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Abstract

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

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

1. Introduction

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

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

2. Animals, material and methods

2.1. Animals and housing

We conducted two experiments, one with 81 bulls (E1; 10/2015 - 11/2016) and one with 71 heifers (E2; 06/2016 – 09/2017). Both trials respected the Swiss laws of animal protection and were authorized by the cantonal veterinary office of Fribourg, Switzerland (No. 2015_21_FR). The 81 bulls used in E1 were the same as reported in Reiche et al. (2020) and Reiche et al. (2019). Half of the animals were disbudded at the age of 52 (E1) and 63 (E2) days using a hot iron under sedation, local anesthesia and systemic analgesia. In both experiments, rearing groups were balanced for behavioural reactivity before disbudding (see section 2.2.), cortisol responses to ACTH (see Reiche et al. (2020)) and body weight. In E1, they were assigned to three rearing conditions (RC) according to their horn status; rearing groups consisted of horned (H+) or disbudded (H-) animals, or were mixed (M; half of the group horned, half disbudded), each with two replicates. The two replicates started one week apart. Bulls were housed in groups of 13-14 per pen on deep litter with access to an outdoor pen (earth floor; deep litter and total space allowance of 3.9 and 9.4 m2/bull), in front of which people passed up to 30 times a day. Straw was distributed manually. Each bull was equipped with an ear chip giving it *ad libitum* access to one of the seven automatic feeding stations (distributing a total mixed ration containing hay, corn silage and 10% concentrates). Each bull had further access to a supplement of 200 g of concentrates at another, single feeding station. In E2, animals were assigned in a similar manner as bulls to two rearing groups (horned and disbudded, no mixed group was present); the two replicates started two weeks apart. Seventeen to 18 heifers per pen were housed in four pens in a deep litter-loose housing barn (deep litter and total space allowance: 3.4 m^2 and 6 m^2/heifer), without access to an outdoor pen. Straw was distributed automatically. Heifers were fed twice a day a similar diet as in E1, distributed by a feed mixer in a 6-m-long feed trough (without fences). Animal caretakers passed up to 4 times a day. In both experiments, animals were weighed every five wks on a scale (Grüter, Eschenbach, Switzerland). The E1 weighing took place in the outdoor pens and the E2 weighing in a 250 m^2 fenced area adjacent to each of the 4 indoor rearing pens. In both experiments, 2-m high, solid wood panels separated rearing pens from each other in order to avoid visual and physical contact between rearing groups.

2.2. Behavioural measurements

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

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

In E1, general activity (GA) was measured over four periods during fattening, February (P1), May (P2), July (P3) and October (P4), that is at 4 ,7, 9 and 12 months of age, respectively, and in E2 two periods, September (P1) and February (P2), that is at 4 and 9 months of age, respectively, by accelerometers (ITIN & Hoch, Liestal, Switzerland). At each period, accelerometers were used during two to four consecutive days. In total, 28 and 36 accelerometers were available and used in E1 and E2, respectively. Within each period, accelerometers were used simultaneously for the animals of two rearing groups of one replicate. For E1, choice of the two rearing groups was balanced for RC over the periods. Accelerometers were fixed laterally at the left hind leg, proximal to the fetlock joint. During measuring periods, position and functioning of devices were checked two times per day (07:00 AM and 04:00 PM), during which the devices were replaced or fixed when necessary. Lying, standing and walking time (sum of durations of all lying, standing and walking bouts, respectively, during a given recording period), and number of stand ups (event where the animal stands, moving from a lying to standing position) have been quantified (Alsaaod et al., 2015). To exploit the recorded data, the software Rumiwatch® converter (version 36, ITIN & Hoch, Liestal, Switzerland), previously validated for posture and locomotion behaviour in cattle housed indoors, was used (Alsaaod et al., 2015).

