

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

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1	Effects of physica	I enrichment items	and social housing	on calves'	growth,
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2 behaviour and response to novelty

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9

10 Highlights

- 11 Access to physical items (e.g. brush, teat) improved growth; pair housing did not.
- 12 **O** Physical items reduced undesirable behaviours, while social housing promoted
- 13 positive behaviours.
- 14 **•** The combination of physical items and social housing showed no further

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,

21	behaviour and fearfulness. Forty-eight calves were randomly allocated to either
22	individual (IP) or pair (PP) pens from 2 days to 8 weeks of age. Half of the calves in
23	each housing treatment were provided with physical enrichment items (stationary
24	brushes, plastic chains, rubber teats and haynets filled with strawberry-scented hay;
25	PE). The remaining calves received no physical enrichment items (NPE). Concentrate
26	consumption was measured daily and calves were weighed at birth and weekly
27	thereafter. When calves were 2 to 5 weeks of age, they were recorded by a camera
28	between 06:00 h and 20:00 h twice weekly, and behavioural data were collected using
29	instantaneous scan sampling at 5-min intervals. Their behavioural responses to a novel
30	environment and a novel object were then assessed at 5 or 6 weeks of age. PE calves
31	tended to have greater average daily gains than NPE calves (mean \pm IQR; 610.6 \pm
32	151.8 g/d vs. 568.8 \pm 77.1 g/d; p = 0.095). PE calves spent more time consuming hay
33	than NPE calves. Among calves in IP pens, PE calves consumed less concentrate
34	than NPE calves. Calves in PE-IP pens had better concentrate feeding efficiency than
35	those in NPE-IP, NPE-PP and PE-PP pens. For home pen behaviours, PE calves
36	showed less frequent non-nutritive sucking than NPE calves (0.802 \pm 0.451% vs. 1.897
37	\pm 0.401% of scans) and less frequent cross-sucking. Furthermore, PP increased or
38	tended to increase the time spent on locomotor play, fixture sniffing, social sniffing,
39	allogrooming and cross-sucking, but tended to decrease non-nutritive sucking
40	compared to IP. No treatment effects were found on behaviour in the novelty tests. In

41	conclusion, physical items may improve calf growth more effectively than social
42	housing does. Physical items and social housing may satisfy diverse natural
43	behaviours and reduce undesirable behaviour in different ways. However, these
44	treatments had no effect on calf fear in novel environment and object tests. The
45	combination of physical items and social housing showed no further improvement in
46	calf welfare.
47	Key words: dairy calf, environmental enrichment, average daily gain, behaviour, fear,
48	welfare
49	
50	1. Introduction
51	In the dairy industry, it is standard practice to raise calves in non-enriched individual
52	pens after birth (Pempek et al., 2016). However, since the living environment fails to
53	meet the needs of calves and restrict their natural behaviours (e.g. Jensen et al.,
54	1998), social housing has been used to improve their welfare (Van De Weerd and Day,
55	2009). Many studies have shown that social housing provides benefits by promoting
56	natural behaviours, reducing undesirable behaviours, and improving emotional states
57	and production performance (e.g. Costa et al., 2015; Pempek et al., 2016). However,
58	social housing has also been suggested to induce new welfare problems, such as
59	greater risk of respiratory disease (Cobb et al., 2014) and increased cross-sucking

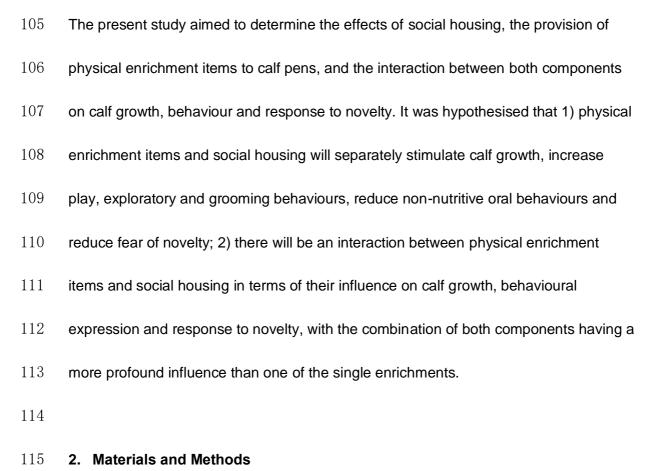
behaviour (Lidfors and Isberg, 2003), which may cause health problems (Größbacher,
2013).

