Human-Animal Interactions in the Early Life of Dairy Calves

– Effect of brushing on behavior and health

Patricia Bilsland

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Swedish University of Agricultural Sciences, SLU
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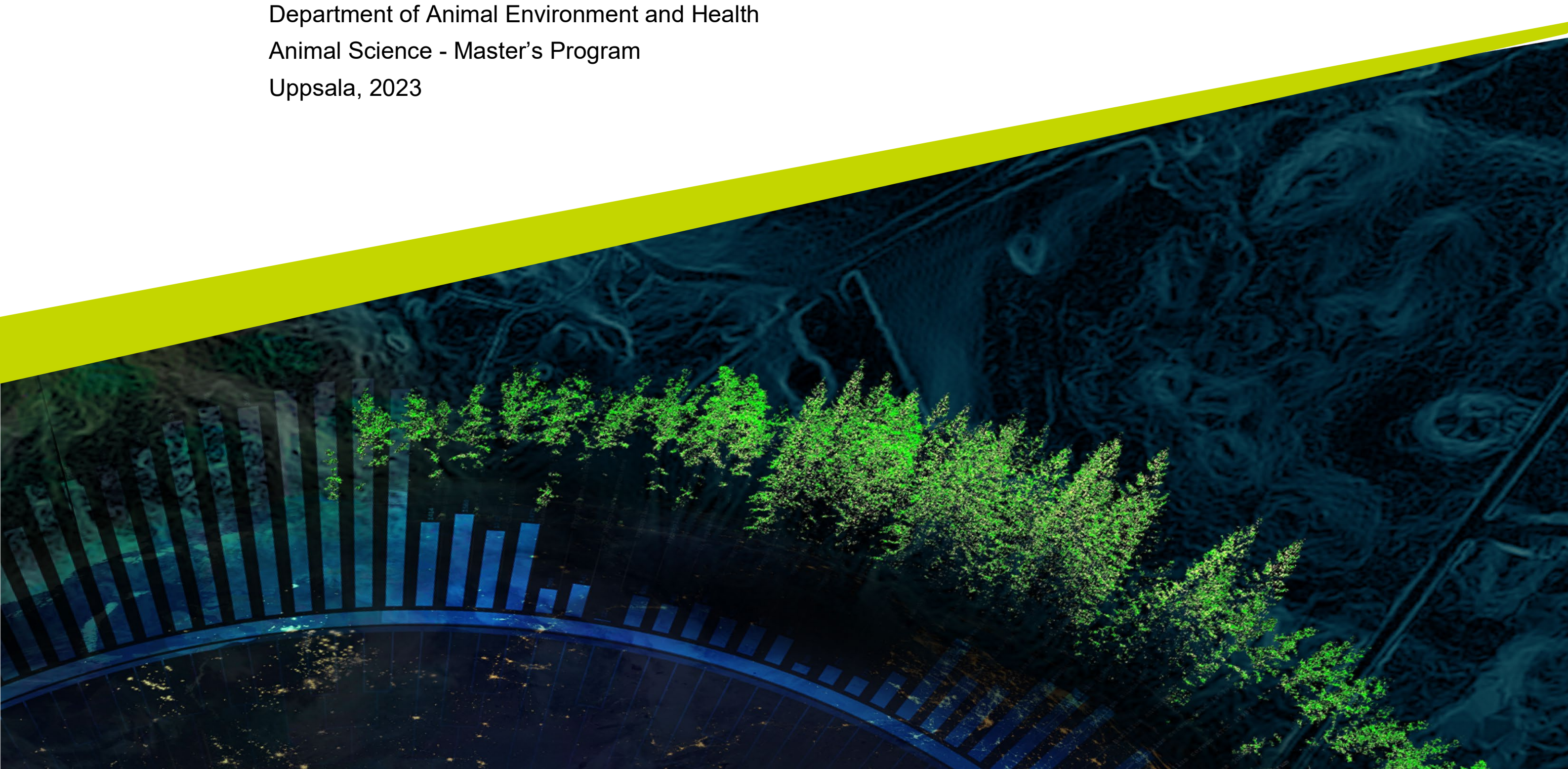

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Effect of brushing on behavior and health

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Abstract

This study was conducted to investigate the influence of positive human-animal interactions and tactile stimulation on the behavior and health of young dairy calves. 16 individually housed calves were divided into two groups: One group received tactile stimulation by manual brushing for 30 minutes twice daily for 10 days, and the control group was housed in the same settings but was not brushed. The behavior of all calves was observed four times daily, before and after brushing sessions. Their health was monitored by means of a physical examination and blood count on the first and last days of the experiment and by daily visual inspections. The hypothesis was that brushed calves would be healthier and display more of the natural playful behavior of young calves compared to their control counterparts. We found, however, that brushed calves were less fearful of the experimenters, while control calves displayed more of a play behavior, kicking, during the observation periods. All calves remained healthy during the experiment. There were some indications that, in terms of blood count, brushed calves could mature earlier, as their cell profile was closer to that of older animals. However, more studies are needed to verify if brushed calves become physiologically mature ahead of controls.

Keywords: dairy calf, tactile stimulation, brushing, human-animal interaction, anthrozoology, health, individual housing, behavior, positive welfare

Dedication

To every animal who touched my life. Thank you for teaching me to love and respect the different.

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Abbreviations

ADG	Average daily gain
B	Brush
C	Control
F	Female
GLM	General linear model
H	Holstein
Hb	Hemoglobin
HAI	Human-animal interaction
HAR	Human-Animal relationship
HR	Heart rate
hsp	Heat shock protein
IOR	Inter observer reliability
M	Male
MCH	Mean corpuscular hemoglobin
RBC	Red blood cell
RR	Respiratory rate
SLU	Swedish University of Agricultural Sciences
SEM	Standard error of the mean
SR	Swedish red

1. Introduction

Interactions between humans and cattle are a daily part of life at a dairy farm, even if operations are becoming increasingly automated. Both parties develop a dynamic relationship that can be positive, neutral or negative (Hemsworth & Coleman 2011). The quality of this relationship may range from very good to very poor, and can trigger, respectively, pleasant emotions, such as joy or relaxation, or unpleasant emotions, such as fear, pain or frustration (Waiblinger et al. 2006; Waiblinger 2017). The behavior of animals towards their handlers or other humans can indicate the quality of the human-animal interactions (HAI) experienced by the animals (Hemsworth & Coleman 2011). There is evidence that animals that live under an undesirable emotional state are not only chronically stressed, which can lead to immune suppression, but also have a higher risk of getting injured or injuring others while trying to avoid humans (Hemsworth & Coleman 2011). The Swedish Work Environment Act mentioned already in 1977 the workers' physical and mental health as well as the need to protect people from ill-health and injuries. Therefore, a good HAI is important not only for the welfare of cattle, but also for stockpeople.

