Gentling dairy calves
– behavioural responses during brushing

Lugn taktil behandling av mjölkkalvar
- beteendemässiga reaktioner under borstning

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Abstract

The human-animal relationship is important from an ethical standpoint as it influences the welfare of both animals and stockpeople. The importance of positive aspects of animal welfare, such as rewards and pleasures, have gained increasing interest in recent years. Focus is shifting from merely reducing aversive experiences for animals, to creating opportunities for pleasurable experiences and rewards. Gentling involves additional positive contact, to that of routine management, and can include stroking, brushing and/or talking in a soft way to the animals.

This study explored the immediate behavioural responses of dairy calves that were gently brushed for 30 minutes two times a day for ten consecutive days. They were housed individually in a controlled environment, to ensure high health status and minimize the effect of disease. Video recordings of the treatment sessions from day 1, 2, 5, and 10 were analysed for the 16 calves included in the study, of which half were submitted to gentle brushing by a human and the rest to a control treatment. Calves that were gently brushed played more often and for a longer time than control calves and exhibited a higher proportion of neck-stretching as well as exploratory behaviour within the pen. Possible reasons for this could be that the calves lived in a barren environment and the availability of social interactions with the handler and the brushing may have been perceived as stimulus for excitement in the calves. Gentling of dairy calves early in life showed immediate positive effect on behaviours possibly indicative of pleasurable experiences, hence improving the animal welfare which emphasizes the significance of a good human-animal relationship.

Since gentling of shelter cats has shown indications of enhanced emotional wellbeing and increased levels of IgA, it would be of interest to further investigate if gentling of dairy calves on commercial dairy farms could induce physiological alterations which improve the mucosal immunity, possibly resulting in healthier cows in less need of treatments with antibiotics during their life and thus prevent antimicrobial resistance and increase the profitability for the farmer.

Keywords: Brushing, human-animal relationship, calves, behaviour, positive emotions, positive welfare

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1. Introduction

The relationship between humans and other animals is of moral significance (Balcombe, 2009) as a good relationship can, for instance, improve welfare by reducing stress responses in dairy cattle (Calderón-Amor *et al.*, 2020; Waiblinger *et al.*, 2004; Lensink *et al.*, 2001), thereby also lowering the risk of injury for both animals and stockpeople. The stockperson's behaviour during handling has been found to influence the mood of dairy calves (Ellingsen *et al.*, 2014). Characteristics of the stockperson, such as being nervous, dominating and aggressive, were related to calves having a more negative mood whereas patient, calm handling with stroking and gentle vocal interactions resulted in higher levels of positive mood in the calves (Ellingsen *et al.*, 2014) which further emphasizes the significance of the link between human and animal behaviour. Beyond that, there are economic incentives for a good human-animal relationship because it may have positive effects on productivity (Hemsworth *et al.*, 2002; Waiblinger *et al.*, 2002; Hemsworth *et al.*, 2000). In addition, there is a rising consumer demand for welfare friendly animal products (More *et al.*, 2017; Bayvel *et al.*, 2012), enabling label production which offers further economic benefits.

Scholarly discussions concerning animal sentience have for long focused on negative aspects, such as pain and suffering (Green & Mellor, 2011; Balcombe, 2009; Yeates & Main, 2008). Contrastingly, the positive aspects; rewards and pleasures, have been far less explored by scientists (Balcombe, 2009; Yeates & Main, 2008). However, the moral significance of pleasure is increasing and it is essential to create opportunities for animals to experience rewards and pleasure, instead of merely working on reduction of aversive experiences (Mellor, 2015; Balcombe, 2009). Suggested feasible ways to, in practice, promote positive emotions are to promote play in young animals and stroke animals or promote social licking, hence increasing the general quality of life (Boissy *et al.*, 2007).

Additional positive contact beyond the routine management, in other words gentling, can involve for instance stroking (Lürzel *et al.*, 2015b; Schmied *et al.*, 2008a; Schmied *et al.*, 2008b), brushing (Boivin *et al.*, 1998; Boissy & Bouissou, 1988), and talking softly (Lürzel *et al.*, 2015b; Waiblinger *et al.*, 2004) as well as stroking or brushing in association to feeding (Waiblinger *et al.*, 2004; Boivin *et* *al.*, 1998). Behavioural indicators of emotion can be used when assessing the influence of interactions between humans and animals (Mendl *et al.*, 2010).

1.1. Tactile interactions and lying behaviour

Positive tactile interactions play a key role in the creation of social bonds and are considered as a source of pleasure, thus, positive emotions (Balcombe, 2009; Boissy *et al.*, 2007). Allogrooming is a type of affiliative behaviour (Boissy *et al.*, 2007; Sato *et al.*, 1993) that also serves a hygienic purpose and is seen in farm animals such as cattle, pigs, and horses but is not as common in sheep (Boissy *et al.*, 2007). Social licking during allogrooming lowers the heart rate of receiving dairy cattle, indicating a calming effect and probable positive affective state (Laister *et al.*, 2011). An appeasing effect of allogrooming has also been mentioned by Reinhardt *et al.* (1986) as recipients stretched the part of the body being groomed, suggesting a pleasurable experience.

Stroking by humans has been suggested as possibly being perceived like intra-specific social licking by cows, especially stroking of body regions often exposed to social grooming (Schmied *et al.*, 2008b). Therefore, human stroking could affect the cow-human relationship in an affiliative and possibly rewarding way similar to the effects of intra-specific social licking on social bonds of cows (Schmied *et al.*, 2008b). Additionally, stroking of the neck of cattle was found to improve the human-animal relationship (Schmied *et al.*, 2008a).

Gentle tactile and vocal interactions can reduce dairy cows' (Lürzel *et al.*, 2018) and dairy heifers' (Lürzel *et al.*, 2016) fear of humans and the heifers likely perceived the treatment as pleasurable. Additional gentle interactions from a stockperson can reduce veal calves' reactivity to people (Lensink *et al.*, 2000a) and handling (Lensink *et al.*, 2000b) and thus improve animal welfare. Being brushed by a human has been suggested to be a source of positive experience for calves (Westerath *et al.*, 2014). In previous research, both pair- and group housed calves have been seen using mechanical brushes (Horvath & Miller-Cushon, 2019; Zobel *et al.*, 2017) and individually housed calves have been seen using stationary brushes (Pempek *et al.*, 2017). Group housed calves used a mechanical brush with consistent bout duration despite increasing age (Horvath & Miller-Cushon, 2019) and the brush use of calves housed individually indicates allogrooming motivation even in the absence of social companionship (Pempek *et al.*, 2017).

Positive effects of gentle tactile interactions have also been seen in other species. Gentling of young lambs of reactive breeds reduced stress when separated from conspecifics and improved the human-animal relationship quality (Caroprese *et al.*, 2012). Reduced fear reactions, with a long-lasting effect up to six months of age, were found in young gentled laboratory rats (Maurer *et al.*, 2008). Shelter cats that were gentled showed indications of enhanced emotional wellbeing by being more likely to have a positively valenced mood (Gourkow *et al.*, 2014) and maintaining positive affect (Gourkow & Phillips, 2015).

Another important aspect concerning the welfare of dairy cows is lying behaviour. Munksgaard and Simonsen (1996) suggested that temporary lying deprivation in cows is aversive and according to Cooper *et al.* (2008), deprivation of lying likely had a negative effect on the emotional state of cows. Furthermore, Fisher *et al.* (2002) showed an increase in plasma cortisol in dairy cows deprived of lying. Consequently, lying is important for resting and relaxation in cattle. The lying posture with head relaxed, on ground or on flank, has been used as an indicator of sleep in cattle (Fukasawa *et al.*, 2018; Norring & Valros, 2016; Hanninen *et al.*, 2008), hence also an indication of relaxation.

1.2. Play behaviour and neck-stretching

Play is suggested to be a promising indicator of positive welfare (Held & Spinka, 2011; Yeates & Main, 2008; Boissy *et al.*, 2007; Jensen *et al.*, 1998). The expression of play behaviour in a gene pool is sustained by its adaptive benefits (Balcombe, 2009). However, in this interaction it is important to acknowledge the proximate role of pleasure, at least in the case of animals with consciousness and emotions (Balcombe, 2009). Exhibition of adaptive behaviour in animals, in this context play behaviour, is thus caused by pleasure which acts as the proximate mechanism (Balcombe, 2009). If environmental conditions are unfavourable (Krachun *et al.*, 2010) or if the calf is compromised, for instance in pain (Mintline *et al.*, 2013), play behaviour is suppressed. Furthermore, play behaviour in calves is dependent on sufficient space (Jensen & Kyhn, 2000; Jensen *et al.*, 1998). Exposure to a companion or a larger pen induced a rebound effect on play behaviour of individually housed calves, indicating deprivation of play when housed without social companionship (Valnickova *et al.*, 2015). There is a likely association between play and a positive affective state (Boissy *et al.*, 2007).

The occurrence of play is spontaneous and unpredictable, consequently, measuring these behaviours is difficult (Balcombe, 2009). However, Boissy *et al.* (2007) have described play behaviour as the least difficult positive emotions indicator to read or interpret and play has been used as a welfare indicator in dairy calves (Mintline *et al.*, 2013; Duve *et al.*, 2012). Schütz *et al.* (2012) found no significant difference in play between calves that received positive or negative handling, both groups were playing (running) during 0.7% of the observation period of 1800 seconds, which corresponds to 12.6 seconds. The spectrum of welfare is widely covered when using play as an indicator as both the absence of bad and the presence of good welfare, can be signalled (Held & Spinka, 2011).