At the age of 10 and 11 months, animals of E1 and E2, respectively, were subjected individually to a Novel object test (NOT). The test arena (Figure 1) had a size of 4 x 4 meters and was bordered by 2-m-high, solid barriers that ensured visual separation from conspecifics. In E1, the arena was erected in the feeding area of the rearing pen, containing a solid floor. In E2, the test arena was erected in the outdoor area, which contained an earth floor with woodchips. All animals of one replicate were tested in the afternoons of two consecutive days, using a design balanced for rearing group. Forty-five min before the test, the whole rearing group was quietly led to the outdoor pen (E1) or the feeding area (E2) of their home pen. The test arena was installed. Fifteen minutes prior to the beginning of the test, animals to be tested were returned to the rearing pen's straw area. The order of tested animals was randomized. Two familiar handlers led the animal to be tested gently towards the test arena. Time from the start of this intervention until the closure of the door of the test arena was recorded for each animal. The test was organised in four phases and started as soon as the door of the test arena was closed behind the test animal. The first phase tested reactivity to isolation in a novel environment and lasted 60 s. The subsequent three phases aimed to study reactivity to the presence of a novel object (NO; a traffic cone). In the second phase (duration: 10 s) the NO was lowered from the ceiling in the middle of the test arena, announced by the ringing of a small bell that had been attached to the cone in order to get the cone noticed by the experimental animal. The third phase started with the NO touching the ground and lasted until the first contact or - in case of no contact - for 120 s (thus phase duration depended on the animal), and the fourth phase started at the first contact and ended 15 s afterwards. Encoded behaviours, test phases and their definitions are listed in Table 1.

Two weeks after the NOT, each rearing group was submitted to a food competition (FC) test, following Terlouw et al. (1991) with modifications. Animals had been overnight fasted, and tests took place between 8:00 and 9:00 AM. In E1, seven buckets containing 200 g of the normal concentrate (equivalent of a daily portion distributed by the feeding station) were positioned in a circle in the middle of the floor of the straw area of the rearing pen. In E2, pre-tests found that the heifers were not attracted by the buckets arranged in this manner, but that they were if the food was distributed in the feeding troughs. The set-up with the buckets may have produced a fear response as the animals were used to trough feeding. The test took therefore place at the feeding trough, which was reduced to half its length using wood panels thus giving access to only half of the animals of one rearing pen at the same time. The test was repeated three times. Prior to each session, two familiar handlers quietly returned the animals to the outdoor pen and the straw area in E1 and E2, respectively. Sessions ended when all the concentrate had been eaten, i.e. after 130-140 s in E1 and 120 s in E2. Behaviour analysis included vicinity to feed (recorded every ten seconds, Table 1) and social interactions, recorded continuously, with actor (initiating the interaction) and receiver (receiving the interaction). Agonistic behaviours recorded involved those without physical contact (threats and avoidances), those with contact, including head-to-head interaction (head-to-head contact, rub, push), head-to-body and body-to-body contact (head butts and pushes to body, or pushing with the body), and non-agonistic interactions (head on back, head on hindquarters, mounting) (Table 1). Agonistic interactions were identified as "successful" when they caused displacement of the receiver and "unsuccessful" if not.

All behavioural tests were filmed by video cameras (Panasonic, Osaka, Japan). Behaviour analyses were carried out using the software The Observer (version 11, Noldus, Wageningen, The Netherlands).

2.3. Statistical analysis

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

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

Statistical analyses were performed using linear mixed models of the R environment (Bates et al., 2014, Core, 2018). Rearing condition, period (where present) and their interactions were introduced as fixed factors; replicate of the experiment, day and animal as random factors. Models met the assumption of normally distributed residuals and homogeneity of variances (visual check of residual-versus-covariable- and residual versus-fitted-plots). Significance of fixed factors was analysed by partial F-tests (type-III-ANOVA). Pairwise comparisons were performed on least-squares means with adjustment for multiple comparisons following Benjamini and Hochberg (1995). Rarely observed behaviours during FC with mean occurrences <0.2 /min were coded as binary variables and analysed by logistic regression for binomial data using logit-transformation. Agonistic and non-agonistic activities in the FCT have been analysed as totals as well as the separate activities composing them (Table 1). Totals that differed significantly between RC were subsequently analysed by linear regression, using the various activities composing them as covariable, to identify which activity contributed significantly to the RC effect. If the activity was a rarely observed behaviour (see above) it was introduced as a binary covariable. Rearing condition and replicate were introduced as fixed factors; if RC was no longer significant after introduction of covariables, it was considered that the covariable(s) explained the RC effect.