A common practice in dairy farms is to separate the calf from the cow soon after, or a few days after birth, and house them in individual pens until weaning without any physical contact with another animal. However, this is perceived as unnatural and even cruel by consumers who are increasingly aware of production practices (Busch et al. 2017; Hötzel et al. 2017). There have been justifications to separate the calves from their dams, such as less suffering for the animals when the pair is separated before a bond is formed, protecting the young calf from pathogens circulating in the herd, controlling colostrum and milk intake by the calf (Hötzel et al. 2017), and having more milk left for sale. Additionally, many dairy farms were built/designed to house calves individually before weaning, as this has been thought to provide a better environment for the calves. However, in natural systems calves are not isolated. They have physical contact and develop relationships not only with their dam, but also with other animals in the herd (Whalin et al. 2021). It is believed that the physical contact and play are important for the physical training and social development of the calf in its herd (Whalin et al. 2021).

Cattle are social animals, and the natural curiosity of young calves may offer an opportunity of positive interaction with humans (Lürzel et al. 2016). Allogrooming, or reciprocal licking, is frequently observed between "friends" in a herd and it also plays an important role in the maternal bond (von Keyserlingk & Weary 2007; Johnsen et al. 2015). It has been shown to lower the heart rate of the receiving animal, which could possibly indicate a calming effect (Laister et al. 2011). Studies indicate that cows may in part perceive human stroking of body regions often licked akin to social licking (Schmied et al. 2008), and calves seem to perceive being brushed by a person as positive (Westerath et al. 2014). Dairy heifers that had been stroked during the first 2 weeks of life had a higher weight gain compared to controls (Lürzel et al. 2015), while beef calves that had been gently touched during the first 3 weeks of life were less fearful of humans and showed less stress-related behavior at slaughter (Probst et al. 2012). Additionally, animals that are habituated to being gently handled by humans are less likely to display physiological responses to stress that could lower their immunity (Waiblinger et al. 2006). Researchers have even found a correlation between milk yield and fear of humans, where cows approached less an experimenter in farms with low yield than in farms with high yield (Breuer et al. 2000). Thus, human factors play an important role in the productivity and welfare of farm animals (von Keyserlingk et al. 2009), and potentially the efficiency of the production system (Hemsworth & Coleman 2011; Lindahl et al. 2016).

While it is well documented that stress can negatively influence immunity, studies with different species are showing that positive emotions can positively influence immunity: Detillion et al. (2004) found that positive social interactions protected hamsters against stress and promoted wound healing, and Gourkow et al. (2014) reported that gentle stroking of anxious shelter cats induced positive emotions that were accompanied by an increase in salivary IgA and reduced incidence of upper respiratory disease. Similarly, 30 days old dairy Holstein heifers experiencing positive emotions had higher salivary IgA concentrations than a similar group experiencing negative emotions (Lv et al. 2018). Also, human couples with a better relationship had higher levels of oxytocin and faster wound healing (Gouin et al. 2010) and physical contact with dairy cows as part of an animals assisted intervention improved clinical signs of depression in clinically depressed people (Pedersen et al. 2012).

As mentioned previously, many dairy farms house calves in individual pens until weaning as that was thought to be the best way to rear healthy calves. But research is now looking at positive welfare, which means offering animals more than just the mitigation of suffering. It encompasses positive emotions, positive affective engagement, quality of life and even happiness (Lawrence et al. 2019). What can be done to improve the life of healthy calves raised in individual pens? What happens when animals are happy? Will providing calves the tactile stimulation/physical contact from which they are deprived influence their behavior or health? These are relevant questions, especially as the welfare and health of animals and humans are interconnected - One Health/One Welfare (Pinillos 2018).

Thus, the objective of this project was to investigate the effect of a positive HAI and tactile stimulation, offered by means of manual brushing, on the health and behavior of young dairy calves separated from the dam at birth and housed in individual pens. The hypothesis was that brushed calves would be healthier and display more of the natural playful behavior expected of young calves compared to their control counterparts.

2. Materials and Method

The experiment was carried out in August 2019 at the Swedish Livestock Research Centre, Lövsta lantbruksforskning, belonging to the Swedish University of Agricultural Sciences, SLU, and located in Uppsala, Sweden. This project is part of a larger research on the welfare and health of dairy calves, and all procedures had been duly approved and authorized by the Ethics Committee under permit Dnr5.8.18-08933/2019.

2.1. Animals, facilities, and experimenters

Animals

We used 16 calves, 10 males and 6 females, born at the farm from the production herd. There were 13 Swedish Red and 3 Holstein calves, with a mean age at the first day of the experiment of 9.75±0.892 days (mean ± SEM - standard error of the mean), ranging from 4 to 14 days of age; mean birth weight of 36.13±1.41 kg, ranging from 26.1 to 46.0 kg; and mean weight on the first day of the experiment of 44.44±1.12 kg, ranging from 37.4 to 53.6 kg. To keep the age range of the calves in the experiment as low as possible, we used two batches of eight calves.

Facilities

The animals were housed individually in an experimental barn. They were brought to individual pens soon after birth, fed colostrum, weighed and ear tagged. All calves stayed in the same pen for the entire duration of the experiment. There were 16 pens (95 cm x 148 cm) built with wooden pallets especially for the trial (Figures 1 and 2).
The calves were placed consecutively by order of birth facing each other on alternating sides of the stable (Figure 3).

The first eight pens were separated from the last eight by a curtain, so that calves in the second batch would not see what was going on while the experiment was being conducted with the first batch (Figures 3 and 4). Each calf could see the calf opposite it and glimpse the movement of its neighbor through the slits between boards, but not reach or lick their neighbor. The pens were numbered 1 to 16 and Treatment and Control pens were assigned facing each other on alternating sides, as shown in Figure 3.

Figure 1– Pens built with pallets

Figure 2 – Picture of an individual pen

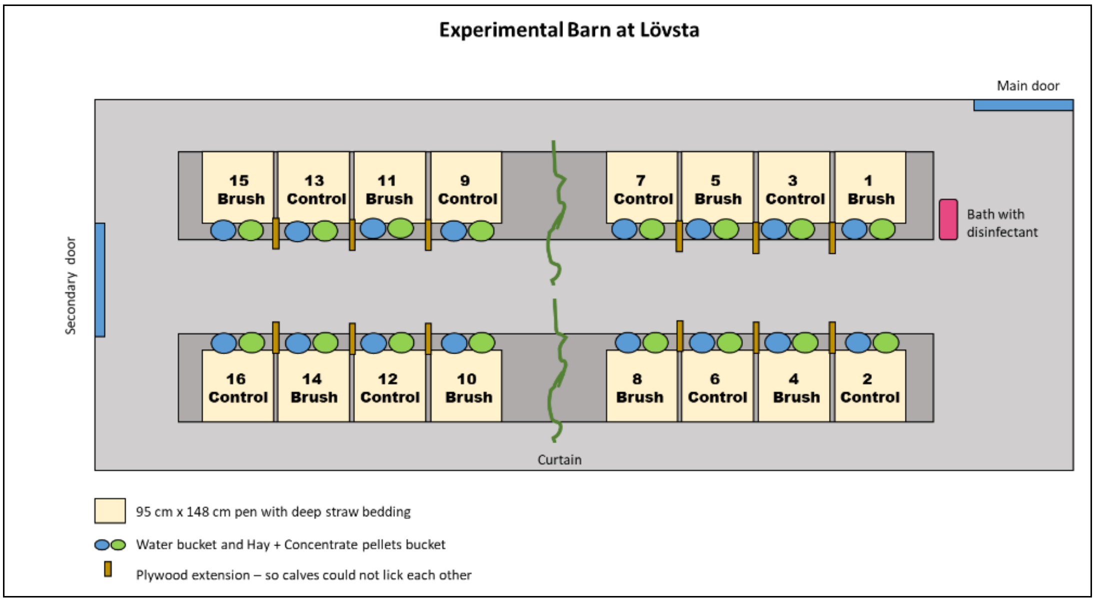

Figure 3 – Diagram of the experimental barn. The numbers of the pens indicate placement of calves by order of birth.