During social licking (Laister *et al.*, 2011) and brushing or stroking by a human (Lürzel *et al.*, 2015a; Westerath *et al.*, 2014; Bertenshaw & Rowlinson, 2008), stretching of the neck is commonly observed in cattle. Neck-stretching has been used as an indicator of positive affective states in heifers stroked by a human and was interpreted to signal enjoyment (Lange *et al.*, 2020). Further, neck-stretching in dairy heifers that had been brushed by a human was suggested to reflect pleasure in a study by Bertenshaw and Rowlinson (2008).

1.3. Exploratory behaviour

Exploration is for most animal species a behavioural need as they are motivated to perform exploratory behaviour (Boissy *et al.*, 2007). However, it is closely linked to and suppressed by fear (Boissy *et al.*, 2007). For these reasons, exploration could be of interest regarding positive affective states of animals and as it seems gathering information is to a certain degree self-reinforcing (Boissy *et al.*, 2007). It is a behaviour suggested to be continually present, but information gathering is interrupted, by for instance high levels of hunger, and animals then exhibit behaviour that reduces the primary need in the most efficient way (Inglis *et al.*, 2001). An example is optimal foraging behaviour in the case of a certain, high level of food deprivation (Inglis *et al.*, 2001). Exploration can indicate that no other immediate needs are present as well as be an ongoing pleasurable activity, if the theory about information primacy is correct (Boissy *et al.*, 2007).

Examples of exploratory behaviour for calves are sniffing and licking of fixtures (Bertelsen & Jensen, 2019). Calves have also been observed sniffing and licking at a human (Li *et al.*, 2020). In a novel human test, calves that had been brushed by a human early in life tended to rub their forehead and lick more at the person than control calves (Li *et al.*, 2020). Repeated licking of inanimate objects is common for calves in the absence of their dam (Margerison *et al.*, 2003). As previously stated, allogrooming can reduce heart rate and have an appeasing effect of the receiving dairy cattle. Therefore, similar effects could possibly be seen during brushing by a human and perhaps result in more time spent lying, thus indirectly decrease the expression of exploratory behaviour.

The positive emotional states that, as it seems, cause or are required for expression of play behaviour, neck-stretching, and exploration underline the importance of these behaviours as strong candidates to indicate positive animal welfare.

Therefore, it is of interest to investigate the behavioural effects of gentle tactile interaction in relation to positive affective states in animals.

1.4. Aim and hypotheses

1.4.1. Aim

The aim of this study was to investigate the immediate behavioural effects of tactile stimulation by brushing dairy calves in early life.

1.4.2. Hypotheses

1. Tactile stimulation will significantly increase the level of relaxation in calves
    a. Tactile stimulation will significantly shorten the latency for the calves to lie down over the treatment sessions
    b. The time spent lying will be significantly higher, and the time spent standing and moving will be significantly lower, for calves that received tactile stimulation in comparison to control calves
    c. The time spent lying down with head relaxed will be significantly higher for calves that received tactile stimulation than for control calves
    d. The time spent lying down with head relaxed on handler will be significantly increased over the treatment sessions for calves that received tactile stimulation
2. Tactile stimulation will lead to a positive affective state in dairy calves
    a. Tactile stimulation will significantly increase expression of play behaviour in calves, in comparison to control treatment
    b. Tactile stimulation will significantly increase expression of neck-stretching in calves, compared to control treatment
3. Tactile stimulation will significantly decrease calves' exploration of the surrounding compared to control treatment
    a. The proportion of exploratory behaviours within and outside of the pen, respectively, will be significantly lower for calves that received tactile stimulation than for control calves

b. Tactile stimulation will significantly decrease exploration of feed/water buckets, compared to control treatment

c. Tactile stimulation will significantly decrease sniffing and licking on fixtures, respectively, in comparison to control treatment

d. Calves receiving tactile stimulation will be sniffing/licking on their handler significantly more than they will be sniffing/licking on fixtures

2. Material and methods

2.1. Animals

This experiment included 16 Swedish Holstein (H) calves and Swedish Red (SR) calves, both males and females, see table 1. They were born from the middle of June 2019 to the beginning of August 2019 and divided into two batches (1 and 2), balanced in accordance to their birth date. Because some calves were born later than expected, batch one consisted of two smaller batches; 1A and 1B. Age at the start of the experiment varied from four to 14 days, with mean age of ten days.

Table 1. Information on the breed, sex and age of the calves in the experiment

Batch	Animal number	Breed	Sex	Age at start of experiment (days)
1A	1	SR	Male	14
1A	2	SR	Male	14
1A	3	SR	Female	12
1A	4	SR	Male	10
1A	5	SR	Male	7
1B	6	H	Female	7
1B	7	SR	Female	4
1B	8	H	Male	4
2	9	SR	Female	14
2	10	H	Female	13
2	11	SR	Male	12
2	12	SR	Male	12
2	13	SR	Male	12
2	14	SR	Female	7
2	15	SR	Male	8
2	16	SR	Male	7

2.2. Housing and management

Ethical approval for the study was obtained from the Ethical Committee of Animal Experimentation in Uppsala, Sweden (5.8.18-08933/2019). The experiment was carried out at the Swedish Livestock Centre, Lövsta, in Uppsala, Sweden in July and August of 2019. The calves were individually housed with deep straw bedding in pens built especially for this experiment in a tie-stall to ensure a controlled environment. The pens measured 95 cm x 148 cm and were built with pallets used as walls and a steel bar gate in the front with the bottom part in plywood. Water and hay/concentrate buckets were hanging on the outside. At the front, in between but outside of each pen, a plywood partition/extension was mounted to prevent physical contact between calves, see figure 1. The barn was divided with a tarpaulin curtain to prevent the second batch of calves from seeing what was going on during the experimental period of the first batch, see figure 2. The only light sources in the barn were white tube lamps, they were lit continuously but the light was dimmed between 10 PM and 5 or 6 AM.

Figure 1. Individual pens in experimental barn

Figure 2. Overview of experimental barn

Calves were removed from their mother shortly after birth and moved to individual pens in the experimental barn. They were given colostrum and whole milk from the dam until they were three days old. From four days of age all calves received warm whole milk. Vaccination against ringworm was carried out in accordance with the schedule on the farm. The calves remained in their pens until the day after the treatment period of ten days was over. Males went to beef production and females entered the herd on the farm.

2.2.1. Feeding

In accordance with the routine practices of the farm, the calves were fed whole milk in teat buckets twice daily as well as water, hay and concentrate pellets offered *ad libitum*, the latter two mixed together in the same bucket. The calves were fed prior to the researchers arriving to the barn in the morning and after they had left in the afternoon, approximately at 7 AM and 5 PM respectively.

2.2.2. Hygiene measures

Only the main door was used to enter and exit the barn, see figure 3. The staff and researchers stepped in a bath with disinfectant placed in the entrance. The researchers wore disposable gloves when touching the calves and when entering a pen, they also wore disposable booties covering their boots. All the calves in the brush treatment group had their own brush.

To decrease the risk of cross contamination, all calves had their own water and hay/concentrate buckets. The teat buckets were not personal because they were disassembled and thoroughly washed and sanitized after every use. The farm staff cleaned the barn in the morning and afternoon, around 7 AM and 5 PM, before the researchers arrived and after they had left, following the same hygiene procedures as mentioned above.

2.3. Experimental design

The calves were divided into two treatment groups; brushing and control, see figure 3. In accordance with date of birth the calves were placed in the pens numbered 1-16, in ascending order. The experimental period was ten consecutive days and each day consisted of a morning and afternoon session. To balance the distribution of male and female calves between the different parts of the barn (pens with odd and even numbers), calves 14 and 15 were switched.

Batch 1B had one day without treatments between day 7-8 and two days between day 8-9. Batch 2 had one day without treatments between day 2-3. The second batch of calves did not receive the treatments on day 10, for practical reasons. Instead, they received extra treatment sessions distributed on days 3, 4, 7, and 8, to ensure that all calves in the brushing group were treated the same amount of time.

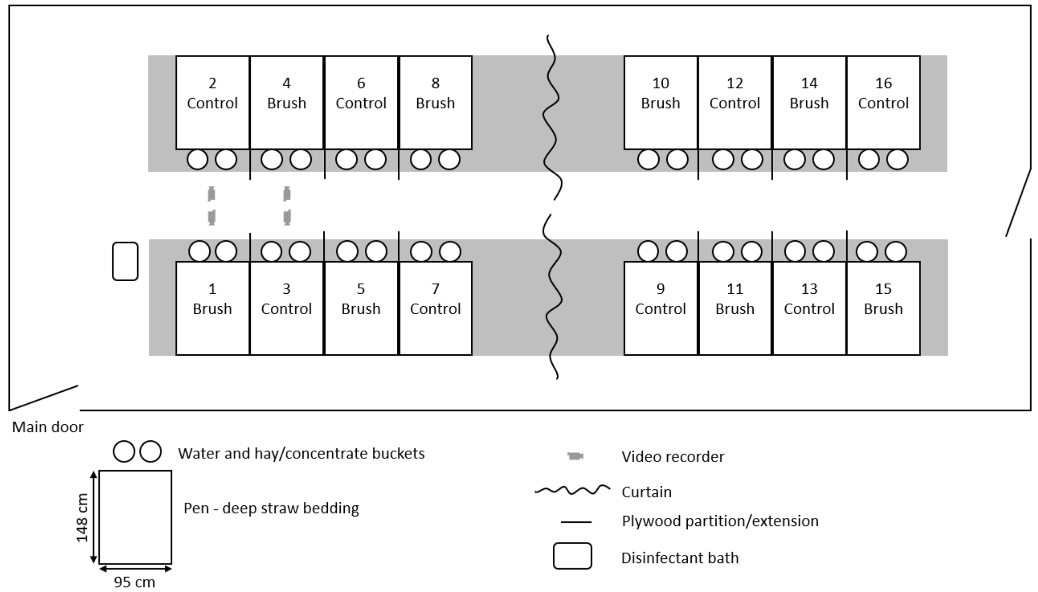

Figure 3. Experimental barn - Swedish Livestock Centre Lövsta

The calves were weighed, and blood samples for analysis of oxytocin and cortisol were collected, on the first and last days of treatment, see table 2. Saliva samples for analysis of IgA were collected on the first, fifth and tenth day. The heart rate of the calves was monitored during one of the treatment sessions each day, and once outside of the treatment sessions, for all calves in the brushing group and the control calves of the first batch. However, neither weight, blood/saliva samples or heart rate were part of this report. The present report focuses on behaviour of the calves.

