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Master Thesis

'Behavioural differences of horned and disbudded dairy cows'

submitted by

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Abstract

The presence of horns in cattle and their functions received public attention in recent years. The debate mainly questions the necessity and consequences of the commonly executed procedure of disbudding, i.e. the destruction of horn-forming tissue. It is well known that disbudding promotes pain and behavioural changes in calves and affects the animals' emotional state. However, knowledge on potential long-term effects, e.g. behavioural differences between horned and disbudded adult animals are scarce. Therefore, using an explorative approach this thesis aimed at investigating the behavioural reactivity of horned and disbudded dairy cows in situations including new environments and different forms of isolation and confinement.

In the frame of a feeding experiment, a total of 20 Swiss Braunvieh dairy cows (of which 10 were horned (H+) and 10 disbudded (H-)) were observed in 6 different (test) situations on in total 9 occasions. Behavioural observations were carried out when the animals were **isolated** (conducted twice: (i) physical and visual isolation in an Arena Test followed by a Human Approach Test, (ii) visual isolation in a Respiration chamber) and under **confinement** ((i) head fixation (conducted twice), (ii) physical confinement in a scale and (iii) mounting of a heart rate monitoring belt). Additionally, the animals' **activity** after arrival at the experimental farm was measured through accelerometers. Due to the explorative approach, results were described descriptively only.

Results revealed situation dependent differences between horned and disbudded cows. No differences of activity in the new environment were found. Under isolation, behavioural differences between H- and H+ animals were found, but no clear pattern emerged. Under confinement, H+ animals tended to show greater agitation by showing more stepping and spending more time with arched back during physical confinement in the scale. Additionally, H+ animals showed more pulls and tail movements when the head was fixated. Spearman correlations for behavioural expression across repeated test situations were higher for H+ animals regarding vocalizations under isolation and tail movements during head fixation. However, H- animals tended to show a higher consistency in the number of zone changes in the Arena test.

In conclusion, the present data did not reveal clear differences between horned cows and cows that had been disbudded as calves. To shed more light on the effect of horns or potential long-term effects of disbudding on the behaviour of dairy cows, more research is needed which should take the limitations of this study such as small sample size and lack of information on the animals' overall time budgets into account.

Zusammenfassung

Das Halten behornter Rinder und die Funktionen des Horns wurden in den letzten Jahren verstärkt öffentlich diskutiert. Die Debatte dreht sich dabei vor allem um die Fragen der Notwendigkeit und die Folgen des häufig durchgeführten Enthornens, wodurch das hornbildende Gewebe zerstört wird. Es ist gut belegt, dass das Enthornen bei Kälbern Schmerzen auslöst, zu Verhaltensänderungen führt und den affektiven Zustand der Tiere beeinflusst. Untersuchungen über Verhaltensunterschiede zwischen behornten und enthornten adulten Tieren sind jedoch rar. Mittels eines explorativen Ansatzes befasste sich diese Arbeit daher mit dem Vergleich des Verhaltens behornter und enthornter Milchkühe in Situationen, die die Tiere mit neuen Umgebungen und verschiedenen Formen der Isolation und Fixierung konfrontierten.

Im Rahmen eines Fütterungsexperiments wurden insgesamt 20 Schweizer Braunvieh-Milchkühe (davon 10 behornt (H+) und 10 enthornt (H-)) in 6 verschiedenen (Test-) Situationen zu insgesamt 9 Zeitpunkten beobachtet. Verhaltensbeobachtungen wurden durchgeführt, während die Tiere **isoliert** waren (zweimal beobachtet: (i) physische und visuelle Isolation in einem Arena-Test, gefolgt von einem Annäherungstest durch eine unbekannte Person, (ii) visuelle Isolation in einer Respirationskammer) und unter **Fixierung** ((i) Fixierung des Kopfes (zweimal durchgeführt), (ii) physische Fixierung in einer Waage und (iii) Anlegen eines Herzfrequenzüberwachungsgurts). Zusätzlich wurde die **Aktivität** der Tiere nach ihrer Ankunft am Versuchsbetrieb mit Hilfe von Beschleunigungsmessern aufgezeichnet. Aufgrund des explorativen Ansatzes wurden die Ergebnisse lediglich deskriptiv ausgewertet.

Die Ergebnisse zeigten situationsabhängige Unterschiede zwischen behornten und enthornten Milchkühen. Keine Unterschiede wurden hinsichtlich der Aktivität der Tiere in der neuen Umgebung festgestellt. In Isolation wurden Verhaltensunterschiede zwischen H- und H+ Tieren beobachtet, aber es ergab sich kein klares Muster. Unter Fixierung neigten H+ Tiere zu größerer Unruhe; H+ Tiere traten mehr auf der Stelle während physischer Fixierung in der Waage und krümmten gleichzeitig ihren Rücken länger als H- Tiere. Darüber hinaus zeigten H+ Tiere mehr Zerr- und Schwanzbewegungen während der Kopffixierung. Spearman-Korrelationen für beobachtete Verhalten in wiederholten Testsituationen ergaben höhere Verhaltenskonsistenzen bei H+ Tieren hinsichtlich ihrer Vokalisation in Isolation und ihrer Schwanzbewegungen während der Kopffixierung. H- Tiere zeigten jedoch tendenziell eine höhere Konsistenz bei der Anzahl der Zonenwechsel im Arena-Test.

Zusammenfassend lässt sich sagen, dass die vorliegenden Daten keine eindeutigen Unterschiede zwischen behornten und als Kalb enthornten Milchkühen erkennen ließen. Ein besseres Verständnis der Bedeutung von Hörnern oder von Langzeiteffekten des Enthornens sollte durch weitere Untersuchungen erzielt werden, die auch die Limitierungen dieser Studie hinsichtlich Stichprobengröße und fehlender Information über die Zeitbudgets der Tiere berücksichtigen.

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1. Introduction

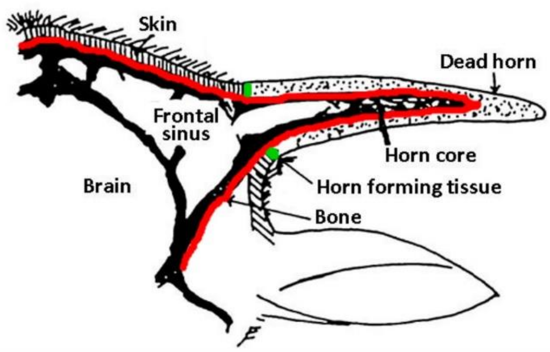
The presence of horns in cattle and their functions are widely discussed (e.g., reviewed by Knierim, Irrgang and Roth, 2015) and also received public attention in recent years (e.g., Hornkuh, 2018). The focus of debates mainly questions the necessity and consequences of the commonly executed procedure of disbudding. Disbudding means the destruction of horn-forming tissue, which is, independently of gender, present in every not-polled-bred-calve. There are several ways to disbud animals which have different popularities around the globe. The preponderant form of disbudding in the European Union for example is through the application of heat by using a hot-iron (Gottardo *et al.*, 2011; Cozzi *et al.*, 2015). Disbudding is a very common procedure, but its frequency has been shown to be influenced by the production system. On a European level, highest frequencies of disbudding were reported for dairy and suckler calves (Cozzi *et al.*, 2015). In the European dairy milk industry approximately 81% of the animals are reported to be disbudded (ALCASDE, 2009). Reasons for disbudding are mainly related to safety concerns that come with horned animals using their horns in confrontational situations against other animals, barn equipment or against staff during handling situations (Gottardo *et al.*, 2011; Kling-Eveillard *et al.*, 2015). The elevated frequency of human-animal interaction in dairy herds explains the higher prevalence of disbudding in this production system.

Due to the very common execution of disbudding, it can be seen as standard procedure in conventional agriculture. However, laws concerning the procedure of disbudding differ throughout Europe. In Austria, for example, the animal welfare legislation permits disbudding of calves in the first six weeks of life and under the use of sedation, local anaesthetic, and postsurgical pain treatment. Additionally, disbudding must be executed by a qualified person or a veterinarian (1. THV, Anlage 4).

Nevertheless, according to a European-wide survey with over 600 farmers as participants, disbudding without pain treatment appeared to still be a common practice, since only 30% of surveyed farms stated to use pain treatment after the procedure (Cozzi *et al.*, 2015). A survey focusing on the prevalence of disbudding practices in Northern Italy, interviewing 639 farmers, found similar results with an overall prevalence of disbudding without pain treatment in 85.5% of interviewed farmers (Gottardo *et al.*, 2011).

Short-term effects (up until two weeks) of disbudding, with and without pain treatment, have been repeatedly researched in the past. Studies have shown that disbudding promotes pain in calves (Adcock and Tucker, 2018, 2020), results in behavioural changes (Mintline *et al.*, 2013; Adcock, Cruz and Tucker, 2020) and affects the animals‘ emotional state (Neave *et al.*, 2013). Studies focusing on behavioural differences between horned and disbudded adult animals, or potential long-term effects of the absence of horns, are rather rare, though. Nevertheless, looking further into potential effects the elimination of horns might have, might be of interest since there are several functions and roles that are attributed to the horn of which for some more scientifical evidence is available than for others (Knierim, Irrgang and Roth, 2015). Some of these functions and roles of the horns are linked to the horns' anatomy. To explain this in more detail, the anatomy of horns in cattle will first be briefly described in the following paragraph.

Horn forming tissues lie at the top of the animals' head of every not-polled-bred calve, positioned above the animals' eyes. This tissue starts to form horn-buds during the first two months of the animals' life. Horn buds are free-floating above the skull first and, if not eliminated through disbudding, start to attach to the skull as the calf grows older. With time, the horn-buds grow into horns. Once grown, the outside of the horn consists of keratin while the inside mainly consists of bone. Horns start to be pneumatised through the caudal frontal sinus which connects the horns to the frontal sinus. The dermis at the basis of the horn is supplied by nerves (cornual nerve). This nerve gets blocked by applying local anaesthesia around the horn buds when disbudding is performed with pain treatment. Furthermore, horns are supplied by blood vessels which reach as far into the horn as the frontal sinus; Only the very end of the horn is considered as 'dead' horn tip (see *Figure 1* and *Figure 2*). Horns continue to grow throughout the animals' life (Spengler Neff, Hurni and Streiff, 2015; Hornkuh, 2018; Engel, 1969).



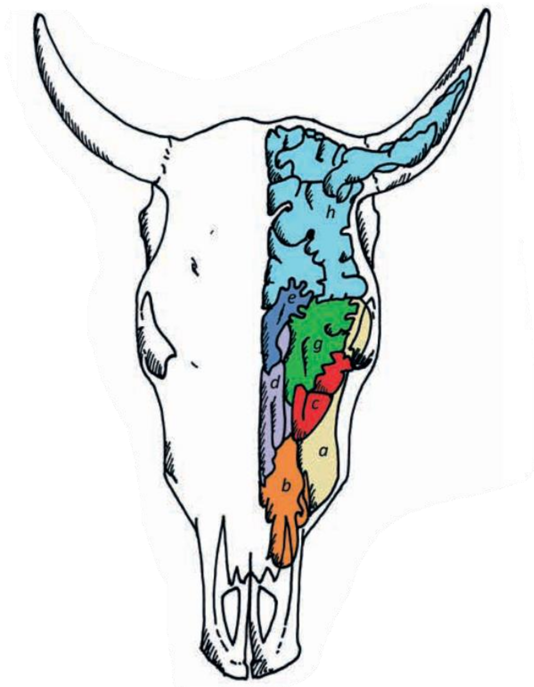


Figure 1 and Figure 2: The graphs show the connection between horns and the frontal sinus of the animals.
Picture 1 shows a section of the animal's head showing one horn and ear; visible is the horn which is attached to the skull of the animal and directly linked to the frontal sinus. The area where the horn forming tissue used to be before growing into horns is marked in green. The 'dead' tip of the horn consists of keratin only and is neither vascularized nor innervated. Source: http://animalethicsri.weebly.com/horns-or-antlers.html.
Picture 2 shows the whole skull of a horned cattle. One half of the skull is sectioned and labelled with letters from a-h and different colours which sections the area between the animals' nose and horn. It shows the horn as part of the (e, g, h) frontal sinus cavity (light- and dark blue and green) which is linked to the (d) upper nasal concha (purple), the (c) temporal

bone cavity (red), (b) palate cavity (orange) and the (a) maxillary sinus (yellow) Source: Spengler Neff, Hurni and Streiff, 2015.

One of the functions linked to the horns' anatomy is the potential influence of horns on the animals' thermoregulation. This is based on the horn's direct link to the frontal sinus, which makes the horn a possible player in the animals' nasal heat exchange (Spengler Neff, Hurni and Streiff, 2015; Hornkuh, 2018). In addition to that, cattle in hot climate regions tend to grow bigger horns than in colder climates (e.g., Watussi-cattle which are a local breed in eastern Africa with big horns) (Spengler Neff, Hurni and Streiff, 2015). Furthermore, some authors attribute to the horns a role in the animals' digestion process. This is because all naturally horned animals are ruminants and therefore, besides the presence of horns, share physiological and anatomical characteristics that come with rumination (Spengler Neff, Hurni and Streiff, 2015; Hornkuh, 2018). Additionally, due to the direct connection between the hollow part of the horn and the frontal sinus, which leads to the paranasal sinus, the by-passing of digestion gases during the rumination process is mentioned as another reason that horns may play a role in the digestive processes. These roles of the horn are not very well scientifically researched, though.

Nevertheless, scientific research showed that another function of the horn is embedded in the communication between animals by using the horns as communicative tool. Lutz *et al.* (2019) investigated agonistic behaviours of horned and disbudded dairy cows under different space allowances in an outdoor arena on 12 farms (of which half had horned and the other half disbudded animals). They selected a group of 20 animals at each farm and observed 6 focal animals of different ranks (two of low, medium, and high rank) at four different space allowances (5, 8, 12 and 15m² per cow) for one hour on 4 consecutive days.