Figure 4 – View of the experimental barn with curtain separating animals from first and second batches.

The type of bedding was deep straw to minimize the interaction required for cleaning – new clean straw was added as needed, with no predetermined schedule or amount of straw (if the pen looked dirty or damp, the experimenters would add more straw). The barn was lit continuously by white tube lamps, although from 10 PM to 5 or 6 AM the illumination in the barn was dimmed as only four lamps remained on. The average daytime temperature in the barn during the experimental period was 20^0C, with the highest recording being 26^0C in an exceptionally hot day and the lowest being 18^0C.

All pens were equipped with two buckets, one for water and one for hay and starter pellets. These buckets were numbered so they could be used exclusively by each calf. The pen gate also had a holder for the milk bucket, but that bucket was removed as soon as the calf drank all the milk. As the teat buckets (milk buckets) were washed with soap and disinfected after every meal, following the farm's routine, they were not marked for each calf. They were, however, used exclusively by the calves in the experiment and separated from the other milk buckets in the farm. The feeding schedule followed the farm's usual protocol, with three meals of colostrum soon after birth. If the quality of the colostrum of the dam was considered not good enough (threshold of 20% Brix), the calf received colostrum from a bank or another cow. After the initial meals, the calves received colostrum twice daily for the first 3 days. After that, the calves were fed milk from the milk tank twice daily until weaning. Water, hay, and starter pellets were available *ad libitum*. The feeding and cleaning of the barn were done by farm staff before the arrival of the experimenters in the morning and in the afternoon after the data collection was finished for the day. The experimenters added water, hay, pellets, and bedding if needed during the day.

In addition to individual buckets, other hygiene measures were used to decrease the possibility of disease: a tub containing disinfectant was placed at the entrance of the barn so boots could be cleaned; each calf in the brush group had its own brush; and the experimenters always wore disposable booties and gloves when entering a pen.

The calves were vaccinated against ringworm by farm staff according to the farm's regular schedule, which is every ten days starting in the first week of life, with bull calves receiving 2 doses (they are usually sold to beef farmers at approximately two weeks of age) and heifer calves receiving 3 doses.

Experimenters

The experimenters were both female. They wore dark green twill overalls and green rubber boots. They entered the barn at approximately 8:30 in the morning and were the only people in the barn until 15:30 in the afternoon. Farm staff entered before and after that period to clean and feed the calves.

2.2. Design

The animals were randomly allocated according to birth order (Figure 3) into one of two groups: Brush (B), where calves would receive tactile stimulation by being brushed by an experimenter for 30 minutes twice a day for 10 days; or Control (C), where calves would not receive tactile stimulation or experience physical contact with a human – they would just be able to see and hear the experimenters in the barn. The experiment started when 5 animals had finished the colostrum period. For this reason, Batch 1 (containing 8 animals) was divided into two sub-batches. On the first day of the experiment, before the arrival of the experimenters, the calves were weighed and had a blood sample taken from the jugular vein by farm staff. The same was done on the last day of the experiment.
Behavior observations were conducted daily during the experimental period for all calves. All calves were physically examined in the first and last days, and checked (visually) daily for signs of disease.

2.2.1. Treatment

Brushing Procedure

Brushing was done with a dandy brush (one for each calf) and a circular wrist movement, trying to simulate the action of a cow licking its calf. The calf was not forcefully brushed, but gently habituated to brushing. The calf was free to move around the pen and to lie down or stand. The entire body of the calf could be brushed, as long as the calf did not show signs of fear of discomfort at being brushed. If the calf backed away from the experimenter or the brush, brushing stopped until the calf was calm again. Calves were always brushed by the same experimenter and the experimenters wore disposable gloves and booties when entering each pen.

The treatment session started with the experimenter standing in front of the pen for one minute, showing the brush to the calf. Then she entered the pen and started counting the 30 minutes brushing time following the procedure described above. Once the 30 minutes ended the experimenter exited the pen. Each experimenter brushed two calves per day, that is four brushing sessions. The order of brushing was changed each day to avoid a bias of time of the session, so the calf that was brushed first one day, was brushed last the next day.

Control Procedure

Nothing was done with the control animals. They could see and hear what was going on in the barn, but they had no interaction with the experimenters, except for the placement of a heart monitoring strap once a day (description under topic 2.2.5. Additional Data Collection)

2.2.2. Behavior Observations

The calves were observed in different periods of the day applying instantaneous observations. The first group of observations, the Simple Instantaneous Observations, was done four times a day (approximately 8:30 am, 11:30 am, 12:45 pm and 15:30 pm). The experimenter walked quickly by the pens and registered the behavior that was happening (from the ethogram) at the instant she looked at each calf. Each round of observations lasted less than 1 minute. The second group of observations, the Detailed Instantaneous Observations, took longer time and started at approximately 8:30 am and 11:30 am, immediately after the first group of observations was done. Here the experimenter stood in front of each pen for five consecutive minutes and recorded every five seconds the behavior (from the ethogram) of the calf at that instant. The ethograms used for recording the behaviors were developed considering the expected behavior of young calves housed individually. The description of each behavior in the ethograms is presented in Tables 1 and 2.

Table 1: Description of calf behaviors recorded using Simple Instantaneous Observations.

Standing	Standing with entire body (and head) inside the pen.
Lying	Recumbent position, alert or not, and not actively performing any other behavior.
Head out	Standing with its head out of the pen, without actively performing any other behavior.
Drinking	Head inside the water bucket and swallowing water.
Eating	Head in the feed bucket and selecting and chewing hay or feed pellets.
Self-grooming	Licking or scratching itself (without the aid of fixtures).
Sniffing	Sniffing any part of the pen (inside or outside), gate or bucket.
Licking	Licking (or biting) any part of the pen (inside or outside), gate or bucket.
Object play	Uses any of the fixtures in or out of the pen to interact with.
Individual play	Jerks its head, shakes its head, bucks, jumps or kicks.

Table 2: Description of calf behaviors recorded using Detailed Instantaneous Observations.

