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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 ($\chi^2=3.282$, $df=1$, $p=0.070$). 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

physical environments (Bolt et al., 2017), all of which are considered stressors, compromising their performance and welfare; signs of this include a growth check (Chua et al., 2002; Vieira et al., 2010), increased undesirable social behaviours (Kerr and Wood-Gush, 1987) and increased distress responses (Weary et al., 2008).

Based on the reality of periodic changes in management and environments including those at regrouping after weaning, calves need to learn how to respond to and utilise complex environments (Horvath, 2019). Since calves with better cognitive abilities are able to show more flexible behaviour (Gaillard et al., 2014) and increased behavioural flexibility can help calves better adapt to environmental changes (Horvath, 2019), calves' adaptive capacity may depend on their cognitive ability. Social housing in the pre-weaning period has been widely studied as a means to improve calves' capacity to adapt to environmental changes since this may be a sensitive period in brain development and can impact later behavioural flexibility (Meagher et al., 2015), which measures an individual's ability to adjust their behaviour in response to environmental cues (Coppens et al., 2010). Calves who are socially housed before weaning show higher tolerance to unfamiliar animals later in life with less aggressive interactions but more non-agonistic interactions (Veissier et al., 1994). The feed intake behaviours in post-weaning home pens including latency to start feeding and time spent at a feeder, concentrate consumptions and weight gains are all improved by pre-weaning social housing (Vieira et al., 2010).

Physical enrichment is similarly suggested to alter social skills and abilities to cope with stressors (Weary et al., 2008). Studies in piglets have shown that physical enrichment improved piglets' performance in a cognitive ability test (Grimberg-Henrici et al., 2016), increased feed consumption in the first two days in post-weaning pens (Oostindjer et al., 2010) and might reduce aggression in post-weaning pens (Kutzer et al., 2009). However, in calves, the effect of pre-weaning physical enrichment on their ability to adapt to weaning and regrouping has not yet been explored. Since physical enrichment may enable pre-weaning

calves to garner more experience dealing with external stimuli, it is expected to positively impact calves' adaptive capacity in later life.

In addition, research on the combined effect of social housing and physical enrichment on animals' adaptive capacity to environmental changes is growing. For example, physical enrichment and social housing mitigated piglets' weaning stress and reduced their post-weaning aggression (Ko et al., 2020). In calves, although combined methods have not been explored, it is expected that the combination of social housing and physical enrichment may further improve their cognitive ability and adaptability to environmental changes, since both components may stimulate calves in different ways (Mandel et al., 2016).

The effect of different emotions on cognitive functioning is an important research area in animals, which may contribute to understanding the mechanisms underlying individual variation in cognitive performance (Broom, 2010). Barren environments may lead to prolonged high levels of stress hormones (e.g. glucocorticoids) in livestock, which can affect neurons within the hippocampus (Lupien et al., 1998). For this reason, researchers have mainly focused on the impact of negative emotions induced by poor environmental conditions on cognitive ability (e.g. dairy calves: Gaillard et al., 2014; broiler chickens: Tahamtani et al., 2018). However, the positive emotion of curiosity is also considered an intrinsic factor underpinning cognitive performance (Kidd and Hayden, 2015). In orang-utans, exploratory behaviour is assessed as the indicator of curiosity to predict their problem-solving abilities (Damerius et al., 2017). In young horses, exploratory behaviour towards novel objects correlates with increased learning capability (Christensen et al., 2021). However, in calves, whether curiosity levels are correlated with cognitive ability is still unknown.

The first aim of the present study was to determine the effects of providing physical 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

treatments in blocks according to their date of birth such that each housing type was represented within each of eight groups (i.e. the six calves born first were assigned to the first group, the next six calves born were assigned to the second group, etc). Within each group, this meant that two calves were assigned into individual pens (2.4 m² each) and four calves were assigned into two pair pens (4.8 m² each). One individual pen and one pair pen within each group were provided with physical enrichment items: one net filled with strawberry-scented ryegrass hay, one rubber teat, one plastic chain, and one stationary brush for individual pens; one net filled with strawberry-scented ryegrass hay and two of all other items for pair pens to reduce competition for items (see Zhang et al. 2021 for more details). The aim of these items was to satisfy foraging, sucking, and grooming motivations of young calves (see Mandel et al., 2016). The rest of the individual and pair pens were not provided with the physical enrichment items. All pens were bedded with deep straw. All calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets until 49 days of age. A total of 5 L/d of milk was offered to each calf until 14 days of age, followed 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 days of age, calves received 2.5 L milk at 07:00 h only. Thereafter, calves were weaned at 57 days of age. In the pre-weaning and weaning period, all calves had ad libitum access to concentrate (VITA concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and water. Calves had auditory and visual contact with one another and limited tactile contact with neighbours through the gap between the panel bars (120 mm, large enough for calves' muzzles to go through the gap).

