The effect of training first-lactation heifers to the milking parlor on the behavioral reactivity to humans and the physiological and behavioral responses to milking and productivity

M. A. Sutherland¹ and F. J. Huddart

AgResearch Ltd., Ruakura Research Centre, Hamilton 3240, New Zealand

ABSTRACT

The objectives of this study were (1) to determine whether training heifers before calving would modulate the behavioral reactivity to humans and the behavioral and physiological responses to milking routines after calving, and (2) whether heifer temperament would affect this response. The behavioral reactivity of heifers to humans was assessed prepartum using 4 behavioral tests (restraint, exit speed, avoidance distance in the paddock and arena, and a voluntary approach test). These behavioral tests were repeated immediately after and 12 wk after training. Temperament was defined based on exit time from a restraint device, and heifers were denoted as either low (LR; $n = 20$) or high (HR; $n = 20$) responders. Two weeks before calving, half the LR and HR heifers were selected randomly for training to the milking parlor, whereas the other heifers were left undisturbed in the paddock. Training consisted of 4 sessions conducted over 2 consecutive days and involved introducing the heifers to the milking platform, associated noises, and human contact. During the first 5 d of lactation, behavioral and physiological data were collected from all heifers, including behavior during cup attachment, plasma cortisol concentrations, and residual milk volumes. Milk production data were collected over the entire lactation period. Training reduced the avoidance distance of LR but not HR heifers, and trained HR heifers stepped more than trained LR heifers during a restraint test. The behavioral response of heifers to the restraint test was positively correlated with milk yield, milking duration, and residual milk volume. Trained LR heifers flinched, stepped, and kicked more during attachment of milking clusters than did untrained LR heifers. During the first week of lactation, total milk yield was lower in trained than untrained HR heifers, and maximum flow rates were higher and residual milk volumes were lower in trained

than untrained heifers, irrespective of heifer temperament. Plasma cortisol concentrations were lower on d 5 of lactation than on d 1 and were higher in HR heifers after milking than before milking. Over the first 8 mo of lactation, milking duration was lower and average flow rates were higher in trained than untrained heifers. These results suggest that trained heifers may have experienced less distress during the first week of lactation, but the effect of training on the behavioral and physiological responses to milking appeared to be influenced by heifer temperament.

Key words: behavior, temperament, heifer, training

INTRODUCTION

During the first days of lactation, first-lactation heifers are exposed to several novel experiences associated with the milking process and often have had limited experience with humans before calving. Fear of humans and novelty can cause stress and reduce productivity in dairy cattle (Bruckmaier et al., 1993; Rushen et al., 1999). Furthermore, primiparous heifers may experience stress during early lactation that can result in increased heart rate, cortisol release, and disturbed milk ejection (Van Reenen et al., 2002). Heifers may also kick off milking clusters, step more during milking, and display other behaviors that interfere with milking efficiency and increase the chance of injury to milkers (Willis, 1983) and the risk of mastitis (Ivemeyer et al., 2011). The performance of stepping and kicking behaviors by heifers during the first week of lactation has been shown to correspond to their level of experience with humans and the milking process in a nonlinear dynamic (Bremner, 1997). Handling or training that reduces heifers' fear of humans, the milking process, or both, before calving could therefore reduce the performance of stepping and kicking behaviors in the milking parlor, especially early in lactation. Several strategies have been studied, including positive handling of heifers and familiarizing them with the milking parlor before calving (Bremner, 1997; Hemsworth et al., 1989; Bertenshaw et al., 2008). Positive handling of heifers around

Received November 30, 2011.

Accepted September 3, 2012.

¹Corresponding author: mhairi.sutherland@agresearch.co.nz

the time of calving reduced the number of flinch, step, and kick responses performed during the first 20 wk of lactation, mean milk cortisol concentrations, and the avoidance distance in response to a stationary human (Hemsworth et al., 1989). Handling and experience in the milking parlor before calving reduced the frequency of movement and kicking during milking in the first week of lactation compared with heifers that received no handling or familiarization period (Bremner, 1997). Positive handling before or at the time of calving appears to reduce heifers' fear of humans and their behavioral responses to the milking process; however, few studies have investigated the effect of training heifers to the milking parlor on the behavioral and physiological responses to milking during early lactation.

The human-animal relationship can affect the welfare, health, and productivity of farm animals and is particularly important for dairy cattle, which interact with humans regularly. Numerous tests have been used to assess the human-animal relationship, and these tests can fit into 3 categories: moving human, stationary human, and restraint (Waiblinger et al., 2006). The behavioral reactivity of dairy cows in response to an approaching or a stationary human has been associated with the behavioral response to milking and milk production. Dairy cattle that had a smaller avoidance distance in response to an approaching human stepped less during milking (Rousing et al., 2004), and dairy cattle that had a shorter approach distance toward a stationary human had higher milk yields, milk fat levels, and milk protein levels (Breuer et al., 2000). Furthermore, gentle, positive handling can reduce the behavioral reactivity of cows to humans and reduced the avoidance distance of heifers to an approaching human (Boissy and Bouissou, 1988; Schmied et al., 2008). Therefore, reducing the behavioral reactivity of heifers to humans using techniques such as handling could potentially reduce their behavioral response to milking and improve milk production.

The term "temperament" can be used to describe the relatively stable differences observed in the behavioral predisposition of animals (Strelau 1987). The most commonly used test to assess temperament in beef cattle is the exit speed test (Kilgour et al., 2006; Curley et al., 2008), and this test has been used in dairy cattle (Gibbons et al., 2011). Variations in cattle temperament, measured using an exit speed test, were related to variations in functional characteristics of the hypothalamic-pituitary-adrenal axis (Curley et al., 2008), productivity (Petherick et al., 2009), and carcass quality (King et al., 2006). It would therefore be of interest to know whether behavioral reactivity to humans or temperament influences the physiological and behavioral responses to milking and milk productivity

early in a heifer's lactation. It would also be of interest to know whether responses to training differ depending on the temperament of an animal. The objectives of this study were to determine whether training before calving would modulate behavioral reactivity to humans and the behavioral and physiological responses of first-lactation heifers during introduction to milking routines after calving, and whether these responses would be affected by temperament.

