Hoofing it outdoors:

Evaluating the effect of low frequencies of outdoor access on gait and hoof health of non-clinically lame cows housed in restricted movement environments through innovative measures.

BY

SHABNAZ MOKHTARNAZIF

Department of Animal Science

McGill University, Montreal

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ABSTRACT

Technologies in dairy farms enable precise data collection, facilitating the detection of subtle changes in cow behaviour and health. They can help improve cows' welfare by identifying early signs of health issues and minimizing their dire effects. Although increasing movement opportunities through regular outdoor access can benefit cows in restricted movement environments, its daily implementation can be challenging. Moreover, there have been few studies on the effect of outdoor access on non-lame cows. The objectives of this study were 1) to evaluate the effect of low frequencies of outdoor access on the gait and hoof health of non-clinically lame cows and 2) by using human-based and innovative technologies observe both clinical and subclinical effects of outdoor access. We hypothesized that low frequencies of outdoor access would have limited effects on cows' gait and hoof health, however by the use of technology we would be able to detect the subclinical changes in cows' gait traits and hoof condition.

Thirty-six tie-stall-housed Holstein dairy cows blocked by parity and DIM (6 blocks) and provided 1h of outdoor access in 2 treatments (EX1=1d/week and EX3=3d/week) for 5 weeks. Data were collected before (Pre-trial), after (Post-trial), and 8 weeks after (Follow-up) outdoor provision. We assessed cows' gait using a 5-point visual scoring system and technologies. We attached 20 reflective markers to cows to measure their gait variables through 3D motion analysis. Pressure plates were used to measure the kinetics of a cow's gait while she stood still on them. Clinical claw lesions and dimensions were assessed at the trimming chute. For subclinical signs of lesions, the average coronary band surface temperatures in dorsal view were measured using an infrared thermography camera. Data were analyzed using mixed effect models with block, period, treatment, and the period×treatment interaction as fixed effects ($\alpha<0.05$), and either cow or claw nested within cow as random effects.

Average gait scores at Pre-trial, Post-trial, and Follow-up were 2.13 ± 0.55, 1.98 ± 0.61, and 2.02 ± 0.51, respectively, with no effect of time or treatment in the overall gait score. Cows showed higher stance time ($P<0.05$) in Post-trial (0.73 ± 0.01s) compared to 0.70 ± 0.01s in both Pre-trial and Follow-up with no effect of treatment ($P>0.05$). Only EX3 cows applied higher pressure (20-30 N/cm2) on pressure plates ($P<0.05$) in Post-trial and Follow-up. Only non-severe lesions (sole hemorrhage, n=39, and white line hemorrhages, n=2) were observed during the study. No treatment or time effects were found for hoof lesions (P>0.05). Average hoof temperature was lower (P < 0.05) in the Post-trial (27.7 ± 0.25°C) and Follow-up (27.6 ± 0.27°C) compared to Pre-trial (29 ± 0.26°C) with no effect of treatment (P > 0.05). Sole length and width, and claw length increased from Pre-trial to Post-trial (P<0.0.5) with no change (P>0.05) in claw angle. Treatment did not affect claw conformation (P>0.05).

Gait scoring and clinical hoof health assessment did not detect discernible difference between the effect of outdoor access for EX1 and EX3 cows. With the use of technology, we effectively identified favourable impacts of outdoor access in both treatment groups. Following the implementation of outdoor access, cows showed more confidence in walking and standing with higher stance time and the application of more pressure on their claws. The reduction in the hoof temperature suggests a potential decrease in pain and discomfort in their hooves with a positive effect on claw wear and growth rate. In alignment with our hypothesis, although no clinical effects of outdoor access were noted, our findings indicate that technologies such as 3D motion analysis, pressure platforms, and thermography can be useful in the detection of subtle changes in gait and hoof health. These findings suggest that even minimal outdoor access, as low as 1h/week, holds the potential to yield favourable effects on the gait and hoof health of cows.

RÉSUMÉ

L'utilisation de technologies en ferme permet une collecte précise des données, facilitant ainsi la détection de variations dans le comportement et la santé des vaches. Offrir davantage d'opportunités de mouvement grâce à un accès régulier à l'extérieur peut être bénéfique pour le bien-être des vaches vivant dans des environnements où les mouvements restreints. Peu d'études ont été menées sur l'effet de l'accès à l'extérieur sur la démarche et la santé des pieds et membres des vaches non boiteuses. L'objectif de cette étude était d'évaluer l'effet de faibles fréquences d'accès à l'extérieur sur la démarche et la santé des pieds et membres de vaches non boiteuses en utilisant des technologies innovantes pour observer des effets subcliniques fins.

Trente-six vaches Holstein en lactation, logées en stabulation entravée, ont été regroupées par parité et jours en lactation (6 groupes) et ont bénéficié d'un accès à l'extérieur d'une heure dans le cadre de deux traitements (EX1 = 1 jour/semaine et EX3 = 3 jours/semaine) pendant 5 semaines. Les données ont été collectées avant (pré-essai), après (post-essai) et 8 semaines après (suivi) la mise en place de l'accès à l'extérieur. Nous avons évalué le score de démarche des vaches à l'aide d'un système de notation visuelle à 5-points et de technologies. Nous avons fixé 20 marqueurs réfléchissants sur les vaches pour mesurer les variables de démarche par le biais de l'analyse de mouvement en 3D. Des plateformes d'analyse de pression ont été utilisées pour mesurer la cinétique de la démarche des vaches lorsqu'elles étaient immobiles dessus. Les lésions des onglons et leurs dimensions ont été évaluées à la table de parage. Pour les signes subcliniques de lésions, la température moyenne de la surface des bandes coronaires a été mesurée en vue dorsale à l'aide d'une caméra à thermographie infrarouge. Les données ont été analysées à l'aide de modèles à effets mixtes avec le bloc, la période, le traitement et l'interaction période×traitement en tant qu'effets fixes ($\alpha<0.05$), et soit la vache soit l'onglon emboîté dans la vache en tant qu'effet aléatoire.

Les scores moyens de démarche lors des phases pré-essai, post-essai et de suivi étaient respectivement de 2,13±0,55, 1,98±0,61 et 2,02±0,51, sans effet du temps ou du traitement sur le score global de démarche. Les vaches ont montré un temps de station debout plus long ($P<0,05$) post-essai (0,73±0,01 sec) par rapport à 0,70±0,01 sec à la fois lors de la phase pré-essai et de suivi, sans effet du traitement ($P>0,05$). Seules les vaches EX3 ont exercé une pression plus élevée (20-30 N/cm2) sur la plaque d'analyse de pression ($P<0,05$) après l'essai et lors du suivi.

Seules des lésions non graves (hémorragies de la sole, n=39, et hémorragies de la ligne blanche, n=2) ont été observées au cours de l'étude. Aucun effet du traitement ou du temps n'a été observé sur les lésions des onglons ($P>0,05$). La température moyenne des onglons était plus basse ($P<0,05$) post-essai (27,7±0,25 °C) et lors du suivi (27,6±0,27 °C) comparé à la phase pré-essai (29±0,26 °C), sans effet du traitement ($P>0,05$). La longueur et la largeur de la sole, ainsi que la longueur de l'onglon, ont augmenté entre le pré et post-essai ($P<0,05$), sans changement ($P>0,05$) dans l'angle de l'onglon. Le traitement n'a eu aucun effet sur la conformation des onglons ($P>0,05$).

L'évaluation de la démarche et l'évaluation clinique de la santé des onglons n'ont pas détecté d'effets discernables de l'accès à l'extérieur pour les vaches EX1 et EX3. Nos résultats indiquent que des technologies telles que l'analyse du mouvement en 3D, les plateformes d'analyse de pression et la thermographie permettent la détection fine des changements de la démarche et de la santé des pieds. Un accès minimal à l'extérieur, aussi faible qu'une heure par semaine, a le potentiel d'avoir des effets favorables sur la démarche et la santé des pieds et membres des vaches.

DEDICATION

I humbly dedicate this thesis to the courageous women and men of Iran who have tragically lost their lives or unjustly found themselves imprisoned in their unwavering struggle for freedom and equality. They stand as shining examples of resilience and determination, embodying the spirit of "Women, Life, Freedom." May their unwavering commitment inspire us all to continue advocating for a world where these fundamental rights are upheld without compromise.

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CONTRIBUTION OF AUTHORS

In this thesis, the authors of the manuscript are:

Shabnaz Mokhtarnazif (primary author), Elsa Vasseur (supervising author), Elise Shepley (co-supervising author), Amir Nejati (contributing author), and Gabriel Machado Dallago (contributing author).

Shabnaz Mokhtarnazif was the primary author of each chapter of the manuscript and conducted the experiment described in Chapter 3-4-5. Shabnaz conducted the search and screening in Chapter 2. Shabnaz conducted the experiment in Chapter 3, for which she performed all live and video observations with processing, compiling, and handling data. Elsa Vasseur supervised the primary author and reviewed and co-authored all chapters. Elsa co-conceptualized all chapters and co-designed the experiment in chapters 3-4-5 and obtained the funding for the research work. Elise Shepley co-supervised the primary author, and reviewed, and co-authored chapter 2. Elise also co-conceptualized, co-designed, reviewed and co-authored the experiment presented in chapters 3-4-5 and 4. Amir Nejati co-conceptualized, co-designed, reviewed, and co-authored, the experiment presented in chapters 3-4-5. Gabriel Machado Dallago co-conceptualized the design of the model and reviewed the analytics and results interpretation in chapters 3-4-5.

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LIST OF ABBREVIATIONS

2D: 2-Dimensional

3D: 3-Dimensional

30Secr: 30 seconds recording

AAT: American Academy of Thermography

AIC: Akaike Information Criteria

AT: Ambient temperature

AVForce : Average force

AVPressure : Average pressure

Avr : Average

BRT: Background radiated temperature

CB: Coronary band

C.D.I.C: Canadian Dairy Information Center

CHDL: Claw horn disruption lesions

COP: Center of pressure

DIM: Days in milk

EX1: Treatment with 1d/week of outdoor access

EX3: Treatment with 3d/week of outdoor access

FP: Force plate

fps: Frame per second

GRF: Ground reaction force

ICAR: International Committee for Animal Recording

IP: Image processing

IRT: Infrared thermography

LCB: Lateral coronary band

LZ4: Zone 4 of lateral claw

Max: Maximum

MCB: Medial coronary band

MForce: Maximum force

Min: Minimum

MMA: Methyl methacrylate

Mpressure: Maximum pressure

MZ4: Zone 4 of medial claw

NFACC: National Farm Animal Care Council

NRS: Numeric rating system

PMS: Pressure mapping systems

PRT: Predicted radiated temperature

ROI: Region of interest

ROM: Range of motion

SC: Screenshot

THI: temperature-humidity Index

VA: Video analyzing

WDP: Weight distribution platforms

Z0: Zone 0 of sole

Z10: Zone 10 of sole

CHAPTER 1 GENERAL INTRODUCTION

The use of technology in dairy farming is often referred to as "precision dairy farming" or "smart dairy farming” (Borchers and Bewley, 2015; Akbar et al., 2020). Implementation of technologies has rapidly evolved in recent years, revolutionizing dairy management, and enhancing overall efficiency, productivity, and animal welfare (Akbar et al., 2020; Cockburn, 2020). Technologies have been used in dairy farms to monitor many aspects of the cows’ life, including activity level, milk yield and milk components, temperature, the incidence of mastitis, and other disorders (Borchers and Bewley, 2015). Using technologies such as sensor-based systems can help farmers to monitor cows’ behaviour and performance in real time, which can help them to locate cows in need of medical attention in the early stages and improve the overall welfare and health of their cows (Akbar et al., 2020; Cockburn, 2020). The timely identification of diseases and health anomalies helps to prevent them from worsening, leading to more effective, cost-efficient, and less distressing treatment for cows.

In developed countries, while the total number of dairy farms is dropping, the number of cows per farm is rising, requiring more complex herd management and individual monitoring. (Barkema et al., 2015). Welfare in dairy cows includes 3 main factors: biological functioning, such as good health and performance; affective state, such as pain-free and positive experiences; and how much they can live according to their nature, such as being provided with grazing opportunities (von Keyserlingk et al., 2009). With the intensification of dairy farms, changes in housing and management have led to an increase in certain welfare risk factors, such as indoor confinement without access to pastures, and in welfare animal outcomes, such as the prevalence of mastitis and lameness (Barkema et al., 2015). The main housing types in Canadian dairy farms are tie-stalls and free stalls, with tie-stalls representing about 73% of the non-robotic milking farms (C.D.I.C., 2022). Restrictive housing systems like tie-stalls limit cows movement opportunities and prevent them from fully expressing their natural behavioural repertoire (Shepley et al., 2020). In addition, they have been associated with some poor animal welfare outcomes such as poor claw conformation (Corazzin et al., 2010), lying comfort (Shepley et al., 2019), and reproductive performance (Borchers et al., 2017), as well as high prevalence of lameness (Popescu et al., 2013). Studies looking at pasture provision in tie-stall cows found that when cows were untethered in the pasture they expressed more natural behaviours, such as exploration (Loberg et al., 2004), lying, and estrus behaviours (Mee and Boyle, 2020). Pasture provision has also been found to have positive effects on cows' health as it can reduce the risk of lameness and mastitis (Mee and Boyle, 2020). While providing pasture access is not always feasible, providing additional space and complexity, such as partial outdoor access to cows in confined housing systems, can result in improved animal welfare outcomes such as higher locomotor activities (Shepley et al., 2020) and reduced lameness and leg injuries prevalence (Popescu et al., 2013; Palacio et al., 2023). Cows who had access to the outdoors also exhibited improved affective states, fostered better relationships with humans, and reduced their fearfulness, ultimately leading to improved handling (Aigueperse and Vasseur, 2021). As a result of such studies, effective 1 April 2027, the new Canadian Code of Practice for the Care and Handling of Dairy Cattle will make provision for regular exercise access and freedom of movement for tethered cows during their production cycle mandatory (NFACC, 2023); however, more research is needed to understand the level of outdoor access provision necessary to see an improvement in cow welfare.

Lameness denotes the expression of painful conditions that lead to compromised movement or a deviation from normal gait or posture (Van Nuffel et al., 2015) and is known as one of the main welfare issues faced by dairy farms (von Keyserlingk et al., 2009). Lameness has been reported to be in the top three conditions observed in culled cows (Vogel et al., 2018) and can cause significant economic losses due to the reduction in milk yield and reproductive performance, veterinary and treatment costs, and increased risk of culling (Huxley, 2013). The mean prevalence of lameness reported in studies worldwide was reported as 22.8% with a range of 5.1% to 45% (Thomsen et al., 2023). Results regarding the effect of outdoor access on cows' lameness often contradict, and although in some studies no effect was observed (Loberg et al., 2004; Chapinal et al., 2010b), others reported positive effects, namely the reduction of lameness in cows after the provision of outdoor access (Hernandez-Mendo et al., 2007; Popescu et al., 2013; Palacio et al., 2023). As reviewed by Shepley et al. (2020), these inconsistencies may be due to differences in housing systems, frequency and duration of outdoor access, and cow conditions. Most of the studies investigating the effect of outdoor access were conducted either on cows housed in free stalls or on cows that showed clinical signs of lameness. There is limited knowledge on the effect of outdoor access on the gait and hoof health of non-clinically lame cows housed in movement-restricted environments like tie-stalls. In a previous study conducted in our lab (Nejati, 2021), the effect of high frequency (5d/week) of short duration (1h/day) of outdoor access on gait and hoof health of non-clinically lame cows was examined and the results showed that the overall gait score of cows was reduced by 1 score (from a 5-point scaling system, in which 1 means perfect gait and 5 means severe lameness) after 5 weeks of outdoor access. However, as mentioned by Smid et al. (2021), there are some barriers for farmers regarding the provision of outdoor access such as labour and management difficulties, including protecting cows from wildlife and weather extremities, upholding biosecurity protocols, and intensifying labour demands for the regular movement of cows to and from pastures. Consequently, the implementation of 5d/week outdoor access regimen might not be viable for all Canadian farms due to the mentioned reasons. Thus, it is imperative to conduct more research in order to understand what level of outdoor access would be necessary to see an overall improvement in cow gait and hoof health.

Clinical conditions can include conditions that look like a disease but are not and can be referred to symptomatic patients without dysfunction meeting diagnostic criteria for a disease (Boorse, 1997; Tresker, 2020). It can be defined as a condition in which this patient is diagnosed based on the presence of symptoms but does not have a disease. Therefore, in this context clinical lameness is defined as a condition in which a cow shows symptoms of limping and obvious deviations from normal gait, and they might need to be visited and treated at this level. In this condition the cow can be diagnosed as “lame” using human-based techniques like the score of more than 3 in a 5-point locomotion scoring system (1 means normal gait and 5 means severe lameness) or have visible disorders in their hooves. Based on Merriam-Webster (2022b) dictionary, subclinical means “not detectable or producing effects that are not detectable by the usual clinical tests”, therefore we define subclinical lameness as a condition in which cows are in early stages of lameness (i.e., scores less than 3) but not showing visible signs of lameness.

