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Randomized clinical trial investigating the effect of exercise and standing on concrete prior to first calving on time to first lameness event in dairy heifers

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ABSTRACT

This controlled clinical trial investigated if an intervention immediately before the first calving event could reduce lameness incidence in pasture-based dairy heifers. Seven hundred ninety heifers across 6 farms in the Waikato region of New Zealand were randomly enrolled into treatment or control groups at a ratio of 1:1. The treatment consisted of heifers walking approximately 1 km from pasture along the farm race, standing on concrete for one hour, and then walking back to their paddock. This occurred once a day, 5 times a week, for 5 wk before calving. The control heifers were managed solely at pasture before calving. Just before calving, both groups were bought together and managed as one group for the remainder of the study. Heifers were followed for up to 28 wk, with fortnightly lameness scores collected to identify animals with a lameness score of ≥2 (lameness score 0–3). Lameness could also be diagnosed by the farmers, who had no formal lameness scoring training. The primary outcome of interest was time to first lame event. Secondary outcomes included milk solid production, change in body condition score during early lactation, time from onset of breeding season until conception, feasibility of the regimen and change in sole soft tissue thickness and profile. From a total of 782 heifers that had data collected on the outcomes, 102 (13.0%) individual first lameness events were recorded, 53 in heifers in the treatment group and 49 in control heifers. Of those 102 lameness events, 51 were first diagnosed by farmers. No apparent differences were detected in the hazard rate for time to first lame event between heifers in the 2 treatment groups. Treatment heifers had a 1.12 times hazard rate (95% confidence interval: 0.65–1.95) of a lame event compared with control heifers. No associations were identified between heifers in the 2 groups for any of the secondary outcome measures. However, farmers did report that the intervention was practical and easy to implement. It is possible that the intervention did not challenge the hoof enough, and that longer duration and distances walked may have resulted in a different outcome. Although no improvement in lameness outcomes were reported, no negative effects during and after the intervention were noted in animals in the intervention group. Further research into the area of lameness prevention is needed as there are few evidence-based solutions available to reduce lameness incidence in pasture-based systems.
Key words: lameness, prevention, pasture-based, heifers

INTRODUCTION

Lameness is one of the major production-limiting diseases, and, arguably, the most important animal welfare condition affecting New Zealand dairy cattle when prevalence (Fabian et al., 2014), incidence (Gibbs, 2010) and duration of the disease (Laven et al., 2008) are considered.

A recent review focusing on control of lameness in dairy heifers stressed the importance of focusing on heifers to control lameness (Bell and Randall, 2021), and a compelling case can be made of why insults that occur to the hoof of a heifer can have profound effects on lameness later in life (Hirst et al., 2002; Randall et al., 2016; Bell and Randall, 2021). Hirst et al. (2002) reported a 3.2 times hazard (95% CI: 1.3–7.5) of claw horn lameness in the second lactation in animals that were lame as heifers compared with those that were not lame as heifers. A negative feedback loop adds further challenge to lameness control, where a case of lameness is associated with pathological changes within the hoof that in turn predisposes cattle to future cases of lameness (Newsome et al., 2016; Wilson et al., 2021).

This suggests that an increased amount of effort should be aimed at either reducing the risk of lameness in the first lactation, or at the very least extending the time to first lame event, with particular emphasis around the transition period for the heifer (Bell and Randall, 2021). Although several control methods and

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recommendations have been published (Vermunt and Parkinson, 2002; Potterton et al., 2012; Bell and Randall, 2021), many of these recommendations are based on observational studies rather that interventional studies (e.g., Vermunt and Parkinson, 2002), and practical implementation by farmers for many of these strategies has been challenging (Bell et al., 2009; Barker et al., 2012; Bruijnis et al., 2013). This is likely a bigger challenge in pasture-based dairy systems as there are fewer practical options available to help reduce the effect of lameness (Hund et al., 2019). Of the 10 foot-health intervention categories that were proposed by Bell and Randall (2021), only 4 are likely to apply to pasture-based systems (genomic selection, heifer transition and early postcalving management, fortnightly mobility scoring and prompt effective treatment, and the development of the digital cushion with exercise and hard tracks or flooring). Only these 4 are applicable because the vast majority of dairy cows in New Zealand are never housed and the rates of prophylactic hoof-trimming are low, as described in Bryan et al. (2012).

The negative effect of housing dairy cattle on hard flooring for an extended period of time around calving on hoof health has been demonstrated in several studies (Vermunt and Greenough, 1996; Webster, 2001; Laven and Livesey, 2004; Bergsten et al., 2015), demonstrating that housing management before, and after calving can have a profound effect on future lameness events. There is also evidence that an adaptive process can occur within the hoof in response to environmental stressors, and the timing of when this occurs appears critical (Bergsten and Frank, 1996; Bergsten et al., 2015; Randall et al., 2016).

It is currently unknown what effect precalving transition management has on postpartum hoof health in dairy heifers that have spent the majority of their lives at pasture. The transition period for heifers is relatively unique in New Zealand, in that they have spent usually the majority, if not all of their life before first calving at pasture and a large proportion (>90%) of them calve over a short time frame of approximately 2 mo. Yet after calving, these animals are exposed to a host of new environmental (e.g., concrete yards, farm races of varying quality) and management stressors (e.g., introduction to new staff, mixing with other older cows, often in large groups (>300 animals) and other cows, introduction to feed sources other than grazed pasture, walking long distances along farm races), in addition to the unavoidable changes that calving has on the hoof (Webster, 2001; Tarlton et al., 2002; Knott et al., 2007). Biochemical changes associated with parturition alter and weaken the connective tissue involved with the suspensory apparatus of the pedal bone within the hoof capsule, resulting in greater laxity of the pedal bone within the hoof capsule in primiparous heifers compared with maiden heifers of a similar age (Tarlton et al., 2002). This laxity increases the loading pressure of the pedal bone and can result in damage and disorganization to the adjacent corium. Although this is a natural and unavoidable phenomenon, the clinical effect can be modified by the environment the cattle are housed in (Knott et al., 2007). The combination of parturition and housing heifers on concrete has been associated with greater sole lesion scores in the 3 mo after parturition, compared with heifers housed on straw yards around the time of parturition (Knott et al., 2007). Dairy cattle in New Zealand stand on concrete for shorter timer periods than housed cattle, with the majority of the exposure occurring immediately before milking on a collecting yard and during milking. However, exposure to concrete is still an important risk factor for lameness in these cattle (Hund et al., 2019).

Conditioning the hooves of heifers to standing on concrete and walking on tracks around the transition period could allow the hoof to adapt to the stresses it will be exposed to after calving. Gard et al. (2015) reported that weaned bull calves reared on a race consisting of mixed terrain with enforced exercise for 4 mo adapted by significantly increasing digital cushion volume compared with calves managed on a small grass paddock. This is relevant as digital cushion volume has been reported as one of the key predictors for lameness risk in dairy cows (Bicalho et al., 2009). No similar studies have been undertaken in pregnant heifers. However, exercise has been shown to increase hoof wear, and thus, horn growth in pregnant dry cows. Black et al. (2017) reported that in cows that were housed in freestalls, adding an exercise regimen (walking for 1.5 h on concrete alleyways) before calving increased both hoof growth and wear, resulting in more equal hoof horn growth and wear than in cows housed in the same freestalls, but without exercise. Black et al. (2017) concluded that their exercise regimen did not impair hoof health and may have improved it. Thus, prepartum exercise may directly increase the ability of the hoof to protect against external trauma. It may also have an indirect effect; Bergsten et al. (2015) reported that heifers reared on hard surfaces for one year before calving had sole hemorrhage before calving, but fewer sole lesions after calving. This is consistent with the findings of Randall et al. (2016) that heifers with the presence of moderate sole and white line hemorrhage immediately before calving were associated with a significant reduction in lifetime lameness risk, inferring that the development of hoof hemorrhages before calving is associated with hoof resilience.