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

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

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

For synchrony analysis, we used scan-samples of posture (lying vs standing) at 20-min intervals following Stoye et al. (2012). The Kappa coefficient is an indicator of behavioural synchrony on the group level, i.e. whether behaviours of a group are more synchronized than expected by chance, and corrects for differences related to group size. They were calculated for each RC and replicate and within mixed groups, for horned and disbudded subgroups, following Rook and Penning (1991), with K = 1 when complete synchrony is observed and K=0 when the observed proportion of synchrony is equal to the expected. Kappa coefficients were compared between periods and rearing groups using linear mixed models as described above. Using the same scan samples, Ruckstuhl's indices were calculated following Ruckstuhl (1999) for each animal, and express the degree to which an individual synchronises with the rest of the group. The Ruckstuhl index does not correct for group size, and consequently, we have only compared the values for similar group sizes (i.e. horned vs disbudded bulls within the mixed groups were not compared).

Means are presented with standard errors (± SE), unless otherwise specified.

3. Results

3.1. Spontaneous behaviour

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

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

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

3.2. Novel object test

Results are presented in Table 3. In E1, most differences were found between H+ and H- bulls. Horned bulls needed less time to be driven in the test arena, spent more time in zone 3 and changed less often the zone during isolation (phase 1) than H- bulls. During lowering of the NO (phase 2), H+ and M bulls spent less time in zone 2 and H+ bulls more time in zone 4 than H- bulls, but time spent in zones near the exit (total time spent in zone 1 and zone 2) did not differ ($p=0.12$) between RC (71.2±6.4, 60.4±6.9 and 76.7±6.4 for H+, M and H- bulls, respectively). Latency to sniff the NO did not differ according to RC and ranged from 0 to 114 s (mean: 19.9 s). One M bull did not touch the NO within 120 s. Compared to H- and M bulls, H+ bulls tended to spend more time sniffing the NO after the first contact (phase 4). In phase 4, within mixed RC, horned bulls spent less time in zone 2 (MH+: 10.91 ± 7.37 %; MH-: 27.91 ± 8.21 %; $P<0.05$) and less time in locomotion (MH+: 20.33 ± 5.67 %; MH-: 36.36 ± 6.80 %; $P<0.05$) than disbudded bulls.

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

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

In E2, H+ heifers spent less time during phase 1 looking elsewhere and more time sniffing elsewhere than H- heifers. In phase 2, H+ heifers tended to look longer at the exit door than H- heifers. Latency of heifers that touched the NO ranged from 0 to 104 s (mean: 14.9 s). Five heifers of each RC did not touch the NO within 120 s. In phase 3, H+ heifers changed more often the zone and tended to spend less time looking at the NO than H- heifers. In phase 4, H+ heifers spent more time in zone 2, and tended to spend more time moving and less time looking elsewhere than H- heifers.

Most correlations concerned variables relative to contact with the NO (Table 4). Sniffing the NO and immobility in phases 1 and 4 were positively correlated with each other. Latency to sniff the NO was positively correlated with time spent in sniffing the exit and number of zone changes (phase 3), and negatively with looking at the NO (phase 3). The plot of a principal component analysis of robustly correlated variables, explaining 72.0 % of the variation, presented a similar distribution of the variables as the PCA plot of E1, apart from that the first axis did only include locomotion in phases 1 and 4.

3.3. Food competition

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

In E2, 388 interactions were recorded (31% mounting or head on back or hindquarters, 29% pushing with the body, 18% pushing with the head, 7% head-to-head interactions, 11 % threat or avoiding, 4% head butts). Sixty and 55 heifers out of 71 initiated and received at least one interaction; number of initiated and received interactions ranged from 0 to 13 and from 0 to 9, respectively. Horned heifers expressed greater numbers of total agonistic interactions without contact. When introduced as covariables in the linear regression analysis, levels of threats ($P<0.001$) and avoidances ($P<0.001$) explained together 90.4 % of the variability in total agonistic interactions without contact (with similar contributions) and removed the RC effect ($P = 0.80$). Compared to H- heifers, more H+ heifers tended to express head-to-body interactions and they tended to greater total levels of agonistic activities (Table 5). Levels of head-to-body interactions ($P < 0.001$) and agonistic interactions without contact ($P < 0.001$) explained to 41.7 % of the variability in total agonistic interactions and removed the RC effect ($P = 0.86$). Horned heifers were further more successful in displacing other animals agonistically (Table 5). In both experiments, body weight was not correlated with vicinity and frequency of behaviours.