Based on the definitions of the clinical and subclinical lameness, assessment of lameness can be categorized in two groups: 1) human-based or clinical assessment: the gait of cows can be clinically assessed by looking at cows’ walking traits (i.e., flexion of their joints, symmetry in their gait, cows posture, etc.) to score their gait, or cows can be tilted in the trimming chute to directly look for hoof lesions (Flower et al., 2005). 2) Technology-based or subclinical assessment: subclinical assessment means using non-routine diagnostic tests to find the results that are not usually paying attention to using technologies like Magnetic resonance imaging. ultrasound imaging, etc. (Rosamond and Couper, 2022). There have been some technologies developed to investigate changes in cows' gaits (i.e., 2D and 3D gait analysis, force and pressure plates, accelerometers, etc.) and hoof condition (e.g., hoof thermography). These systems have been developed to detect slight changes and subclinical signs of lameness before they become visible to human eyes (Alsaaod et al., 2015a; Alsaaod et al., 2019b; Bradtmueller et al., 2023; Nejati et al., 2023). The possible lack of effect of outdoor access provision in some studies might be attributed to the possibility that the impact of outdoor conditions was subtle and subclinical, making it difficult to be visible through clinical assessments.

CHAPTER 2 LITERATURE REVIEW

2.1. Kinematics Technologies and Their Use in Leg and Hoof Health Assessment

Assessment of gait kinematics is one of the automated lameness detection methods for animals in which the main focus is on the motion of the joints, their angles, segment orientation, and posture during walking (Alsaaod et al., 2019b; Zhong et al., 2021). The history of kinematics analysis of cow gait goes back to the 1990s, in which Herlin and Drevemo (1997) compared the angles range of motion in cows kept only in indoor housing with ones who had access to pasture for about 3 months using the Trackeye motion analysis software (Innovative Vision AB, Linktiping, Sweden). Different technologies have been used for dairy cow kinematic measurements, including pressure and force platforms, accelerometers, and vision-based systems (reviewed by Bradtmueller et al. (2023) and Nejati et al. (2023)). Vision-based systems are the main methodology used for kinematic investigation in cows. In these systems, videos and sequential images are used to analyze the gait. Video and picture analysis can be done either using commercially available software which can track and analyze motion, manually measuring kinematic variables from videos and images, or using machine learning and algorithms. These systems are mainly adapted from what has been used in studies evaluating humans' and horses' gait.

Based on the scoping review done by Nejati et al. (2023), vision-based systems can be categorized into two classes:

1. **Video analysis (VA):** In this technique, videos or consecutive images are used to track and digitize anatomical landmarks of cows (e.g., hooves, limb joints, etc.) by using special software. Defining the anatomical regions of interest can be manually added to the videos in the software program or by attaching markers to those parts directly on the cow, which are automatically tracked by the software.
2. **Image processing (IP):** In this method, numbers of still images are taken from videos to analyze the motion and posture of cows at the desirable frames of walking. To facilitate analysis, some operations such as background subtraction might be applied to the images. The motion or posture of the anatomical region(s) of interest is then processed either manually or using machine learning algorithms.

In vision-based systems, digital video cameras and 3-dimensional (3D) depth sensors, have been used with recording rates between 15-60 frames per second (fps). Most studies used only one camera recording cows walking from one side (2-dimensional; 2D); however, 3D cameras have been used recently, mainly by installing the cameras on the ceiling of the return alley from the milking parlour or other similar allies to capture videos of cows for 3D depth analysis of the spine (Viazzi et al., 2014; Pezzuolo et al., 2018). For example, Abdul Jabbar et al. (2017) used the 3D depth camera located above the cows at a height of 3.69 meters from the ground and captured at least 2 complete gait cycles, while the cows were exiting the parlour. They studied hip joint movements to assess the asymmetry in cows' gait and they were able to differentiate between cows with a gait score of 1 and those with a score of 2 or more on a 5-point scoring system with high sensitivity and specificity (i.e., 100% and 75%, respectively).

Lameness and painful hoof lesions alter cows' normal gait and posture (Flower et al., 2006), so investigating kinematic variables might be a useful method to detect lameness in the early stages, as it can detect changes before clinical signs become visible. In a systematic review done by Bradtmueller et al. (2023), variables measured in kinematics studies are categorized into two main groups: spatial measurements and temporal measurements. Spatial measurements are defined as measurements that look at the distance between two points (either two anatomical parts on the cow's body or the floor and a part of the cow's body) or the degree of joint mobility are assessed. Temporal measurements assess the time and duration of the specific gait phases such as stride, stance or swing, bipedal and tripodal support (Tijssen et al., 2021). Some of these kinematic variables and internal and external factors affecting them have been investigated in the literature and are presented in **Table 2.1-1**.

2.1.1. Normal Gait Characteristics

Walking is a locomotor activity characterized by the sequential bending and extension of the joints, resulting in the movement of the limbs. During walking, a cow initiates the swing phase by shortening the limb through bending hip and knee/hock joints and flexing short digital flexor muscles, leading to the lifting of the limb and the detachment of the hoof from the ground. Subsequently, through the gradual extension of the joints, the limb contacts the ground again. Prior to fully bearing weight on the limb, the cow assesses the stability of the floor. Once the floor is deemed stable, the support phase commences, where the limb remains in contact with the ground, which will be followed by the subsequent swing phase (Hildebrand, 1989; Van Nuffel et al., 2015). In a typical gait pattern, the stride lengths and intervals are generally consistent, whereby the rear limb typically lands either directly at the position of the front foot or slightly ahead of it (i.e., track-up, see section 2.1.2.3). In this gait pattern, the duration of the supporting phase tends to be longer than that of the swing phase. Additionally, during the swing phase of two consecutive strides, there is generally less than a 50% overlap between the legs (Van Nuffel et al., 2015). In a normal gait, triple support (or the time the stride is supported by three limbs on the ground and only one limb swinging) is only 18% of the stride (Flower et al., 2005).

Table 2.1-1 Kinematic variables measured in studies, techniques (VA: Video Analysis, IP: Image processing), and the type of the camera used and its view (table adapted from Bradtmueller et al., 2023 and Nejati et al., 2023)

	Variable	Definition	Technique	Camera type
Spatial	Stride length	The horizontal distance between two consecutive hoofprints of the same limb (Flower et al., 2005; Blackie et al., 2011)	VA (Flower et al., 2005; Carvalho et al., 2007)/ IP (Jiang et al., 2019)	2D Camera, Side view
	Stride height	The greatest change in height or vertical position happens between two consecutive hoof strikes of the same limb (Flower et al., 2005)	VA (Flower et al., 2005)	2D Camera, Side view
	Track up	The hind hoof's distance relative to the position of the fore hoof (Song et al., 2008)	VA (Flower et al., 2007; Yamamoto et al., 2014) IP (Song et al., 2008; Schmid et al., 2009)	2D Camera, Side view
	Back posture	The distance and the angle created in the back line between the withers and tail bone (Aoki et al., 2006; Poursaberi et al., 2010; Blackie et al., 2013)	VA (Aoki et al., 2006; Blackie et al., 2013) IP (Poursaberi et al., 2010; Viazzi et al., 2014; Van Hertem et al., 2018)	2D Camera, Side view (Aoki et al., 2006; Poursaberi et al., 2010; Blackie et al., 2013; Viazzi et al., 2014)

				3D Camera, Overhead (Viazzi et al., 2014)
	Joints' range of motion	The ability of the joint to extend and flex during the walking which can affect gait's smoothness and rhythm (Blackie et al., 2011)	VA (Carvalho et al., 2007; Blackie et al., 2011) IP (Pluk et al., 2012)	2D Camera, Side view
	Head bob	Deviation between the lowest and highest position of the lower jaw during each step (Mokhtarnazif et al., 2020)	VA (Blackie et al., 2013) IP (Mokhtarnazif et al., 2020)	2D Camera, Side view
Temporal	Speed	Division of the distance to the time (Blackie et al., 2011; Mokhtarnazif et al., 2020)	VA (Flower et al., 2005; Aoki et al., 2006; Blackie et al., 2011; Yamamoto et al., 2014; Mokhtarnazif et al., 2020)	2D Camera, Side view
	Support phase	The time the hoof is in touch with the ground and does not move (Kang et al., 2020)	IP (Kang et al., 2020)	2D Camera, Side view
	Tri- and bipedal support	Duration of simultaneous stance phase of two (bipedal) or three (tripodal) limbs (Tijssen et al., 2021)	VA (Flower et al., 2005; Tijssen et al., 2021)	2 Camera, Side view

2.1.2. Spatial Variables:

2.1.2.1. Stride Length

Stride length is defined as the horizontal distance between two consecutive hoofprints of the same limb (Flower et al., 2006; Blackie et al., 2011). Normal stride length can differ between cows based on a number of cow characteristics, including the cow's breed, size, and age and it has been reported in a range from 1.39 – 1.7 meters (Flower et al., 2005; Blackie et al., 2011; Mokhtarnazif et al., 2020). Hoof and leg health can impact the length of the stride, it has been reported that cows suffering from sole ulcers have shorter strides compared to cows with no lesions (Flower et al., 2005; Blackie et al., 2013). Although the severity of lesions and locomotion score had a negative impact on the cows' stride length (Blackie et al., 2007; Zhao et al., 2018), when different lesions were compared, only cows with sole ulcer had significant shorter strides (Flower et al., 2005; Blackie et al., 2013). It seems that painful lesions result in shorter strides (Flower et al., 2006). Other than lameness and leg injuries, some internal and external factors such as motivation, stage of lactation, or hoof trimming can influence a cow's gait. For example, when the cows' motivation to walk was studied, it suggested that, regardless of the cows' locomotion score, greater motivation resulted in greater stride length (Mokhtarnazif et al., 2020). Cows showed smaller strides in the later stages of lactation which might be due to the weight of the calf in the uterus (Van Nuffel et al., 2016). Aoki et al. (2006) investigated the effect of trimming on gait variables and they found that hoof trimming can increase the length of cows' stride to more than 10 cm two days after hoof trimming.

Environmental factors including lighting and floor type can influence cows' gait, as they showed shorter strides in dark environments and wet floors (Van Nuffel et al., 2016). Proper friction and traction between the hoof and ground also influence the stride, with cows walking on rubber mats having longer stride lengths compared to cows walking on concrete (Flower et al., 2007; Franco-Gendron et al., 2016). Franco-Gendron et al. (2016) compared stride length and other gait characteristics while cows walking on rubber mats, concrete and four types of methyl methacrylate (MMA) resin aggregate flooring, they found that stride length was significantly higher on rubber mats compared to concrete and two types of MMA which had a higher dynamic coefficient of friction.

2.1.2.2. Stride Height

Stride height is another variable often measured in kinematics studies. It is defined as the greatest change in the height or vertical position that occurs between two consecutive hoof strikes of the same limb. Similar to stride length, floor type and some lesions like sole ulcers affect stride heights, cows lifted their feet in lower levels when they had sole ulcers or walked on harder flooring like concrete, compared to non-sole-ulcer cows and the ones walked on rubber mats (Flower et al., 2005; Flower et al., 2007). Alsaaod et al. (2017) found that cows lift their foot higher when they are affected with heel erosion compared to the ones with digital dermatitis, they suggested that it might be due to pain caused by stretch of the affected skin during the toe-off. Other cow-related factors such as udder fill also influence the stride height, for example, Flower et al. (2006) found that when cows were leaving the milking parlour, they had greater stride heights compared to when they walked towards the parlour, which is probably because of their motivation to go to their home pen to get the fresh feed and bore less weight due to the udder engorgement.

2.1.2.3. Track-up

Track-up or tracking distance, also known as “track-way” (Song et al., 2008) or “stride overlap” (Flower et al., 2007), is a spatial variable defined as the hind hoof's distance relative to the position of the fore hoof along the walking direction. Track-up is usually calculated by subtracting the rear hoof's position from the position of the front hoof (Song et al., 2008), with a smaller distance denoting better tracking. Flower and Weary (2006) described the perfect gait as the one in which the rear leg lands on or in front of the front leg hoofprint. Song et al. (2008) found a strong correlation between track-up value and gait score in dairy cows. They found that in cows with a gait score of less than 2 (based on a continuous 5-point scale, with a lower score referring to sounder cows), the track-up value can be negative or comparatively lower than lame cows with a gait score closer to 5 (6.1cm and 22cm, respectively).

Changes in track-up can be the result of the changes in the cows’ stride length and therefore it is similarly affected by several factors. For example, some claw injuries, such as sole ulcer, can negatively affect cows’ tracking-up (Flower et al., 2006; Flower et al., 2007). Also, the surfaces cows walk on influence the stride overlap. For example, when Flower et al. (2007) investigated the effect of soft and hard flooring on some kinematics variables, they found that cows walking on rubber mats have significantly better tracking-up compared to the ones walking on concrete in cows with and without sole ulcers. The track-up value for cows without sole ulcers walking on concrete was 4 times worse than non-sole ulcer cows walking on rubber mats.

2.1.2.4. Back Posture

Back posture, or back curvature, is another spatial variable assessment in kinematics studies. The spine of the cow is generally flat in non-lame and arched in more severely lame cows (Sprecher et al., 1997; Flower and Weary, 2006). As it is one of the most easily detectable signs of lameness in dairy cattle, it has been used in many human-based visual locomotion scoring systems. In kinematics studies, back posture is usually measured as the distance and the angle created in the back line between the withers and tail bone (Aoki et al., 2006; Poursaberi et al., 2010; Blackie et al., 2013). Blackie et al. (2013) also measured the height of the spine vertebrae of interest (T3, T7, L1, L4, lumbosacral joint and tail head) from the ground. Mokhtarnazif et al. (2020) measured the back arch as the area of the semicircle formed by the back curvature (i.e., the line formed by the vertebrae interest on the back) and the straight line between the withers and lumbosacral joint (i.e., the shortest possible distance between vertebrae). In the all-aforementioned studies, the measurement of back posture was conducted during weight-bearing instances.

Poursaberi et al., (2010; 2011) used an image processing technique to predict lameness using back posture and head position. In the first study, they automatically extracted the back arch from pictures and used a circle to pass through 3 points on the cow's back (shoulder, hip, and middle point of these two areas) and calculated its radius, then the back arch calculated by taking the reciprocal of the radius, represented 1/radius, and expressed in 1/pixel units. This method was able to detect lameness – defined as a score of 2 or more in a 3-point scaling system – in cows with an accuracy of 96% (Poursaberi et al., 2010). In the second study, they tried to use back posture for early lameness detection in 1,200 cows enrolled from commercial farms. By calculating the angles between the neck, the highest point of the back and tail and their head position, they were able to classify lameness in three classes (healthy, moderately lame and lame) with more than 97% rate of success (Poursaberi et al., 2011). In contrast, Blackie et al. (2013) did not find any changes in back postures between cows with a locomotion score of 1 to 3 (i.e., sound to non-severely lame cows, out of the 5-point scoring system) and relatively small changes in cows with sole ulcers. The reason might be due to the fact that they did not use cows with severe lameness, therefore cows may have been able to mask the signs of pain and discomfort. Back posture can also be influenced by internal and external factors like their motivation to walk, calving, and hoof trimming (Aoki et al., 2006; Blackie et al., 2011; Mokhtarnazif et al., 2020).

2.1.2.5. Joint Range of Motion

The range of motion (ROM) in joints is defined as the ability of the joint to extend and flex during walking, which can affect the smoothness and rhythm of gait. It can be measured by calculating the difference between the maximum and minimum degree of the joint (Blackie et al., 2011). Pluk et al. (2012) measured the ROM of cows' legs by calculating the total rotation the leg undergoes while the hoof is in contact with the ground. To do so, they calculated the "touch angle" by measuring the leg's angle with the ground the first time it is connected to the ground and the "release angle", which is defined as the angle between the leg and the ground at the time the hoof leaves the ground (or the end of the leg movement). The ROM is then determined by subtracting these two angles. The ROM can be measured in different limb joints (stifle/shoulder, hock/knee, fetlock, and coffin) though fetlock, hock and knee are the most common joints investigated in the studies.

Studies show that the ROM of different limb joints may be useful in classifying dairy cow locomotion scores. Pluk et al. (2012) found that lame cows have significantly smaller ROM compared to non-lame cows and touch and release angles are different in different locomotion scores, therefore they used these measurements to classify lameness. They scored cows using a 3-scoring point system and found that by combining ROM and leg angles they could classify cows based on their locomotion score with 65% accuracy. When they tried to classify cows with locomotion scores of 1 and 2 in one group (non-lame) and 3 in another group (lame), they reached 81.5% accuracy. In contrast to these results, Blackie et al., (2011; 2013) did not find a strong relationship between cows' locomotion score and fetlock and hock ROM. Tijssen et al. (2021) found that ROM is different in the hind and front limbs, reporting front limbs as having an 80-degree ROM compared to a 60-degree ROM found in the hind limbs, with steeper decline and incline before and after lifting the hoof. Studies showed that hoof trimming can help cows walk smoother by reducing the hock and knee angle at the beginning of the support phase, showing that limbs can bend more and land less vertically (Aoki et al., 2006; Tanida et al., 2011). Trimming can also increase the extension of the fetlock joint from 157° to 162° and help the cows move less stiffly (Carvalho et al., 2007). The type of flooring on which cows walk can significantly impact the range of motion exhibited by their joints. Specifically, cows walking on concrete surfaces with higher levels of friction tend to exhibit a greater ROM in their joints (Flower et al., 2007)

2.1.3. Temporal Variables

Cows walking speed and the time they spend on triple support or during the swing phase are known as important indicators for revealing abnormalities in dairy cow gait. Walking speed can be measured either by the time a cow spends walking a known distance (Mokhtarnazif et al., 2020) or by dividing the stance length by the stance time (Blackie et al., 2011). The normal walking speed reported for cows is about 1.35 ± 0.15 m/s (Van Nuffel et al., 2015), though it can range from 0.9 m/s (Flower et al., 2006; Mokhtarnazif et al., 2020) to 1.75 m/s (Blackie et al., 2011). Different factors can affect a cow's walking speed. Cows considered lame or have higher locomotion scores usually walk slower than ones with normal gait (Blackie et al., 2011; Mokhtarnazif et al., 2020). Lame cows might reduce their walking speed due to the pain they experience. They may exhibit hesitation in placing their injured hoof on the ground, leading them to take more cautious steps in an attempt to minimize their pain while walking.