63	One further way of improving animal welfare is to provide physical enrichment (Boissy
64	et al., 2007) through altering the complexity of animals' enclosure or adding physical
65	items to the enclosure (Bloomsmith et al., 1991). For calves, enhancing the complexity
66	of their enclosure can stimulate the expression of natural behaviour and reduce
67	undesirable behaviour (e.g. Jensen et al., 1998). However, the effect of adding
68	physical items to the enclosure on calves' behaviours is rarely studied. Pempek et al.
69	(2017) indicated that adding physical items (teat, brush, "lollie" and chain) to calves'
70	hutches promoted the expression of locomotor play. Horvath et al. (2020) illustrated
71	that the provision of a brush reduced total time engaged in non-nutritive oral
72	behaviours but increased time engaged in grooming. Ude et al. (2011) found that after
73	adding teats into standard pens, calves showed reduced non-nutritive oral behaviours.
74	
75	As well as benefits to calves' behaviours, physical items may also improve calves'
76	growth. For instance, Horvath et al. (2020) indicated that the provision of hay tended to
77	increase solid feed intake and average daily gain of calves during weaning. Mandel et
78	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.

82	Furthermore, physical items have effects on responses to novelty in many farm
83	animals. Fear, which can increase risk of injury and decrease biological functioning
84	(Meehan and Mench, 2002), is a common emotion for animals when they face novelty
85	(Forkman et al., 2007). It can be expressed by behaviours such as active defence,
86	passive avoidance, expressive movements and alarm calls in novelty tests (Erhard and
87	Mendl, 1999; Forkman et al., 2007). Adding relevant items to the enclosure has been
88	shown to affect fear responses, as seen through reducing avoidance and freezing of a
89	novel object in domestic chicks (Jones and Waddington, 1992) and reducing latency to
90	approach a person in piglets (Rodarte et al., 2004). However, the effect of adding items
91	to the enclosure on calves' emotional states is less well known.
92	
93	Whilst the individual effects of social housing or physical items on the improvement in
94	animal's welfare has been widely studied in many species, investigation into the
95	combination of both components is still limited. However, a number of studies in
96	laboratory rats have demonstrated that the application of both social housing and
97	physical items had diverse and non-additive behavioural effects in open-field and novel
98	object tests (e.g. Zimmermann et al., 2001; Schrijver et al., 2002), and improved the

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



- 116 2.1. Ethics statement

117 The study was performed at the Centre for Dairy Research, University of Reading

118 (CEDAR), Reading, UK. All procedures complied with guidelines for the Ethical

119 Treatment of Animals in Applied Animal Behaviour and Welfare Research (Sherwin et

120 al., 2017), and UK and EU laws governing research in animals.

- 121
- 122 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.

128

129	Calves were assigned into eight blocks (six calves in each) according to their date of
130	birth. Within block, calves were randomly allocated to either individual (IP) or pair (PP)
131	pens. Half of the calves in each housing treatment were provided with physical
132	enrichment items (PE): one stationary brush, one plastic chain, one rubber teat and
133	one haynet filled with strawberry-scented ryegrass hay for IP; one haynet filled with
134	strawberry-scented ryegrass hay and two of all other items for PP. Physical enrichment
135	items were chosen based on the motivations hypothesized to be inadequately fulfilled
136	in standard housing. Remaining calves received no additional physical enrichment
137	items (NPE). The area of an IP and a PP was 2.4 m^2 and 4.8 m^2 , respectively; the

138 whole area of each pen was covered with deep straw and fresh straw were added daily 139 into each pen after morning milk feeding.

