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1 Effects of horn status on behaviour in fattening cattle in the field and during reactivity tests

2

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9

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11

12 Abstract

13 Disbudding is in the short-term invasive and needs pharmacological pain treatment but it facilitates  
14 animal management. The present study investigates the longer-term consequences on behaviour to  
15 evaluate possible effects on animal welfare. Experiment 1 (E1) used 81 bulls up to the age of 12 and  
16 experiment 2 (E2) 71 heifers up to the age of 11 months. Half of the animals was disbudded at about  
17 2 months of age. Different rearing conditions (RC) were compared, with animals housed in groups  
18 containing i) only animals with horns, ii) only disbudded animals, or iii) mixed (half with horns, and half  
19 disbudded; only in E1). Each rearing condition had two replicates. The effects of RC were studied on  
20 general activity and synchrony at 4 and 9 months (E1 and E2), and 7 and 12 months (only E1) of age.  
21 In E2 and during the last measuring period of E1, disbudding and mixing modified physical activity in  
22 the field. Behaviour during a novel object test (NOT) and a food competition test (FCT) were studied  
23 at 10 and 11 months of age, for E1 and E2, respectively. During the FCT, compared to disbudded,  
24 horned (unmixed) animals showed more agonistic interactions with contact in E1, and more agonistic  
25 behaviour without contact in E2. In the NOT, disbudded (mixed and unmixed) animals of E1 presented  
26 more fear-related reactions compared to horned animals while in E2, the opposite was found. In the  
27 NOT and FCT, mixed groups had intermediate levels for behaviours influenced by horn status. In

28 conclusion, the behavioural traits influenced by horn status appeared to be at least partly associated  
29 with agonistic behaviour and fear, and may influence welfare status. However, depending on the  
30 experiment and the test, different negative and positive effects on welfare were found. The mixing of  
31 horned and disbudded animals in rearing groups may also modify the behavioural consequences of  
32 horn status.

33

34 Key words (AABS max. 6): Horns; Beef cattle; **General activity; Behavioural reactivity;** Competition

## 35 1. Introduction

36 Disbudding, the removal of horn buds in young calves, facilitates animal management, particularly  
37 when animals are in groups in confined environments. For example, at slaughter, bruising was lower  
38 in groups of genetically polled or dehorned cattle compared to groups containing horned cattle  
39 (Meischke et al., 1974, Shaw et al., 1976). On the other side, the intervention is invasive and the pain  
40 and short-term stress responses are well described (reviewed by Herskin and Nielsen (2018), Casoni et  
41 al. (2019)). There is also some information on the longer-term consequences of horn status with  
42 respect to behaviour. Compared to cows and heifers without, cows and heifers with horns expressed  
43 more agonistic interactions, and, associated with this, had higher dominance ranks (Sambraus, 1969,  
44 Bouissou, 1972, Lutz et al., 2016). In another study, horned cows showed similar levels of agonistic  
45 behaviour, but a greater proportion of agonistic behaviour without contact than hornless cows (Lutz  
46 et al., 2019). Fordyce et al. (1988) reported that horned adult cattle had calmer behavioural responses  
47 to handling. Finally, in waiting pens, groups of horned dairy cows showed slightly greater locomotor  
48 activity, than groups of hornless cows (Lutz et al., 2019).

49 To our knowledge, the effects of horn status on behaviour under other conditions, such as during  
50 reactivity tests, or spontaneous general activity in the field, are lacking. The two studies presented  
51 here investigated the effect of disbudding on the behaviour of young male and female cattle, during  
52 reactivity tests and in the field. The animals were reared in mixed and unmixed groups with respect to  
53 horn status to take into account rearing contexts as they may occur in practice. The objective was to  
54 enlarge our knowledge of the longer-term behavioural consequences of disbudding and to refine our  
55 interpretation of its effects in terms of animal welfare.

56

## 57 2. Animals, material and methods

### 58 2.1. Animals and housing

59 We conducted two experiments, one with 81 bulls (E1; 10/2015 - 11/2016) and one with 71 heifers  
60 (E2; 06/2016 – 09/2017). Both trials respected the Swiss laws of animal protection and were authorized  
61 by the cantonal veterinary office of Fribourg, Switzerland (No. 2015\_21\_FR). The 81 bulls used in E1

62 were the same as reported in Reiche et al. (2020) and Reiche et al. (2019). Half of the animals were  
63 disbudded at the age of 52 (E1) and 63 (E2) days using a hot iron under sedation, local anesthesia and  
64 systemic analgesia. In both experiments, rearing groups were balanced for behavioural reactivity  
65 before disbudding (see section 2.2.), cortisol responses to ACTH (see Reiche et al. (2020)) and body  
66 weight. In E1, they were assigned to three rearing conditions (RC) according to their horn status;  
67 rearing groups consisted of horned (H+) or disbudded (H-) animals, or were mixed (M; half of the group  
68 horned, half disbudded), each with two replicates. The two replicates started one week apart. Bulls  
69 were housed in groups of 13-14 per pen on deep litter with access to an outdoor pen (earth floor; deep  
70 litter and total space allowance of 3.9 and 9.4 m<sup>2</sup>/bull), in front of which people passed up to 30 times  
71 a day. Straw was distributed manually. Each bull was equipped with an ear chip giving it *ad libitum*  
72 access to one of the seven automatic feeding stations (distributing a total mixed ration containing hay,  
73 corn silage and 10% concentrates). Each bull had further access to a supplement of 200 g of  
74 concentrates at another, single feeding station. In E2, animals were assigned in a similar manner as  
75 bulls to two rearing groups (horned and disbudded, no mixed group was present); the two replicates  
76 started two weeks apart. Seventeen to 18 heifers per pen were housed in four pens in a deep litter-  
77 loose housing barn (deep litter and total space allowance: 3.4 m<sup>2</sup> and 6 m<sup>2</sup>/heifer), without access to  
78 an outdoor pen. Straw was distributed automatically. Heifers were fed twice a day a similar diet as in  
79 E1, distributed by a feed mixer in a 6-m-long feed trough (without fences). Animal caretakers passed  
80 up to 4 times a day. In both experiments, animals were weighed every five wks on a scale (Grüter,  
81 Eschenbach, Switzerland). The E1 weighing took place in the outdoor pens and the E2 weighing in a  
82 250 m<sup>2</sup> fenced area adjacent to each of the 4 indoor rearing pens. In both experiments, 2-m high, solid  
83 wood panels separated rearing pens from each other in order to avoid visual and physical contact  
84 between rearing groups.

85

## 86 2.2. Behavioural measurements

87 The behavioural measurements and tests were organised similarly for the two experiments, but were  
88 not identical, due to technical constraints.

89 One week before disbudding, calves were subjected to reactivity tests in order to balance rearing  
90 groups for behavioural reactivity. In both experiments, a 3-min open-field test was conducted on  
91 individual calves at the age of 6 (E1) and 7 (E2) weeks in test arenas of 4 x 4 m. In E1, calves were  
92 subjected to an additional 4-min group reactivity test to evaluate the approach behaviour to an  
93 unknown human who had entered the rearing pen in which calves were housed in groups of 15-16  
94 calves between arrival at the farm and grouping.