Standing	Standing in a stationary manner with entire body (and head) inside the pen.
Moving	Walking inside the pen.
Lying	Recumbent position, alert or not, and not actively performing any other behavior.
Head out	Standing with head out of the pen, without actively performing any other behavior.
Drinking	Head inside the water bucket and swallowing water.
Eating	Head in the feed bucket and selecting and chewing hay or feed pellets.
Self-grooming	Licking or scratching itself (without the aid of fixtures).
Scratching	Scratching any part of the body against any part of the pen, gate or bucket.
Sniffing	Sniffing any part of the pen (inside or outside), gate or bucket.
Licking	Licking (or biting) any part of the pen (inside or outside), gate or bucket.
Seeking interaction	Head out of the gate and either jerking head or trying to reach observer with tongue.
Object play – Straw	Manipulates straw with tongue, licks it, sniffs it, chews it, scratches it or shuffles its nose in it.
Object play – Butt	Presses head against any part of the pen or gate.
Object play – Object	Uses any object of the pen, gate or buckets to play with.
Individual play - Jump	Moves upwards taking all four legs off the ground.
Individual play – Jerk Head	Moves its head vertically (up and down) while with entire body inside the pen.
Individual play – Shake head	Moves head horizontally (from one side to the other) with entire body inside the pen.
Individual play – Buck	Lifts rump and both hind legs simultaneously off the ground.
Individual play - Kick	Shoots one hind leg off the ground.

As mentioned previously, behavior observations were done four times a day. Simple and detailed instantaneous observations in the morning - when the experimenters entered the barn and soon after the last morning brushing session (approximately 8:30 am and 11:30 am), and simple instantaneous observations in the afternoon – when the experimenters entered the barn after lunch and after the last brushing session (approximately 12:45 pm and 15:30 pm).

Both experimenters conducted observations and balanced the sequence of observations (order in which the calves were observed) so all calves were exposed to the same amount of visual contact with the experimenters throughout the experiment. Inter-observer reliability (IOR) was tested for simple and detailed instantaneous observations by calculating the index of concordance, that is, the percentage of agreements between both observers over a certain number of observations conducted simultaneously (Martin & Bateson 2007).

2.2.3. Health

The health of the calves was monitored by means of physical examinations on the first and last days of the experimental period, as well as daily visual inspections. Blood samples were collected on the first and last experimental days. In this experiment the calves were expected to remain healthy and no challenge to the immune system was conducted. Additionally, as the focus of the experiment was tactile stimulation, the health assessments were made taking that into account. This means that the examinations were done with as minimal physical contact as possible: only the most easily palpated lymph nodes were checked, and no thorough auscultation of heart and lungs was made.

Blood

On the first and last days of the experiment, and prior to the arrival of the experimenters, farm staff weighed all calves and collected blood samples of each animal. Whole blood was collected from the jugular vein of the calves into vacuum tubes (Vacutainer®) with EDTA for a Blood Count to investigate the number of white blood cells (WBC - neutrophils, eosinophils, lymphocytes, monocytes, and basophils), red blood cells (RBC) and hemoglobin (Hb) in each sample. The tubes with blood were stored at room temperature until they could be sent to the lab (Clinical Pathology Laboratory at the University Animal Hospital - UDS) for analysis (less than 5 hours from collection).

Physical examination

A basic physical examination was conducted by a veterinarian on the morning of the first and of the last days of the experiment. This included checking posture, alertness, vital signs (heart rate, respiratory rate, breathing pattern), rectal temperature, palpation of prescapular and precrural lymph nodes, palpation of navel, skin tent test to check hydration and inspection of oral, ocular, and vulvar mucosa for color and discharge. Hind legs, tail and pen were also inspected for signs of diarrhea. On the other days of the experiment there was a brief visual inspection for signs of alterations in posture, alertness, presence of cough, ocular or nasal discharge or diarrhea. As mentioned previously, the examination was brief as to limit the tactile stimulation to the calves.

Rectal temperature was measured with a digital thermometer and respiratory rate and heart rate were assessed with the aid of a stethoscope. Reference ranges for normal calf respiratory rate (RR), heart rate (HR) and rectal temperature were 20-50 mov/min, 90-112 bpm and 38.5 – 39.5^{0}C, respectively (Allan J. Roussel 2009). Prescapular and precrural lymph nodes were palpated and recorded using the following score system: 0 = Normal or slightly enlarged 1 = enlarged, warm, painful, fluctuant or solid; 2 = enlarged, warm, painful, fluctuant or solid and suppurating. Eyes and nostrils were checked for discharge and marked as: 0 = none or fluid discharge; 1 = serous discharge; 2 = mucoid, purulent or bloody discharge. Hydration was assessed by pulling the skin of the mid-neck of the calf and assessing the tent: 0 = skin tent < 2 seconds; 1 = skin tent between 2 and 3 seconds; 2 = skin tent $\geq$ 4 seconds. When feces were found in a pen they were marked in relation to consistency using the following score: 0 = firm or slightly sticky; 1 = paste; 2 = watery.

2.2.4. Statistical analyses

Behavior observations

The detailed instantaneous observations totalized 18,240 registrations of behaviors recorded during the experimental period (9,120 for C calves and 9,120 for B calves). When compiled by day for the statistical analysis there were 152 observations read per behavior; the data included 16 clusters (animals) and a correlation matrix dimension of 10 (behaviors). These were analyzed using the GENMOD procedure, with Poisson distribution, log link function and a working correlation matrix.

The simple instantaneous observations totalized 581 behavior occurrences recorded during the experimental period (290 for C calves and 291 for B calves).

These were analyzed using the GLIMMIX procedure, with binomial distribution, logit link function and diagonal variance matrix. Both models of behavioral observations considered treatment, breed, gender, and experimental day (except for some behavior with very few recordings) as fixed factors and were analyzed using SAS version 9.4.

Physiological parameters and health

The statistical analyses of physiological parameters were done using Minitab 18®. The data on blood was analyzed with GLM (General Linear Model), using the blood parameter as response and breed, gender and treatment as fixed factors. When investigating the change from the first to last day in the blood collected, GLM was used for the response "difference between last day and first day samples". The change in RR and HR was analyzed using a paired t-test.

2.2.5. Additional data collected

The calves were submitted to other procedures during the experiment. These are not included in this thesis but are briefly described below.

Sample Collection

Saliva samples were collected on days 1, 5 and 10 after the first morning behavior observations and before the first brushing session. The experimenters held each calf still, opened its mouth with one hand and with the other rolled a cotton pad (from a Salivette® tube) held with a forceps over and under the tongue of the calf. The saliva-soaked pads were then placed in the sterile tubes and centrifuged. The samples were frozen for later analysis of IgA.

Immediately after the end of the experiment (late afternoon of the tenth day), the calves were anesthetized for the placement of a catheter in the jugular vein so a series of blood samples could be collected the following day. These samples should be used to assess, in addition to blood cell profile, immunologic, stress and positive welfare markers.

Video recording

All brushing sessions were filmed. Cameras on 1.10 m high tripods were placed back-to-back, so the calf being brushed and the control calf housed in the pen directly opposite it could be filmed simultaneously. An analysis of the behavior of the calves during these video recordings has been published in a different thesis (Linder 2020).