Results revealed that horned animals showed fewer interactions with body contact than disbudded animals and therefore displayed a different pattern of communicative behaviour. The authors concluded that horned animals communicate effectively through positioning their head and therefore don't necessarily need body contact for successful communication (Lutz *et al.*, 2019). Independently of horn status, results showed that fewest agonistic interactions took place under the highest space allowance per cow. The authors concluded that to mitigate agonistic interactions, independently of horn status, greater space allowances would be beneficial for herds of dairy cows which is simply due to the provision of more space to avoid peers. Related to this, it has been shown that horned animals tend to generally keep greater inter-individual distances to peers which results in greater space requirements of horned animals, e.g. under loose-housing conditions (Knierim, Irrgang and Roth, 2015; Spengler Neff, Hurni and Streiff, 2015; Lutz *et al.*, 2019). According to these findings, under the aspect of concerns of injuries of peers related to the keeping of horned animals, disbudding could be prevented by providing more space in barns.

Nevertheless, these findings appear to not be applicable to all horned cattle since in a study comparing agonistic behaviours of horned and disbudded heifers and bulls in a food competition test, differences related to the animals' sex in terms of communication with or without body contact were observable (Reiche, Dohme-Meier and Claudia Terlouw, 2020). While horned heifers supported the results of Lutz *et al. (*2019) by showing more interactions without body contact compared to disbudded heifers, the opposite effect was found for horned bulls (Reiche, Dohme-Meier and Claudia Terlouw, 2020). The effect of sex might be related to hormonal differences, as stated by the authors.

Reiche, Dohme-Meier and Terlouw (2020) also investigated differences between horned and disbudded heifers and bulls in other (test) situations. Summarized it can be said that the authors found differences of spontaneous behaviours (lying down, walking, standing) under loose-housing conditions during 'day-to-day' circumstances between horned and disbudded animals. Horned animals, heifers as well as bulls, showed more active behaviour, meaning that they spent more time standing and walking in the barn than disbudded animals, as measured by accelerometers. During a novel object test, horned heifers performed more zone changes than disbudded heifers while the opposite was observed for horned and disbudded bulls.

In conclusion, it can be said that behavioural differences between horned and disbudded heifers and bulls are observable under different test-conditions and in terms of their spontaneous activity. Furthermore, differences concerning the animals' ways of communication were found between horned and disbudded dairy cows. Still, found differences appear to be situation-, and partly dependent on the animals' sex (Reiche, Dohme-Meier and Terlouw, 2020; Lutz *et al.*, 2019). Whether found differences can be traced back to the absence of horns or potentially show long-term effects of disbudding still needs to be ascertained. Nevertheless, there are no studies investigating behavioural differences between horned and disbudded adult animals in situations that are not related to social interactions and their ways of communication. Due to the observable differences between horned and disbudded heifers and bulls in Reiche, Dohme-Meier and Terlouw (2020) possible behavioural differences under different (test) situations in adult animals led to the aim of this thesis.

Aim of the thesis

Within the context of a feeding experiment related to the potential thermoregulatory role of horns (which will be briefly described under *2.2 Experimental design of the feeding experiment*), the possibility arose to observe horned and disbudded dairy cows in different (test) situations. Due to the scarcity of earlier studies and hence a lack of clear hypothesis, an explorative approach was chosen for this study which runs under the global research question:

'Are there differences between horned and disbudded dairy cows in their behavioural reactivity in selected situations including new environments and different forms of isolation and confinement?'

2. Animals, Material and Methods

All behavioural observations were carried out during a feeding experiment with horned and disbudded dairy cows, which had been authorized by the veterinary office of the canton Zurich, Switzerland (ZH127/2020).

2.1 Animals and housing conditions

Observations took place between March and July 2021 at the experimental farm AgroVet - Strickhof (Eschikon 27, 8315 Lindau) in Switzerland, Kanton Zürich (ZH). In total 20 Swiss Braunvieh dairy cows with similar genetic indices (for milk yield and fitness) were used in this experiment. Half of the cows were horned (H+) while the other half had been disbudded as calves (H-). The 20 cows originated from 18 different farms. 16 of the animals originated from farms with permanent tethering during winter and grazing during summer, whereas four cows originated from free range barns (out of which three cows were disbudded and one horned). All animals were in their second or third lactation and started the experiment at 150 ± 30 DIM. The mean body weight was 635 ± 41 kg for H- and 641 ± 48 kg for H+. The cows were pregnant at the start of the experiment, except for one cow that was successfully inseminated during the experiment. Two cows aborted during the experiment of which one was excluded from the experiment after the abortion.

The animals were assigned to five batches of four cows each. Each batch lasted for 36 days and a maximum of two batches were at the experimental farm at the same time. All animals were transported by the same transport company and handled by the same carrier. Prior to transport, animals underwent a veterinary check. All cows were clinically healthy at arrival on the farm. Before entering the experimental barn, the cows' claws were washed and disinfected. Additionally, an accelerometer (MSR logger) was attached to every cow's left hindleg (for details see chapter *(i) Activity after Arrival*). Afterwards, all animals were led into the tie-stall barn. Animals were tethered using collars and a pneumatic cow trainer was installed above each animal. The stalls were equipped with a rubber mattress and an additional layer of straw on top. The corridor behind the stalls was made of iron manure grids (visible in *Figure 3* and *4*). Partitions between each cow were made of metal bar loops of approximately 1.5-meter length; physical contact between neighbouring animals was possible, mostly in the head area though. In the head area, each cow had access to hay in an individual manger; each animal also had access to alfalfa pellets or concentrates, provided in a feeding trough located on its left side. Each animal had access to an individual drinker on its right side (see *Figure 4* and *5*). Every second day, cows were individually provided access to an outdoor loafing area for an approximate timespan of 15-20 minutes.


Figure 3 and Figure 4: Pictures showing the tie-stall area and design of the stalls where cows were housed during the experiment (except when in the respiration chambers); visible are the rubber mats with chopped straw on top, iron grids at the end of the lying area, partitions between animals and the pneumatic cow trainer.

2.2 Experimental design of the feeding experiment

As explained above, all behavioural observations were carried out in conjunction with a feeding experiment, which will be briefly described in the following paragraph.

The feeding experiment was designed as a crossover trial over the two animal groups (H- and H+) with two isoenergetic diets. The cows received either: (i) ad lib. hay supplemented with alfalfa pellets (90:10, C-) or (ii) restricted amounts of hay and concentrate (70:30, C+). Within the period of 36 days which the animals spent at the experimental farm, all animals underwent five experimental periods. The first period consisted of 5 days after arrival, which served as an acclimatization period during which all cows received the C- diet. The second phase lasted for 10 days and was an adaptation period where animals were assigned to either the C- or the C+ diet. The third period was a sampling period of 5.25 days in a respiration chamber (brand: No Pollution, Industrial Systems Ltd., Edinburgh, UK). Afterwards, the diet was changed. The fourth and fifth period corresponded again to a 10-day adaptation phase and a 5.25-d sampling period in the respiration chamber. Four respiration chambers were available at the experimental farm. The equipment of the four respiration chambers was similar to the tie-stalls in the barn (see *Figure 5 and 6*), except that the animals were individually housed with visual, but no physical contact. To habituate the animals to the respiration chambers before entering the respiration chamber for the first sampling period, each animal spent between 4 to 8 hours in the respiration chamber on one day of the first adaptation period.

Figure 5 and Figure 6: Pictures of the equipment of the respiration chamber as well as video stills of all chambers with cows.

2.3 Observed situations and behavioural tests

2.3.1 Overview

The animals were exposed to six different situations/tests; four of the six situations were repeated once so that data collection took place on in total 9 occasions. All tests and observations took place within a time frame of 21 days for each batch, starting directly after the animals' arrival at Strickhof and the end of the first crossover period, i.e., when the animals were released from the respiration chamber after the first sampling period (see *Figure 7*). Throughout data collection, the animals were handled by the same persons.

Whenever possible, behaviour tests were conducted in the morning (around 10 a.m.) or around noon (approximately 11:30 a.m. to 1:00 p.m.), since these were the quietest times of the day at the experimental farm, to avoid environmental noise as much as possible. For every conducted test, signs asking for silence were attached to entrance doors to the barns. During all human-animal interactions that were necessary as part of the test, humans moved in a gentle way and loud talking was avoided. Whenever additional interaction with the animal was needed, for example to redirect loose animals in the needed direction (e.g., move them through a door), this was done using gentle touching of the animal rather than loud acoustic signals.

2.3.2 Detailed description of the observed situations and behavioural tests

Table 1 gives an overview on all observed situations/tests with selected literature references describing similar situations for some of the observed situations. This is followed by a more detailed description.

Table 1: Overview on the observed situations considered for this study. The reference section refers to literature with similar behavioural tests and observations.

Situation	Description	References
(i) Activity after arrival	The animals' activity after arrival at Strickhof is measured for one hour using a MSR logger.	
(ii) Arena Test (AT)	This test shows the animal's behaviour in a new and unknown surrounding in isolation. In the arena test, the animal is observed while being in an outdoor arena. Animals were subjected to the AT on two occasions: 1) Two days after arrival at the experimental farm 2) After the first sampling period in the respiration chamber (21st day at the experimental farm)	(R. Kilgour 1975; R. J. Kilgour, Melville, and Greenwood 2006)
	A human approach test, a test to measure the animal's fearfulness towards humans, was conducted subsequently after AT1 and AT2. In this test, the animal is actively approached by a known human and the animals' behaviour towards this approach is observed.	(R. J. Kilgour, Melville, and Greenwood 2006; Waiblinger, Menke, and Fölsch 2003; Ebinghaus, Ivemeyer, Knierim 2018; Dodzi and Muchenje 2011)
(iii) Weighing (W)	This situation shows the animal's behaviour under confinement. The animal is led into the scale and observed for 2 minutes. After two minutes, the front door of the scale is opened, and the individual exit speed of the animal is measured using a stopwatch.	(Hirata et al. 2016; R. J. Kilgour, Melville, and Greenwood 2006) (Hirata et al. 2016; R. J. Kilgour, Melville, and Greenwood 2006; Dodzi and Muchenje 2011)
(iv) Fixation (HABFix)	HAB:Fix shows the animal's reaction to its head being fixed using a head collar. The animal remains in its individual stall in the barn and the head gets fixated for two minutes. HAB:Fix happened twice for every animal (HABfix1 and HABfix2).	(Graunke *et al.*, 2013)

Situation	Description	References
(v) PolarBelt (HABPolar)	This situation shows the animal's behaviour when a PolarBelt, a device to measure heart rate, is attached to the animal's back for the first time and left on for two minutes.	
(vi) Respiration Chamber	This situation shows the animal's behaviour in new surroundings, in which physical but not visual contact to neighbouring animals is deprived. The animal's behaviour in the respiration chamber is observed for one hour. This is done on two occasions: 1) The first time the animal enters the respiration chamber (habituation to the chamber within the first adaptation period of the feeding experiment) 2) The animal enters the respiration chamber for the second time (start of the first sampling period) Additionally, data from accelerometers is considered for situation 1.	

Figure 7 shows a timeline of all executed tests and observed situations. Per batch, activity after arrival, Arena Tests (AT) 1 and 2, Human Approach Test (HAT) 1 and 2 and Respiration chamber 2 were executed on specific days of the experiment. However, Weighing, Habituation to fixation of the head (HABfix) 1 and 2, Habituation to the belt measuring heart rate (HABpolar) and Respiration chamber 1 did not take place on the same days, but were executed within the marked time frame (between day 4 and 12) for all batches.

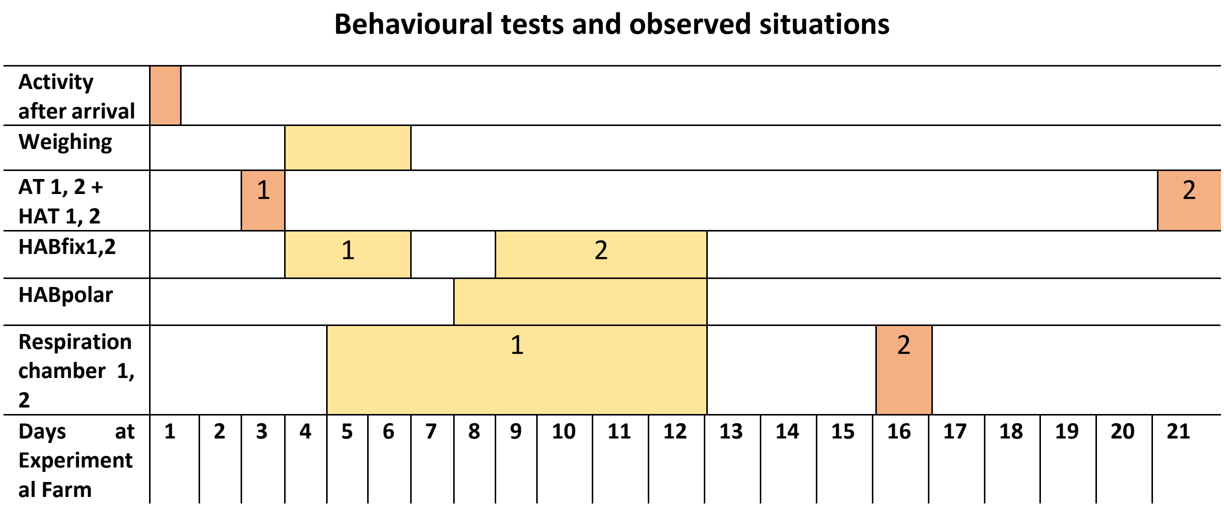


Figure 7: Timeline with all behavioural tests and observed situations; Some tests and observations were conducted at the same dates at the experimental farms for all batches (namely activity after arrival, AT1 + HAT1, Respiration chamber 2 and AT2 and HAT2) whereas others were conducted on no fixed days but within the animals' 4th to 12th day at the experimental farm (weighing, HABfix1, 2, HABpolar, Respiration chamber 1) and in the marked time-frames of the respective tests and situations.