Table 2. Timeline of behavioural observations, and other parameters not included in the present report, over the experimental period for the different calf batches

Batch/Treatment day	1	2	5	9	10
1	B+H+S+W	B+H	B+H+S		B+H+S+W
2	B+H+S+W	B+H	B+H+S	B+H+S+W	

B Behavioural observations
H Heart rate monitoring
S Saliva sample
W Weighing and blood sample

During the experiment, visual health checks were conducted daily to assess health status and detect disease, in particular diarrhoea and respiratory problems. On day one and ten, the health examination was more thorough and required touching the calves. The health checks were adapted to minimize physical contact with the calves, as tactile stimulation was an important part of the experiment. The farm staff were instructed to minimize contact/interaction with the calves to the limit necessary, especially during manipulation of the calves during weighing and vaccination, as the resulting stress may affect the calves' behaviour.

2.3.1. Treatments

The calves in the brushing group were gently brushed twice a day by a handler, totalling 60 min of tactile stimulation per day (30 minutes in the morning and 30 minutes in the afternoon). Control calves did not experience tactile stimulation by the handlers. The exact time for AM and PM sessions varied, but time in between the AM and the PM session for each calf was approximately three hours. Each calf in the brushing group was matched with a specific control calf and constituted a pair.

Gentling procedure

The brushing treatment was done by two experienced female handlers and aimed to be a positive social experience. The handlers were assigned specific calves to brush. Thus, the calves were brushed by the same handler during all treatment sessions. Before each brushing session, the handler stood in front of the pen presenting the brush to the calf for one minute. Then, the handler entered the pen, brushed the calf for 30 minutes, and left the pen.

The calves which were submitted to brushing were respected and brushed according to their acceptance. They could do what they wanted to and move as they wished, within the limits of the pen. Individual differences in the timing to accept physical contact and preferred body regions were considered. The brushing was natural, rhythmic and slow (one brush/second), and the movement of the brush was supposed to mimic the licking of its mother, for and against the direction of the hair coat.

The calves which were in the control treatment were not brushed and followed the routine practices from the farm. The handler did not enter control calves' pens during the time of the session. However, they entered the pens of the control calves in the first batch shortly before one of the sessions each day as they had to mount the heart rate monitoring equipment.

2.4. Behavioural observations

The field work of this experiment was carried out earlier, summer of 2019, and the work of the present study began here with the behavioural observations. Behavioural responses during treatment sessions were video recorded on all treatment days, i.e. day 1-10, using video cameras (Sony handycam) for both the brush and the control group, resulting in approximately 160 hours of video material.

The limited time frame of this master thesis meant that behavioural observations could not be carried out from videos of all treatment days. To determine the possible number of days and sessions to include, a pilot study was conducted and the mean time for analysing a video was used to calculate how many sessions could be included. Following this, the decision was that behavioural observations would be carried out on both morning and afternoon recordings from treatment days 1, 2, 5, and 10, for all 16 calves. This was to include the behavioural responses in the beginning, middle and end of the experiment. Since calves 9-16 did not receive treatment on day 10, data from day 9 for these calves were analysed instead. However, because of technical problems with the recorders, only 101 sessions from these days were available for analysis instead of 128. The missing videos were randomly distributed between calves, treatments and batches. The video material was analysed using the software Mangold Interact® Program Version 18.1.4.4 and Microsoft Excel.

The 30 minutes of treatment started after the handler had entered the pen, when the brush touched the calf. If the brush was out of view when the handler held the brush and reached for the calf, the session began from the moment that a rhythmic movement of the calf being brushed could be seen. Each control calf was matched with a specific brush calf. Hence, the start time of each treatment session for control animals was the same as for its corresponding brush session. However, the sessions for control calf 6 occurred on different days than the brushing sessions for calf 5 as they belonged to different batches (1B and 1A). If the handler stopped brushing the calf and ended the treatment session before 30 minutes had passed, behavioural observations ended the instant the brush was no longer in contact with the calf, after the last brush stroke. If the brush was out of view when this happened the observations ended when the rhythmic brushing movement could no longer be seen.

Behavioural observations were carried out with two different recording methods, continuous recording and instantaneous time sampling. Five different behaviour categories were analysed; main behaviours, play behaviours, latency to lie down, exploratory behaviours, and other behaviour. Ethograms were developed, one for each observation method, see table 3 and 4. The order of behaviour observations were systematically varied for animal ID, time of day, date, day of treatment, and treatment.

2.4.1. Continuous recording

Main behaviours and play behaviours were observed through continuous recording, measuring both frequency and duration. The latency for the calf to lie down was also determined from the continuous recording. The total time for all the main behaviours corresponded to the duration of the treatment session. For definitions of behaviours and information about mutually exclusive behaviours, see table 3.

Table 3. Definitions of the behaviours recorded with continuous method from video recordings

Behaviour and head position	Definition	References
Main behaviours		
Standing [ag]	Standing still on all four legs without locomotion or moving only one limb	Modified from Hanninen *et al.* (2003)
Lying [ah]	Lying down in any resting position	
- Lifted [b]	Head is lifted with support from the neck	Modified from Veissier *et al.* (2002)
- Relaxed [b]		
- - On ground [c]	Chin on the litter	Modified from Veissier *et al.* (2002)
- - On handler [c]	Chin on the handler	Modified from Veissier *et al.* (2002)
- - On flank [c]	Head on or against its flank	Modified from Hanninen *et al.* (2003)
- Out of view [b]	The calf´s head is out of view	
Locomotion [ag]	The calf moves forward, backward, upward, or sideways in an upright position using two or more of its limbs	
Play behaviour [f]		
Butting handler [d]	Pushing the head towards the handler in a playful manner while standing up	Modified from Jensen *et al.* (1998)
Butting fixtures [d]	Pushing the head towards the interior, e.g. steel bars or wall, in a playful manner while standing	Jensen *et al.* (1998)
Jumping [eh]	Forelegs lift off the ground in an upward movement with an elevated forepart of the body and it may be followed by hind legs lifting off the ground	Jensen *et al.* (1998)
Buck [eh]	One or both hind legs kick backwards after the body has ascended sharply from forepart to rear part	Modified from Jensen *et al.* (1998)
Kick [eh]	One hind leg lifts off the ground and extends backwards or sideways	Mintline *et al.* (2012)
Straw play [deh]	While kneeling down on the forelegs the calf pushes or rubs the head, throat or neck in the straw in a playful manner	Jensen *et al.* (1998)
Head shaking [d]	Consecutive and quick head movements	Schmied *et al.* (2008b)
Latency to lie down [fg]	Time until the calf lies down in any resting position, with abdomen in contact with litter	

[abcdefgh] Behaviours with common elevated letter are mutually exclusive

2.4.2. Instantaneous time sampling

The location of the head, exploratory behaviours and neck-stretching were observed using instantaneous time sampling with a 30 second sample interval. The score is expressed as the proportion of all sample points in which the behaviour occurred. Details about definitions of behaviours and mutually exclusive behaviours are shown in table 4.

Table 4. Definition of the behaviours recorded with instantaneous time sampling method from video recordings

Behaviour	Definition	References
Location of head		
Within pen [ab]	The calf´s head is inside the pen, not sticking the tip or more of the muzzle out of the pen	
Outside pen [ac]	Tip or more of the calf´s muzzle is outside the pen	Modified from Weary and Chua (2000)
Exploratory behaviours		
- In bucket [bd]	Muzzle is in feed bucket or water bucket	
- Sniffing fixtures [d]	Muzzle is in contact with or less than 5 cm from an object within or outside of the pen, i.e. litter, steel bars, water or hay/concentrate bucket, or wall (not when lying down with head relaxed)	Modified from Duve and Jensen (2011)
- Licking fixtures [d]	The calf´s tongue is in contact with or sucking on an object within or outside of the pen, i.e. steel bars or wall	Modified from Duve and Jensen (2011)
- Sniffing handler [cd]	Muzzle is in contact with or less than 5 cm from the handler or the brush	Modified from Duve and Jensen (2011)
- Licking handler [cd]	The calf´s tongue is in contact with or sucking on the handler, the handler´s clothes or the brush	Modified from Schmied *et al.* (2008b)
- Nothing [d]	The calf is not doing any of the other behaviours	
- Out of view [d]	The calf´s muzzle is out of view (not considered when muzzle is in bucket)	
Other behaviours		
Neck-stretching [d]	Neck and head are positioned in an outstretched line, directed up, down or forward	Schmied *et al.* (2008b)

[abcd] Behaviours with common elevated letter are mutually exclusive

2.5. Statistical analysis

Data management was conducted using Microsoft Excel, Office 365 ProPlus (version 2002, build 12527.20880). Statistical analysis was performed using the software Minitab® (© 2020 Minitab, LLC, version 19.2020.1). The significance level was set to 0.05.