Tijssen et al. (2021) suggested the normal stance duration of 0.5 – 1 second. The presence of a sole ulcer can increase the average stance and stride duration of four legs in cows (Flower et al., 2007). Other factors including hoof trimming, and floor type influence these temporal variables. For example, cows showed higher walking and stepping rates when they were compared two days before and after trimming, which might be the result of better limb posture (i.e., higher rate of joints bending during landing and lifting of the hooves) after the trimming (Aoki et al., 2006). Similarly, differences in walking and stepping rates were reported when rubber and concrete flooring was compared, with cows walking faster with less time on triple support while walking on softer flooring (Flower et al., 2007). Kang et al. (2020) found that there is a strong correlation between the difference in the supporting phase and the degree of lameness in cows. The supporting phase is defined as the time the hoof is in touch with the ground and does not move. In this study, Kang et al. (2020) used the difference between the maximum and minimum time of support phases of all four legs to assign the degree of lameness severity, they found that the supporting phase of all four hooves was relatively similar in non-lame cows (score 1), which was in contrast with mildly lame and lame cows (score 2 and 3 in a 3-point visual scaling system) that showed differences in the supporting phase between the four hooves. They found that there is a strong positive correlation between the difference between the maximum and minimum time cows spent on the supporting phase and the degree of lameness ($r = 0.86$), which especially had the ability to detect the lame limb as they had lower supporting phase time. The authors suggested it is due to cows tending to bear less weight on the affected limb, therefore they reduce the supporting phase time of that limb and increase the time they bear weight on their non-affected limbs.

2.1.4. Other Gait Variables

To a lesser extent, other gait variables, including head bobbing, leg swing, and gait asymmetry, have been investigated in lameness detection studies. For example, Zhao et al. (2018) used leg swing and the asymmetry of the gait between the limbs of one side by calculating the irregularity of the gait in time and the length of their strides resulting in dysrhythmic steps to categorize cows in three different classes of lameness. To do this, they recorded cows from a side view and categorized them as score 1 if there was no sign of lameness, score 2 if only one sign of lameness was observed and score 3 if two or more signs of lameness were visible in cows. They found that gait asymmetry was the only variable significantly changed between scores 1 and 2, while other gait traits including walking speed, tracking up, stride length, and tenderness (calculated based on the swing time of the front leg) could only be used to differentiate the score 3 from scores 1 and 2. Head bobbing and back posture together can be used as postural traits in lameness detection, as lame cows show more deviation in their head position while walking compared to sound cows (Mokhtarnazif et al., 2020).

2.1.5. Accuracy of Kinematics Measurements in Lameness Detection

Vision-based systems in lameness detection showed promising accuracy in lameness detection. Schlageter-Tello et al. (2014) mentioned a specificity of more than 80% and sensitivity of 39-90% for automatic lameness detection systems when they were validating with human-based visual gait scoring. The authors suggested the reason for this wide range of specificity might be two-fold. First, it might be related to the ability of the automated systems to better recognize lame cows rather than non-lame cows. Second, the gold standard for validating these systems is usually visual gait scoring, which can have poor reliability and agreement if observer training is lacking (Cutler et al., 2017). They also mentioned that, because most of these studies were conducted in experimental farms with controlled conditions (such as controlling cows traffic, light, temperature, etc.), the accuracy of the systems might be overestimated. Zhao et al. (2018) found that, in the kinematics investigation of the gait, different gait variables can have different weights in lameness detection. They showed that asymmetry of gait alone has an accuracy of 67% in lameness categorization, and by adding in the variables of reduction in walking speed and tracking up, the accuracy increased to almost 90%. However, adding other variables such as asymmetry of stance time, stride length and tenderness did not change the accuracy considerably.

Studies showed that machine learning algorithms helped the accuracy of lameness detection using kinematics variables. Viazzi et al. (2013) used the decision tree learning method to classify lameness degree in cows based on their back posture. A total of 76% of cows were classified successfully through this method, with 83% true positive and only 22% false positive. Kang et al. (2020) used deep learning technology to classify cows based on their supporting phase. Not only did they reach up to 96% accuracy in categorizing cows' lameness degree, but the authors could also detect the lame limb with 93% accuracy. Karoui et al. (2021) reached more than 99% prediction accuracy using a deep learning framework when comparing only lame and non-lame cows. However, when 3D coordinates of cows' anatomical points were used to predict the exact locomotion score of cows (in a 5-point scaling system in which cows scored from 1 (sound) to 5 (severely lame) with 0.5 intervals) the accuracy decreased to 0.41 (Bradtmueller, 2022). The walking characteristics of cows and also the accuracy of the algorithms can be influenced by different factors including cows' size, their party and lactation stage and external factors such as weather conditions, passing interval of the cows in front of the camera, etc. (Van Nuffel et al., 2016; Van Hertem et al., 2018; Zhao et al., 2018).

2.2. Kinetic Technologies and Their Use in Gait and Hoof Health Assessment

Kinetics is a subdivision in the study of biomechanics that investigates the connection between forces and torques that act on the body during its motion (Hall, 2019). The main variables measured in kinetics studies are force, pressure and the weight loaded on the floor. These variables can be measured while cows are walking (i.e., dynamic) or standing (i.e., static) on force or pressure plates.

Technologies used to measure kinetic variables can be divided into 3 main categories (Nejati et al., 2023): force platforms (FP), pressure mapping systems (PMS) and weight distribution platforms (WDP). Ground reaction force (GRF), vertical pressure and weight are the primary measurements gathered by these technologies and these variables can also be used to record certain temporal and kinematic-type measurements, such as stance time, swing time, and walking speed (Bradtmueller et al., 2023). In the force plates (FPs), force transducers or load cells are used to measure vertical or three-dimensional ground reaction forces applied on the top of the platform (Dunthorn et al., 2015). As reviewed by Nejati et al. (2023), FPs can be 0.9 to 2 meters long and can be used in multiple numbers to measure bilateral or individual forces of hooves, their data recording rates varied between 50-2000 Hz (mostly 100-250 Hz). Pressure Mapping Systems (PMSs) consists of a network of pressure sensors. They can be used as an alternative or complementary device to force plates (Nejati et al., 2023). Pressure platforms are unique as they can identify multiple hoof-prints of different limbs in a single passage (Bradtmueller et al., 2023). PMSs can be used for both dynamic and static measurements, they can be as small as a square with an area of 605.16 cm^2 to use only on one foot to a plate that is almost 5 meters long. Weight distribution platforms (WDPs) work with weighting units, and they need cows to walk on them to calculate the weight distribution between the four legs. WDPs can be installed in automatic milking systems or other places. The units can be 31-91 length in shape, and if they are installed in automatic milking systems, usually the units used for rear legs are bigger than the front legs (Bradtmueller et al., 2023; Nejati et al., 2023). Variables measured from this technology have been categorized as “weight distribution” by Bradtmueller et al. (2023), which are mainly based on the weight distributed on each limb separately, the ratio of the weight each limb bears and/or the difference between them.

Kinetics variables can help us assess cows’ gait by comparing the differences in the force or pressure applied by each foot. As it will be discussed in sections 2.2.1 and 2.2.2, claw lesions or abnormalities in specific gaits can cause the cow to load less weight on that specific limb while walking due to the discomfort or the pain on the hoof (Schulz et al., 2011). Different variables measured by using these technologies are presented in **Table 2.2-1**.

2.2.1. Normal Gait Characteristics and Factors Affecting Kinetics of Gait.

In the 1800s, Jules Etienne Marey was the first scientist to use pressure transducers to analyze gait cycles in humans (Baker, 2007), and since the early 2000s, the use of kinetic technologies for gait assessments increased in dairy cows (Nejati et al., 2023). These sensor technologies allowed us to investigate deviations from normal gait due to internal (e.g., hoof disorders, udder fills, etc.) and external (e.g., floor type, indoor housing vs. outdoor housing) conditions (Fischer et al., 2022).

The shape and size of the claw are influenced by the competition between the hoof growth rate and its abrasion known as the growth:abrasion rate, which determines the distribution of the weight within the claws (Loberg et al., 2004; Bergsten et al., 2015). When cows are walking, the shock from the ground reaction force is usually absorbed by digital cushions and suspensory apparatus (Rutland, 2021). The keratinized part of the sole and the bulbs are in contact with the ground when cows are walking and standing. As the walls of the hoof are the hardest part of the sole, in a balanced hoof, they bear most of the cow's weight (Van der Tol et al., 2002; Rutland, 2021). Van der Tol et al. (2002) found that the distribution of the maximum pressure is different between the front and rear legs. In the front limbs, the heel of the medial claws usually bears the maximum pressure, whereas in the hind limbs, weight-bearing is mainly observed on the toe of the lateral claws. Abnormalities in claw shape (e.g., overgrown toes with shallow angles) disturb the gait's biomechanics and the distribution of weight within the claws, shifting the weight from the walls to the sole and the heel can increase the risk of sole ulcers and heel horn erosions in cows (Bergsten, 2001; Telezhenko et al., 2008). For example, as claws grow longer, the claw angle and the contact area of the sole to the ground decreases (Ouweltjes et al., 2009).

Morphologically the structural integrity of walls is critical to bear the majority of the weight load, and only a minimal amount of weight-bearing should be distributed to the heels (Nuss and Paulus, 2006). However, any abnormal changes in the contact area, like a flattened sole, can disrupt this balance and shift the distribution of pressure from the walls to the sole and/or heels (Carvalho et al., 2005; Medina-González et al., 2022).

Some factors can influence the weight distribution between cows' claws. For example, studies showed that in untrimmed cows, more pressure was applied on the lateral claw of the hind limb compared to the medial claw (van der Tol et al., 2004; Fischer et al., 2021). Pressure distribution is also different when cows are standing, walking or running. Oehme et al. (2019) found an increasing trend in the contact area and pressure loaded on claws when cows are walking compared to the times they are standing. Similarly, the contact area and the maximum and average pressure applied to claws increased during running and trotting (Fischer et al., 2022). There are environmental factors that can imbalance the ratio of wear and growth in soles. These environmental factors include:

1. **Floor type:**

Flooring plays a significant role in the wear and growth ratio of the claw horn tissue. Hard floors, such as concrete, can cause changes in the shape of the claws (e.g., flat soles), which disrupt pressure distribution from the hoof walls to the soles and bulbs (van der Tol et al., 2004; Telezhenko et al., 2008). Telezhenko et al. (2008) used three different flooring systems (i.e., solid rubber mats, solid mastic asphalt and slatted concrete) to compare changes in weight and pressure distribution in cows. They observed that cows on concrete bore most of their weight on the bulb and wall of their claws and the weight carried out by the sole zone was greater in these cows compared to cows kept on other flooring systems. Changes in weight and pressure distribution can be the result of changes in the contact area, for example in this study, they showed that the contact area of cows kept in asphalt increased more than other types of floorings. Although abrasive flooring like asphalt can increase contact area and reduce the average pressure on the claw because of the increase in the area of the sole has contact with the ground, it can lead to a shift in the pressure distribution from walls to more sensitive areas like the sole. In another study conducted by Oehme et al. (2019), they compared the pressure of the claws on rubber mats and concrete while cows were walking (i.e., dynamic) or standing (i.e., static). They found that the maximum pressure can be 30% higher and that the average pressure was significantly increased on concrete compared to rubber mats (57.33 ± 11.77 N/cm^2 and 36.32 ± 7.77 N/cm^2, respectively).

Table 2.2-1 Definition of the variables measured by force plates (FP), pressure mapping systems (PMS) and weight distribution platforms (WDP) (Table adapted from (Bradtmueller et al., 2023)

Variable	Definition	Unit	Device
Ground Reaction Force (GRF)	Average of ground reaction forces of the limb normalized by cow's weight (Carvalho et al., 2005; Liu et al., 2011), it can be measured as vertical, longitudinal and mediolateral GRF (Thorup et al., 2014)	N/kg	FP : (Carvalho et al., 2005; Liu et al., 2011; Thorup et al., 2014) PMS : (Oehme et al., 2018; Oehme et al., 2019)
Maximum force	The highest force measured by each sensor (Ouweltjes et al., 2009) or the peak of GRF normalized by animal weight (Liu et al., 2011)	N/kg	FP : (Walker et al., 2010; Liu et al., 2011) PMS: (Ouweltjes et al., 2009)
Force asymmetry	The balance of force between left and right limb (Van Nuffel et al., 2009)	N/A	FP : (Liu et al., 2011; Thorup et al., 2014) PMS : (Van Nuffel et al., 2009; Van Nuffel et al., 2013)
Impulse	The integral of force with respect to the stance time (i.e., the area under the curve of the force plotted against time) (Bockstahler et al., 2009; Coetzee et al., 2014) which can be used as a variable for pelvic symmetry (Liu et al., 2011) or comparing lame and non-lame limbs (Kleinhenz et al., 2019)	S	FP: (Liu et al., 2011) PMS: (Kleinhenz et al., 2019)
Moment of force (torque)	The net moments generated by the forces which signifies the rotational effect made by a force applied at a certain distance from a pivot point or axis (Silva and Ambrósio, 2002) which can be calculated in vertical, longitudinal and mediolateral GRF (Thorup et al., 2014)	Nm	FP: (Thorup et al., 2014)

Weight distribution	The ratio of the weight loaded by each limb	%	PMS: (Walker et al., 2010) WDP: (Pastell et al., 2010; Nechanitzky et al., 2016)
Contact area	Total contacted area of the claw to the ground (Ouweltjes et al., 2009) also can defined as the area loaded per claw zone (Oehme et al., 2018)	m^2	PMS : (Ouweltjes et al., 2009; Oehme et al., 2018)
Pressure	Pressure (force/ area) applied by cow while standing (static) and/or walking (dynamic) (Oehme et al., 2019) which can be measured either as average of pressure during phases of stance (van der Tol et al., 2003) or as center of pressure in different directions (Ouweltjes et al., 2009)	N/m^2	PMS: (van der Tol et al., 2003; Ouweltjes et al., 2009; Oehme et al., 2018; Oehme et al., 2019)
Maximum pressure	The maximum of the pressure loaded per foot during walking or at different stance phases (van der Tol et al., 2003; Oehme et al., 2019)	N/m^2	PMS : (van der Tol et al., 2003; Oehme et al., 2019)
Average variation	The average of the weight distributed between limbs (Neveux et al., 2006)	Kg	WDP: (Neveux et al., 2006)
Standard deviation of weight	A method for determination of weight shifting between limbs (Bradtmueller et al., 2023)	Kg	WDP : (Nechanitzky et al., 2016; Alsaaod et al., 2019a)

The weight and pressure distribution within claws can be influenced directly by the flooring type cows walk on. The growth of claw horn tissue can increase on the abrasive floor as cows load more pressure on the hard floor, which increases the blood circulation on the hoof (Vermunt and Greenough, 1995). However, at the same time, the wear of the claw horn tissue increases as well, though it is mostly on the undesirable anatomical part (i.e., shifting the weight-bearing place from hoof walls to their soles) resulting in claw malformation (Vermunt and Greenough, 1995). On the other hand, when the floor is too soft (i.e., rubber mats) with no exposure to abrasive flooring such as concrete or asphalt, can result in too small of an amount of wearing of claw horn tissue, disrupting the ratio between growth and wear and leading to overgrowth of the toe, resulting in the cow shifting her weight to bulbs (van der Tol et al., 2004). Recently Fischer et al. (2022) used an innovative mobile pressure sensor system which attached directly to the cow's claws to compare the contact area and average and maximum pressure of hind limbs on rubber mats and solid concrete surface flooring without abrasive grit or any protective coating on the surface. Similar to previous studies, they found that the average and maximum pressure is greater on concrete when cows are walking while the contact area is lower. They investigated these parameters when the cow was performing different types of movement like jumping and trotting. The authors found that cows put more pressure on their claws while they were jumping or running. They also looked at the pattern and shape of the center of the pressure (COP) of each hind limb and each claw for the first time in individual cows. Although they did not find any effect of the flooring on the pattern of the COP in individual claws, they observed more diversity in the COP of limbs of cows when they walked on the concrete compared to the time they walked on rubber mats, which probably suggesting variations in pressure distribution, which can increase the risk of mechanical injuries of hooves.

2. Hoof trimming:

One of the main purposes of hoof trimming in dairy cows is to reshape the claws to shift the weight from the sensitive part of claws (i.e., soles and heel bulbs) to stronger parts like walls (Nuss and Paulus, 2006). In one study, the effect of hoof trimming on the pressure and contact area was investigated and the authors found that hoof trimming can increase the contact area of hind feet by 45%, which reduced the average pressure loaded on their contact area by 30% and shifted the force distribution between lateral and medial claw (van der Tol et al., 2004). In an ex-vivo study, Fischer et al. (2021) also found that trimming can reduce the pressure applied to the lateral claw by more than 30% and increase the contact area of the medial claw by as much as 18.4%. They introduced the wedge-shaped incision towards the sole tip in their trimming method, which reduced the pressure applied to the bulb and zone 4 of the claw (the site of the sole ulcer). In contrast, Carvalho et al. (2005) found no significant effect of hoof trimming on the distribution of peak pressure, although they found that the peak pressure increased by 10% on the sole of the medial claw of the front foot and 6% in the lateral claw. As mentioned, improper balance of weight in the hooves can be a risk factor for some claw lesions (Tussaint-raven, 1985). For example, over-pressing the heels can damage the horn tissue and cause lesions in that area (van der Tol et al., 2003). The incidence of lameness directly affects the weight bearing and pressure distribution in cows. To reduce the ground reaction force and the pain caused by normal weight-bearing on the affected limb, the cow shifts the weight from the affected limb and/or claw to other limbs and/or the opposite claw (Schulz et al., 2011; Coetzee et al., 2014).