MATERIALS AND METHODS

Animals

Forty primiparous, pregnant Friesian-cross dairy heifers in late gestation were selected for this study. Heifers were selected from a research herd consisting of 120 heifers, based on the results from a human avoidance distance test conducted in the paddock (see description of test below). The 20 heifers with the shortest and the 20 with the longest avoidance distances were selected. These selection criteria were used to achieve a group of heifers that displayed low and high levels of reactivity to humans. The study was conducted from May to September 2010 on a research farm near Hamilton, New Zealand. Heifers calved from June until September. All procedures involving animals were approved by the Ruakura Animal Ethics Committee under the New Zealand Animal Welfare Act 1999.

Behavioral Reactivity Testing

At the start of the study, all 40 heifers were assessed by an avoidance distance test in the paddock. At this time, they were also subjected to tests of restraint, exit speed, and avoidance distance (in an arena) and to a voluntary approach test (test 1). These latter behavioral tests were repeated 2 wk later (test 2) before calving but after training, and a third time after calving (test 3: 12 wk after the second testing period). All behavioral testing was conducted between 1000 and 1400 h, and the tests were performed over consecutive days during each test period. Once all heifers were subjected to the restraint and exit speed tests, they were tested in the voluntary approach test, followed by the avoidance distance test (in the arena). Animals were tested in the restraint and exit speed tests on different days than the approach and avoidance distance tests. The tests were performed in this order during each testing period, and on each test day, the animals tested were balanced for treatment (untrained and trained) and temperament [low responder (**LR**) and high responder (**HR**)].

All behavioral testing over the entire study was conducted by the same 3 handlers with experience in

handling dairy cattle and performing these behavioral tests. All 3 handlers were unfamiliar with the experimental animals before the first testing. The same person was used as the stationary person in the voluntary approach test, and another handler was consistently used to move the heifers to the avoidance distance test arena for all 3 testing periods for all 40 animals. On the morning of the testing days, all 40 heifers were quietly moved by experienced stockpersons from the paddock where they were grazed to the holding area associated with the milking parlor. Movement of heifers between testing areas was conducted by 1 of the 3 handlers involved in the behavioral testing, using only quiet voices and gentle tactile force if necessary.

The avoidance distance test was conducted twice in the paddock by all 3 handlers, 4 d apart, using the methodology modified from Fisher et al. (2000). The average avoidance distance was calculated for each heifer. To perform the test, the person would make eye contact with the focal heifer from directly in front of the animal and approximately 10 m away. The person then approached the animal at a speed of approximately 0.5 m/s, with an arm held out 20 to 30 cm from the leg, and palm and eyes cast down. When the focal animal began to move away from the person, the person would immediately stop, move backward a distance of 10 m, and then repeat the process. The avoidance distance was recorded as the distance from where the person stood when the cow moved on the second approach to the cow's nearest front hoof just before moving away. Distance was measured using 1-m strides (calibrated at the start of every testing day) to the nearest 0.5 m.

The restraint test and exit speed tests were conducted using procedures modified from Gibbons et al. (2011; restraint) and Curley et al. (2008; exit speed). The restraint test was performed by moving a heifer into a squeeze chute (crush). Once the entry gate was shut behind the heifer, the sides of the device were adjusted so they just touched the sides of the fullest part of the heifer's abdomen. Each heifer was held in the crush for 2 min before being released. The total number of hoof lifts (front and back hooves) performed during the restraint period was recorded. Before opening the exit gate, the sides of the crush were moved further apart so as not to impede the ability of the heifer to exit.

The time taken for each heifer to exit the crush and traverse a fixed distance was recorded. An exit speed unit (specifically designed for AgResearch Ltd. by technical staff at the University of Waikato, Hamilton, New Zealand) was used. The unit consisted of 2 light beams and reflectors and 1 readout unit. The first light beam was set up just outside the exit gate of the crush, and the second light beam was set 2.25 m from the exit gate. Both beams were set 0.8 m above ground level

so that they would be triggered by the heifer's body as it exited the restraint area. Once the heifer passed through both light beams, the exit time was recorded in seconds from the readout unit. One handler stood approximately 1 m away from the right-hand side of the crush and behind the shoulder of the animal and the other handler stood behind and on the left-hand side of the crush, during both the restraint and exit speed tests. The same 2 handlers were located in the same positions during testing on all testing days and for all 40 animals.

The voluntary approach test was performed using a test modified from Hemsworth et al. (1989). A focal animal was moved into a fenced arena (7 × 5 m), where a person was already sitting on a chair at the far end. The arena floor (concrete) was marked in 1-m increments from the front of the seated person. The focal animal could not see other animals during the testing period. The person sat still for 5 min in a nonaggressive pose (eyes cast down, palms on lap), and the closest distance the heifer's front hooves were from the seated person when it stood (all 4 hooves stationary for 2 s) was recorded. At the conclusion of the voluntary approach test, the seated person slowly stood up, gained eye contact with the animal, and carried out an avoidance distance test using the same protocol as when measuring avoidance distance in the paddock (except that the distance at the start of the approach distance was not 10 m but constrained by the pen dimensions).

The heifers were then ranked based on their exit time from the crush. Heifers with an exit time of more than 2 s were denoted as LR ($n = 20$) and those with an exit time of less than 2 s were denoted as being HR ($n = 20$).

General Procedures and Experimental Design

Training. Before calving, heifers were allocated randomly to 1 of 2 treatment groups (trained and untrained), balanced for predicted calving date and temperament category ($n = 10$ per group). Heifers allocated to the trained treatment group then received a training program to the rotary milking parlor over 2 consecutive days, whereas those in the other treatment group were left undisturbed in the paddock. All heifers calved within 9 wk of each other, so the period between training and calving ranged from 1 to 10 wk.