Any reduction in the risk of lameness or a delay to the development of the first case of lameness would

Table 1. Lameness scoring descriptors used in a randomized clinical trial investigating the effect of precalving exercise and time standing on concrete on time to first postpartum lame event (adapted from DairyNZ Lameness Scoring system)

Lameness score	Descriptor
0	Walks with even weight bearing on all 4 limbs at a similar walking speed to a person, with regular stride length and rhythm. Straight back line at all times and head held in line or slightly below the backline and steady when walking.
1	Walks unevenly but reduced weight bearing on affected limb not easily obvious. May have uneven stride length or rhythm. Backline straight when standing but may be mildly arched when walking.
2	Lame, with decreased stride length on affected limb. Weight bearing reduced on affected limb, which can easily be identified. Walking speed slower than normal. Backline often arched when standing and walking and head bobs up and down when walking.
3	Severely lame, with minimal or no weight bearing on affected limb. Reluctant to move and cannot keep up with the healthy herd. Backline arched when standing and walking and often large head movements up and down when walking.

result in a long-term reduction in lameness risk across the whole of an animal's productive life. However, to be acceptable on farm, such interventions need to be practical and achievable, as well as effective. Our hypotheses were that exposure to concrete and farm tracks via an exercise regimen immediately before calving in pasture-based heifers would increase sole soft tissue thickness and that this modification would reduce lameness risk postcalving. The primary objective of this study was to assess whether exposure to standing on a concrete surface and exercise on tracks over a 5-wk period immediately before calving affected the hazard of lameness in first-lactation dairy heifers. Secondary objectives included assessing the effect on sole soft tissue thickness and sole profile, along with associations with milk solid production, change in BCS during early lactation, time from onset of breeding until conception, and the feasibility of the regimen.

METHODS

The study was a partially blinded, randomized clinical intervention study conducted on a convenience sample of 6 commercial dairy farms in the Waikato region of New Zealand. All animal procedures were approved by the Ruakura Animal Ethics Committee (AgResearch, Hamilton, New Zealand), application number 14955. The study was designed and conducted in accordance with REFLECT guidelines (O'Connor et al., 2010).

Sample size calculations for total heifer numbers were based on the primary outcome objective, time to first lameness case. As this was a novel study, there was minimal information available on which to base calculations. The assumptions were that 25% of the control animals would have a lameness score (**LS**; Table 1) $\geq$2 (lameness score range: 0–3) at least once during their first 7 mo of lactation, and that the intervention would reduce this by 25% [hazard rate ratio (**HR**) = 0.75]. Clustering of animals within farm was accounted for by including a design effect of 1.2. With an 80% power and a 95% confidence, 293 animals in both groups would be required (586 in total). Based on the herd-sizes of the enrolled farms, a total of 795 heifers were enrolled. This accounted for animal drop-out and issues with missing data; not every heifer was able to be identified on every scoring occasion due to logistical issues observing animal identification when scoring lameness. Sample size calculation was as per methods stated in Therneau and Grambsch (2000) and implemented using the epi.sscomps() function in epiR package within the statistical software R, using version 4.1.0 (2021; https://r-project.org). The secondary outcome of distance from pedal bone to sole surface, as measured by ultrasound, required 28 animals, 14 in each treatment group, to be able to detect a 1.3 mm difference in sole soft tissue thickness, with a standard deviation of 1.2 mm (as described by Laven et al., 2012a), with a power of 80% and a 95% confidence.

Study Farms

This study was conducted on a convenience sample of 6 dairy farms from the Waikato region of New Zealand from June 2020 to February 2021. The farm enrollment criteria consisted of the farm being 100% spring-calving with cows managed at pasture, undertake individual cow milk sampling including testing for weight of fat and protein (kg) on 4 occasions throughout lactation (as commonly practiced in the New Zealand dairy system for herd improvements) and undertake pregnancy diagnosis with ultrasound to confirm conception date. The heifers had to be present on the dairy farm for at least 5 wk before the planned start of calving and had to have spent the majority of their lives (>95%) on grazed pasture and had minimal exposure to concrete up to the stage of enrollment. The farmers also needed to have the necessary willingness and resources to conduct and participate in a long-term interventional study of this type. Larger herd sizes (>450 lactating cows) were preferred when convenience sampling. In

total, 8 farms were contacted from a list of dairy clients of the veterinary practice that the primary author was employed at and that the primary author thought may fit the above criteria, with 6 farmers confirming they would participate in the study. The 2 farmers that declined did so due to the perceived additional workload required to administer the intervention. Farmers were required to record any time that heifers spent standing on concrete additional to the intervention, and to report any lameness that they noticed for any heifers through the study period. Study dates were referred to relative to 2 major farm calendar dates used in the New Zealand dairy system; the date when the first heifer was predicted to start calving, and the planned start of mating for the herd. The length of time between these 2 dates are not the same for every farm, with some New Zealand dairy farms having heifers begin to start calving 1 to 2 wk before the multiparous cows. The period between the planned start of calving for the heifers and the planned start of mating on the enrolled study farms ranged from 82 to 94 d, with a median of 84 d.

Study Animals

The study population consisted of all pregnant nulliparous dairy heifers present on the enrolled study farms at the time of the initiation of the study (approximately 5–6 wk before the start of the 2020 calving period for each herd). The sample population was selected from the study population after the heifers with the following exclusions were removed; any heifer with a LS $\geq$2 on the day of enrollment, any heifer with a BCS $<$4 (using a 10-point scoring system; Roche et al., 2004) on the day of enrollment, and any animal that the farmer stated would not go on to be used for milk production. Postinclusion removals consisted of animals that were identified as lame by the farmer during the intervention, any that calved at least 3 wk before expected calving date (defined as aborted), or any systemic illness that the farmer reported to the study investigator before the animal calving. Enrolled animals were followed until approximately 6 to 7 mo of lactation (culminating with the 15th LS event on each farm), or removal from the herd (whichever occurred first). The one exception was for the secondary outcome of milk production, where data from enrolled animals was collected up until the end of their first lactation.

Randomization

Heifers were randomly allocated into intervention or control groups at a ratio of 1:1 on each farm. Randomization blocking involved BCS. A list of study animals for each farm was created with animals grouped on BCS on the day of enrollment in 0.5 unit increments. For each BCS group, animals were ordered by smallest to largest tag number. A coin was tossed by the lead technician to decide which treatment group the first animal in each block was allocated to. The next animal was then randomized into the alternate group, with this pattern continuing for the remaining of the BCS group.

For the sole profile and sole soft tissue thickness measurements, 30 heifers from 2 farms were selected, 20 from one farm and 10 from the other. The 2 farms were selected based on convenience, as they had the best handling and chute facilities. Animals within the farms were ordered within treatment group and expected calving date, and the study population consisting of animals with expected calving dates in the second week of the seasonal calving period for the heifers on each farm. The planned sample population consisted of the first 10 animals in each treatment group from farm 1 and the first 5 from farm 2. However, due to a recoding issue, 11 treatment animals and 9 control animals were enrolled on farm 1.

