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A calm companion lowers fear in groups of dairy cows

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ABSTRACT

Dairy cows are generally calm and compliant, but some management procedures can make cows fearful or stressed. Not only are fearful cattle a threat to human safety, but fear is also detrimental to animal welfare and productivity. This study aimed to test whether fear in small groups of dairy cattle could be attenuated by the presence of a calm and experienced companion. Twenty-seven dairy cows from a Swedish agricultural school participated in the study. The study included a standardized fear-eliciting stimulus, which was 3 sudden, repetitive openings of a red and white umbrella. Demonstrator cows (n = 9) were selected based on age to ensure that all demonstrators were older than the naïve test cows (n = 18). Of these 9 demonstrator cows, 6 were selected as untrained (i.e., habituated to the presence of the test person) and 3 were selected as trained demonstrators (i.e., additionally habituated to the fear-eliciting stimulus). The remaining 18 test cows comprised 6 test-cow groups of 3 cows each, which were their own controls, resulting in a crossover design; 3 groups were tested with a trained demonstrator first and then with an untrained demonstrator, and vice versa for the other 3 groups, resulting in a total of 12 trials (4 sub-treatments). Response variables were heart rate increase from baseline, behavioral reaction indicative of fear, and latency to resume feeding after exposure to the fear-eliciting stimulus. The study found a calming effect of a trained demonstrator on test cows' heart rate but not on latency to resume feeding or behavioral reaction. Post hoc analyses revealed a carryover effect on latency, indicating that test cows who were accompanied by an untrained demonstrator first had longer latencies than cows in all other sub-treatments. Adding a calm, experienced cow to groups of dairy cattle may mitigate fear and thereby improve welfare and safety.
Key words: social cognition, welfare, cattle management, habituation, farm safety

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INTRODUCTION

Injuries inflicted on humans and animals are a worldwide challenge in dairy production (e.g., Erkal et al., 2008; Douphrate et al., 2013; Lindahl et al., 2016). Animal-related accidents account for an estimated one-fourth of all occupational accidents in Swedish agriculture (Pinzke and Lundqvist, 2007); on average, one death per year occurs when handling cattle. In the United States, Layde et al. (1996) reported that cattle were involved in the majority of animal-related injuries, and the risk of livestock handling injuries was increased on larger operations (>10 workers; Douphrate et al., 2006). With the current trend toward increasing farm size worldwide (Barkema et al., 2015), this incident rate is expected to rise. Dairy cows are usually calm and docile, but when spooked or stressed, they often react unpredictably and violently (e.g., flight, backing, kicking and charging; Boivin et al., 1994; Müller and von Keyserlingk, 2006; Mazurek et al., 2011), and thus become dangerous to handle, imposing a threat to human safety (Grandin, 1999). Not only are fearful cows a threat to human safety, but fear is also detrimental to their welfare and productivity (Hemsworth and Coleman, 1998). On a larger scale, this means that fear-eliciting situations can affect the sustainability of dairy production through lower productivity (i.e., lower growth, yield, and reproduction), which subsequently increases the risk of culling, and thereby reduces the longevity (shorter life in the herd) of the dairy cow (Hemsworth and Coleman, 1998; Hemsworth, 2003; Mota-Rojas et al., 2020a). Hence, there is great interest in identifying efficient ways of reducing fear in dairy cows from the perspectives of human safety, animal welfare, and sustainability.

The literature suggests that fear reactions are generally socially contagious (e.g., Griffin, 2004). Under natural circumstances, reacting with fear toward stimuli that other herd mates have been fearful toward could save your life, and is likely an evolutionarily stable strategy (Griffin, 2004; Cooper and Albentosa, 2005). Fear reactions can, however, also be socially attenuated (e.g., horses: Christensen et al., 2008; Rørvang et al., 2015, sheep: Da Costa et al., 2004), which is also likely an adaptive strategy. Although studies on cattle are limited, studies on calves show that socially housed calves are less reactive toward social and environmental novelty (De Paula Vieira et al., 2012) and show less food neophobia (Costa et al., 2014). Results on heifers additionally suggest that heifers benefit from an experienced companion when learning how to graze (Costa et al., 2016). The mere presence of a calm companion has also been found to lower fear in naïve heifers through social buffering, allowing the naïve individual to moderate its response to fear-eliciting stimuli (Boissy and LeNeindre, 1990). Boissy and LeNeindre (1990), however, tested cows at a demonstrator-to-observer ratio of 1:1, meaning that for every one inexperienced cow, one trained (i.e., experienced/calm) companion would be needed, and it is currently unknown whether an increase in the observer-to-demonstrator ratio affects the transmission of information as, for example, shown in pigeons (Lefebvre and Giraldeau, 1994). In practice, it would be more feasible to make sure that a few cows can handle a particular situation and then calm the rest of the group, compared with training half of the cows. In horses, inclusion of adults in groups of newly weaned foals lowered behavioral and physiological measures of stress (Erber et al., 2012; Henry et al., 2012), and, in practice, it is common to include older and experienced horses in groups of younger and naïve horses. Thus, the potential may exist to explore this vertical transfer of safety information in cattle, which is currently unknown.

This study aimed to investigate whether fear in groups of young dairy cows can be attenuated by the presence of a calm companion, with a demonstrator-to-observer ratio of 1:3. The hypotheses were that cows tested with a trained, calm demonstrator would (1) react with milder behavioral reactions when exposed to a fear-eliciting stimulus, (2) have lower heart rates (**HR**) during the exposure, and (3) have shorter latencies to resume feeding after the exposure, compared with cows accompanied by an untrained demonstrator.

MATERIALS AND METHODS

Ethics

The details of the experiment were evaluated and approved by the Board for Animals in Research and Teaching at Swedish University of Agricultural Sciences (SLU), Sweden (permit ID number: 5.8.18–06784/2020). Of the 3 human experimenters who participated in the study, 2 had an education in responsible use and treatment of animals used in research and functioned as supervisors for the third experimenter (MSc student). All procedures and care for the animals complied with national legislation on animal experimentation by the Swedish Ministry (Act no L 150 29 March 2019; SJVFS, 2019); met the ARRIVE guidelines (Kilkenny et al., 2010); and complied with the ethical guidelines proposed by the Ethical Committee of the International Society of Applied Ethology (Duncan et al., 2013). The director of the agricultural school and the staff involved were informed orally about, and agreed to, the details of the study.

COVID-19 Precautions

This experiment was carried out during the summer of 2021, and the COVID-19 pandemic was still ongoing. Of the 3 experimenters involved in the study, one came from Denmark to Sweden, and was tested by PCR before entry to Sweden (with a negative result). When on farm, the experimenters wore only clean clothes and disinfected boots. Hands were washed and disinfected regularly during training and testing, and the experimenters maintained at least a 1-m distance from all other staff on the farm.

