

Effects of physical enrichment items and social housing on calves' growth, behaviour and response to novelty

Article

Accepted Version

Zhang, C., Juniper, D. T. and Meagher, R. K. (2021) Effects of physical enrichment items and social housing on calves' growth, behaviour and response to novelty. Applied Animal Behaviour Science, 237. 105295. ISSN 0168-1591 doi: 10.1016/j.applanim.2021.105295 Available at https://centaur.reading.ac.uk/96709/

It is advisable to refer to the publisher's version if you intend to cite from the work. See <u>Guidance on citing</u>.

To link to this article DOI: http://dx.doi.org/10.1016/j.applanim.2021.105295

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur



CentAUR

Central Archive at the University of Reading Reading's research outputs online

1 Effects of physical enrichment items and social housing on calves' growth, 2 behaviour and response to novelty Chenyu Zhanga, Darren T. Junipera, Rebecca K. Meaghera, b,* 3 4 ^a School of Agriculture, Policy and Development, University of Reading, Reading, RG6 5 6EU, UK 6 ^b Department of Animal Science and Aquaculture, Dalhousie University, Truro, NS, 7 B2N 5E3, Canada 8 *Corresponding author. E-mail address: Rebecca.Meagher@dal.ca. 9 10 **Highlights** 11 • Access to physical items (e.g. brush, teat) improved growth; pair housing did not. 12 Physical items reduced undesirable behaviours, while social housing promoted 13 positive behaviours. 14 The combination of physical items and social housing showed no further 15 improvement. 16 • The treatments had no effect on calf fear in novel environment and object tests. 17 18 **Abstract** 19 The objective of this study was to determine the effects of social housing, the provision 20 of physical enrichment items, and the interaction between the two on calf growth,

behaviour and fearfulness. Forty-eight calves were randomly allocated to either individual (IP) or pair (PP) pens from 2 days to 8 weeks of age. Half of the calves in each housing treatment were provided with physical enrichment items (stationary brushes, plastic chains, rubber teats and haynets filled with strawberry-scented hay; PE). The remaining calves received no physical enrichment items (NPE). Concentrate consumption was measured daily and calves were weighed at birth and weekly thereafter. When calves were 2 to 5 weeks of age, they were recorded by a camera between 06:00 h and 20:00 h twice weekly, and behavioural data were collected using instantaneous scan sampling at 5-min intervals. Their behavioural responses to a novel environment and a novel object were then assessed at 5 or 6 weeks of age. PE calves tended to have greater average daily gains than NPE calves (mean ± IQR; 610.6 ± 151.8 g/d vs. 568.8 ± 77.1 g/d; p = 0.095). PE calves spent more time consuming hay than NPE calves. Among calves in IP pens, PE calves consumed less concentrate than NPE calves. Calves in PE-IP pens had better concentrate feeding efficiency than those in NPE-IP, NPE-PP and PE-PP pens. For home pen behaviours, PE calves showed less frequent non-nutritive sucking than NPE calves (0.802 ± 0.451% vs. 1.897 ± 0.401% of scans) and less frequent cross-sucking. Furthermore, PP increased or tended to increase the time spent on locomotor play, fixture sniffing, social sniffing, allogrooming and cross-sucking, but tended to decrease non-nutritive sucking compared to IP. No treatment effects were found on behaviour in the novelty tests. In

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

conclusion, physical items may improve calf growth more effectively than social housing does. Physical items and social housing may satisfy diverse natural behaviours and reduce undesirable behaviour in different ways. However, these treatments had no effect on calf fear in novel environment and object tests. The combination of physical items and social housing showed no further improvement in calf welfare.

Key words: dairy calf, environmental enrichment, average daily gain, behaviour, fear, welfare

1. Introduction

In the dairy industry, it is standard practice to raise calves in non-enriched individual pens after birth (Pempek et al., 2016). However, since the living environment fails to meet the needs of calves and restrict their natural behaviours (e.g. Jensen et al., 1998), social housing has been used to improve their welfare (Van De Weerd and Day, 2009). Many studies have shown that social housing provides benefits by promoting natural behaviours, reducing undesirable behaviours, and improving emotional states and production performance (e.g. Costa et al., 2015; Pempek et al., 2016). However, social housing has also been suggested to induce new welfare problems, such as greater risk of respiratory disease (Cobb et al., 2014) and increased cross-sucking

60 behaviour (Lidfors and Isberg, 2003), which may cause health problems (Größbacher, 2013).

62

63

64

65

66

67

68

69

70

71

72

73

61

One further way of improving animal welfare is to provide physical enrichment (Boissy et al., 2007) through altering the complexity of animals' enclosure or adding physical items to the enclosure (Bloomsmith et al., 1991). For calves, enhancing the complexity of their enclosure can stimulate the expression of natural behaviour and reduce undesirable behaviour (e.g. Jensen et al., 1998). However, the effect of adding physical items to the enclosure on calves' behaviours is rarely studied. Pempek et al. (2017) indicated that adding physical items (teat, brush, "lollie" and chain) to calves' hutches promoted the expression of locomotor play. Horvath et al. (2020) illustrated that the provision of a brush reduced total time engaged in non-nutritive oral behaviours but increased time engaged in grooming. Ude et al. (2011) found that after adding teats into standard pens, calves showed reduced non-nutritive oral behaviours.

74

75

76

77

78

As well as benefits to calves' behaviours, physical items may also improve calves' growth. For instance, Horvath et al. (2020) indicated that the provision of hay tended to increase solid feed intake and average daily gain of calves during weaning. Mandel et al. (2016) illustrated providing hay in a net could extend calves' feeding duration and

increase the naturalness of calves' feeding behaviour, since animals often prefer to work for a reward.

Furthermore, physical items have effects on responses to novelty in many farm animals. Fear, which can increase risk of injury and decrease biological functioning (Meehan and Mench, 2002), is a common emotion for animals when they face novelty (Forkman et al., 2007). It can be expressed by behaviours such as active defence, passive avoidance, expressive movements and alarm calls in novelty tests (Erhard and Mendl, 1999; Forkman et al., 2007). Adding relevant items to the enclosure has been shown to affect fear responses, as seen through reducing avoidance and freezing of a novel object in domestic chicks (Jones and Waddington, 1992) and reducing latency to approach a person in piglets (Rodarte et al., 2004). However, the effect of adding items to the enclosure on calves' emotional states is less well known.

Whilst the individual effects of social housing or physical items on the improvement in animal's welfare has been widely studied in many species, investigation into the combination of both components is still limited. However, a number of studies in laboratory rats have demonstrated that the application of both social housing and physical items had diverse and non-additive behavioural effects in open-field and novel object tests (e.g. Zimmermann et al., 2001; Schrijver et al., 2002), and improved the

animals ability to cope with social challenges (Pietropaolo et al., 2004). Although little is known in calves, since social housing and physical items improve animal welfare by providing social contact (Costa et al., 2016) and increasing environmental complexity (Bloomsmith et al., 1991) separately, it might be expected that calves' welfare may be further improved by the combination of both components.