Heart rate monitoring

All brushed calves and half of control calves had heart rate (HR) and heart rate variability (HRV) assessed during brushing sessions by means of a sensor (Polar®) placed on the left ventral lateral aspect of the thorax, right behind the elbow, and held in place by an elastic strap. The calves wore the sensor in only one of the daily brushing sessions, and the order was alternated every day, so that one day the calf would wear the sensor in the morning, the next day in the afternoon. Additionally, one recording of heart rate (for each of the calves that were monitored) was made during the experimenters' lunch break, when the barn was empty of humans. The calves were habituated to the strap before the first brushing.

3. Results

This section presents the more significant findings from the experimental period as pertaining this thesis (behavior observations and general health assessments). As mentioned previously, under section 2.2.5. Additional Data Collection, more than the data concerning this thesis was collected during the experiment, and that will not be presented.

3.1. Calf Behavior

Calf behavior was assessed via instantaneous observations (simple and detailed) and the results expressed as mean proportion of occurrences and standard error. To ensure that the two observers were recording behaviors the same way, the inter-observer reliability (IOR) was calculated as an index of concordance. The index of concordance for detailed instantaneous observations was 92.3% and for simple instantaneous observations 82.5%, calculated respectively over 1200 (20 independent assessments with 60 records) and 80 records for each observer. The IOR was considered acceptable for the observations as they exceed the threshold of 70% (Martin & Bateson 2007). There is a natural difference between observers while recording behaviors. This could be explained by an inherent tendency to record more apparent or interesting behaviors even if they happen slightly before or after the exact sampling time (Martin & Bateson 2007).

3.1.1. Detailed instantaneous observations

A statistically significant difference was found in the proportion of the individual play behavior "Kicking" between C and B calves, where C calves displayed higher levels of the behavior than B calves ($p=0.0343$; chi-square 4.48). Experimental day could not be factored into the model for this behavior due to the small number of occurrences.

A tendency towards difference in mean proportion between treatments was found for Standing, where B calves spent proportionately more time standing than

C calves (p=0.077; chi-square 3.12); and Head Out, with B calves having a head out proportionately more than C calves (p=0.0641, chi-square 3.43). For Head Out there was also a trend towards difference in proportion of the behavior between breeds, with Holsteins displaying more of the behavior than Swedish Reds (p=0.0611; chi-square 3.51).

There was a tendency towards difference in Sniffing between breeds, with Holsteins sniffing proportionately more than Swedish Reds (p=0.0704; chi-square 3.27). There was also a tendency towards difference in proportion of Playing with Straw for gender, with females playing more with straw than males (p=0.0970; chi-square 2.75), and for gender*treatment, where C females played more than B females, while B males played more with straw than C males (p=0.0768; chi-square 3.13). Table 3 shows a summary of the statistical differences found in the detailed instantaneous observations.

Table 3: Summary of statistical differences found in detailed instantaneous observations

Behavior	**Mean proportion ± SEM**		**p-value**
	Control	**Brush**	
Individual play – Kicking	0.000693±0.000144	0.000106±0.000092	0.0343
Standing	0.01626±0.004255	0.03550±0.003343	0.0770
Head out	0.06727±0.01226	0.1240±0.01217	0.0641
	Holstein	**Swedish Red**	
Head out	0.1083±0.01025	0.07708±0.009188	0.0611
Sniffing	0.05266±0.004396	0.03583±0.004175	0.0704
	Female	**Male**	
Playing with straw	0.02867±0.003445	0.01979±0.004088	0.0970
	Control Female	**Brush Female**	
Playing with straw	0.03677±0.004125	0.02235±0.004256	0.0768
	Control Male	**Brush Male**	
Playing with straw	0.01393±0.004476	0.02811±0.008320	0.0768

Where: SEM = Standard error of the mean.

3.1.2. Simple instantaneous observations

A statistically significant difference was found between genders for the behavior Lying, where males were observed lying more often than females (p=0.0141); between genders and between treatments for Head Out, where females had their head out proportionately more than males, (p= 0.0264) and B calves had their head out proportionately more than C calves (p=0.0351). A tendency towards difference between treatments was found for Standing, where C calves displayed this behavior proportionately more than B calves (p=0.0925).

Table 4 shows a summary of the statistical differences found in the simple instantaneous observations.

Table 4: Summary of statistical differences found in the simple instantaneous observations

Behavior	Mean proportion ± SEM		p-value
	Female	**Male**	
Lying	0.5289±0.03640	0.6465±0.03447	0.0141
Head out	0.2391±0.03138	0.1543±0.02498	0.0264
	Control	**Brush**	
Head out	0.1574±0.02588	0.2349±0.02912	0.0351
Standing	0.005956±0.4283	0.002880±0.2077	0.0925

Where: SEM = Standard error of the mean.

3.2. Calf health

The calves were overall healthy during the experiment. The experimental farm at Lövsta has a very low calf mortality: of the 260 calves born at the farm in 2019, only two died before eight weeks of age (farm records). Nevertheless, additional hygiene measures were used to decrease even further the risk of diseases that could influence the outcomes of the experiment.

3.2.1. Physical examinations and daily inspections

All health checks were conducted in the morning after the behavior observations were recorded.

<u>General Health</u>

None of the calves presented noteworthy alterations on vital signs, posture, or alertness. There was variation in the shape and size of lymph nodes, but no calf presented hardened, painful, or hot lymph nodes on palpation (all scored 0).

Four of the calves in the experiment had mild health issues, but none of them required treatment. These are presented in Table 5.

Table 5: Description of health issues observed during the experimental period

Calf	Health issue
1 (Brush)	Prostration, slight dehydration (score 1) and fever one day after vaccination (rectal temperature 40.3^{0}C).
3 (Control)	Area of alopecia and erythema (no pruritus or discomfort observed) in the ventral thoracic area (Figure 5). More straw was added to her pen, the irritation of the skin decreased, and the coat regrew.
10 (Brush)	Pale mucosal membranes and occasional cough; no other significant alterations were observed.
14 (Brush)	Lump on the left side of the maxilla area (Figure 6). The lump was initially firm and painful on palpation and seemed to be related to the subcutaneous tissue. After a couple of days, the lump was no longer painful, it became softer and started decreasing in size.

Figure 5: Picture showing area of alopecia and erythema on ventral thoracic region of calf 3.

Figure 6: Red arrow points to lump on left maxilla of calf 14.

The total number of observations of feces for all calves was 32, with 21 of them scoring 0 and 11 scoring 1 (consistency of paste). Of the observations with score 1, 5 were on the first day of the experiment, 1 on the last day of the experiment, 2 on the day following vaccination, 1 on a day where saliva samples were collected, and 2 in control calves with no apparent difference on the day's routine. Figures 7 and 8 show the typical aspect of the feces during the experiment (score 0).

Figure 7 and Figure 8: Typical aspect of feces during the experiment (Score 0)

Physiological Parameters

The mean temperature of the calves on the first day of the experiment was 38.88±0.266 ^{0}C (mean ± standard error of the mean), while the mean temperature on the last day was 38.77±0.274 ^{0}C.