On the first day of training, in the morning (session 1), heifers to be trained were brought into the dairy yards. They were encouraged onto the rotary platform using good stockmanship practices; no loud voices or objects were used to force the animals. If necessary, the stockperson would use a quiet voice and gentle, tactile force with the hands to encourage the heifers to

move. Heifers traveled around to the exit point, where a stockperson encouraged them to back off the rotary platform by putting an arm in front of the animals' faces and pushing them backward. Each heifer dismounted from the platform, turned around, and moved down an alley back to the main holding yard. This process was repeated 2 more times. In the afternoon on the first day (session 2), heifers were loaded and exited the rotary platform 3 times using the same procedure as in session 1, but during this session, heifers were also introduced to routine noises and objects such as the radio, cup sprayer, and platform washer. On the second day in the morning (session 3), heifers were loaded and exited the rotary platform 3 times, using the same procedures as in session 2, but during this session, they were also introduced to the sounds of the milking machine, had teat spray applied, and were touched gently high on the escutcheon (above the udder) and back legs. Finally, on the second day in the afternoon (session 4), heifers were loaded and exited the rotary platform 3 times using the same procedure as in session 3, but were exposed to further gentle handling or rubbing of the escutcheon, back of the legs, and top of the udder, progressing until the teats and bottom of the udder had been touched.

First Week of Lactation. Each heifer was brought in for milking within 24 h of calving. During the first 5 d of lactation, heifers were kept together in a separate colostrum herd before being moved into a herd that consisted of primiparous and multiparous cows. Behaviors in the milking parlor were observed and recorded during morning milkings for the first 5 d of lactation. During attachment of the milking cluster, cows were given an overall score based on the performance of flinching, stepping, or kicking (**FSK**) behaviors using a 4-point scale (1 = no hind foot movement, heifer may flinch, shiver or do nothing at all; 2 = hind leg lifted no higher than 20 cm, step or shuffle of a hind leg; 3 = hind leg lifted higher than 20 cm, step or forward kick of a hind leg; 4 = backward kick of hind leg). The most extreme behavior performed during each milking period was the score given to that animal. The order in which the cows entered the milking parlor was also recorded.

On each of d 1 to 5 of lactation, all heifers were given an oxytocin challenge and residual milk volume was measured. Once heifers finished voluntary milk ejection, the milking cluster was removed, and 2 mL (40 USP units) of oxytocin (Butocin, Bomac Laboratories Ltd., Auckland, New Zealand) was given by intramuscular injection. One minute after injection, the milking cluster was reattached, the residual milk was collected, and its volume recorded. Residual milk as a percentage of total volume was also calculated. Milk samples were

collected over the entire milking period during the first 5 d of lactation, and somatic cells were enumerated.

Blood samples (10 mL) were collected from all heifers via the coccygeal vein immediately before and after milking on the first and fifth day of lactation. Blood samples were collected from heifers while they were restrained in a holding area that was within close proximity to the milking parlor. Blood was collected into Vacutainer tubes (BD, Franklin Drive, NJ) containing heparin. Blood samples were centrifuged for 12 min at $1,300 \times g$ at 4°C, and plasma was aspirated and stored at -20°C until analyzed. Cortisol concentrations were measured using a commercially available RIA kit (Siemens Cortisol-A-Count Kit, Cruinn Diagnostics Ltd., Dublin, Ireland).

The research farm used a WestfaliaSurge milking system (GEA Farm Technologies, Cambridge, New Zealand) and the associated DairyPlan C21 herd and parlor management software (GEA Farm Technologies). This was used to record production data at each milking (milk yield, milking duration, and average milking flow rate) during the first 8 mo of lactation.

Statistical Analysis

Data were analyzed by ANOVA using the mixed models procedure of SAS, version 9.1 (SAS Institute Inc., Cary, NC). The exit time recorded during the first testing period (test 1) was used to separate the heifers into 2 reactivity groups of 20 cows each. Heifers with an exit time of more than 2 s were denoted as being LR, and those with an exit time of less than 2 s were denoted as being HR. However, 1 LR, trained heifer was removed from the study because of illness. Correlation coefficients were calculated between the behavioral responses of heifers to the different reactivity tests (averaged over all 3 tests) and the behavioral response to milking and milk production measures using Spearman's rank coefficient of correlation in SAS (SAS Institute Inc.).

The analysis was a factorial randomized block fitting 2 reactivity categories, 2 training treatments, and the interaction between reactivity category and training. The main effects included in the model were day, reactivity category, and training treatment. Multiple comparisons were calculated using the PDIFF option in SAS. The average production value of each heifer was calculated from the New Zealand Animal Evaluation records and used as a covariate in the analysis of milk production data because animals could not initially be randomized to treatments based on production records given that they were first-lactation heifers. All data were tested for constant variance and departures from

Table 1. The behavioral responses (LSM $\pm$ SEM) of untrained, low responder (LR; $n = 10$), trained, LR ($n = 9$), untrained, high responder (HR; $n = 10$) and trained, HR ($n = 10$) heifers to reactivity tests performed before training (test 1), immediately after training (test 2), and 12 wk after training (test 3)

Reactivity test	Untrained		Trained		SEM	
	LR	HR	LR	HR	LR	HR
Test 1						
Restraint (steps)	19.9 ^x	20.3 ^x	23.2 ^x	38.9 ^x	5.48	5.34
Exit time (s)	3.1 ^{a,x}	1.6 ^{b,x}	3.0 ^{a,x}	1.5 ^{b,x}	0.65	0.63
Voluntary approach in arena (zone)	2.8 ^x	3.3 ^x	2.8 ^x	4.2 ^x	0.59	0.58
Avoidance distance in arena (m)	4.6 ^x	5.8 ^x	3.9 ^x	5.2 ^x	0.46	0.45
Test 2						
Restraint (steps)	19.2 ^{a,x}	21.4 ^{a,x}	14.9 ^{a,x}	46.2 ^{b,x}	5.48	5.34
Exit time (s)	3.5 ^{a,x}	1.8 ^{b,x}	2.5 ^{a,x}	1.5 ^{b,x}	0.65	0.63
Voluntary approach in arena (zone)	3.1 ^x	3.0 ^x	2.9 ^x	4.0 ^x	0.59	0.58
Avoidance distance in arena (m)	4.8 ^{a,x}	4.2 ^{a,x}	2.8 ^{b,y}	4.7 ^{a,x}	0.46	0.45
Test 3						
Restraint (steps)	3.0 ^y	3.7 ^y	5.7 ^y	10.0 ^y	5.48	5.34
Exit time (s)	5.8 ^{a,y}	3.6 ^{b,y}	6.2 ^{a,y}	3.6 ^{b,y}	0.65	0.63
Voluntary approach in arena (zone)	3.2 ^x	3.8 ^x	2.4 ^x	4.0 ^x	0.59	0.58
Avoidance distance in arena (m)	3.0 ^{ab,y}	2.5 ^{a,y}	2.1 ^{a,y}	4.2 ^{b,x}	0.46	0.45

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

^{x,y}For each reactivity test, means within a column with different superscripts differ ($P < 0.05$).

normal distribution using the univariate procedures of SAS. Cortisol and SCC data were log-transformed to equalize the variance to meet the normality assumptions of the analysis. Data displayed in the graphs, tables, and text are actual data summarized by least squares means $\pm$ standard errors of the mean. Statistical significance was determined at $P < 0.05$.

