordquist, R.E., Van der Staay, F.J.,
603 2016. Effects of environmental enrichment on cognitive performance of pigs in a spatial
604 holeboard discrimination task. Anim. Cogn. 19, 271-283. https://doi.org/10.1007/s10071-015-
605 0932-7.
606 Horvath, K.C., 2019. Influences of the early environment on behavioral development,
607 cognition, and performance in dairy calves, University of Florida.
608 Hulbert, L.E. and Moisá, S.J., 2016. Stress, immunity, and the management of calves. J.
609 Dairy Sci. 99, 3199-3216. http://dx.doi.org/10.3168/jds.2015-10198.
610 Jafari, M., Ansari-Pour, N., 2019. Why, when and how to adjust your P values? Cell J. 20,
611 604. https://doi.org/10.22074/cellj.2019.5992.

612 Jensen, M.B., 2003. The effects of feeding method, milk allowance and social factors on milk
613 feeding behaviour and cross-sucking in group housed dairy calves. Appl. Anim. Behav. Sci.
614 80, 191-206. https://doi.org/10.1016/S0168-1591(02)00216-2.
615 Jensen, M.B., Larsen, L.E., 2014. Effects of level of social contact on dairy calf behavior and
616 health. J. Dairy Sci. 97, 5035-5044. https://doi.org/10.3168/jds.2013-7311.
617 Jensen, M.B., Vestergaard, K.S., Krohn, C.C., 1998. Play behaviour in dairy calves kept in
618 pens: the effect of social contact and space allowance. Appl. Anim. Behav. Sci. 56, 97-108.
619 https://doi.org/10.1016/S0168-1591(97)00106-8.
620 Jensen, M.B., Vestergaard, K.S., Krohn, C.C., Munksgaard, L., 1997. Effect of single versus
621 group housing and space allowance on responses of calves during open-field tests. Appl.
622 Anim. Behav. Sci. 54, 109-121. https://doi.org/10.1016/S0168-1591(96)01183-5.
623 Kerr, S., Wood-Gush, D., 1987. The development of behaviour patterns and temperament in
624 dairy heifers. Behav. Processes 15, 1-16. https://doi.org/10.1016/0376-6357(87)90029-5.
625 Kidd, C., Hayden, B.Y., 2015. The psychology and neuroscience of curiosity. Neuron 88,
626 449-460. https://doi.org/10.1016/j.neuron.2015.09.010.
627 Ko, H.L., Chong, Q., Escribano, D., Camerlink, I., Manteca, X., Llonch, P., 2020. Pre-
628 weaning socialization and environmental enrichment affect life-long response to regrouping
629 in commercially-reared pigs. Appl. Anim. Behav. Sci. 229, 105044.
630 https://doi.org/10.1016/j.applanim.2020.105044.
631 Kutzer, T., Bünger, B., Kjaer, J.B., Schrader, L., 2009. Effects of early contact between non-
632 littermate piglets and of the complexity of farrowing conditions on social behaviour and
633 weight gain. Appl. Anim. Behav. Sci. 121, 16-24.
634 https://doi.org/10.1016/j.applanim.2009.08.004.
635 Lensink, J., Veissier, I., Boissy, A., 2006. Enhancement of performances in a learning task in
636 suckler calves after weaning and relocation: Motivational versus cognitive control?: A pilot
637 study. Appl. Anim. Behav. Sci. 100, 171-181. https://doi.org/10.1016/j.applanim.2005.11.021.
638 Lupien, S.J., De Leon, M., De Santi, S., Convit, A., Tarshish, C., Nair, N.P.V., Thakur, M.,
639 McEwen, B.S., Hauger, R.L., Meaney, M.J., 1998. Cortisol levels during human aging predict