The present study aimed to determine the effects of social housing, the provision of physical enrichment items to calf pens, and the interaction between both components on calf growth, behaviour and response to novelty. It was hypothesised that 1) physical enrichment items and social housing will separately stimulate calf growth, increase play, exploratory and grooming behaviours, reduce non-nutritive oral behaviours and reduce fear of novelty; 2) there will be an interaction between physical enrichment items and social housing in terms of their influence on calf growth, behavioural expression and response to novelty, with the combination of both components having a more profound influence than one of the single enrichments.

2. Materials and Methods

2.1. Ethics statement

The study was performed at the Centre for Dairy Research, University of Reading (CEDAR), Reading, UK. All procedures complied with guidelines for the Ethical

Treatment of Animals in Applied Animal Behaviour and Welfare Research (Sherwin et al., 2017), and UK and EU laws governing research in animals.

2.2. Animal, housing and feeding

Forty-eight male Holstein Friesian calves were included in this study from 2 days of age until 8 weeks of age. When calves were born, 6 litres colostrum was offered to each calf three times within 24 hours of birth. Birth weight, ID and date of birth of the newborn calves were recorded. Calves with birth weights below 35 or above 55 kg were excluded, as well as any calves that were not drinking milk on their own by day 4.

Calves were assigned into eight blocks (six calves in each) according to their date of birth. Within block, calves were randomly allocated to either individual (IP) or pair (PP) pens. Half of the calves in each housing treatment were provided with physical enrichment items (PE): one stationary brush, one plastic chain, one rubber teat and one haynet filled with strawberry-scented ryegrass hay for IP; one haynet filled with strawberry-scented ryegrass hay and two of all other items for PP. Physical enrichment items were chosen based on the motivations hypothesized to be inadequately fulfilled in standard housing. Remaining calves received no additional physical enrichment items (NPE). The area of an IP and a PP was 2.4 m² and 4.8 m², respectively; the

whole area of each pen was covered with deep straw and fresh straw were added daily into each pen after morning milk feeding.

This trial was completed in two cohorts (24 calves in each cohort). Within each cohort, pens were arranged in three rows, so that the calves' visual contact in between rows could be limited by the 2-metre wide passages; calves' physical contact with their neighbours within one row could be limited to the gap between the panel bars. All calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets from 2 days of age to 49 days of age. 2.5 litres per feeding (L/f) of milk was offered to each calf until 14 days of age, followed by 3 L/f from 15 to 42 days of age and 2.5L/f between 43 and 49 days of age. During 50-56 days of age, 2.5 L/f milk was fed to each calf only in the morning. Calves had *ad libitum* access to concentrate (VITA concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and water throughout the study period.

2.3. Growth

Daily concentrate intake was measured by weighing the daily provision of concentrate and the daily collection of concentrate refusals in each pen until 8 weeks of age. All calves were weighed weekly until 8 weeks of age using a wheeled scale. Daily concentrate intake and average daily gain were calculated by averaging across the

entire period. Calves' concentrate feeding efficiency was estimated by the ratio between average daily gain and daily concentrate intake.

2.4. Home Pen Behaviours

Home pen behaviours were recorded by a CCTV (Transit-PTZ, Revader Security Ltd, UK) for 14 hours (06:00 h-20:00 h) twice per week when calves were 2, 3, 4, and 5 weeks of age. Video recordings were watched using instantaneous scans at 5-min intervals. The frequencies of calves' behaviours as listed and defined in Table 1 were recorded.

2.5. Novelty tests

Following home pen behavioural observations, an environmental novelty test was conducted one day before the novel object test. Both tests were conducted one calf at a time. A wheeled scale was used to move each test calf between its home pen and the test arena $(4.0 \times 4.0 \text{ m}^2)$. The test arena was set up at two different places for the calves in the first and second cohorts. When arriving at the entry of the test arena, the calf was lightly tapped on the hindquarters to encourage it to enter the test arena, in which the calf could not see any other calves. Both tests were recorded by either CCTV or webcam (C525, Logitech International S.A, Switzerland). Video recordings were continuously watched. The recorded behaviours for both tests are defined in

Table 2. The novelty of the arena and the object can lead to fear reactions in calves (Horvath et al., 2017). When calves feel fearful of the test arena or novel object, they are typically reluctant to touch the pen fixtures or object, defecate and vocalize more, and show sudden movements (Jensen et al., 1999).

The environmental novelty test started when the door of the test arena was fully closed. Each calf stayed in the test arena for 15-min. For the novel object test, once entry into the test arena calves were allowed to habituate for 5-min. Following the period of habituation a novel object (a white bucket or a traffic cone, used for alternate blocks of calves) was lowered to the centre of the test arena on a pulley. The calf remained in the pen with the novel object for 10-min.

2.6. Statistical analysis

All data were analysed using Minitab 18 (Minitab, LLC, USA). Significant differences were declared at p \leq 0.05 and a trend at 0.05 < p \leq 0.10.

For growth, a general linear model (GLM) was used to determine the effect of forms of enrichment on daily concentrate intake, average daily gain and concentrate feeding efficiency. Factors in the model included physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between these two factors. Calves' birth weight

was used as a covariate. The residuals of daily concentrate intake and concentrate feeding efficiency were not normally distributed, and thus it was square root transformed before analysis.

201

202

203

204

205

206

207

208

209

210

211

212

213

214

215

216

217

200

198

199

For home pen behaviours and the both novelty tests, video recordings were played with Windows Media Player (Microsoft Corporation, US) and data recorded by one observer. In order to determine the inter-observer reliability, another observer watched the home pen behaviour videos of eight calves by randomly choosing one week from 2, 3, 4, and 5 weeks of age for each calf. For both novelty tests, eight calves' videos of environment novelty test and eight calves' videos of novel object test were randomly selected and watched by another observer who was blind to the hypothesis under test. A Pearson correlation was used to compare the reliability between the two observers, which suggested strong positive relationships (home pen behaviour: r=0.995, p<0.001; environmental novelty test: r=0.999, p<0.001; novel object test: r=0.999, p<0.001) and good reliability. For novel object test, the videos were also watched for latency to contact by one of two other observers who were blind to treatment to ensure that data were reliable. Mixed effects model (MEM), GLM or binary logistic regression (BLR) were used to analyse the calves' behaviours in the three tests. For the behaviours analysed by BLR, in order to fit in the regression model, the data of the behaviours were converted to binary by coding any values greater than zero as "1". False

discovery rate (FDR) was used to solve multiple testing issues by calculating adjusted p values (Jafari and Ansari-Pour, 2019).