Intervention

Animals were drafted into either intervention or control groups, as per randomization procedure, 5 to 6 wk before the planned start of calving for the heifers on each farm. On each study farm, animals were then managed separately in their respective treatment groups until the time of calving for the animal, or once the intervention period had finished (whichever occurred first). Heifers in the intervention group were required to walk along the farm race from the paddock that they had been allocated by the farmer on a given day, to the concrete collecting yard, or concrete feed pad. The distance walked varied depending on the day and the farm, but was designed to have the animals walk a total of approximately 1.0 km (range: 0.5–1.5 km) along tracks per intervention day. The intervention animals then stood on concrete without feed for approximately one hour (minimum one hour, maximum 1.5 h), after which they were allowed to walk back to their paddock. Water was available for heifers when on the concrete surface. The intervention took place once a day, 5 d every calendar week for 5 wk (a total of 25 intervention days over a 35-d period) and was carried out by farm staff. Farmers were financially incentivized to participate in the study, to recompense them for the cost of the additional labor required to implement the intervention. Spot audits on all farms were conducted at least weekly by the lead technician to ensure that the intervention was being conducted. Control animals were managed as per normal farm practice during the

5-wk intervention period, and for 4/6 farms, heifers were kept permanently at pasture. Two farms operated the practice of standing animals off. This describes a practice where cattle are moved to a firm surface (in this case, concrete) in advance of inclement weather so as to protect sword from poaching damage caused by animals walking on wet pasture. These 2 farms used this practice sparingly during the intervention phase (twice on one farm, and once on the other). When this occurred, both treatment and control animals were moved to a concrete surface at the same time and for the same duration (up to 12 h), and for heifers in the intervention group, the intervention did not take place the following day. For all farms, feed during the intervention phase consisted solely of grazed pasture. Quantity and quality of pasture that was allocated to the heifers in their daily paddock was not measured as part of the trial and it was left to the individual farmers to make management decisions on this. However, they were instructed to manage the feeding of the 2 treatment groups such that animals in the treatment and control groups received a similar quality and quantity of grazed pasture.

At the completion of the intervention phase, just before the planned start of calving, the 2 treatment groups were merged in preparation for parturition. Once the intervention had ended, farmers managed the heifers as per their standard farm practices, and on all farms, heifers calved at pasture. On all farms, heifers were milked in a herd separate to the main mixed-age cow herd. One farm milked their heifers once a day during the mating period (a set time period to mate cattle, approximately 10–11 wk long, starting approximately 12 wk after the start of calving), and another farm milked their heifers once a day from the start of mating onwards. The remaining 4 farms milked heifers twice a day throughout the entire lactation.

The primary investigator (WM) was not blinded during the intervention, as he was aware which animals were in the intervention group when assisting with the conditioning and exercise enrollment and intervention. However, the technicians assessing the LS were blinded to treatment groups, as all heifers were managed together in one herd after calving. Before the analysis being undertaken, the treatment groups were recoded by an independent operator (i.e., treatment was changed to group X and control was changed to group Y). This allowed the biometrician to be blinded to the treatment groups during statistical analysis. Farm staff did not know which treatment group the heifers were enrolled in once lactation had commenced, although the nature of the intervention did not allow blinding while it was being administered.

Data Collection

Fortnightly LS data from heifers in milk was collected by one of 2 trained technicians, starting 2 wk after the first heifer in each herd had calved and continuing for 15 LS events. LS was based on the DairyNZ 0-to-3 grading system (Table 1) and was conducted as animals were leaving the milking parlor on a flat even concrete surface. The time when the LS data were collected could be at either the morning milking or the afternoon milking and varied between and within farms for practical reasons (e.g., on one farm lack of lighting made it difficult to visualize heifers walking after morning milking in spring, or if the primary technician had another appointment in either the morning or afternoon). The technicians were LS-trained by the authors JH and WM, and were re-assessed 4 times within the first month of the study by the lead researcher, WM. Formal training consisted of a morning of LS theory and watching of prerecorded videos, of which the technicians were quizzed on. Then during the afternoon, the 2 technicians and JH and WM scored >200 animals walking postmilking. Assessment of drift was not carried out during the observation period, and reliability measures were not assessed.

Farm staff were instructed to record the animal number and date of all cases of lameness that they diagnosed on farm. These farmer-recorded lameness cases were analyzed as lame, regardless of their LS status during routine data collection. No formal instructions were given to the farmers by the study authors on how to observe lame animals.

All heifers had BCS measurements collected at 3 time points; at enrollment (BCS1), when 50% of the heifers had been calved at least 4 wk (BCS2) and approximately 2 wk after planned start of mating (BCS3). Animals were scored on a 10-point scale by a DairyNZ certified body condition scorer (Roche et al., 2004).

Data were collected for each heifer on date of birth, breed, individual calving date, and herd removal data up until the point of drying off (~10 mo following calving) by extracting it from the herd recording software Minda Live (Livestock Improvement Cooperation). This software was also used to extract individual milk sample information collected at 4 time points during lactation (at approximately 2, 5, 7, and 9 mo following the herd start of calving). This data provided information on the fat and protein weight of the milk, commonly reported combined in New Zealand as kilograms of milk solids. Trans-rectal ultrasound was used to determine the presence and age of the fetus at approximately 35 d after the end of the mating period by veterinarians, and data on date of conception and pregnancy status

entered into the Infovet platform (Zoetis New Zealand Ltd.).

The animals enrolled for sole profile and sole soft tissue thickness assessment were restrained in a chute and had either left or right hind limbs (left on one of the farms, right on the other farm) lifted on 3 occasions: at enrollment, 7 to 14 d postcalving, and 100 to 110 d postcalving. The hoof was first washed and the widest point of the lateral claw identified. The sole profile was obtained by placing a contour gauge at this position and transposing the profile with pencil onto graph paper. Sole depth from the abaxial aspect of the claw was measured at 10 mm, 20 mm, and 30 mm from the abaxial edge, with vertical measurements from these points to where the abaxial surface would have contacted the ground. The methodology followed that proposed by Tranter and Morris (1992). Ultrasonographic images of the plantar surface were then collected from the lateral claw of the elevated limb with an Easi-scan curvi-linear scanner (BCF Technology Ltd.) following the methodology proposed by Laven et al. (2012b). Briefly, sole soft tissue thickness was defined as the vertical measurement from the sole surface to the tip of the distal phalanx over the midline of the claw, perpendicular to the sole surface. Further details including pictorial illustrations are available in Tranter and Morris (1992) and Laven et al. (2012b).

Statistical Analysis

All analyses were carried out using the statistical software R, using version 4.1.0 (2021; https://r-project.org). The experimental unit of observation was the cow for all analyses. Descriptive statistics for animals from each farm and both treatment groups were described. Counts and proportions of categorical variables were tabulated and continuous variables described with respect to either mean and standard deviation, or median and interquartile range. The proportion of animals removed from the herd during the study period was reported for each farm and for the 2 treatment groups. The proportion of lame animals, and 95% confidence intervals, within each treatment group was reported for the entire observational period.