140

141	This trial was completed in two cohorts (24 calves in each cohort). Within each cohort,
142	pens were arranged in three rows, so that the calves' visual contact in between rows
143	could be limited by the 2-metre wide passages; calves' physical contact with their
144	neighbours within one row could be limited to the gap between the panel bars. All
145	calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets
146	from 2 days of age to 49 days of age. 2.5 litres per feeding (L/f) of milk was offered to
147	each calf until 14 days of age, followed by 3 L/f from 15 to 42 days of age and 2.5L/f
148	between 43 and 49 days of age. During 50-56 days of age, 2.5 L/f milk was fed to each
149	calf only in the morning. Calves had ad libitum access to concentrate (VITA
150	concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and water
151	throughout the study period.
152	
153	2.3. Growth
154	Daily concentrate intake was measured by weighing the daily provision of concentrate
155	and the daily collection of concentrate refusals in each pen until 8 weeks of age. All
156	calves were weighed weekly until 8 weeks of age using a wheeled scale. Daily
157	concentrate intake and average daily gain were calculated by averaging across the

8

- 158 entire period. Calves' concentrate feeding efficiency was estimated by the ratio
- 159 between average daily gain and daily concentrate intake.
- 160
- 161 2.4. Home Pen Behaviours
- 162 Home pen behaviours were recorded by a CCTV (Transit-PTZ, Revader Security Ltd,
- 163 UK) for 14 hours (06:00 h-20:00 h) twice per week when calves were 2, 3, 4, and 5
- 164 weeks of age. Video recordings were watched using instantaneous scans at 5-min
- 165 intervals. The frequencies of calves' behaviours as listed and defined in Table 1 were
- 166 recorded.
- 167
- 168 2.5. Novelty tests

169 Following home pen behavioural observations, an environmental novelty test was 170conducted one day before the novel object test. Both tests were conducted one calf at 171a time. A wheeled scale was used to move each test calf between its home pen and 172the test arena $(4.0 \times 4.0 \text{ m}^2)$. The test arena was set up at two different places for the 173calves in the first and second cohorts. When arriving at the entry of the test arena, the 174 calf was lightly tapped on the hindquarters to encourage it to enter the test arena, in 175which the calf could not see any other calves. Both tests were recorded by either 176 CCTV or webcam (C525, Logitech International S.A, Switzerland). Video recordings 177 were continuously watched. The recorded behaviours for both tests are defined in

178	Table 2. The novelty of the arena and the object can lead to fear reactions in calves
179	(Horvath et al., 2017). When calves feel fearful of the test arena or novel object, they
180	are typically reluctant to touch the pen fixtures or object, defecate and vocalize more,
181	and show sudden movements (Jensen et al., 1999).
182	
183	The environmental novelty test started when the door of the test arena was fully
184	closed. Each calf stayed in the test arena for 15-min. For the novel object test, once
185	entry into the test arena calves were allowed to habituate for 5-min. Following the
186	period of habituation a novel object (a white bucket or a traffic cone, used for alternate
187	blocks of calves) was lowered to the centre of the test arena on a pulley. The calf
188	remained in the pen with the novel object for 10-min.
189	
190	2.6. Statistical analysis
191	All data were analysed using Minitab 18 (Minitab, LLC, USA). Significant differences
192	were declared at $p \le 0.05$ and a trend at $0.05 .$
193	
194	For growth, a general linear model (GLM) was used to determine the effect of forms of
195	enrichment on daily concentrate intake, average daily gain and concentrate feeding
196	efficiency. Factors in the model included physical enrichment items (NPE or PE), social
197	housing (IP or PP) and the interaction between these two factors. Calves' birth weight