Situations (ii) to (v) were recorded using a camera (Panasonic SD99) and when filmed from two angles, a smart phone was additionally installed. Since the Respiration chambers were equipped with cameras that were filming from top front (see *Figure 6* and *7*), recordings from these cameras were used for the observations in the respiration chambers.

(i) Activity after arrival: All animals arrived at the experimental farm and entered the barn around noon (between 12 and 2 p.m.). To measure the animals' activity, an accelerometer (model MSR145W2D (MSR, 2019)) was attached to the animals left hind leg before entering the barn. The data loggers continuously recorded acceleration and cumulated acceleration data for the respective time frame of one hour after the animals' arrival was considered as activity measure and evaluated using 'R' (Simmler, 2019). *Figure 8* shows a picture of the used device.

Figure 8: The used logger MSR145W2D; source: https://www.msr.ch/en/product/wifi-data-logger-msr145w2d/

(ii) Arena Test (AT): The arena (*Figure 9* and *Figure 10*) was approximately 8.5 m long and 4.2 m wide; it was installed in the outdoor area adjacent to the tie-stall barn. For visual separation from the surroundings, wooden walls of approximately 2.1 meters height were installed surrounding the arena on all sides, except for the side which consisted of the building's wall. The arena was divided into eight zones of equal size and had two entrance doors. One entrance allowed access from the barn (leading into zone 1) and the other from outside (leading into zone 8). The floor was slatted in six of the eight zones (zone 1-3; and zone 6-8), and these zones were the ones closest to the entrance doors. In zones 4 and 5 the floor was solid. Cameras were arranged on two points, filming from two different angles from outside of the wooden walls (*Figure 9*). The arena was cleaned between every animal; before the first animal entered the arena, the floor was wettened to ensure similar conditions.

All animals were tested individually on two different occasions (1 and 2). During the respective testing time span of 10 minutes several different behaviours were observed. The observed behaviours and their definitions were the same for both test situations (1 and 2) and are described in *Table 2*. Since animals entered the Arena Test twice in different phases of the experiment, conditions prior to the tests differed and are described in the following.

In preparation for the first testing occasion (AT 1), approximately 45 minutes prior to the test, a head-collar was attached to the animal's head. A corridor barred with strings, leading from the individual places of the cows in the tie-stall barn to the arena, was installed aiming at allowing the animals to walk to the arena at their own pace. The animal was released from tethering and walked to the arena, using human guidance only, when necessary, namely when the animal stopped or turned around. The time from releasing the animal until closing the entrance door of the arena was measured using a stopwatch. The Arena Test started as soon as the door between tie-stall and arena was shut behind the animal.

For the second occasion (AT 2), the animals entered the arena after they had been released from the respiration chambers. Before releasing the animals, a head collar was attached, and animals walked freely at their own pace to the arena, using human guidance only under the same conditions as described for AT1. Since the respiration chambers were in a different building than the tie-stall barn, the animals had to walk an approximate distance of 100 meters between the respiration chamber and the test arena. Contrary to AT1, where animals entered the arena through zone 1, coming from the barn, animals entered the arena in AT2 through the opposite door, i.e., in zone 8. Once the door was shut gently behind the animal, the Arena Test 2 started.

Table 2 Observed behaviours in the arena test and their definitions; behaviours in italic indicate point events.

Observed behaviour	**Definition**
Transition between zones	*A transition between zones was recorded when both front legs of the animal entered another zone.*
Vocalization	*Open: vocalisation with an open muzzle.*
	Closed: vocalisation with a closed muzzle.
Excretion	*Defecation or urination.*
Interaction with wall	Sniffing, licking, rubbing, touching the wall.
Head out of sight	Head not visible (e.g., if one camera failed).

A human approach test was conducted in the arena after the cows had stayed there for 10 min (immediately after AT 1 and 2). The human conducting the test entered the arena for both situations through the same gate, via zone 8 (see *Figure 10)*. After gently opening the gate, entering, and closing the gate, the approaching person aimed to establish an approximate distance of three meters to the animal. Afterwards, the person aimed to approach the animal as described by Waiblinger, Menke, and Fölsch (2003) at a pace of one step per second, executing an 'Active Human Approach Test'. To ensure a uniform walking speed, the approaching person wore headphones emitting an audible signal every second. During the approach, the person's right hand was held straight in front of its body at an average angle of 45° (Waiblinger, Menke and Fölsch, 2003) and the left hand was held behind its back. As soon as the animal started to withdraw, the distance between the animal's head and the approaching person's hand was measured using a digital distance measuring device which was held in the right-hand. Depending on the distance between the cow and the approaching person when the cow showed signs of withdrawal, a score from 0 to 6 points was assigned (see *Table 3)*.
If the person failed to establish a 3-meter distance before approaching the animal, because the cow followed the person through the arena, the test was terminated after approximately one minute. The person then stopped, turned towards the cow, and tried to stroke her for an approximate duration of 10 seconds. When the cow tolerated to be touched for a duration of 10 seconds, the highest score (6) was assigned (see *Table 3*).

Figure 9: Picture of the arena viewed from one of positioned cameras outside of the wooden walls.

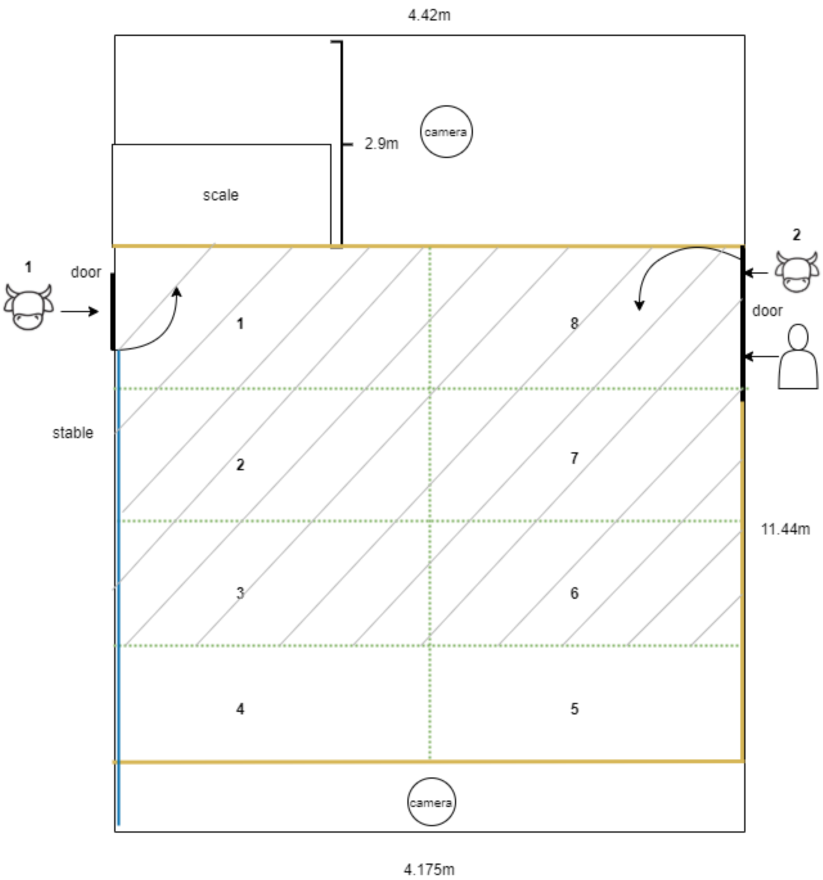


Figure 10: Outline of the outdoor area for the arena and human approach tests; the test arena was divided into eight zones of equal size. Lines through the zones indicate slatted concrete floors whereas blank zones indicate solid concrete flooring. *Brown lines represent wooden walls whereas the blue line represents the building wall of the tie-stall barn. Cows entered the*

arena through different doors for the two test situations (1, 2). The human entered the arena for HAT1 and 2 via the same door. Two cameras were installed outside of the wooden walls to film the test situations.

Table 3 Behavioural scores used in the Human Approach Test.

Scale	**Description of the cows' behaviour (adapted from Dodzi and Muchenje, 2011)**
0	Cow avoided the approaching person at >2m
1	Cow avoided the approaching person at a distance between 2 and 1 m
2	Cow avoided the approaching person at a distance between 1 and 0.5 m
3	Cow avoided the approaching person at a distance of <0.5m
4	Cow accepted the touch of the person on head
5	Cow tolerates touch for 10s on head
6	Cow approaches person

(iii) Weighing

The scale was located directly adjacent to the barn (see *Figure 10*) which made it possible to release the animals from their stalls under the same conditions as for the AT. Animals walked voluntarily to the scale and the test started when the animal successfully had boarded the scale, and the front and rear door had been closed. The animal remained in the scale for two minutes. During this time, the animal was filmed from the left side from the animal's perspective. Observed behaviours are described in *Table 4*. *Figure 11* shows a cow in the scale during weighing.

Table 4 Observed behaviours while weighing and their definitions; observed behaviours in italic indicate point events.

Observed behaviours	**Definitions**
Head positions	Head above scale (at least the eyes of the animal exceed the top of the scale).
	Head at ground (muzzle is below carpal joints).
	Levelled head (head held in a position between 'at ground' and 'above scale').
Head movements	*Turning the head resulting in an angle between approx. 45° and 90° from a front facing position.*
	Turning the head resulting in an angle of >90°.
Vocalization	*Open: Vocalization with open muzzle.*
	Closed: Vocalization with closed muzzle.

Movement	*Stepping: included lifting the leg up until the pastern bone, lifting or dragging of the hoof on the floor; the leg can be replaced again on the same spot or be moved to a different area.*
	Slipping: while the body of the animal doesn't move forward, the claws of one leg move forward for at least one claw-length but stay in contact with the floor.
Back position	Arched back: the back line is not levelled with the animals' hip and shoulder.
	Straight back: back line levels with hip and shoulder.
Excretion	*Defecation or urination.*

Figure 11: A horned cow during weighing showing levelled head position and an arched back.

After the behavioural observations in the scale, a person gently approached the front door of the scale to release the animal. When the front door was opened the animal was free to leave the scale at its individual pace. The time it took the animal to leave the scale was measured using a stopwatch. The exit time was assessed for two phases (see *Table 5)*.

Table 5: Phases defined for assessing the time when the cows exit the scale

Phase 1	Timespan from opening the scales front door until the animals' muzzle passed the door.
Phase 2	Timespan between the end of phase 1 and the animal leaving the scale, with the moment of the last claw stepping from the scale. Phase 2 was capped at 60 seconds.

(iv) Habituation – head fixation (HABfix)

A head collar was attached approximately 15-20 minutes prior to testing. The person executing the test stepped on the mattress of the animal's place in the tie-stall barn and approached the animal from its left side. The person then fixated the animal's head to the right-hand side from the animals' perspective using the string of the head collar (see *Figure 12*). The behavioural observation started when the string was attached to the bar by a nod and the person gently stepped away from the animal. The test lasted for two minutes. Two cameras, one from the front and one from the back, recorded the behaviour. After the test period, the animal's head was released, and the collar was removed from the animal's head. All animals were observed during fixation twice within the second phase of the experiment on two non-consecutive days, except for one batch where testing on consecutive days was inevitable. *Table 6* shows the observed behaviours and their definitions. *Figure 12* shows one cow during HABfix.

Table 6: Observed behaviours in the fixation test and their definitions. Observed behaviours in Italic indicate point events.

Observed behaviours	**Definitions**
Pull	*Cow pulls on the string; it is a fast movement directed to the back or side.*
Vocalisation	*Open: Vocalization with open muzzle.*
	Closed: Vocalization with a closed muzzle.
Leg movement	*Stepping: included lifting the leg up until the pastern bone, lifting or dragging of the hoof on the floor; the leg can be placed again on the same spot or be moved to a different area.*
	Kicking: front or rear leg is lifted above the height of the pastern bone in a fast movement which might be directed towards the rear or the animals' belly.
Tail movement (Hintze *et al.*, 2020)	Hanging: tail hangs straight downwards, minimal movements of tails and movements because of the tail being fixated are possible; it does not include lifting tails or tails laying on the back of the animal; if tail is not hanging or in motion (as defined below), no events for tails are recorded.
	In motion: active movement of the tail, it moves from one side to the other; tip exceeds the height of the focal knee of the animal; every movement to the side is counted as one movement.
Excretion	*Defecation or urination.*

Initially, the ethogram contained two more behaviours concerning the string of the head collar. It was the intention to establish the time the animal spent leaning into the string, resulting in a stretched rope, compared to a loose rope without leaning into it. Unfortunately, these behaviours had to be removed from the ethogram due to unsatisfactory intra-observer reliability (see *2.4 Data analysis*).

Figure 12: H- cow during HABfix with the head fixated with a rope.

(v) Habituation - Polar belt (HABpolar)

The person executing the test approached the animal with the Polar belt in her hand, approaching the animal from the left. The Polar belt was gently placed on the animal's back, the person walked to the other side of the animal and fixed the belt. The test started as soon as the observer stepped away from the animal. The test lasted for two minutes with, again, two installed cameras filming from the front and back of the animal. Observed behaviours are described in *Table 7*.

Table 7: Observed behaviours during HABpolar and their definitions. Observed behaviours in Italic indicate point events.