The primary outcome was the number of days from the end of the intervention to first case of lameness (either LS >1/3 or farmer diagnosed lameness); any subsequent lameness events were not analyzed. The outcome variable was right censored to either the last LS event on a farm or when an animal was removed from the herd, whichever occurred first. Data were initially presented in the form of Kaplan-Meier curves, with treatment group or farm as the predictor, respectively, to visualize the survival curve between farms and animals in each treatment group. To assess the statistical association between treatment group and time to first lameness case, a Cox proportional hazard model was developed, with ties handled using the Efron approximation. The predictor of interest was treatment group; breed was included as a possible confounding variable. It was also of interest to assess whether the effect of treatment on lameness risk differed between farms, so initially a farm × treatment interaction term was included into the model. Both breed and treatment group were included in the initial multivariable model, as was the farm × treatment interaction term; starting with the interaction term, variables were removed from the multivariable model if partial likelihood ratio test statistic between 2 nested models had a $P > 0.05$. If the farm × treatment interaction term was removed, then farm was treated as a frailty term, to account for clustering of animals within farm. Results from the model were reported as HR with associated 95% confidence intervals. Proportional hazards assumption was assessed using a global statistic based on Schoenfeld residuals, and scaled Schoenfeld residuals plotted over time for each variable with a smoothing line and 95% confidence interval to visually assess any deviation from proportional hazards. The proportional hazards of animals in the 2 treatment groups within each farm (farm × treatment interaction) were also assessed using the methods described above to ensure that the assumption of proportional hazards was constant over each farm. Deviance residuals from the final model >3 were further investigated as potential outliers. The effect of influential observations were assessed with dfbetas for each model coefficient. Any dfbetas >0.10, or any observations that had dfbetas substantially greater than the bulk of the observations, were investigated further. If any reason was found to remove these observations (e.g., data entry issue), then they were removed. Otherwise, they remained in the model.

Secondary outcomes included time from the date where the herd started mating to conception, herd removal, BCS, and milk fat + protein (kg of milk solids). Sole profile and sole soft tissue thickness from the examined subset of animals were also analyzed.

Reproductive data were recoded as the number of days from the herd start of mating to conception date and was analyzed as time-to-event data. The methodology was the same as described for time to first case of lameness above, with the same model building strategy and predictors assessed. All nonpregnant animals were right censored to the median mating length of the farms (77 d).

Milk solids per cow per herd-test day was analyzed using mixed linear regression techniques. As there were repeated temporal herd-test measurements for each

heifer, heifer was included as a random intercept term, and a first-order autoregressive correlation structure for herd-test number within heifer was included in the error term. Farm was included as a fixed effect to avoid model complexities of 3 levels of hierarchy. Predictors of interest were treatment group, breed, DIM, farm, and herd-test number. A saturated initial multivariable model was produced, as per recommendations of Zuur et al. (2009). Variance inflation factors for all variables were produced before any variable selection was conducted, as it was likely that DIM and herd-test number would be highly correlated. If the variance inflation factor was >4 for either of those 2 variables, then herd-test number would not be included into the model. Variables were then removed from the model if the likelihood ratio test between 2 nested models was $P > 0.05$, until all variables remaining had a likelihood ratio test $P \leq 0.05$. Treatment group was forced into the model as the predictor of interest. Various polynomials, including the Wilmink function, were assessed for improved model fit over a linear term for DIM; the DIM variable with the model with the lowest Akaike information criterion was selected as the DIM function. Standardized residuals >3 from the final model were investigated further to assess if there was any reason to remove them (e.g., data error entries). Cook's distances were produced for each observation from the final model and ranked in order from largest to smallest. Any of those that were substantially greater than the bulk of the observations were reported and had their raw data investigated further. The assumptions of homoscedasticity and normality of residuals were assessed graphically.

Body condition scores at each time point were tabulated, and the BCS change between first and third BCS (BCS 1 to BCS 3) and second and third BCS (BCS 2 to BCS3) reported. Data were collapsed into reductions in BCS of >0.5 or ≤0.5 units. For each binary outcome, BCS 1 to BCS 3, and BCS 2 to BCS 3, a mixed logistic regression model was used to assess the relationship between treatment group and BCS change over time. The only predictor included was treatment group; farm was included as a random effect.

Sole soft tissue thickness (mm) was analyzed using linear regression, with the outcome for each heifer the difference between the measurements at the second (postcalving) and first (enrollment) visit, and between the third (mating) and first visit, respectively. A single model accounting for repeated sole measurements over time was produced. The hypothesis was that the intervention altered the sole soft tissue thickness over time. As such, an interaction between treatment and timing of measurement (postcalving or mating) term was forced into the final model. This interaction term also accounted for the clustering within animal, so no random effect of animal was needed. It was also possible that the effect of treatment may have been modified by farm. A 3-way interaction term between farm, treatment, and timing remained in the model if the log-likelihood ratio test between 2 nested models was $P \leq 0.05$. If this was not met, then the 3-way interaction term was removed, and farm assessed for its inclusion into a multivariable model. No other predictors were included in the model. Data were reported as estimated marginal means and 95% confidence intervals adjusted for multiple pairwise comparisons using the Šidák correction. Linear regression assumptions of homoscedasticity and normality of residuals were assessed, as well as linearity for the continuous predictor, sole soft tissue thickness at enrollment. The outcome for sole profile was the difference of the distance vertically between the 30 mm and 10 mm measurement from one lateral hind claw on a given measurement day. Linear regression was conducted to assess the association between treatments over time on the sole profile of the claw. Inclusion of farm in the model, and model reporting was as per sole soft tissue thickness.

RESULTS

Of the 795 animals in the study population, 790 were enrolled into the study (Figure 1). Of those, 397 were randomly enrolled into the treatment group and 393 into the control. A total of 782 heifers remained in the study at the completion of the intervention phase, and this was the denominator for all analyses, unless otherwise stated. Two animals were diagnosed as lame during the intervention phase; one from the treatment group and one from the control group. Three hundred seventy-nine treatment animals and 376 control animals completed the study. Descriptive statistics for the 790 enrolled heifers in both treatment groups are presented in Table 2. Seventeen animals were culled, and 3 died, during the course of the study period (up to approximately mid-February, 2021); 11 of these were control animals and 9 were in the treatment group. The number of removed animals per farm varied from zero to 12, with a median of 3 removed animals per farm.

Lameness Outcomes

Fifteen lameness assessments were conducted on each farm, starting between July 14 and August 6, 2020, and continuing until between January 25 and February 19, 2021, depending on the start of calving of the farm. Due to the range of calving dates, heifers had a median of 14 lameness assessments, with a range of 12 to 15. A total of 102/782 (13.0%; 95% CI: 10.9–15.6%)