Animals and Treatment Groups

Twenty-seven dairy cows participated in the study. All cows were residents of the agricultural school Naturbruksskolan Uddetorp (Skara, Sweden), and hence were familiar with each other. The oldest cows of the herd were selected as demonstrator cows (n = 9, mean age = 5.8 yr, range = 4–9 yr), and the youngest cows of the herd were selected as test cows (n = 18, mean age = 2.5 yr, range = 2–3 yr). This selection was done to ensure the naïve test cows were at least 2 yr younger than the demonstrators, because attenuation of fear (i.e., oblique social transmission or transmission of information from adults to unrelated juveniles; Cavalli-Sforza et al., 1982) is affected by age, with older and more experienced adults functioning as repositories of social information (McComb et al., 2001). The cows were a mixture of Swedish Holstein (n = 13), Swedish Red (n = 12), and Jersey (n = 2), and the uneven distribution of breeds reflected availability on the farm. All cows were in different stages of lactation, and cows that had recently calved were given at least 1 wk to recuperate before joining the experiment, and cows expected to calve within 1 wk from the day of testing were excluded. In addition to grass from pasture, cows had access to a partial mixed ration provided indoors (ad libitum), with extra concentrate based on the individual production. Water was also available ad libitum.

The naïve test cows were divided into 6 test cow groups, each consisting of 3 cows. The group size (n = 4; 3 test cows plus 1 demonstrator cow) was chosen based on a previous horse study (Rørvang and Christensen, 2018) to have comparable results. The groups were balanced according to age, lactation number, breed, kin (no related individuals were tested together), and previous treatment in the preceding study (J. Stenfelt, Y. Yngvesson, and M. V. Rørvang, unpublished data). After the first trial, one test cow (Swedish Red, 2 yr old) came into heat and had to be excluded from the second trial due to mounting behavior and safety concerns. In order to maintain a demonstrator to observer ratio equal to 1:3, a replacement cow (Swedish Holstein, 3 yr old) participated in the second trial. The demonstrator cows were either trained (n = 3) or untrained (n = 6) (for training criteria, see section "Preparation and Training of Cows"). The naïve test cows were their own controls, and each test cow group was tested once with a trained demonstrator and once with an untrained demonstrator. Each untrained demonstrator cow was tested with a naïve test cow group only once to avoid any habituation in subsequent testing. Each trained demonstrator was tested with a naïve test cow group twice as each trained demonstrator was trained to never react during testing. The order in which the groups were tested (i.e., with the trained or untrained demonstrator in the first trial and vice versa in the second trial) was balanced to account for potential effects of repeated exposure and previous experience. This resulted in a crossover design with a total of 36 exposures over 18 test cows, 12 trials, and 4 sub-treatments: untrained A (n = 9) → trained B (n = 9), and trained A (n = 9) → untrained B (n = 9).

Experimental Arena

The experimental arena was constructed in a designated area of the cows' normal pasture (Figure 1). The runway (35 m long) led the cows from the indoor barn to the start box (approximately 27 m^2, Figure 1), from where each cow group could be led collectively into the experimental arena (approximately 288 m^2, Figure 1) in a standardized manner. The runway had a mixture of sand and wood chips, constructing a nonslippery bedding, when leading cows to and from the barn. The start box and experimental arena mainly had a bedding of dry dirt. Inside the test arena, the 4 yellow feed buckets (volume: 26 L, height: 30 cm, diameter: 39 cm, Tubtrugs; Red Gorilla) were placed in a half-circle, each 2 m away from the test person (i.e., 1.5 m away from the fear-eliciting stimulus) and approximately 1.1 m apart. A video camera on a 1.5-m-high tripod was placed outside the fence and recorded all trials. The height of the yellow feed buckets allowed the cows to see above the edge when feeding from the bucket (Figure 2A).

Preparations and Training of Cows

Three test persons were part of the experiment. These persons were not part of the usual on-farm staff but were familiar to all cows, and all cows were used to being handled and touched by the 3 individuals. Before this experiment, all cows participated in a social-learning study where they were trained to associate the yellow feed bucket with food (J. Stenfelt, Y. Yngvesson, and M. V. Rørvang, unpublished data). This training was used in this experiment, and all cows were pretested to ensure they complied with a pretest criterion: walking directly to the yellow feed bucket upon release into the arena and feeding from the yellow feed bucket for at least 30 s. In addition to the pretesting mentioned above, the untrained demonstrator cows (n = 6) were habituated to the arena and the presence of the test person standing in the half-circle (Figure 1); the trained demonstrator cows (n = 3) were additionally systematically habituated or desensitized (Pearce, 2008) to the fear-eliciting stimulus: 3 sudden, repetitive openings of a red and white umbrella (Supplemental Video S1; https://osf.io/uwk8z/; Rørvang, 2022). The habituation process was done by exposing the cows to the umbrella sufficiently far away to not elicit any fear reaction. The cows could then approach the umbrella, which was moved away, until reaching their yellow feed buckets. This was repeated until all demonstrator cows could reliably approach the umbrella while it was opened repeatedly. The procedure required a maximum of 3 repetitions. The colors of the yellow feed bucket and the red and white umbrella were chosen, based on knowledge of cattle color vision (Dabrowska et al., 1981; Jacobs et al., 1998), to ensure that neither blended in with the surrounding colors (brown/gray dirt and green vegetation). The habituation criterion for untrained demonstrators was approaching and feeding from the yellow feed bucket for a minimum of 30 s immediately after release into the arena with the test person present. The habituation criterion for trained demonstrators was the same as for untrained demonstrators, but, in addition, showing no behavioral reaction to the fear-eliciting stimulus (i.e., flight reaction, backing, vocalizations or lifting the head; Supplemental Video S1).