220

221

222

223

224

225

226

227

228

229

230

231

232

233

234

235

236

218

219

For home pen behaviours, time spent consuming concentrate and hay, ruminating, fixture sniffing, non-nutritive sucking and social sniffing were analysed by MEM. The fixed factors were physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between the two factors. The random factor was calves' ID number. The covariates were calves' birth weight, age, average temperature of the barn during the testing days and milk refusal during the testing days. The residuals of time spent consuming concentrate and hay, ruminating, non-nutritive sucking and social sniffing were not normally distributed or did not meet the assumption of homogeneity of variance, and thus these variables were square root transformed before analysis. In addition, locomotor play, fixture scratching, tongue rolling, allogrooming, social play and cross-sucking were analysed by BLR. The categorical predictors were physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between the two factors. Continuous variables included in the analysis were calves' birth weight, age, average temperature of the barn during testing days, and milk refusal during testing days. Fixture play and straw play were not analysed because they were rarely expressed.

In the novelty tests, fixture touching and abrupt movement in the environmental novelty test, and object touching and latency to first contact with the object in the novel object test were analysed by GLM. Factors included physical enrichment items (NPE or PE), social housing (IP or PP), the interaction between the two factors, arena locations and objects; object was only included as a factor in the novel object test. The covariate was average temperature of the barn during the testing day. Variables, with the exception of latency to first contact with the object in the novel object test, were square root or logarithm transformed before analysis as residuals were not normally distributed. Defecation bout, sudden neck movement and vocalization in the environmental test, and defecation bout, abrupt movement and vocalization in the novel object test were analysed by BLR. Categorical predictors included physical enrichment items (NPE or PE), social housing (IP or PP), the interaction between the two factors, arena locations and objects; object was only included as a factor in the novel object test. The continuous variable was average temperature of the barn during the testing day. Sudden neck movement in the novel object test was not analysed because calves rarely showed this behaviour.

254

255

256

257

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

One calf's data for daily concentrate intake, average daily gain and concentrate feeding efficiency was discarded due to an abscess on its tongue. In addition, on one occasion home pen behaviours for two calves were only recorded for 14 hours due to a

technological problem. As a result of an abscess on one calf's tongue and navel inflammation in another calf, the data from 2, 3, 4, and 5 weeks of age for the former calf and the data from 3, 4, and 5 weeks of age for the latter calf were discarded before analysis. Moreover, the data from two calves in both novelty tests were discarded before analysis because one calf had an abscess on its tongue and the other one was familiar with the test arena and the novel objects due to his pen location.

3. Results

3.1. Growth

Physical enrichment items and social housing had interactions, resulting in a significant difference in daily concentrate intake ($F_{1,42} = 7.01$, p = 0.011; Figure 1). Examination of the means by Tukey's multiple comparison test demonstrated that for calves in IP pens, the provision of PE reduced their daily concentrate intake, but not for calves in PP pens.

Calves in PE pens tended to have greater average daily gains when compared with those in NPE pens ($F_{1,42}$ = 2.92, p = 0.095; Figure 2). In contrast, calves in IP and PP pens showed similar rates of average daily gain (mean ± IQR; 577.7 ± 70.1 g/d vs. 601.8 ± 141.9 g/d; $F_{1,42}$ = 0.97, p = 0.331).

Physical enrichment items and social housing had interactions, resulting in a significant difference in concentrate feeding efficiency ($F_{1,42} = 8.04$, p = 0.007; Figure 3). Examination of the means by Tukey's multiple comparison test demonstrated that calves in PE-IP pens had better concentrate feeding efficiency than those in NPE-IP, NPE-PP and PE-PP pens.

3.2. Home pen behaviour

Feed intake related behaviours, including hay intake, concentrate intake, and ruminating are shown in Table 3. Hay intake behaviour was significantly more frequent for calves in PE pens than calves in NPE pens (3.138 \pm 2.142 % vs. 2.202 \pm 1.063 % of scans; $F_{1,39.58} = 8.51$, p = 0.006). Physical enrichment items and social housing tended to have interactions on the time spent consuming concentrate ($F_{1,38.92} = 3.74$, p = 0.061), with calves in PE-IP pens showed reduced time spent consuming concentrate compared with those in NPE-IP pens.

There were no interactions between physical enrichment items and social housing with respect to the incidence of the natural and undesirable behaviours. Calves in PP pens expressed more fixture sniffing (Table 3) than those in IP pens ($5.765 \pm 2.643 \%$ vs. $4.263 \pm 2.128 \%$ of scans; $F_{1,42.03} = 16.66$, adjusted p < 0.001). Social sniffing was more frequent for calves in PP pens than calves in IP pens ($0.686 \pm 0.070 \%$ vs. 0.157

 \pm 0.045 % of scans; F_{1,41.82} = 43.23, adjusted p < 0.001). In terms of non-nutritive sucking, calves in PE pens were observed to show less non-nutritive sucking than those in NPE pens (0.802 \pm 0.451 % vs. 1.897 \pm 0.401 % of scans; F_{1,40.75} = 26.22, adjusted p < 0.001), and calves in PP pens tended to show less non-nutritive sucking than those in IP pens (1.096 \pm 0.452 % vs. 1.503 \pm 0.477 % of scans; F_{1,41.42} = 3.59, adjusted p = 0.098).

PE tended to suppress the expression of fixture scratching (Table 4) compared with NPE (adjusted p = 0.078). PP increased or tended to increase the expression of locomotor play and allogrooming in comparison with IP (adjusted p = 0.065; adjusted p = 0.059). For cross-sucking, PE suppressed the expression of this behaviour compared with NPE (adjusted p = 0.012), while PP increased the frequency of this behaviour in comparison with IP (adjusted p < 0.001).

3.3. Novelty tests

In the environmental novelty test, calves in PE pens and NPE pens showed similar durations of fixture touching (316.3 \pm 0.5 vs. 331.0 \pm 1.0 seconds; $F_{1,40}$ = 0.17, adjusted p = 0.908) and similar frequencies of abrupt movement (0.312 \pm 1.000 vs. 1.040 \pm 3.000 bouts; $F_{1,40}$ = 3.41, adjusted p = 0.288), defectaion (adjusted p = 1.000), sudden neck movement (adjusted p = 1.000) and vocalization (adjusted p = 1.152). Calves in

PP pens and IP pens showed similar durations of fixture touching (303.8 \pm 0.6 vs. 344.6 \pm 0.3 seconds; F_{1,40} = 1.33, adjusted p = 0.640) and similar frequencies of abrupt movement (0.466 \pm 1.000 vs. 0.803 \pm 2.000 bouts; F_{1,40} = 0.73, adjusted p = 0.498), defecation (adjusted p = 0.625), sudden neck movement (adjusted p = 1.220) and vocalization (adjusted p = 0.752). In addition, physical enrichment items and social housing had no interactions on these behavioural responses.