Due to, for example, technical problems with the cameras 32 videos were shorter than the 30 minutes of treatment, of which 26 were brushing videos and six were control. Video lengths of those not long enough varied in length between 5 minutes and 47 seconds and 29 minutes and 58 seconds. However, only 2% of the videos were shorter than ten minutes and 94% of the videos were longer than 29 minutes. Therefore, data were upscaled to represent the same time span, 30 minutes. For the 30 remaining brushing videos and 39 control videos, the behavioural observations were stopped at exactly 30 minutes.

Analyses were executed using Mixed Effects Models with mean value per treatment session (30 minutes) for each calf and each day of treatment or One-way ANOVA with mean value per treatment session for each calf. Further analyses of significant results from Mixed Effects Model were performed using Tukey Pairwise Comparisons.

2.5.1. Continuous recording

Data for the different main behaviours were analysed using Mixed Effects Model with calf as random factor, and the fixed factors treatment (brushing or control) and day of treatment (1, 2, 5 and 10) as well as the interaction between treatment and day of treatment. Play behaviour data were analysed using One-way ANOVA with the fixed factor treatment (brushing or control).

Data for latency to lie down were analysed using a Mixed Effects Model with the random factor calf and fixed factors treatment and day of treatment as well as the interaction between the fixed factors. Data for latency to lie down were edited before analysis. Latencies of zero seconds were removed as those observations represented calves already lying down when behavioural observations started. In cases of calves never lying down during a treatment session, the latency to lie down was set to 1800 seconds.

2.5.2. Instantaneous time sampling

Data for exploratory behaviours and other behaviour were analysed using One-way ANOVA with treatment as a fixed factor. The exception was the data for testing if sniffing and licking handler differed from sniffing and licking fixtures for brushing group. In that case the fixed factor was behaviour.

Prior to statistical analyses, data for exploratory behaviours were processed to consider the different number of behaviours possible to perform within (four) and outside (three) of the pen. The mean proportions of the behaviours were divided by the number of exploratory behaviours possible to exhibit within and outside of the pen respectively.

3. Results

3.1. Behaviour during tactile stimulation

3.1.1. Continuous recording

Results from the continuous recording method are presented as the mean duration in seconds ± standard error of the mean per treatment session (a session corresponds to 30 minutes or 1800 seconds). However, for the mean duration per occurrence of lying with head relaxed and play behaviour, the mean ± standard deviation of the mean per treatment session is given.

Main behaviours

The mean duration of lying for calves in the brushing group (985 ± 112) was significantly lower ($F_{1,14}$=15.83; *P*=0.001) than it was for the control group (1631 ± 118). There was also a tendency towards a significant difference between individual calves in the amount of time they spent lying (Z=1.59; *P*=0.055). Day of treatment had no significant effect on duration of lying ($F_{3,37}$=2.32; *P*=0.091). However, there was a significant interaction effect between treatment and day of treatment ($F_{3,37}$=3.21; *P* =0.034) and the mean duration of lying for brushing group on treatment day two was significantly shorter than for the other treatment days for both brushing and control group. The Mixed Effects Model showed an outlier for control animal 7 on treatment day one, but the model was outlier robust. Data of lying duration over the different treatment days between the brushing and control groups are illustrated in figure 4.

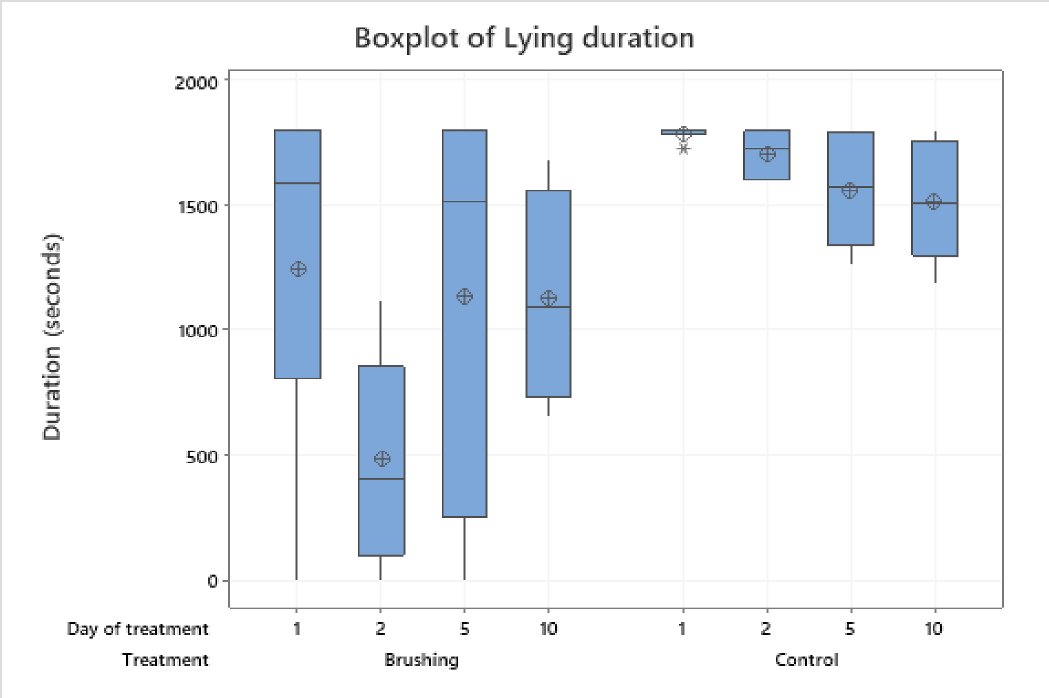

Figure 4. Boxplot of lying duration for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

The mean total duration of lying with head relaxed for the brushed calves (462 ± 115) was significantly lower ($F_{1,15}$=5.05; *P*=0.040) than for the control calves (834 ± 120). The mean duration of lying with head relaxed on the first treatment day (890 ± 121) was significantly longer ($F_{3,38}$=3.56; *P*=0.023) than on treatment day ten (503 ± 111). No interaction effect was found for the fixed factors ($F_{3,38}$=1.09; *P*=0.364). There was a significant difference between calves in the duration of lying with head relaxed (Z=1.80; *P*=0.036). However, Tukey Pairwise comparisons showed no significant differences between calves. Figure 5 illustrates data of lying with head relaxed over the treatment days for the brushing and control groups.

There was no significant difference (F=0.00; *P*=0.971) in mean duration per occurrence for lying with head relaxed between the brushed calves (114 ± 76) and the control calves (115 ± 40).

There was a tendency towards a significant difference ($F_{3,20}$=2.72; *P*=0.072) in mean duration of lying with head relaxed on handler between treatment days 1 (1.2 ± 2.9), 2 (1.7 ± 2.7), 5 (25.6 ± 2.7), and 10 (8.6 ± 2.7). Data for lying with head relaxed on the handler were not normally distributed and had three outliers, hence data were transformed using natural logarithm. The results were back transformed using natural antilog.

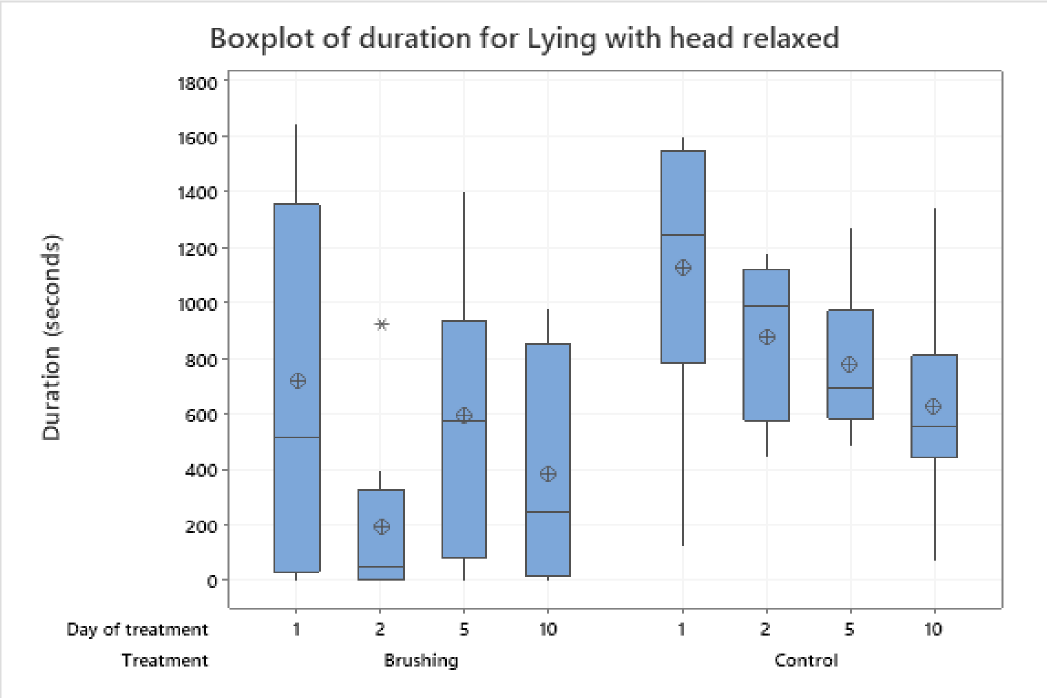

Figure 5. Boxplot of the duration of lying with head relaxed for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

The mean total duration of standing for calves in the brushing group (734 ± 103) was significantly higher ($F_{1,14}$=15.41; *P*=0.001) than it was for the control group (149 ± 108). Day of treatment had no significant effect on duration of standing ($F_{3,37}$=2.20; *P*=0.105). However, calves in the brushing group spent significantly longer time standing ($F_{3,37}$=2.98; *P*=0.044) on the second treatment day in comparison to the other treatment days, as well as in comparison to all the treatment days for the control group. Data of standing duration over the different treatment days between the brushing and control groups are illustrated in figure 6. There was a tendency towards a significant difference between calves in the amount of time they spent standing (Z=1.61; *P*=0.054). Results showed an outlier for calf 7 on the first day of treatment, but the model was outlier robust.