95 In E1, general activity (GA) was measured over four periods during fattening, February (P1), May (P2),  
96 July (P3) and October (P4), that is at 4, 7, 9 and 12 months of age, respectively, and in E2 two periods,  
97 September (P1) and February (P2), that is at 4 and 9 months of age, respectively, by accelerometers  
98 (ITIN & Hoch, Liestal, Switzerland). At each period, accelerometers were used during two to four  
99 consecutive days. In total, 28 and 36 accelerometers were available and used in E1 and E2, respectively.

100 Within each period, accelerometers were used simultaneously for the animals of two rearing groups  
101 of one replicate. For E1, choice of the two rearing groups was balanced for RC over the periods.

102 Accelerometers were fixed laterally at the left hind leg, proximal to the fetlock joint. During measuring  
103 periods, position and functioning of devices were checked two times per day (07:00 AM and 04:00  
104 PM), during which the devices were replaced or fixed when necessary. Lying, standing and walking  
105 time (sum of durations of all lying, standing and walking bouts, respectively, during a given recording  
106 period), and number of stand ups (event where the animal stands, moving from a lying to standing  
107 position) have been quantified (Alsaad et al., 2015). To exploit the recorded data, the software  
108 Rumiwatch® converter (version 36, ITIN & Hoch, Liestal, Switzerland), previously validated for posture  
109 and locomotion behaviour in cattle housed indoors, was used (Alsaad et al., 2015).

110 At the age of 10 and 11 months, animals of E1 and E2, respectively, were subjected individually to a  
111 Novel object test (NOT). The test arena (Figure 1) had a size of 4 x 4 meters and was bordered by 2-m-  
112 high, solid barriers that ensured visual separation from conspecifics. In E1, the arena was erected in  
113 the feeding area of the rearing pen, containing a solid floor. In E2, the test arena was erected in the  
114 outdoor area, which contained an earth floor with woodchips. All animals of one replicate were tested  
115 in the afternoons of two consecutive days, using a design balanced for rearing group. Forty-five min

116 before the test, the whole rearing group was quietly led to the outdoor pen (E1) or the feeding area  
117 (E2) of their home pen. The test arena was installed. Fifteen minutes prior to the beginning of the test,  
118 animals to be tested were returned to the rearing pen's straw area. The order of tested animals was  
119 randomized. Two familiar handlers led the animal to be tested gently towards the test arena. Time  
120 from the start of this intervention until the closure of the door of the test arena was recorded for each  
121 animal. The test was organised in four phases and started as soon as the door of the test arena was  
122 closed behind the test animal. The first phase tested reactivity to isolation in a novel environment and  
123 lasted 60 s. The subsequent three phases aimed to study reactivity to the presence of a novel object  
124 (NO; a traffic cone). In the second phase (duration: 10 s) the NO was lowered from the ceiling in the  
125 middle of the test arena, announced by the ringing of a small bell that had been attached to the cone  
126 in order to get the cone noticed by the experimental animal. The third phase started with the NO  
127 touching the ground and lasted until the first contact or - in case of no contact - for 120 s (thus phase  
128 duration depended on the animal), and the fourth phase started at the first contact and ended 15 s  
129 afterwards. Encoded behaviours, test phases and their definitions are listed in Table 1.

130 Two weeks after the NOT, each rearing group was submitted to a food competition (FC) test, following  
131 Terlouw et al. (1991) with modifications. Animals had been overnight fasted, and tests took place  
132 between 8:00 and 9:00 AM. In E1, seven buckets containing 200 g of the normal concentrate  
133 (equivalent of a daily portion distributed by the feeding station) were positioned in a circle in the  
134 middle of the floor of the straw area of the rearing pen. In E2, pre-tests found that the heifers were  
135 not attracted by the buckets arranged in this manner, but that they were if the food was distributed in  
136 the feeding troughs. The set-up with the buckets may have produced a fear response as the animals  
137 were used to trough feeding. The test took therefore place at the feeding trough, which was reduced  
138 to half its length using wood panels thus giving access to only half of the animals of one rearing pen at  
139 the same time. The test was repeated three times. Prior to each session, two familiar handlers quietly  
140 returned the animals to the outdoor pen and the straw area in E1 and E2, respectively. Sessions ended  
141 when all the concentrate had been eaten, i.e. after 130-140 s in E1 and 120 s in E2. Behaviour analysis  
142 included vicinity to feed (recorded every ten seconds, Table 1) and social interactions, recorded

143 continuously, with actor (initiating the interaction) and receiver (receiving the interaction). Agonistic  
144 behaviours recorded involved those without physical contact (threats and avoidances), **those with**  
145 **contact, including** head-to-head interaction (head-to-head contact, rub, push), head-to-body and  
146 body-to-body contact (head butts and pushes to body, or pushing with the body), and non-agonistic  
147 interactions (head on back, head on hindquarters, mounting) (Table 1). **Agonistic** interactions were  
148 identified as “successful” when they caused displacement of the receiver and “unsuccessful” if not.  
149 All behavioural tests were filmed by video cameras (Panasonic, Osaka, Japan). Behaviour analyses were  
150 carried out using the software The Observer (version 11, Noldus, Wageningen, The Netherlands).

151

### 152 2.3. Statistical analysis

153 *General activity.* Various interventions (feeding, straw distribution, cleaning, control of  
154 accelerometers) took place between 8-11 AM and 4-5 PM and these periods were therefore excluded  
155 from the statistical analysis. Consequently, analysed periods started at 11:00 AM and **ended** at 8:00  
156 AM the following day (total of 20h). Duration of each activity was summed and expressed in minutes  
157 per animal and per day. Number of stand-ups were also summed and expressed per animal and per  
158 day. Only complete 20h days without missing data were included in **the** statistical analysis.

159 *Reactivity tests.* For statistical analysis of NOT, ranked data were used. Animals that did not touch the  
160 object were removed from the analyses of phases 3 and 4. For the FCT, for each animal, the number  
161 of each behaviour/animal/minute and the percentage of time for each of the vicinities relative to the  
162 food (number of observations where the animal was in a certain vicinity divided by the number of total  
163 observations) were calculated for each of the **three** sessions. For the statistical analysis, means of the  
164 **three** sessions were used.