In the novel object test, calves in PE pens and NPE pens showed similar durations of object touching (21.1 \pm 19.7 vs. 12.8 \pm 51.0 seconds; F_{1,39} = 0.87, adjusted p = 0.446) and latency to first contact with the object (135.7 \pm 139.0 vs. 256.8 \pm 560.0 seconds; F_{1,39} = 3.36, adjusted p = 0.375), and similar frequencies of abrupt movement (adjusted p = 0.295), defecation (adjusted p = 1.000) and vocalization (adjusted p = 0.258). Calves in PP pens and IP pens showed similar durations of object touching (15.9 \pm 62.1 vs. 17.0 \pm 25.5 seconds; F_{1,39} = 0.01, adjusted p = 1.131) and latency to first contact with the object (198.3 \pm 534.5 vs. 194.1 \pm 476.5 seconds; F_{1,39} < 0.01, adjusted p = 0.950), and similar frequencies of abrupt movement (adjusted p = 0.828), defecation (adjusted p = 0.200) and vocalization (adjusted p = 1.495). In addition, physical enrichment items and social housing had no interactions on these behavioural responses.

4. Discussion

Physical enrichment items offered some benefits for growth and supressed nonnutritive oral behaviours. Social housing had no effect on calves' growth but promoted,
or tended to promote, some positive behaviours. Physical enrichment and social
housing had non-additive effects on calves' growth and home pen behaviour. Physical
enrichment items, social housing and the interaction between these two factors had no
effect on calves' behavioural responses in the novelty tests.

4.1. Growth and feeding effects

In the present study, physical enrichment tended to increase calves' average daily gain, but reduced individual calves' daily concentrate intake, resulting in improved feeding efficiency. This contrasts with the findings of Pempek et al. (2017), who reported that furnished pens had no effect on calves' concentrate intake and weight gain. The difference may be attributed to the provision of roughage to calves.

According to Pempek et al. (2017), calves had no access to hay or other roughage, but in this study, ryegrass hay was provided *ad libitum* to calves. While calves without physical enrichment only consumed ryegrass hay from hay racks, strawberry-scented ryegrass hay was also provided to physically enriched calves from haynets. Since animals can use their sensorial perceptions to choose palatable feeds (Baumont, 1996) and some aromas can increase the palatability of hay (Cannas et al., 2009), the

strawberry aroma in this study might have stimulated calves to eat more hay.

Strawberry was chosen because red berry flavouring was previously found to be a preferred aroma for dairy cattle (Meagher et al., 2017). As observed, calves with physical enrichment items showed a higher frequency of hay intake, which may result in increased consumption of hay and increased average daily gain compared with non-physically enriched calves.

Altogether, the increase in roughage intake in calves with physical enrichment items, and better concentrate feeding efficiency in individual enriched pens, are likely to be economically beneficial on farms. Improved feed conversion efficiency is an important objective for profitable dairy operations (Bach et al., 2007). Oostindjer et al. (2010) also demonstrated that physical enrichment (straw, wood shavings, peat, and branches) positively affected the feed conversion efficiency for piglets. These results may be attributed to the reduced stress in physically enriched living environments (Barnett et al., 1983).

In contrast to the effect of physical enrichment, social housing had no effect on calves' daily concentrate intake, average daily gain and concentrate feeding efficiency in this study. However, previous studies found that social housing increased weight gain in calves (Tapki, 2007; Jensen et al., 2015; Pempek et al., 2016). This is likely owing to

increased concentrate intake through social learning or social facilitation (Costa et al., 2015). In other words, the presence of other calves near the bucket or sight of them eating would increase the likelihood of calves paying attention to feed and perform similar behaviours; calves could also learn where to find concentrate and how to consume it by observing, or interacting, with calves showing those behaviours. One potential reason for the lack of treatment effect in this study is the different housing design. When researchers previously studied the effect of social housing on calves' growth, they compared calves in grouped environments with calves in individual environments with only auditory contact or auditory and visual contact. For instance, Jensen et al. (2015) positioned adjacent pens 1.5-metre apart to prevent physical contact between calves in different pens. However, in the current experiment, calves had auditory, visual and limited physical contact with their neighbours. Therefore, calves in individual pens may imitate or learn how to consume concentrate from their pair-housed neighbours. Jensen and Larsen (2014) similarly demonstrated that calves in individual pens with limited physical contact with their neighbours and calves in paired pens had similar daily concentrate intake and average daily gain.

394

395

396

397

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

4.2. Home pen behaviour

Expression of locomotor play tended to be higher in pair-housed calves than in individually-housed calves. As play behaviour typically reflects an absence of negative

affective states, or indicates increased positive experience (Held and Špinka, 2011), social housing may provide a more pleasurable living environment for calves. Jensen et al. (1998) also showed that social stimulation might lead to the appearance of locomotor play. However, calves in individual and paired pens showed similarly low frequencies of social play. One reason for this phenomenon could be the later emergence of social play in the calves' life, with limited amounts occurring in the first few weeks (Jensen et al., 1998). In addition, physical enrichment items had no effect on locomotor or social play, which may indicate that these types of play are not stimulated by external objects.

Sniffing is a type of exploratory behaviour which is motivated by the animals need gather environmental information (Westerath et al., 2009). The expression is perceived to be intrinsically pleasant or self-rewarding (Boissy et al., 2007). In the present study, calves in paired pens showed more fixture sniffing than those in individual pens. This result may indicate that housing calves in pairs may be an effective way to release calves' exploratory motivation and stimulate them to explore their living environment. The increased expression of social sniffing in pair-housed calves may also corroborate this view. By contrast, physical enrichment items had no effect on calves' exploratory behaviour, maybe because the additional items attracted the calves' attention so that they spent more time exploring additional items rather than the rest of the environment

(e.g. Zobel et al., 2017).

Allogrooming and fixture scratching are body care behaviours (Kohari et al., 2007), which help maintain hygiene of the animal's body by removing debris or ectoparasites (Rich, 1973). Moreover, allogrooming is important in forming or maintaining social relationships between calves (Færevik et al., 2007). In this study, social housing tended to increase the expression of allogrooming, but physical enrichment items had no effect on this behaviour. This result agreed with previous studies conducted by Tapki (2007), and Horvath and Miller-Cushon (2019). The former showed that social housing encouraged calves to express allogrooming voluntarily. The latter suggested that physical enrichment item (brush) had no effect on this natural behaviour. The result from this study may demonstrate that allogrooming does not relate to stimuli from external items. In addition to social body care behaviour, fixture scratching was expressed less in physically enriched calves, which might be a consequence of the existence of other more suitable scratching items in physically enriched pens.