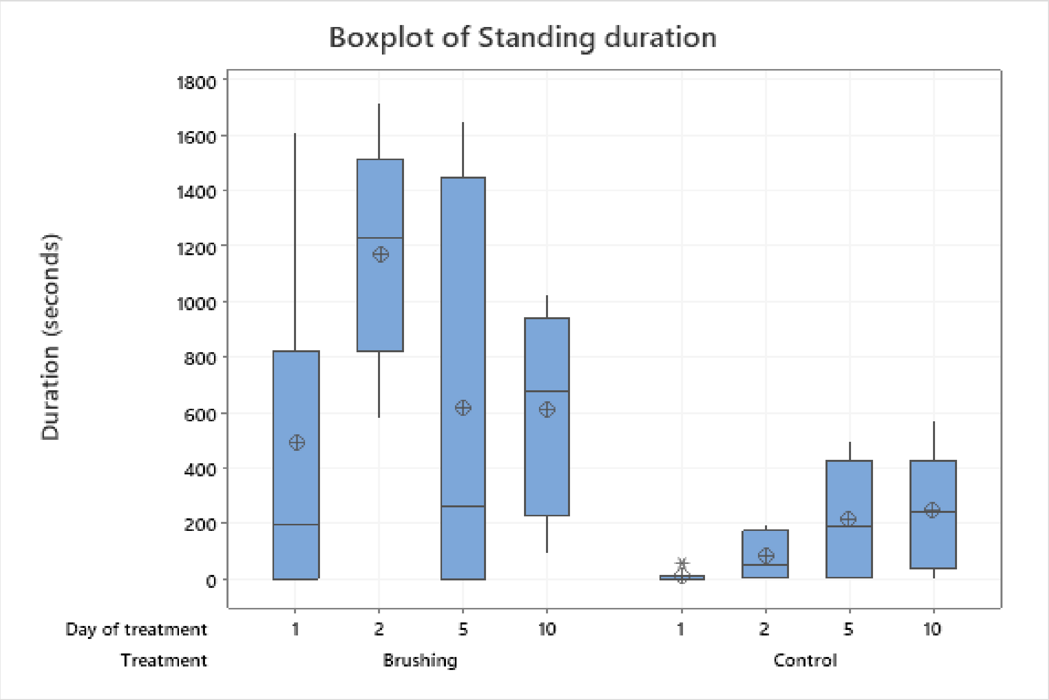

Figure 6. Boxplot of standing duration for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

Brushed calves (75 ± 12) were moving for a significantly longer mean duration ($F_{1,14}$=10.73; P=0.005) than control calves (19 ± 12). On the second day of treatment (79 ± 12) calves had significantly longer mean duration of locomotion ($F_{3,36}$=4.68; P=0.007) than on the other treatment days (day 1: 37 ± 13; day 5: 37 ± 11; day 10: 37 ± 11). Further, the calves that received the brushing treatment were moving significantly more ($F_{3,36}$=8.21; P<0.001) on day two of treatment compared to the other days, as well as in comparison to the control calves on all treatment days. There was a significant difference between calves in the time they spent moving (Z=1.83; P=0.033), although the Tukey Pairwise comparisons did not show any significant difference between calves. The normal probability plot of residuals indicated that data of locomotion were not normally distributed, and an Outlier Test showed two outliers, brushing calf 10 on treatment day ten and control calf 7 on the first day of treatment. Data were edited to exclude the two outliers as the model was not outlier robust. The normal probability plot of residuals indicated that the edited data were closer to showing a normal distribution than when including the outliers (P=0.05). Data of locomotion duration over the different treatment days between the brushing and control groups are shown with the edited data in figure 7.

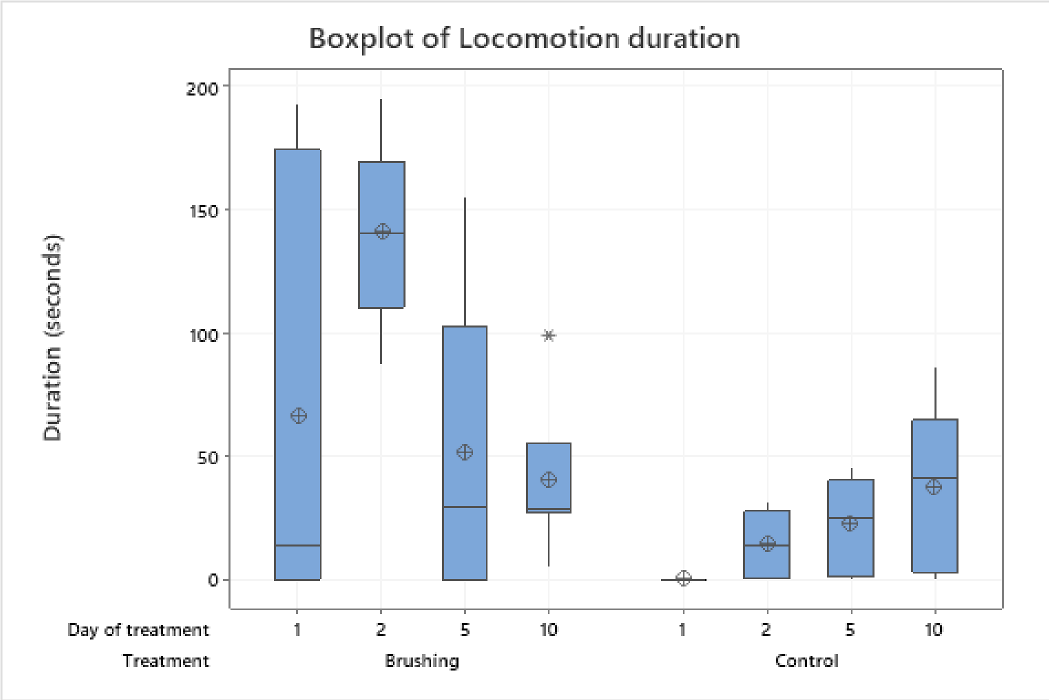

Figure 7. Boxplot of locomotion duration for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

The time budget of main behaviours for both treatment groups were as follows in descending order; lying, standing, locomotion. The time budget of main behaviours for the treatment groups are illustrated in figure 8.

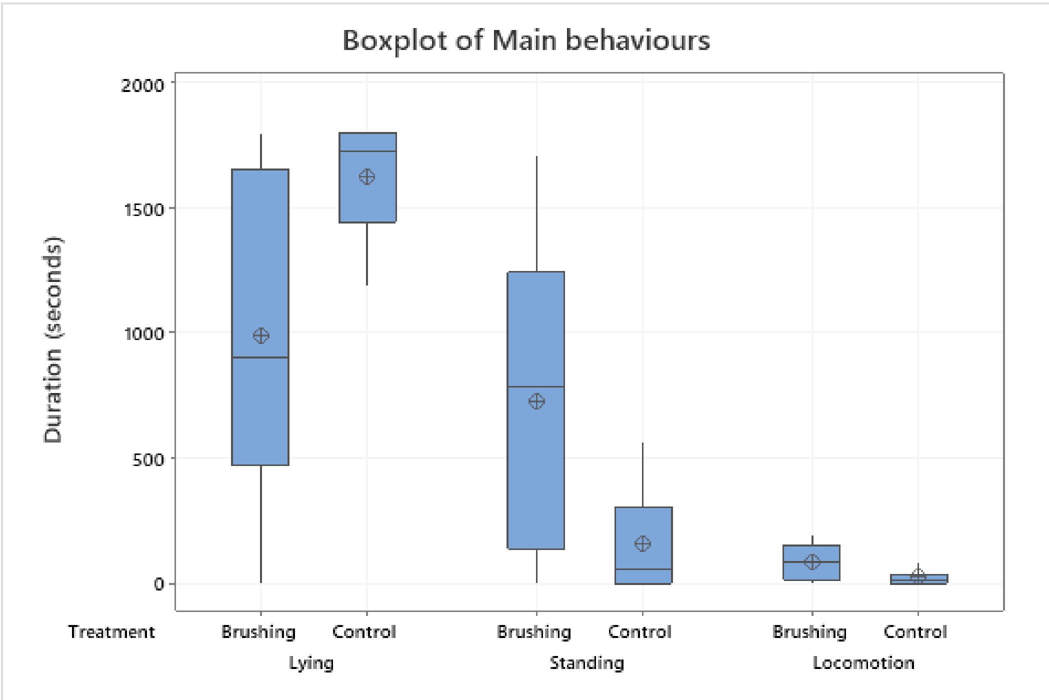

Figure 8. Boxplot of lying, standing, and locomotion durations for dairy calves in the brushing (N=8) and control (N=8) groups

Play behaviour

The brushed calves (25.3 ± 16.6) played for a significantly longer (F=16.40; *P*=0.001) mean duration than control calves (1.3 ± 2.1). The play behaviours with longest mean durations per treatment session for the brushing group were butting handler (18.4 ± 15.0), jumping (3.2 ± 3.8), and butting fixtures (2.5 ± 3.5). For the control group the corresponding behaviours were butting fixtures (0.7 ± 1.6), jumping (0.39 ± 0.6), and head shaking (0.1 ± 0.3). Data on duration of play behaviour over the different treatment days for the brushing and control groups are shown in figure 9. The residual versus fits plot indicated that data did not have equal variance. Control animal 12 was an outlier, but the model was robust against it.