Observed behaviours	**Definitions**
Head movement	*Turning the head resulting in an angle between approx. 45° and 90° from a front facing position.*
	Turning the head >90°.
	Interaction with the belt: the animal touches, sniffs or licks the belt.
Vocalization	*Differentiated between open and closed vocalization.*

Feeding	Feeding: animal interacts with hay or concentrate; the animal's head is lowered in the feeding area; lifting of the head while chewing and subsequently returning to interaction with feed are also considered as 'feeding'.
	Drinking: animal drinks from the drinker.
	Ruminating: animal chews the cud.
Leg movement	*Stepping: included lifting the leg up until the pastern bone, lifting or dragging of the hoof on the floor; the leg can be placed again on the same spot or be moved to a different area.*
	Kicking: front or rear leg is lifted above the height of the pastern bone in a fast movement which might be directed towards the rear or the animals' belly.
Tail movement (Hintze *et al.*, 2020)	Hanging: tail hangs straight downwards, minimal movements of tails and movements because of the tail being fixated are possible; it does not include lifting tails or tails laying on the back of the animal; if tail is not hanging or in motion (as defined below), no events for tails are recorded.
	In motion: active movement of the tail, it moves from one side to the other; tip exceeds the height of the focal knee of the animal; every side is counted as one movement.
Excretion	*Defecation or urination.*

(vi) Respiration chamber

Observations in the respiration chamber were done on two occasions: (1) when animals entered the respiration chamber for the first time (habituation to the respiration chamber within the second period of the feeding experiment) and (2) when entering the respiration chamber the second time, namely for the first sampling period. The four respiration chambers were arranged as follows: respiration chambers 1 and 2 as well as chambers 3 and 4 were directly adjacent to each other. Between the chambers 2 and 3 there was a pathway of approximately one meter width. Since visual contact between animals in chamber 2 and 3 was possible, solely animals in chambers 1 and 4 had only one neighbouring animal.

All tests took place in the morning (entering the respiration chambers between 7 and 8:30 a.m.) except for one batch which was habituated to the chambers later in the morning (around 11:30 a.m.). Prior to the test, the animal was driven from the barn to the respiration chamber by a stockperson using a head collar and a lead rope. The walking distance between the tie-stall and the respiration chamber was approximately 100 meters, crossing the outdoor arena. The observation started when all four animals of one batch had entered and had been tethered in their individual respiration chamber and lasted for one hour. Videos from the respiration chamber were generated through installed cameras in the chambers. Videos were without audio. Observed behaviours are described in *Table 8*.

Table 8: Observed behaviours and their definitions for the observation time in the respiration chamber. Behaviours in Italic indicate point events.

Observed behaviours	Definitions
Head orientation	Front: the animals head faces the front or is oriented within an angle of approximately 45° to either side.
	Neighbouring animal: the animals head is oriented to the side with a neighbouring animal (head turned between approximately 45° to 90°).
	Other side (=the side not oriented to a neighbouring animal): the animal's head is oriented to the side but not towards a neighbouring animal.
	Back: the head is oriented towards the animals' back in an angle of >90°.
Activity of head	*The sum of changes between head orientation (front, neighbouring animal, the opposite side, or the back).*
Pull	*Cow pulls on the string; it is a fast movement directed to the back or side.*
Vocalization	*Indirect assessment of vocalization identified through contraction of the animals' belly, the tilted head into the nape and stretched neck.*
Excretion	*Defecation or urination.*
Feeding	Feeding: animal interacts with provided hay or concentrate; the animals' head is lowered in the feeding area; lifts of the head while chewing and subsequent returns to interaction with feed are also considered as 'feeding'.
	Rumination: animal chews the cud.
	Drinking: animal drinks from the drinker.
Body position	Standing: animal stands on all four legs.
	Lying: animals' body is lowered to the ground.

In addition to the behavioural observations, acceleration data was obtained from MSR loggers (see *(i) Activity after arrival*) for the first situation in the respiration chamber. Acceleration data from the second observation in the respiration chamber had to be excluded from the evaluation due to technical issues.

2.4 Data analysis

All situations recorded on video (Arena Tests 1, 2, Weighing, HabFix 1 and 2, HabPolar and Respiration chamber 1, 2) were evaluated using the freeware software BORIS (Friard and Gamba, 2016). The behavioural observations resulted in either durations or frequencies. In some cases, the full observation time was not available due to unforeseen events (e.g., failure of one camera). If up to 30% of the observation time was missing, frequency data were extrapolated for the full timespan, while durations were always expressed as percentage of the exact observation time span. If less than 70% of the observation time were available (e.g., due to one camera overheating during test situations) or where unforeseen events made the testing situations not comparable to the rest of the videos (e.g., door not closing properly for AT or unforeseen and unplanned human-animal interaction during observation time), the video recordings were completely excluded from analysis. This led for example to the exclusion of 5 videos for AT2.

Videos were randomized using the Excel randomization function 'RAND()'. The videos were evaluated by one person only. To test the intra-observer-reliability of video scoring, at least 25% of the videos of one situation were evaluated twice and tested for their Cohen's Kappa value; in the case of observations in the respiration chambers, only the first 10 minutes of the videos were considered. Reliability testing was done using the Cohen's Kappa function in BORIS. A buffer time frame of one second was used by the program when checking agreement between coded events. Values may lie between -1 and +1 and values from +0.41 were considered as at least 'fair' agreement (Landis and Koch, 1977).

Generated Cohen's Kappa values for with BORIS evaluated situations are presented in *Table 9*.

Table 9: Cohen's Kappa values for the different observed situations/tests with behavioural observations.

Situation	**Mean kappa value**	**Range of values**
Arena Test (1, 2)	0.77	0.51-0.93
Weighing	0.66	0.51-0.83
HabFix (1, 2)	0.69	0.44-0.83
HabPolar	0.68	0.62-0.85
Respiration chamber (1, 2)	0.81	0.74-0.93

The statistical approach focused on descriptive statistics and linear correlations after Spearman (using 'R'; Revelle, 2021). Correlations after Spearman focused on the consistency of the most frequently observed behaviours within repeated tests and observations.

To compare groups (H- vs. H+), data was illustrated as boxplots using 'R' (Wickham, 2016). Tests for significance were dismissed due to the explorative approach and since this work aimed at generating a general picture of behavioural differences between horned and disbudded dairy cows as well as due to the small sample size.

3. Results

Results are described as group median and lower (25th) and upper (75th) quartile (median; 25th, 75th quartile). Spearman correlation coefficients focus on consistencies of the most frequently observed behaviours within repeated situations (see *(vii) Consistencies of selected behaviours*).

(i) Activity after arrival

During the first hour in the novel environment (tie-stall), all animals were standing for the whole observation period. H- and H+ animals showed similar activity, expressed as cumulated acceleration over the one hour measuring period (H-: 194; 171, 209; H+: 204; 194, 237).

(ii) Arena Test

Arena Test 1 The median time it took the animals to walk themselves from their individual stalls to the arena was 01:27 minutes (01:02, 02:09 minutes) for H- (n_{H-}=10) and 01:54 minutes (01:32, 02:21) for H+ (n_{H+}=10).

Frequencies of behaviours of the observed animals (n_{H-}=10; n_{H+}=9) in AT1 are presented in *Figure 13.* Vocalization (= sum of open and closed vocalization) was the most frequently observed behaviour for both groups. Median of vocalizations for H- cows during the whole 10-minute observation period was 4.5 (2.0, 9.0), while it was 1.0 (0.0, 12.9) for H+ animals. Open vocalization was observed more frequently than closed vocalization for both animal groups. 3.9 (1.0, 9.0) vocalizations with an open muzzle were observed for H- cows while H+ animals were observed to vocalize with an open muzzle 0.0 (0.0, 4.0) times. Closed vocalization showed similar frequencies for H- and H+.
With a median of 0.5 (0.0, 1.0), H- cows showed slightly less excretions (=sum of urination and defecation) than H+ cows (2.0; 1.0, 2.0). Defecation was observed more often compared to urination for both animal groups and largely accounted for the pattern regarding total excretions.
H- animals spent 14.0% (11.3%, 20.6%) of the observation time in interaction with the wall while H+ animals were observed to spend 18.0% (11.8%, 21.8%) of observation time interacting with the wall.
Medians of transitions between zones were 65.4 (47.4, 79.7) and 76.6 (24.9, 80.9) transitions for H- and H+ cows. Transitions between zones showed positive associations (r_s > 0.5) with excretion (r_{H-}

=0.81; rH+=0.57), vocalizations (rH-=0.8; rH+=0.75) and time spent in interaction with the wall (rH-=0.75; rH+=0.5).

Independently of horn status, most zone changes were observed during the first two minutes of AT1 (see *Figure 14*). Transitions between zones decreased to a similar extent for both animal groups during the subsequent 8 minutes, resulting in a zone change median in minute 10 of 2.5 (0.0, 5.5) for H- animals and 3.5 (1.5, 4.5) for H+ animals.

During AT1, the animals spent most of the time in zone 1 (H-: 13.3%; 10.0%, 14.7%; H+: 29.6%; 16.2%, 35.2%) or zone 8 (H-: 12.6%; 11.1%, 14.8%; H+: 15.5%; 12.3%, 16.9%). H- animals spent least of the observation time in zones 4 and 5 (zone 4: 4.4%, 2.3%; 13.9%; zone 5: 5.2%, 3.4%; 9.0%). H+ animals spent least of the observation time in zone 4 and 6 (zone 4: 5.1%, 0.0%; 9.4%; zone 6: 5.48 %; zone 6: 4.6%, 3.8%; 7.8%).

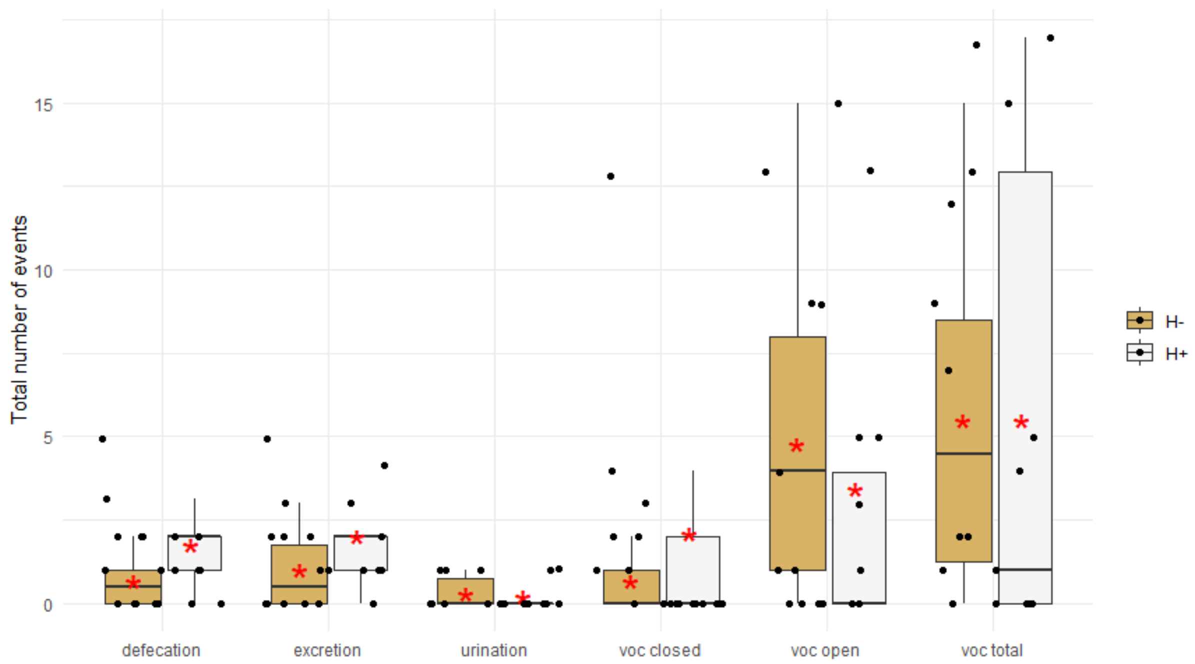


*Figure 13: Boxplots of the number of events (defecation, urination, excretion=sum of defecation and urination, vocalization with open (voc. open) and closed (voc. closed) muzzle as well as total vocalization=sum of voc. open and voc. closed)) observed in H- (n=10) and H+ (n=9) cows during 10minutes of the first Arena Test (AT1); *=mean number of events.*

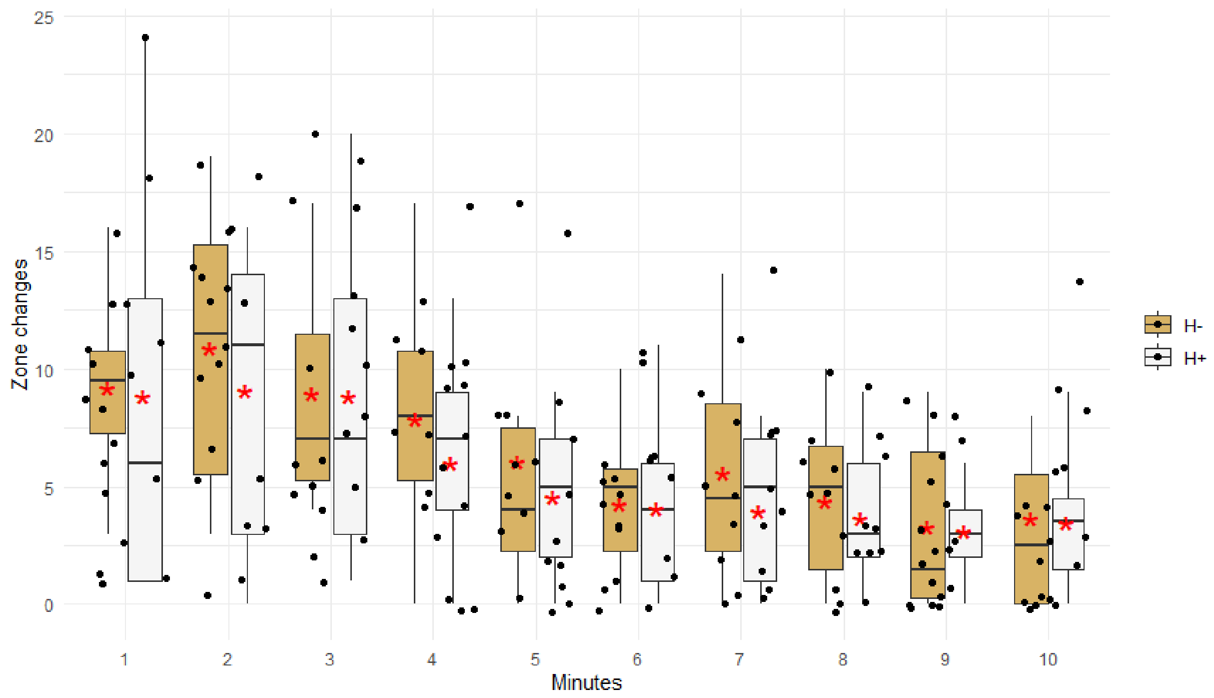


*Figure 14: Boxplots of the transitions between zones over time (in minutes) for H- (n=10) and H+ (n=9) cows during the first Arena Test (AT1); *=mean number of zone changes.*

Arena Test 2 Observed frequencies of behaviours during AT2 (n_{H-} = 7; n_{H+} = 8) are displayed in *Figure 15.* Independently of horn status, the most frequently observed behaviour was vocalisation (=sum of open and closed vocalization). H- animals vocalized 3.0 (1.5, 12.4) times during the whole observation period, while H+ animals vocalized 7.0 (3.2, 10.3) times during AT2. Open vocalization was observed more often than closed vocalization for both animal groups. H- cows vocalized 3.0 (0.0, 11.8) times and H+ 4.6 (1.5, 7.7) times with an open muzzle. Closed vocalization showed a median of 1.0 (0.0, 1.0) vocalization for H- and 2.5 (0.0, 4.0) vocalizations for H+ cows. Excretion was almost never observed for both animal groups.