Non-nutritive sucking, cross-sucking and tongue rolling are considered as non-nutritive oral behaviours, which are non-functional and harmful (Le Neindre, 1993; Jensen, 2003; Garner, 2005). Non-nutritive sucking may be considered as redirected sucking behaviour (De Passillé et al., 1992). Calves have a strong motivation for suckling.

Therefore, in the absence of their dam or a teat, they may redirect this behaviour toward elements in their environment. This is different from calves' behaviour in nature, and might be an indication of frustration (Leruste et al., 2014). Cross-sucking is an abnormal behaviour, which is a redirection from milk suckling behaviour toward the ear, tail, navel, prepuce, or other body parts of other calves (Leruste et al., 2014), and can lead to hair loss, inflammation and diseases in the receiver (Jensen, 2003). Tongue rolling is considered as a stereotypic behaviour indicating frustration or lack of stimulation (Leruste et al., 2014, Mason and Latham, 2004). In the present study, calves in paired pens tended to show less non-nutritive sucking but showed more cross-sucking than those in individual pens. This result agrees with that of Pempek et al. (2016), whose study showed that although non-nutritive sucking was observed more often among individually-housed calves, calves housed in pairs appeared to redirect this behaviour to their companion as cross-sucking. Physically enriched calves show less non-nutritive sucking and cross-sucking than non-physically enriched calves. This was shown by Veissier et al. (2002), whose study suggested that providing a teat after milk intake reduced non-nutritive sucking, while Newberry (1995) demonstrated that the occurrence of cross-sucking behaviour was reduced when calves were presented with dry rubber nipples following milk intake. In addition, the expression of tongue rolling was not affected by physical enrichment items or social housing. This may be because tongue rolling is directly related to feeding and ruminating behaviours (Webb

438

439

440

441

442

443

444

445

446

447

448

449

450

451

452

453

454

455

456

et al., 2012). In this study, although the frequency of concentrate intake, hay intake and ruminating were affected by different treatments, the frequency of feed intake behaviours was similar across all treatments.

4.3. Response to novelty

Neither physical enrichment nor pair housing were found to affect calves' behavioural responses in either novelty test. These findings agree with previous studies showing no effect of social housing (Jensen and Larsen 2014) or the provision of physical complexity to a standard hutch (Pempek et al. 2017) on calves' behavioural responses to social and environmental novelty. This phenomenon may indicate the static environment created by providing additional objects was not complex enough to elicit emotional change in novel situations. Therefore, a more complex and dynamic environment is probably needed in future studies to reduce calves' fearfulness. In terms of the effect of social housing, Leruste et al. (2014) found that some behavioural responses of calves (e.g. vocalization and exploratory behaviour) in individual pens with tactile contact were similar to those of pair-housed calves, which may indicate that individual housing with tactile contact may result in similar fearfulness in pair housed calves.

5. Conclusion

Provision of physical enrichment improved calves' growth by promoting intake of roughage and increasing weight gain and concentrate feeding efficiency. In contrast, social housing was less effective at improving calves' growth. Provision of physical enrichment reduced calves' non-nutritive oral behaviours, while social housing had a positive impact on play, exploratory, and social behaviours. However, neither treatment affected calves' fear of novelty. In conclusion, physical enrichment items and social housing may satisfy calves' needs in different ways, but the combination of both components did not further improve calves' welfare.

Declaration of Competing Interest

None.

Acknowledgements

This research did not receive any specific grant income from funding agencies in the public, commercial, or not-for-profit sectors. We gratefully acknowledge support from the Department of Animal Sciences, University of Reading. We also thank the staff at CEDAR for assistance and animal care, especially Barney Jones for his guidance and Nikki Crisp, Rafal Szoka, Alistair Smith, Tomasz Szoka, Edel Madden, Debbie Cockman and Chris Fawdry for their help and support.

498 References

- Bach, A., Giménez, A., Juaristi, J., Ahedo, J., 2007. Effects of physical form of a starter for dairy
- replacement calves on feed intake and performance. J. Dairy Sci. 90, 3028-3033.
- 501 https://doi.org/10.3168/jds.2006-761.
- Barnett, J., Hemsworth, P., Hand, A., 1983. Effects of chronic stress on some blood parameters in the
- 503 pig. Appl. Anim. Ethol. 9, 273-277. https://doi.org/10.1016/0304-3762(83)90007-X.
- Baumont, R., 1996. Palatability and feeding behaviour in ruminants. A review. Annales de Zootechnie
- 505 45, 385–400. https://doi.org/10.1051/animres:19960501.
- Bloomsmith, M.A., Brent, L.Y., Schapiro, S.J., 1991. Guidelines for developing and managing an
- environmental enrichment program for nonhuman primates. Lab. Anim. Sci. 41, 372-377.
- Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B.,
- 509 Dimitrov, I., Langbein, J., 2007. Assessment of positive emotions in animals to improve their welfare.
- 510 Physiol. Behav 92, 375-397. https://doi.org/10.1016/j.physbeh.2007.02.003.
- 511 Cannas, A., Mereu, A., Decandia, M., Molle, G., 2009. Role of sensorial perceptions in feed selection
- 512 and intake by domestic herbivores. Ital. J. Anim. Sci. 8, 243-251.
- 513 https://doi.org/10.4081/ijas.2009.s2.243.
- 514 Cobb, C., Obeidat, B., Sellers, M., Pepper-Yowell, A., Hanson, D., Ballou, M., 2014. Improved
- performance and heightened neutrophil responses during the neonatal and weaning periods among
- outdoor group-housed Holstein calves. J. Dairy Sci. 97, 930-939. https://doi.org/10.3168/jds.2013-
- 517 6905.
- Costa, J., Meagher, R., Von Keyserlingk, M., Weary, D., 2015. Early pair housing increases solid feed
- intake and weight gains in dairy calves. J. Dairy Sci. 98, 6381-6386. https://doi.org/10.3168/jds.2015-
- 520 9395.
- 521 Costa, J.H.C., von Keyserlingk, M.A.G., Weary, D.M., 2016. Invited review: Effects of group housing of
- dairy calves on behavior, cognition, performance, and health. J. Dairy Sci. 99, 2453-2467.
- 523 https://doi.org/10.3168/jds.2015-10144.
- De Passillé, A., Metz, J., Mekking, P., Wiepkema, P., 1992. Does drinking milk stimulate sucking in
- 525 young calves? Appl. Anim. Behav. Sci. 34, 23-36. https://doi.org/10.1016/S0168-1591(05)80054-1.
- 526 Erhard, H.W., Mendl, M., 1999. Tonic immobility and emergence time in pigs—more evidence for
- behavioural strategies. Appl. Anim. Behav. Sci. 61, 227-237. https://doi.org/10.1016/S0168-
- 528 1591(98)00196-8.
- Færevik, G., Andersen, I.L., Jensen, M.B., Bøe, K.E., 2007. Increased group size reduces conflicts and
- strengthens the preference for familiar group mates after regrouping of weaned dairy calves (Bos
- taurus). Appl. Anim. Behav. Sci. 108, 215-228. https://doi.org/10.1016/j.applanim.2007.01.010.
- Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E., Jones, R., 2007. A critical review of fear
- 533 tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav 92, 340-374.
- 534 https://doi.org/10.1016/j.physbeh.2007.03.016.
- Garner, J.P., 2005. Stereotypies and other abnormal repetitive behaviors: potential impact on validity,
- 536 reliability, and replicability of scientific outcomes. ILAR J. 46, 106-117.
- 537 https://doi.org/10.1093/ilar.46.2.106.