The mean frequency of play behaviour for brushed calves (17.6 ± 10.4) was significantly higher (F=19.71; *P*=0.001) than for control calves (1.2 ± 1.2). However, the residual versus fits plot indicated that data did not have equal variance.

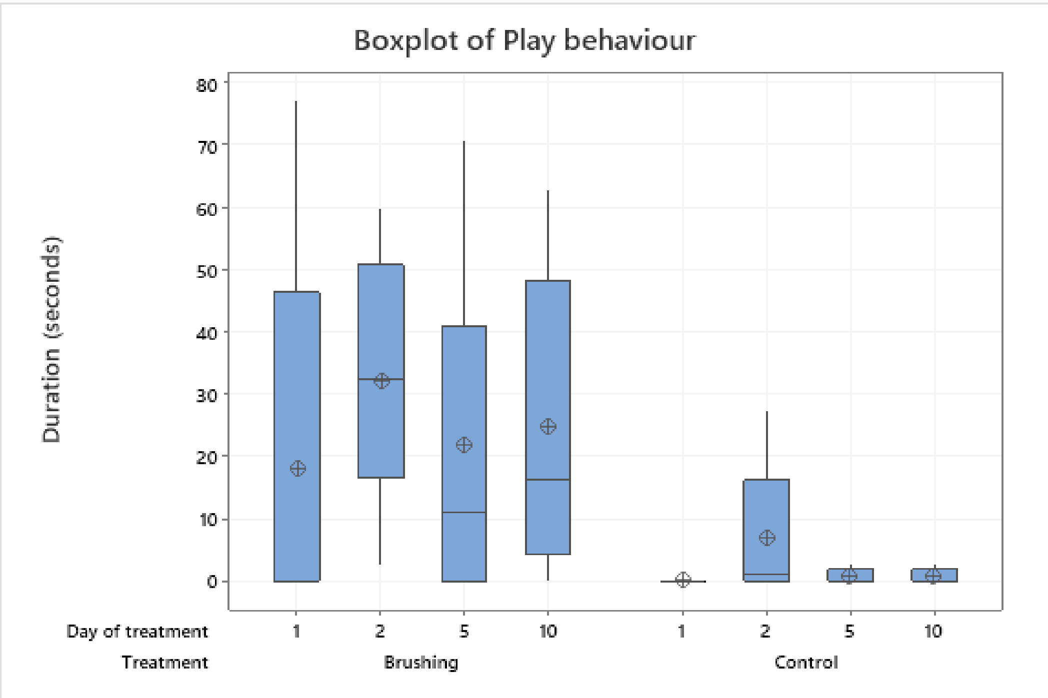

Figure 9. Boxplot of play behaviour duration for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

Latency to lie down

The mean latency to lie down during a treatment session for the brushing group (879 ± 109) was significantly longer ($F_{1,20}$=15.49; P=0.001) than for the control group (168 ± 144). Day of treatment did not have a significant effect on latency to lie down ($F_{3,26}$=0.35; P=0.788) and there was no significant interaction between treatment and day of treatment ($F_{3,26}$=1.47; P=0.245). Data on latency to lie down for the treatments over the different treatment days are illustrated in figure 10. In three cases calves did not lie down during either the morning or afternoon session of a day and in 19 cases the calves were already lying when the session started.

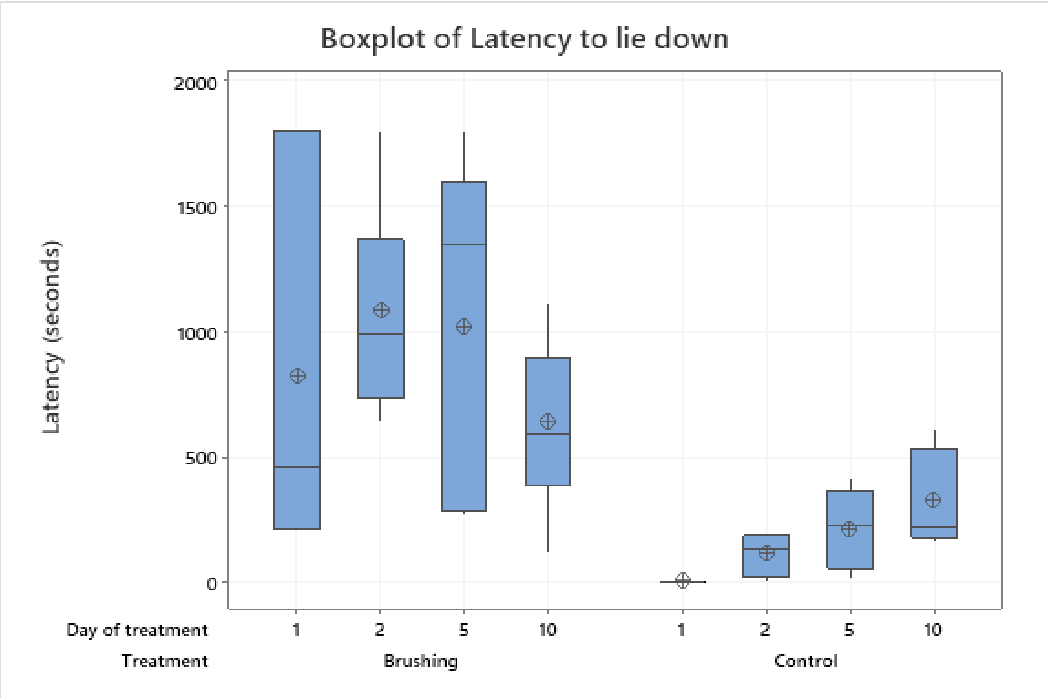

Figure 10. Boxplot of latency to lie down for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

3.1.2. Instantaneous time sampling

Results from the instantaneous time sampling method are presented as the mean proportion of all sample points in which the behaviour occurred ± standard deviation of the mean per treatment session. However, for the mean proportion of sniffing/licking fixtures versus sniffing licking handler, the mean ± standard error of the mean per treatment session is given. Following any corrections to ensure that the time covered was exactly 30 min, the mean score for the number of occasions the calf was observed performing the behaviour was divided by 61 to obtain the proportion of time spent in that behaviour.

Exploratory behaviour

The mean proportion of exploratory behaviour within the pen was significantly higher (F=16.48; P=0.001) for brushing group (0.049 ± 0.022) than for the control group (0.015 ± 0.008).

There was no significant difference (F=1.65; P=0.219) in the mean proportion of exploratory behaviour outside of the pen between the brushing group (0.019 ± 0.009) and the control group (0.012 ± 0.012).

There was no significant difference (F=0.42; P=0.527) in the mean proportion of time spent with head in bucket between the brushing group (0.040 ± 0.022) and the control group (0.030 ± 0.036).

There was a tendency towards a significant difference (F=3.43; P=0.085) between the brushing group (0.054 ± 0.030) and control group (0.033 ± 0.012) in the mean proportion of time they spent sniffing fixtures.

There was no significant difference (F=1.11; P=0.313) in the mean proportion of time spent licking fixtures between the brushing group (0.014 ± 0.011) and control group (0.021 ± 0.015). Data had two outliers (control calf 3 and brushing calf 4) and were not normally distributed. Following the removal of the two outliers the data showed a normal distribution, although the treatment effect was not significant regardless of whether the outliers were included or not.

The brushed calves spent a significantly higher ($F_{1,7}$=11.74; P=0.011) mean proportion of the time sniffing/licking on the handler (0.139 ± 0.019) than on the fixtures (0.076 ± 0.019). Data of sniffing/licking fixtures and sniffing/licking handler are illustrated in figure 11.

Figure 11. Boxplot of sniffing and licking on fixtures and handler for dairy calves in the brushing (N=8) group

Other behaviour

The mean proportion of neck-stretching was significantly higher ($F=38.54$; $P<0.001$) for the brushing group (0.042 ± 0.017) than for the control group (0.003 ± 0.004). The residual versus fits plot indicated that data did not have equal variance. The mean proportions of neck-stretching over the different treatment days and groups are shown in figure 12.