H- animals interacted 11% (5.7%, 14.3%) of the observation time with the wall, whereas H+ animals spent 14.2% (9.6%, 19.6) of the observation time in interaction with the wall.

Median transitions between zones amounted to 46.4 (34.0; 49.8) in H- animals and 58.4 (47.2; 77.9) in H+ animals. Transitions between zones showed positive associations (rs > 0.5) with vocalizations (rH-=0.79; rH+=0.66) and time spent in interaction with the wall for H+ (r=0.5) and weak positive correlations for H- (r=0.43). Since excretions were rarely observed during AT2, no associations were observable between transitions between zones and excretions for H- animals while weak positive associations were found for H+ animals (r=0.17).

Transitions between zones were highest in the first two minutes (see *Figure 16*), independently of horn status. Within the subsequent test minutes, transitions between zones decreased until minute 9 and increased slightly in minute 10 for both animal groups.

H- animals spent most of the observation time in zone 2 (19.0%, 10.8%; 24.0%) and zone 1 (18.5%, 4.7%; 22.0%), while H+ animals spent most of the observation time in zone 1 (16.6%, 10.7%; 20.2%) and zone 7 (15.0%, 10.3%; 18.8%). H- and H+ animals spent least of the observation time in zone 4 (H-: 0.2%, 0.0%; 5.8%; H+: 1.4%, 0.6%; 3.9%) and zone 5 (H-: 1.8%, 0.0%; 2.4%; H+: 3.0%, 0.6%; 7.0%).

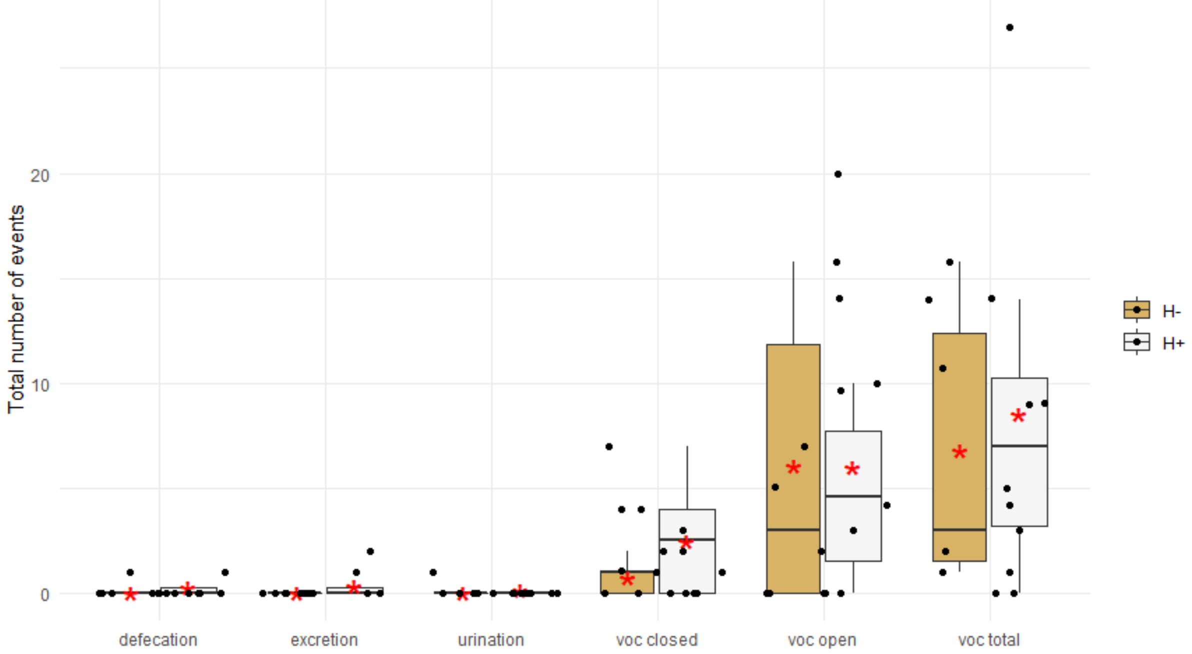


*Figure 15: Boxplots of the number of events (defecation, urination, excretion=sum of defecation and urination, vocalization with open (voc. open) and closed (voc. closed) muzzle as well as total vocalization=sum of voc. open and voc. closed)) observed in H- (n=7) and H+ (n=8) cows during 10minutes of the second Arena Test (AT2); *=mean number of events.*

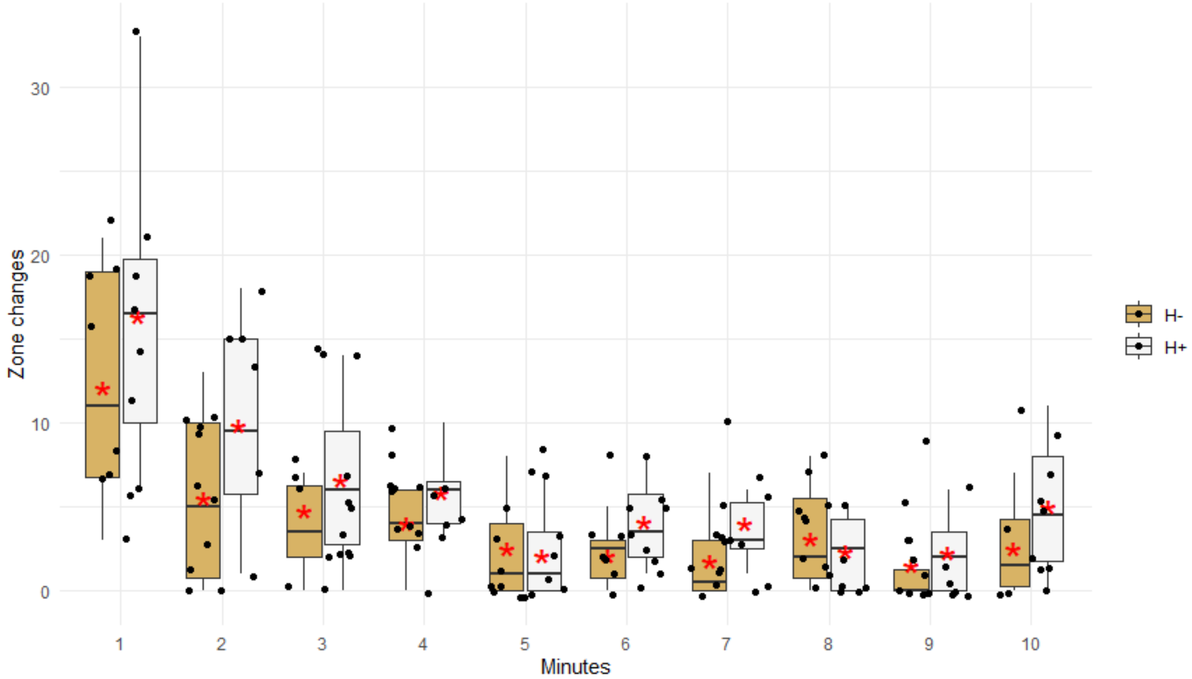


*Figure 16: Boxplots of the transitions between zones over time (in minutes) for H- (n=7) and H+ (n=8) cows during the second Arena Test (AT2); *=mean number of zone changes.*

Human approach Test

Table 10 shows the distribution of scores assigned during HAT1 and HAT2 for H- and H+ animals. The highest score (6) was assigned to most animals, independently of horn status. In HAT1, 80% of H- and H+ scored 6 points in HAT1, whereas in HAT2, the highest score was attributed to 60% of H- animals. Most of the animals approached the person as soon as the latter entered the test arena. It was often not possible to establish the approach-distance to the animal.

Table 10 Percentages of scores assigned (1-6) during HAT1 (n_{H-}=10; n_{H+}=10) and HAT2 (n_{H-}=10; n_{H+}=8) for H- and H+ animals.

	HAT 1		**HAT 2**	
Scores	**H- n=10**	**H+ n=10**	**H- n=10**	**H+ n=8**
1	0	0	0	0
2	0	0	0	0
3	20 %	0	10 %	0
4	0	20 %	30 %	11%
5	0	0	0	0
6	80 %	80 %	60%	89%

(iii) Weighing

When standing for two minutes in the scale (n_{H-}=10; n_{H+}=9), animals of both animal groups kept their head levelled during most of the observation time (H- 66.1%; 59.3%, 77.8%; H+ 74.7%; 61.0%, 84.1%). H- spent 17.7% (13.0%, 20.5%) of observation time and H+ 11.1% (5.5%, 20.5%) of observation time with their head at ground. The least of the observation time, H- and H+ animals spent with their head above the scale (H- 6.4%; 2.3%, 33.3%; H+ 8.3%; 5.8%, 11.9%).

Looking left or right was similar for H- and H+ animals (see *Figure 17*). The median number of times turning the head >90° was 4.1 (3.6, 6.1) times for H- animals and 6.4 (5.8, 8.6) times for H+ animals.

Stepping was the most frequently observed leg movement; the median number of stepping showed 6.8 (3.0, 16.4) events for H- and 34.6 (23.3, 48.4) events for H+ animals. Slipping was never observed for H- animals (0.0%; 0.0%, 0.0%), while two H+ animals were observed slipping.

Back positions were not visible for all animals, therefore five animals had to be excluded from this evaluation (n_{H-} = 7 and n_{H+} = 8). Expressed as percentage of observation time, H- and H+ animals spent more time arching their back than keeping it straight (H-: 59.0%; 38.3%, 84.5%; H+: 85.8%; 64.2%, 100.0% of observation time). Vocalizations and excretions were observed on very few occasions and did not differ between groups.

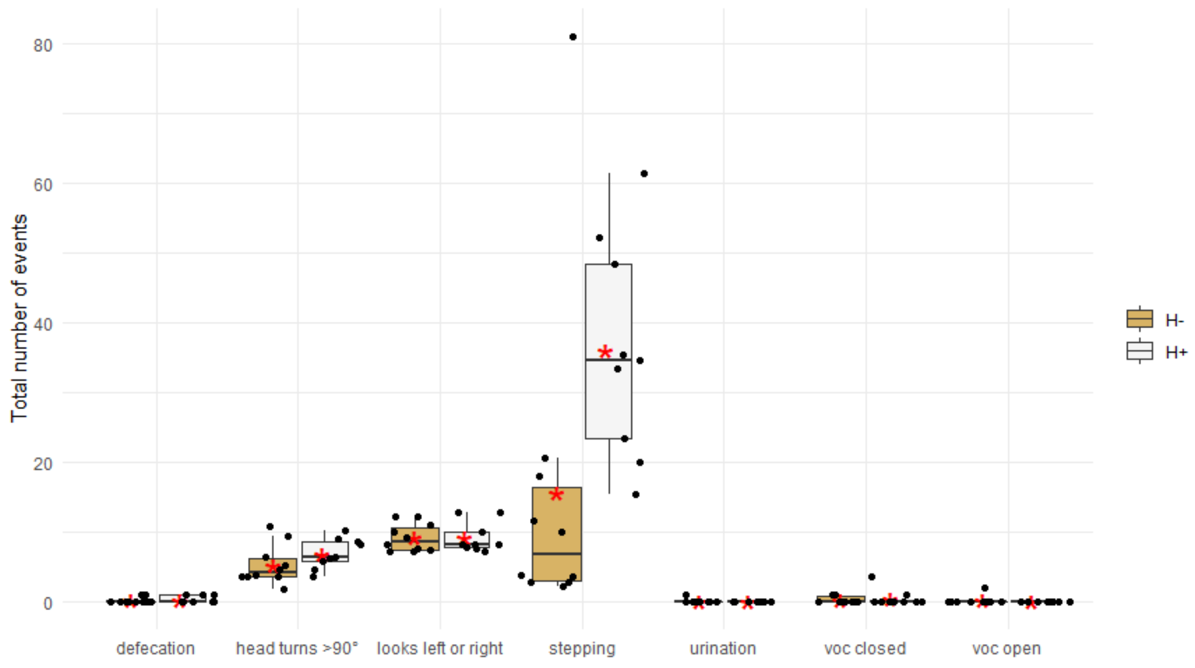


*Figure 17: Observed total frequencies of behaviours (defecation, head turns >90°, looking left or right, stepping, urination, voc. closed, voc. open) during the 2 minutes observation time while weighing the animals for H- (n=10) and H+ (n=9); *=mean number of events.*

Figure 18 shows the exit duration for H- and H+ (n_{H-} = 10; n_{H+} = 9) animals by exit phase. The median for exit phase 1 was 1 second (1.0; 2.0 seconds) for both animal groups. In exit speed phase 2, the median was at 7.5 seconds (5.3; 8.8) and 6.0 seconds (4.0; 10.0) for H- and H+ animals.