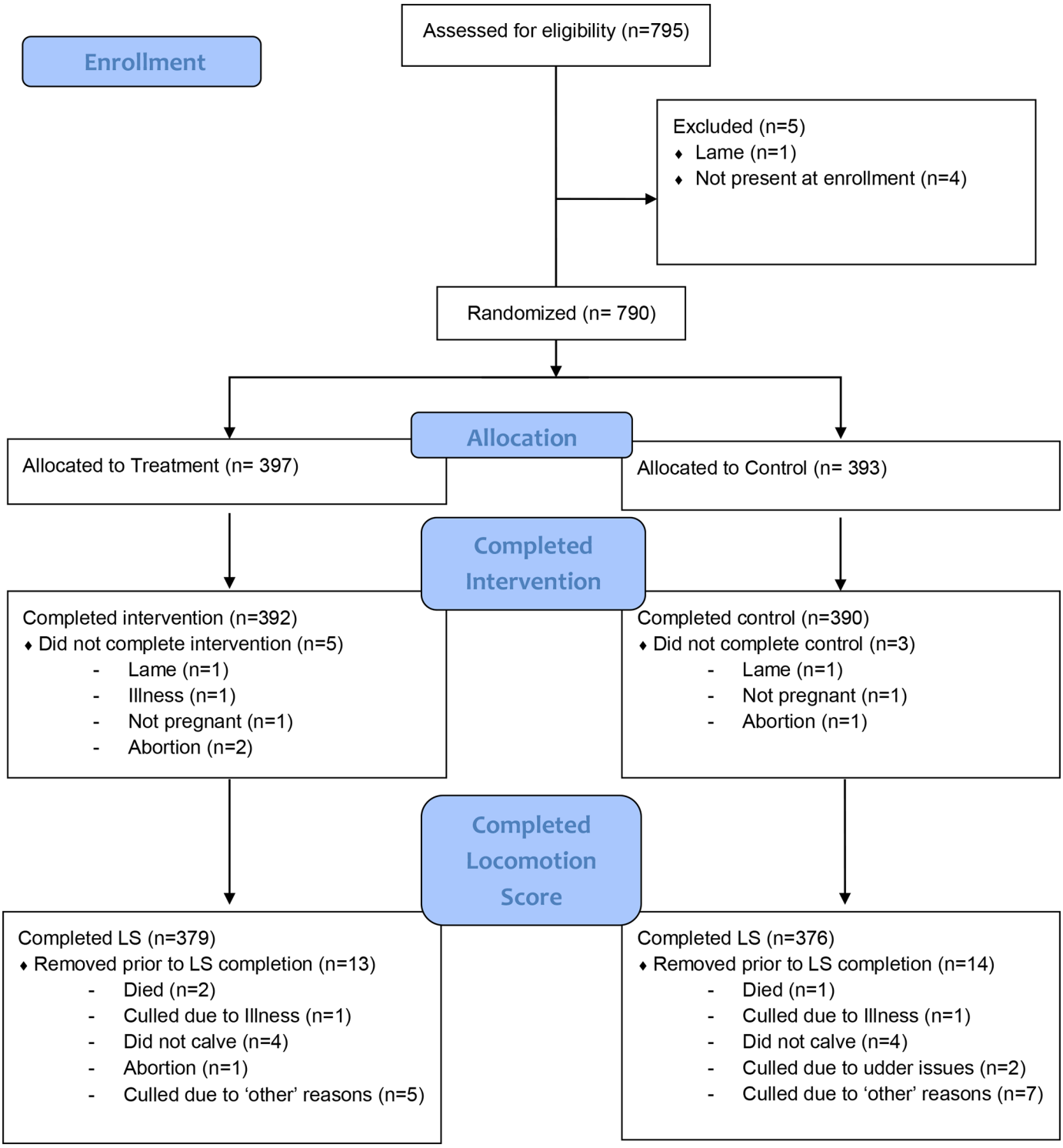

Figure 1. Flowchart outlining reasons for inclusion, exclusion, and removal of animals from a randomized clinical trial investigating the effect of exercise and time standing on concrete on time to first lame event. Only animals that completed lameness scoring were included in the analysis. LS = locomotion score.

Table 2. Descriptive statistics for 790 enrolled heifers, across 6 farms, in a randomized study investigating the effect of exercise and standing on concrete precalving, on the risk of lameness during lactation; number of heifers in each category presented, with percentage in parentheses

Variable	Level	Control	Treatment	Overall
Farm	1	95 (50.3)	94 (49.7)	189
	2	58 (50.4)	57 (49.6)	115
	3	55 (49.1)	57 (50.9)	112
	4	41 (49.4)	42 (50.6)	83
	5	51 (50.0)	51 (50.0)	102
	6	93 (49.2)	96 (50.8)	189
BCS	≤4.5	49 (47.1)	55 (52.9)	104
	5	65 (46.4)	75 (53.6)	140
	5.5	207 (49.4)	212 (50.6)	419
	≥6	72 (56.7)	55 (43.3)	127
Breed	Holstein-Friesian	60 (48.0)	65 (51.6)	125
	Jersey	87 (49.4)	89 (50.6)	176
	Kiwi cross and other	230 (50.0)	230 (50.0)	460
	NA[1]	16 (55.2)	13 (44.8)	29

[1]Animals did not have breed data available as they had been culled or removed from the study before study completion.

heifers were diagnosed as lame between the end of the intervention phase and the end of the LS phase; 51 of these were diagnosed during LS events, and the other 51 were diagnosed by farmers, before the technician visit. Fifty-three of the lame animals belonged to the treatment group (13.6%; 95% CI: 10.6–17.4%), and 49 to the control group (12.6%; 95% CI: 9.6–16.2%).

The data were compatible with there being no effect of treatment group on the hazard of lameness with the hazard rate in the treatment group being 1.12 times (95% CI: 0.65–1.95; $P = 0.94$) the hazard of a case of lameness in the control group (Figure 2). If the true hazard ratio was 0.65, we would almost never (2.5% of the time) expect a HR as large as we observed. Thus HR <0.65 are contradicted by the data. If the true HR was >1.95, we would almost never (2.5% of the time) expect an HR as small as we observed. Thus HR >1.95 are contradicted by the data. Breed was not associated with hazard of lameness ($P = 0.52$). There was no evidence of a farm × treatment interaction ($P = 0.71$). The proportional hazard assumptions were not violated for treatment, nor were they violated for groups of animals in the 2 treatment groups within each farm. Four observations were identified with deviance residuals >3; no reason was identified to exclude these observations. No influential observations were identified.

Reproductive Outcomes

A total of 754 heifers had pregnancy data; 20 heifers were removed from the herd before pregnancy testing, and 8 had had missing pregnancy test data. The data were compatible with no difference in the hazards of pregnancy between treatment and control animals ($P = 0.72$). The hazards of pregnancy in the treatment heifers was 0.97 times (95% CI: 0.84–1.13) the hazards of pregnancy in the control animals.

Milk Production

Milk test data were collected for 2,892 individual samples from 758 study heifers. The outputs of the mixed linear regression model can be found in Table 3. Heifers in the treatment group produced 0.010 (95% CI: 0.016–0.036) kg of milk solids/cow per day less than control heifers across the entire lactation, but the data were compatible with no difference between the groups ($P = 0.46$). As there were few herd-test measurements within the first 50 d of lactation, a linear term for DIM provided a better fit for the model than polynomial functions such as the Wilmink lactation function. DIM and herd-test number demonstrated collinearity issues (variance inflation factor for DIM of 5.6), and therefore, only DIM was included in the model. Farm, breed and DIM were all associated with daily MS production (Table 3). There were 3 observations that had Cook's distance >0.060 (at least 20% greater than the next closest observation). All 3 of these were the first herd-test value from Jersey animals from the same farm, 2 in the control group and one in the treatment group. No reason was found to exclude these values. No outlier observations were identified, and there were no deviations from homoscedasticity or normality or residuals.