In addition to this training, cows were habituated (Pearce, 2008) to wearing an elastic girth with wireless HR sensors (Polar equine H10). All 3 test persons participated in this habituation process. From 1 to 3 d before testing, each cow would be trained inside the

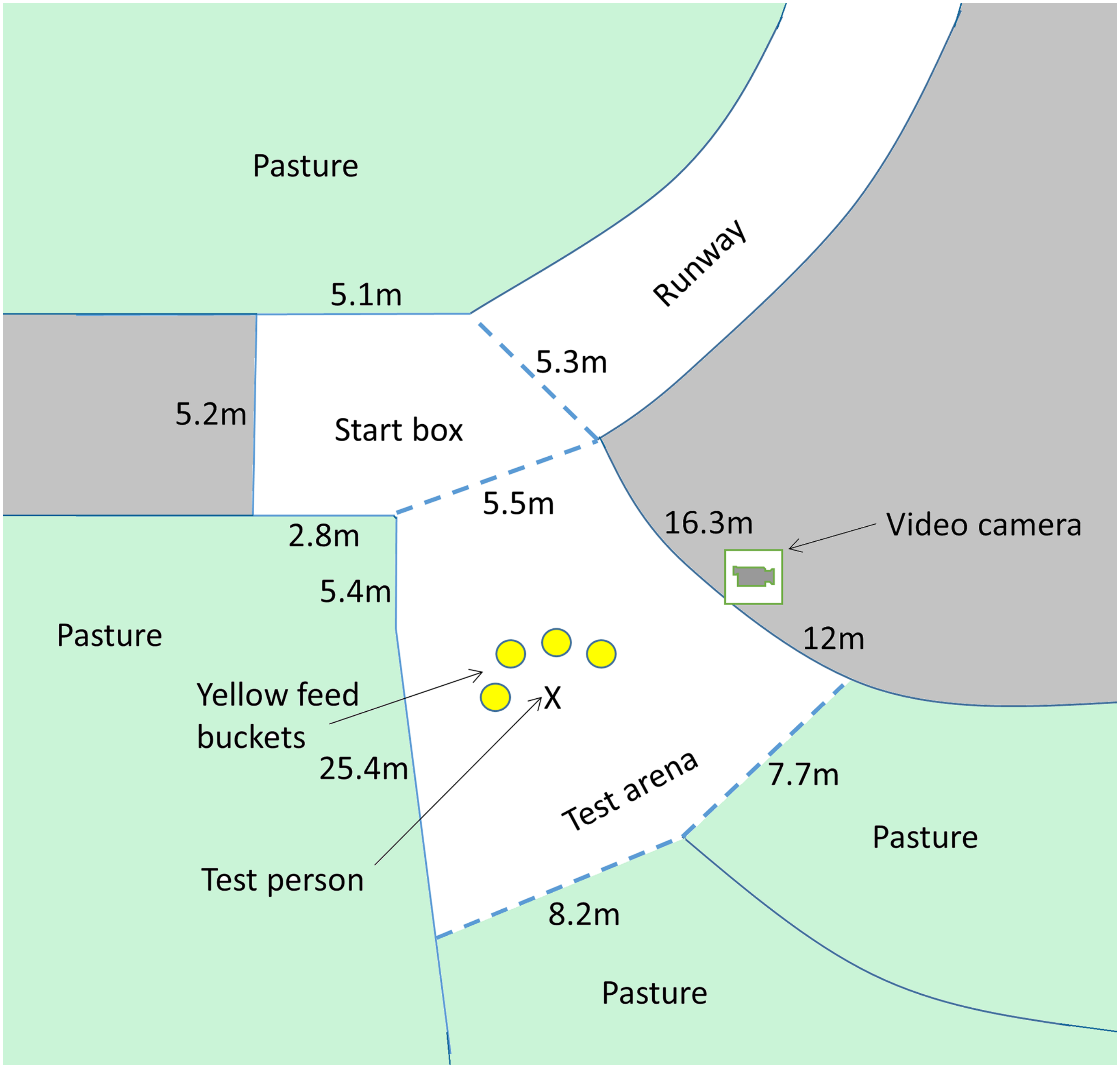

Figure 1. Top view of the experimental venue. The venue was invisible to all other cows, both those on pasture and those in the stable (not included in the experiments). The runway led the cows to the start box, from where they were ($n = 4$, each) led into the test arena as a group. In the arena, the 4 yellow feed buckets were placed in a half-circle 2 m away from the test person. A video camera on a tripod (height: 150 cm) was placed outside the fencing and recorded each test. The dashed blue lines illustrate where the fence could be opened. When a test had ended, the cow group would be led out to pasture, out of sight of the next cow group.

barn, either standing on the slatted floor or inside a cubicle, depending on her own preference. More hesitant and shy cows were trained from a cubicle with a rope on the back of the rails to keep the cow inside the cubicle for safety reasons. The first step of the habituation process was to scratch the cow on her back, stomach, and behind the shoulder blade, where the girth would subsequently be placed (Figure 2B). Second, the girth was placed on the cow's back and fastened loosely around her belly. The third step was to tighten the girth gradually and fasten a wristwatch receiver to the girth. The cows were given time to habituate to