640 hippocampal atrophy and memory deficits. Nat. Neurosci. 1, 69-73.
641 https://doi.org/10.1038/271.
642 Mandel, R., Whay, H.R., Klement, E., Nicol, C.J., 2016. Invited review: Environmental
643 enrichment of dairy cows and calves in indoor housing. J. Dairy Sci. 99, 1695-1715.
644 https://doi.org/10.3168/jds.2015-9875.
645 Martin, J.E., Ison, S.H., Baxter, E.M., 2015. The influence of neonatal environment on piglet
646 play behaviour and post-weaning social and cognitive development. Appl. Anim. Behav. Sci.
647 163, 69-79. https://doi.org/10.1016/j.applanim.2014.11.022.
648 Meagher, R.K., Daros, R.R., Costa, J.H., Von Keyserlingk, M.A., Hötzel, M.J., Weary, D.M.,
649 2015. Effects of degree and timing of social housing on reversal learning and response to
650 novel objects in dairy calves. PLoS One 10, e0132828.
651 https://doi.org/10.1371/journal.pone.0132828.
652 Mikuš, T., Marzel, R., Mikuš, O., 2020. Early weaning: new insights on an ever-persistent
653 problem in the dairy industry. J. Dairy Res. 87, 88-92.
654 https://doi.org/10.1017/S0022029920000503.
655 Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of
656 captive environments. Appl. Anim. Behav. Sci. 44, 229-243. https://doi.org/10.1016/0168-
657 1591(95)00616-Z.
658 Nilsson, S.R., Alsiö, J., Somerville, E.M., Clifton, P.G., 2015. The rat's not for turning:
659 Dissociating the psychological components of cognitive inflexibility. Neurosci. Biobehav.
660 Rev. 56, 1-14. https://doi.org/10.1016/j.neubiorev.2015.06.015.
661 Oostindjer, M., Bolhuis, J., Mendl, M., Held, S., Gerrits, W., Van den Brand, H., Kemp, B.,
662 2010. Effects of environmental enrichment and loose housing of lactating sows on piglet
663 performance before and after weaning. J. Anim. Sci. 88, 3554-3562.
664 https://doi.org/10.2527/jas.2010-2940.
665 Raussi, S., Niskanen, S., Siivonen, J., Hänninen, L., Hepola, H., Jauhiainen, L., Veissier, I.,
666 2010. The formation of preferential relationships at early age in cattle. Behav. Processes 84,
667 726-731. https://doi.org/10.1016/j.beproc.2010.05.005.

668 Rojas-Ferrer, I., Thompson, M.J., Morand-Ferron, J., 2020. Is exploration a metric for
669 information gathering? Attraction to novelty and plasticity in black-capped
670 chickadees. Ethology 126, 383-392. https://doi.org/10.1111/eth.12982.
671 Tahamtani, F.M., Pedersen, I.J., Toinon, C., Riber, A.B., 2018. Effects of environmental
672 complexity on fearfulness and learning ability in fast growing broiler chickens. Appl. Anim.
673 Behav. Sci. 207, 49-56. https://doi.org/10.1016/j.applanim.2018.04.005.
674 Van de Weerd, H.A. and Day, J.E., 2009. A review of environmental enrichment for pigs
675 housed in intensive housing systems. Appl. Anim. Behav. Sci. 116, 1-20.
676 https://doi.org/10.1016/j.applanim.2008.08.001.
677 Veissier, I., Boissy, A., De Passillé, A.M., Rushen, J., Van Reenen, C., Roussel, S.,
678 Andanson, S., Pradel, P., 2001. Calves' responses to repeated social regrouping and
679 relocation. J. Anim. Sci. 79, 2580-2593. https://doi.org/10.2527/2001.79102580x.
680 Veissier, I., Gesmier, V., Le Neindre, P., Gautier, J.Y., Bertrand, G., 1994. The effects of
681 rearing in individual crates on subsequent social behaviour of veal calves. Appl. Anim.
682 Behav. Sci. 41, 199-210. https://doi.org/10.1016/0168-1591(94)90023-X.
683 Vieira, A.D.P., Von Keyserlingk, M., Weary, D., 2010. Effects of pair versus single housing
684 on performance and behavior of dairy calves before and after weaning from milk. J. Dairy
685 Sci. 93, 3079-3085. https://doi.org/10.3168/jds.2009-2516.
686 Weary, D.M., Jasper, J., Hötzel, M.J., 2008. Understanding weaning distress. Appl. Anim.
687 Behav. Sci. 110, 24-41. https://doi.org/10.1016/j.applanim.2007.03.025.
688 Wood-Gush, D.G.M., Vestergaard, K., 1993. Inquisitive exploration in pigs. Anim. Behav. 45,
689 185-187. https://doi.org/10.1006/anbe.1993.1017.
690 Zhang, C., Juniper, D.T., Meagher, R.K., 2021. Effects of physical enrichment items and
691 social housing on calves' growth, behaviour and response to novelty. Appl. Anim. Behav.
692 Sci. 105295. https://doi.org/10.1016/j.applanim.2021.105295.
693 Zobel, G., Neave, H.W., Henderson, H.V., Webster, J., 2017. Calves use an automated
694 brush and a hanging rope when pair-housed. Animals 7, 84.
695 https://doi.org/10.3390/ani7110084.