The mean respiratory rate (RR) for all the calves on the first day was 57±3.32 movements per minute (mov/min), while on the last day 37.13±2.77 mov/min. There was a statistically significant difference between both days (p-value 0.001 – paired t-test). However, when this comparison was made for each group of calves, the reduction in respiratory rate was more significant for B calves than C calves, as in B calves the RR on the first day was 62.00±5.55 and the last day 34.00±4.47, with a p-value of 0.009 compared to C calves, where the mean RR on the first day was 52.00±3.02 and on the last day 40.25±3.17 mov/min, with a p-value of 0.055.

The mean heart rate (HR) on the first day was 139 ± 2.86 beats per minute (bpm), while on the last day it was 135.25 ± 6.92 bpm. Although there was no statistically significant difference in a paired t-test between HR in the first and last days of the experiment when all calves were considered, there was trend towards difference when the comparison was made between the first and last days of B calves (p-value = 0.068, with HR on the first day being 138.5±4.72 and on the last day 122.75±5.46). Table five summarizes the statistical differences found for physiological parameters:

Table 6: Summary of statistical differences found in physiological parameters

Physiological parameter	First Day	Last day	p-value
Respiratory rate (mov/min) – all calves	57±3.32	37.13±2.77	0.001
Respiratory rate (mov/min) – B calves	62.00±5.55	34.00±4.47	0.009
Respiratory rate (mov/min) – C calves	52.00±3.02	40.25±3.17	0.055
Heart rate (bpm) – B calves	138.5±4.72	122.75±5.46	0.068

Where: B = Brush; mov/min = movements per minute; bpm = beats per minute

Blood

Blood from samples collected from all calves on the morning of the first and of the last days of the experiment was analyzed and white blood cells (WBC - neutrophils, eosinophils, lymphocytes, monocytes and basophils), red blood cells (RBC), hemoglobin (Hb) and mean corpuscular hemoglobin (MCH). Most samples were within the normal references used by the lab conducting the analyses (Jain, N, 1986), the exceptions are listed on Table 7.

Table 7: Blood samples with parameters not within normal ranges, by calf, experimental group, and day.

Blood parameter	Level	Calf (experimental group)	Day
WBC	Low	4 (B)	First day
	Low	2 (C); 4 (B); 10 (B)	Last day
RBC	Low	10 (B); 12 (C)	First day
	Low	12 (C)	Last day
Hb	Low	10 (B), 12 (C)	First day
	Low	1(B); 4 (B); 10 (B), 12 (C)	Last day
MCH	Low	1 (B); 10 (B)	Last day

Where: WBC = White blood cells; RBC = Red blood cells; Hb = Hemoglobin; MCH = Mean corpuscular hemoglobin

There was a significant statistical difference in samples collected on the first day of the experiment in WBC for gender (p=0.007), with females having a higher WBC count than males, and treatment (p=0.021), where Control calves had a higher WBC count than Brush calves; in Neutrophils for gender (p=0.008), where females had more neutrophils than males; in Eosinophils for breed (p=0.004), where Holsteins had more eosinophils than Swedish Reds and gender (p=0.025) where females had more eosinophils than males; and in Lymphocytes for treatment (p=0.006) where Control calves had a higher lymphocyte count than Brush calves. No significant differences were found for any of the parameters measured in blood samples collected on the last day of the experiment.

However, when the change in blood parameters between the first and last days of the experiment was compared, there was a higher tendency towards increase in lymphocytes (p=0.087) for B calves; towards different basophil profile for breed (p=0.091) with an increase in the number of basophils for H calves, and a decrease for SR calves; and towards a different RBC (p=0.088), where B calves showed an increase the mean number of RBC while C calves showed a mean decrease in RBC. There was also a significant difference in the decrease in MCH for gender (p=0.039), with females having a greater mean reduction than males. Table 5 summarizes the statistical differences found in blood parameters.

Table 8: Summary of statistical differences found in blood parameters

Physiological parameter	**Mean ± SEM**		**p-value**
First day			
	Female	**Male**	
WBC (cells/l)	10.428±0.641x10^9	7.621±0.649x10^9	p=0.007
Neutrophils (cells/l)	4.569±0.407 x10^9	2.851±0.412x10^9	p=0.008
Eosinophils (cells/l)	0.1135±0.188 x10^9	0.0494±0.0190x10^9	p=0.025
	Control	**Brush**	
WBC (cells/l)	10.095±0.658x10^9	7.954±0.596 x10^9	p=0.021
Lymphocytes (cells/l)	4.908±0.325x10^9	3.599±0.295 x10^9	p=0.006
	Holstein	**Swedish Red**	
Eosinophils (cells/l)	0.0279±0.0263 x10^9	0.13±0.0133x10^9	p=0.004
Last day (No significant differences were found)			
Change between first and last days			
	Control	**Brush**	
Lymphocytes (cells/l)	(↑) 0.116±0.357x10^9	(↑) 0.927±0.324x10^9	p=0.087
RBC (cells/l)	(↓) 0.772±0.616 x10^{12}	(↑) 0.626±0.559x10^{12}	p=0.088
	Female	**Male**	
MCH (pg)	(↓) 1.403±0.175	(↓) 0.864±0.177	p=0.039
	Holstein	**Swedish Red**	
Basophils (cells/l)	(↑) 0.0278±0.0258 x10^9	(↓) 0.0273±0.013 x10^9	p=0.091

Where: SEM = Standard error of the mean; WBC = White blood cells; RBC = Red blood cells; and MCH = Mean corpuscular hemoglobin; ↑ = increase; ↓ = decrease.

Weight Gain

The weight of C and B calves presented as mean±SEM on the first and last days of the experiment was 43.52±1.48 kg and 45.36±1.71 kg, and 51.79±1.30 kg and 53.17±2.07 kg, respectively. The wait gain for C calves was 8.262±0.463 kg and for B calves it was 7.813±0.738 kg. The average daily gain (ADG) for C calves was 0.7735±0.0444 kg and for B calves 0.7564±0.0742. No significant difference was found between experimental groups, genders, or breeds.

4. Discussion

In this experiment we were not able to confirm a link between tactile stimulation and improved health or a more natural play behavior of young calves. Neither the simple nor the detailed instantaneous observations reflected the initial expectation that brushed calves would be more playful, and the small number of calves in a period of life when their blood cells are in constant change makes it harder to draw conclusions about health and immunity, especially without a challenge. However, the experiment showed that the tactile contact with the calves decreased their fear of the experimenters, suggesting a positive HAI.

4.1. Behavior

The hypothesis was that brushed calves, having received more tactile and social stimulation would be more playful, inquisitive, active, and less fearful of humans. This, however, was not entirely what happened during the observation periods and the hypothesis was only partially accepted.