165 Statistical analyses were performed using linear mixed models of the R environment (Bates et al., 2014,  
166 Core, 2018). Rearing condition, period (where present) and their interactions were introduced as fixed  
167 factors; replicate of the experiment, day and animal as random factors. Models met the assumption of  
168 normally distributed residuals and homogeneity of variances (visual check of residual-versus-  
169 covariable- and residual versus-fitted-plots). Significance of fixed factors was analysed by partial F-tests



170 (type-III-ANOVA). Pairwise comparisons were performed on least-squares means with adjustment for  
171 multiple comparisons following Benjamini and Hochberg (1995). Rarely observed behaviours during FC  
172 with mean occurrences  $<0.2$  /min were coded as binary variables and analysed by logistic regression  
173 for binomial data using logit-transformation. **Agonistic and non-agonistic activities in the FCT have**  
174 **been analysed as totals as well as the separate activities composing them (Table 1). Totals that differed**  
175 **significantly between RC were subsequently analysed by linear regression, using the various activities**  
176 **composing them as covariable, to identify which activity contributed significantly to the RC effect. If**  
177 **the activity was a rarely observed behaviour (see above) it was introduced as a binary covariable.**  
178 **Rearing condition and replicate were introduced as fixed factors; if RC was no longer significant after**  
179 **introduction of covariables, it was considered that the covariable(s) explained the RC effect.**

180 Only effects observed in both replicates are considered robust and are presented **in the Results section.**

181 The XLstat Addinsoft (Version 2017.2) was used for Spearman correlations and Principal Component  
182 Analyses (PCA), based on individual data (with Spearman rank correlation matrix as input data). PCA  
183 was used to obtain graphical representations and general overview of behavioural tendencies in the  
184 NOT. **PCAs represent only correlations whose  $\rho > 0.40$  and if  $\rho$  and significance did not depend on the**  
185 **factor replicate; i.e. if introduction of the factor replicate in the analysis (ANCOVA) did not remove the**  
186 **significance of the correlation. Final PCAs contain only robustly correlated variables with loadings  $>0.50$**   
187 **or  $<-0.50$ .**

188 **For** the open field tests (E1 and E2) and human approach tests (E1) carried out before disbudding, 4  
189 PCAs, one per replicate of each experiment, were produced to summarise behavioural responses. The  
190 first two axes of the final models explained between 53.1 and 70.5% of the total variability for each  
191 replicate of each experiment. The ranked individual scores on the first two axes of the PCA were  
192 balanced across rearing groups (within replicate and experiment) and consequently, similar between  
193 rearing groups (analysis of variance:  $P > 0.80$ ).

194 For synchrony analysis, we used scan-samples **of posture (lying vs standing)** at 20-min intervals  
195 following Stoye et al. (2012). The Kappa coefficient is an indicator of behavioural synchrony on the  
196 group level, i.e. whether behaviours of a group are more synchronized than expected by chance, and

197 corrects for differences related to group size. They were calculated for each RC and replicate and within  
198 mixed groups, for horned and disbudded subgroups, following Rook and Penning (1991), with  $K = 1$   
199 when complete synchrony is observed and  $K=0$  when the observed proportion of synchrony is equal  
200 to the expected. Kappa coefficients were compared between periods and rearing groups using linear  
201 mixed models as described above. Using the same scan samples, Ruckstuhl's indices were calculated  
202 following Ruckstuhl (1999) for each animal, and express the degree to which an individual synchronises  
203 with the rest of the group. The Ruckstuhl index does not correct for group size, and consequently, we  
204 have only compared the values for similar group sizes (i.e. horned vs disbudded bulls within the mixed  
205 groups were not compared).

206 Means are presented with standard errors ( $\pm$  SE), unless otherwise specified.

207

### 208 3. Results

#### 209 3.1. Spontaneous behaviour

210 In both experiments, daily activities of lying, standing and walking were influenced by the measuring  
211 period (Table S1). In E1, rearing condition had no effect in the first three measuring periods. In the  
212 fourth measuring period (Table 2), M bulls tended to spend more time lying and spent less time walking  
213 than H+ and H- bulls. Disbudded bulls showed least stand ups and spent less time walking than horned  
214 bulls. No differences were found between horned and disbudded bulls within mixed RC ( $P>0.39$ ).

215 In E2, H+ heifers spent less time lying, more time standing, and tended to spend less time walking than  
216 H- heifers (Table 2).

217 In E1, depending on replicate and RC, Kappa coefficients ranged between 0.17 – 0.64 and increased  
218 over time (Table S2). In E2, whereas Kappa coefficients in H+ heifers decreased slightly from the first  
219 to the second measuring period, those of H- heifers decreased markedly (Table S2; interaction effect:  
220  $P<0.05$ ).

221

#### 222 3.2. Novel object test

223 Results are presented in Table 3. In E1, most differences were found between H+ and H- bulls. Horned  
224 bulls needed less time to be driven in the test arena, spent more time in zone 3 and changed less often  
225 the zone during isolation (phase 1) than H- bulls. During lowering of the NO (phase 2), H+ and M bulls  
226 spent less time in zone 2 and H+ bulls more time in zone 4 than H- bulls, but time spent in zones near  
227 the exit (total time spent in zone 1 and zone 2) did not differ ( $p=0.12$ ) between RC ( $71.2\pm6.4$ ,  $60.4\pm6.9$   
228 and  $76.7\pm6.4$  for H+, M and H- bulls, respectively). Latency to sniff the NO did not differ according to  
229 RC and ranged from 0 to 114 s (mean: 19.9 s). One M bull did not touch the NO within 120 s. Compared  
230 to H- and M bulls, H+ bulls tended to spend more time sniffing the NO after the first contact (phase 4).  
231 In phase 4, within mixed RC, horned bulls spent less time in zone 2 (MH+:  $10.91 \pm 7.37$  %; MH-:  $27.91$   
232  $\pm 8.21$  %;  $P<0.05$ ) and less time in locomotion (MH+:  $20.33 \pm 5.67$  %; MH-:  $36.36 \pm 6.80$  %;  $P<0.05$ ) than  
233 disbudded bulls.

234 Most correlations were found between behaviours recorded during phases 1 and 4 (Table 4). A positive  
235 correlation between time spent immobile (phase 4) and time spent touching the NO (phase 4) was  
236 found for all RC. For H+ and H- bulls, more time spent sniffing elsewhere (phase1), was correlated with  
237 more time spent in zone 4 (phase 1) and more time spent immobile (phase 4). Immobility (phase 1)  
238 was positively correlated with time spent touching the NO (phase 4), amongst others. For H- and M  
239 bulls, time spent in zone 1 (phase1) was negatively correlated with time spent interacting with the NO  
240 (phase 4), and number of zone changes in phases 3 and 4 were positively and negatively, respectively,  
241 correlated with latency to sniff the NO and with time spent sniffing elsewhere (phase 1). A positive  
242 correlation between time spent sniffing elsewhere (phase 1) and time spent touching the NO (phase  
243 4) was found for H+ and M bulls.

244 **The Principal Component Analysis based on correlated variables of the NOT of E1 explained 60.5% of**  
245 **the variance (Figure S1).** Lower levels of locomotion (percentage of time and zone changes) were  
246 associated with higher levels of explorative activities of the environment in phase 1 and the NO in  
247 phase 4 on the first axis. The second axis shows that a longer latency to sniff the NO was associated  
248 with more zone changes during that latency (phase 3).