Tables

Table 1. Area sizes (m^2) of eight post-weaning home pens

Sector	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8
Lying area	67.2	67.2	67.2	67.2	42.7	24.5	24.5	24.5
Feeding area	28.8	28.8	28.8	28.8	18.3	28.5	10.5	10.5
Whole area	96.0	96.0	96.0	96.0	61.0	53.0	35.0	35.0

Table 2. Ethogram of behaviours in post-weaning home pens (adapted from Zhang et al., 2021)

Behaviour	Definition
TMR intake	Heading through the feed barrier and chewing
Ruminating	Chewing without TMR and straw
Exploring	Sniffing, licking or sucking ground or any fixture in the pen
Play	Engaging in a gallop, leap, jump, buck, kick or turn, putting the forelegs on other calves' back or rubbing the forehead against other calves' forehead without pushing (Veissier et al., 1994; Jensen et al., 1998)
Fixture scratching	Putting head, neck or body in contact with any fixture in the pen and slightly moving back and forth or up and down
Social sniffing	Putting muzzle in contact with or less than one muzzle length from other calves with neck not relaxed
Allogrooming	Putting tongue out of mouth and in contact with head, neck or body of other calves
Lying next to familiar calves	Lying down with the head's distance to any lying calves who were companions or neighbours in pre-weaning pens being less than 30 cm (Færevik et al., 2007)

Lying next to unfamiliar calves	Lying down with the head's distance to any lying calves who were not companions and neighbours in pre-weaning pens being less than 30 cm (Færevik et al., 2007)
Cross-sucking	Sucking or biting toward ear, mouth, navel, scrotum, prepuce, or other body parts of other calves
Agonistic behaviour	Pushing, butting or chasing other calves, or displacing other calves from their feeding places or lying places (Færevik et al., 2007)
Other behaviours	Such as lying alone, standing, walking and drinking water

700

701 **Table 3.** Variables in post-weaning home pens (mean ± SE) analysed using generalized linear mixed models. Sample sizes were pre-weaning
702 physical enrichment PE, n = 22 calves; pre-weaning non-physical enrichment NPE, n = 24 calves; pre-weaning pair housing PP, n = 31 calves
703 and pre-weaning individual housing IP, n = 15 calves