RESULTS

Behavioral Reactivity Testing

Before training, avoidance distances in the paddock differed ($P < 0.05$) between LR (3.6 ± 0.27 m) and HR (4.0 ± 0.26 m) heifers. Over all 3 behavioral reactivity test periods, heifers categorized as LR tended ($P = 0.091$) to step less in the crush (LR: 14.3 ± 2.24 steps; HR: 23.4 ± 2.18 steps), took longer ($P < 0.001$) to exit the crush (LR: 4.0 ± 0.26 s; HR: 2.3 ± 0.26 s), had a shorter ($P < 0.05$) avoidance distance in the arena (LR: 3.5 ± 0.19 m; HR: 4.4 ± 0.18 m), and had a shorter ($P < 0.05$) voluntary approach distance in the arena (LR: 2.9 ± 0.24 zone; HR: 3.7 ± 0.24 zone).

The responses of heifers to the restraint, exit speed, avoidance distance, and voluntary approach tests changed between test periods (Table 1). The numbers of steps taken by untrained and trained heifers while in the crush were similar ($P > 0.05$) between tests 1 and 2 but were lower ($P < 0.001$) in test 3. Immediately after training, trained HR heifers stepped more in the crush than untrained HR ($P < 0.05$) and LR ($P < 0.05$) heifers. Twelve weeks after training, no difference ($P >$

0.05) was observed in the number of steps displayed by LR and HR untrained and trained heifers.

The time taken for untrained and trained heifers to exit the crush was similar ($P > 0.05$) between tests 1 and 2 but was longer ($P < 0.001$) in test 3. Untrained and trained HR heifers took less ($P < 0.05$) time than LR untrained and trained heifers to exit the crush regardless of the test period.

The distance within which heifers voluntarily approached the stationary human did not differ ($P > 0.05$) between test periods and was not affected by temperament. The avoidance distances of heifers in the arena in response to an approaching human did not differ ($P > 0.05$) between untrained and trained heifers in test 1. Trained LR heifers displayed shorter ($P < 0.05$) avoidance distances in the arena in tests 2 and 3 than in test 1, but the avoidance distance did not change ($P > 0.05$) over time for trained HR heifers. The avoidance distances of untrained LR and HR heifers in the arena were shorter ($P < 0.05$) in test 3 than in tests 1 and 2.

A Spearman's rank correlation test was used to determine the relationship between the behavioral reactivity of heifers to humans and the behavioral response to milking and milk production measures (Table 2). Heifers that stepped more while restrained in the crush had higher milk yields ($P < 0.001$), longer milking durations ($P < 0.05$), and higher residual milk volumes ($P < 0.05$). No relationship was observed between the behavioral response to the exit speed, human avoidance distance, and voluntary approach distance tests and the behavioral response to milking and milk production measures.

Table 2. Spearman rank correlation coefficients between the behavioral response of heifers to humans using restraint, exit speed, human avoidance distance, and voluntary approach tests and the behavioral response to milking and milk production measures during the first week of lactation

Item	Restraint	Exit speed	Human avoidance distance (paddock)	Human avoidance distance (arena)	Voluntary approach distance
Behavioral response to milking					
FSK score ¹	0.22	0.02	0.23	0.08	0.06
Milking order	0.17	0.22	0.15	-0.08	0.10
Milk production measure					
Milk yield	0.97*	0.18	0.04	0.22	0.06
Milking duration	0.41*	0.15	0.02	0.09	0.02
Average milk flow rate	0.05	-0.11	-0.12	0.10	0.18
Maximum milk flow rate	0.13	-0.15	-0.17	0.10	0.06
Residual milk volume	0.39*	-0.14	0.17	0.27	0.23
Percentage residual milk volume	0.28	-0.15	0.16	0.24	0.21

¹FSK = flinch, step, and kick score.

* $P < 0.05$.

First Week of Lactation

Behavioral Response to Milking. By d 5 of lactation, the FSK score was lower ($P < 0.05$) than on previous days (d 1: 2.7 ± 0.13 ; d 2: 2.6 ± 0.13 ; d 3: 2.8 ± 0.13 ; d 4: 2.6 ± 0.13 ; d 5: 2.1 ± 0.13). Over the first 5 d of lactation, the FSK score was higher ($P < 0.001$) in trained LR than in untrained LR heifers, but did not differ ($P > 0.05$) between untrained and trained HR heifers (Table 3). Untrained heifers had a higher ($P < 0.01$) position in the milking order than did trained heifers, regardless of the day of lactation and heifer temperament (untrained: 3.6 ± 0.22 ; trained: 2.8 ± 0.22).

Residual Milk Volumes. Residual milk volumes and the residual milk volume percentages decreased ($P < 0.001$) from d 1 to 5 of lactation, irrespective of the training treatment and heifer temperament (residual milk volume: d 1: 2.2 ± 0.33 kg; d 2: 1.6 ± 0.33 kg; d 3: 1.3 ± 0.33 kg; d 4: 1.1 ± 0.33 kg; d 5: 0.7 ± 0.33 kg; residual milk volume percentage: d 1: $25.8 \pm 3.42\%$; d 2: $17.1 \pm 3.42\%$; d 3: $12.3 \pm 3.42\%$; d 4: $9.5 \pm 3.42\%$; d 5: $6.2 \pm 3.42\%$ of total volume].