In the detailed instantaneous observations, the only behavior with a significant statistical difference between groups was Individual Play – Kicking, where C calves displayed a higher proportion of the behavior than B calves. Kicking is a type of locomotor play displayed by young animals in natural environments, especially when there is a restriction of space (Rushen & de Passillé 2014). Play is considered an indicator of good welfare, as it has been observed to decrease when animals are in pain or hungry and increase when they are in positive social environments (Größbacher et al. 2020). It was hypothesized that B calves would perform more play behaviors than C calves, as it has been shown that calves who had social interaction (albeit with cattle) were more playful and less fearful of restraint when compared to calves housed individually from birth (Duve et al. 2021). This hypothesis, however, could not be accepted for the observation periods. Yet this was different during the brushing period. An analysis of the recordings of the brushing sessions (Linder, 2020) showed that brushed calves played more frequently and for longer periods than control calves.

There was a tendency (meaning that it is likely that a significant statistical difference would have been found had a larger number of animals been used in the experiment) towards B calves displaying a higher proportion of behaviors standing and head out than C calves. This could suggest that B calves were more interested and had a shorter avoidance distance towards the experimenters compared to C calves. Additionally, in a parallel study from the same experiment, Linder (2020) found that B calves were standing and/or moving for a significantly longer periods of time than C calves, and they had a significantly longer latency to lie down during brushing sessions. Brushing is perceived as pleasurable by calves (Westerath et al. 2014) and stroked calves have been shown to have decreased fear and lower avoidance distance from humans (Probst et al. 2012), and zero avoidance distance towards the experimenter (Lürzel et al. 2016).

Holstein calves seem to be less fearful and more inquisitive than Swedish Red calves, as there was a tendency towards more Head Out and Sniffing behaviors for Holsteins. Female calves seem to be more inquisitive than males, as they showed a tendency towards displaying a greater proportion of the behavior of Playing with Straw than males. When that behavior was analyzed for gender and treatment together, the results were different, as C females tended to play more with straw that B females, while B males tended to play more with straw than C males. In a large review of studies on calf behavior in natural settings (Whalin et al. 2021) the authors found reports of female beef calves at pasture following their mothers more often than male calves, and male calves initiating more play behavior (such as mounting and pushing) than females. Interestingly, cows with female calves were faster to leave a start box to meet their offspring than cows with male calves, suggesting a stronger bond between cows and female offspring (Svensson 2022). Perhaps female calves also follow their dams to learn how to explore the environment, and this could explain the tendency we observed for females towards a higher exploratory behavior. Unfortunately, Linder (2020) did not separate the behaviors recorded during the brushing sessions by gender. It would have been interesting to see if she had also found differences in play and exploratory behavior by gender.

The simple instantaneous observations showed more significant differences between experimental groups. Male calves were observed Lying proportionately more than female calves, while females had their Head Out proportionately more than males. As mentioned previously, beef heifer calves followed their mother more often than bull calves in a free-ranging setting (Lidfors & Jensen 1988). Perhaps females seek more social interaction than males, which could explain the findings from this experiment. This could also reflect the result of an indirect genetic selection of both breeds (Holstein and Swedish Red) for behavior, as docile females are preferable for dairy production. When experimental groups were compared, B calves showed significantly more of the Head Out behavior than C calves. Again, this behavior could indicate that B calves were less fearful of the experimenters and displayed a shorter avoidance distance. There was a trend for C calves to be Standing proportionately more than B calves. Calves likely have competing motivations, with fear of humans on one side fighting with curiosity and exploration on the other (Hemsworth & Coleman 2011). When the experimenters entered the barn the calves were possibly interested and stood, but B calves and females took the extra steps forward and put their head out of the pen, decreasing the distance between themselves and the experimenters. This approach response displayed by the calves could be reflecting a positive affective state (Ede et al. 2019) and a good HAI.

Having animals that are not fearful of humans is important for the welfare of both cattle and stockpeople. Stockpeople have an important role in the welfare of the animals they care for. Finding ways to improve HAI can have benefits for farmers in terms of increased efficiency, productivity and health of animals as well as decrease in injuries resulting from fear (Rushen, Jeffrey et al. 2008) and improve job satisfaction. Modern production systems are becoming more technological, but animals still have some degree of interaction with humans. Farms with good HAI will have animals that can be more efficiently handled, and workers will have a more positive return for their input.

4.2. Health

The daily inspections focused on checking for signs of diarrhea or respiratory disease, which together with septicemia are the main health concerns of young dairy calves (McGuirk 2008; Torsein et al. 2011). Only calves that had been born in "clean" areas of the farm and when there had been no birthing problems entered the experiment. Additionally, sanitary measures, such as individual milk buckets and nipples, individual starter, hay and water buckets, individual brushes, and use of gloves and booties by experimenters entering the pens, were taken. The farm at Lövsta has a low calf mortality - 0.7% for calves up to 8 weeks, according to farm records, compared to the Swedish mortality rate of 3.1% up to 90 days of age (Svensson et al. 2006) - and not surprisingly, the experimental calves were overall healthy during the experiment. There were minor health issues, but none of the calves required treatment.

Physiological Parameters

The rectal temperature of the calves was within the normal range when the animals were examined in the first and last days of the experiment.

The RR was higher than normal in the first day and within the normal range in the last. A likely explanation is that on the first day the calves were over excited/stressed/fearful, not being habituated to humans. Interestingly, the reduction in RR between first and last days observed in B calves was more statistically significant than that for C calves, suggesting that B calves were likely less fearful of having a person approach their pen.

Conversely, the HR of all calves was above normal range (for animals at rest) on both days it was measured. A possible explanation for this elevation could be stress/fear of being touched on the first day for both groups of calves, and perhaps stress/fear for C calves and excitement for B calves on the last day. Nevertheless, HR reflects the arousal of the animal (von Borell et al. 2007), not necessarily the quality of their emotions as it may rise with negative or positive emotions.

Blood

We found some differences in blood count for breed, gender, and treatment on the first day of the experiment. Interestingly, this did not happen with samples collected on the last day of the experiment. However, when we compared the change that happened with each individual calf between both samplings, some trends were observed. Even though there was no significant statistical difference in the number of leukocytes, B calves tended to show a larger increase in the number of lymphocytes between the first and last days of the experiment compared to C calves. There was also a tendency towards difference for erythrocytes with B calves having a mean increase in RBC while C calves had a mean decrease in RBC. The MCH estimates the amount of Hb in red blood cells, and its decrease could suggest iron deficiency (Smith 2009). In our experiment, female calves had a greater decrease in MCH than male calves; even if most calves had hemoglobin (Hb) below 100g/dl on the last day, suggesting anemia. The incidence of anemia in young calves is high in many herds and does not seem to reflect on disease or growth rate of the calves (Smith 2009). This is naturally corrected when the calves increase their intake of solid feed.