Variables	Mean ± SE		Adjusted p-value[1]	Mean ± SE		Adjusted p-value[1]
	PE	NPE	PE vs. NPE	PP	IP	PP vs. IP
TMR intake (%^{2})	21.3 ± 1.8	21.9 ± 1.7	0.775	22.2 ± 1.6	21.0 ± 1.8	1.104
Ruminating (%^{2})	20.9 ± 1.4	18.9 ± 1.4	0.429	19.5 ± 1.3	20.3 ± 1.5	0.817
Exploring (%^{2})	7.5 ± 0.3	5.5 ± 0.3	<0.001*	6.9 ± 0.3	6.1 ± 0.4	0.372
Social sniffing (%^{2})	1.3 ± 0.2	0.6 ± 0.2	<0.001*	0.9 ± 0.1	1.0 ± 0.2	0.931
Fixture scratching (%^{2})	0.2 ± 0.1	0.3 ± 0.1	0.691	0.2 ± 0.1	0.2 ± 0.1	1.152
Allogrooming (%^{2})	0.3 ± 0.1	0.1 ± 0.1	0.414	0.2 ± 0.1	0.2 ± 0.1	0.991
Lying next to familiar calves (%^{2})	10.5 ± 1.3	9.3 ± 1.3	0.632	12.0 ± 1.1	7.8 ± 1.4	0.032*
Lying next to unfamiliar calves (%^{2})	6.0 ± 1.9	6.3 ± 1.9	0.854	6.6 ± 1.8	5.8 ± 2.0	0.947
Cross-sucking (%^{2})	1.4 ± 0.5	1.4 ± 0.5	0.839	1.8 ± 0.4	1.0 ± 0.5	0.008*

Agonistic behaviour (%[2])	0.1 ± 0.1	0.1 ± 0.1	1.302	0.1 ± 0.1	0.1 ± 0.1	0.783

[1]Adjusted p-values were calculated using false discovery rate (FDR).

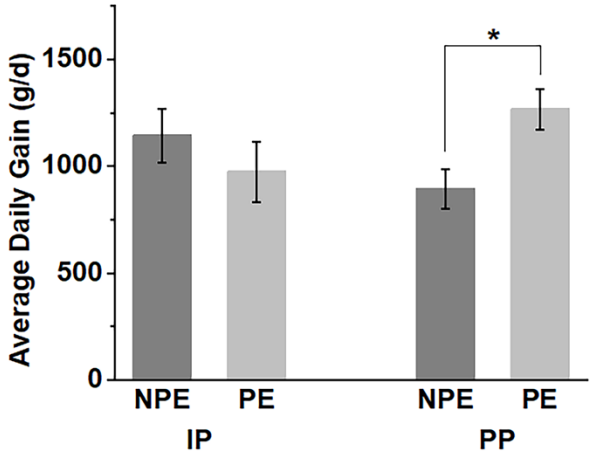
[2]% indicated percentage of observations.

*Indicated a significant difference.

Table 4. Discrimination indices (mean ± SE) of post-weaning calves for 15-min and 60-min retention times analysed using generalized linear mixed models. Sample sizes for 15-min retention time were pre-weaning physical enrichment PE, n = 21 calves; pre-weaning non-physical enrichment NPE, n = 19 calves; pre-weaning pair housing PP, n = 27 calves and pre-weaning individual housing IP, n = 13 calves; sample sizes for 60-min retention time were pre-weaning physical enrichment PE, n = 19 calves; pre-weaning non-physical enrichment NPE, n = 19 calves; pre-weaning pair housing PP, n = 25 calves and pre-weaning individual housing IP, n = 13 calves

Variables	Mean ± SE		p-value	Mean ± SE		p-value
	PE	NPE	PE vs. NPE	PP	IP	PP vs. IP
15-min	0.191 ± 0.189	-0.190 ± 0.206	0.070	0.026 ± 0.172	-0.025 ± 0.220	0.806
60-min	0.282 ± 0.145	0.052 ± 0.142	0.265	0.276 ± 0.122	0.059 ± 0.160	0.288

Figure caption



715

716 **Figure 1.** Means (± SE) of average daily gain measured on days 1 and 7 in post-weaning home pens for calves from pre-weaning non-enriched
717 individual pens (NPE-IP; n = 8 calves), pre-weaning physically enriched individual pens (PE-IP; n = 7 calves), pre-weaning non-enriched pair
718 pens (NPE-PP; n = 16 calves) and pre-weaning physically enriched pair pens (PE-PP; n = 15 calves). Asterisk (*) indicated a significant
719 difference.

720