Residual milk volumes and residual milk volume percentages were lower ($P < 0.001$) in trained than untrained heifers, irrespective of temperament (residual milk volume: untrained: 1.8 ± 0.21 kg; trained: 1.0 ± 0.22 kg; residual milk volume percentage: untrained: $18.6 \pm 2.17\%$; trained: $9.8 \pm 2.22\%$ of total volume).

SCC. Somatic cell counts decreased ($P < 0.001$) over the first 5 d of lactation, regardless of the training treatment or temperament (d 1: $2,751 \pm 384.7$ cells $\times 10^3$ /mL; d 2: $1,441.21 \pm 378.2$ cells $\times 10^3$ /mL; d 3: $1,286 \pm 373.5$ cells $\times 10^3$ /mL; d 4: 993 ± 373.5 cells $\times 10^3$ /mL; d 5: 418 ± 373.5 cells $\times 10^3$ /mL). Overall mean SCC during the first 5 d of lactation were greater ($P <$

0.05) in trained LR than untrained LR and trained HR heifers (Table 3).

Plasma Cortisol Concentrations. On d 5 of lactation, cortisol concentrations were lower ($P < 0.001$) than on d 1 of lactation (d 1: 14.3 ± 0.91 ng/mL; d 5: 7.1 ± 0.90 ng/mL). Overall, trained heifers had lower ($P < 0.05$) cortisol concentrations than untrained heifers (untrained: 11.4 ± 0.89 ng/mL; trained: 10.0 ± 0.92 ng/mL). Furthermore, cortisol concentrations after milking were greater ($P < 0.005$) than premilking concentrations in HR heifers, but not in LR heifers, regardless of the day of lactation (Figure 1). No interaction ($P > 0.05$) was observed between training treatment and temperament, regardless of the day of lactation.

Milk Production

First Week of Lactation. Total milk yields were affected ($P < 0.05$) by the training treatment and temperament during the first 5 d of lactation (Table 3). Total milk yields were lower ($P < 0.001$) in trained HR heifers than in untrained HR heifers, but milk yields did not differ ($P > 0.05$) between untrained and trained LR heifers. Average and maximum milk flow rates increased ($P < 0.05$) during the first week of lactation and were higher ($P < 0.005$) on d 5 than on d 1 of lactation (average milk flow rate: d 1: 1.1 ± 0.09 kg/min; d 2: 1.3 ± 0.09 kg/min; d 3: 1.4 ± 0.09 kg/min; d 4: 1.6 ± 0.09 kg/min; d 5: 1.8 ± 0.09 kg/min; maximum milk flow rate: d 1: 2.7 ± 0.20 kg/min; d 2: 3.1 ± 0.20 kg/min; d 3: 3.2 ± 0.20 kg/min; d 4: 3.3 ± 0.20 kg/min; d 5: 3.6 ± 0.20 kg/min). Furthermore, overall maximum milk flow rates were higher ($P < 0.05$) in trained than untrained heifers, regardless of temperament and the day of lactation (untrained: 3.0 ± 0.13 kg/min; trained: 3.4 ± 0.13 kg/min). The training treatment or

Figure 1. Plasma cortisol concentrations (ng/mL; LSM $\pm$ SEM) of low responder (LR; $n = 19$) and high responder (HR; $n = 20$) heifers before and after milking. Means accompanied by an * differ at $P < 0.05$.

temperament had no effect ($P > 0.05$) on spontaneous milk yield or milking duration.

First 8 mo of Lactation. Milking durations were longer ($P < 0.001$) in untrained LR than trained LR heifers over the first 8 mo of lactation (Table 3). Furthermore, milking durations were longer ($P < 0.001$) in untrained LR than trained HR heifers. Overall, milking durations were shorter ($P < 0.001$) in trained than untrained heifers (untrained: 359.7 ± 7.10 s; trained: 312.5 ± 7.10 s) and shorter ($P < 0.005$) in HR than LR heifers (LR: 351.6 ± 7.13 s; HR: 321.3 ± 7.07 s). Average milk flow rates were higher ($P < 0.001$) in trained than untrained heifers (untrained: 2.0 ± 0.5 kg/min; trained: 2.4 ± 0.05 kg/min). However, training treatment or temperament had no effect ($P > 0.05$) on average daily milking yields.

DISCUSSION

In the present study, we assessed the effect of training on the behavioral reactivity of heifers to humans and the behavioral and physiological responses to the milking process. Furthermore, the interaction between training and heifer temperament was investigated. Training reduced the distance at which LR heifers avoided an approaching human in the arena and increased the behavioral response of HR heifers to the restraint test. Training appeared to reduce the stress experienced by heifers during the first week of lactation, as indicated by reduced residual milk volumes and cortisol concentrations. Furthermore, training appeared to affect the behavioral response to milking and milk production measures of heifers differently depending on

Table 3. The behavioral responses and milk production measures, residual milk volumes, and SCC (LSM $\pm$ SEM) of untrained, low responder (LR; $n = 10$), trained, LR ($n = 9$), untrained, high responder (HR; $n = 10$), and trained, HR ($n = 10$) heifers during lactation

Measure	LR			HR			P-value			
	Untrained	Trained	SEM	Untrained	Trained	SEM	Training	Temperament	Training $\times$ temperament	
Milking behavior										
Flinch, step, kick score	2.2 ^a	2.8 ^b	0.12	2.5 ^{ab}	2.6 ^b	0.12	0.004	0.840	0.017	
Milking order	3.5	3.1	0.32	3.7	2.4	0.31	0.010	0.395	0.154	
First 5 d of lactation										
Daily yield (kg)	8.4	8.7	0.35	8.8	8.2	0.36	0.690	0.982	0.265	
Total milk yield (kg)	10.3 ^b	9.8 ^b	0.29	10.6 ^b	8.8 ^a	0.30	0.001	0.167	0.049	
Milking duration (s)	551.5	554.5	23.98	574.2	514.0	24.80	0.240	0.713	0.222	
Average milk flow rate (kg/min)	1.4	1.4	0.08	1.4	1.5	0.08	0.411	0.506	0.377	
Maximum milk flow rate (kg/min)	2.7	3.4	0.18	3.3	3.4	0.18	0.038	0.151	0.186	
Residual milk (kg)	1.7	1.4	0.30	1.8	0.6	0.31	0.001	0.185	0.916	
Residual milk (% of total volume)	19.2	12.1	3.14	18.0	7.6	3.23	0.001	0.174	0.996	
SCC (cells $\times 10^3$ /mL)	885.7 ^a	1,927.2 ^b	340.66	1,516.3 ^{ab}	1,181.9 ^a	333.21	0.438	0.629	0.002	
First 8 mo of lactation										
Daily yield (kg)	10.1	10.4	0.10	10.4	10.4	0.11	0.217	0.191	0.402	
Milking duration (s)	383.6 ^b	315.2 ^a	9.96	335.8 ^a	309.9 ^a	10.61	0.001	0.009	0.055	
Average milk flow rate (kg/min)	1.9	2.4	0.07	2.1	2.4	0.07	0.001	0.074	0.365	