2.2.2. Using Kinetic Technologies for Lameness Detection

For more than two decades, sensitive pressure and force platforms have been used in studies for bovine lameness detection (Alsaaod et al., 2019b). In one study in which lameness was induced in beef calves (aged between 4-6 months), it was found that the degree of lameness was negatively correlated with the force and pressure contact applied by animals on the sensitive pressure and force mats (Coetzee et al., 2014). Dunthorn et al. (2015) updated their 1-dimensional parallel force plates to the 3D system with 7 transducers to measure vertical, lateral, and longitudinal forces when cows leave the milking parlour. Their results showed that by using three dimensions, the device can predict lameness with a sensitivity and specificity of more than 90%, but they could not get similar results when using combinations of 1 or 2 dimensions. These results were similar to a previous study done by Liu et al. (2011) where they used a 1-dimensional, vertical force plate to predict lameness in cows. Their prediction model had a sensitivity of 52% and specificity of 89%; however, they found that ground reaction force (GRF) was lower in lame cows compared to sound cows. When investigating lame cows (i.e., cows had higher pain reaction test response, lesion severity and locomotion score) only half of the cows

(48%) showed a reduction in the vertical force of the affected limb. Cows might reduce the force applied to the affected limb while walking to reduce the pain, however, this reduction might not be only in the vertical direction (Dunthorn et al., 2015).

An additional technique for lameness identification in cows is to evaluate the weight distribution between the legs, as lame cows take more steps with rear legs while standing on the weighing platform and had more asymmetry in weight bearing on their rear legs (Pastell and Kujala, 2007; Chapinal and Tucker, 2012). Pastell et al. (2008) used weight and force sensors to compare the weight distribution between legs when cows were standing in the milking parlour as well as force distribution while walking. They found that cows load lower force and weight on the lame leg. To mitigate the pain, cows tried to keep most of the weight on the contralateral leg. In contrast, non-lame cows loaded weight and force evenly between their legs (Pastell et al., 2008; Chapinal and Tucker, 2012). Studies have demonstrated that the standard deviation of weight distribution on the legs, the discrepancy in average weight loaded by the rear legs, and the ratio of the weight between legs are effective indicators for detecting lameness in cows (Pastell and Kujala, 2007; Nechanitzky et al., 2016). Nonetheless, if the results of these variables are merged with other measurements such as lying time, lying bouts, and walking speed, they can improve the precision of the lameness detection methods (Chapinal et al., 2010a; Nechanitzky et al., 2016).

2.3. Infrared Technology and Its Use in Leg and Hoof Health Assessment

Surface temperature in disease evaluation was first used by Hippocrates, in the fifth century, B.C., when he used his sense of touch to subjectively evaluate the coldness or warmth of his patients' skin. In the 16th century, Santorio (1561-1636) invented the oral thermometer to objectively measure body temperature (Pearce, 2002). Centuries passed, and now we have more advanced technologies that enable us to measure body temperature more accurately without any direct contact with patients, like infrared thermography (IRT).

Infrared thermography is an accurate, non-invasive technique that helps us to visually evaluate the electromagnetic radiations emitted from any object warmer than absolute zero (-375 ℃). These radiations are in the infrared section of the electromagnetic spectrum with wavelengths from 9-11µm and cannot be seen with the naked eyes (Speakman and Ward, 1998).

Thus, the IRT camera converts the thermal energy into electronic video signals and creates an image either in false colours or grayscale which represents the energy level (i.e., the surface temperature) of the object (Meola and Carlomagno, 2004; Casas-Alvarado et al., 2020). In IRT devices, certain features can have significant impacts on the quality of thermal scans and the data obtained from them. In the following sections, we will explore some of these features and discuss their effects.

1. **Emissivity:**

   The ability of the surface of an object to emit heat by radiation is known as the emissivity (Merriam-Webster, 2022a). Emissivity is defined as a value between 0 to 1, where 0 means no emission and a perfect reflection of the energy and 1 means a perfect emission (Soroko and Howell, 2018). In animals, the emissivity of skin is dependent on the type, thickness, density, and colour of their coat. For example in thermograms from zebras, black strips were about 10℃ warmer than white ones (McCafferty, 2007). Based on our knowledge so far, the exact emissivity number of the skin of livestock animals such as cattle, horses, ovine, etc., has not been demonstrated (McManus et al., 2022). The emissivity of the bare skin in humans is calculated as 0.98 (Steketee, 1973), though in thermography studies of animals - based on the region of interest (ROI), the recommended value by the camera's manufacturer and skin condition - emissivity was reported in these research articles ranging from 0.93 (Nikkhah et al., 2005; Renn et al., 2014) to 1 (Soroko et al., 2014). In most of the articles reviewed in this study, emissivity was recorded between 0.95 to 0.98 (**Table 2.4-1**).

2. **Resolution:**

   The resolution of the camera is defined as the ability of the camera to provide detailed images in a higher number of pixels (Loebich et al., 2007). In thermographic pictures, a thermal profile is made based on the data represented by each pixel. The higher resolution allows IRT cameras to detect smaller objects from higher distances and also have a more detailed thermal profile from the interested region (McManus et al., 2022). The minimum resolution suggested by the American Academy of Thermography (AAT) is 640 × 480 pixels for IRT cameras (Turner et al., 2016).

3. **Sensitivity:**

   Thermal sensitivity or noise equivalent temperature difference means the lowest temperature difference the IRT camera can capture. This measurement shows how well the IRT camera can distinguish between very small differences in thermal radiation of the object, the lower the number shows higher the sensitivity of the IRT camera (McManus et al., 2022), based on the AAT recommendations, camera sensitivity should be less than 50 mK in 30°C (Turner et al., 2016), however most studies either not mentioned the camera sensitivity or not accurately reported it (**Table 2.4-1**). The accuracy of the temperature read by IRT depends on device calibration, the angle and distance of the camera to the ROI (McManus et al., 2022), usually the distance of 0.5 m to 1 m between the camera and the object is recommended (McManus et al., 2022). Church et al. (2014) found that increasing the distance of the camera to the object from 0.5 to 3 m would reduce the temperature read by the camera up to 2 °C. In articles that studied lameness and leg injuries, thermal pictures were taken from 30 cm (Wilhelm et al., 2015; Anagnostopoulos et al., 2021; Kim and Cho, 2021) to 2 m (Nikkhah et al., 2005; Gloster et al., 2011; Oikonomou et al., 2014; Renn et al., 2014; Salles et al., 2016; Arican et al., 2018). Jiao et al. (2016) found that an angle of more than 45° can significantly affect the temperature read by IRT. An angle that is orthogonal to less than 45° is recommended when IRT is used (Soroko and Howell, 2018; McManus et al., 2022).

Not only can camera features impact IRT output, but climate and environmental factors can also directly affect thermography pictures. Direct sunlight, wind, relative humidity and, most importantly, the ambient temperature can compromise the use of IRT. Alsaaod and Büscher (2012) found that the mean temperature of the coronary band of the cow hoof and the control skin above that were positively correlated with the ambient temperature. Landgraf et al. (2014) tried to quantify the effect of ambient temperature, relative humidity, temperature-humidity index (THI), wind speed and air pressure on different anatomical parts of sheep (eye) and dairy cows (udder and claws). They concluded that ambient temperature and THI had the most influence on IRT camera output. The average temperature of the cows' claws had the most correlation with ambient temperature ($r = 0.83$) and the maximum temperature of the eye was the least correlated with ambient temperature ($r = 0.5$). In this study, there was no correlation between airspeed and eye temperature, which was in contrast with the findings of Church et al. (2014) which indicated reductions in eye temperature as the wind speed increased. Other studies found that eye temperature had a relatively stable temperature in different conditions and was least affected by ambient temperature compared to other body regions, therefore serving as a good indicator of the core body temperature (Gloster et al., 2011; Poikalainen et al., 2012; Salles et al., 2016).

To minimize the effect of ambient temperature, researchers tried to find models (Landgraf et al., 2014; Lin et al., 2018) and normalization methods (Nikkhah et al., 2005; Lu et al., 2011; Nejati, 2021). In some studies, ambient temperature – either with or without the inclusion of relative humidity – was added to the statistical model (Alsaaod et al., 2015b; Rodríguez et al., 2016; Edwards, 2019) as either a fixed effect (Harris-Bridge et al., 2018) or covariate (Marti et al., 2015; Orman and Endres, 2016). Recently, Cook et al. (2021) accounted for ambient temperature's impact on thermography readings using linear regression analyses to establish a significant relationship between air temperature (AT) and background radiated temperature (BRT) of the IRT images. The BRT was then used as a proxy for AT to calculate the predicted radiated temperature (PRT) for the animals, and the difference between the observed radiated temperature and PRT was termed the residual radiated temperature, which was included as a variable in the statistical model. It is also suggested that because of the correlation between ambient and background temperature, it can be used to calculate the residual radiated temperature if for any reason the ambient temperature could not be calculated during the thermal scanning (Schaefer and Cook, 2022).

Infrared thermography has been used in large animal medicine and husbandry to assess various aspects of animal health and well-being. Infrared thermography allows for the evaluation of factors such as health status, stress levels, and management and housing conditions (Kunc and Knizkova, 2012; Rekant et al., 2015; Soroko and Howell, 2018). Infrared thermography is used to measure the skin temperature of the interested area which can be a reflection of the blood circulation (Casas-Alvarado et al., 2020), and can be affected by the activity level (Douthit et al., 2014), stress and physiological changes (Holmes et al., 2003; McGreevy et al., 2012; Bartolomé et al., 2013), presence of inflammation or infectious (Infernuso et al., 2010; Alsaaod et al., 2014), and the environment (Church et al., 2014). Infrared thermography can be used to evaluate animal performance. For example, IRT has been employed to predict the performance of racehorses, with results indicating that more successful horses tend to have relatively higher skin temperatures (Soroko et al., 2014). In livestock, IRT has also been utilized to assess milk production and meat quality (Kunc and Knizkova, 2012; Horcada Ibáñez et al., 2019). While the use of IRT for pregnancy detection has shown limited success (Rekant et al., 2016), it has demonstrated promising results in estrus detection (Perez Marquez et al., 2019, 2021). Additionally, IRT can be a valuable technique in pain management and surgery, with applications in detecting postoperative pain and monitoring anesthesia and surgical procedures (Czaplik et al., 2017; Küls et al., 2017; Casas-Alvarado et al., 2020). Evaluating stress in animals using IRT is another important application of the technology. Eye temperature has often been used as an indicator of body temperature and shown to be positively correlated with heart rate (Bartolomé et al., 2013; Redaelli et al., 2019).

2.3.1. IRT in Leg and Hoof Health Assessment

Infrared thermography has been used for lameness detection (Alsaaod et al., 2015a). For example, Kim and Cho (2021) used IRT in 66 horses to investigate the thermal distribution of soles with and without abscesses and found that soles with an abscess have higher temperatures than those without. According to Turner (2001), a difference in temperatures between symmetrical parts of bodies of more than 1℃ can be a sign of inflammation. Due to the high accuracy of IRT cameras (± 0.01℃), this technology can detect inflammation approximately 2 weeks before the clinical signs appear (Head and Dyson, 2001; Schaefer et al., 2007). Therefore, IRT can be a valuable tool in the detection of lameness and hoof injuries in cows. It offers a non-invasive method to assess the health of the legs and hooves. By using an IRT camera, various regions of the hoof and leg can be scanned, allowing for the identification of potentially injured hooves without the need for direct examination in a trimming chute. However, it is important to consider the influence of environmental factors and the cleanliness of the area on the reliability of IRT readings. Therefore, certain preparations may be necessary before scanning the region of interest (ROI). In the following sections, we will discuss the different regions of interest and the preparation methods for IRT scanning, various statistical descriptors used to record temperature readings in these areas, and finally the accuracy of IRT in detecting lameness.

2.3.1.1. Regions of Interest (ROI) and Views

One of the main factors in detecting lameness is to find a proper region of interest (ROI) and take the scans from the views that are more susceptible to inflammation and better show the thermal changes. Assessment of coronary band temperature is one of the most common regions for lameness detection and it has been used in cattle, horses, sheep, and goats. Thermal scans are usually taken from dorsal and lateral views of the coronary band (**Table 2.4-1**). The use of the coronary band for lameness detection offers several advantages (Alsaaod et al., 2015a). Firstly, the region has well-developed vascularization and blood circulation, allowing it to reflect changes in the blood flow of the claw. This makes it possible to detect inflammation and other alterations in blood circulation by analyzing the temperature of the coronary band. Secondly, obtaining IRT scans from the coronary band is easier and does not require any restrictions, resulting in less labour and reduced stress for the animals. Lastly, the coronary band's abundant vascularization and lack of hair make it easily identifiable in IRT images.

Another view used in IRT for lameness detection is the sole view of the hooves. The temperature of the sole has been used in detecting hoof abscesses in horses (Kim and Cho, 2021), evaluating locomotion score (Oikonomou et al., 2014; Rodríguez et al., 2016) and measuring digital cushion thickness (Oikonomou et al., 2014). From this view, the skin between the dewclaws and heel can be evaluated as well.

The recorded temperature of hooves can be significantly different based on the time of the day and the side and the temperature of the region the measurements are taken from (D'Alterio et al., 2011); therefore, different regions might have different predictive values in lameness detection. For example, Gelasakis et al. (2021) used both coronary band and heel temperatures from the sole view to detect white line disease and foot rot in sheep. The authors found that, although the temperature difference between ambient and heel temperatures has higher sensitivity in foot rot detection compared to the coronary band (83.3% and 51.9% respectively), using the temperature difference between the coronary band and the ambient temperature had a lower rate of false negative (specificity of 79.7% vs. 47.8%). Another study compared using heel and coronary band temperatures for digital dermatitis detection in dairy cows (Harris-Bridge et al., 2018). In this study, the temperature of both the coronary band and heel were significantly higher in cows affected by digital dermatitis, though the difference between sound and lame hooves was more evident by heel temperature. In terms of the relation between locomotion score and hoof thermography, Rodríguez et al. (2016) found that IRT can distinguish non-lame cows from severely lame cows in all interior, posterior and sole views.

2.3.1.2. Preparation of ROI for IRT

Whether to clean the ROI before IRT scans or not is critical. The claws of the cows are the part of the body that is in contact with the floor and can be contaminated by the manure and slurry running on the floor. On the other hand, cleaning claws either by running water or just rubbing them with a clean cloth can affect their surface temperature. There were more studies that did some type of cleaning prior to scanning compared to ones with hooves left dirty and unwashed. (**Table 2.4-1**). Stokes et al. (2012) compared using IRT in three different situations: 1) unclean foot while standing, 2) cleaned with running water and dried foot with a paper towel while standing and 3) cleaned foot while lifted in trimming chute. They found that the first situation (i.e., not-lifted uncleaned foot) has higher sensitivity and specificity for lesion detection compared to others.

Other than the cleanliness of the feet, the cows' posture, and the time they had been in that posture can influence the thermograms. For example, cows showed higher temperatures when they were lying with their feet under their body compared to the time they stood on concrete (Gloster et al., 2011). In some studies, after preparing the setup, the authors waited from 10 seconds (Munsell, 2006) to 10-20 minutes (Nikkhah et al., 2005; Arican et al., 2018) before scanning.

2.3.1.3. Statistical Descriptors

The thermography images are typically uploaded into special software so the data can be handled, and temperature values can be extracted. Based on the software, the user can obtain information such as the temperature of one specific location of interest or determine the maximum, minimum, average, and standard deviation of the ROI temperature. Maximum temperature is the variable that has been most reported in the literature (Harris-Bridge et al., 2018), followed by average and minimum temperatures (**Table 2.4-1**). Harris-Bridge et al. (2018) compared the maximum, mean, 90^{th} and 95^{th} percentile of temperature range, coefficient of variation and standard deviation of the temperature values for digital dermatitis detection in the heel and coronary band area. They concluded that, although maximum temperature might be the most accurate variable, other statistical descriptors such as the 95th percentile can be useful and, if they are used along with maximum temperature, can increase the accuracy, and reduce the false positive of the test in lameness detection.

The possible reasons behind the popularity of the use of maximum temperature in the current literature are 2-fold. First, when the interested region is selected, the max temperature is the only variable that would not affect the accuracy of selection. For example, the skin in the coronary band or heel usually has the highest temperature, therefore the area surrounding them can cause misreading for the minimum and average temperature if the anatomical region of interest is not selected correctly or uniformly between all observations. Moreover, it has been mentioned that the effect of environmental conditions such as ambient temperature is lower on the maximum temperature compared to minimum and average temperature (Landgraf et al., 2014; Byrne et al., 2017; Uddin et al., 2020).