198	was used as a covariate. The residuals of daily concentrate intake and concentrate
199	feeding efficiency were not normally distributed, and thus it was square root
200	transformed before analysis.
201	
202	For home pen behaviours and the both novelty tests, video recordings were played
203	with Windows Media Player (Microsoft Corporation, US) and data recorded by one
204	observer. In order to determine the inter-observer reliability, another observer watched
205	the home pen behaviour videos of eight calves by randomly choosing one week from 2,
206	3, 4, and 5 weeks of age for each calf. For both novelty tests, eight calves' videos of
207	environment novelty test and eight calves' videos of novel object test were randomly
208	selected and watched by another observer who was blind to the hypothesis under test.
209	A Pearson correlation was used to compare the reliability between the two observers,
210	which suggested strong positive relationships (home pen behaviour: r=0.995, p<0.001;
211	environmental novelty test: r=0.999, p<0.001; novel object test: r=0.999, p<0.001) and
212	good reliability. For novel object test, the videos were also watched for latency to
213	contact by one of two other observers who were blind to treatment to ensure that data
214	were reliable. Mixed effects model (MEM), GLM or binary logistic regression (BLR)
215	were used to analyse the calves' behaviours in the three tests. For the behaviours
216	analysed by BLR, in order to fit in the regression model, the data of the behaviours
217	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).

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

238	In the novelty tests, fixture touching and abrupt movement in the environmental novelty
239	test, and object touching and latency to first contact with the object in the novel object
240	test were analysed by GLM. Factors included physical enrichment items (NPE or PE),
241	social housing (IP or PP), the interaction between the two factors, arena locations and
242	objects; object was only included as a factor in the novel object test. The covariate was
243	average temperature of the barn during the testing day. Variables, with the exception of
244	latency to first contact with the object in the novel object test, were square root or
245	logarithm transformed before analysis as residuals were not normally distributed.
246	Defecation bout, sudden neck movement and vocalization in the environmental test,
247	and defecation bout, abrupt movement and vocalization in the novel object test were
248	analysed by BLR. Categorical predictors included physical enrichment items (NPE or
249	PE), social housing (IP or PP), the interaction between the two factors, arena locations
250	and objects; object was only included as a factor in the novel object test. The
251	continuous variable was average temperature of the barn during the testing day.
252	Sudden neck movement in the novel object test was not analysed because calves
253	rarely showed this behaviour.
254	
255	One calf's data for daily concentrate intake, average daily gain and concentrate feeding
256	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

258	technological problem. As a result of an abscess on one calf's tongue and navel
259	inflammation in another calf, the data from 2, 3, 4, and 5 weeks of age for the former
260	calf and the data from 3, 4, and 5 weeks of age for the latter calf were discarded before
261	analysis. Moreover, the data from two calves in both novelty tests were discarded
262	before analysis because one calf had an abscess on its tongue and the other one was
263	familiar with the test arena and the novel objects due to his pen location.
264	
265	3. Results
266	3.1. Growth
267	Physical enrichment items and social housing had interactions, resulting in a significant
268	difference in daily concentrate intake ($F_{1,42}$ = 7.01, p = 0.011; Figure 1). Examination of
269	the means by Tukey's multiple comparison test demonstrated that for calves in IP
270	pens, the provision of PE reduced their daily concentrate intake, but not for calves in
271	PP pens.
272	
273	Calves in PE pens tended to have greater average daily gains when compared with
274	those in NPE pens ($F_{1,42}$ = 2.92, p = 0.095; Figure 2). In contrast, calves in IP and PP
275	pens showed similar rates of average daily gain (mean \pm IQR; 577.7 \pm 70.1 g/d vs.
276	$601.8 \pm 141.9 \text{ g/d}; \text{ F}_{1,42} = 0.97, \text{ p} = 0.331$).
277	

278 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).

280 Examination of the means by Tukey's multiple comparison test demonstrated that

- 281 calves in PE-IP pens had better concentrate feeding efficiency than those in NPE-IP,
- 282 NPE-PP and PE-PP pens.