Body Condition Score

The BCS change from BCS 1 to BCS 3, and BCS 2 to BCS 3 is presented in Table 4. The data were compat-

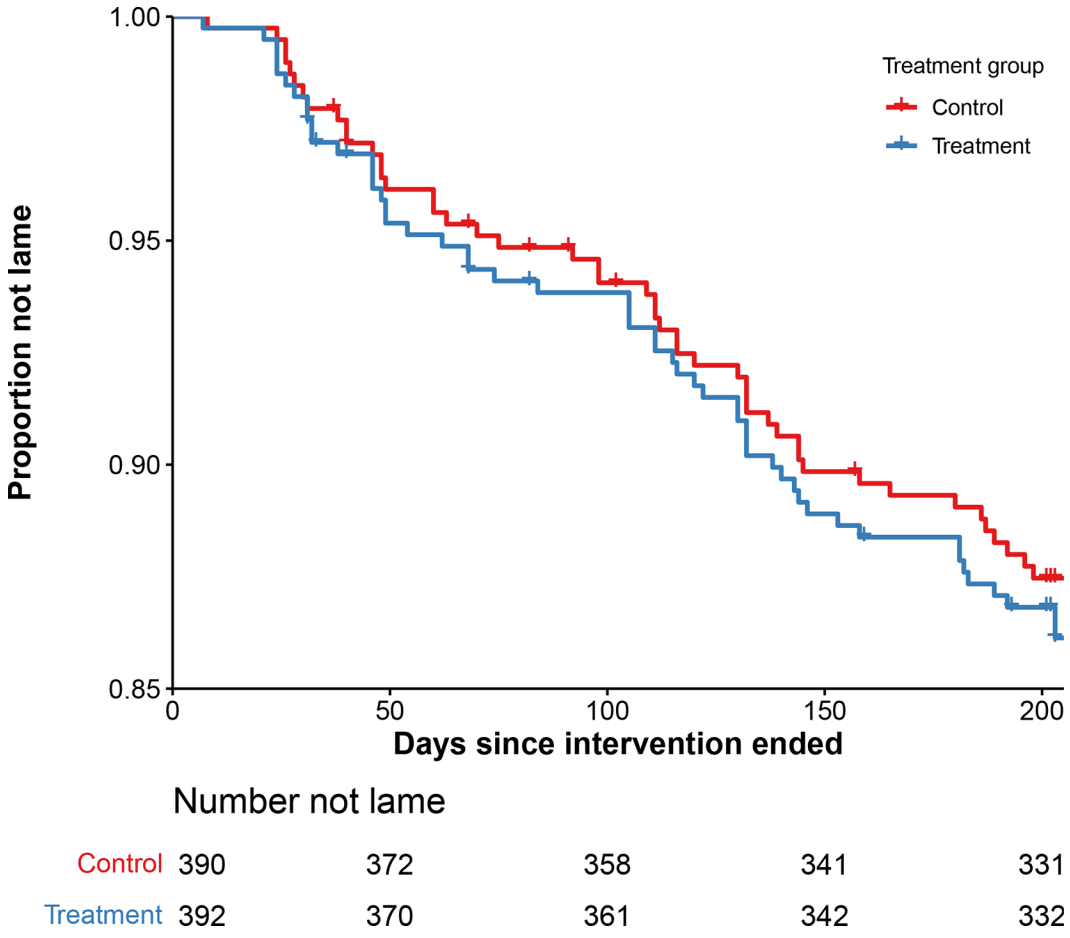

Figure 2. Kaplan-Meier survival plot for treatment group and time in days since the end of intervention to first case of lameness from a randomized clinical trial investigating the effect of exercise and time standing on concrete on time to first lame event. Lameness was defined as either lameness score (LS) >1 by a trained technician, or diagnosed by farmer. The + represent censored nonlame animals removed from the herd before the end of the study. A risk table of the number of heifers that remain eligible for first case of lameness at 50-d increments throughout the study period is presented below the Kaplan-Meier plot.

Table 3. Output from mixed linear regression model with kilograms of milk solids/heifer per day as the outcome (n = 2,892 samples from 758 heifers); animal was included as a random intercept and an autoregression correlation structure for herd-test within animal included in the error term

Variable	Category	Coefficient	SE	Lower 95% CI	Upper 95% CI	*P*-value
Intercept		1.967	0.026			
Treatment	Control	Referent				0.46
	Treatment	−0.010	0.013	−0.036	0.016	
Farm	1	Referent				<0.001
	2	0.007	0.027	−0.045	0.058	
	3	−0.321	0.028	−0.376	−0.267	
	4	−0.201	0.038	−0.275	−0.126	
	5	−0.253	0.026	−0.303	−0.203	
	6	−0.587	0.027	−0.639	−0.535	
Breed	Friesian	Referent				<0.001
	Kiwi cross and other	0.026	0.021	−0.015	0.067	
	Jersey	−0.091	0.032	−0.154	−0.028	
DIM		−0.003	0.000	−0.003	−0.003	<0.001

Table 4. The unit of BCS change at 2 time intervals between treatment and control heifers from randomized study investigating the effect of exercise and standing on concrete precalving

		Unit of BCS change						
Interval	Category	−0.5	0	0.5	1	1.5	2	2.5
BCS 2–BCS 3[1]	Control	14	160	137	13	0	0	0
	Treatment	16	148	138	14	0	0	0
BCS 1–BCS 3[2]	Control	2	8	66	150	107	18	0
	Treatment	0	16	74	144	98	20	1

[1]The numerical difference between BCS measured approximately 4 wk postcalving and BCS measured approximately 2 wk after the start of mating (~14 wk after the completion of the intervention).

[2]The numerical difference between BCS measured at the initiation of the intervention (5–6 wk before the herd expected calving start date) and BCS measured approximately 2 wk after the start of mating (~14 wk after the completion of the intervention).

ible with no difference between treatment groups in the number of animals that lost >0.5 BCS from the second and third BCS assessment ($P = 0.24$) and from the first to third BCS assessment ($P = 0.36$).

Sole Soft Tissue Thickness and Sole Profile

A total of 28 (14 of which were treatment) and 25 (11 treatment) heifers had sole soft tissue thickness measurements at the second and third examination, respectively. Due to recording errors with saving of images, there were only 2 treatment heifers from farm 2 that had measurements at the third examination. A 3-way interaction existed between farm, examination number and treatment group ($P = 0.05$; Figure 3). There was no difference between treatment and control heifers on farm 1 at the calving and mating measurements, nor between calving and mating. However, treatment heifers from farm 2 had 3.37 (95% CI: 0.24–6.49) mm greater sole soft tissue thickness at the mating visit compared with the calving visit, and also had 3.82 (95% CI: 0.92–6.71) mm greater increase in sole soft tissue thickness than treatment heifers from farm 1. On farm 1 heifers had a reduction in sole soft tissue thickness from enrollment to calving of 1.22 (95% CI: 0.04–2.42) and 1.48 (95% CI: 0.42–2.55) mm for control and treatment heifers, respectively.

At the postcalving visit, there were 30 heifers, 16 of which were in the treatment group with sole-profile measurements. Twenty-nine heifers, 15 of those in the treatment group, had sole profile measurements at mating. Farm was not included in the final multivariable model as it did not modify the effect of treatment ($P = 0.45$), nor was it associated with sole profile overall ($P = 0.39$). The data were compatible with treatment having no effect on sole profile at calving ($P = 0.77$) or mating ($P = 1.0$). Averaged across both treatment and control groups, at calving heifers had 1.58 mm (95% CI: 0.85–2.30) smaller difference between measurements taken 30 mm and 10 mm from the abaxial hoof margin compared with measurements collected at enrollment. There was less variation in sole profile between calving and mating (mean difference 0.19; 95% CI: −0.55 to 0.92 mm).

DISCUSSION

This article reports findings from a randomized controlled trial, conducted and reported in accordance with the REFLECT guidelines (O'Connor et al., 2010), investigating the effects of exercise and standing on concrete precalving in pasture-based dairy heifers on time to first lame event. No associations were identified between treatment group with respect to time to lame event, nor any of the secondary outcomes. Despite not finding clear evidence of differences between heifers in the treatment and control groups, we believe it is important that study outcomes are reported to inform future research in the important areas of study for dairy cow health and welfare.