2.3.2. The Accuracy of IRT in Lameness Detection

To be able to use the IRT on commercial dairy farms, high accuracy of IRT in disease or abnormalities detection such as lameness is critical. Studies are struggling to find the specific thresholds for temperature differences or changes that result in both high sensitivity and high specificity. The threshold has been described as; 1) the temperature difference between the ROI and the control area or other anatomical part within the animal (Alsaaod and Büscher, 2012; Alsaaod et al., 2014), 2) the temperature difference between the extracted temperature from the ROI and the ambient temperature (Gelasakis et al., 2021; Coe and Blackie, 2022), or 3) the difference from the average temperature taken from healthy hooves (Byrne et al., 2018; Coe and Blackie, 2022). Two studies used the area under the curve to report their threshold (Anagnostopoulos et al., 2021; Altay and Albayrak Delialioğlu, 2022). Most studies, however, used a specific temperature to define the threshold. For example, 16.6℃ has been used as a threshold in the sole of dairy cows (Rodríguez et al., 2016) and 36.8 ℃ in the interdigital space of sheep (Talukder et al., 2015). It is good to keep in mind that the difference between thresholds might also be due to the aim of the study like finding a specific lesion (Harris-Bridge et al., 2018) or comparing locomotion scores given to cows (Coe and Blackie, 2022).

In the defined thresholds, the sensitivity and specificity of IRT in lameness detection could be as low as 28.1% (Orman and Endres, 2016) and 39.2% (Lin et al., 2018), respectively. Byrne et al. (2018) used IRT to validate its use in the detection of hooves infections in sheep. They found that, when using the difference between maximum hoof temperature and the average of the coldest hooves on the same day, IRT can detect infections with 92% sensitivity and 91% specificity. One of the challenges in using IRT is that some methods have high sensitivity, though do not have high specificity. This poses an issue, especially in its practical application on commercial dairy farms, as it would result in more labour due to non-lame cows being taken to be checked or treated where no actual issue exists. For example, when Stokes et al. (2012) used IRT on unwashed hooves, they found a sensitivity and specificity of 80% and 73%, respectively, while after cleaning the hooves, the sensitivity increased to more than 90% but the specificity decreased to less than 55%. Similar to determining the threshold, the accuracy of the use of hoof temperatures in lameness detection depends on many factors including the ROI (Rodríguez et al., 2016), the objective of the study (Gloster et al., 2011; Orman and Endres, 2016), the selected threshold (Main et al., 2012; Byrne et al., 2018) and the device used (Coe and Blackie, 2022).

2.4. OBJECTIVES

2.4.1. Overall Objectives

The objective of this study was to evaluate the effect of low frequency of outdoor access on gait and hoof health of non-lame cows housed in movement-restricted environments.

2.4.2. Specific Objectives

This study consists of 2 specific objectives:

1. Evaluating the effect of 1 hour and 3 hours per week of outdoor access on gait and hoof health of non-clinically lame cows housed in tie-stalls using both human-based techniques for clinical assessment and technology-based techniques for subclinical assessments. We hypothesized that low frequency of outdoor would have limited impact on the gait and hoof health of non clinically lame cows but will improve their gait in subclinical levels.

2. Evaluating the use of technologies in detecting slight changes in gait and hoof health of cows after the provision of outdoor access. We hypothesized to observe the subclinical effect of outdoor access on the gait and hoof health of cows by the use of technology.

Table 2.4-1 Specifications and settings used in IRT for lameness detection in different species.

Reference	Species	Statistical descriptors of temperature	ROI	Preparation before IRT	Camera Distance from IOR	Emissivity	Sensitivity	Accuracy	Resolution (pixels)
(Altay and Albayrak Delialioğlu, 2022)	Cattle	Min, Max, Avr.	HS	N/A	N/A	N/A	N/A	N/A	N/A
(Coe and Blackie, 2022)	Cattle	Max, Residual temp.	Heel bulbs and below in hind foot	No washing or cleaning	50 cm	0.95	N/A	N/A	N/A
(Thomas et al., 2022)	Cattle	Max	CB, Area between heel bulbs	Clean with water, brush and dried with paper towel	50 cm	0.98	N/A	± 2%	320 × 240
(Kim and Cho, 2021)	Equine	Average	HS	Brush	30-50 cm	0.97	N/A	N/A	N/A
(Gelasakis et al., 2021)	Sheep	Max	CB, HH, HS	Gentle removing of debris	about 50 cm	0.95	0.06° C	± 2°C	320 X 240

(Werema et al., 2021)	Cattle	Max	CB, above CB, below accessory digit, interdigital space	No preparation	N/A	0.95	N/A	N/A	N/A
(Akin and Ozturan, 2021)	Cattle	Max, Min, Avr.	HS	Clean with pressured water and dried with paper towel	50 cm	0.95	N/A	±0.01°C	N/A
(Anagnostopoulos et al., 2021)	Cattle	Max	IDS between the heel bulbs	No washing and cleaning, wipe manure from soles quickly	30 cm	0.95	N/A	N/A	N/A
(Kroustallas et al., 2021)	Pigs	Max	Lower limb parts	Washed and dried with paper towel	70-90 cm	0.98	<0.06 °C	±2%,	320 X 240,
(Fabbri et al., 2020)	Cattle	Absolute Avr.	Central, IDS and lateral part of hind foot.	Cleaned and trimmed	70 cm	0.98	0.08 °C (at 30 °C),	±2 °C.	320 × 240
(Reppert et al., 2020)	Goat	Maximum	CB	N/A	1 m	N/A	N/A	N/A	N/A
(Harris-Bridge et al., 2018)	Cattle	Max, mean, 90^{th} percentile, 95^{th} percentile,	CB and pastern	No cleaning	1 m	0.98	±0.04 °C	± 1%	spatial resolution = 0.65

		coefficient of variation and standard deviation							mrad. Camera resolution= 640 X 480 pixels
(Gianesella et al., 2018)	Cattle	Absolute Avr.	Central area, IDS, lateral and medial claw of the hind foot	Cleaned and trimmed	70 cm	0.98	0.08°C (at 30°C)	±2°C	spatial resolution (IFOV) of 1.3 mrad, Camera resolution = 320 × 240
(Arican et al., 2018)	Cattle	N/A	CB	Clean from debris and moisture	1.5-2 m	N/A	N/A	N/A	N/A
(Bobić et al., 2018)	Cattle	Max, Min, Avr.	CB	Cleaned of impurities	1 m	0.95	N/A	N/A	N/A
(Byrne et al., 2018)	Sheep	Max and average	CB to the dew claw	N/A	70 cm	0.98	<0.03 °C	±2 °C	320 × 240
(Bobić et al., 2017)	Cattle	Max, Min and Avr.	Coronary band	Cleansed of impurities.	1 m	0.95	N/A	N/A	N/A
(Byrne et al., 2017)	Cattle	Max, Min and Avr.	Udder, eye, hooves	N/A	80 cm (udder), 90 cm (eye), 1.2 m (hooves)	0.98	<0.03°C	±2°C	320 × 240

(Rodríguez et al., 2016)	Cattle	Avr.	IDS, HS, HH and CB	Washed and dried with paper towel	40 cm	0.95	N/A	N/A	N/A
(Orman and Endres, 2016)	Cattle	Arithmetic Avr.	CB	No washing, cleaned from debris	50 cm	0.98	N/A	2% of reading or ±2°C	160 × 120
(Salles et al., 2016)	Cattle	Max, Avr.	Eye, left front leg, right and left flank, and forehead	N/A	2 m (Whole body), 20 cm (eye, forehead, and cranial left foreleg), 1 m (Caudal left foreleg)	0.98	N/A	N/A	N/A
(Alsaaod et al., 2015b)	Cattle	The difference of Max temp. between medial and lateral claws	CB of lateral and medial claws	No washing and cleaning, exclude dirty feet	50 cm	0.95	N/A	±0.01°C	N/A
(Marti et al., 2015)	Cattle	N/A	Claw area	N/A	N/A	N/A	N/A	N/A	N/A
(Talukder et al., 2015)	Sheep	Max, Min and Avr	IDS	No cleaning and washing	50 cm	0.98	N/A	N/A	N/A
(Wilhelm et al., 2015)	Cattle	Avr.	Claw area	Trim to clean	30 cm	1	N/A	N/A	N/A

(Oikonomou et al., 2014)	Cattle	Spot exact temp.	HS and lateral hind foot	Trimmed and cleaned	2 m	0.95	N/A	N/A	N/A
(Alsaaod et al., 2014)	Cattle	Max	CB of lateral and medial claws	N/A	50 cm	0.95		± 0.01	
(Douthit et al., 2014)	Equine	Avr. Temp of for hooves	1 cm below coronary band	N/A	N/A	N/A	N/A	N/A	N/A
(Renn et al., 2014)	Cattle	Max	limbs	Used dry limb	2 m	0.93	0.12°C	N/A	N/A
(Soroko et al., 2014)	Equine	Max, Min and Avr	Distal limbs, and the dorsal aspect of the back	Brush dirt and mud	1 m	1	N/A	N/A	640 X 480
(Alsaaod and Büscher, 2012)	Cattle	Max, Min and Avr	CB, skin above CB	Wash with pressured water and dried using paper towel	50 cm	0.98	N/A	N/A	1,280 × 1,024
(Poikalainen et al., 2012)	Cattle		udder, feet, and areas with skin injuries	N/A	60 cm	0.95	0.1°C	N/A	N/A

(Stokes et al., 2012)	Cattle	Max	Pastern	No washing and cleaning + washing, cleaning and dry with cloth	Using camera marker to set the distance	N/A	N/A	N/A	N/A
(Gloster et al., 2011)	Cattle	Max	CB, left eye	N/A	1-2 m	0.95	N/A	± 2°C	N/A
(D'Alterio et al., 2011)	Sheep	Avr ± SD	IDS	N/A	50 cm	0.98	N/A	N/A	N/A
(Munsell, 2006)	Cattle	Max, Avr	CB in hind foot	Brush to remove dirt	1 m	N/A	50 mK at 30 °C	± 2 °C	N/A
(Nikkhah et al., 2005)	Cattle	Max	CB of lateral and medial claw	Clean from debris	1.5-2 m	0.93	N/A	N/A	N/A

Abbreviations:

Max = maximum, Min = minimum, Avr = average, CB= coronary band, HS= hoof sole, HH= hoof heel, IDS = interdigital space

CHAPTER 3 – Materials and Methods

3.1. Ethical statement

All procedures and the use of animals were approved by the Animal Care Committee of McGill University and affiliated hospitals and research institutes (Protocol #2016-7794).

3.2. Study Design

Thirty-six lactating Holstein cows housed in the tie-stall Dairy Complex of McGill University were enrolled in this study from November 2021 to February 2022 (i.e., experimental period). All 36 cows were housed in 4 adjacent rows in the barn and milked twice daily at 12 h intervals. Cows were grouped into 6 blocks based on parity (primiparous and multiparous) and days in milk (DIM) in which cows were grouped as early lactation: 0-100 DIM, mid-lactation: 101-200 DIM and late lactation: 201-305 DIM. Within each block, cows were randomly assigned to one of the two treatments (3 cows/treatment): Exercise1X (Ex1) for which cows had access to the outdoors one day a week (n=18) with the average parity of 2.11 ± 1.05 and DIM of 133 ± 65.55 and Exercise3X (Ex3) for which cows had access to outdoor three days per week (n=18), the average parity and DIM for latter group were 2.11 ± 0.99 and 111.5 ± 57.41, respectively. The trial was conducted over 5 consecutive weeks from November 1st to December

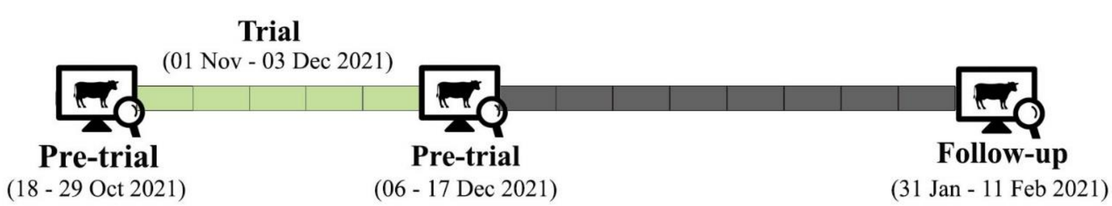


Figure 3.2-1 This figure shows the timeline of the study and the three data collection points.

3rd, 2021. On each day of outings, cows had access to an outdoor pasture-based exercise yard for 1 hour, from 10-11 AM. The pasture-based exercise yard, located adjacent to the barn, was equally divided into 6 paddocks each measuring 117 m^2 (9 m x 13 m). For the first outing, each block was randomly assigned to 1 paddock and subsequently changed paddocks weekly in a clockwise rotation. Gait scoring, 3D motion analysis, kinetics measurements, claw lesion assessment, hoof thermography, and claw conformation measurements were assessed at 3 data collection points: before the trial (Pre-trial), at the end of the trial (Post-trial) and 8 weeks after the trial (Follow-up; **Figure 3.2-1**)

3.3. Gait Analysis

3.3.1. Animal Selection and Passage Criteria

In this study, gait evaluations were conducted at all data collection points – Pre-trial, Post-trial, and Follow-up – utilizing both clinical visual gait scoring and an objective and subclinical, technologically assisted three-dimensional (3D) motion analysis system. Due to logistical constraints, a subsample of cows was selected for gait analysis. The subsample selection was based on our previous experience of cows with gait analysis and their ease of handling: of the 36 cows, ones that had experience with gait analysis in the previous 2 months and who were known to be easy to handle were automatically selected. To complete the selection, additional cows with no exposure to gait analysis in the previous 2 months were selected during habituation to the experimental environment while being walked in the test corridor. A trained handler led the cows with a halter out of their stall to the designated experimental area where they were walked in a straight test corridor that measured 5 m in length. After walking for 5 minutes in the experimental area, cows who had not calmed down were excluded from the subsampling. Finally, a subsample of 22 cows out of the 36 (i.e., 11 cow from Ex1 and 11 from Ex 3 treatments) were selected for gait analysis in the pre-trial and post-trial data collection points. However, only 21 cows were recorded in the follow up data collection point as 1 cow was culled by that time.

In each data collection point, cows were walked for a minimum of 5 passages along the 5 m test corridor with concrete flooring covered with rubber mat, ensuring that the cow walked in a straight line at a consistent pace without running, stopping, defecating, or urinating for at least one passage. If a usable passage was not obtained after 30 minutes, the cow was returned to her stall for the remainder of the day. The cow was tested again on the last day of that collection point, and it was considered “missing data” if the passages did not meet the criteria listed. As needed, a bucket filled with grain was placed at least 1 m in front of the cow while she was walking. To encourage cows to walk and make sure they would walk in a straight line with monotonous speed, additional handlers were sometimes used (i.e., one handler positioned behind the cow, to encourage the cow to move forward, and/or 1-2 additional persons standing adjacent to the corridor to guide the cows to walk in a straight line or reducing their walking speed).

3.3.2. Visual Gait scoring

For visual gait scoring, also known as visual locomotion scoring, the cows' passages were recorded using two high-performance cameras (Basler Ace, Ahrensburg, Germany) fixed to the ceiling with a perpendicular view of the corridor (one from the right side; one from the rear). A total of 65 passages (2x22 recordings from pre- and post-trial and 21 recordings from follow-up) were recorded, resulting in 130 recordings (65 passages x 2 views recorded/passage) available for gait analysis. All recordings were coded with numbers and randomized so that the trained observer was unaware of the animal's treatment group and data collection point. The observers were trained by an expert member of our lab on gait scoring using video recordings and SOPs made in our lab. The weighted kappa inter and intra observer reliability was 0.82 and 0.64, respectively, for the overall gait score and 0.39 and 0.37 for the six gait attributes (**Table 3.3-1**). Six gait attributes (swinging out, back arch, tracking-up, joint flexion, asymmetric gait, and reluctance to bear weight; **Table 3.3-1**) were visually scored using rearview camera for swinging out and side view for other attributes. The rearview camera was used for swinging out as it provided better vision on the swinging of the legs. Scores were assigned to each trait based on Shepley and Vasseur (2021a) using a 0-5 scale with 0.5 intervals, where 0 represents the soundest and 5 the most severe that trait might be seen in the cow. An overall gait score was also assigned, based on (Flower and Weary, 2006), on a 5-point scale (Numeric rating scale, NRS) with 0.5 increments, with 1 indicating the perfect gait and 5 indicating severe lameness. Cows were categorized as non-lame (NRS < 3), moderately lame (NRS = 3), lame (NRS = 4), and severely lame (NRS = 5).

Table 3.3-1. Gait attribute definitions and their associated ratings (0-5, described by Shepley and Vasseur (2021a) and adapted from (Flower and Weary, 2006))

Gait Attribute	**Definition**	**Scoring Scale with 0.5 intervals**	
		0	5
Swinging out	Description of visual gait variables and the corresponding endpoints of a visual analog scale	Hind legs moving in straight line during the swing phase	Pronounced, circular motion of the hind legs during the swing phase

Arch back	The shape of the spine when the cattle walks	Flat spine	Convex arch between the withers and tailbone
Tracking up	The gap between the imprint left behind the front hoof and the new imprint formed from the rear hoof.	Hind hoof falls in imprint left by the front hoof of the same side	Hind hoof falls short of the imprint left by the front hoof of the same side
Joint flexion	Related to the flexions and extensions of the limb while the cow is moving	All limbs flex and extend easily	All limbs are stiff and limited in their range of motion
Asymmetric step	How even the stepping pattern of a cow is	Equal steps: cow places her hooves in an even “1, 2, 3, 4” rhythm	Not equal; cow places her hooves in an uneven rhythm
Reluctance to bear weight	How evenly the cow distributes her weight when walking	Bears weight equally over all legs	Uneven weight bearing between legs

3.3.3. 3D Motion Gait Analysis

To enhance the evaluation of gait and to observer subclinical and slight changes, a comprehensive 3D motion analysis of gait was performed to obtain precise and detailed measurements of various gait attributes.