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

their temperament. These results suggest that training can reduce the reactivity of heifers to humans and reduce the stress experienced by heifers during the first week of lactation; however, animal temperament may influence the effect of training on heifers.

In the present study, heifers were categorized as either LR or HR on the basis of their response to an exit speed test as a means to assess temperament. The exit speed test has commonly been used to assess animal temperament in beef cattle (Kilgour et al., 2006; Curley et al., 2008), and recently, it has been used to assess temperament in dairy cattle (Gibbons et al., 2011). The exit speed test can also be used to assess the behavioral response of animals to humans. Waiblinger et al. (2006) categorized the tests used to assess the human-animal relationship according to the degree of human-animal interaction during the test: (1) the animal is free to approach a stationary human (e.g., voluntary approach test), (2) the animal is approached by a human (e.g., avoidance distance test), and (3) the animal is handled or restrained in a restraint device or by a human (e.g., exit speed test). In the present study, a voluntary approach test and a human avoidance distance test were also used to assess the behavioral reactivity of heifers to humans. Low-responding heifers stepped less in a restraint test, had a shorter avoidance distance in the paddock and arena, and had a closer voluntary approach distance toward a stationary human. Kilgour et al. (2006) and Gibbons et al. (2011) also found positive correlations between the response of cattle to an exit speed test and a human avoidance distance test. All these tests measure the behavioral response of animals to humans; therefore, a strong correlation between these tests could be expected, but the strongest correlation found was between the avoidance distance test (in the arena) and the voluntary approach test ($r = 0.50$). Therefore, the behavioral response of heifers may reflect motivations other than fear of humans alone depending on the test; for example, the response to the voluntary approach test may be influenced by other motivations, such as exploration (Waiblinger et al., 2003).

The 4 behavioral tests used in the present study were selected on the basis that they assessed the responses of heifers to differing degrees of human interactions. Furthermore, these behavioral tests have previously been used to assess the behavioral responsiveness of dairy cattle to humans (Waiblinger et al., 2003; Windschnurer et al., 2008; Gibbons et al., 2011). The same 4 tests were used to measure the behavioral reactivity of heifers before, immediately after, and again 12 wk after training to assess the effect of training and familiarization to the milking process on the behavioral responses of heifers to humans. Overall, the behavioral response to restraint, exit speed, and avoidance distance in the

arena were markedly reduced by test 3, but the voluntary approach distance of a heifer to a stationary human did not change over time. In beef cattle, the time taken to exit a crush increased and avoidance distance decreased, but the distance at which an animal would approach a stationary human did not change between testing periods (Kilgour et al., 2006), which is consistent with the results of the present study. Conversely, Gibbons et al. (2011) found that exit times decreased slightly from the first to second testing period, but then increased again. It is likely that the reduction in behavioral reactivity in heifers to humans over time in the present study is a response to the increased level of human-animal interactions associated with lactation and milk harvesting, which would have resulted in habituation and a reduced fear of humans and the milking process.

In the present study, training reduced the avoidance distance that LR heifers displayed toward an approaching human but had no effect on the avoidance distance of HR heifers. Furthermore, training increased the number of steps performed by HR heifers in response to the restraint test. Schmied et al. (2008) found that stroking different body parts of dairy cows for 3 wk reduced the avoidance distance of animals in response to an approaching human. Hemsworth et al. (1989) found that heifers that were handled positively at the time of calving were more likely to approach a stationary human and spend more time in close proximity to a human than unhandled heifers. These results suggest that the training regimen used in the present study was sufficient to partially reduce the behavioral response of LR heifers to humans, but may have increased the behavioral response of HR heifers to humans. The HR heifers may have actually found the intense training regimen used in the present study aversive to some degree. Therefore, more comprehensive training regimens may be necessary that include a higher degree of positive human contact to reduce the behavioral response of heifers to humans, and heifers that appear to have a higher level of fear toward humans at the time of handling may need more training than animals that show less fear toward humans.

Exit speed from a crush has been associated with adrenal responsiveness (Curley et al., 2008), productivity (Petherick et al., 2009), and carcass quality (King et al., 2006) in beef cattle. In the present study, exit time was not associated with any milk production measures; however, the number of steps performed by heifers in response to being restrained in a crush was highly correlated with milk yield. Willis (1983) and Rousing et al. (2004) found that stepping during milking was positively associated with milk yield in dairy cows. However, in the present study, no association between

the FSK response to milking, the behavioral response to the restraint test, or milk yield was found, suggesting that the motivation for heifers to step while restrained in a crush may be different from the motivation to step during the milking process.

The behavioral responses of heifers to milking during the first week of lactation were higher in trained LR heifers but remained similar between trained and untrained HR heifers. This varies from the results of Hemsworth et al. (1989), who found that heifers handled at the time of calving displayed lower FSK behaviors in response to milking. Bremner (1997) found that heifers that had either the most or the least familiarization with handling before calving were less likely to move or kick during the first week of lactation. Bremner (1997) suggested that as animals become familiarized with people and the milking process, behavioral reactivity (e.g., FSK) initially increases until they become tamer (or habituated) and reactivity consequently decreases. Gibbons et al. (2011) also stated that very tame and very fearful animals are both more likely to stand stationary in response to a stressor. These results in combination with the behavioral responsiveness of LR and HR to the reactivity tests suggest that the training regimen used in the present study may have been sufficient to reduce the fear of LR heifers to humans, but not HR heifers. Because of the wide range of methods used to measure stepping and kicking behaviors in response to milking described in the literature, it is difficult to compare studies directly, and some methodologies may be more sensitive to measuring the responsiveness of heifers to humans than others. However, the training regimen used in the present study was not sufficient to markedly reduce the behavioral response of heifers to milking during the first week of lactation; therefore, it would be interesting to investigate what components of training (e.g., handling or familiarization to the milking environment) are more effective at reducing the behavioral response of heifers to the milking process.