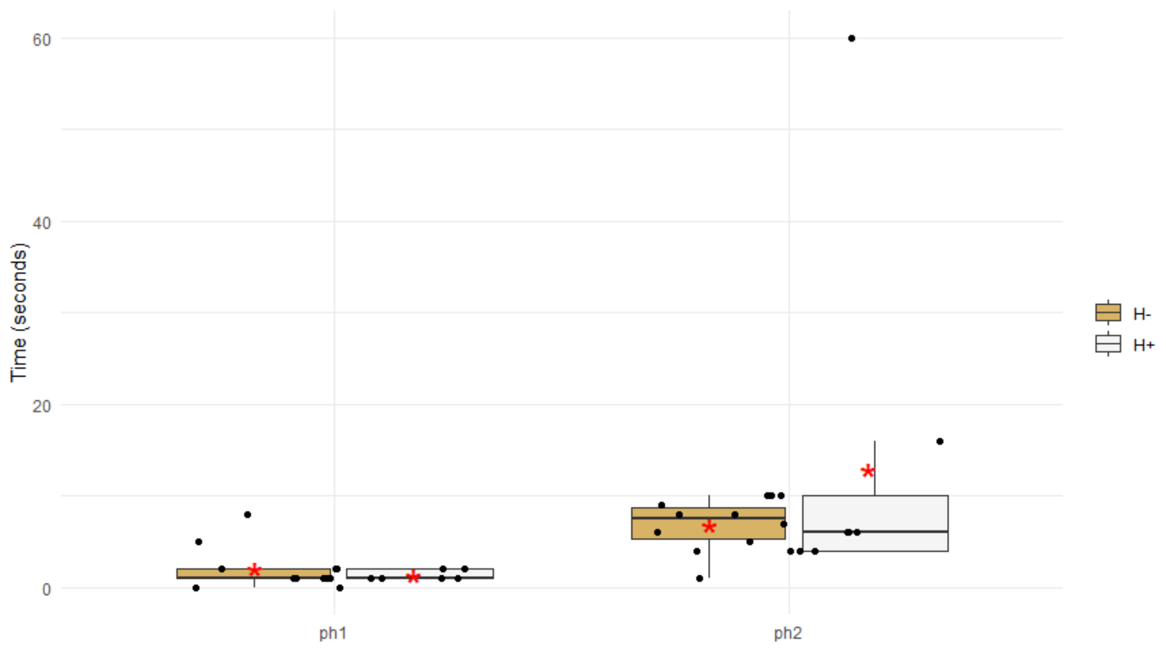


*Figure 18: Exit durations for H- (n=10) and H+ (n=9) by exit phase (phase 1 and 2) measured in seconds; *=mean time.*

(iv) HABfix

HABfix 1 *Figure 19* shows frequencies of behaviours observed during HABfix 1 (n_{H-}=10; n_{H+}=10). Stepping was the most frequently observed behaviour during HABfix1 and was similar for H- (20.9; 10.0, 23.5) and H+ (20.5; 12.8, 21.6). Medians of pulls were 7.6 (5.1, 13.7) for H- and 10.8 (7.5, 12.6) for H+ animals.

H- animals showed a median of 0.5 (0.0, 1.7) tail movements while 2.9 (0.5, 4.4) tail movements were observed for H+ animals. During HABfix 1, the tails of the animals were mostly hanging for both animal groups (H- 96.0%; 64.3%, 99.2%; H+ 80.5%; 70.5%, 91.5%).

Vocalizations were never and kicking and excretions only very rarely observed.

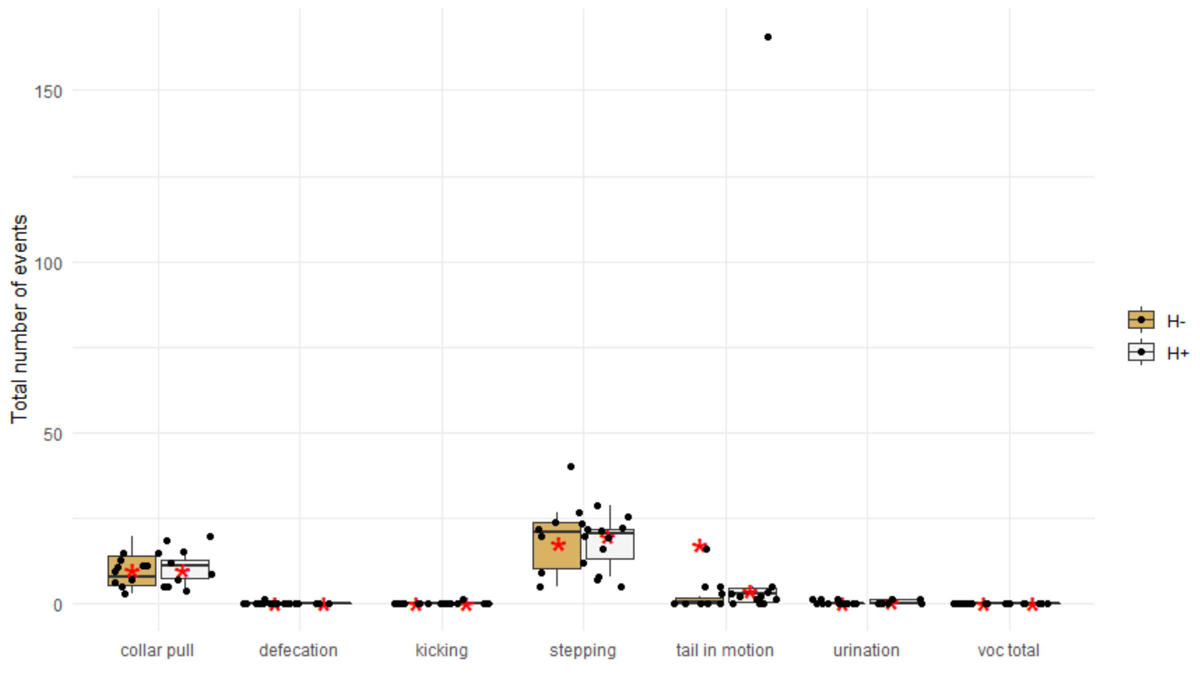


*Figure 19: Observed total frequencies of behaviours (collar pull, defecation, kicking, stepping, tail in motion, urination, voc. total=sum of vocalization open and vocalization closed) during the 2 minutes observation time of HABfix1 for H- (n=10) and H+ (n=10); *=mean number of events.*

HABfix 2 During HABfix 2 (n_{H-}=10; n_{H+}=10), the most frequently observed behaviour was stepping (H- 18.0; 13.1, 24.4; H+ 21.4; 10.7, 34.3) (see *Figure 20*). Medians of pulls were 6.5 (5.1, 11.1) for H- and 10.5 (6.8, 13.1) for H+ animals.

H- animals moved the tail 0.9 times (0, 3.7) and H+ animals 1.5 times (0.2, 2.7). The animals' tails were hanging for most of the observation time and both animal groups (H- 89.9%; 69.6%, 97.9%; H+ 94.3%; 80.1%, 97.8%). Vocalizations, kicking, and excretions were never observed.

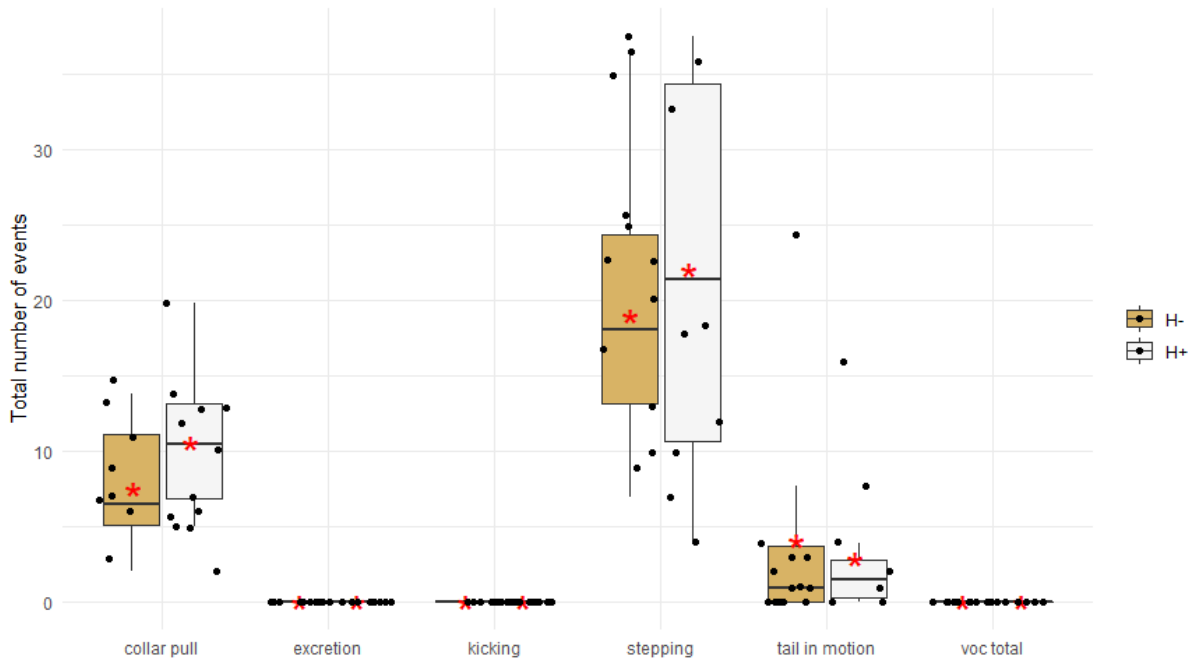


*Figure 20: Observed total frequencies of behaviours (collar pull, excretion=sum of defecation and urination, kicking, stepping, tail in motion, voc. total=sum of vocalization open and vocalization closed) during the 2 minutes observation time of HABfix2 for H- (n=10) and H+ (n=10); *=mean number of events.*

(v) HABpolar

During HABpolar, the most frequently observed behaviour was stepping, independently of horn status. Frequencies were similar for both animal groups (H-: 8.9; 4.4, 15.2; H+: 9.3; 5.7, 14.2). The animals' tails were hanging during most of the observation time for both animal groups (H-: 91%; 85.3%, 94.7%; H+: 91.9%; 69.0%, 97.7%). H- animals moved their tails 4.0 times (0.2, 4.6) and H+ animals 2.5 times (1.2, 4.9).

Independently of horn status, animals turned their head to the left or right around 5 times during the test (H-: 5.0; 3.9, 5.7; H+: 5.9; 4.0, 6.7) and showed similar turns of their head >90° (H-: 2.9; 0.0, 3.0; H+: 2.0; 1.2, 3.7). Interactions with the belt were rarely observed but similar for H- (1.0; 0.0, 2.0) and H+ (1.5; 0.0, 2.0). Feeding, drinking, and ruminating was hardly observed and showed a median of 0.0 (0.0, 0.0) for all behaviours and both animal groups.

(vi) Respiration chamber

Respiration chamber 1 All H- and H+ animals (n_{H-}=10; n_{H+}=9) were standing for the whole observation period. H- animals spent 48.2% (20.6%, 52.9%) of observation time feeding while H+ animals spent 30.9% (21.8%, 50.4%) of observation time feeding. Drinking was rarely observed, and the median of time spent drinking was at 1.0% (0.3%, 3.5%) of observation time for H- animals and at 2.0% (0.2%, 3.9%) for H+ animals.

Independently of horn status, animals spent most of the observation time facing the front (H-: 71.0%; 58.6%, 79.9%; H+: 74.9%; 62.2%, 77.1%). With about 17% of observation time, H- and H+ animals spent similar amounts of time facing the neighbouring animal(s). H- animals spent 5.3% (4.0%, 6.8%) of the observation time facing the side with no neighbouring animal while this was the case for 10.6% (9.1%, 21.2%) of observation time for H+ animals. H- and H+ animals spent similar amounts of time with their head oriented to the back of the respiration chamber.

Frequencies of behaviours are displayed in *Figure 21*. Medians of changes in head position were 141 (112, 192) for H- animals and 162 (105, 176) for H+ animals. H- animals pulled more often (31.5; 19.5, 34.0) than H+ animals (22.0; 17.0, 34.0). The median numbers of total vocalizations were 29.0 (10.3, 49.3) for H- and 15.0 (3.0, 26.0) for H+ animals. The number of excretions was similar for H- and H+ animals.

Cumulated acceleration (n_{H-} =8; n_{H+}=8) amounted to 385 (244; 527) and 283 (237; 422) for H- and H+ animals (see *Figure 22*).