249 In E2, H+ heifers spent less time during phase 1 looking elsewhere and more time sniffing elsewhere  
250 than H- heifers. In phase 2, H+ heifers tended to look longer at the exit door than H- heifers. Latency  
251 of heifers that touched the NO ranged from 0 to 104 s (mean: 14.9 s). Five heifers of each RC did not  
252 touch the NO within 120 s. In phase 3, H+ heifers changed more often the zone and tended to spend  
253 less time looking at the NO than H- heifers. In phase 4, H+ heifers spent more time in zone 2, and  
254 tended to spend more time moving and less time looking elsewhere than H- heifers.  
255 Most correlations concerned variables relative to contact with the NO (Table 4). Sniffing the NO and  
256 immobility in phases 1 and 4 were positively correlated with each other. Latency to sniff the NO was  
257 positively correlated with time spent in sniffing the exit and number of zone changes (phase 3), and  
258 negatively with looking at the NO (phase 3). The plot of a principal component analysis of robustly  
259 correlated variables, explaining 72.0 % of the variation, presented a similar distribution of the variables  
260 as the PCA plot of E1, apart from that the first axis did only include locomotion in phases 1 and 4.

261

### 262 3.3. Food competition

263 Across sessions, percentage of time spent in vicinity 1 ranged from 0 to 80.7% in E1 and from 0 to 100%  
264 in E2. In E1, 817 interactions were recorded (56.3% pushing with the head, 15.5% mounting or head  
265 on back or hindquarters, 13.3% pushing with the body, 6.2% head-to-head interactions, 4.4% head  
266 butts, 4.3% threat or avoiding). Horned unmixed bulls showed greater frequency of total agonistic  
267 interactions, than H- bulls (Table 5). This was essentially due to interactions with contact, particularly  
268 head-to-body and head-to-head interactions: their combined introduction as covariables (both at  
269  $P < 0.001$ ) explained 73.9 % of the variability of total agonistic behaviour in the linear regression  
270 analysis, and removed the effect of RC ( $P = 0.20$ ). Unmixed H+ bulls were relatively less successful in  
271 displacing other animals compared to unmixed H- bulls (Table 5). No differences were found within M  
272 groups ( $P > 0.26$ ).

273 In E2, 388 interactions were recorded (31% mounting or head on back or hindquarters, 29% pushing  
274 with the body, 18% pushing with the head, 7% head-to-head interactions, 11 % threat or avoiding, 4%  
275 head butts). Sixty and 55 heifers out of 71 initiated and received at least one interaction; number of

276 initiated and received interactions ranged from 0 to 13 and from 0 to 9, respectively. Horned heifers  
277 expressed greater numbers of total agonistic interactions without contact. When introduced as  
278 covariables in the linear regression analysis, levels of threats ( $P < 0.001$ ) and avoidances ( $P < 0.001$ )  
279 explained together 90.4 % of the variability in total agonistic interactions without contact (with similar  
280 contributions) and removed the RC effect ( $P = 0.80$ ). Compared to H- heifers, more H+ heifers tended  
281 to express head-to-body interactions and they tended to greater total levels of agonistic activities  
282 (Table 5). Levels of head-to-body interactions ( $P < 0.001$ ) and agonistic interactions without contact ( $P$   
283  $< 0.001$ ) explained to 41.7 % of the variability in total agonistic interactions and removed the RC effect  
284 ( $P = 0.86$ ). Horned heifers were further more successful in displacing other animals agonistically (Table  
285 5). In both experiments, body weight was not correlated with vicinity and frequency of behaviours.

286

### 287 3.4. Relationships between tests

288 For both E1 and E2, correlations were found between tests, but they were not consistent, that is, they  
289 differed between rearing conditions and/or repetition, and are therefore not presented.

290

## 291 4. Discussion

292 Part of the studied behavioural traits differed between horned and disbudded young fattening cattle.  
293 In the field, horned heifers and bulls aged 12 months expressed more standing and walking  
294 respectively, than when disbudded. Horn status influenced further frequency and forms of agonistic  
295 behaviour during the FCT, explorative and fear-related behaviour during the NOT, but the exact effects  
296 varied between experiments.

297 The correlations and principal component analyses show that in both experiments, greater levels of  
298 exploration of the NO were associated with greater levels of standing immobile. Increased interaction  
299 with the NO was also associated with shorter durations of sniffing (E2) and staying close to (E1) the  
300 exit door, behaviours indicative of lower fear (Bourguet et al., 2010). The activity profile including  
301 exploration of the NO and low interest for the exit door may therefore express that the balance  
302 between fear and curiosity tips towards the latter (Bourguet et al., 2010). In E1, compared to H+ bulls,

303 H- bulls spent less time sniffing the NO, while more time was needed to drive them towards the test  
304 arena (Lensink et al., 2001, Bourguet et al., 2010). Hence, in E1, H- bulls appeared more fearful  
305 compared to H+ bulls. In E2, H+ heifers tended to look less at the NO, and had more zone changes;  
306 both activities were associated with a longer latency until sniffing the object and they may be  
307 interpreted as an expression of greater fear (Boissy and Bouissou, 1995). Hence, in E2, presence of  
308 horns appears to be associated with greater fearfulness.

309 Negative correlations were found between exploration of the NO and locomotion in both E1 and E2.  
310 Most other studies found that increased exploration, including of a novel object, was associated with  
311 increased locomotion (Le Neindre, 1989, de Passillé et al., 1995, Van Reenen et al., 2005, Graunke et  
312 al., 2013, Foris et al., 2018), contrary to the present study. A possible explanation is that in contrast to  
313 these earlier studies, in our study, locomotion during the different phases was socially motivated due  
314 to the vicinity of the other members of the rearing group.

315 The different results obtained in the NOT in E1 and E2, may be related to the differences between the  
316 two experiments, for example density and group size in the rearing pen, or the slightly different NOT  
317 set-ups. It is also likely that gender played a major role. For example, in E2, heifers appeared more  
318 fearful as the percentage of animals that did not touch the NO at all was greater than in E1. This gender  
319 effect may be testosterone related, as demonstrated by Boissy and Bouissou (1994).

320 During the FCT, levels of agonistic interactions were much lower in E2 than in E1, with the most  
321 frequent agonistic activity being pushing with the head in E1 and mounting on the back in E2. In  
322 coherence with certain studies (Bouissou, 1972, Lutz et al., 2016) but in contrast to others (Lutz et al.,  
323 2019), horned bulls and heifers showed more agonistic behaviour. In E1, the increase was largely  
324 explained by increased frequencies of physical interactions, particularly head-to-body exchanges in H+  
325 bulls. In addition, more H+ bulls expressed head-to-head contacts. In contrast, in E2, H+ compared to  
326 H- heifers showed a greater proportion of agonistic behaviour without contact, which is in line with  
327 Lutz et al. (2019). In E1, in the unmixed groups, displacements attempts of disbudded animals were  
328 more successful than horned animals, while the opposite effect was found for E2. Despite these  
329 effects, the horn status did not modify the time spent in the food area in E1 or E2. An obvious reason

330 is that the test disposition produced a ceiling effect, that is, all the places giving access to the food  
331 were maximally occupied. In contrast, in the mixed rearing groups, horn status did not influence either  
332 vicinity to food or **initiated** interactions, unlike in Bouissou (1972).