283

284 **3.2**. Home pen behaviour

285 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 %

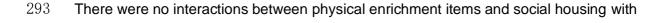
of scans; F_{1,39.58} = 8.51, p = 0.006). Physical enrichment items and social housing

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

291 concentrate compared with those in NPE-IP pens.

292



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 ± 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 ± 0.070 % vs. 0.157

298	\pm 0.045 % of scans; F _{1,41.82} = 43.23, adjusted p < 0.001). In terms of non-nutritive
299	sucking, calves in PE pens were observed to show less non-nutritive sucking than
300	those in NPE pens (0.802 \pm 0.451 % vs. 1.897 \pm 0.401 % of scans; F_{1,40.75} = 26.22,
301	adjusted p < 0.001), and calves in PP pens tended to show less non-nutritive sucking
302	than those in IP pens (1.096 \pm 0.452 % vs. 1.503 \pm 0.477 % of scans; $F_{1,41.42}$ = 3.59,
303	adjusted $p = 0.098$).
304	
305	PE tended to suppress the expression of fixture scratching (Table 4) compared with
306	NPE (adjusted $p = 0.078$). PP increased or tended to increase the expression of
307	locomotor play and allogrooming in comparison with IP (adjusted $p = 0.065$; adjusted p
308	= 0.059). For cross-sucking, PE suppressed the expression of this behaviour compared
309	with NPE (adjusted $p = 0.012$), while PP increased the frequency of this behaviour in
310	comparison with IP (adjusted $p < 0.001$).
311	
312	3.3. Novelty tests
313	In the environmental novelty test, calves in PE pens and NPE pens showed similar
314	durations of fixture touching (316.3 \pm 0.5 vs. 331.0 \pm 1.0 seconds; F _{1,40} = 0.17, adjusted
315	p = 0.908) and similar frequencies of abrupt movement (0.312 \pm 1.000 vs. 1.040 \pm
316	3.000 bouts; $F_{1,40} = 3.41$, adjusted p = 0.288), defecation (adjusted p = 1.000), sudden
317	neck movement (adjusted $p = 1.000$) and vocalization (adjusted $p = 1.152$). Calves in

318	PP pens and IP pens showed similar durations of fixture touching (303.8 \pm 0.6 vs.
319	344.6 \pm 0.3 seconds; F _{1,40} = 1.33, adjusted p = 0.640) and similar frequencies of abrupt
320	movement (0.466 ± 1.000 vs. 0.803 ± 2.000 bouts; $F_{1,40}$ = 0.73, adjusted p = 0.498),
321	defecation (adjusted $p = 0.625$), sudden neck movement (adjusted $p = 1.220$) and
322	vocalization (adjusted p = 0.752). In addition, physical enrichment items and social
323	housing had no interactions on these behavioural responses.
324	
325	In the novel object test, calves in PE pens and NPE pens showed similar durations of
326	object touching (21.1 \pm 19.7 vs. 12.8 \pm 51.0 seconds; F _{1,39} = 0.87, adjusted p = 0.446)
327	and latency to first contact with the object (135.7 \pm 139.0 vs. 256.8 \pm 560.0 seconds;
328	$F_{1,39}$ = 3.36, adjusted p = 0.375), and similar frequencies of abrupt movement (adjusted
329	p = 0.295), defecation (adjusted $p = 1.000$) and vocalization (adjusted $p = 0.258$).
330	Calves in PP pens and IP pens showed similar durations of object touching (15.9 \pm
331	62.1 vs. 17.0 \pm 25.5 seconds; F _{1,39} = 0.01, adjusted p = 1.131) and latency to first
332	contact with the object (198.3 \pm 534.5 vs. 194.1 \pm 476.5 seconds; $F_{1,39}$ < 0.01, adjusted
333	p = 0.950), and similar frequencies of abrupt movement (adjusted $p = 0.828$),
334	defecation (adjusted $p = 0.200$) and vocalization (adjusted $p = 1.495$). In addition,
335	physical enrichment items and social housing had no interactions on these behavioural
336	responses.