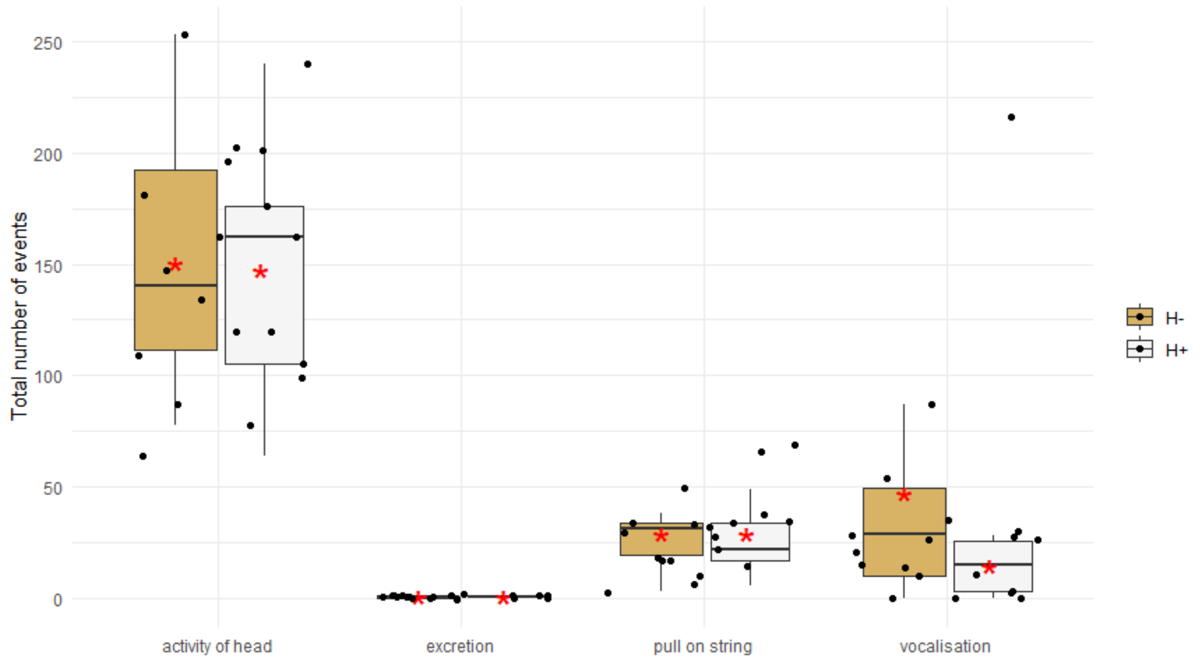


*Figure 21: Observed total frequencies of behaviours (activity of head, excretion=sum of defecation and urination, pull on string, and vocalization) during the one-hour observation time of Respiration chamber 1 for H- (n=10) and H+ (n=9); *=mean number of events.*

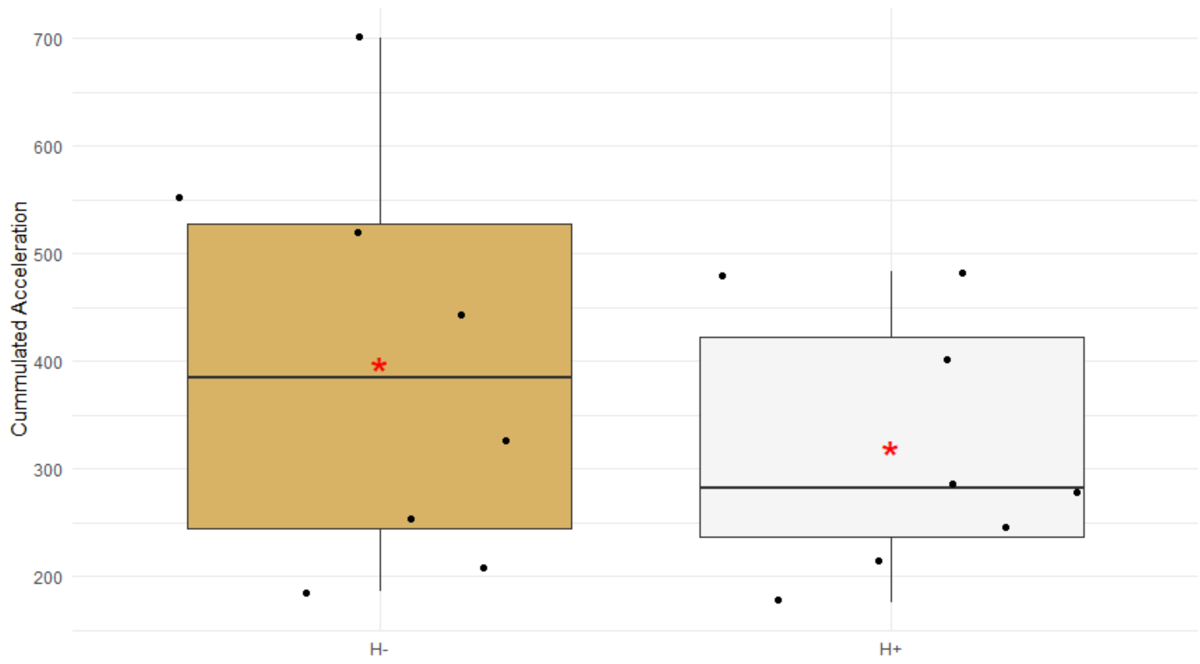


*Figure 22: Acceleration data from the MSR logger showing cumulated acceleration of the one-hour observation time during Respiration chamber 1 for H- (n=10) and H+ (n=9); *=mean cumulated acceleration.*

Respiration chamber 2 All animals (n_{H-} = 9; n_{H+} = 10) were standing during the observation time, except for one H- cow who lied down for the last 10 minutes of observation (H- and H+: 100.0%, 100.0%; 100.0%). H- animals and H+ animals spent 38.9% (38.5%, 53.7%) and 44.6% (42.0%, 52.6%) of observation time feeding. Time spent drinking was similar for H- (3.1%; 0.8%, 4.0%) and H+ (3.8%; 1.6%, 6.7%).

With a median of 63.0% and 68.7%, the most frequently observed head position was facing the front for H- and H+ animals. H- animals spent 23.7% (8.9%, 26.0%) of observation time facing the neighbouring animal, while H+ animals spent 17.2% (15.6%, 27.8%) of observation time facing the neighbouring cow. The time spent oriented to the opposite side of the neighbouring cow as well as the time spent with the head oriented to the back were similar for H- and H+ animals.

Frequencies of behaviours are presented in *Figure 23*. Medians of changes in head positions amounted to 128 (113, 146) changes for H- animals and 1120 (112, 153) for H+ animals. Pulls were similar for H- and H+ cows. Vocalizations were less frequently observed in H- 5.0 (2.0, 11.0) than in H- animals 10.5 (1.3, 24.8). Excretions were rarely observed in both groups.

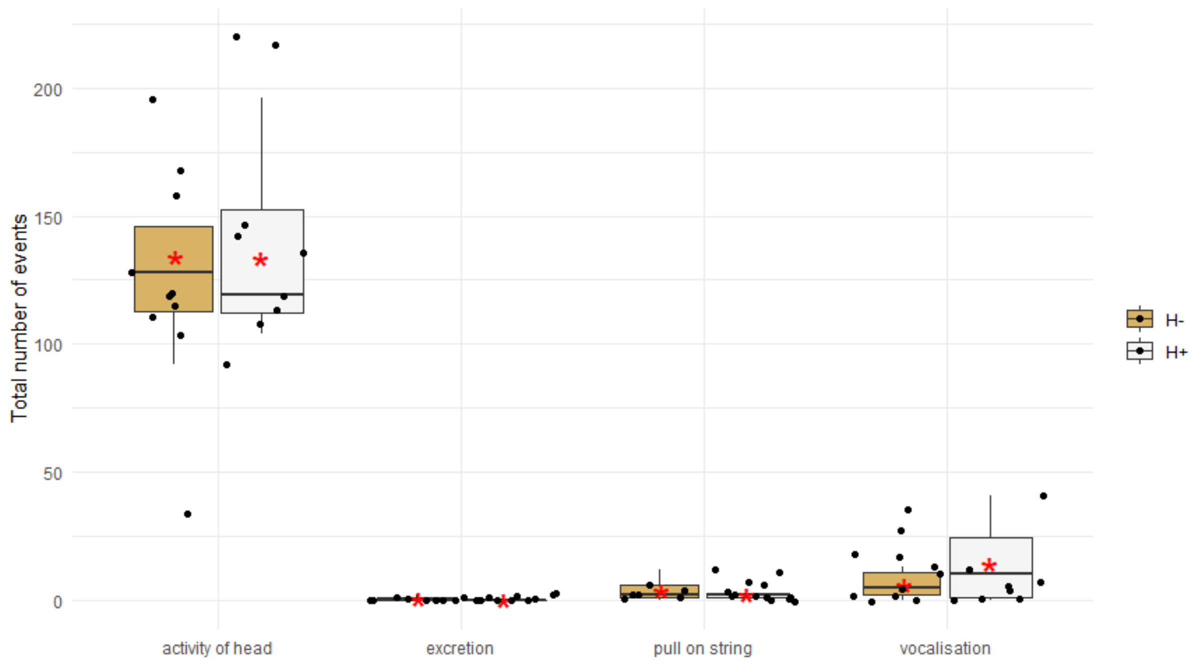


*Figure 23: Observed total frequencies of behaviours (activity of head, excretion=sum of defecation and urination, pull on string, and vocalization) during the one-hour observation time of Respiration chamber 2 for H- (n=9) and H+ (n=10); *=mean number of events.*

(vii) Consistency of selected behaviours

Three tests and situations were repeated once (AT, HABfix and Respiration chamber). The most frequently observed behaviours in the respective situations were correlated after Spearman to check for behavioural consistency within animal groups.

Consistencies of selected behaviours in AT1 and AT2

Correlations between test sessions AT1 and AT2 for selected behaviours are displayed in *Table 11*. Positive associations were found for zone changes, explorative behaviours (=interaction with the wall), and vocalizations, while no associations were found for excretion behaviour. Correlation coefficients were higher (zone changes), similar (exploration) or lower (vocalization) for H- compared to H+ animals.

Table 11: Spearman correlations of behaviours between sessions (Zone changes, Explorative behaviour (=Interaction with wall), Vocalization and Excretion) between AT1 and AT2 for H- and H+ animals.

H- H+	AT2: Zone changes	AT2: Explorative beh.	AT2: Vocalization	AT2: Excretion
AT1: Zone changes	0.71 0.33			
AT1: Explorative beh.		0.64 0.76		
AT1: Vocalization			0.39 0.76	

AT1: Excretion				- 0.11

Consistencies of selected behaviours in HABfix1 and HABfix2

Correlations between selected behaviours in HABfix1 and HABfix2 are displayed in *Table 12*. Events of stepping showed positive associations for both H- and H+, while correlations for tail movements were weak in H- animals (r=0.13) but clearly positive for H+ animals (r=0.84). For pull behaviours, again only for H+ animals a meaningful, but negative association was found.

Table 12: Spearman correlations of behaviours between sessions (Stepping, Pull and Tail movement) observed during HABfix1 and HABfix2 for H- and H+ animals.

H- H+	HABfix2: Stepping	HABfix2: Pull	HABfix2: Tail movement
HABfix1: Stepping	0.53 0.59		
HABfix1: Pull		0.00 -0.60	
HABfix1: Tail movement			0.13 0.84

Consistencies of selected behaviours in Respiration chamber 1 and 2

Correlations between selected behaviours in Respiration chamber 1 and Respiration chamber 2 are displayed in *Table 13*. Positive associations were found for both animal groups for vocalization and pull behaviours. Vocalizations showed weak positive associations for H- animals (r=0.39) and clear positive associations for H+ animals (r=0.71) while the opposite was observed for pulls (r_{H-}=0.61; r_{H+}=0.12). Activity of the head (=changes between head positions) showed positive associations for H- (r=0.65) and negative associations for H+ (r=-0.57).

Table 13: Spearman correlations of behaviours between sessions (Vocalization, Pull and Activity of head) observed during Respiration chamber 1 and 2 for H- and H+ animals.

H- H+	Respi2: Vocalizations	Respi2: Pull	Respi2: Activity of head
Respi1: Vocalizations	0.39 0.71		
Respi1: Pull		0.61 0.12	
Respi1: Activity of head			0.65 -0.57

4. Discussion

Besides the exposure to a new environment (Activity after arrival), the tests and situations the animals were subjected to can be grouped in two categories: (i) isolation (physical: Respiration chamber 1, 2;

physical and visual: AT 1, 2) and (ii) confinement (physical: Weighing; head fixation: HABfix 1 and 2, confinement through a belt: HABpolar). Since activity after arrival revealed no differences between animal groups, only behavioural tests and observations of isolation and confinement will be discussed in more detail in the respective category. The statistical approach focused on descriptive statistics hence the discussed behavioural differences might not be of statistical significance. The discussion of results will be followed by an elaboration on the consistency of behaviours and finally refer to the limitations of the present study.

4.1 Isolation

Arena Test and Respiration Chamber Movement was measured through different parameters in the two test situations confronting the animals with isolation (AT and Respiration chamber). While transitions between zones were counted in the Arena Tests, MSR loggers measured acceleration during Respiration chamber1 (Acceleration-data for Respiration chamber 2 had to be excluded from the evaluation). Cumulated acceleration showed a higher activity of H- animals during the first observation in the Respiration chamber while more movement, expressed through more transitions between zones, was observed for H+ animals in both Arena Tests. While previous experience of the animals may not affect the recorded activity in the Respiration chamber, there is one factor that needs consideration in relation to the expression of movement in the Arena Test. All animals, independently of horn status, spent most of the observation time in zones nearest to the entrance doors, especially so during AT1. Even though there were no group differences observable, some animals appeared rather immobile which might be traced back to the animals' unfamiliarity with slatted floor, which were present in the zones closest to the entrance doors. Previous experience of the individual animals with slatted floors is not known, but possibly this not only influenced the time animals spent in respective zones but simultaneously influenced the performed transitions between zones. If animals with previous in-/experience with slatted floor were not balanced across the two groups, this might have biased the results, making interpretation challenging and uncertain. To assess the potential influence of the floor conditions on the mobility of the animals, either the inclusion of recording the time spent walking and time spent immobile (e.g., as described in Reiche, Dohme-Meier, and Terlouw 2020) or a different form of evaluation (e.g., QBAs – Qualitative Behaviour Assessment) would have been of interest. Results might have shown more transitions between zones with less inter-animal deviation if solid floor conditions were ensured.