333 **The different experimental conditions may explain differences in behaviour and effect of horn status**  
334 **between E1 and E2. The greater agonistic tendencies of bulls may be related to the effects of**  
335 **testosterone; heifers receiving testosterone showed more agonistic behaviour (Boissy and Bouissou,**  
336 **1994), or to the earlier and more stable social structuring in female than in male young cattle**  
337 **(Sambras, 1978, Bouissou, 1985, Hall, 1986). In addition, the heads of the animals were less accessible**  
338 **in E2, which reduces agonistic interactions at the feeding place (Bouissou et al., 1970, DeVries and von**  
339 **Keyserlingk, 2006) and which may also have contributed to the more frequent targeting of the**  
340 **hindquarters (Wierenga, 1990).**

341 Behavioural synchrony **is an indicator of social cohesion (Gibbons et al., 2010). Field observations**  
342 **found an increase over time in E1 indicating that social cohesion increased over time, which is coherent**  
343 **with earlier observations on stable rearing groups of young bulls (Mounier et al., 2006a, Gibbons et al.,**  
344 **2010). Horn status or mixed status of the group had however no influence.**

345 **Observations in the field found further that in both experiments, disbudding modified physical activity.**  
346 In E1, at the age of 12 mo, unmixed H+ bulls had greater daily walking times and more frequent stand  
347 ups **than H- bulls**. Throughout E2, compared to H- heifers, H+ heifers expressed greater daily standing  
348 and shorter daily lying times, while **their walking time tended to be less**. In an earlier report, **slightly**  
349 **greater physical activity, including walking, was observed in horned compared to disbudded dairy cows**  
350 **of different breeds kept in groups under different space allowances (Lutz et al., 2019).**

351 Locomotion **may be motivated by the avoidance of dominant conspecifics and it has been suggested**  
352 **that horned cattle walk more in order to avoid group members (Oester, 1977, Lensink and Leruste,**  
353 **2006). Increased locomotion due to agonistic pressure in horned bulls is coherent with our observation**  
354 **that during the food competition test H+ bulls presented more head-to-head interactions. The absence**  
355 **of differences between RC in activity levels in the field in E1 before the age of 12 months may be**  
356 **related to the less strong hierarchical structure in groups of young bulls of this age compared to heifers**

357 (Samraus, 1978, Bouissou, 1985, Hall, 1986). If horn status influences avoidance behaviour, it will  
358 influence activity levels more particularly in hierarchically structured herds. Based on this hypothesis,  
359 social tensions are expected to be lowest in the mixed bulls, as they had the lowest activity levels at  
360 the age of 12 months. In coherence with this, we suggested earlier that the social hierarchy of the  
361 mixed groups was less strong, as removal of half of the animals from each rearing group for slaughter,  
362 produced less pronounced stress reactions in the mixed, than the other groups (Reiche et al., 2019).  
363 Greater activity levels may also be related to different muscle functioning; proteomic analyses of  
364 muscles of the bulls of E1 found higher levels of many proteins in unmixed horned compared to  
365 disbudded bulls, including structural proteins involved in the functioning of the contractile system  
366 (Mato et al., 2018).

367

368 The absence or removal of horns presents several welfare advantages relative to animal management  
369 and security. However, possible consequences on behaviour need also attention and this was the  
370 purpose of this study. Welfare is considered to be good if an animal is in a physical and mental state in  
371 which negative emotions are absent (Veissier and Boissy, 2007). Negative emotions are caused by fear  
372 and pain, amongst others. The behavioural traits influenced by horn status appeared to be at least  
373 partly associated with agonistic behaviour and fear, and may therefore influence welfare status.  
374 However, our results do not identify uniformly advantages or disadvantages. In the field, in E1, H+ bulls  
375 showed increased locomotion, possibly due to agonistic pressure, which is coherent with their greater  
376 total number of agonistic interactions during the FCT, compared to H- bulls. Other effects depended  
377 on the experiment and were thus context dependent. During the FCT, horned animals presented more  
378 agonistic interactions with contact in E1, and more agonistic behaviour without contact in E2.  
379 Increased agonistic activity with physical contact may be more painful; in this respect partly opposite  
380 effects were found in E1 and E2. Similarly, in E1, during the NOT, horned animals appeared less fearful  
381 compared to horned animals while in E2, the opposite was found. Hence, results indicate that  
382 behaviours related to negative emotions such as fear and aggression were influenced by disbudding,



383 but the effects of disbudding depend on the type of observation, in the field, during the FCT or NOT,  
384 and the experiment.

385

386

## 387 5. Conclusion

388 The results of the present study show that in young bulls and heifers between 4 and 12 months of age,  
389 disbudding did not influence levels of observable agonistic behaviour but it did influence physical  
390 activity. It affected further fear-related and certain expressions of agonistic behaviour, but differently  
391 depending on the experiment. Overall, results indicate that consequences of disbudding on cattle  
392 behaviour may be long-lasting. However, the effects were small and given their context dependency,  
393 they do not clearly indicate overall negative or positive effects on animal welfare in terms of  
394 behavioural or social consequences. The context dependency of the results are coherent with existing  
395 literature and illustrate the multifactorial character of agonistic and more largely, social behaviour.  
396 Further study is necessary to refine our knowledge on the welfare consequences of the absence of  
397 horns.

398

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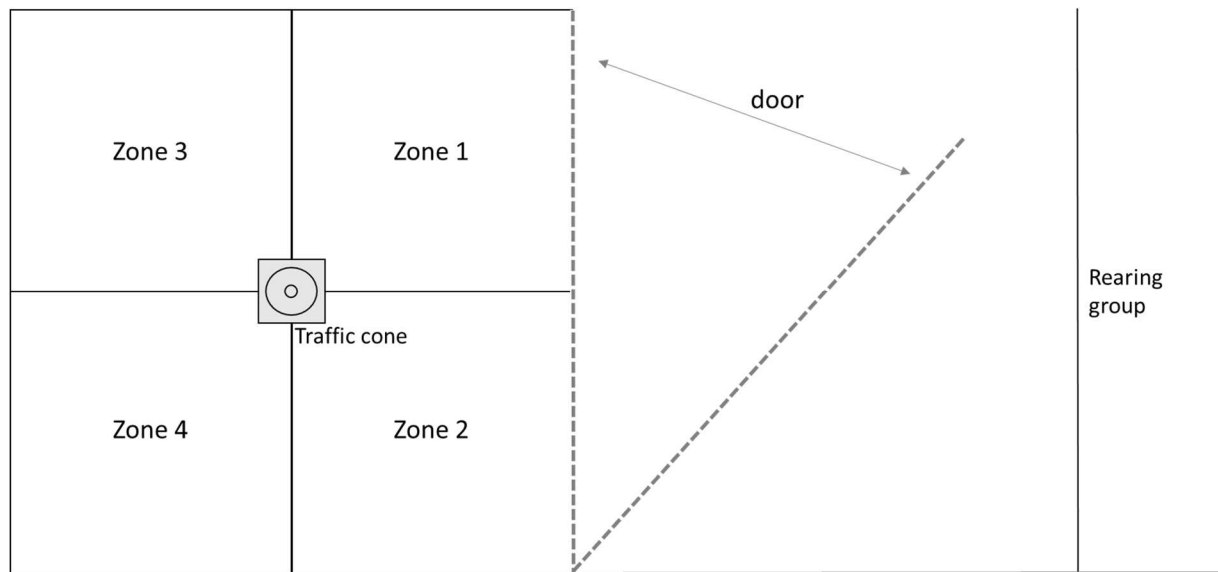
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504