Central inhibition of milk ejection is the result of inhibition of oxytocin release from the pituitary gland and can be abolished by giving exogenous oxytocin (Bruckmaier, 2005). Central inhibition of milk ejection in dairy cattle is often associated with emotional stressors, such as being milked in a novel environment (Bruckmaier et al., 1993) or being milked for the first time, in the case of primiparous heifers (Van Reenen et al., 2002). In the present study, heifers were given oxytocin to measure residual milk volume to assess whether milk ejection was disturbed. Residual milk volume decreased over the first week of lactation regardless of the training treatment or temperament, suggesting that all animals experienced some level of central inhibition of milk ejection on the first day of lactation,

which rapidly decreased by d 5 of lactation, probably because of animals becoming habituated to the milking process. Van Reenen et al. (2002) also found that residual milk volumes decreased by 16% between d 2 and 4 of lactation in primiparous heifers. Furthermore, in the present study, residual milk volumes were lower in trained than untrained heifers, suggesting that the central mechanisms associated with milk ejection were less disturbed in trained heifers. Rushen et al. (1999) found that residual milk volumes were increased in cows when they were milked in the presence of a person who had previously treated them aversively. These results provide some evidence that training reduced the distress experienced by heifers during the first week of lactation even though this was not associated with a reduction in the behavioral response to milking.

In the present study, plasma cortisol concentrations increased during milking, but this was significant only for HR heifers, suggesting that HR heifers found the experience of milking more distressful than LR heifers. In addition, plasma cortisol concentrations were lower on the fifth day of lactation relative to the first day of lactation. Van Reenen et al. (2002) also observed that cortisol concentrations increased during milking in heifers and that the cortisol response to milking was less by d 4 of lactation. Therefore, it appears as though by d 4 or 5 of lactation, heifers are already habituating to the milking process, which may suggest that they are experiencing less distress. This is also supported by the lower residual milk volumes observed in heifers over the first week of lactation in the present study and by Van Reenen et al. (2002). In the present study, cortisol concentrations were lower in trained than untrained heifers, suggesting that trained heifers may have experienced less distress during milking. Heifers that were handled at the time of calving also had reduced milk cortisol concentrations (Hemsworth et al., 1989). Exposing heifers to the milking environment and increased human interaction before or at calving may therefore reduce the fear responses of these animals to milking. Furthermore, residual milk volumes were lower in trained than untrained heifers in the present study, which may reflect a lower stress response to milking. However, the release of cortisol during milking in dairy cows is essential for normal lactogenesis (Tucker, 1981), so changes in cortisol concentrations may also reflect normal physiological functioning rather than being an indication of distress, especially considering the low concentrations found in the present study. The lower cortisol levels and residual milk volumes observed in trained heifers in the present study, however, suggest that training reduced the distress experienced by these animals during the first week of lactation.

During the first week of lactation, training affected milk production in LR and HR heifers differently. It is interesting that spontaneous milk yields (milk ejection before exogenous oxytocin administration) were not affected by the training treatment or temperament; however, total milk volumes were lower in trained than untrained HR heifers but did not differ between LR heifers. These differences in total milk volumes can be explained by differences in residual milk volume. Even though the result was not significant, trained HR heifers had a lower residual milk volume than untrained HR and all LR heifers, suggesting that the central mechanisms associated with milk ejection were not disturbed to the same extent in trained HR heifers (Bruckmaier et al., 1993). Alternatively, the reduced residual milk volume produced by trained HR heifers was due to peripheral inhibition. This occurs when the action of oxytocin on the contraction of smooth muscle in the milk duct is inhibited by the action of catecholamines, even in the presence of normal oxytocin release from the pituitary gland (Bruckmaier, 2005). Peripheral inhibition can be induced experimentally by administering an α -adrenergic receptor agonist before milking, resulting in a reduction in total milk yield, which is the result of the blockage of milk flow into the cistern rather than the teats (Bruckmaier et al., 1997). Furthermore, the inhibitory effect of an α -adrenergic receptor agonist on milk ejection is not reversed by giving an injection of oxytocin (Bruckmaier et al., 1997), which is in agreement with the lower residual milk volume produced by trained HR heifers in the present study. It is unclear why training would cause peripheral inhibition in HR but not LR heifers, unless training, especially the short and intense training regimen that was used in the present study, caused HR heifers to have a greater fear response at the time of milking (e.g., increased heart rate and release of catecholamines). Another possibility is that, by chance, the HR heifers selected in this study to be trained were naturally low producers. It is impossible to select heifers based on milk production data, so it is not possible to know if that was the cause of the present results, but individual heifer productivity values for milk production were used as a covariate in the data analysis to help control for this possibility.

Milk production measures (milk yield, milking duration, and average milk flow rates) were recorded over the entire lactation period to determine whether training treatment or temperament affected milk production in the long term. In the present study, overall milk yield was not affected by training treatment or temperament, which is consistent with the results from the first week of lactation. After the first week of lactation, residual milk volume was not measured, so it is not possible to know whether differences in residual milk volumes

between trained and untrained HR heifers during the first week of lactation persisted throughout lactation. Milking durations were longer in untrained LR than in trained LR and HR heifers over the entire lactation period. Differences in milking duration are not consistent with observations during the first week of lactation or milk yield, and the reasons for this are unclear. Furthermore, average milk flow rates were higher in trained than untrained heifers, which is not consistent with the pattern of average daily milk yield but is consistent with the shorter milking durations observed in trained heifers. More research is needed to explore the relationships between heifer temperament and training and the associated influences on milk ejection. In the present study, trained heifers had lower cortisol levels and residual milk volumes, suggesting that these animals may have experienced less distress during the first week of lactation. Furthermore, training appeared to affect the behavioral reactivity of heifers to humans, the behavioral response to milking, and milk production measures differentially depending on temperament. In the future, it would be interesting to evaluate different training regimens on the behavior, physiology, and milk production of heifers in relation to temperament.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance from AgResearch (Hamilton, New Zealand) staff Suzanne Dowling and Gemma Worth, and DairyNZ (Hamilton, New Zealand) staff Bruce Sugar. This study was funded by DairyNZ.