This study was powered to detect a 25% reduction in the hazard of lameness from an assumed baseline hazard of 25%. This a priori hazard rate reduction equated to an absolute reduction in lameness incidence of ~6%, and was selected as a large enough difference that may appeal to farmers to implement the intervention. With the apparent lameness incidence risk in the control group being half that expected before the study (12.6 vs. 25.0%), an HR of ~0.5 for lameness in animals in the intervention group compared with animals in the control group would have been required to detect an absolute difference of ~6%. Post hoc power calculation revealed that this study had a 93% probability of detecting that difference, if it indeed existed. Therefore, we have reasonable confidence that this intervention, in its current form at least, is unlikely to result in a biologically meaningful reductions in the rate of lameness in first-lactation dairy heifers. It is possible that both

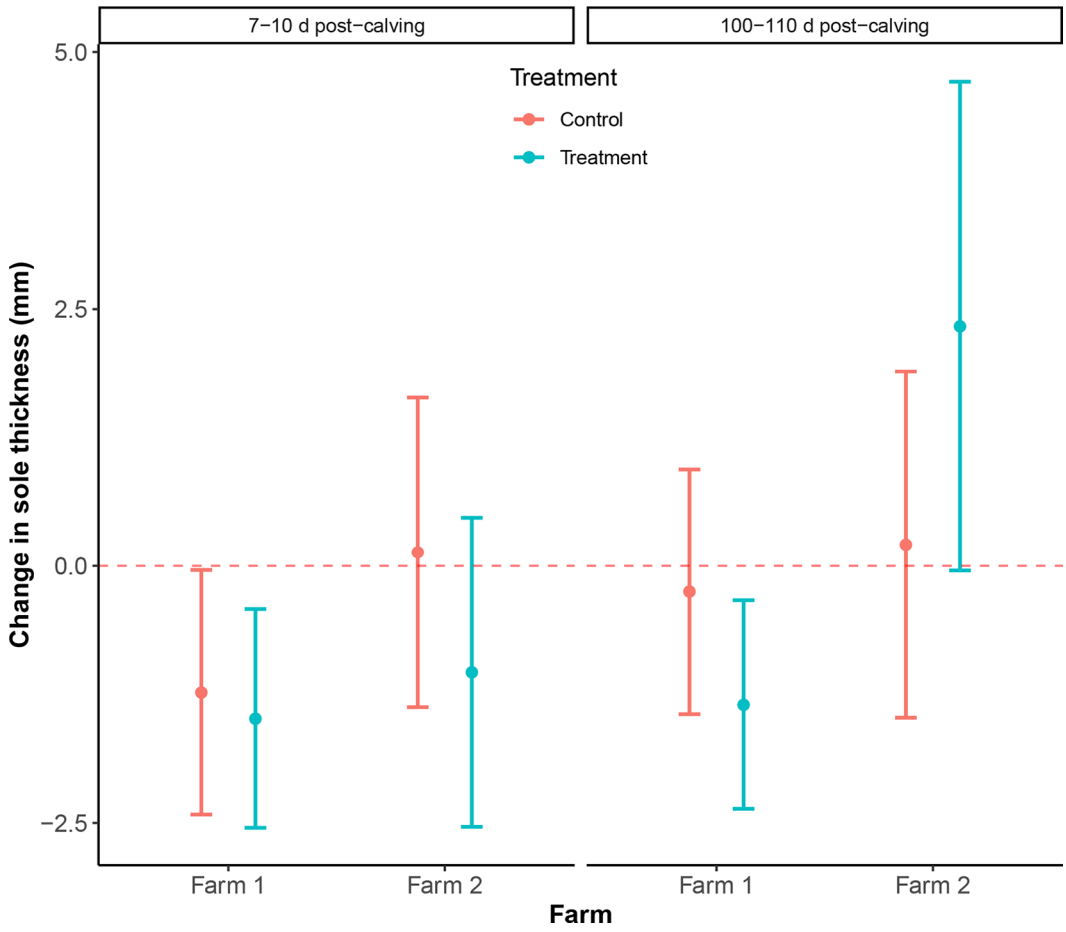

Figure 3. Error plot of estimated marginal means of the differences (mm) in the distance between the sole surface and the tip of the pedal bone, from the initial measurement at enrollment. Two outcome measurements were collected; 7 to 10 d postcalving and just before the start of mating on 2 of the 6 enrolled farms. Outputs are from a linear regression model, with 95% confidence intervals adjusted for multiple comparisons.

the distances walked, and the length of time standing on concrete, were not great enough to elicit a response in the hoof. This statement is supported by the findings of sole soft tissue thickness and sole profile in the current study where there was no difference in sole soft tissue thickness or sole profile after calving between intervention and control animals. Although there was a 3-way interaction present between farm, treatment group and time, due to this only involving 2 treatment animals on one of the farms, it needs to be corroborated in future studies before much weight is applied to it. It was expected that even if a reduction in hazards of lameness was not observed that the intervention should at least result in sole measurement changes over time, however we found no effect on sole concavity or sole soft tissue thickness. The lack of treatment effect on these measures adds weight to the argument that the intervention may not have been sufficient to promote changes in the hoof. As this was a proof-of-concept study, the study could have been designed with a more extreme version of the intervention, where heifers were expected to walk much longer distances and stand on concrete for extended periods of time (>6 h/d). The authors considered it important to not exaggerate the time on concrete and potentially cause harm. Although prolonged exposure to hard surfaces in the transition period alone has not been investigated before, enough evidence exists on the negative associations between hard surfaces around calving and claw horn health (Webster, 2001; Laven and Livesey, 2004; Knott et al., 2007; Bergsten et al., 2015) that erring on the side of caution was appropriate for the first clinical study of its kind. Importantly, the intervention was also designed to be practical for farmers to implement. The one hour standing time enabled time for staff to have a break or do small jobs around the milking parlor. In addition, the intervention was only conducted on 25 d across a period of 35 d to allow some flex in the staffing schedule. Requiring longer intervention times per day and for it to be conducted every day for an extended time

period would have likely resulted in poor uptake of the intervention on farm, regardless of the outcome.

There is little argument that the management and housing environment before and after calving can have an effect on hoof health and lameness risk (Bergsten and Frank, 1996; Webster, 2001; Laven and Livesey, 2004; Knott et al., 2007; Bergsten et al., 2015). Bergsten et al. (2015) investigated one of the more extreme examples using a 2 × 2 factorial design investigating hard and soft flooring with one intervention occurring a year before calving and a second intervention occurring for an additional 12 mo after first calving in dairy heifers. By the end of the first intervention, in the second year of life, the proportion of white line hemorrhages was greater in heifers wintered on hard surfaces than in heifers wintered on soft surfaces. After the second year of the intervention, white line and sole lesions, along with LS, were significantly greater in heifers managed postcalving in a cubicle yard with slatted concrete floors compared with those managed in a yard with slatted rubber floors. Importantly, an interaction was reported between the 2 treatment groups and type of flooring. Heifers managed on hard surfaces as pregnant heifers and soft surfaces after they had calved had a lower prevalence and severity of sole and white line hemorrhage compared with the other 3 combinations of housing management (soft-hard, soft-soft, hard-hard). Furthermore, heifers managed on soft flooring when pregnant and hard surfaces after calving had the highest prevalence and severity of claw horn lesions, a group most similar to conditions experience in pasture-based systems. The inference from this study is that exposure of the hoof during the rearing period to surfaces that increase wear, and thus stimulate hoof growth (Telezhenko et al., 2009; Laven et al., 2012a), and the prevalence of mild-moderate hoof horn hemorrhages is actually beneficial to hoof health postcalving. This is consistent with the clinical findings of Randall et al. (2016) where heifers with mild white lesions, zero to 2 mo before first calving had a 66% reduction in odds of lifetime lameness compared with heifers with no white line lesions precalving.