3.4. Relationships between tests

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

4. Discussion

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

The correlations and principal component analyses show that in both experiments, greater levels of exploration of the NO were associated with greater levels of standing immobile. Increased interaction with the NO was also associated with shorter durations of sniffing (E2) and staying close to (E1) the exit door, behaviours indicative of lower fear (Bourguet et al., 2010). The activity profile including exploration of the NO and low interest for the exit door may therefore express that the balance between fear and curiosity tips towards the latter (Bourguet et al., 2010). In E1, compared to H+ bulls, H- bulls spent less time sniffing the NO, while more time was needed to drive them towards the test arena (Lensink et al., 2001, Bourguet et al., 2010). Hence, in E1, H- bulls appeared more fearful compared to H+ bulls. In E2, H+ heifers tended to look less at the NO, and had more zone changes; both activities were associated with a longer latency until sniffing the object and they may be interpreted as an expression of greater fear (Boissy and Bouissou, 1995). Hence, in E2, presence of horns appears to be associated with greater fearfulness.

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

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

During the FCT, levels of agonistic interactions were much lower in E2 than in E1, with the most frequent agonistic activity being pushing with the head in E1 and mounting on the back in E2. In coherence with certain studies (Bouissou, 1972, Lutz et al., 2016) but in contrast to others (Lutz et al., 2019), horned bulls and heifers showed more agonistic behaviour. In E1, the increase was largely explained by increased frequencies of physical interactions, particularly head-to-body exchanges in H+ bulls. In addition, more H+ bulls expressed head-to-head contacts. In contrast, in E2, H+ compared to H- heifers showed a greater proportion of agonistic behaviour without contact, which is in line with Lutz et al. (2019). In E1, in the unmixed groups, displacements attempts of disbudded animals were more successful than horned animals, while the opposite effect was found for E2. Despite these effects, the horn status did not modify the time spent in the food area in E1 or E2. An obvious reason is that the test disposition produced a ceiling effect, that is, all the places giving access to the food were maximally occupied. In contrast, in the mixed rearing groups, horn status did not influence either vicinity to food or initiated interactions, unlike in Bouissou (1972).

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

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

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

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

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

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

5. Conclusion

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

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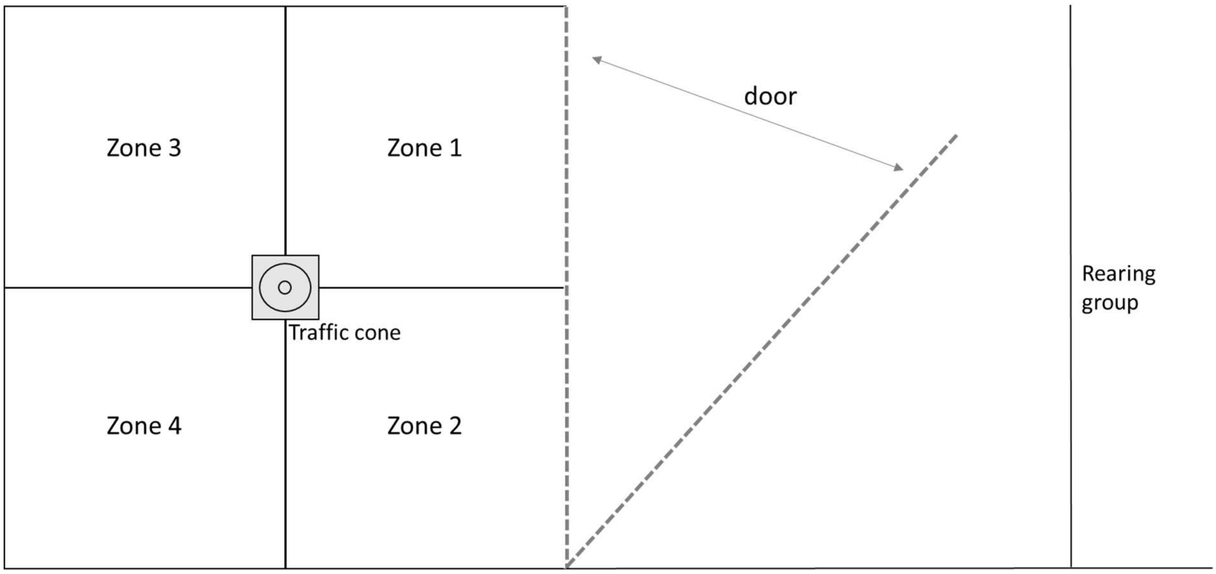