338	4.	Dic	cuss	inn
220	4.	012	LUSS	1011

339	Physical enrichment items offered some benefits for growth and supressed non-
340	nutritive oral behaviours. Social housing had no effect on calves' growth but promoted,
341	or tended to promote, some positive behaviours. Physical enrichment and social
342	housing had non-additive effects on calves' growth and home pen behaviour. Physical
343	enrichment items, social housing and the interaction between these two factors had no
344	effect on calves' behavioural responses in the novelty tests.
345	
346	4.1. Growth and feeding effects
347	In the present study, physical enrichment tended to increase calves' average daily
348	gain, but reduced individual calves' daily concentrate intake, resulting in improved
349	feeding efficiency. This contrasts with the findings of Pempek et al. (2017), who
350	reported that furnished pens had no effect on calves' concentrate intake and weight
351	gain. The difference may be attributed to the provision of roughage to calves.
352	According to Pempek et al. (2017), calves had no access to hay or other roughage, but
353	in this study, ryegrass hay was provided ad libitum to calves. While calves without
354	physical enrichment only consumed ryegrass hay from hay racks, strawberry-scented
355	ryegrass hay was also provided to physically enriched calves from haynets. Since
356	animals can use their sensorial perceptions to choose palatable feeds (Baumont, 1996)
357	and some aromas can increase the palatability of hay (Cannas et al., 2009), the

358	strawberry aroma in this study might have stimulated calves to eat more hay.
359	Strawberry was chosen because red berry flavouring was previously found to be a
360	preferred aroma for dairy cattle (Meagher et al., 2017). As observed, calves with
361	physical enrichment items showed a higher frequency of hay intake, which may result
362	in increased consumption of hay and increased average daily gain compared with non-
363	physically enriched calves.
364	
365	Altogether, the increase in roughage intake in calves with physical enrichment items,
366	and better concentrate feeding efficiency in individual enriched pens, are likely to be
367	economically beneficial on farms. Improved feed conversion efficiency is an important
368	objective for profitable dairy operations (Bach et al., 2007). Oostindjer et al. (2010) also
369	demonstrated that physical enrichment (straw, wood shavings, peat, and branches)
370	positively affected the feed conversion efficiency for piglets. These results may be
371	attributed to the reduced stress in physically enriched living environments (Barnett et
372	al., 1983).
373	

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

378 increased concentrate intake through social learning or social facilitation (Costa et al., 379 2015). In other words, the presence of other calves near the bucket or sight of them 380 eating would increase the likelihood of calves paying attention to feed and perform 381 similar behaviours; calves could also learn where to find concentrate and how to 382 consume it by observing, or interacting, with calves showing those behaviours. One 383 potential reason for the lack of treatment effect in this study is the different housing 384 design. When researchers previously studied the effect of social housing on calves' 385 growth, they compared calves in grouped environments with calves in individual 386 environments with only auditory contact or auditory and visual contact. For instance, 387 Jensen et al. (2015) positioned adjacent pens 1.5-metre apart to prevent physical 388 contact between calves in different pens. However, in the current experiment, calves 389 had auditory, visual and limited physical contact with their neighbours. Therefore, 390 calves in individual pens may imitate or learn how to consume concentrate from their 391 pair-housed neighbours. Jensen and Larsen (2014) similarly demonstrated that calves 392 in individual pens with limited physical contact with their neighbours and calves in 393 paired pens had similar daily concentrate intake and average daily gain. 394