- 538 Größbacher, V., 2013. Non-nutritive sucking, health situation and identification of risk factors in
- group-housed dairy calves in Austria., Department of Sustainable Agricultural Systems, University of
- Natural Resources and Life Sciences.
- Held, S.D., Špinka, M., 2011. Animal play and animal welfare. Anim. Behav. 81, 891-899.
- 542 https://doi.org/10.1016/j.anbehav.2011.01.007.
- Horvath, K., Allen, A., Miller-Cushon, E., 2020. Effects of access to stationary brushes and chopped
- hay on behavior and performance of individually housed dairy calves. J. Dairy Sci. 103, 8421-8432.
- 545 https://doi.org/10.3168/jds.2019-18042.
- Horvath, K., Fernandez, M., Miller-Cushon, E.K., 2017. The effect of feeding enrichment in the milk-
- 547 feeding stage on the cognition of dairy calves in a T-maze. Appl. Anim. Behav. Sci. 187, 8-14.
- 548 https://doi.org/10.1016/j.applanim.2016.11.016.
- Horvath, K., Miller-Cushon, E., 2019. Characterizing grooming behavior patterns and the influence
- of brush access on the behavior of group-housed dairy calves. J. Dairy Sci. 102, 3421-3430.
- 551 https://doi.org/10.3168/jds.2018-15460.
- Jafari, M., Ansari-Pour, N., 2019. Why, when and how to adjust your P values? Cell J. 20, 604-607.
- 553 http://doi.org/10.22074/cellj.2019.5992.
- Jensen, M.B., 2003. The effects of feeding method, milk allowance and social factors on milk feeding
- behaviour and cross-sucking in group housed dairy calves. Appl. Anim. Behav. Sci. 80, 191-206.
- 556 https://doi.org/10.1016/S0168-1591(02)00216-2.
- Jensen, M.B., Duve, L.R., Weary, D., 2015. Pair housing and enhanced milk allowance increase play
- behavior and improve performance in dairy calves. J. Dairy Sci. 98, 2568-2575.
- 559 https://doi.org/10.3168/jds.2014-8272.
- Jensen, M.B., Larsen, L.E., 2014. Effects of level of social contact on dairy calf behavior and health. J.
- 561 Dairy Sci. 97, 5035-5044. https://doi.org/10.3168/jds.2013-7311.
- Jensen, M.B., Munksgaard, L., Mogensen, L., Krohn, C.C., 1999. Effects of housing in different social
- environments on open-field and social responses of female dairy calves. Acta Agric. Scand. Sect. A
- Anim. Sci. 49, 113-120. https://doi.org/10.1080/090647099424178.
- Jensen, M.B., Vestergaard, K.S., Krohn, C.C., 1998. Play behaviour in dairy calves kept in pens: the
- effect of social contact and space allowance. Appl. Anim. Behav. Sci. 56, 97-108.
- 567 https://doi.org/10.1016/S0168-1591(97)00106-8.
- Jones, R.B., Waddington, D., 1992. Modification of fear in domestic chicks, Gallus gallus domesticus,
- via regular handling and early environmental enrichment. Anim. Behav. 43, 1021-1033.
- 570 https://doi.org/10.1016/S0003-3472(06)80015-1.
- Kohari, D., Kosako, T., Fukasawa, M., Tsukada, H., 2007. Effect of environmental enrichment by
- providing trees as rubbing objects in grassland: grazing cattle need tree-grooming. Anim. Sci. J. 78,
- 573 413-416. https://doi.org/10.1111/j.1740-0929.2007.00455.x.
- Le Neindre, P., 1993. Evaluating housing systems for veal calves. J. Anim. Sci. 71, 1345-1354.
- 575 https://doi.org/10.2527/1993.7151345x.
- Leruste, H., Brscic, M., Cozzi, G., Kemp, B., Wolthuis-Fillerup, M., Lensink, B., Bokkers, E., Van Reenen,
- 577 C., 2014. Prevalence and potential influencing factors of non-nutritive oral behaviors of veal calves
- on commercial farms. J. Dairy Sci. 97, 7021-7030. https://doi.org/10.3168/jds.2014-7917.