Figure 1

Schematic representation of the test arena and set-up used for the novel object test in experiments 1 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	Definition: 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
Agonistic interactions (successful and unsuccessful)		
Behaviour with contact		
Head-to-head contact		
	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
Head-to-body contact		
	Head butts	Hitting/Butting the receiver with head, horns or hornbase
	Pushing with the head	Pushing head against receiver
Body-to-body contact		
	Pushing with the body	Pushing parts of the body (other than the head) against receiver
Behaviour without contact [a]		
	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)
Successful agonistic interaction		Any agonistic interaction with displacement of the receiver
Unsuccessful agonistic interaction		Any agonistic interaction without displacement of the receiver
Non-agonistic interactions		
	Head on back	Head on the receivers' back
	Head on hindquarters	Head on the receivers' hindquarters
	Mounting	Mounting receiver on croup, back or neck

[a] Threatening and avoiding were defined to be mutually exclusive to avoid counting an event twice.

Table 2

Means, standard errors and effects of behaviours related to general activity by experiment, period, rearing condition and their interactions.

Experiment 1 (n=81)	P4			p-values		
	H+ (n=27)	M (n=27)	H- (n=27)	Per	RC	Per×RC
Laytime (min/d)	716.6[a] ± 27.4	755.7[b] ± 2.3	712.7[a] ± 12.2	**<0.0001**	0.17	*0.064*
Standtime (min/d)	420.0 ± 10.4	405.3 ± 5.1	432.2 ± 7.9	**<0.0001**	0.11	0.34
Walktime (min/d)	62.1[c] ± 4.3	39.4[a] ± 1.2	53.3[b] ± 2.6	**<0.0001**	0.73	**<0.0001**
Standups (number/d)	13.2[b] ± 0.4	13.6[b] ± 0.3	11.4[a] ± 0.2	**<0.0001**	0.46	**<0.0001**

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 linear 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.

Experiment 2 (n=71)	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[a] ± 6.1	735.0[ab] ± 7.0	762.9[b] ± 3.4	809.6[c] ± 3.7	**<0.0001**	**<0.0001**	**<0.0001**
Standtime (min/d)	449.6[c] ± 5.9	432.9[b] ± 6.6	415.5[b] ± 3.2	366.8[a] ± 3.5	**<0.0001**	**<0.0001**	**<0.0001**
Walktime (min/d)	28.9[b] ± 0.7	32.4[c] ± 0.7	21.9[a] ± 0.4	23.9[a] ± 0.5	**<0.0001**	*0.092*	*0.055*
Standups (number/d)	15.9 ± 0.3	16.6 ± 0.4	11.6 ± 0.2	12.0 ± 0.2	**<0.0001**	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 linear 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^{b} ± 4.41	24.5^{ab} ± 4.71	15.9^{a} ± 2.4	8.60	**0.014**	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	*0.099*	43.8 ± 3.6	40.9 ± 3.3	0.03	0.86
Number of zone changes	5.4^{a} ± 0.60	7.3^{ab} ± 0.67	8.0^{b} ± 0.7	8.22	**0.016**	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^{a} ± 2.5	36.3^{b} ± 2.3	6.77	**0.009**
Sniffing elsewhere (%)	32.0 ± 4.8	29.3 ± 3.9	24.8 ± 2.8	0.76	0.68	35.7^{b} ± 3.0	26.2^{a} ± 2.4	9.02	**0.003**
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^{a} ± 5.0	14.5^{a} ± 5.0	36.0^{b} ± 6.7	7.97	**0.019**	31.5 ± 5.0	23.2 ± 6.7	0.88	0.35
Zone 3 (%)	12.8^{b} ± 4.4	28.1^{b} ± 5.6	21.6^{ab} ± 6.3	6.35	**0.042**	18.0 ± 3.2	24.7 ± 4.9	0.45	0.50
Zone 4 (%)	16.0^{b} ± 5.3	11.5^{ab} ± 4.1	1.8^{a} ± 1.2	6.74	**0.034**	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	*0.094*
	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^{b} ± 1.2	2.8^{a} ± 0.6	4.97	**0.026**
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	*0.097*
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^{b} ± 5.1	15.5^{a} ± 3.8	10.93	**<0.001**
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	*0.050*
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	*0.099*
Sniffing NO (%)	31.31^{a} ± 4.33	22.20^{a} ± 3.90	18.46^{a} ± 2.56	6.19	**0.045**	23.5 ± 4.4	21.5 ± 3.3	0.14	0.71
Time to push animal in test arena	20.38^{a} ± 1.25	24.48^{ab} ± 1.92	29.93^{b} ± 2.66	6.44	**0.040**	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.