REFERENCES

- Bertenshaw, C., P. Rowlinson, H. Edge, S. Douglas, and R. Shiel. 2008. The effect of different degrees of 'positive' human-animal interaction during rearing on the welfare and subsequent production of commercial dairy heifers. *Appl. Anim. Behav. Sci.* 114:65-75.
- Boissy, A., and M. Bouissou. 1988. Effects of early handling on heifers' subsequent reactivity to humans and to unfamiliar situations. *Appl. Anim. Behav. Sci.* 20:259-273.
- Bremner, K. J. 1997. Behavior of dairy heifers during adaptation to milking. *Proc. N. Z. Soc. Anim. Prod.* 57:105-108.
- Breuer, K., P. H. Hemsworth, J. L. Barnett, L. R. Matthews, and G. J. Coleman. 2000. Behavioural response to humans and the productivity of commercial dairy cows. *Appl. Anim. Behav. Sci.* 66:273-288.
- Bruckmaier, R. M. 2005. Normal and disturbed milk ejection in dairy cows. *Domest. Anim. Endocrinol.* 29:268-273.
- Bruckmaier, R. M., D. Schams, and J. W. Blum. 1993. Milk removal in familiar and unfamiliar surroundings: Concentrations of oxytocin, prolactin, cortisol and β -endorphin. *J. Dairy Res.* 60:449-456.
- Bruckmaier, R. M., O. Wellnitz, and J. W. Blum. 1997. Inhibition of milk ejection in cows by oxytocin receptor blockade, α -adrenergic receptor stimulation and in unfamiliar surroundings. *J. Dairy Res.* 64:315-325.
- Curley, K. O., Jr., D. A. Neuendorff, A. W. Lewis, J. J. Cleere, T. H. Welsh Jr., and R. D. Randel. 2008. Functional characteristics

- of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. *Horm. Behav.* 53:20–27.
- Fisher, A. D., C. A. Morris, and L. R. Matthews. 2000. Cattle behaviour: Comparison of measures of temperament in beef cattle. *Proc. N. Z. Soc. Anim. Prod.* 60:214–217.
- Gibbons, J. M., A. B. Lawrence, and M. J. Haskell. 2011. Consistency of flight speed and response to restraint in a crush in dairy cattle. *Appl. Anim. Behav. Sci.* 131:15–20.
- Hemsworth, P. H., J. L. Barnett, A. J. Tilbrook, and C. Hansen. 1989. The effects of handling by humans at calving and during milking on the behavior and milk cortisol concentrations of primiparous dairy cows. *Appl. Anim. Behav. Sci.* 22:313–326.
- Ivemeyer, S., U. Knierim, and S. Waiblinger. 2011. Effect of human-animal relationship and management on udder health in Swiss dairy herds. *J. Dairy Sci.* 94:5890–5902.
- Kilgour, R. J., G. J. Melville, and P. L. Greenwood. 2006. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans, restraint and novelty. *Appl. Anim. Behav. Sci.* 99:21–40.
- King, D. A., C. E. Schuehle Pfeiffer, R. D. Randel, T. H. Welsh Jr., R. A. Oliphint, B. E. Baird, K. O. Curley Jr., R. C. Vann, D. S. Hale, and J. W. Savell. 2006. Influences of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. *Meat Sci.* 74:546–556.
- Petherick, J. C., V. J. Doogan, B. K. Venus, R. G. Holroyd, and P. Olsson. 2009. Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. *Appl. Anim. Behav. Sci.* 120:28–38.
- Rousing, T., M. Bonde, J. H. Badsberg, and J. T. Sørensen. 2004. Stepping and kicking behaviour during milking in relation to response in human-animal interaction test and clinical health in loose housed dairy cows. *Livest. Prod. Sci.* 88:1–8.
- Rushen, J., A. A. Taylor, and A. M. de Passillé. 1999. Domestic animals' fear of humans and its effect on their welfare. *Appl. Anim. Behav. Sci.* 65:285–303.
- Schmied, C., X. Boivin, and S. Waiblinger. 2008. Stroking different body regions of dairy cows: Effects on avoidance and approach behavior towards humans. *J. Dairy Sci.* 91:596–605.
- Strelau, J. 1987. The concept of temperament in personality research. *Eur. J. Personal.* 1:107–117.
- Tucker, H. A. 1981. Physiological control of mammary growth, lactogenesis, and lactation. *J. Dairy Sci.* 64:1403–1421.
- Van Reenen, C. G., J. T. N. Van der Werf, R. M. Bruckmaier, H. Hopster, B. Engel, J. P. T. M. Noordhuizen, and H. J. Blokhuis. 2002. Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. *J. Dairy Sci.* 85:2551–2561.
- Waiblinger, S., X. Boivin, V. Pedersen, M. V. Tosi, A. M. Janczak, E. K. Visser, and R. B. Jones. 2006. Assessing the human-animal relationship in farmed species: A critical review. *Appl. Anim. Behav. Sci.* 101:185–242.
- Waiblinger, S., C. Menke, and D. W. Fölsch. 2003. Influences on the avoidance and approach behaviour of dairy cows towards humans on 35 farms. *Appl. Anim. Behav. Sci.* 84:23–39.
- Willis, G. L. 1983. A possible relationship between the flinch, step and kick response and milk yield in lactating cows. *Appl. Anim. Ethol.* 10:287–290.
- Windschnurer, I., C. Schmied, X. Boivin, and S. Waiblinger. 2008. Reliability and inter-test relationship of tests for on-farm assessment of dairy cows' relationship to humans. *Appl. Anim. Behav. Sci.* 114:37–53.