The hypothesis of this experiment was that positive HAI and brushing would positively influence the health of the calves, but the number of calves in this experiment was too small to draw conclusions based on changes to the blood count, especially as the calves were all clinically healthy. Breed, gender, and ambient temperature (ježek et al. 2011), as well as animal excitement, muscular activity, time of sampling, nutrition and others (Schalm & Jain 1986) seem to play a role in the blood count of cattle. This is understandable as leucocytes in the blood are only a portion of the total population and many factors could trigger fluctuations (Roland et al. 2014). Also, there seem to be marked individual differences in the leukocyte count (Schalm & Jain 1986), meaning that results should not be the single basis of a diagnosis or prognosis (Roland et al. 2014), or in the case of the present experiment, causative conclusions. Calves have a high erythrocyte count at birth and then show a decreasing trend to the lowest levels at around 3 weeks of age (Brun-Hansen et al. 2006), after which the levels rise again. Hb and MCH are usually high at birth and decrease in the first month of age. The newborn calf has more neutrophils than lymphocytes, but the ratio changes in the first week of life (Schalm & Jain 1986; Roland et al. 2014), and the number of lymphocytes starts increasing so that at about 3 months of age lymphocytes represent 80% of the circulating leucocytes (Roland et al. 2014). Thus, it was interesting to find B calves showed a tendency towards increase in production of erythrocytes and lymphocytes (natural development of the species) possibly ahead of C calves.

Weight

No statistical difference was found between experimental groups in terms of weight gain, which is in line with the findings from a study about positive interactions in veal calves (Lensink et al. 2000). Conversely, in a study conducted with dairy heifers (Lürzel et al. 2015), calves that received positive interaction (including stroking) had a higher average daily gain compared to controls that received minimal interaction with people (interaction limited to farm routine). However, the experimental period in the dairy heifer study was longer (8 to 86.2 ± 5.1 days of age) than in the present study. It would be interesting to learn if positive interactions can influence the onset of rumination, and that, under a longer time span, could explain the increase in weight gain observed by Lürtzel *et al*. (2015).

4.3. General comments and suggestions for future research

This experiment raised interesting questions that could be addressed in future studies: 1) Could tactile stimulation and social contact help trigger an earlier development of blood parameters? A larger number of animals would be needed to test this, especially as the blood cell profile of calves is changing at this age. An immunological challenge could also provide insightful information about the influence of tactile stimulation on health and immunity. 2) Could brushing trigger an earlier onset of rumination compared to calves that were not brushed? We observed that some of the calves started ruminating during the experimental period. Not surprisingly, as this seems to be the age in which calves with access to pasture start ruminating (Whalin et al. 2021). It could be interesting to learn if brushing and/or social contact (HAI) stimulated the development of the calves' digestive system – licking by the cow has been linked to faster urinating and defecating of neonatal calves (Metz & Metz 1986). No data on rumination was collected during this experiment, but perhaps further studies could investigate if brushing or social contact (HAI) could influence the onset of rumination. Earlier rumination could improve the uptake of nutrients (including iron) in the feed and stimulate the immune system. If brushing leads to earlier rumination, perhaps this could be an explanation for the increase in the number of erythrocytes and lymphocytes observed in B calves.

One of the limitations of the present study was that with the manual brushing we could not separate social interaction from physical/tactile stimulation, so we can't know if the results we found are due to the social interaction between the calves and the experimenters, the physical action of the brush on the calf triggering physiological reactions or a combination of those. Perhaps a future study could add different experimental groups, such as one in which calves have no contact (visual, auditory, olfactory or tactile) with the experimenters, and one where the tactile stimulation is provided by mechanical brushing, thereby removing the influence of social interaction (HAI). Another limitation was the small number of animals in the study. It was not viable operationally, economically, and even ethically to have more animals in the experiment. Nevertheless, it provided some insights into interesting directions for future studies on the subject.

There is some research, mainly from Asia, trying to find markers that could link positive emotions to increased resistance to stressors. One of these markers is the expression of heat shock protein (hsp). The enhanced expression of hsp70 observed in farm animals that had positive interactions with humans seems to be associated with increased resilience to environmental stressors and disease (Zulkifli 2013). It could be interesting to assess these markers in future studies of tactile stimulation and positive welfare of calves. Farm animals will occasionally experience some sort of negative interaction with humans or stressful event, such as vaccination, medical procedures and even mixing of groups. Therefore, it is important to study how their environment and HAI can increase their resilience to these stressors and improve their welfare.

It is becoming increasingly clear that the welfare of stockpeople and farm animals go hand in hand. The concept of One Health (World Health Organization-WHO, 2021) recognizes that people, animals, plants, and the environment they share are all interconnected. The One Welfare framework (Pinillos 2018) adds to the One Health as it acknowledges the interdependency between the welfare of humans, animals, and environment, and encourages a multidisciplinary approach to research in the field of agriculture and animal production. More research is needed to help improve the quality of life, productivity, and sustainability in our farms, but the future looks promising and exciting when envisioning all possibilities that could rise from collaboration between different areas of expertise.

5. Conclusions

Brushed calves were less fearful of humans compared to control calves, although the latter displayed more of a play behavior, Kicking, during the observation periods than the former. There was some indication to suggest that, in terms of blood cell profile, brushed calves could mature earlier. However, more studies are needed to verify if brushed calves become physiologically mature ahead of controls.

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8. Appendix – Popular scientific summary

What happens with the behavior and health of a baby dairy calf when it gets a cuddle?

Consumers are increasingly concerned about the conditions in which farm animals are raised. Much is said about animal abuse and the consequences of negative human-animal interactions to the welfare of production animals. But what happens when the animals are well cared for? What happens when animals have a chance to experience positive emotions? Will that influence their health? Will it influence their behavior? In this experiment we wanted to know if a positive interaction with a human, offered by means of a tactile stimulation (brushing), would affect the behavior and/or health of young dairy calves.

Cows lick their calves from the moment they are born, as well as their preferred animals in the herd (allogrooming). That is considered part of social interaction repertoire for the species. Previous research has shown that brushing is perceived as positive by calves. We chose brushing, simulating the cow licking its calf, as the method to provide positive interaction between a human (experimenter) and a young calf.

So, we divided a group of 16 young calves housed in individual pens from birth into two groups: one that would be brushed 30 minutes twice a day for ten days, and one that would be in the same barn, but not brushed. We examined the animals for health and collected blood samples on the first and last days of the experiment. We observed the behavior of all calves four times daily, before and after each brushing session.

The calves that were brushed were less fearful of the experimenters compared to the calves that only had visual contact with them. This was shown by their behavior (coming forward towards the experimenter) as well as physiological markers, such as respiratory rate and heart rate. Animals that are not afraid of humans will likely be more efficiently handled, more productive, healthier and have a lower risk of getting injured or injuring someone. This is important not only for the welfare of the cattle, but of the stock people as well. Nevertheless, the calves that were not brushed showed more of one play behavior (kicking) during the observation periods. Play behavior is considered a marker of good welfare, as scared, hungry, or sick calves do not play. However, a parallel study analyzing the video recordings of the brushing sessions found that the brushed calves actually moved more, explored more the pen, and played more than the non-brushed calves. This suggests that the play behavior of the brushed calves was displayed when they had the physical/social contact with the experimenters.

All calves were overall healthy during the study. We found some indication that calves that were brushed had a blood cell profile physiologically maturing ahead of the calves that had no physical contact, but that would have to be tested in another experiment with a larger number of animals. A follow-up to this study could also be to investigate if brushed calves start ruminating before calves that are not brushed. Further research is needed to explore these interesting and important topics for the welfare of dairy calves and of the humans who care for them.