Experiment 1		H+ (n=27)	H- (n=27)	M (n=26)	R2 (%)	P Var
Zone 1 phase 1 (%)	Number of zone changes phase 4	**0.56**	**0.47**		33.21	**<0.001***
	Interacting with NO phase 4 (%)		**-0.46**	-0.29	11.84	**0.014**
Zone 4 phase 1 (%)	Sniffing elsewhere phase 1 (%)	**0.41**	**0.74**		29.92	**<0.001**
	Number of zone changes phase 4	**-0.43**	**-0.59**		22.47	**<0.001**
Sniffing elsewhere phase 1 (%)	Immobile phase 4 (%)	**0.56**	0.34		27.52	**<0.001***
	Number of zone changes phase 4		**-0.48**	**-0.57**	40.80	**<0.001***
	Touching NO phase 4 (%)	0.26		**0.41**	9.75	**0.009**
Immobile phase 4 (%)	Touching NO phase 4 (%)	**0.45**	**0.78**	**0.72**	45.09	**<0.001**
Immobile phase 1 (%)	Touching NO phase 4 (%)	**0.51**	**0.42**		26.16	**<0.001**
Number of zone changes phase 3	Latency to sniff NO (s)		**0.77**	**0.74**	52.58	**<0.001**

Experiment 2		H+ (n=31)	H- (n=30)	R2 (%)	P Var
Immobile phase 1 (%)	Immobile phase 4 (%)	**0.57**	**0.42**	29.46	**<0.001***
	Sniffing NO phase 4 (%)	**0.58**	**0.41**	20.89	**<0.001**
Looking at NO phase 3 (%)	Latency to sniff NO (s)	**-0.64**	**-0.59**	36.47	**<0.001**
Sniffing exit phase 3 (%)		**0.68**	**0.57**	34.04	**<0.001**
Number of zone changes phase 3		**0.58**	**0.85**	55.64	**<0.001**
Immobile phase 4 (%)	Sniffing NO phase 4 (%)	**0.52**	**0.58**	23.98	**<0.001**

* 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 occurence 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				
Number of total agonistic interactions/min	1.51^{b} ± 0.12	1.29^{ab} ± 0.13	1.04^{a} ± 0.11	4.22	**0.018**	-	-
% of animals expressing behaviour <u>without</u> contact	*44.4%*	*22.2%*	*25.9%*	-	-	*1.41*	*0.16*
Number of agonistic interactions <u>with</u> contact/min	1.42^{b} ± 0.12	1.24^{ab} ± 0.13	0.98^{a} ± 0.11	3.73	**0.029**	-	-
% of animals expressing head-to-head-interactions	*62.9%*	*37.0%*	*18.5%*	-	-	*3.17*	***0.002***
Nb of head-to-body-interactions/min	1.05^{b} ± 0.09	1.00^{ab} ± 0.11	0.71^{a} ± 0.09	3.98	**0.023**		
% of animals expressing body-to-body-interactions	*62.9%*	*51.9%*	*70.4%*	-	-	-0.64	0.52
Number of non-agonistic interactions/min	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^{b} ± 0.05	0.56^{ab} ± 0.06	0.42^{a} ± 0.05	3.12	**0.049**	-	-

b) Experiment 2

	H+		H-	F-value	p-value RC	z	p-value H+ vs H-
	36		35				
Number of total agonistic interactions/min	1.10 ± 0.12		0.78 ± 0.11	3.78	*0.056*	-	-
% of animals expressing behaviour <u>without</u> contact	*52.80%*		*25.70%*	-	-	*-2.29*	***0.022***
Number of agonistic interactions <u>with</u> contact/min	0.89 ± 0.11		0.69 ± 0.10	1.80	0.18	-	-
% of animals expressing head-to-head-interactions	*19.4%*		*25.7%*	-	-	*0.63*	*0.53*
% of animals expressing head-to-body-interactions	*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	-	-
Number of non-agonistic interactions/min	0.43 ± 0.07		0.42 ± 0.07	0.00	0.99	-	-
Number of successful agonistic interactions/min	0.68^{b} ± 0.08		0.39^{a} ± 0.06	6.66	**0.012**	-	-
% of animals expressing unsuccessful agonistic interactions	*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.