- Lidfors, L., Isberg, L., 2003. Intersucking in dairy cattle—review and questionnaire. Appl. Anim. Behav.
- 580 Sci. 80, 207-231. https://doi.org/10.1016/S0168-1591(02)00215-0.
- Mandel, R., Whay, H.R., Klement, E., Nicol, C.J., 2016. Invited review: Environmental enrichment of
- 582 dairy cows and calves in indoor housing. J. Dairy Sci. 99, 1695-1715.
- 583 https://doi.org/10.3168/jds.2015-9875.
- Mason, G., Latham, N., 2004. Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator?
- 585 Anim. Welf. 13, S57-69.
- Meagher, R.K., Weary, D.M., von Keyserlingk, M.A., 2017. Some like it varied: Individual differences in
- 587 preference for feed variety in dairy heifers. Appl. Anim. Behav. Sci. 195, 8-14.
- 588 https://doi.org/10.1016/j.applanim.2017.06.006.
- Meehan, C., Mench, J., 2002. Environmental enrichment affects the fear and exploratory responses
- 590 to novelty of young Amazon parrots. Appl. Anim. Behav. Sci. 79, 75-88.
- 591 https://doi.org/10.1016/S0168-1591(02)00118-1.
- Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of captive
- 593 environments. Appl. Anim. Behav. Sci. 44, 229-243. https://doi.org/10.1016/0168-1591(95)00616-Z.
- Oostindjer, M., Bolhuis, J., Mendl, M., Held, S., Gerrits, W., Van den Brand, H., Kemp, B., 2010. Effects
- of environmental enrichment and loose housing of lactating sows on piglet performance before and
- after weaning. J. Anim. Sci. 88, 3554-3562. https://doi.org/10.2527/jas.2010-2940.
- Pempek, J., Eastridge, M., Proudfoot, K., 2017. The effect of a furnished individual hutch pre-weaning
- 598 on calf behavior, response to novelty, and growth. J. Dairy Sci. 100, 4807-4817.
- 599 https://doi.org/10.3168/jds.2016-12180.
- Pempek, J.A., Eastridge, M.L., Swartzwelder, S.S., Daniels, K.M., Yohe, T.T., 2016. Housing system may
- affect behavior and growth performance of Jersey heifer calves. J. Dairy Sci. 99, 569-578.
- 602 https://doi.org/10.3168/jds.2015-10088.
- Pietropaolo, S., Branchi, I., Cirulli, F., Chiarotti, F., Aloe, L., Alleva, E., 2004. Long-term effects of the
- periadolescent environment on exploratory activity and aggressive behaviour in mice: social versus
- 605 physical enrichment. Physiol. Behav 81, 443-453. https://doi.org/10.1016/j.physbeh.2004.02.022.
- Rich, G., 1973. Grooming and yarding of spring-born calves prevent paralysis caused by the Rocky
- Mountain wood tick. Can. J. Anim. Sci. 53, 377-378. https://doi.org/10.4141/cjas73-057.
- Rodarte, L.F., Ducoing, A., Galindo, F., Romano, M.C., Valdez, R.A., 2004. The effect of environmental
- manipulation on behavior, salivary cortisol, and growth of piglets weaned at 14 days of age. J. Appl.
- 610 Anim. Welf. Sci. 7, 171-179. https://doi.org/10.1207/s15327604jaws0703_3.
- Schrijver, N.C., Bahr, N.I., Weiss, I.C., Würbel, H., 2002. Dissociable effects of isolation rearing and
- environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol.
- 613 Biochem. Behav. 73, 209-224. https://doi.org/10.1016/S0091-3057(02)00790-6.
- Sherwin, C., Christiansen, S., Duncan, I., Erhard, H., Lay, D., Mench, J., O'Connor, C., 2017. Ethical
- Treatment of Animals in Applied Animal Behaviour Research.
- Tapki, İ., 2007. Effects of individual or combined housing systems on behavioural and growth
- 617 responses of dairy calves. Acta Agric. Scand. Sect. A Anim. Sci. 57, 55-60.
- 618 https://doi.org/10.1080/09064700701464405.
- Ude, G., Georg, H., Schwalm, A., 2011. Reducing milk induced cross-sucking of group housed calves

- 620 by an environmentally enriched post feeding area. Livest. Sci. 138, 293-298.
- 621 https://doi.org/10.1016/j.livsci.2010.12.004.
- Van De Weerd, H.A., Day, J.E.L., 2009. A review of environmental enrichment for pigs housed in
- 623 intensive housing systems. Appl. Anim. Behav. Sci. 116, 1-20.
- 624 https://doi.org/10.1016/j.applanim.2008.08.001.
- Veissier, I., De Passillé, A., Després, G., Rushen, J., Charpentier, I., Ramirez De La Fe, A., Pradel, P.,
- 626 2002. Does nutritive and non-nutritive sucking reduce other oral behaviors and stimulate rest in
- 627 calves? J. Anim. Sci. 80, 2574-2587. https://doi.org/10.1093/ansci/80.10.2574.
- Webb, L.E., Bokkers, E.A., Engel, B., Gerrits, W.J., Berends, H., van Reenen, C.G., 2012. Behaviour and
- welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration
- 630 adjusted for similar growth. Appl. Anim. Behav. Sci. 136, 108-116.
- 631 https://doi.org/10.1016/j.applanim.2011.12.004.
- Westerath, H.S., Laister, S., Winckler, C., Knierim, U., 2009. Exploration as an indicator of good welfare
- in beef bulls: an attempt to develop a test for on-farm assessment. Appl. Anim. Behav. Sci. 116, 126-
- 634 133. https://doi.org/10.1016/j.applanim.2008.08.012.
- Zimmermann, A., Stauffacher, M., Langhans, W., Würbel, H., 2001. Enrichment-dependent
- differences in novelty exploration in rats can be explained by habituation. Behav. Brain Res. 121, 11-
- 637 20. https://doi.org/10.1016/S0166-4328(00)00377-6.

- Zobel, G., Neave, H.W., Henderson, H.V., Webster, J., 2017. Calves use an automated brush and a
- hanging rope when pair-housed. Animals-Basel 7, 84-95. https://doi.org/10.3390/ani7110084.

641 Tables

642

Table 1. Ethogram of the home pen behaviours

Category	Behaviour	Definition			
Feeding & ruminating	Concentrate intake	Heading in or above the concentrate bucket and chewing			
	Hay intake	Chewing hay from the hay rack or haynet			
	Ruminating	Chewing without concentrate, straw or hay			
Exploratory behaviour	Fixture sniffing	Putting muzzle in contact with or less than one muzzle length from any fixture in the pen with neck not			
		relaxed			
Play	Locomotor play	Engaging in a gallop, leap, Jump, buck-low, buck-high, buck-kick or turn.			
	Straw play	Kneeling down on the two forelegs and butting straw, or rubbing head or neck in straw in a playful manner			
	Fixture play	Standing and butting head against any fixture in the pen in a playful manner			

Grooming	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				
Non-nutritive oral behaviour	Non-nutritive sucking	Licking, sucking or biting any fixture of the pen				
	Tongue rolling	Making a repeated rolling and stretching of the tongue outside or sometimes inside open mouth				
	Cross-sucking	Sucking or biting toward ear, mouth, navel, scrotum, prepuce, or other body parts of other calves				
Social behaviour	Social sniffing	Putting muzzle in contact with or less than one muzzle length from other calves with neck not relaxed				
	Social play	Mounting other calves, running with other calves or butting head against head, neck or body of other calves				
		in a playful manner				
	Allogrooming	Putting tongue out of mouth and in contact with head, neck or body of other calves				
Others	Other behaviours	Such as lying down, standing, walking and drinking water				

Table 2. Ethogram of the recorded behaviours in the environmental novelty test (ENT) and the novel object test (NOT)

Test(s)	Behaviour	Definition
ENT	Fixture touching ¹	Sniffing, licking or sucking the testing arena while standing or walking
ENT, NOT	Defecation ²	The bouts of defecation
ENT, NOT	Abrupt movement ²	Showing an abrupt movement in a reverse direction from the area being explored
ENT, NOT	Sudden neck movement ²	Showing a sudden neck movement such as a startle reflex while exploring
ENT, NOT	Vocalization ²	Vocalizing with mouth opened or closed
NOT	Latency to first contact with the object ¹	Time interval from lowering the object to the centre of the test arena to touching the object
NOT	Object touching ¹	Sniffing, licking, sucking or butting the object while standing or walking

 $^{^{1}}$ The time duration of the behaviour was recorded.