505 Figure 1

506 Schematic representation of the test arena and set-up used for the novel object test in experiments 1

507 and 2. Dotted lines represent the two positions of the mobile entrance/exit door (closed vs. open)

**Table 1**a) Definitions of test phases and **observed** behaviours during **the novel object test**.

Phases	Start	End
1 - Isolation	Closure of the arena's door	60 s after start
2 - Introduction of the object	Start of lowering down the NO	NO touches the ground
3 - Time until first contact	NO touches the ground	First contact with NO/ in case of no contact: 120s after start
4 - Time after first contact (only if NO was touched)	First contact with NO	15 s after start
Behaviour	Definition	
Zone 1-4	Two front legs in the respective zone	
Zone change	Animal passes from one zone to another	
Looking to exit/ elsewhere/ NO	Head oriented (distance > 20 cm) toward exit/ other than exit or NO/ NO	
Sniffing exit/ elsewhere/ NO	Head oriented toward exit/ other than exit or NO / NO (distance < 20 cm)	
Immobile	Animal standing, four feet at the ground	
In motion	Animal walking or running	
Muzzle on barrier (exit/elsewhere)	Muzzle on barrier	
Latency	Time between NO touches the ground and first contact with NO	
Contact NO	Licking or rubbing NO	
Touch NO	Sniffing, licking or rubbing NO	
Interaction NO	Looking at, sniffing, licking or rubbing NO	

**Table 1**

**b) Definitions and categories of behaviours observed during the food competition test.**

Vicinity to feed		Definition	
		Experiment 1	Experiment 2
Vicinity 1		Head in or above a bucket	Head in or above the feeding trough
Vicinity 2		Animal standing between eating animals, distance between head of the animal and the nearest bucket <50 cm	Animal between or next to/besides eating animals, distance between head of the animal and the nearest point of the feeding trough <100 cm
Vicinity 3		Distance between the animal's front legs and the nearest bucket less or equal to the length of an animal	Distance between the animal's front legs and the nearest point of the feeding trough < two lengths of an animal
Vicinity 4		Distance between the animal's front legs and the nearest bucket greater than the length of an animal	Distance between the animal's front legs and the nearest point of the feeding trough > two lengths of an animal
Category	Social interaction	Definition	
<i>Agonistic interactions (successful and unsuccessful)</i>			
<i>Behaviour with contact</i>			
<i>Head-to-head contact</i>			
	Head-to-head contact	Foreheads of two animals against each other without pushing	
	Head-to-head rub	Foreheads of two animals rubbing against each other without pushing	
	Head-to-head push	Foreheads of two animals against each other, pushing with force	
<i>Head-to-body contact</i>			
	Head butts	Hitting/Butting the receiver with head, horns or hornbase	
	Pushing with the head	Pushing head against receiver	
<i>Body-to-body contact</i>			
	Pushing with the body	Pushing parts of the body (other than the head) against receiver	
<i>Behaviour without contact<sup>a</sup></i>			
	Threatening	Looking (head lowered) at or swinging head in the direction of the receiver which avoids or withdraws	
	Avoiding	Animal avoids or withdraws when another animal approaches (when the latter does not threaten or look at it)	
<i>Successful agonistic interaction</i>		<i>Any agonistic interaction with displacement of the receiver</i>	
<i>Unsuccessful agonistic interaction</i>		<i>Any agonistic interaction without displacement of the receiver</i>	
<i>Non-agonistic interactions</i>			
	Head on back	Head on the receivers' back	
	Head on hindquarters	Head on the receivers' hindquarters	
	Mounting	Mounting receiver on croup, back or neck	

<sup>a</sup> Threatening and avoiding were defined to be mutually exclusive to avoid counting an event twice.

<b>Table 2</b>							
Means, standard errors and effects of behaviours related to general activity by experiment, period, rearing condition and their interactions.							
<b>Experiment 1 (n=81)</b>	P4			p-values			
	H+ (n=27)	M (n=27)	H- (n=27)	Per	RC	Per×RC	
Laytime (min/d)	716.6 <sup>a</sup> ± 27.4	755.7 <sup>b</sup> ± 2.3	712.7 <sup>a</sup> ± 12.2	<b>&lt;0.0001</b>	0.17	<i>0.064</i>	
Standtime (min/d)	420.0 ± 10.4	405.3 ± 5.1	432.2 ± 7.9	<b>&lt;0.0001</b>	0.11	0.34	
Walktime (min/d)	62.1 <sup>c</sup> ± 4.3	39.4 <sup>a</sup> ± 1.2	53.3 <sup>b</sup> ± 2.6	<b>&lt;0.0001</b>	0.73	<b>&lt;0.0001</b>	
Standups (number/d)	13.2 <sup>b</sup> ± 0.4	13.6 <sup>b</sup> ± 0.3	11.4 <sup>a</sup> ± 0.2	<b>&lt;0.0001</b>	0.46	<b>&lt;0.0001</b>	
P4 - Period 4 (12 months of age). Means of periods 1-3 are presented in table S1, as rearing conditions within the first three periods were not statistically different from each other. Test statistics and p values are derived from <b>linear</b> mixed models. Wald chisquare values of significant results (P<0.05) ranged from 38.08 to 307.78, that of the tendency (P=0.064) was 11.90 and those of not significant results (P>0.10) ranged from 0.62 to 6.77.							
<b>Experiment 2 (n=71)</b>	P1		P2		p-values		
	H+ (n=36)	H- (n=35)	H+ (n=36)	H- (n=35)	Per	RC	Per×RC
Laytime (min/d)	721.8 <sup>a</sup> ± 6.1	735.0 <sup>ab</sup> ± 7.0	762.9 <sup>b</sup> ± 3.4	809.6 <sup>c</sup> ± 3.7	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Standtime (min/d)	449.6 <sup>c</sup> ± 5.9	432.9 <sup>b</sup> ± 6.6	415.5 <sup>b</sup> ± 3.2	366.8 <sup>a</sup> ± 3.5	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
Walktime (min/d)	28.9 <sup>b</sup> ± 0.7	32.4 <sup>c</sup> ± 0.7	21.9 <sup>a</sup> ± 0.4	23.9 <sup>a</sup> ± 0.5	<b>&lt;0.0001</b>	<i>0.092</i>	<i>0.055</i>
Standups (number/d)	15.9 ± 0.3	16.6 ± 0.4	11.6 ± 0.2	12.0 ± 0.2	<b>&lt;0.0001</b>	0.47	0.99
P1 - Period 1 (4 months of age); P2 - Period 2 (9 months of age)							
H+, H-, M - Unmixed horned, unmixed disbudded and mixed rearing group, respectively; Per - Period; RC - Rearing condition. P-values are derived from partial F-tests for parameters used in linear mixed models. Within a line, different superscripts indicate that means are statistically significant (pairwise comparisons of least-squares means). Bold and italic letters indicate significance and tendency, respectively. Test statistics and p values are derived from <b>linear</b> mixed models. Wald chisquare values of significant results (P<0.05) ranged from 16.62 to 172.62, those of tendencies from 2.85 to 3.69 and those of not significant results (P>0.10) ranged from 0.0001 to 0.52.							