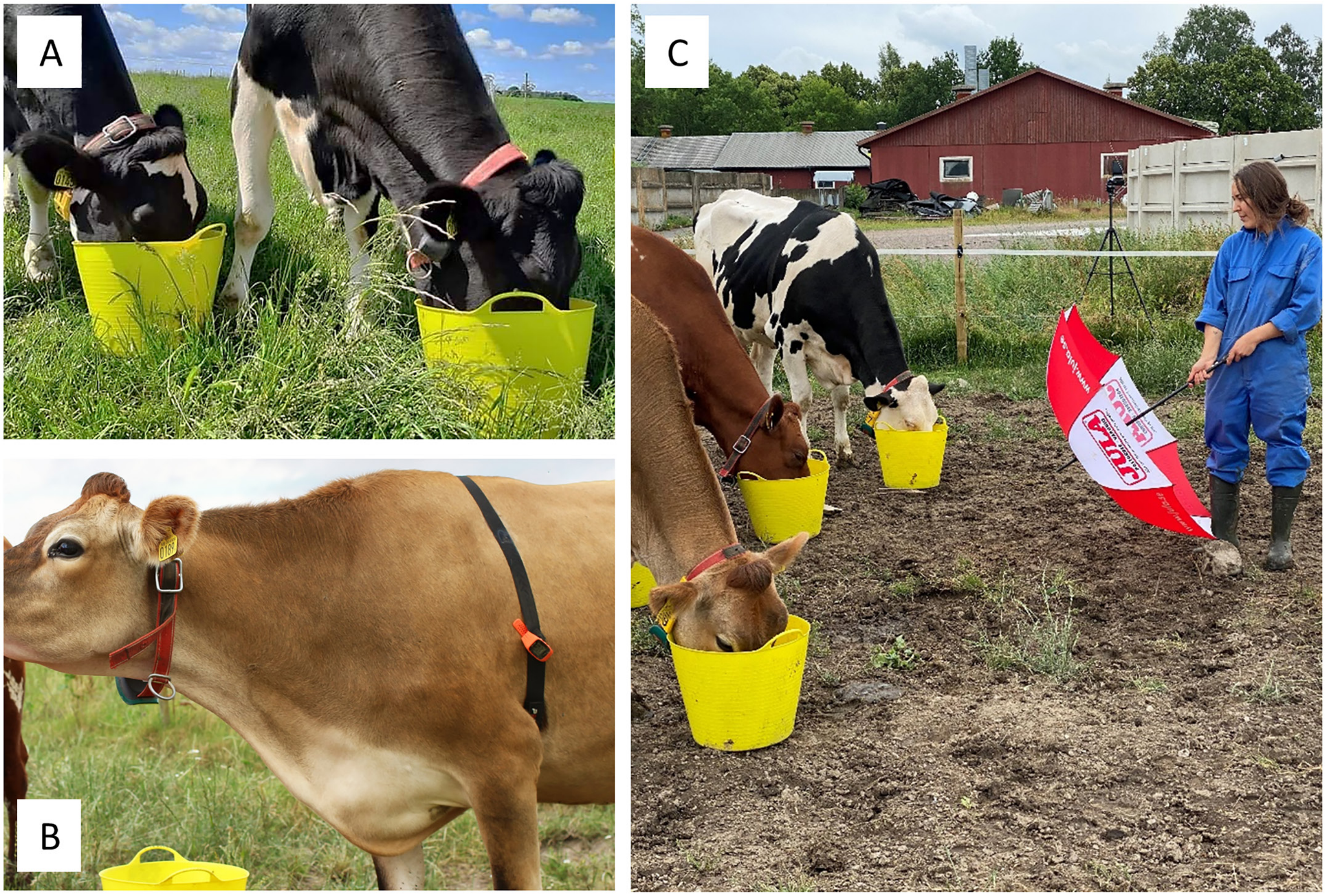

Figure 2. (A) The yellow feed buckets (volume: 26 L, height: 30 cm, diameter: 39 cm) that allowed the cows to look over the edge while feeding. (B) A cow wearing a heart rate monitor, attached by an elastic girth with electrodes behind the left shoulder and left elbow, and a wristwatch receiver attached to the girth (orange clock). (C) The fear-eliciting stimulus—the red/white umbrella was opened and closed 3 times in a row, after which it was kept open by the test person for the remainder of the test (2 min). The video camera can be seen in the background (behind the fence), with cows feeding from the yellow buckets in a half-circle (all ~1.5 m away from the umbrella).

the girth, after which it was calmly removed. On the test day, additionally, lukewarm water and ultrasound gel (Apotekets Klinik Eksplorationsgel, Apoteket) were applied on the areas where the HR sensors were situated: behind the shoulder blade and behind the elbow, to optimize conductance between skin and electrodes. After this, the cows would have at least 15 min (mean = 37.4, range = 15–46 min) wearing the HR monitor before the test commenced.

Testing

Testing was conducted over 3 consecutive days. Each test session started after either the morning milking or afternoon milking, with a midday break to avoid testing during the midday heat. Testing in the first half of the day (0900 to 1200 h) and the second half of the day (1730 to 2030 h) was balanced between treatments, and none of the groups were tested twice on the same day. During testing, the ambient outdoor temperature was (mean ± SE) 20 ± 3°C, and the weather conditions were either partly cloudy or sunny. At the start of each test session, the farm staff would assist in sorting the cows to be tested from the rest of the herd in the indoor barn, following normal procedures used on the farm to avoid stress. The test group was separated from the herd in the section closest to the outdoor access and remained in visual and physical contact with the rest of the herd when indoors. The group had access to feed and water ad libitum and to cubicles (1 per cow). After separation, the groups had 15 min to adjust to the separation before being fitted with the HR monitors (see section "Preparations and Training of Cows"). One HR monitor per test cow (and no HR monitor on the demonstrator cow due to limited equipment) was fitted. The fitting of all HR monitors was performed by

Table 1. The immediate behavioral reaction during exposure to the fear-eliciting stimulus was scored on a scale from 0 (no reaction) to 3 (flight reaction)

Score	Code	Description of immediate behavioral reaction
0	None	No reaction
1	Head up/vigilance	Raises head, may interrupt eating, no steps[1]
2	Back	As for head up, but includes backing away from bucket, maximum of 3 steps[1]
3	Flight	Flees from the bucket (run or walk) abruptly, more than 3 steps[1]

[1]Modified for cow behavior from Rørvang et al. (2018).

the same test person. After fitting of the monitors, the specific cows to be tested were led out into the outdoor runway with their respective demonstrator cow (either a trained or an untrained demonstrator; Figure 1). The group was then led through the runway (distance: ~35 m) to the start box, where they waited until the test person was in position behind the 4 yellow feed buckets in the test arena. The group was released collectively into the test arena and went directly to the yellow feed buckets (as tested during pretesting, distance: ~16 m). After feeding for 30 s and when all cows in the group had their heads in the buckets, the fear-eliciting stimulus was released; that is, the umbrella was opened 3 times in a row (Supplemental Video S1) and then left open for the remainder of the trial (Figure 2C). The umbrella opening was done by an experienced person (umbrella opening was trained before the experiments to ensure consistency and uniformity) who was not blind to the treatments (as the reaction of the demonstrator cow was visible). The total duration of the trial was 2 min (as per Rørvang and Christensen, 2018) from the first opening of the umbrella. The opening of the umbrella was performed by the same test person as during the habituation process. After the test ended, the group was collectively led out on pasture, out of sight from the test arena.

Data Editing and Behavioral Observations

The experiment was recorded on video for later extraction of immediate behavioral reaction (Table 1) and latency to return to the feed buckets (in seconds). Cows that did not return to the feed buckets within the trial duration (2 min) were assigned a latency of 120 s [n = 1, from the first untrained group (untrained A) and n = 1, from the second untrained group (untrained B)]. Heart rate was recorded with Polar Equine RS800CX (R–R recordings; Polar Electro Oy), which consisted of an Equine Wearlink with a W.I.N.D. transmitter (Polar H10) and a wristwatch receiver [Polar M430 (n = 1) or Polar Unite (n = 2)]. This HR monitoring system was originally developed for horses but older versions have been validated on dairy cows (Hopster and Blokhuis, 1994). The data were downloaded using the online software Polar Flow (https://flow.polar.com). Because of recording errors, likely caused by low conductance between electrode and skin, 5 HR files were lost: 1 from trained A, 1 from trained B, 1 from untrained A, and 2 from untrained B. Demonstrator cows were not included in the analysis; thus, the total sample size was 18 cows for each treatment, of which half were repetitions (as each cow was tested with both treatments; total n = 36). The maximum HR during exposure to the fear-eliciting stimulus was determined for each cow. A baseline HR was also determined for each cow in the period preceding the trial (i.e., inside the barn). This baseline mean HR was measured over 10 min, at least 5 min after the equipment was fitted, and before cows were led out from the barn. In this period, the cows were standing or walking inside the barn to simulate more or less normal conditions. The difference between baseline HR and maximum HR during the trial was used to calculate the spike in HR following exposure to the fear-eliciting stimulus, resulting in the response variable "HR_diff," which was used in the analysis.