As this was a novel experiment, it is difficult to directly compare findings to any previous research. Although several studies had investigated the effect of different housing environments pre- and postcalving in dairy heifers (Vermunt and Greenough, 1996; Webster, 2001; Laven and Livesey, 2004; Knott et al., 2007; Bergsten et al., 2015), only 2 investigated an intervention that incorporated both an exercise and housing component (Gard et al., 2015; Black et al., 2017). The most comparable study, with respect to intervention, was conducted on recently weaned bull calves split into 2 groups (Gard et al., 2015); the control group was managed on a small grass paddock, and the treatment group on a race consisting of mixed terrain for 4 mo. The treatment calves were also fed at opposite ends of the access way to stimulate exercise. At the conclusion of the study, calves in the treatment group had 37% greater digital cushion volumes (measured by magnetic resonance imaging and computed tomography scan) compared with calves in the control group, suggesting the adaptive capability of the hoof to respond to the animal's environment and degree of movement. Black et al. (2017) also investigated an exercise regimen during the precalving period but in housed dry cows rather than heifers at pasture. Cattle in the exercise group over the dry period had equal horn growth and wear at the cranial aspect of the claw compared with the cows that were not exercised, which had an increase in horn growth relative to wear at that point. The authors reported no association between treatment and LS; however, this study would have been severely underpowered to find such an association if it did truly exist (n = 60 across 3 treatment groups). Even with these 2 studies, comparisons to the current study and extrapolation to pasture-based dairy heifers are difficult. All of the enrolled animals in the study conducted by Black et al. (2017) had completed at least one lactation, and were thus more likely to have had previous lameness issues and Gard et al. (2015) enrolled 2-mo-old weaned bull calves. Given the association previously reported between age (Newsome et al., 2016), and parturition (Tarlton et al., 2002) on hoof health, respectively, the findings of this current study probably need to be interpreted in isolation.

Another limitation of comparing the aforementioned studies with the current study is that the primary outcomes were not clinical lameness events. Rather, they focus on hoof factors that increase the risk of lameness, such as digital cushion volume (Gard et al., 2015), hoof horn hemorrhage (Vermunt and Greenough, 1996; Bergsten et al., 2015) and net hoof growth (Telezhenko et al., 2009; Black et al., 2017). Despite recommendations and reviews existing for lameness control (Vermunt and Parkinson, 2002; Potterton et al., 2012; Bell and Randall, 2021), implementation of control programs remains a major issue (Bell et al., 2009; Barker et al., 2012; Main et al., 2012). Observing a reduction in clinical lameness and the pride of having a healthy, nonlame herd was one of the major motivating factors for controlling lameness from a survey of 222 UK dairy farmers (Leach et al., 2010). Yet only 25% of 500 surveyed Dutch dairy farmers believed that subclinical hoof disorders (such as hoof horn hemorrhage) were animal welfare concerns (Bruijnis et al., 2013), thus, likely reducing motivation to implement a control strategy directed toward reducing the incidence of such lesions. To promote behavioral

change from farmers, the authors wanted the primary outcome to be clinical lameness. Hence, the focus during the current study was to enroll as large a number of heifers as possible and concentrate predominantly on LS outcomes. This did, however, come at the expense of recording lesion incidence. With this lack of lesion information, we cannot be confident that all of the reported lameness cases were due to claw horn lesions. However, they were unlikely to be digital dermatitis, as the within-herd prevalence of digital dermatitis in the Waikato region in New Zealand has been reported to be 3.2% (Yang et al., 2019), and within those animals <1% were reported to be clinically lame (A. Yang, Department of Veterinary Epidemiology, Nanjing Agricultural University, China; personal communication)

It is unknown if the nonrandomly selected 6 study farms are representative of the total population, and the degree to which the results can be extrapolated to heifers in other pasture-based systems in unknown. An overall lameness incidence risk of 12% in the control animals is below the median lameness incidence reported from all cows from 43 farms in the South Island of New Zealand (Gibbs, 2010) and could be considered low. Accurate lameness incidence and prevalence for all lactating animals were not available on any of these farms, so any inferences on overall lameness risk could not be made. However, in the first author's opinion, the facilities and track maintenance on all 6 farms could be considered good. It is possible that in farms with a high baseline lameness incidence, where poor management of tracks and concrete were responsible for much of the lameness, that an intervention such as this may have had a greater effect on the time to first lame event (either positively or negatively). Animals were managed within treatment groups during the intervention phase. Although it was attempted to manage these in a similar manner, apart from the intervention itself, it was still possible that the diet and external environment may have differed slightly between the 2 groups. Although unlikely, this may have biased the association toward the null hypothesis. These limitations withstanding, the internal validity of the study likely remained high, with large numbers of heifers involved, and strong adherence to protocol intervention and follow up.

Measurement bias was a possibility in this study due to the 2 different methods of diagnosing lame cattle, trained technician and farmer. No formal reliability measurements between technicians were carried out, nor was the possibility of observer drift assessed. There currently is also no formal LS training program available in New Zealand, so the authors produced their own training based on combined experiences. These factors could have resulted in misclassification bias. As the technician was blinded to the treatment groups, any misclassification would have likely erred toward the null. However, we do not believe that this would have had a large effect on this study, as the technicians were experienced in LS and involved in lameness studies with LS as outcomes running in parallel. Furthermore, only 2 technicians were used, with one technician conducting 80% of scoring events, limiting the interobserver error common with LS (Schlageter-Tello et al., 2014). The other source of potential misclassification derived from farmer reporting of lameness. Half of the lame animals were first identified by farmers. This was an important source of lameness diagnosis and was included as the authors did not want an animal to be lame for up to 14 d in between LS visits. Once animals are identified by a farmer as lame, they are often removed from the milking herd and managed separately. These animals are then often not milked at every milking and thus, may not have been presented at a LS assessment visit. Although not formally trained in scoring, all of the farm managers in the 6 enrolled farms were experienced stockpersons, and all of them diagnose and treat their lame cattle on farm. Although it was possible a farmer could have recorded the numbers of the animals as they were conducting the intervention, the likelihood of this was very low. It was far more likely that the farmer did not know what treatment group the animals belonged to, so again, if there was any risk of bias, it was toward the null.

Despite the data being compatible with no association between treatment group and lameness rates, we can also conclude that the intervention produced no deleterious effects both during the intervention, and for up to 7 mo after the cessation of the intervention. There was no increase in lameness risk during the intervention, with only 2 heifers identified as lame during the intervention period, one in the treatment group and one in the control group. After the intervention, no biologically relevant differences were found between the treatment groups with respect to BCS, MS and reproductive indices. Furthermore, the farmers all reported that the intervention was easy to implement, and, on at least 3 of the farms, the farmers believed the intervention heifers became accustomed to the intervention and anecdotally reported that the heifers appeared quiet and settled with human interaction.

Enough prior evidence exists to suggest that management of heifers before calving can have a large effect on the subsequent hoof health. Therefore, rather than conclude that transition management in the New Zealand situation cannot reduce the effect of lameness, it is more likely that this current method was not sufficient to elicit a response. This information is critical as it gives us confidence that future investigations in this research topic should involve increasing the time on

concrete or increasing the exercise component. We urgently require other practical science-based methods to proactively reduce the incidence of lameness in pasture-based dairy cows.

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