**Table 3**

Means, standard errors and effects of observed behaviours during Novel Object tests by experiment and rearing condition.

		Experiment 1					Experiment 2			
		H+	M	H-	Wald chisquare	p-value RC	H+	H-	Wald chisquare	p-value RC
Isolation		n=27	n=27	n=27			n=36	n=35		
	Zone 1 (%)	29.3 ± 4.5	32.4 ± 3.9	37.1 ± 4.4	1.89	0.39	33.3 ± 2.9	33.6 ± 3.5	0.11	0.74
	Zone 2 (%)	17.5 ± 3.3	23.1 ± 3.0	25.8 ± 3.3	3.99	0.14	23.0 ± 2.6	25.3 ± 2.9	0.09	0.76
	Zone 3 (%)	31.4 <sup>b</sup> ± 4.41	24.5 <sup>ab</sup> ± 4.71	15.9 <sup>a</sup> ± 2.4	8.60	<b>0.014</b>	25.9 ± 3.4	20.1 ± 2.8	1.94	0.16
	Zone 4 (%)	21.9 ± 4.2	19.9 ± 3.7	21.2 ± 4.6	0.11	0.95	17.8 ± 1.7	21.0 ± 3.8	0.24	0.62
	In motion (%)	36.2 ± 3.9	38.8 ± 2.9	43.6 ± 2.8	4.62	<i>0.099</i>	43.8 ± 3.6	40.9 ± 3.3	0.03	0.86
	Number of zone changes	5.4 <sup>a</sup> ± 0.60	7.3 <sup>ab</sup> ± 0.67	8.0 <sup>b</sup> ± 0.7	8.22	<b>0.016</b>	11.0 ± 9.6	11.3 ± 1.0	0.16	0.69
	Looking elsewhere (%)	28.2 ± 4.4	22.1 ± 2.2	22.6 ± 2.3	0.53	0.77	29.2 <sup>a</sup> ± 2.5	36.3 <sup>b</sup> ± 2.3	6.77	<b>0.009</b>
	Sniffing elsewhere (%)	32.0 ± 4.8	29.3 ± 3.9	24.8 ± 2.8	0.76	0.68	35.7 <sup>b</sup> ± 3.0	26.2 <sup>a</sup> ± 2.4	9.02	<b>0.003</b>
Lowering down the NO		n=27	n=27	n=27			n=36	n=35		
	Zone 1 (%)	56.8 ± 6.9	45.9 ± 7.0	40.6 ± 6.0	2.97	0.23	24.5 ± 5.1	32.6 ± 5.3	0.95	0.33
	Zone 2 (%)	14.4 <sup>a</sup> ± 5.0	14.5 <sup>a</sup> ± 5.0	36.0 <sup>b</sup> ± 6.7	7.97	<b>0.019</b>	31.5 ± 5.0	23.2 ± 6.7	0.88	0.35
	Zone 3 (%)	12.8 <sup>b</sup> ± 4.4	28.1 <sup>b</sup> ± 5.6	21.6 <sup>ab</sup> ± 6.3	6.35	<b>0.042</b>	18.0 ± 3.2	24.7 ± 4.9	0.45	0.50
	Zone 4 (%)	16.0 <sup>b</sup> ± 5.3	11.5 <sup>ab</sup> ± 4.1	1.8 <sup>a</sup> ± 1.2	6.74	<b>0.034</b>	26.0 ± 4.8	19.5 ± 4.5	1.16	0.28
	Looking to exit (%)	15.4 ± 3.6	10.1 ± 2.3	12.2 ± 4.1	0.76	0.69	8.5 ± 1.4	6.1 ± 1.6	2.81	<i>0.094</i>
		only bulls with latency < 120 s					only heifers with latency < 120 s			
With object before contact		n=27	n=26	n=27			n=31	n=30		
	In motion (%)	48.9 ± 5.3	45.7 ± 4.8	50.3 ± 6.0	0.41	0.81	47.8 ± 6.4	46.6 ± 6.9	0.08	0.78
	Number of zone changes	2.1 ± 0.23	3.0 ± 0.7	2.4 ± 0.47	0.11	0.95	4.8 <sup>b</sup> ± 1.2	2.8 <sup>a</sup> ± 0.6	4.97	<b>0.026</b>
	Looking to NO (%)	38.5 ± 7.6	42.9 ± 7.7	48.5 ± 8.0	0.53	0.77	27.4 ± 6.4	42.2 ± 6.8	2.75	<i>0.097</i>
After contact		n=27	n=26	n=27			n=31	n=30		
	Zone 1 (%)	19.4 ± 4.7	34.7 ± 7.2	24.1 ± 6.1	2.29	0.32	30.7 ± 4.8	41.3 ± 7.1	0.59	0.44
	Zone 2 (%)	28.5 ± 6.4	21.1 ± 5.9	23.0 ± 5.8	1.05	0.59	34.0 <sup>b</sup> ± 5.1	15.5 <sup>a</sup> ± 3.8	10.93	<b>&lt;0.001</b>
	In motion (%)	31.7 ± 5.8	30.4 ± 4.8	30.1 ± 4.3	0.13	0.94	45.2 ± 4.5	33.1 ± 4.9	3.84	<i>0.050</i>
	Number of zone changes	2.2 ± 0.2	2.3 ± 0.2	2.5 ± 0.2	0.88	0.64	3.7 ± 0.4	3.3 ± 0.5	1.72	0.19
	Looking elsewhere (%)	12.6 ± 2.7	12.8 ± 2.7	12.1 ± 2.5	0.15	0.93	21.9 ± 3.7	29.7 ± 4.0	2.72	<i>0.099</i>
	Sniffing NO (%)	31.31 <sup>a</sup> ± 4.33	22.20 <sup>a</sup> ± 3.90	18.46 <sup>a</sup> ± 2.56	6.19	<b>0.045</b>	23.5 ± 4.4	21.5 ± 3.3	0.14	0.71
	Time to push animal in test arena	20.38 <sup>a</sup> ± 1.25	24.48 <sup>ab</sup> ± 1.92	29.93 <sup>b</sup> ± 2.66	6.44	<b>0.040</b>	24.6 ± 1.6	29.8 ± 2.9	1.11	0.29

H+, H-, M - Unmixed horned, unmixed disbudded and mixed rearing group, respectively; RC - Rearing condition; NO - Novel object.

P-values for rearing condition are derived from Wald chisquare test statistics for parameters used in linear mixed models on ranked data. Within a line, different superscripts indicate that means are statistically significant (pairwise comparisons of least-squares means). Bold and italic letters indicate significance and tendency, respectively.