Statistical Analyses

All statistical analyses were performed using the R software, version 4.1.0 ("Camp Pontanezen"; R Foundation for Statistical Computing, 2021). All *P*-values were evaluated as significant according to a significance level of 5% and for tendencies at 10%.

Behavioral reactions were analyzed in an ordinal mixed-effects model as the response variable was ordinal (0, 1, 2, or 3) using the adaptive Gauss-Hermite quadrature approximation using the package "ordinal" (Christensen, 2015). Treatment (categorical variable: trained vs. untrained demonstrator) was included as a fixed factor, and group (categorical variable with levels 1–12) as well as cow ID (categorical variable) were included as random factors to account for repeated measures of each cow. A control parameter was also included [control = clmm.control(useMatrix = T)] to calculate the variance-covariance matrix and ensure an optimized method to find estimates of the parameters.

Latencies and HR (HR_diff) were analyzed in mixed-effect models for normal data using package "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017). The models included the same fixed and random effects as for behavioral reaction. Analysis of variance was used to extract the overall effect of treatment in all 3 models.

Because of the high correlation between treatment and demonstrator reaction (the trained demonstrator never reacted and the untrained demonstrator reacted in all but one trial), demonstrator reaction was not included in the analyses. The nature of the crossover design posed a high chance of a carryover effect between treatments (from trained to untrained and vice versa). A post hoc analysis was thus carried out to elucidate whether any of the sub-treatments differed (untrained A, trained B, trained A, and untrained B). New models were fitted for all 4 response variables using the same method as previously described, but with a new fixed effect variable for treatment (now: categorical variable with 4 levels: untrained A, untrained B, trained A, trained B). Pairwise comparisons from these new models were performed using contrasts (package "emmeans" by Lenth, 2021). These pairwise comparisons were chosen to test whether the effect of having a trained or an untrained demonstrator, respectively, in the first trial affected the response when tested subsequently with the opposite treatment; that is, (1) whether cows exposed to an untrained demonstrator in their first trial reacted more fearfully in the next trial despite the presence of the calm companion, and (2) whether the effect of a calm companion in the first trial diminished in the subsequent trial because of exposure to the fearful companion. Last, the post hoc pairwise comparisons were used to compare the fearfulness level from each sub-treatment.

All of the models were run both with and without the cow that came into heat as well as the cow who replaced her. As there were no differences in the results, both cows were kept in the final models. Last, the behavioral reaction (converted to a numerical scale of 0–3; see Table 1) and mean and maximum HR were analyzed using Spearman correlation analyses to investigate the association between the behavioral fear reaction and increase in HR.

RESULTS

Behavioral Reaction

None of the trained demonstrator cows reacted during the test, but the untrained demonstrator cows reacted in all but one trial (otherwise, reactions ranged from 1 to 3, median = 2.25). The immediate behavioral reactions of the test cows are summarized in Figure 3A and B, divided both by overall treatment and by sub-treatment. There was large variation but only test cows with a trained demonstrator cow received the score 0 (no reaction). Four cows sniffed the umbrella after being exposed to the opening, 1 from the trained A group, 1 from untrained A group, and 2 from the trained B group. Sample videos illustrating the immediate behavioral reactions according to treatment can be found in the supplemental files (Supplemental Video S2, with trained demonstrator cow, and Supplemental Video S3, with untrained demonstrator cow; https://osf.io/uwk8z/; Rørvang, 2022). The fitting of the ordinal model showed that treatment had no overall effect on the immediate behavioral reaction (ordinal mixed-effects model: estimate ± SE = −0.82 ± 0.69, z-value = −1.20, $P = 0.19$). The post hoc pairwise comparisons (using the fitting of the same model but with sub-treatments as fixed effect) showed that none of the sub-treatments had any effect on the immediate behavioral reaction (Figure 3B).

Latency to Resume Feeding

The analysis of latencies showed that treatment had no overall effect on the latency to resume feeding after being frightened (Gaussian mixed-effects model: $F_{\mathrm{df}} = 1.71_1$, $P = 0.24$). The post hoc pairwise comparisons, using the sub-treatments as fixed effect in the model, showed that latency to resume feeding was significantly higher for the cow groups that were tested with an untrained companion in the first trial (Table 2). The carryover effects are shown in Figure 4.

HR Increase

Heart rates generally increased from baseline during trial (Figure 5). The model fitting of HR (HR_diff) showed that treatment had a significant overall effect on the increase in HR during the trials (Gaussian mixed-effects model: $F_{\mathrm{df}} = 4.60_1$, $P = 0.047$). The post hoc pairwise comparisons, however, showed that sub-treatment had no effect on the HR spikes (all P-values >0.1, Figure 6). The behavioral reactions were not correlated with mean or maximum HR during the trials (Spearman correlation: $r_{\mathrm{HRmean}} = 0.28$, $P_{\mathrm{HRmean}} = 0.29$, $r_{\mathrm{HRmax}} = 0.28$, $P_{\mathrm{HRmax}} = 0.31$) or with the increase in HR (Spearman correlation: $r = 0.22$, $P = 0.23$).

DISCUSSION

This study aimed to investigate whether fear in groups of young dairy cows could be attenuated by the presence of a calm companion, with a higher demon-