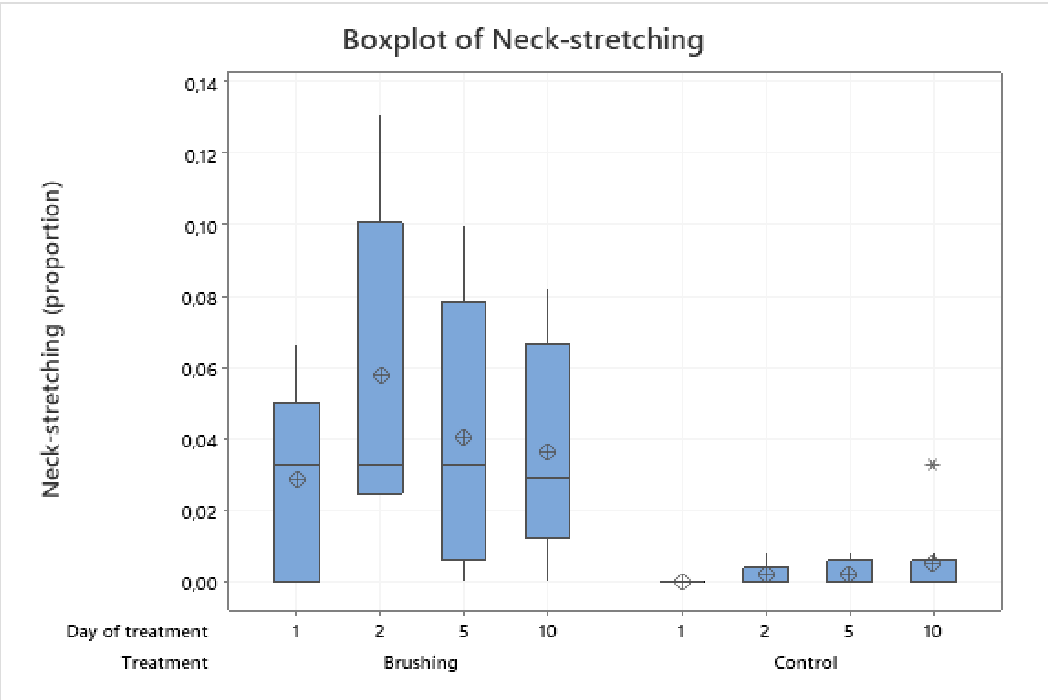

Figure 12. Boxplot of neck-stretching for dairy calves in the brushing (N=8) and control (N=8) groups over the different treatment days

4. Discussion

The results of the present study reaffirm the significance of a good human-animal relationship as it was shown that gentling of dairy calves early in life has immediate positive effect on behaviours possibly indicative of pleasurable experiences.

It was found that calves that were brushed had significantly longer mean durations of standing, locomotion and play behaviour. They also played more often and showed more neck-stretching and exploratory behaviour, in particular sniffing and licking more at the handler than at the fixtures. The brushed calves had a longer mean latency to lie down and shorter mean duration of lying with their heads relaxed.

In the following sections, these behaviours will be discussed in more detail. The discussion will end with some reflections about the methodology and some considerations for future research.

4.1. Main behaviours and latency to lie down

The results of this study do not support the hypotheses about a reduced latency to lie down over the brushing sessions nor increased mean duration of lying and decreased mean duration of standing and locomotion. Furthermore, brushed calves were lying with their head relaxed for a shorter time than control calves, which is in contradiction to the hypothesis. Possible reasons for this could be that the calves lived in a barren environment and the availability of social interactions with the handler and the brushing may have been perceived as stimulus for excitement in the calves. Thus, the brushing treatment possibly prolonged the latency to lie down and resulted in more active behaviours, such as play behaviours, which would explain the reduced duration of lying and increased duration of locomotion and/or standing as well as the reduced time spent lying with head relaxed.

Similar effects of being exposed to a social companion have been seen in individually housed calves in which a rebound effect on play behaviour was seen (Valnickova *et al.*, 2015). Further, the mean duration per occurrence for lying with head relaxed was not affected by treatment, which could be a possible indication that calves do not experience brushing as disturbing. Additionally, the hypothesised increase in duration of lying with head relaxed on handler over the treatment days was not in accordance with the result as no significant difference was seen. Overall, these results bring no evidence of a calming effect of tactile interactions in contrast to existing research of Laister *et al.* (2011), where tactile interactions in form of allogrooming lowered heart rate of receiving cattle. Although, the heart rate data for the calves in this current study have not yet been analysed.

4.2. Play behaviour

The results, increased expression of play behaviour during brushing by a human, are in accordance with the hypothesis. Play has, as previously stated, been suggested as a strong candidate for indication of positive animal welfare (Held & Spinka, 2011; Yeates & Main, 2008; Boissy *et al.*, 2007; Jensen *et al.*, 1998), thus validating an indication of a possible positive affective state of the calves during brushing. If the calves would have perceived brushing as negative, the exhibition of play would likely have been non-significant between brushing and control group or even reduced because play is suppressed if the calf is compromised (Mintline *et al.*, 2013).

If the increase in play was actually triggered by the brushing or by the mere presence of a gentle human in the pen is difficult to know. However, if the control group would have had a human present in the pen, but not brushing or interacting with the calf, this question could be answered. On the other hand, even though the human does not interact with the calf, it is probably inevitable that the calf interacts with the human. Thus, it would have been interesting to have two control groups, one in which a human was present in the pen and one without. It would clarify to what extent the change in play behaviour was due to the brushing, the mere human presence in the pen, or both. Regardless, being brushed by a human still significantly increased play behaviour in the calves.

The mean total duration of play behaviour per observation of 30 minutes was 25.3 seconds for the brushed calves in this current study, which was significantly longer than for the control calves, and is longer than the 12.6 seconds per 30 minutes of observation reported by Schütz *et al.* (2012) where no significant difference was seen between the treatments. In that study, the calves were, unlike the present study, not gentled and they were housed in groups. The calves received positive or negative handling and play was stimulated by adding fresh straw (Schütz *et al.*, 2012). In the current study, the calves received 10 hours of gentle brushing per individual. The shorter duration (5 minutes) as well as total time (5 hours) of positive handling in the Schütz *et al.* (2012) study, in combination with the fact that the calves were housed in groups of five and received treatment as a group, could be reasons why the authors did not see any significant effect on play between the treatments. Therefore, it seems like gentling of individual dairy calves, for a longer duration as well as total time, is a more effective method to induce expression of behaviours indicative of positive emotion, and thus improve animal welfare, than just positive handling.

The behavioural observations in this report were part of a larger experiment, which included other measurements. Another part of the experiment, not included in the present study, concerned heart rate monitoring. All calves in the brushing group and the control calves in the first batch were monitored. Hence, control calves (number 1-8) had heart rate monitors around their thorax during one of the treatment sessions each day, and once outside of the time of treatment sessions to record basal heart rate levels. Recording the heart rate data involved manipulating the calves twice a day, mounting and dismounting of the equipment (four times on the day of recording basal heart rate levels). The calves were habituated to the equipment for five minutes before recording of the first treatment session. However, the possibility of social interactions with the researcher during the mounting procedure prior to treatment sessions may have led to excitement for these calves. This could have been followed by play behaviours and potentially affected the results of the study, meaning possibly increasing durations of standing and/or locomotion as well as play. Though, only half of the calves in the control group were exposed to heart rate monitoring in contrast to all of the calves in the brushing group. With that said, together with the highly significant increase in play for the brushed calves, the heart rate monitoring probably did not affect the outcome to any great extent.

In addition to the heart rate monitoring, the calves were weighed, and blood samples were collected on the first and last treatment days. Saliva samples were collected on the first, fifth and tenth day of treatment. These procedures may also have influenced the behaviour of the calves, possibly by reducing the expression of play, but the effect was likely negligible as all calves experienced the procedures and a significant treatment effect was seen for play.

4.3. Exploratory behaviour

Gentling increased the proportion of exploratory behaviour within the pen, which contradicts the hypothesised decrease. The brushing did not have any significant effect on exploration with head outside of the pen, nor on the proportion of time that the calves had their heads in the water or feed buckets, thus it did not significantly decrease the behaviours as was hypothesised. However, expected from the treatment was an overall calming effect with some predicted expression of play, but it seems the treatment was likely perceived as a greater stimulus for active behaviours than expected. Hence, there was more exploration of the handler and fixtures.

The significantly higher proportion of sniffing/licking handler in comparison to sniffing/licking fixtures along with the tendency to a higher proportion sniffing fixtures for the brushed calves may in part explain the significant increase in exploration within the pen, but not outside the pen. Moreover, exploratory behaviour directed towards the handler could only be carried out when the calves' heads were inside the pen, hence it influenced the final proportion of exploration within the pen. This seems to be the case even if the number of behaviours possible to perform within and outside of the pen were taken into account before the statistical analysis.

4.4. Other behaviour

The hypothesis of significant increase of neck-stretching for the brushed calves was confirmed and further consolidates the possible pleasurable experience of being brushed as it is a behaviour shown both during social licking between cows (Laister *et al.*, 2011) and during brushing or stroking by a human (Lürzel *et al.*, 2015a; Westerath *et al.*, 2014; Bertenshaw & Rowlinson, 2008). Neck-stretching has been suggested to reflect pleasure in dairy heifers (Bertenshaw & Rowlinson, 2008) and Lange *et al.* (2020) used neck-stretching as an indicator of positive emotions in heifers. The significant increase in neck-stretching for the brushed calves can therefore possibly be indicative of calves perceiving the brushing as positive, which is also in line with previous work by Westerath *et al.* (2014) in which neck-stretching while being brushed contributed to the authors concluding that brushing was rated a positive experience by calves.

4.5. Human-animal interactions

The immediate behavioural responses of dairy calves being brushed, for instance the increased expression of play behaviour and neck-stretching, could possibly indicate that brushing was perceived as a positive experience. Gentling of lambs has previously been shown to enhance the human-animal relationship (Caroprese *et al.*, 2012) and, if the human-animal relationship is improved through brushing of calves early in life, it could have implications for the daily management on dairy farms. Previous studies have shown that a lower fear of the stockpeople can entail reduced risk of injuries for both cows and humans (Calderón-Amor *et al.*, 2020; Waiblinger *et al.*, 2004; Lensink *et al.*, 2001). Furthermore, as suggested in existing research, a good human-animal relationship may also improve dairy cattle productivity (Hemsworth *et al.*, 2002; Waiblinger *et al.*, 2002; Hemsworth *et al.*, 2000). For these reasons, brushing calves could possibly be used as a management tool on dairy farms.