395 4.2. Home pen behaviour

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

398	affective states, or indicates increased positive experience (Held and Špinka, 2011),
399	social housing may provide a more pleasurable living environment for calves. Jensen
400	et al. (1998) also showed that social stimulation might lead to the appearance of
401	locomotor play. However, calves in individual and paired pens showed similarly low
402	frequencies of social play. One reason for this phenomenon could be the later
403	emergence of social play in the calves' life, with limited amounts occurring in the first
404	few weeks (Jensen et al., 1998). In addition, physical enrichment items had no effect
405	on locomotor or social play, which may indicate that these types of play are not
406	stimulated by external objects.
407	
408	Sniffing is a type of exploratory behaviour which is motivated by the animals need
408 409	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
409	gather environmental information (Westerath et al., 2009). The expression is perceived
409 410	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,
409 410 411	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
409 410 411 412	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
 409 410 411 412 413 	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.
 409 410 411 412 413 414 	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

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

420	Allogrooming and fixture scratching are body care behaviours (Kohari et al., 2007),
421	which help maintain hygiene of the animal's body by removing debris or ectoparasites
422	(Rich, 1973). Moreover, allogrooming is important in forming or maintaining social
423	relationships between calves (Færevik et al., 2007). In this study, social housing
424	tended to increase the expression of allogrooming, but physical enrichment items had
425	no effect on this behaviour. This result agreed with previous studies conducted by
426	Tapki (2007), and Horvath and Miller-Cushon (2019). The former showed that social
427	housing encouraged calves to express allogrooming voluntarily. The latter suggested
428	that physical enrichment item (brush) had no effect on this natural behaviour. The
429	result from this study may demonstrate that allogrooming does not relate to stimuli from
430	external items. In addition to social body care behaviour, fixture scratching was
431	expressed less in physically enriched calves, which might be a consequence of the
432	existence of other more suitable scratching items in physically enriched pens.
433	
434	Non-nutritive sucking, cross-sucking and tongue rolling are considered as non-nutritive
435	oral behaviours, which are non-functional and harmful (Le Neindre, 1993; Jensen,
436	2003; Garner, 2005). Non-nutritive sucking may be considered as redirected sucking
437	behaviour (De Passillé et al., 1992). Calves have a strong motivation for suckling.

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

458 et al., 2012). In this study, although the frequency of concentrate intake, hay intake and

459 ruminating were affected by different treatments, the frequency of feed intake

460 behaviours was similar across all treatments.

461

462 4.3. Response to novelty

463 Neither physical enrichment nor pair housing were found to affect calves' behavioural 464 responses in either novelty test. These findings agree with previous studies showing no 465 effect of social housing (Jensen and Larsen 2014) or the provision of physical 466 complexity to a standard hutch (Pempek et al. 2017) on calves' behavioural responses 467 to social and environmental novelty. This phenomenon may indicate the static 468 environment created by providing additional objects was not complex enough to elicit 469 emotional change in novel situations. Therefore, a more complex and dynamic 470 environment is probably needed in future studies to reduce calves' fearfulness. In 471 terms of the effect of social housing, Leruste et al. (2014) found that some behavioural 472 responses of calves (e.g. vocalization and exploratory behaviour) in individual pens 473 with tactile contact were similar to those of pair-housed calves, which may indicate that 474 individual housing with tactile contact may result in similar fearfulness in pair housed 475 calves. 476

110

477 **5.** Conclusion

478	Provision of physical enrichment improved calves' growth by promoting intake of
479	roughage and increasing weight gain and concentrate feeding efficiency. In contrast,
480	social housing was less effective at improving calves' growth. Provision of physical
481	enrichment reduced calves' non-nutritive oral behaviours, while social housing had a
482	positive impact on play, exploratory, and social behaviours. However, neither treatment
483	affected calves' fear of novelty. In conclusion, physical enrichment items and social
484	housing may satisfy calves' needs in different ways, but the combination of both
485	components did not further improve calves' welfare.
486	
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Tables

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

- ⁶⁴⁵ ¹The time duration of the behaviour was recorded.
- ²The frequency of the behaviour was recorded.