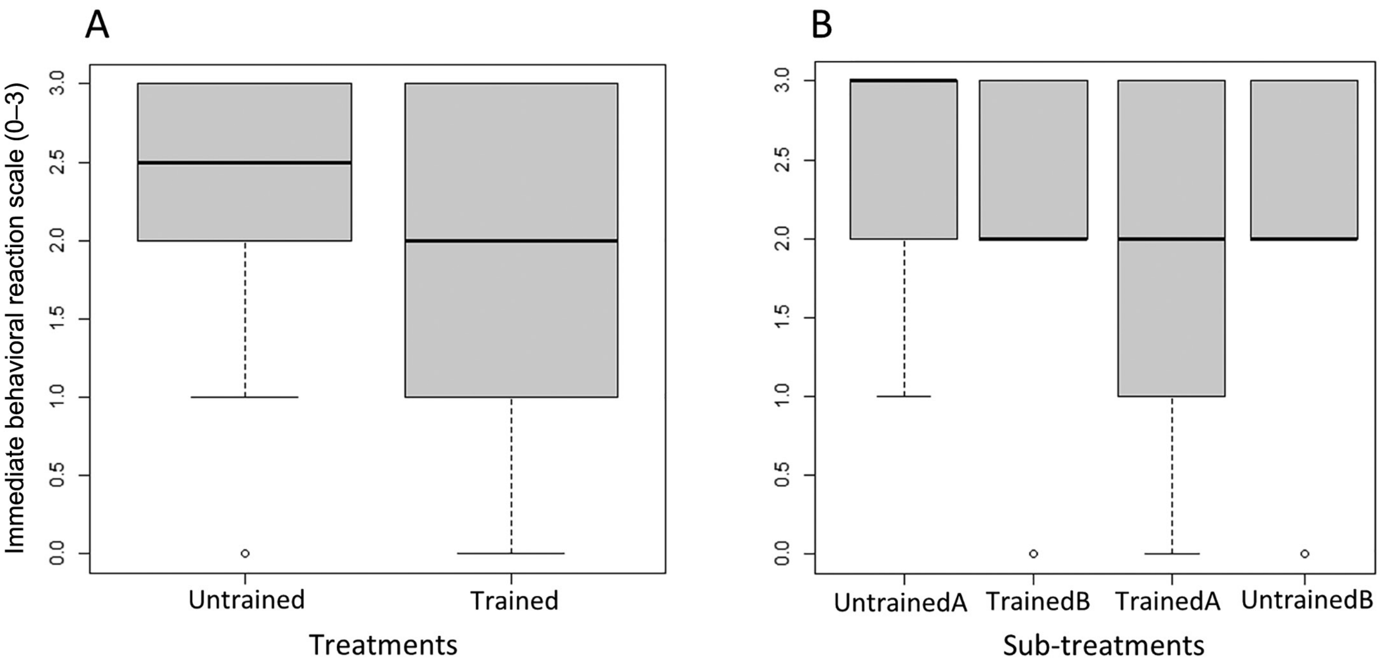

Figure 3. Boxplots of immediate behavioral reactions on a scale (see Table 1) from 0 (no reaction) to 3 (flight reaction) shown by (A) treatment: untrained (untrained demonstrator cow) and trained (trained demonstrator cow), and (B) sub-treatment: untrained A, trained B, trained A, and untrained B (see section "Treatments"). The boxes represent the 25% and 75% quartiles, the black lines inside the boxes represent the median, and the error bars indicate the minimum and maximum values.

strator-to-observer ratio (1:3) than previously tested. The hypotheses were that cows tested with a trained, calm demonstrator would have a milder behavioral reaction when exposed to the fear-eliciting stimulus, lower HR during the exposure, and shorter latency to resume feeding after the exposure. Although there was a numerical difference between untrained and trained groups with a demonstrator for behavioral reaction, this difference was nonsignificant; hence, this hypothesis was not confirmed. Our results did confirm the hypothesis that the HR increase would be lower for the cow groups who had a trained demonstrator. The post hoc analysis confirmed that cows accompanied by an untrained demonstrator cow in their first trial had significantly longer latencies to resume feeding than cows in all other sub-treatments.

The unexpected lack of effect of treatment on behavioral reaction to the fear-eliciting stimulus is in accordance with recent findings in horses (Ricci-Bonot et al., 2021), but contrasts with other findings in horses (Rørvang and Christensen, 2018), even though the same fear-eliciting stimulus (i.e., umbrella opening) was used in all cases. Ricci-Bonot et al. (2021) tested horses using both the novel object test and the umbrella test and found that a calm companion reduced the naïve horses' behavioral response in the novel object test but not in the umbrella test. Conversely, HR recovery time was affected by a calm companion in the umbrella test but

Table 2. Post hoc pairwise comparisons of the sub-treatments

Comparison	Estimate	SE	df	*t* ratio	*P*-value
Untrained A – untrained B	1.63	0.47	10.9	3.45	0.024*
Untrained A – trained A	1.53	0.47	10.9	3.25	0.033*
Untrained A – trained B	1.17	0.37	4.8	3.15	0.091†
Untrained B – trained A	−0.09	0.36	4.4	−0.25	0.99

[1]Untrained A = naïve cows accompanied with an untrained demonstrator in their first exposure; untrained B = naïve cows accompanied with an untrained demonstrator in their second exposure; trained A = naïve cows accompanied with a trained demonstrator in their first exposure; and trained B = naïve cows accompanied with a trained demonstrator in the second exposure.

*$P < 0.05$, †$P < 0.1$.