3.3.3.1. Animal Preparation

Similar to Bradtmueller (2022), spherical reflective markers were attached to 20 anatomical parts on the cow’s body (4 markers on each leg and 4 on the back) prior to walking cows in the test corridor. The joint markers were placed at the coffin and fetlock of all four legs, the carpal and elbow of the front legs, as well as the corresponding joints of the hind legs, meaning the hock and stifle joints (i.e., 16 markers). Four markers were placed on the back of each cow, one on the withers (i.e., highest thoracic vertebrae), one on the spinous process of 13th thoracic vertebrae, one on the area where the last lumbar vertebrae meet the sacrum and one on the tailbone or sacrococcygeal joint. To make the procedure repeatable and ensure marker placement was consistent between and within cows, a 10 x 10 cm stencil laminated paper was used to identify the anatomical landmarks and highlight them with either an ink marker or by shaving the area. To attach the reflective markers on cows' body, duct tape was first attached directly to the assigned anatomical landmark; then markers were placed on top of the duct tape using double-sided tape. The tail switch was restrained using a cloth strap to prevent marker masking during cows walking.

3.3.3.2. Recording and Calibration

For 3D gait analysis, the cows' passages were recorded using 6 high-performance cameras (Basler Ace, Ahrensburg, Germany) attached to the ceiling (i.e., 3 cameras on the right and 3 cameras on the left side of the test corridor) at 2.3 m from the ground such that each camera view overlapped with at least 2 other camera views. All cameras were wired to a desktop computer, and videos were captured using CONTEMPLAS TEMPLO capture engine (CONTEMPLAS GmbH, Kempten, Germany) to ensure synchronization of all videos. Cameras were set to record videos 60 frames per second (fps), with 350 shutters per second and gain of 75, image sizes and position of area of interest were 1280 width x 800 height pixels. To ensure accuracy of the video data upon analysis, a calibration video was recorded every day of testing before the first recording. A calibration device with 24 spherical reflective markers was placed in the middle of the test corridor (volume dimension: 196,84 cm x 196,84 cm x 196,84 cm) and positioned such that all 24 markers were visible by all 6 cameras to record the calibration videos. A total of 65 passages (2 x 22 records from Pre- and Post-trial and 21 recordings from Follow-up) were recorded.

3.3.3.3. Data Extraction and Preparation

To track the markers, the videos recorded by CONTEMPLAS TEMPLO were exported to the Vicon Motus (Version 10.0.1, Vicon motion systems Inc. Oxford, UK) software. In the first step, marker positions, definitions of angles based on the marker positions (e.g., hock angle defined as the angle made by fetlock, hock, and stifle markers), and events (i.e., the time of start and end points of each passage, toe-off and landing of each limb) were labelled in the software. In the next step, the markers from the calibration video for each day were tracked, then 6 videos of each passage were exported to the software. A passage was defined as the time a cow started to walk in a straight line in the test corridor to the time she left the corridor, which consisted of 2 to 3 full strides. Out of 20 markers, only 11 were digitized and tracked using Motus Software. These included markers attached to the coffin joints of all four legs (4 markers), fetlock, hock, and stifle joints of the rear legs (6 markers), and the one on the withers (1 marker). This enabled for the calculation of track-up (cm), stride length (cm), stride time (s), velocity (cm/s), and hock joint's range of motion for each passage (**Table 3.3-2**). The default coordinate system for our experiment is determined by the position of the calibration frame within the measuring space (also referred to as the measuring volume). The X-axis represents the horizontal longitudinal axis, corresponding to the cows' walking direction. The Y-axis indicates the horizontal transverse axis, which is perpendicular to the cows' walking direction, while the Z-axis signifies the vertical axis (**Figure 3.3-1**). In cases where the default X-axis in Motus was not aligned with the cow's walking direction, motion tracking (positions) of the withers' marker on the Y-axis (transverse axis) was used to adjust the direction of the calibration frame, rotating it 15° to 25° around the Z (vertical) axis (**Figure 3.3-2**). Marker tracking for each leg started at few frames before the first landing of the hoof (toe-on) and continued for a few frames after the last time the cow lifted her hoof from the ground (toe-off). In each passage, all toe-ons and toe-offs of each leg were recorded as an event.

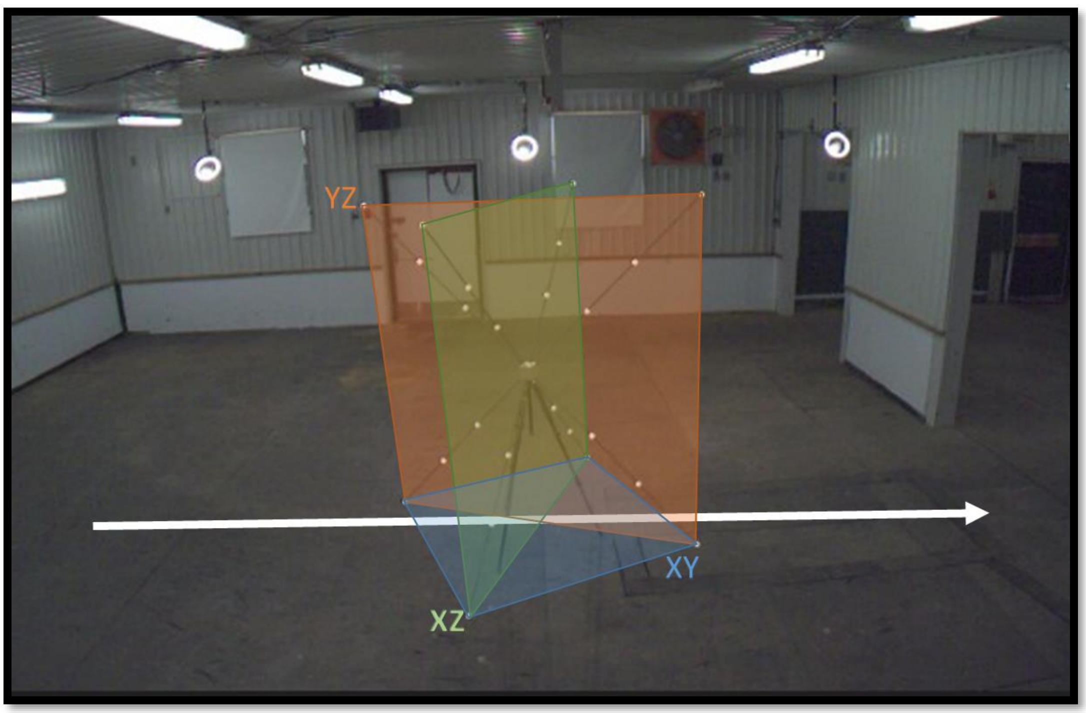


Figure 3.3-1 This figure represents the calibration device and X-Y-Z axis based on the calibration device position. The white arrow shows the cow's walking direction

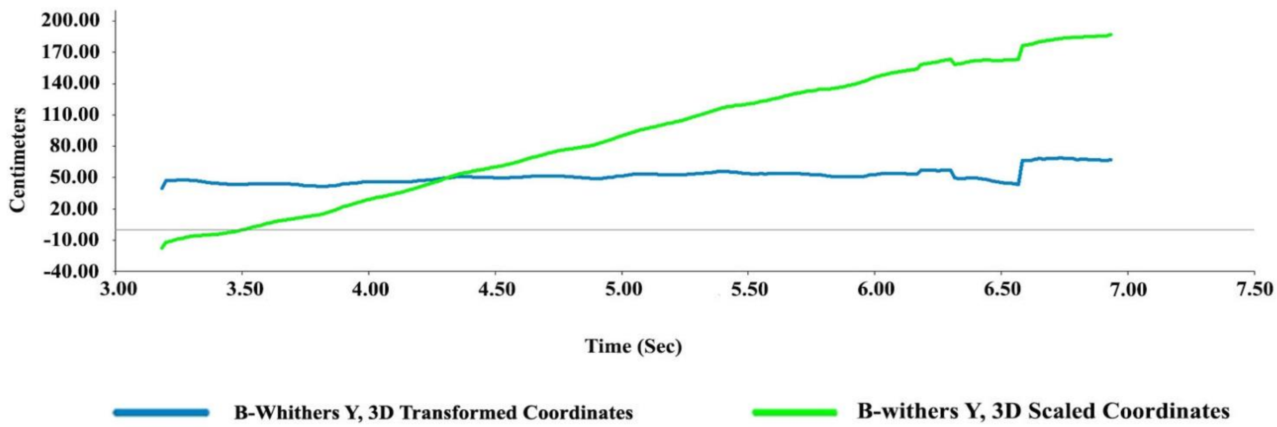


Figure 3.3-2 This graph displays the position (distance in cm from the origin) of the cows' withers' marker on the transverse axis of the measuring space over time (in seconds) during the passage, showcasing the cows' walking direction. The green line (3D scale coordinates) represents the cow's withers' position on the transverse axis before correction (default coordinate), while the blue line (3D transformed coordinates) illustrates the cow's withers' position on the same axis after correction.

After correcting the calibration, the coordinates of each marker in the 3D axis and hock angles in each frame were extracted from the software into Excel files. Based on the coordinates, the 11 variables of interest were then calculated as described in **Table 3.3-2**.

Table 3.3-2 The kinematic variables, their definitions and the method used to calculate them based on the data provided by the Vicon Motus software (Version 10.0.1, Vicon motion systems Inc. Oxford, UK)

Variable[1]	Definition
Track upX (cm)	The average distance between the coordinates of coffin marker of fore limb at toe-on events and coffin marker of the ipsilateral rear limb in X axis (longitudinal axis, or the cow's walking direction)
Track upXY (cm)	The average distance between the coordinates of coffins markers of fore limb at toe-on event and coffin marker of the ipsilateral rear limb in XY

	axis (the diagonal line connecting longitudinal (X) and Transverse (Y) axis)
Stride length (cm)	The average distance between the two consecutive stride strikes (coordinate of coffin marker at the toe-on event) of the same limb in XY axis
Stride time (s)	The number of frames between two consecutive toe-on events of the same hoof ÷ 60
Velocity (cm/s)	Stride length ÷ stride time
Stance time (s)	The number of frames between toe-on and the next toe-off ÷ 60
Hock angle (Min)	The lowest number recorded from the angle created between the fetlock, hock, and stifle markers during walking from the first toe-on to the last toe-off
Hock angle (Max)	The highest number recorded from the angle created between the fetlock, hock, and stifle markers during walking from the first toe-on to the last toe-off
Hock angle range of motion (ROM)	The difference between Maximum and Minimum hock angles
Hock angle (Med)	The median of the numbers recorded from the angle created between the fetlock, hock, and stifle markers during walking from the first toe-on to the last toe-off of each passage
Hock angle (Avr)	The average of hock angel at all toe-on and toe-off events of each passage

[1]Abbreviations:

Min = Minimum, Max = Maximum, Med = Median, Avr = Average

3.3.4. Kinetic Assessment of Gait

Kinetic analysis of the gait, which serves as another tool for subclinical and objective measurement of gait, was conducted during all data collection points (i.e., Pre-trial, Post-trial, and Follow-up) on the subsample of cows described in the gait analysis section. In each data collection points, a trained handler led the cows with a halter out of their stalls to the designated experimental area, where cows were individually tied in the kinetic pen such that the cow was standing on the 2 pressure plates (FootScan®, Teckscan Inc. MA, USA) parallel to one another with the left legs (front and hind) on one plate and the right legs (front and hind) on the other. Plates were connected separately to two different portable laptops and the FOOTSCAN software (FootScan®, Teckscan Inc. MA, USA) was used to visualize and capture the data. Data was collected while the cow stood still on the plates. In the first step, 2 screenshots (SC) per plate of the images visualized by the FOOTSCAN software (FootScan®, Teckscan Inc. MA, USA, one per computer) were taken simultaneously through the software, then videos were recorded with the FOOTSCAN software for up to 30s (125 fps) recording (30Srec). The cows had to be still during both the SC and 30Srec data collections. If the cow moved one or more legs the data collection was redone.

Different kinetic variables were measured depending on the data collection strategy (SC vs 30Srec). Contact area (cm^2), as well as maximum and average pressure (N/cm^2) were measured at both SC and 30Srec. Maximum and average force (N) were only measured in 30Srec, whereas pressure distribution ratio was measured using SC data only. The kinetics variables are described in **Table 3.3-3**. Force and pressure at 30Srec were calculated by the software at each frame based on the footprint zones. Since the software is designed based on human anatomy, we first defined each claw as separate zones and then calculated the maximum and average force (N) as well as pressure (N/cm^2) measured by software from all frames in that specific zone.

Table 3.3-3 The kinetics variables and how they were calculated using the data from Footscan software (FootScan®, Teckscan Inc. MA, USA)

Variable[1]	Unit	Definition
Contact_area_SC	cm^2	The area of the hoofprint sensed by the pressure platform at screenshots
Contact_area_30Srec	cm^2	The biggest contact area of the hoofprint sensed by the pressure platform during the 30 seconds recording

MPressure_SC	N/ cm^2	The highest (maximum) pressure measured by the software in the screenshots
AVPressure _SC	N/ cm^2	The average of the pressures measured in the hoofprint of the cow in screenshots
MPressure_30Srec	N/ cm^2	The highest pressure measured by software during the 30 seconds recording
AVPressure_30Srec	N/ cm^2	The average of the pressures measured at all frames
MForce_30Srec	N	The highest force measured by software during the 30 seconds recordings
AVForce_30Srec	N	The average of the forces measured at all frames
pressure distribution	%	The ratio of the pressure distributed between each claw

[1]**Abbreviation:**

SC = Screenshot, 30Srec= 30 seconds recording, MPressure = maximum pressure, AVPressure = Average pressure, MForce= Maximum force, AVForce = average force

Pressure, force, and pressure distribution were calculated directly by the FOOTSCAN software and extracted into an Excel file for further analysis. To measure the contact area, pictures of SC and 30Srec were used and exported to the Adobe photoshop software (Photoshop 2022, version 23.2, Adobe Systems Inc., San Jose, USA). The number of pixels representing the known distance in cm was calculated using the scale provided in the footscan software (FootScan®, Teckscan Inc. MA, USA). Then, the magic wand tool was utilized to select the hoofprint area, followed by the calculation of the contact area using the number of pixels within the selected region.

3.4. Hoof Health Assessment

Cows were restrained in an upright hoof trimming chute for hoof health assessment, including clinical hoof health assessment (section 3.4.1), sole thermography (section 3.4.2) and measuring claw conformation (section 3.4.3). A professional hoof trimmer performed all the hoof trimming during the project. A full hoof trimming using the five-step Dutch method (Toussaint, 1989) was conducted a month before the start of the Pre-trial point. In order to clean hooves before our measurements, sliver horn trimming (in which a thin layer, or “sliver,” of the sole of the hoof is removed to clean the sole and evaluate hoof health and conformation without significantly altering the hoof's overall structure) was performed at the Pre-trial, Post-trial and Follow-up points. A full hoof trimming was conducted after the sliver trimming at follow-up (8 weeks after trial) to investigate possible hoof lesions and pathologies.

3.4.1. Clinical Hoof Health Assessment

Clinical assessment of hoof health was performed on all 36 cows at Pre-trial and Follow-up data collection points. Although possible lesions (i.e., infectious, and non-infectious lesions) were recorded at the Post-trial, data regarding non-infectious lesions was not included for further analysis, due to the pathogenesis of claw horn disruption lesions (CHDL) development. It is estimated that it would take approximately 8 weeks to be able to observe the consequences of metabolic and mechanical tensions on the sole of the hoof (Shearer et al., 2015). Therefore, we considered that the lesions recorded at the Post-trial could not be a reliable representation of the effect of treatment application (i.e., outdoor access).

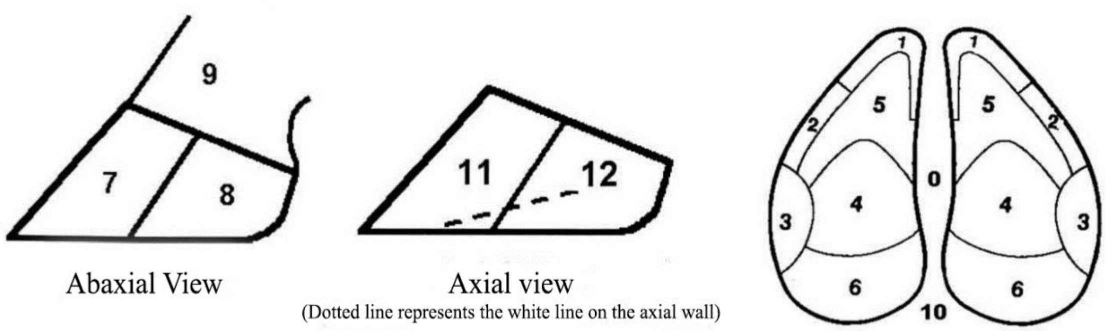


Figure 3.4-1 sole zones used to describe the 12 locations of claw lesions, figure adapted from Shearer et al. (2004)

The incidence of lesions was assessed at the trimming chute by a trained observer who recorded the number and location of lesions (**Figure 3.4-1**). Claw lesions were named and classified based on ICAR Claw Health Atlas (Egger-Danner et al., 2014). Additionally, the combination of Flower and Weary (2006) and Nikkhah et al. (2005) systems were used to create a 5-point scaling system (**Figure 3.4-2**) to score severity of the lesions (0 = no hemorrhage and/or discoloration, 1= slight hemorrhage, 2= moderate hemorrhage, 3= severe hemorrhaged lesion and potential visibility of fresh blood, 4= exposed of the corium, ulcer) in all 8 claws.