Calves within each pre-weaning group were introduced to eight post-weaning home pens (six calves in each) together following the weaning of the youngest calf in the group and were monitored for four weeks. The area of each post-weaning home pen is reported in Table1. The lying area was bedded with deep straw and the feeding area had a concrete floor. Calves had ad libitum access to total mixed ration (TMR; grass silage, maize silage and concentrate) and water throughout the period.

2.2. *Growth*

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

2.3. *Behaviours in post-weaning home pens*

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

2.4. *Cognitive ability test*

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

2.4.1. *Experimental setup*

In this experiment, exploratory behaviour included sniffing, sucking, licking, scratching and butting the objects. A square testing arena (16 m²) was setup with the ground being covered by straw. The arena was equipped with a webcam (C525, Logitech International S.A, Switzerland) to record calves' behaviour. Test objects were placed on the opposite panels of the entrance. The left and right objects were both 50 cm away from the left and right corner and were 90 cm away from the ground. Two sets of objects were used in this experiment, with the first set of three green feeders (26 cm × 26 cm × 19 cm; GN1, GN2, GN3) and one blue bottle (12 cm × 12 cm × 28 cm; BE1) and the second set of three grey feeders (35 cm × 12 cm × 10 cm; GY1, GY2, GY3) and one brown pipe (11 cm × 11 cm × 38 cm; BN1). The testing arena and the objects were cleaned between phases to minimise olfactory traces.

2.4.2. Experimental spatial and object bias

A pilot study was conducted on four consecutive days before the cognitive ability test to assess spatial bias in the arena and bias towards the two sets of objects. Eight male registered pure Holstein calves reared in group pens with birth weight between 35 and 55 kg were used when they were 10 to 12 weeks of age. All calves were individually handled to habituate to the empty arena for 20-min on day 1, followed by individually exploring two identical yellow brushes (11 cm × 13 cm × 32 cm) in the arena for 15-min after 5-min habituation on day 2. On days 3 and 4, calves were handled to individually explore the first set of objects (GN1 vs. BE1) and the second set of objects (GY1 vs. BN1) for 15-min separately after 5-min habituation. The differences of the ratio of exploratory duration towards the left and right yellow brushes, towards the two different objects in the first and second set were tested separately by conducting pair-samples t-tests. The results showed that calves did not have spatial bias ($t = -1.20$, $p = 0.270$) and bias towards the two sets of objects (set one: $t = 0.39$, $p = 0.708$; set two: $t = 0.98$, $p = 0.941$).

2.4.3. Experimental procedures

Each calf was tested for 15-min and 60-min retention times on two consecutive testing days. Calves attended the test within one week after the recording of post-weaning home pen behaviours and took turns to be tested on same testing days.

Each calf was individually handled to habituate to the empty testing arena for 20-min one day before testing. On the first testing day, the following pattern was used: a testing calf habituated to the empty testing arena for 5-min. Then, in the sample phase, the calf was removed and two identical objects (GN1 and GN2 or GY1 and GY2; used for alternate calves at alternate retention time) were placed on the panels, followed by letting the calf back to the testing arena for 15-min to explore both objects. Thereafter, the calf was transported back to its post-weaning home pen. The test phase was initiated after 15-min retention time. Following habituation to the empty testing arena for 5-min (these 5-min were included in the total retention time) a novel object and a familiar object in the same set used in the sample phase (BE1 and GN3 or BN1 and GY3) was placed in the testing arena and the calf was allowed 15-min to explore both objects. For each set of objects, the novel object and familiar object were separately placed on the left and right side of the panel in one test and changed side in the next test. After the test phase, the calf was transported back to its post-weaning home pen. The procedure was repeated on the second testing day with 60-min retention time.