648 **Table 3.** Six home pen behaviours (mean ± IQR) analysed using mixed effects models (MEM)¹. Samples sizes were physically enriched

649 individual PE-IP, n = 7; non-physically enriched individual NPE-IP, n = 8; physically enriched pair PE-PP, n = 16 and non-physical enrichment

650 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

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

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

⁶⁵³ ³Adjusted p-values were calculated using false discovery rate (FDR) to fixture sniffing, social sniffing and non-nutritive sucking.

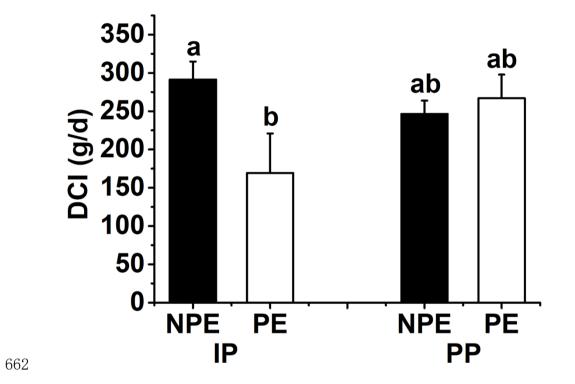
Table 4. Six home pen behaviours (mean ± IQR) analysed using binary logistic regression (BLR)¹. Samples sizes were physical enrichment

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	

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

	Cross-sucking (%)	-1.594	3.067	0.012	<0.001	PE < NPE	PP > IP		
657	¹ Significant difference was declared at adjusted $p \le 0.05$ and a trend at 0.05 < adjusted $p \le 0.10$.								
658	² Adjusted p-values were calculated using false discovery rate (FDR).								
659	³ Whether enriched treatments (tend to) make each behaviour been expressed more likely or less likely.								

661 Figures



663 Figure 1. Back-transformed means (±IQR) of daily concentrate intake (DCI) for calves from non-physically enriched individual pens (NPE-IP; n

- 664 = 8 calves), physically enriched individual pens (PE-IP; n = 7 calves), non-physically enriched paired pens (NPE-PP; n = 16 calves) and
- 665 physically enriched paired pens (PE-PP; n = 16 calves). Different letters (a, b) represent significant differences between treatments at $p \le 0.05$.

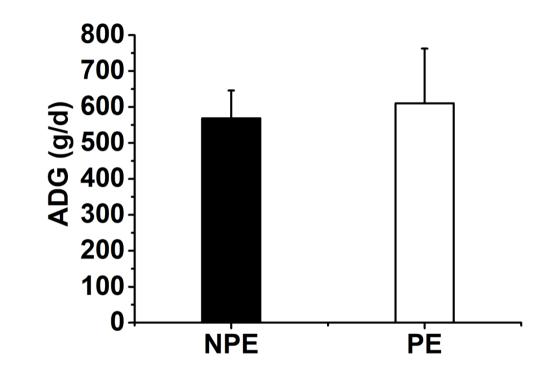
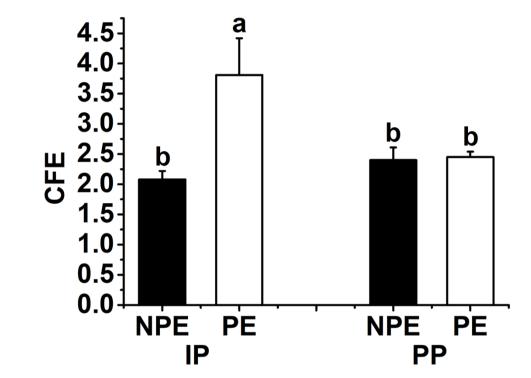


Figure 2. Means (±IQR) of average daily gain (ADG) for a) calves from non-physically enriched pens (NPE; n = 24 calves) and physically

⁶⁶⁹ enriched pens (PE; n = 23 calves).



671

672 Figure 3. Back-transformed means (±IQR) of concentrate feeding efficiency (CFE; grams of gain per gram of concentrate intake) for calves

673 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

significant differences between treatments at p<0.05.