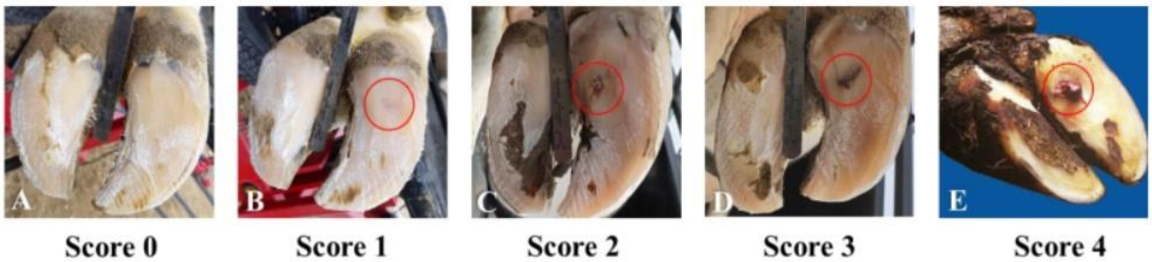


Figure 3.4-2 Sole ulcer is presented in this figure as an example of the scoring system for claw lesions severity. This 5-point scale starts from 0 (picture A) meaning no lesion, to 4 (picture D) meaning ulcer, adapted from Nikkhah et al. (2005) and Flower and Weary (2006). Pictures from A-D were taken by Vasseur's lab, only picture E is courtesy of ICAR Atlas

3.4.2. Subclinical Hoof Health Assessment

Hoof thermography was used for subclinical assessments of hoof health. A Handheld Infrared thermography (IRT) Camera (FlIR E8, Teledyne FLIR LLC, Wilsonville, Oregon, USA) was used to take IRT images. The IRT camera (FLIR E8, Teledyne FLIR LLC, Wilsonville, Oregon, USA) produced a digital photo along with a thermal image. The object temperature range of the IRT camera was –20°C to 250°C with a thermal sensitivity of 0.05°C and an accuracy of ± 2% of reading within this restricted range. The wide-angle lens was 45° × 34°, and the camera resolution was 320 × 240 pixels. Before taking images, object parameters were adjusted on the camera as follows: emissivity value, 0.95; distance from the object, 1 m; and reflected temperature, 20°C. All thermography pictures were taken within a distance of 0.5-1 meter from the area of interest. An IRT image of the cow's eye was taken to use as a control for individual variance in body temperature (Redaelli et al., 2019). IRT images were taken of each hoof from two separate views (i.e., dorsal -coronary band and plantar -sole).

IRT images from the coronary band were taken from the sub-sample of 23 cows (see section 3.3.1) at three data collection points (Pre-trial, Post-trial and Follow-up). The digital format of images taken by the thermal camera was used to evaluate the foot hygiene (hoof, coronary band, and dew claws area) using a 4-point hygiene scoring system ranging from 0 to 3 (**Figure 3.4-3**). In this scale, which was adapted from Schreiner and Ruegg (2003), a foot with a cleanliness level of 0 indicates it is completely clean. A level of 1 signifies a foot that is slightly dirty, with scattered splashes of manure. A level of 2 represents a moderately dirty foot, where the hoof and coronary band area are mostly covered with dirt or manure. Lastly, a level of 3 indicates a foot that is very dirty, being completely covered with dirt or manure. Feet with scores 2 and 3 were dropped from further analysis. At each data collection point (Pre-trial, Post-trial and Follow-up), pictures were taken from cows right after the kinetic recording was done. To reduce any artifacts in the thermograms, the cow was made to not move for at least 1 minute prior to the IRT image being taken. The kinetic pen is in a room with a controlled temperature and no direct sunlight or wind flow. Nevertheless, to consider the possible ambient temperature variation between the data collection points, a thermometer (Mini Thermo-Anemometer, EXTECH instruments, Nashua, NH, USA) was attached to the pole closest to the cow's head position to allow the recording of the time of the scanning and pen temperature at the time of scanning for each cow.

Score 0 **Score 1** **Score 2** **Score 3**

Figure 3.4-3 Foot hygiene scoring scale from 0 (picture A) in which the foot is entirely clean to 3 (Picture D) which shows the foot covered in dirt; adapted from (Schreiner and Ruegg, 2003)

Sole view thermal images were taken for all 36 cows using the same camera (FlIR E8, Teledyne FLIR LLC, Wilsonville, Oregon, USA) after sliver trimming and removal of dirt at the three data collection points (there was a 2–5-minute gap between trimming and taking IRT pictures). The trimming chute was placed in a corridor protected from direct sunlight and wind flow. A thermometer (Mini Thermo-Anemometer, EXTECH instruments, Nashua, NH, USA) was placed at the trimming chute to record the ambient temperature after taking IRT pictures from each cow.

Data from the dorsal view was obtained using Therma-CAM Researcher Professional 2.10 software (FLIR Systems, Inc., Wilsonville, Oregon, USA). The images were first imported to the software, and three regions of interest (ROI) were selected: hoof coronary band area (CB) and coronary band of lateral and medial claws (LCB and MCB, respectively; **Figure 3.4-4**). Maximum, minimum, average, Maximum minus minimum, and standard deviation of the selected regions and cow’s eye were extracted from IRT images.

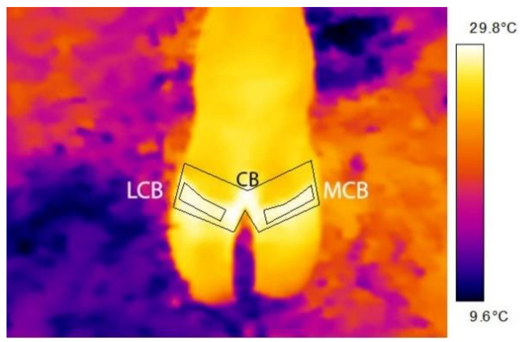


Figure 3.4-4 The ROI selected from dorsal view, CB = hoof coronary band, MCB = medial claw coronary band and LCB = lateral claw coronary band.

To obtain sole view data, pictures were imported to the FLIR TOOLS software (Version 6.4.18039.1003, FLIR Systems, Inc., Wilsonville, Oregon, USA) and four anatomical zones based on the description of Shearer et al. (2004) were selected as ROI: the skin above the claws or zone 10 (Z10), the skin between the claws or zone 0 (Z0), and the most common area for sole ulcer (Oikonomou et al., 2014), which is the zone 4 of both lateral (LZ4) and medial (MZ4) claw. Maximum, minimum, and average temperature of all ROIs were measured for further analysis. (**Figure 3.4-5**)

It has been reported in previous studies (Landgraf et al., 2014; Uddin et al., 2019) that the maximum temperature of the eye remains least affected by ambient temperature, making it a potentially suitable indicator for core body temperature. Therefore, only the maximum temperature of the eye was used for further analysis. Similarly, the hoof's maximum temperature has been found to have a narrower distribution and lower variation across different ambient temperatures (Byrne et al., 2017; Uddin et al., 2020), while the average temperature of the hoof demonstrates greater consistency and precision as it represents numerous pixels' information (Montanholi et al., 2008; Byrne et al., 2017; Cook et al., 2021). Consequently, only these two descriptives (i.e., maximum, and average temperature of IOR in both coronary and sole views) will be reported in the results. The information regarding other variables will be accessible through **Supplementary Table 5.2-7** to **Supplementary Table 5.2-10**.

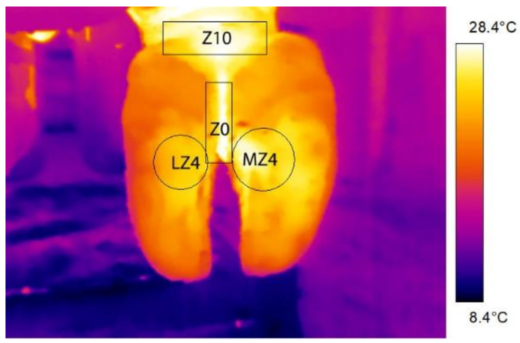


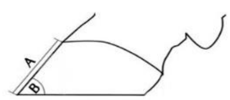
Figure 3.4-5 The ROI selected from the plantar (sole) view; Z10 = Zone 10 or the skin above the claws, Z0 = Zone 0 or the skin between the lateral and medial claw, LZ4 = Zone 4 of the lateral claw and MZ4 = Zone for of the medial claw.

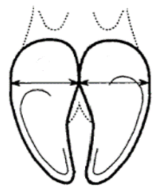
3.4.3. Measuring Claw Conformation

Claw conformation data was collected at the three data collection points for all 36 cows enrolled the study while they were restrained in the hoof trimming chute after the sliver hoof trimming. The measurements were done either live in the trimming chute or using digital pictures taken at the time to be analyzed later. For the live measurements, a trained observer measured claw length as the distance from the horn junction at the coronary band to the apex of the toe at the dorsal view using a ruler. The toe angle was measured as the angle of the dorsal border to the weight-bearing surface using a Digital sliding T-bevel (ANGLE-IZER®, General Tools & Instruments LLC, NJ, USA). Live measurements were only taken from the lateral claw of each hind leg because of the time limitation for evaluating all claws, they bear more weight compared to medial claws, and they are more susceptible to claw lesions (Nuss and Paulus, 2006; Correa-Valencia et al., 2019). Digital pictures of the soles of both the medial and lateral claws of hind limbs were taken using a cellphone rear camera (Samsung Galaxy 8 and iPhone 12 Promax). Sole length and width were measured using the ImageJ software (U. S. National

Institutes of Health, Bethesda, Maryland, USA) as explained in **Table 3.4-1**. The procedure detailed in Laven et al. (2015) was followed. A ruler was placed above the hooves' heels to be used as a scale in digital pictures by measuring the number of pixels in 1cm of the ruler; this scale was used to measure the sole length and sole width based on the number of pixels.

Table 3.4-1 Measurement of claw conformation both live (claw length and toe angle) and using a digital picture (sole length and width), measurements adapted from Vermunt and Greenough (1995) and Laven et al. (2015)

Variables	Figure	Live/digital picture	Claw	Definition
Claw length (cm)	1. 	Live	Lateral	The distance from the horn junction at the coronary band to the apex of the toe (Vermunt and Greenough, 1996)
Toe angle (°)	2. 	Live	Lateral	The angle of the dorsal border to the weight-bearing surface (Vermunt and Greenough, 1996)
Sole length (cm)	3. 	Digital picture	Lateral and medial	The distance between the apex of the toe to the point where ground contact is lost (Laven et al., 2015)
Sole width(cm)	4.	Digital picture	Lateral and medial	The largest distance between the abaxial and axial wall of

		the claw (Laven et al., 2015)

3.5. Statistical Analysis

Linear mixed models were used to assess the effects of outdoor access on different variables. We used block, treatment (EX1 and EX3), and time (Pre-trial, Post-trial and Follow-up) as fixed effects. We used time as a random slope to evaluate the effect of outdoor access over time for individual cows (Lohse et al., 2020) for all variables. We used cow as a random intercept for visual gait scoring. For clinical hoof health assessment, kinetic and conformation measurements, we used claws nested within cows as a random intercept instead of cows. For 3D motion analysis and thermography variables, we used limb nested within cow as a random intercept. For the thermography data, eye maximum temperature and ambient temperature were added as co-variables. The statistical analysis was done using R Core Team (2022)(Version 4.2.2, Vienna, Austria) with RStudio interface (version 2023.03.1, RStudio: Integrated Development for R, PBC, Boston, MA, USA) and the R package *nlme* (Pinheiro et al., 2020). The following linear mixed model was used for visual gait scoring variables including, overall NRS, swing out, arch back, tracking up, joint flexion, asymmetric steps, and reluctance to bear weight:

$$Y_{iojkm} = \mu + block_j + time_K + trt_m + time_k * trt_m + cow_{ojkm} + time_{iojkm} + e_{iojkm}$$

Where Y_{iojkm} is the visual gait scoring outcome variable with the ith random slope of time, oth random intercept of cow nested in jth block, kth time points and mth treatment, μ is the overall mean, $block_j$ is the effect of the jth block (1,2,3,4,5,6), trt_m is the effect of the mth treatment (EX1 and EX3), $time_K$ is the effect of the kth time point (Pre-trial, Post-trial and Follow-up), $time_k * trt_m$ is the interaction effect between mth treatment and Kth time point, cow_{ojkm} is the random intercept of the oth cow nested withing the jth block, kth time point and mth treatment ~ N(0, σ^2), $time_{iojkm}$ is the random slope of ith nested in oth cow, jth block, kth o time point and mth treatment ~ N(0, σ^2) and e_{iojkm} is the random error ~ N(0, σ^2).

For 3D motion analysis variables of track-up, stride length and time, velocity, stance time and hock angles, the following mixed model was used for statistical analysis:

$$Y_{iojkm} = \mu + block_j + time_K + trt_m + time_k * trt_m + limb\ (cow)_{ojkm} + time_{iojkm} + e_{iojkm}$$

Where Y_{iojkm} is the 3D motion analysis outcome variable with the ith random slope of time, oth random intercept of limb nested in cow, jth block, kth of time points and mth treatment, μ is the overall mean, $block_j$ is the effect of jth block (1,2,3,4,5,6), trt_m is the effect of mth treatment (EX1 and EX3), $time_K$ is the effect of kth time point (Pre-trial, Post-trial and Follow-up), $time_k * trt_m$ is the interaction effect between mth treatment and kth time point, $limb\ (cow)_{ojkm}$ is the random intercept of the limb nested in oth cow ,jth block, kth time point and mth treatment ~ N(0, σ^2), $time_{iojkm}$ is the random slope of ith nested in oth cow, jth block, kth time point and mth treatment ~ N(0, σ^2) and e_{iojkm} is the random error ~ N(0, σ^2).

For the kinetic variables of weight distribution, pressure, force and contact area, the following linear mixed model was used:

$$Y_{iojkm} = \mu + block_j + time_K + trt_m + time_k * trt_m + claw\ (cow)_{ojkm} + time_{iojkm} + e_{iojkm}$$

Where Y_{iojkm} is the kinetic analysis outcome variable with the ith random slope of time, oth random intercept of claw nested in cow, jth block, kth time points and mth treatment μ is the overall mean, $block_j$ is the effect of jth block (1,2,3,4,5,6), trt_m is the effect of mth treatment (EX1 and EX3), $time_K$ is the effect of kth time point (Pre-trial, Post-trial and Follow-up), $time_k * trt_m$ is the interaction effect between mth treatment and kth time point, $claw\ (cow)_{ojkm}$ is the random intercept of the claw nested in oth cow ,jth block, kth time point and mth treatment ~ N(0, σ^2), $time_{iojkm}$ is the random slope of ith nested in oth cow, jth block, kth time point and mth treatment ~ N(0, σ^2) and e_{iojkm} is the random error ~ N(0, σ^2).

Claw lesions were considered as a binary variable and were analyzed using the following mixed effect logistic model:

$$Y_{iojkm} = block_j + time_K + trt_m + time_k * trt_m + claw\ (cow)_{ojkm} + time_{iojkm} + e_{iojkm}$$

Where $Y_{iojkm}$is the claw lesion analysis outcome variable with the i^{th} random slope of time, o^{th} random intercept of claw nested in cow, j^{th} block, k^{th} time point and m^{th} treatment $block_j$ is the effect of j^{th} block (1,2,3,4,5,6), trt_m is the effect of m^{th} treatment (EX1 and EX3), $time_K$ is the effect of Kth of time points (Pre-trial, Post-trial and Follow-up), $time_k * \ trt_m$ is the interaction effect between m^{th} treatment and k^{th} time point, $claw \ (cow)_{ojkm}$ is the random intercept of the claw nested in o^{th} cow ,j^{th} block, k^{th} time point and m^{th} treatment ~ N(0, σ^2), $time_{iojkm}$ is the random slope of i^{th} nested in o^{th} cow, j^{th} block, k^{th} time point and m^{th} treatment ~ N(0, σ^2) and e_{iojkm} is the random error.

Thermography data from both dorsal and plantar view were analyzed using the following linear mixed effect statistical model:

$$Y_{jkmnpoi} = \mu + block_j + \ time_K + \ trt_m + \ time_k * \ trt_m + \ AmbT_n + \ eyetemp_p \\ + \ limb \ (cow)_{ojkm} + \ time_{iojkm} + \ e_{iojkm}$$

Where $Y_{iojkm}$is the hoof thermography analysis outcome variable with the i^{th} random slope of time, o^{th} random intercept of limb nested in cow, j^{th} block, k^{th} time point and m^{th} treatment μ is the overall mean, $block_j$ is the effect of j^{th} block (1,2,3,4,5,6), trt_m is the effect of m^{th} treatment (EX1 and EX3), $time_K$ is the effect of k^{th} time point (Pre-trial, Post-trial and Follow-up), $time_k * \ trt_m$ is the interaction effect between m^{th} treatment and k^{th} time point $AmbT_n$ and $eyetemp_p$ are co-variables used for the effect of ambient temperature , and maximum eye temperature, $limb \ (cow)_{ojkm}$ is the random intercept of the limb nested in o^{th} cow ,j^{th} block, k^{th} of time points and m^{th} treatment ~ N(0, σ^2), $time_{iojkm}$ is the random slope of i^{th} nested in o^{th} cow, j^{th} block, k^{th} time point and m^{th} treatment ~ N(0, σ^2) and e_{iojkm} is the random error.