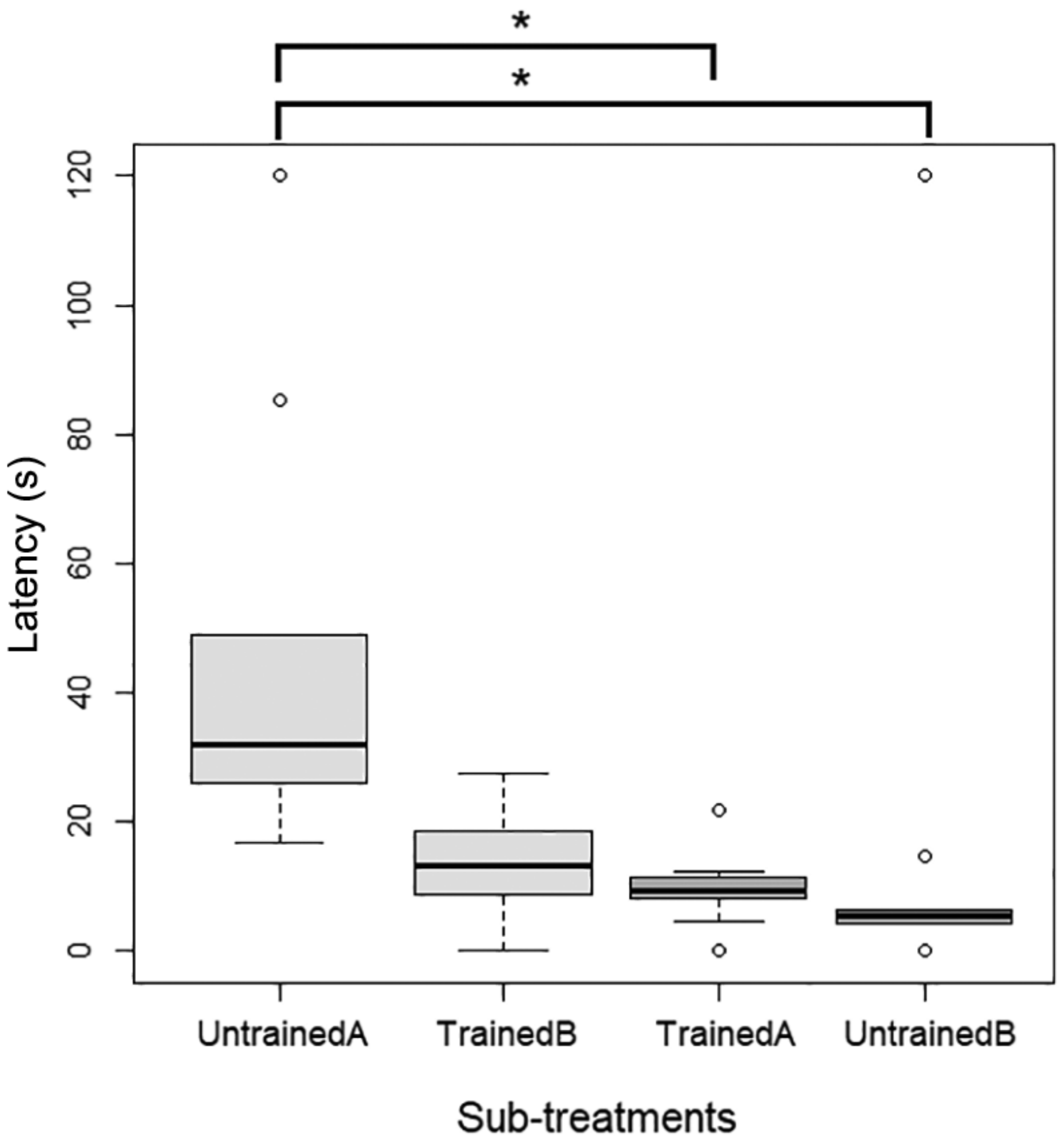

Figure 4. Boxplot of latency to resume feeding (in s), divided by sub-treatment: untrained A, trained B (untrained demonstrator first and trained demonstrator second), trained A, and untrained B (trained demonstrator first and untrained demonstrator second). Boxes illustrate the 25% and 75% quartiles, the black lines inside the boxes represent the median, and the error bars indicate the minimum and maximum values.

not in the novel object test. Our results add support to the theory proposed by Ricci-Bonot et al. (2021) that the nature of a stressor has a differential effect on behavioral and physiological measures of social buffering.

The lack of effect of treatment on behavioral reaction also has to be viewed in the context of the chosen group size and the chosen dairy cow herd. In the current study, the group size (n = 4) and also the demonstrator-to-observer ratio (equal to 1:3) were chosen based on previous results in horses (Rørvang and Christensen, 2018), which ensured comparability. It is likely, however, that our results would have shown a stronger effect of the calm companion at a reduced demonstrator-to-observer ratio (e.g., 1:2 or 1:1). In contrast, we aimed to increase the demonstrator-to-observer ratio (compared with, for example, Boissy and Le Neindre, 1990) to test a situation that might be more feasible on farm; that is, habituating 3 cows for every 1 cow manually trained to be calm, instead of habituating only 1 cow for each trained calm cow. In addition, as the participating dairy cows were residents of the agricultural school, they were used to many stimuli from various origins and of varying nature. For example, the cows participated in local fairs, competitions, and shows, and were used to many visitors on a daily basis. It is therefore likely that this particular herd overall reacted less to the fear-eliciting

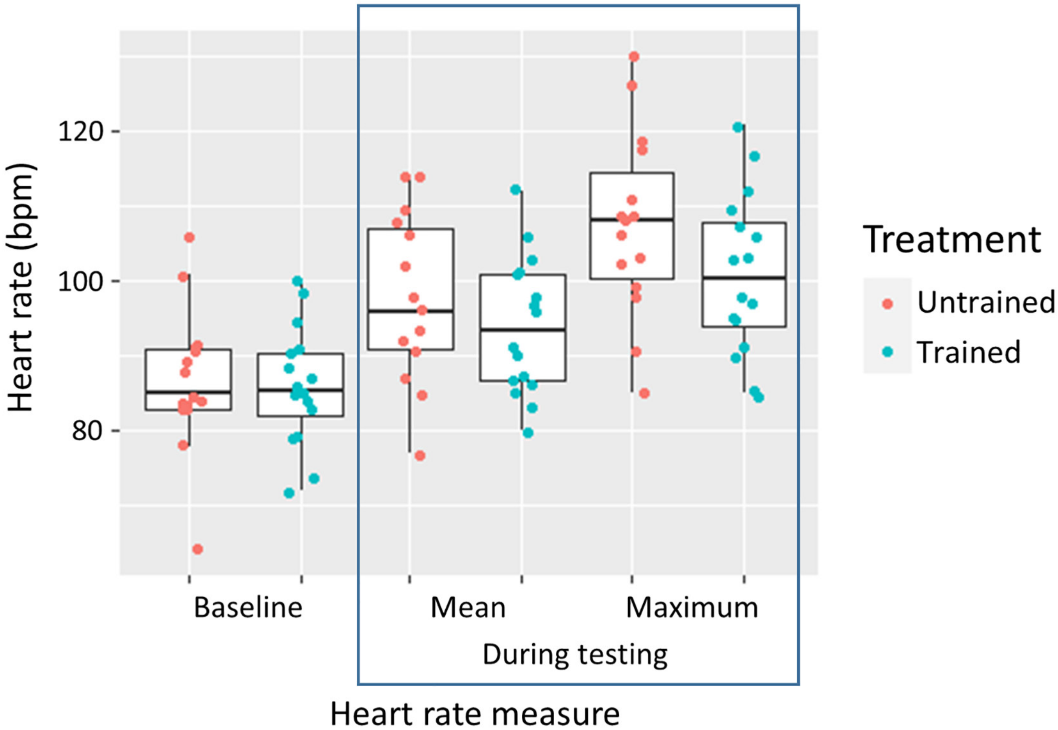

Figure 5. Overview of heart rates (in beats per minute; bpm) before testing (baseline) and during testing (mean and maximum), divided by treatment: trained is indicated in red, and test untrained in blue. Every dot represents one measurement on a cow; the white boxes illustrate the 25% and 75% quartiles, the black lines inside the boxes represent the median, and the error bars indicate the minimum and maximum values.

stimulus, perhaps explaining the nonsignificant result in relation to the behavioral reaction. Testing this setup at another farm is highly recommended.

The lack of effect of treatment could additionally be caused by the choice of demonstrator. Older and more experienced demonstrators have been shown to be more salient demonstrators for younger and more naïve observers (e.g., McComb et al., 2001), hence our choice to use older companions in this experiment. However, it is likely that the mere presence of the older companion could have lowered the overall fear of the group (Rault 2012), which could have contributed to the lack of effect. Because of this, we encourage future experiments to include similarly aged companions to be able to elucidate the effect of age and experience on transfer and alleviation of fear reactions.

Last, this contrasting result in relation to the hypothesis must be evaluated from the point of view that the hypothesis was built on knowledge from research on horses, and the underlying reason for different results could be species-specific. The findings that latency to resume feeding after exposure to the stimulus and an increase in HR were significantly affected by treatment and sub-treatments indicate that dairy cows may not show a behavioral fear response, despite being scared. Such results have also been demonstrated in Icelandic horses (see e.g., Rørvang et al., 2015; Yngvesson et al., 2016). Being scared or stressed without expressing it could be beneficial for dairy cows, who have been domesticated to function in production systems (Tucker, 2017) where traits such as docility, calmness around humans and machinery, and approachability by humans may have been favored. Fear is detrimental to animal welfare (Duncan 1993; Broom, 1991, 2014), and if dairy cows are scared without showing it, we might be unaware of a welfare problem. Thus, it is important to further expand on the studies on fear in cattle and to develop efficient means to alleviate it.