Eight calves' videos in the sample phase were randomly chosen and watched to measure the difference of the ratio of exploratory duration towards the left and right objects to ensure there was no spatial bias to the testing arena by conducting pair-samples t-tests. The results showed that calves did not have spatial bias ($t = -0.924$, $p = 0.386$). In the test phase, we determined discrimination index $[= (T_N - T_F) / (T_N + T_F)]$ as the difference between the exploratory duration of the novel object (T_N) and the familiar object (T_F) divided by the sum of exploratory duration of the novel and familiar objects (Ennaceur and Delacour, 1988). A 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 ($\chi^2 = 3.282$, $df = 1$, $p = 0.070$, Table 4). Individually and pair housed calves showed similar discrimination indices ($\chi^2 = 0.060$, $df = 1$, $p = 0.806$). There were no interactions between physical enrichment items and pair housing with respect to discrimination index ($\chi^2 = 0.837$, $df = 1$, $p = 0.360$).

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

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 (β coefficient \pm SE: 0.092 ± 3.875 ; $t = 0.237$, $p = 0.814$; $n = 40$) and 60-min retention time in cognitive ability test (β coefficient \pm 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

biological functioning, fulfil behavioural requirements, help cope with stressors, reduce frustration, and promote positive emotions (Mandel et al., 2016). Although the initial environment varies between studies, since individual housing is widely used in the dairy industry, many researchers treat this as the baseline and suggest that social housing should be considered a type of environmental enrichment (e.g. Bloomsmith et al., 1991; Mandel et al., 2016). In contrast, others do not categorize social housing as a form of environmental enrichment (e.g. Costa et al., 2016) since calves are gregarious animals, housing them in groups is practice that satisfies their basic needs. In addition, the benefits of group housing have been gradually accepted by an increasing number of farmers, and housing pre-weaning calves in groups is becoming more popular in a number of countries. Therefore, as the dairy industry develops, we would argue that social housing should not be considered a type of environmental enrichment but rather a minimum standard of calves' early environment.

4.1. Growth

The combination of physical enrichment items and pair housing improved or tended to improve calves' average daily gain after weaning and regrouping when compared with either component alone. Although in the present study frequencies of TMR intake and rumination did not show statistical differences between treatments, the mean values for calves from physically enriched pair pens were numerically higher than those from the other treatments. Therefore, the combination of physical enrichment items and pair housing may reduce stress responses towards mixing in novel environments (Kutzer et al., 2009), thus increasing feed consumption and rumination resulting in improved weight gain. In contrast, neither providing physical enrichment items nor pair housing to pre-weaning calves affected their weight gain after weaning and regrouping. This is in agreement with studies in pigs (Oostindjer et al., 2010) and in calves (Duve and Jensen, 2012). These results may be attributed to the tremendous amount of stress that animals are subjected to during environmental change. The sudden transformation to novel environments with new feeds and mixing with unfamiliar

peers may result in considerable stress for calves (Hulbert and Moisé, 2016), especially for those without experiencing both social and external stimuli before and thus, this overshadows the differences arising in their pre-weaning period.

4.2. Behaviours in post-weaning home pens

Exploration is a process of information gathering for animals (Rojas-Ferrer et al., 2020), which may help animals to better control or predict new environments (Wood-Gush and Vestergaard, 1993). In the present study, the expression of exploratory behaviour in post-weaning home pens was promoted by pre-weaning physical enrichment. Since in pre-weaning pens, the provision of additional items may attract calves' attention to explore them (Zobel et al., 2017), the high exploratory motivation of physically enriched pre-weaning calves may persist in post-weaning group pens. The increased expression of exploratory behaviour towards peers (social sniffing) in the same post-weaning home pens for calves with pre-weaning physical enrichment may also corroborate this view. By contrast, pre-weaning pair housing had no effect on calves' exploratory behaviour towards post-weaning home pens and peers in the same pens. This is in contrast with the finding of Jensen et al. (1997), which indicated the lack of social housing early in life could delay exploratory behaviour. The difference may be because in the present study individually housed pre-weaning calves could have olfactory, visual and limited tactile interactions with neighbours in pair pens through the bars of panels and thus, they might have acquired some social experience like that of pair-housed calves.