Nevertheless, H+ animals transitioning more often between zones compared to H- animals aligns partly with findings of an earlier study where more zone changes in H+ heifers compared to H- heifers were observed during a Novel Object Test (Reiche, Dohme-Meier and Terlouw, 2020). More zone changes of H+ animals together with a higher latency to sniff a novel object, were interpreted as greater fearfulness of H+ animals within the test conditions by the authors (Reiche, Dohme-Meier and Terlouw, 2020). However, transitions between zones might either be interpreted as an indicator of fear/stress or as explorative behaviour (reviewed by Forkman *et al.*, 2007). In the present study, both the number of transitions between zones and the time spent in interaction with the wall (indicator for exploration) as well as the number of transitions and excretion and vocalisation (rather stress-indicating behaviours) were positively correlated (r_s>0.5) in AT1 for both animal groups. Therefore, no clear interpretation of H+ animals transitioning more often between zones than H- animals is possible.

Comparing the frequencies of zone changes in AT1 to AT2 for H- and H+ animals, transitions declined for both animal groups. A so-called 'rebound-effect' which characterizes the expression of elevated movement and activity in animals after periods of being restrained of movement which has been described in previous studies (e.g., described for calves in Rushen and de Passillé, 2014 and Jensen, 2001) was therefore not observed. Even though animals went into AT2 straight after spending 5.25 days in the Respiration chamber under behavioural restrictions, H- and H+ animals transitioned less often between zones in AT2 than in AT1. That animals had to walk the approximate distance of 100 meters between the respiration chambers and the Arena might be an influencing factor. Possibly, this distance was sufficient for the animals to express their urge for movement which certainly applied to some animals who jumped and galloped on the way to the arena.

In the present study, vocalization appeared to be the most frequently observed behaviour in situations involving isolation. Only total vocalization (=sum of open and closed vocalization) will be discussed here, as a further differentiation between open and closed vocalization had to be dismissed due to the currently not yet completely understood backgrounds and reasons behind the different forms of vocalizations in cattle (reviewed by: Green, Johnston and Clark, 2018 and Manteuffel, Puppe, and Schön 2004; Watts and Stookey 2000).

While vocalization was more frequently observed in H- animals in Arena Test 1 and Respiration chamber 1, the opposite was the case during Arena Test 2 and Respiration chamber 2. The current, main understanding of vocalization in cattle is, that it is a form of communication between animals and that its frequency is influenced by different external (e.g., isolation, pain, handling, presence of humans, mother-calve separation) and internal stimuli (e.g., physiological state, parturition, emotional state) (Watts and Stookey, 2000; reviewed by: Green, Johnston and Clark, 2018). While cattle vocalize mainly during presumably unpleasant events (e.g., handling procedures) and vocalize rather seldom during pleasant events (e.g. food anticipation) (reviewed by Laurijs *et al.*, 2021), vocalization in assumed negatively valanced situations can be interpreted as an indicator of discomfort (Grandin, 1997; Watts, 1998; Yoshihara and Oya, 2021). Vocalizations during negatively associated situations were also linked to higher levels of salivary cortisol in a previous study (Yoshihara and Oya, 2021), which underlines this assumption. Since social isolation has previously been described as 'a severe psychological stress in cattle' (Boissy and Le Neindre, 1997) due to the animals' gregariousness (reviewed by: Green, Johnston and Clark, 2018), vocalization during isolation to peers can be interpreted as an indicator of discomfort. Nevertheless, no clear pattern of differences in vocalization between H- and H+ animals was observable.

In AT1 H+ animals vocalized less frequently and showed less excretions, which can also be interpreted as an indicator of stress, than H- animals. In AT2, excretion frequency was generally lower and was basically the same for both animal groups while vocalizations increased in H+ animals compared to AT1. Excretions were observed less frequently during Respiration chamber 1 than in AT1. This might indicate that visual and physical isolation in the Arena Test is a greater stressor to the animals than being only physically isolated like in the Respiration chamber. Nevertheless, looking at the frequencies of excretions and vocalizations under isolation, no clear pattern of differences between H- and H+ animals is observable. Adding to the rather unclear pattern and challenging interpretation of behavioural differences between H- and H+ animals under isolation are the observed frequencies of pulls and head changes during the observations in the Respiration chamber. While during Respiration chamber 1 H- animals pulled on the string more frequently than H+ animals, H+ animals changed their head position more often than H- animals. Pulls and head changes declined for both animal groups between Respiration chamber 1 and 2 and showed similar frequencies during Respiration chamber 2. *Figure 24* illustrates the unclear pattern of found behavioural differences between H- and H+ animals in the two observed situations under isolation. Big upwards facing arrows illustrate greater values/more events or higher cumulated acceleration while green bars illustrate equal values.

Isolation				
Situation	**Behaviour**	**Unit**	**H-**	**H+**
Arena Test 1	Zone changes	Events		⬆
	Excretion	Events		⬆
	Vocalisation	Events	⬆	
Arena Test 2	Zone changes	Events		⬆
	Excretion	Events	▬	▬
	Vocalisation	Events		⬆
Respiration Chamber 1	Activity	Cumm. Acc.	⬆	
	Pull	Events	⬆	
	Vocalisation	Events	⬆	
	Excretion	Events	▬	▬
Respiration Chamber 2	Pull	Events	▬	▬
	Vocalisation	Events		⬆
	Excretion	Events	▬	▬

Figure 24: Illustration of observed behavioural frequencies during isolation (Arena Test 1, 2; Respiration chamber 1,2); compared behaviours were mostly observed as Events and activity was measured as cumulated acceleration. Symbols under the section H- and H+ are supposed to illustrate found behavioural differences - big blue upwards facing arrows stand for greater values/more events while small, downwards facing arrows illustrate the counterpart. Green bars illustrate equal values and therefore no differences between H- and H+. The figure shows the rather unclear pattern of behavioural differences between H- and H+ animals under isolation.

Human Approach Test The most influencing factor for the animals' behaviour towards humans in human-approach tests is the human-animal relationship (Waiblinger, Menke and Fölsch, 2003; Waiblinger *et al.*, 2006; Ebinghaus, Ivemeyer and Knierim, 2018). Previous studies have shown that avoidance distances to humans are positively influenced, meaning to result in smaller or no avoidance-distances, by a small ratio between stockperson and cow. This came with specific influencing management criteria of e.g. the farmers' ability to identify cows individually and manual feeding (Waiblinger, Menke and Fölsch, 2003; Petherick *et al.*, 2009; Ebinghaus, Ivemeyer and Knierim, 2018). That animals for the present study originated from small family-led, mainly tie-stall farms with seemingly very caring farmers (some even came to visit their animals during the experiment) might be an explanation for the behaviour shown, meaning that there was mostly no avoidance distance to the approaching human. Most animals even approached the person by themselves making it difficult or impossible to establish a start distance. Therefore, a close and positive human-animal relationship can be assumed for most tested animals.

The animals' behaviour in Human Approach Tests has previously been shown to be a consistent individual measure (Gibbons, Lawrence and Haskell, 2009). However, the low variability in the (lack of) avoidance behaviour does not support the assumption of a highly individual measure. Slightly less H-animals being attributed the highest score during HAT2 may reflect unpleasant experiences with humans made during the time spent in the respiration chambers (e.g., sampling of blood and urine, measuring of body temperature twice a day etc.) before the execution of HAT2. Nevertheless, the effect was very small and not observed for H+ animals.

The HAT for this study was conducted in cows that had been physically isolated from their peers and following a period spent in isolation (AT). Many Human Approach Tests described in literature are conducted in the familiar environment, i.e. in the barn and under the usual herd situation, i.e. in the home pen or at the feeding rack (Waiblinger, Menke and Fölsch, 2003; Petherick *et al.*, 2009; MacKay *et al.*, 2014; Ebinghaus, Ivemeyer and Knierim, 2018) or on pasture (Hirata *et al.*, 2016). Since human approaches in isolated situations might be more stressful for the animals compared to when being done in familiar environments, this could have evoked more diverse and stronger reactions of the animals towards the approaching human. Nevertheless, this was not observable, and most animals still tolerated the touch of the approaching human.

4.2 Physical confinement

While no differences in stepping frequencies between animal groups were observable during HABfix and HABpolar, marked differences were found during weighing with H+ animals showing more stepping behaviour compared to H- animals. Stepping can be interpreted as indicator of restlessness which may indicate that H+ animals felt more agitated under physical confinement conditions in the scale. Since H- and H+ animals for this study were selected for similar body length, this difference might trace back to the presence of horns. According to Knierim, Irrgang, and Roth 2015 and Lutz et al. 2019, horned animals tend to keep greater inter-individual distances to peers than animals without horns and might potentially have generally greater space requirements in their head regions, as suggested by observations in the scale. Besides the elevated frequency of stepping, H+ animals were observed to arch the back slightly longer in the scale compared to H- animals which might also lead back to the greater agitation due to the limited space provision. Furthermore, H+ animals left the scale slightly faster during exit phase 1 which might be due to their greater urge for space. Nevertheless, the results are not comparable with earlier studies, as behavioural differences between horned and disbudded animals have not yet been investigated under individual confinement.

During HABfix1 and HABfix2, H+ animals showed a tendency to more pulls and tail movements while during HABpolar more tail movements were observable for H- cows (illustrated in *Figure 25*). Pull behaviours declined slightly from HABfix1 to HABfix2 for H- animals but remained the same for H+ animals. Therefore, it can be said that H+ animals showed a tendency towards greater behavioural reactions during head fixation and confinement in the scale.

Confinement			
Situation	**Behaviour (Events)**	**H-**	**H+**
HABfix 1	Stepping	—	—
	Pull		↑
	Tail movement		↑
HABfix 2	Stepping	—	—
	Pull		↑
	Tail movement		↑
Weighing	Stepping		↑
HABpolar	Stepping	—	—
	Tail movement	↑	

Figure 25: Illustration of observed behavioural frequencies under confinement (HABfix 1, 2; Weighing; HABpolar); compared behaviours were observed as Events. Symbols under the section H- and H+ illustrate found behavioural differences - big blue upwards facing arrows stand for greater values/more events while small, downwards facing arrows illustrate the counterpart. Green bars illustrate equal values and therefore no differences between H- and H+. It shows that H+ animals expressed a tendency of more behavioural reactions under physical confinement conditions in the scale and head fixation during HABfix.

4.3 Consistency of behaviours

Consistency of behaviours shown in repeated test situations, expressed as correlations of frequency of behaviours, did not follow a clear pattern in the two animal groups. Some behaviours were positively correlated (>0.5) in both animal groups (frequency of stepping during HABfix, time spent in interaction with the wall during ATs), while some behaviours showed only positive correlations for H+ animals (frequency of vocalization in the ATs and Respiration chambers, tail movement during HABfix, head movements in the Respiration chamber) and others only for H- animals (zone changes in the ATs, pull behaviours in the Respiration chamber). Nevertheless, it needs to be considered that only selected behaviours were correlated of which some were challenging to be clearly defined. This was for example the case for the definition of a 'pull' behaviour in the ethogram. This was partly due to the different ways animals executed a 'pull' but also because the fixation, meaning the length of the rope which fixated the animals' head during HABfix, was not standardized.

Consistent behavioural reactions of animals towards stimuli (such as e.g. confrontation with humans or isolation) but also across situations can be used to assess animal temperament (reviewed by Réale *et al.*, 2007). Temperamental animals, often measured through assessing flight speed and crush scores of animals, have been shown to perform lower in productivity measures such as time spent feeding and feed intake, growth rate and carcass weight (Cafe *et al.*, 2011). Since temperament describes the long-term consistency of behavioural reactions of animals towards situations and tests of this study were repeated within a few days, no clear interpretation of temperamental differences of the two animal groups can be made. Nevertheless, the (slight) differences found may indicate temperamental differences between horned and disbudded dairy cows which would need further investigations in future studies.

For the future inclusion of 'pull' behaviours to ethograms, more standardized conditions should be assured and the definition of a 'pull' might benefit from the inclusion of measuring its force (e.g. as described in Graunke *et al.*, 2013).

4.4 Limitations

When interpreting the results of the present study, limitations of the experimental design must be kept in mind. Since the behavioural observations were done additionally to a feeding experiment, behavioural tests had to be aligned with the capacities and possibilities of the given frame of this experiment. The feeding rations were balanced over H- and H+, but nevertheless might still have had an influence on the animals' behaviour and potentially have increased behavioural variability (especially during the observations in the Respiration chamber 2 and (H)AT2). To be able to detect potential behavioural differences between horned and disbudded dairy cows, a broader approach, meaning the observation of more animals, in more diverse tests and situations with the inclusion of behavioural observations in positive/neutral situations might have been beneficial. Including basic behaviours such as time spent resting, feeding, or ruminating might have additionally been beneficial. Furthermore, the rather small sample size must be considered which further reduces the generalizability of the results found. To be able to trace behavioural differences back to either the presence/absence of horns or to potential long-term effects of disbudding, it might also be of interest to include polled animals in future studies.

5. Conclusion

Across the different test situations, no consistent pattern of differences between horned and disbudded animals emerged. The present data therefore do not suggest that the lack of horns or the disbudding experience of the calves has a longer-term effect on the behavioural reactivity. However, the limitations of the study in terms of the specificity of the test situations or the small sample size do not allow to draw general conclusions on a lack of differences in behaviour between the groups. Further research could for example focus on the time budgets (i.e. lying, feeding, rumination etc.) or the social behaviour of the cows and also the use of qualitative behavioural assessment may complement quantitative measures.

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