Latency to resume feeding after being exposed to the fear-eliciting stimulus was not significantly affected by the calm companion, which was unexpected although in accordance with previous findings on horses (Rørvang et al., 2015; Rørvang and Christensen, 2018). Despite the overall lack of effect of treatment on latency to resume feeding, the post hoc analyses showed that latency to resume feeding was significantly higher for cow groups tested with an untrained companion in their first trial, which supported our hypothesis. This finding adds further support to the overarching hypothesis of a calming effect of a trained companion. Interestingly, the post hoc analysis also showed that when tested the second time, both sub-treatment groups (both with a

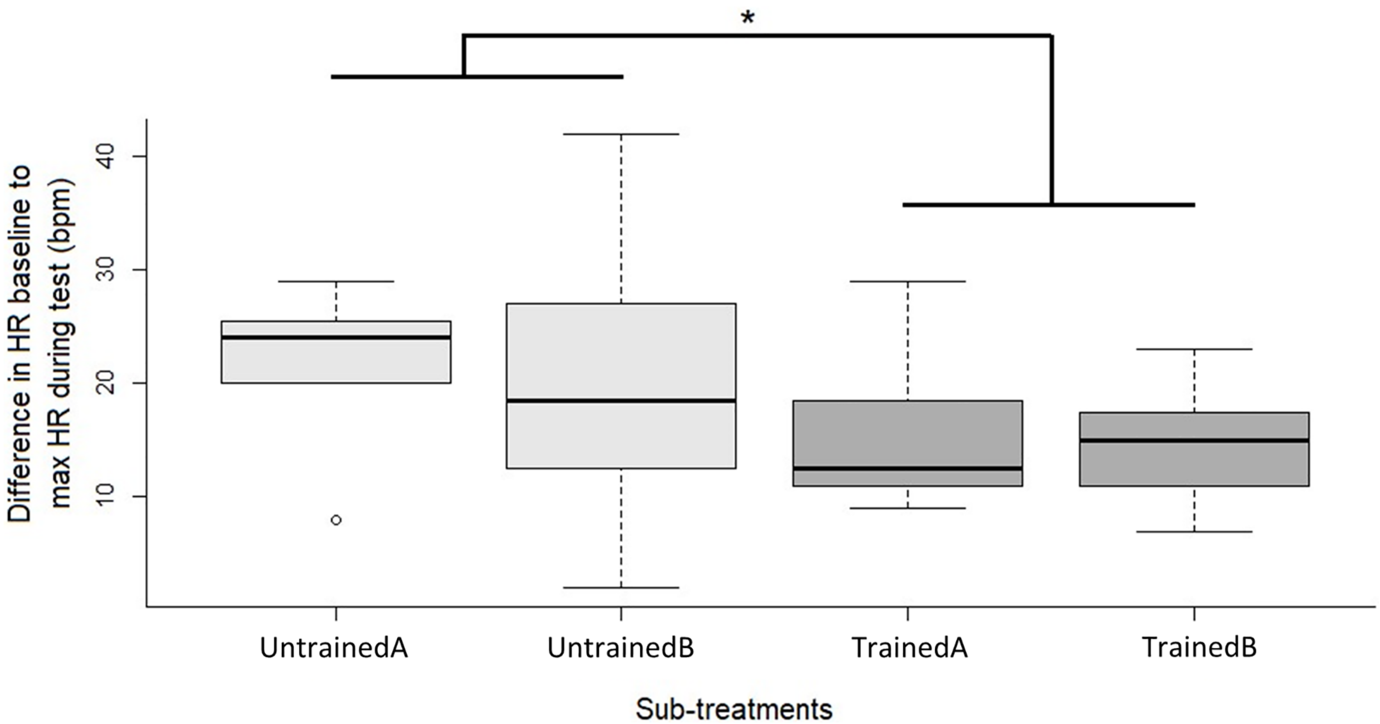

Figure 6. Boxplot of difference in heart rate (HR; beats per minute, bpm) between baseline and maximum during the test, divided by sub-treatment: untrained A, trained B (untrained demonstrator first and trained demonstrator second), trained A, untrained B (trained demonstrator first and untrained demonstrator second). Boxes illustrate the 25% and 75% quartiles, the black lines inside the boxes represent the median, and the error bars indicate the minimum and maximum values. The overall effect of treatment is highlighted by the thick black lines (*$P = 0.047$).

trained and untrained demonstrator) had similar latencies. This indicates that although the groups who had an untrained demonstrator in their first trial had significantly longer latencies, these latencies were reduced to the same level as for those who had the trained demonstrator after one exposure to the trained companion during the fear-eliciting situation. Cows tested with an untrained companion in their first trial could have been affected by this test, leading to a sensitization effect, making them react more fearfully in subsequent testing. This was not, however, the case, as all parameters were lower in the second trial with the trained companion. In addition, the groups who were exposed to the trained demonstrator in their first trial did not show an increase in latency to resume feeding after being tested with the untrained demonstrator. All but one untrained demonstrators reacted; hence, almost all test cows were exposed to a companion expressing fearful behavior when tested with an untrained demonstrator. Therefore, the results collectively suggest that calm companions may be efficient in attenuating fear.

In practical terms, the current findings suggest that cows (especially younger cows) can benefit from having a calm and experienced companion. Using calm companions in groups of dairy cows could thus lower the overall fear of the group, thereby reducing the risk of injuries to both humans and animals and increasing animal welfare, but it could also improve the validity of ethological tests and experiments. The finding that social buffering works in groups (with a demonstrator-to-observer ratio of 1:3) further means that habituation of one group member positively affects the naïve group and could lower the on-farm workload. When such a plan is implemented on the farm, the farm staff could take advantage of this information when familiarizing naïve cows or heifers to new procedures, such as moving to new pastures, hoof trimming, entering a milking parlor or robot, and veterinary treatments, among others. In these situations, staff would only need to train one cow to achieve a calmer group, which could save valuable time in a busy farm schedule.

CONCLUSIONS

In this study on the vertical transmission of safety information, the presence of an older and experienced companion cow lowered HR and latency to resume feeding in younger test cows exposed to a fear-eliciting stimulus. Behavioral reaction was only numerically affected; hence, we encourage more research on social buffering in dairy cow groups in different contexts. If these results are confirmed across other fear-eliciting contexts, they have practical implications on farm, as adding one calm companion to groups of inexperienced dairy cows can reduce fear and, in turn, lower the risk of human injuries and improve animal welfare and productivity.

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