However, it is important to remember that gentling can only improve animal welfare to a certain level. Even more important is to reflect on the initial level of welfare and to what extent it is actually improved? The brushing of young calves is supposed to mimic the licking of its mother. Nevertheless, brushing dairy calves for short periods of time probably cannot accomplish the same effects as the presence and relationship with the dam could achieve, at least not to the same extent. Further, it is difficult to know precisely how the brushing was perceived by the calves. Where on the welfare spectrum did they experience themselves to be before the brushing and how big of an effect did this treatment have on their quality of life?

It was not possible in this study to follow the calves later, so it is not possible to comment on whether the immediate positive behavioural effects that were seen had long lasting effects for these calves. However, if calves are habituated to brushing in early life, it is possible that they could be easier to handle during brushing later in life. For instance, if the stockpeople brushes them in order to remove dirt, it would be preferable that the cows accept, or even better enjoy, being brushed from both a welfare point of view as well as a work environment safety perspective.

4.6. Reflections about the method

To get the most detailed and correct reflection of the behavioural responses of the calves during the treatments, continuous observations of all behaviours would have been the best choice and of course doing observations on all sessions from all treatment days. However, it was reasoned that it would take too long to do continuous observations on all behaviours in the ethograms and that it was more plausible to choose instantaneous time sampling for behaviours which are more commonly occurring. The ethograms could also have included different behaviours, for instance rumination behaviour. It would have been interesting to see if the brushing treatment had any impact on the onset and development of rumination in young dairy cattle and to link this with their weight gain. Although it would probably have required a second camera, with another angle, to be sure if the calf was actually ruminating or merely chewing on straw. Further, the definitions for licking handler and licking fixtures could have been developed in such a way that it was easier to distinguish these behaviours from sniffing handler or fixtures. The definitions of licking handler or fixtures were difficult to use without watching a few seconds before and after the actual sample point. The reason for this was that in many cases when the calf was licking on the handler or the fixtures, the tongue was only out for brief moments and when jumping exactly 30 seconds forward to see a frozen picture it was impossible to determine if the calf was actually sniffing or licking if the tongue was not out and the video was not rewound a few seconds.

In order for the gathered data to be comparable between occasions, that is between treatment sessions that sometimes varied in length, some sort of correction had to be done. If not, the absolute duration of a behaviour would not have been comparable between occasions. To manage this, data from both observation methods were upscaled. The data were divided by the length of, or the number of sample points available for each session and then multiplied with 1800 seconds or 61 sample points, depending on the recording method used for the behaviour. When upscaling data it is assumed that the behaviours are basically the same during the observation period, which means supposing the behaviours in the beginning of the session do not differ from those in the end. This assumption could be problematic if there were many sessions that were very short. However, in the current study that was not the case as there were only two short videos and 94% of the videos were longer than 29 minutes. Hence, the influence of the upscaling on the results is negligible. Before it was decided to upscale the data, other options were considered. The alternatives discussed were to exclude data from videos which were shorter than ten or five minutes or shorten all videos to a certain length, for instance 25 minutes. Since behavioural observations were made on four out of ten treatment days, due to the limited time frame of this thesis, it was reasoned that excluding more data than was already done would probably have had a greater impact on the results than upscaling as the data set was already quite small, therefore the most suitable solution was to upscale the data.

Another possibly more problematic matter, is the data loss of complete sessions due to technical problems with the video recorders. Data from several sessions, of both brushing and control animals, were missing. More specifically, on the first treatment day, data from one of the sessions were missing for calves number 1, 5, 6, and 9-16. Data were missing from both sessions on treatment day one for calves number 2-4 and on treatment day two for calves 2, 3, and 7. Further, data were missing from one of the sessions on treatment day two for calves 6 and 12, on treatment day five for calf 9, as well as on treatment day ten for calf 7. Day one of treatment only had video material from 15 sessions from 13 animals, instead of 32 sessions from 16 animals. There was thus a lack of data from more than half of the sessions from this selected day. Of course, the best scenario would have been to analyse all videos from all days. However, the decisions about the number of days and which days to analyse were based on the limitation in time and the interest in possible behavioural differences over the treatment period. Therefore, it was important that behavioural observations were carried out on sessions from the beginning, middle and end of the experiment. Perhaps, a significant difference between treatment days could have been seen for some behaviours if more or other treatment days with more complete number of videos would have been analysed.

Although the brushing had a significant effect on the mean durations of the different main behaviours, there were tendencies towards significant differences between calves. It is possible that eight calves per treatment group was not enough, at least not for the behaviours without significant treatment effects. The tendencies towards significant differences between individuals for some behaviours may indicate that the brushing was experienced differently by different calves. Another aspect concerning this would be the influence of the handlers who were brushing the calves. Even though they followed the same instructions, they may have brushed the calves in slightly different ways and thus possibly had different effect on the calves. Therefore, it could have been interesting to include the handler in the analysis.

4.7. Future research

Within the frame of this experiment, but not included in this study, saliva samples were collected on the first, fifth and last days of treatment. They are to be analysed for levels of immunoglobulin A (IgA) in relation to gentle brushing of calves by a human. This is, as of now, a knowledge gap to be filled. Previous research on gentled shelter cats has indicated positive effects on emotional wellbeing as cats that received gentle stroking were more prone to be rated a positive mood (Gourkow *et al.*, 2014) and gentling helped cats to sustain a positive affective state (Gourkow & Phillips, 2015). Additional findings in these cat studies indicated that gentling can enhance mucosal immunity as secretion of IgA, analysed from collected stool samples, increased in comparison to control cats. Furthermore, cats within the gentled groups that showed a positive response to the stroking had higher values of IgA (Gourkow & Phillips, 2015; Gourkow *et al.*, 2014). Gentled cats were also less likely to develop upper respiratory disease. The behavioural results of the present study, which indicated a positive affective state in calves that were gentled, are consistent with the research by Gourkow *et al.* (2014) and Gourkow and Phillips (2015).

For the above-mentioned reasons, it would be of considerable interest to proceed with research in this area on calves on commercial dairy farms as well as adult cattle, to investigate if gentling can induce physiological alterations which enhance the immunity of the mucosa. If mucosal immunity in dairy calves can be enhanced by gentling early in life, perhaps it would have positive implications on health and in the long run increase productivity of cows, hence also the profitability of the farm. Another positive aspect would be the possible decrease in the use of antibiotics as well as prevention of antimicrobial resistance, if the health status of farm animals increases. In future studies, it would be interesting to investigate if gentling of dairy calves has any long-term effects, such as reduced need for treatment with antibiotics in their life. Furthermore, if brushed calves would turn out to be heavier, it would be positive for the males that go to meat production and for the profitability of the farmer. In practice, long sessions of gentling calves may not be plausible which is why it would be of interest to explore if positive effects, for instance reduced heart rate, reduced concentration of serum cortisol, increased levels of oxytocin and IgA, higher daily weight gain, and more expression of play behaviour as well as neck-stretching, can be achieved by providing calves access to a stationary or mechanical brush.

5. Conclusions

In conclusion, gentle tactile interaction two times per day, for 30 minutes, for ten consecutive days in early life affected some types of behaviours during treatment in individually housed dairy calves. The significantly increased duration and frequency of play, proportion of exploratory behaviour within the pen, as well as proportion of neck-stretching during treatment indicated a possible immediate positive effect on calves' affective state and thus animal welfare, which emphasizes the significance of a good human-animal relationship. However, when interpreting these results, it is of essence to bear in mind that this study was conducted in a controlled environment to ensure high health status in the calves and minimize any effect of disease. Therefore, the conclusions have high internal validity but low external validity. Calves on regular commercial dairy farms generally do not live under these comprehensive hygiene measures which may entail lower health status than in this study. Consequently, the effects of tactile stimulation may not be seen to the same extent in practice.

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Appendix 1 Popular scientific summary - How do dairy calves respond to brushing?

The welfare of both humans and animals is affected by the relationship between them. Therefore, the human-animal relationship is important from an ethical point of view. In recent years, research on creating opportunities for rewards and pleasure have gained more interest, instead of only focusing on reducing negative experiences for animals. A method commonly used to create positive experiences for animals involves additional positive contact to that of daily management. It is often called gentling and can for example include stroking, brushing and/or talking in a soft way to the animals.

How do dairy calves behave when being brushed? The calves were gently brushed for 30 minutes two times a day for ten consecutive days. They were housed individually on a research farm, to make sure they were as healthy as possible. The treatment sessions were video recorded. Behaviour analyses were done on days 1, 2, 5, and 10 for the 16 calves included in the study. Half of them were submitted to gentle brushing by a human and the rest were not. The brushed calves played more often and for a longer time than control calves during the brushing. They also stretched their necks more often as well as explored more within the pen. Possible reasons for this could be that the calves lived in a barren environment and the presence of the handler and being brushed may have been perceived as stimulus for excitement in the calves. Brushing dairy calves showed immediate positive effect on behaviours that can possibly indicate pleasurable experiences. Hence, improving the animal welfare which emphasizes the importance of a good human-animal relationship.

Enhanced emotional wellbeing have been indicated in gentled shelter cats. They also had an improved immune system and were less likely to get upper respiratory disease. If gentling of dairy calves on commercial dairy farms could improve their immune system, it could possibly result in healthier cows in less need of treatments with antibiotics during their life. This could in turn prevent resistance against antibiotics and increase the profitability for the farmer.