²The frequency of the behaviour was recorded.

Table 3. Six home pen behaviours (mean \pm IQR) analysed using mixed effects models (MEM)¹. Samples sizes were physically enriched individual PE-IP, n = 7; non-physically enriched individual NPE-IP, n = 8; physically enriched pair PE-PP, n = 16 and non-physical enrichment pair NPE-PP, n = 16

Variables	IP		PP		(adjusted) p-value ³		
	PE	NPE	PE	NPE	PE vs. NPE	PP vs. IP	Interaction
Concentrate intake (%) ²	0.738 ± 0.440	1.512 ± 0.259	0.844 ± 0.201	0.990 ± 0.449	0.005	0.253	0.061
Hay intake (%) ²	2.936 ± 2.140	2.024 ± 0.762	3.346 ± 2.465	2.387 ± 1.654	0.006	0.236	0.973
Ruminating (%) ²	6.211 ± 3.091	6.373 ± 3.960	6.639 ± 3.869	6.248 ± 3.736	0.879	0.840	0.714
Fixture sniffing (%)	4.724 ± 3.225	3.802 ± 1.040	5.437 ± 2.256	6.092 ± 2.339	1.079	< 0.001	0.24
Social sniffing (%) ²	0.210 ± 0.104	0.113 ± 0.011	0.678 ± 0.051	0.695 ± 0.055	0.794	< 0.001	0.664
Non-nutritive sucking (%) ²	0.890 ± 0.199	2.276 ± 0.446	0.718 ± 0.318	1.553 ± 0.537	< 0.001	0.098	1.164

¹Significant difference was declared at (adjusted) $p \le 0.05$ and a trend at 0.05 < (adjusted) $p \le 0.10$.

²Square root transformation was applied to the variables. The values of mean ± IQR for the variables are back-transformed.

PE, n = 23; non-physical enrichment NPE, n = 24; pair PP, n = 32 and individual, n = 15

652

653

654

655

656

³Adjusted p-values were calculated using false discovery rate (FDR) to fixture sniffing, social sniffing and non-nutritive sucking.

Table 4. Six home pen behaviours (mean ± IQR) analysed using binary logistic regression (BLR)¹. Samples sizes were physical enrichment

Variables	Coefficient		Adjusted	p-value ²	Effect ³		
-	PE vs. NPE	PP vs. IP	PE vs. NPE	PP vs. IP	PE vs. NPE	PP vs. IP	
Fixture scratching (%)	-1.387	0.000	0.078	1.000	PE < NPE	No	
Locomotor play (%)	1.154	1.108	0.198	0.065	No	PP > IP	
Allogrooming (%)	0.048	0.924	0.933	0.059	No	PP > IP	
Social play (%)	-0.082	0.794	1.093	0.150	No	No	
Tongue rolling (%)	-0.693	0.239	0.298	0.626	No	No	

	Cross-sucking (%)	-1.594	3.067	0.012	<0.001	PE < NPE	PP > IP	
657	¹ Significant difference was	declared at adju	sted p ≤ 0.05 and	a trend at 0.05 < ad	justed p ≤ 0.10.			
658	² Adjusted p-values were ca	alculated using fa	lse discovery rate	(FDR).				

³Whether enriched treatments (tend to) make each behaviour been expressed more likely or less likely.

661 Figures

659

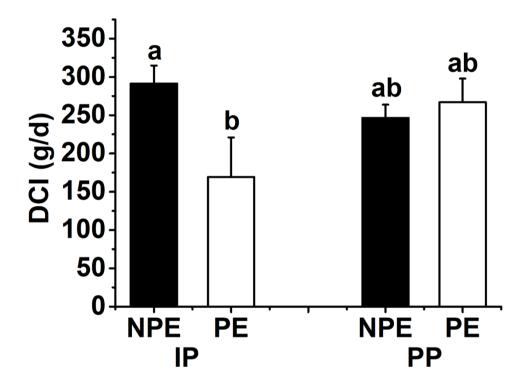


Figure 1. Back-transformed means (±IQR) of daily concentrate intake (DCI) for calves from non-physically enriched individual pens (NPE-IP; n = 8 calves), physically enriched individual pens (PE-IP; n = 7 calves), non-physically enriched paired pens (NPE-PP; n = 16 calves) and physically enriched paired pens (PE-PP; n = 16 calves). Different letters (a, b) represent significant differences between treatments at p≤0.05.

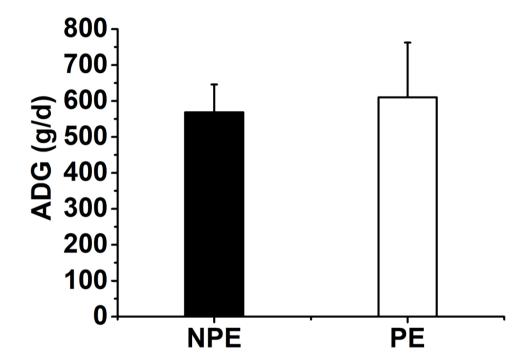


Figure 2. Means (±IQR) of average daily gain (ADG) for a) calves from non-physically enriched pens (NPE; n = 24 calves) and physically enriched pens (PE; n = 23 calves).

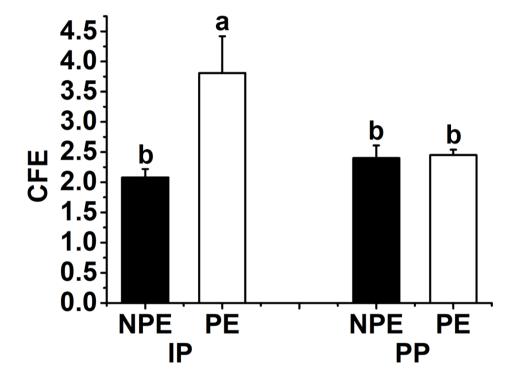


Figure 3. Back-transformed means (±IQR) of concentrate feeding efficiency (CFE; grams of gain per gram of concentrate intake) for calves from non-physically enriched individual pens (NPE-IP; n = 8 calves), physically enriched individual pens (PE-IP; n = 7 calves), non-physically

- 674 enriched paired pens (NPE-PP; n = 16 calves) and physically enriched paired pens (PE-PP; n = 16 calves). Different letters (a, b) represent
- 675 significant differences between treatments at p≤0.05.