Social lying reflects the focal animal's choice of having a social partner during rest (Duve and Jensen, 2012), which may indicate high social tolerance to peers in the same pens (Estevez et al., 2007). The timing of recording calves' behaviours in their post-weaning home pens in the present study was selected to end on 11 after initial introduction to post-weaning home pens because new social relationships can be well established and other activities

normally return to basic levels after this time (Bøe and Færevik, 2003; Færevik et al., 2007). Although pre-weaning pair housing increased the expression of social lying in post-weaning home pens towards the previously familiar calves throughout this period in the present study, this does not mean they have better tolerance. Since calves reared in the same pre-weaning social pens can establish strong bonds with each other (Raussi et al., 2010), the higher expression of social lying in post-weaning home pens towards previously familiar calves may indicate they are maintaining their strong relationships and increasing safety in novel environments (Grignard et al., 2000). Færevik et al. (2007) also found that introducing calves with their companions to a new group, they rested more with familiar companions in the first three days after grouping. Since in the present study pre-weaning pair housing and pre-weaning physical enrichment items did not affect social lying with unfamiliar calves in post-weaning home pens, both methods may not have effects on calves' social capacity after weaning and regrouping.

Cross-sucking, as an undesirable behaviour in calves, may result in hair loss, inflammation and disease in receivers (Jensen, 2003). Since the present study illustrated that pre-weaning pair housing increased the expression of cross-sucking in post-weaning home pens, the higher expression of the undesirable behaviour may reflect frustration in the performing calf (Costa et al, 2016), and thus may indicate poor adaptability of calves with pre-weaning social experience to the weaning situation. In contrast, pre-weaning physical enrichment had no effect on the expression of cross-sucking in post-weaning home pens. Although providing physical enrichment items such as dry teats may redirect calves' cross-sucking motivation to the items and reduce their expression of the non-nutritive oral behaviour in the pre-weaning period (e.g. Newberry, 1995), yet the items obviously do not have a long-term impact on reducing cross-sucking. Appropriate physical enrichment items may be needed for post-weaning calves to redirect their cross-sucking motivation.

Agonistic behaviour usually refers to the negative side of social interactions in animals (Chaloupková et al., 2007), which may result in a tremendous cost to economic efficiency and animal welfare owing to stress and injury (Fraser and Rushen, 1987). In the present study, both pre-weaning pair housing and physical enrichment had no effect on the agonistic behaviour of post-weaning calves. Since calves from the different pre-weaning treatments rarely expressed agonistic behaviour in post-weaning home pens, it may indicate that cattle at a young age have limited motivation for resource monopolisation (Davies and Houston, 1984) and aggression (Veissier et al., 2001).

4.3. *Cognitive ability test*

In the present study, physically enriched calves tended to be better at discriminating familiar and novel objects than non-enriched calves for the 15-min retention time suggesting that physical enrichment might improve calves' object recognition memory ability. Pair housing had no effect on the discrimination indices for the 15-min and 60-min retention times indicating that pair housing might not improve calves' object recognition memory. The results of effect of physical enrichment on calves' memory ability in the current study agree with Martin et al. (2015) in piglets. However, the findings of pair housing not affecting calves' memory ability in the current study are in contrast to those reported by Gaillard et al. (2014). They demonstrated that pair housed calves showed reduced exploration in repeated object recognition test, but individually housed calves did not, which indicated that only pair housed calves learned to recognize the recurring object. The differences in the results of these studies may be attributed to the different housing designs. In the present study, individually housed pre-weaning calves had limited physical contact with neighbours, whilst in the Gaillard et al. (2014) individually housed calves did not have any physical contact with neighbours. Since limited physical contact between calves stimulates the expression of social behaviours and reduces their fear of novel situations (Jensen and Larsen, 2014), it is reasonable to deduce that individually housed calves with limited physical contact with 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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696 **Tables**697 **Table 1.** Area sizes (m²) 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

698

699 **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

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

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

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

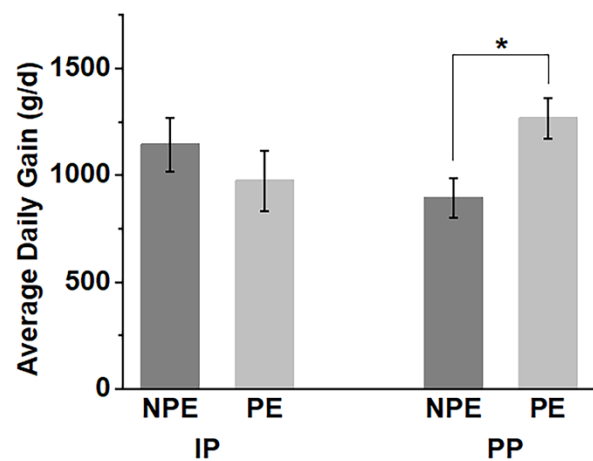
²% indicated percentage of observations.

*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 (\pm 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