**Table 4**

**Correlations found** among behaviours observed during the novel object test by experiment: Spearman correlation coefficients by rearing condition and adjusted determination coefficients and probability values derived from ANCOVA models including different rearing conditions.

		H+ (n=27)	H- (n=27)	M (n=26)	R2 (%)	P Var
<b>Experiment 1</b>						
Zone 1 phase 1 (%)	Number of zone changes phase 4	<b>0.56</b>	<b>0.47</b>		33.21	<b>&lt;0.001*</b>
	Interacting with NO phase 4 (%)		<b>-0.46</b>	-0.29	11.84	<b>0.014</b>
Zone 4 phase 1 (%)	Sniffing elsewhere phase 1 (%)	<b>0.41</b>	<b>0.74</b>		29.92	<b>&lt;0.001</b>
	Number of zone changes phase 4	<b>-0.43</b>	<b>-0.59</b>		22.47	<b>&lt;0.001</b>
Sniffing elsewhere phase 1 (%)	Immobile phase 4 (%)	<b>0.56</b>	0.34		27.52	<b>&lt;0.001*</b>
	Number of zone changes phase 4		<b>-0.48</b>	<b>-0.57</b>	40.80	<b>&lt;0.001*</b>
	Touching NO phase 4 (%)	0.26		<b>0.41</b>	9.75	<b>0.009</b>
Immobile phase 4 (%)	Touching NO phase 4 (%)	<b>0.45</b>	<b>0.78</b>	<b>0.72</b>	45.09	<b>&lt;0.001</b>
Immobile phase 1 (%)	Touching NO phase 4 (%)	<b>0.51</b>	<b>0.42</b>		26.16	<b>&lt;0.001</b>
Number of zone changes phase 3	Latency to sniff NO (s)		<b>0.77</b>	<b>0.74</b>	52.58	<b>&lt;0.001</b>
<b>Experiment 2</b>						
		H+ (n=31)	H- (n=30)		R2 (%)	P Var
Immobile phase 1 (%)	Immobile phase 4 (%)	<b>0.57</b>	<b>0.42</b>		29.46	<b>&lt;0.001*</b>
	Sniffing NO phase 4 (%)	<b>0.58</b>	<b>0.41</b>		20.89	<b>&lt;0.001</b>
Looking at NO phase 3 (%)	Latency to sniff NO (s)	<b>-0.64</b>	<b>-0.59</b>		36.47	<b>&lt;0.001</b>
Sniffing exit phase 3 (%)		<b>0.68</b>	<b>0.57</b>		34.04	<b>&lt;0.001</b>
Number of zone changes phase 3		<b>0.58</b>	<b>0.85</b>		55.64	<b>&lt;0.001</b>
Immobile phase 4 (%)	Sniffing NO phase 4 (%)	<b>0.52</b>	<b>0.58</b>		23.98	<b>&lt;0.001</b>

\* The effect of replicate was significant.

H+, H-, M - Unmixed horned, unmixed disbudded and mixed rearing condition, respectively;  
R2 - adjusted R-squared, P - p-value; Var – explanatory variable; NO - Novel object.

**Table 5**

Means, standard errors, frequencies of occurrence and effects of behaviour **categories of social interactions** during food competition tests by experiment and rearing condition. **Behaviours with mean occurrences <0.2 /min were coded as binary variables (see Statistical Analyses) and therefore, percentages of animals expressing the behaviour are presented. These behaviours were also introduced as binary variables in the regression analyses (see text).**

## a) Experiment 1

	H+	M	H-	F-value	p-value RC	z	p-value H+ vs H-
N	27	27	27				
<b>Number of total agonistic interactions/min</b>	1.51 <sup>b</sup> ± 0.12	1.29 <sup>ab</sup> ± 0.13	1.04 <sup>a</sup> ± 0.11	4.22	<b>0.018</b>	-	-
<i>% of animals expressing behaviour <u>without</u> contact</i>	44.4%	22.2%	25.9%	-	-	1.41	0.16
Number of agonistic interactions <u>with</u> contact/min	1.42 <sup>b</sup> ± 0.12	1.24 <sup>ab</sup> ± 0.13	0.98 <sup>a</sup> ± 0.11	3.73	<b>0.029</b>	-	-
<i>% of animals expressing head-to-head-interactions</i>	62.9%	37.0%	18.5%	-	-	3.17	<b>0.002</b>
Nb of head-to-body-interactions/min	1.05 <sup>b</sup> ± 0.09	1.00 <sup>ab</sup> ± 0.11	0.71 <sup>a</sup> ± 0.09	3.98	<b>0.023</b>	-	-
<i>% of animals expressing body-to-body-interactions</i>	62.9%	51.9%	70.4%	-	-	-0.64	0.52
<b>Number of non-agonistic interactions/min</b>	0.25 ± 0.08	0.23 ± 0.06	0.24 ± 0.08	0.13	0.87	-	-
Number of successful agonistic interactions/min	0.68 ± 0.08	0.66 ± 0.10	0.58 ± 0.08	0.29	0.75	-	-
Number of unsuccessful agonistic interactions/min	0.66 <sup>b</sup> ± 0.05	0.56 <sup>ab</sup> ± 0.06	0.42 <sup>a</sup> ± 0.05	3.12	<b>0.049</b>	-	-

## b) Experiment 2

	H+	M	H-	F-value	p-value RC	z	p-value H+ vs H-
	36		35				
<b>Number of total agonistic interactions/min</b>	1.10 ± 0.12		0.78 ± 0.11	3.78	0.056	-	-
<i>% of animals expressing behaviour <u>without</u> contact</i>	52.80%		25.70%	-	-	-2.29	<b>0.022</b>
Number of agonistic interactions <u>with</u> contact/min	0.89 ± 0.11		0.69 ± 0.10	1.80	0.18	-	-
<i>% of animals expressing head-to-head-interactions</i>	19.4%		25.7%	-	-	0.63	0.53
<i>% of animals expressing head-to-body-interactions</i>	6.3%		4.3%	-	-	-1.76	0.078
Nb of body-to-body-interactions/min	0.38 ± 0.06		0.41 ± 0.07	0.01	0.94	-	-
<b>Number of non-agonistic interactions/min</b>	0.43 ± 0.07		0.42 ± 0.07	0.00	0.99	-	-
Number of successful agonistic interactions/min	0.68 <sup>b</sup> ± 0.08		0.39 <sup>a</sup> ± 0.06	6.66	<b>0.012</b>	-	-
<i>% of animals expressing unsuccessful agonistic interactions</i>	58.3%		40.0%	-	-	-1.54	0.12

H+, H-, M - Unmixed horned, unmixed disbudded and mixed rearing group, respectively; RC - Rearing condition.

P-values for rearing condition are derived from F test statistics for parameters used in linear mixed models on ranked data. Within a line, different superscripts indicate that means are statistically significant (pairwise comparisons of least-squares means). Z-values with associated p-values are shown for data analyzed by binomial logistic regression. Bold and italic letters indicate significance and tendency, respectively.