Residual analysis was conducted on all models to assess the assumptions of homoscedasticity, independence, and normal distribution of within-group errors, as well as to verify the normal distribution and independence of random effects. This graphical analysis followed the procedures outlined by (Pinheiro and Bates, 2000). Serial correlation structures were also examined to account for potential dependencies among observations over time. The correlation structures evaluated included general, autoregressive of order 1, and compound symmetry. The model selection process utilized the Akaike Information Criteria (AIC) to identify the best-fitting model for the data. Due to the complexity of the models, some models for some variables did not converge, therefore, to fix this issue, we deleted the random slope of time for that specific model and then tested the models for the serial correlation structure (**Supplementary Table 5.2-1**). To assess the statistical significance of fixed effects in the models, the ANOVA test was employed, with a significance level of $\alpha < 0.05$. Estimated effects were evaluated using marginal means, and Bonferroni P-value adjustment was applied to account for multiple comparisons of means.

CHAPTER 4 - RESULTS

4.1. Gait Analysis

4.1.1. Visual Gait Scoring

The overall NRS and 6 gait attributes did not significantly change from Pre-trial to Post-trial and Follow-up data collection points and between treatment groups ($P > 0.05$). There was also no significant effect of time*treatment for the overall gait score or the attributes ($P > 0.05$). Our results indicated that cows started the trial with low overall gait scores (2.16 ± 0.16 and 2.02 ± 0.16 for EX1 and EX3, respectively) and showed a numerical reduction of 0.2-0.3 in Post-trial (i.e., 1.84 ± 0.16 and 1.75 ± 0.16 in EX1 and EX3, respectively). This was consistent eight weeks after the provision of outdoor access, with cows displaying a similar numerical reduction to 1.91 ± 0.16 and 1.75 ± 0.16 for EX1 and EX3, respectively, at Follow-up (**Figure 4.1-1**). The same results were observed in 3 gait attributes (i.e., track-up, joint flexion and reluctance to bear weight). No cows with NRS > 3 were enrolled in the study, and no cows got an overall gait score of 3.5 or more during the study. Only 3 cows in Pre-trial (2 from EX1 and 1 from EX3) and 1 cow from EX3 in Post-trial were moderately lame (NRS = 3) across the study.

4.1.2. 3D Motion Gait Analysis

Our results regarding the 3D motion analysis revealed significant changes in stride time and stance time over time ($P < 0.05$). The average stride time of all cows exhibited an increase from 1.09 ± 0.01s in Pre-trial to 1.13 ± 0.01s in Post-trial and returned to the initial value of 1.09 ± 0.01s at Follow-up ($P = 0.007$). The average stance time demonstrated the same pattern with an increase of 0.03s from Pre-trial to Post-trial, followed by a reduction in Follow-up ($P = 0.003$, **Supplementary Table 5.2-5**). There was no significant effect of treatment or time in treatment for any of the mentioned variables. There was no effect of treatment, time, or time * treatment for stride length, velocity, track-up values in the X and XY axis and hock angle values throughout the study (**Supplementary Table 5.2-5**).

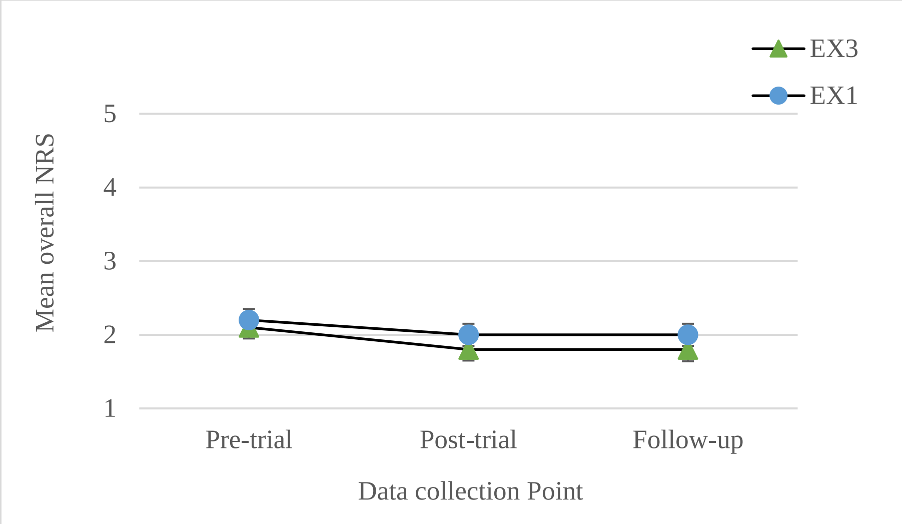


Figure 4.1-1 The average overall gait score (NRS) of both treatment groups at the three data collection points did not significantly change from Pre-trial to Post-trial and Follow-up data collection points and between treatment groups ($P > 0.05$).

4.1.3. Kinetic Assessment of Gait

Our results regarding the distribution of pressure revealed that there was no effect of treatment, time, or time * treatment on the mean distribution of pressure between the two treatment groups (EX1 and EX3) and from Pre-trial to Post-trial and Follow-up. However, the screenshots (SC) of the contact area claws made on the pressure plates showed a reduction from Post-trial to Follow-up (26.8 ± 0.62 cm^2 and 24.8 ± 0.67 cm^2, respectively, $P = 0.0015$). The contact area measured by the 30 seconds recording (30Srec) showed non-significant increase from Pre-trial to Post-trial (28.5 ± 0.69 cm^2 and 30.1 ± 0.66 cm^2, respectively, $P = 0.07$) and reduced to 27.5 ± 0.68 cm^2 ($P = 0.001$) in Follow-up, with no significant different between the mean contact area in Pre-trial and Follow-up.

The mean maximum and average pressure applied by claws in both SC and 30Srec showed no significant effect of time (Pre-trial, Post-trial and Follow-up) or treatment (EX1 and EX3, $P > 0.05$). However, the average pressure in SC and maximum pressure in 30Srec showed significant effects of time*treatment. The average pressure in SC (AVPressure_SC) of EX3 increased from Pre-trial to Post-trial and Follow-up (36.0 ± 3.02, 46.7 ± 2.62 and 51.8 ± 3.67 N/cm^2, respectively, P = 0.014). The same results were observed in the maximum pressure of 30Srec (MPressure_30Srec) of cows in EX3 treatment, with the pressure loaded by claws increasing from 62.7 ± 6.17 N/cm^2 in Pre-trial to 81.6 ± 5.56 N/cm^2 and 91.2 ± 6.21 N/cm^2 in Post-trial and Follow-up, respectively (P = 0.04). Although there was no effect of time * treatment for other pressure variables (i.e., MPressure_SC and AVPressure_30Srec), they increased numerically from Pre-trial to Post-trial and Follow-up.

The analysis of force data from the pressure plate revealed no significant effect of treatment (EX1 and EX3), time (Pre-trial, Post-trial and Follow-up) or time * treatment on the mean maximum and average force applied during 30Srec.

4.2. Hoof Health Assessment

4.2.1. Clinical Hoof Health Assessment

A total of 41 claw lesions were recorded at the two data collection points (i.e., Pre-trial = 22 sole hemorrhage and Follow-up = 17 sole hemorrhages and 2 white line hemorrhages). Thirty-nine of the lesions were sole hemorrhages that occurred at zone 4 (**Figure 3.4-1**), there were 30 lesions with a severity of 1 (76.9%), 8 lesions with a severity of 2 (20.5%) and only one sole hemorrhage had a severity of 3 (2.6%). Only two white line hemorrhages were observed at the Follow-up, with the severity of 1 and 2 at zone 3. The prevalence of lesions was similar for EX1 (5.56% and 6.62%) and EX3 (9.72% and 6.94%) in Pre-trial and Follow-up (P = 0.47). There was no effect of time, treatment, or time * treatment on the severity of the lesions (P > 0.05).

4.2.2. Subclinical Hoof Health Assessment

The results of the analyzed data regarding coronary band thermography revealed that the mean maximum temperature of lateral (LCB) and medial (MCB) claw reduced from Pre-trial (LCB = 31.7 ± 0.29 °C and MCB = 31.4 ± 0.35 °C) to Post-trial (LCB = 30.0 ± 0.27 °C and MCB = 29.9 ± 0.28 °C) (P < 0.05), although the maximum temperature in lateral coronary band (LCB) was lower in Follow-up compared to Pre-trial (30.1 ± 0.29 °C, P = 0.001). This effect was not seen in the maximum temperature of the medial claw (i.e., MCB, 30.4 ± 0.25 °C, P = 0.16).

Similar results were observed in the mean average temperature of the hoof coronary band (CB) and lateral and medial claws (LCB and MCB). The average temperature of the coronary band of all cows showed a reduction of 1.5 to 2 °C from Pre-trial (29.1 ± 0.26 °C, 30.3 ± 0.28 °C and 30.0 ± 0.34 °C, in CB, LCB and MCB, respectively) to 27.6 ± 0.25 °C, 28.7 ± 0.26 °C and 28.3 ± 0.27 °C in Post-trial and 27.7 ± 0.24 °C, 28.4 ± 0.25 °C and 28.4 ± 0.25 °C in Follow-up for CB, LCB and MCB, respectively ($P < 0.05$). However, no effect of time was observed for the maximum temperature of the CB ($P > 0.05$).

Our results regarding sole temperature demonstrated increasing in the mean maximum temperature of Z10 and Z0 from Pre-trial (26.0 ± 0.36 °C and 28.1 ± 0.41°C, respectively) to Follow-up (29.0 ± 0.36 °C and 30.8 ± 0.41 °C, $P < 0.05$). Although the maximum temperature of these two zones increased in the Post-trial (28.2 ± 0.35 °C and 29.3 ± 0.40 °C, in Z10 and Z0, respectively), this increase was only significant in the Z10 ($P < 0.0001$). Similar results were observed in the mean average temperatures of Z10 and Z0. They increased from Pre-trial (22.4 ± 0.33 °C and 23.8 ± 0.35 °C) to Post-trial (25.2 ± 0.32 °C and 24.9 ± 0.34 °C, $P < 0.05$) and Follow-up (25.6 ± 0.33 °C and 25.2 ± 0.35 °C), with no significant difference between Post-trial and Follow-up. Opposite to what has been observed in the skinny part of the sole (i.e., Z10 and Z0), a reduction of 1.3 °C to 2.2 °C was observed in the maximum and average temperature of the LZ4 and MZ4 from Pre-trial (maximum temperature = 23.8 ± 0.30 °C and 23.7 ± 0.32 °C and average temperature = 22.1 ± 0.26 °C and 22.8 ± 0.28 °C for LZ4 and MZ4, respectively) to Follow-up ($P < 0.05$, **Supplementary Table 5.2-9**). The maximum and average temperature of the LZ4 and MZ4 showed a non-significant increase from Pre-trial to Post-trial ($P > 0.05$) and a significant reduction from Post-trial to Follow-up ($P < 0.05$, **Supplementary Table 5.2-9**)

No significant effect of treatment or time * treatment was observed for either maximum and average temperatures of the coronary band and sole views in all ROIs.

4.2.3. Measuring Claw Conformation

Our findings regarding the sole measurements revealed an increase in the sole width, sole length, and claw length from Pre-trial (5.23 ± 0.04 cm, 8.92 ± 0.08 cm, and 8.03 ± 0.06 cm) to Post-trial (5.47 ± 0.04 cm, 9.46 ± 0.08 cm, and 8.43 ± 0.06 cm, $P < 0.05$). Interestingly, sole dimensions showed reductions in both sole width (5.29 ± 0.04 cm, $P = 0.004$) and sole length

(9.35 ± 0.10 cm, P = 1.0). However, claw length showed a significant increase from Post-trial to Follow-up (8.90 ± 0.06 cm, P < .0001). There was no effect of treatment or time * treatment for the aforementioned variables. Similarly, claw angle measurements showed no significant effect of treatment, time, or time * treatment during the study.

CHAPTER 5 – Discussion

5.1. Gait analysis

5.1.1. Visual Gait Scoring

The limited provision of outdoor access (either 1h or 3h per week) did not lead to a reduction in the overall NRS, nor did it improve any of the six gait attributes of cows in both treatment groups (EX1 and EX3) during the study. This finding was in accordance with our hypothesis that the level of outdoor access provided may not be sufficient to see improvements through clinical assessment. These results contrast with the findings of Nejati (2021), who reported a 1 to 1.2-score reduction in overall NRS and three gait attributes (i.e., track-up, asymmetric steps and reluctance to bear weight) in non-lame cows housed in tie-stalls using a 5-point scoring system. While both studies were similar in terms of space allowance, experiment duration, and daily outdoor access duration, the key difference lies in the frequency of outdoor access: in Nejati (2021), cows had more frequent outdoor exposure, being given access to the exercise yard five days per week over a five-week period. Our results also differed from those of Shepley and Vasseur (2021a), who found an improvement in joint flexion for non-lame tie-stalled cows when housed in a deep-bedded loose pen during 8 weeks of their dry-off period . The findings from these studies suggest that granting cows increased movement opportunities can lead to clinical improvements in their gaits. The influence of exercise access and enhanced movement opportunities on cows' gaits could be tied to the duration and frequency of outdoor access (Shepley and Vasseur, 2021b). Moreover, their initial gait and lameness status and baseline fitness could also play a pivotal role in determining the outcome of exercise provision. In the context of lameness prevalence influenced by outdoor access, Corazzin et al. (2010) found that summer grazing led to a reduction in lameness prevalence amongst cows housed in tie-stalls. Furthermore, Palacio et al. (2023) noted that even though cows' access to the outdoors might be limited to several hours per week during the winter, they exhibited lower levels of lameness compared to cows without any outdoor access. These findings align with past research which found that cows in different housing systems show lower levels of lameness when provided with outdoor access (Regula et al., 2004, Bielfeldt et al., 2005, Hernandez-Mendo et al., 2007, Popescu et al., 2013)

The primary objective of our study was to investigate the effects of less frequent outdoor access on cows without any signs of clinical lameness. We aimed to determine whether limited movement opportunities might influence the gait of tie-stall cows. Our results indicated a minor and non-significant reduction of 0.2-0.3 in the overall gait score and some specific gait attributes, namely track-up, joint flexion, and reluctance to bear weight. These findings align with our initial assumptions: we did not expect to observe significant changes through our visual gait scoring since this method might not possess the sensitivity required to detect them. To identify more subtle and subclinical changes in gait, we employed technologies like 3D motion analysis.

5.1.2. 3D Motion Gait Analysis

The 3D motion analysis of cow gait revealed a temporary positive effect of limited outdoor access on both stance and stride time. Cows spent more time on both stance and stride time at Post-trial compared to Pre-trial, regardless of their treatment group. However, all returned to their initial values during the Follow-up data collection period.

Telezhenko (2007) introduced the term "locomotion comfort," which means the physical and mental satisfaction cows would feel when walking on specific surfaces, potentially leading to enhanced natural gait behaviours. An increase in the stance time – the supporting phase, during which the cow's hoof is in contact with the ground without moving – might be a sign of an enhancement in the locomotion comfort of the cows (Alsaaod et al., 2017). For lame cows, this stance time is generally shorter in the affected limb than in a sound limb. This is likely due to the cow's attempt to minimize discomfort by reducing the time her affected limb spends bearing weight, in contrast to the longer duration she allows her sound limbs to be grounded. (Kang et al., 2020). Liu et al. (2011) also found that cows with clinical lameness (score 4 and 5 in a 5-point scoring system) had shorter stride times compared to cows with a locomotion score of 3 or less. We suggest that the observed increase in stance time across both our treatment groups might stem from the improved confidence and/or locomotion comfort that the cows attained after the 5-week experiment. Even the modest provision of outdoor access – frequencies as brief as 1 to 3 hours per week – appeared to have positive effects when releasing cows from their individual stalls. This finding holds implications for dairies where cows are housed in movement-restricted environments. For these establishments with limited resources to facilitate extended outdoor access for cows, even minimal efforts to augment movement opportunities can enhance the animals' locomotion comfort.

Research exploring gait kinematics across different flooring types has shown that cows walking on more comfortable surfaces, such as rubber floors, demonstrate reduced stride times and increased walking speeds compared to harder surfaces like concrete or mastic asphalt (Flower et al., 2007; Franco-Gendron et al., 2016). Additionally, the presence of painful lesions, such as sole ulcers, and increased locomotion scores are associated with longer stride times and slower walking speeds (Flower et al., 2007; Maertens et al., 2011). Notably, in these cited studies, alterations in stride time were consistently linked to changes in walking speed. In contrast, our study revealed that although stride time increased, there was no variation in walking speed or stride length across all data collection points. Typically, in non-lame cows, the stride time is composed of approximately two-thirds stance time and one-third swing time (Alsaaod et al., 2017), or the period when the hoof is raised and moving forward between lifting off and landing again. Similarly, our findings underscore that the increase in stride time was predominantly driven by an extended stance time. Furthermore, while an extended stride time is generally seen as an indication of a deteriorating gait (Flower et al., 2005; Flower et al., 2007; Franco-Gendron et al., 2016), in our context, this increase was chiefly due to adjustments in the stance time, not due to changes in the swing time. This observation strengthens our prior interpretation regarding extended stance time as a result of locomotion comfort and confidence.

Both stance and stride times exhibited a similar reduction to their respective Pre-trial values at the Follow-up data collection point. This decline could be attributed to the cows being confined again without access to the outdoors and having limited opportunities to move, leading to a potential loss of their walking confidence. Contrary to these results, when cows were granted a higher frequency of outdoor access (5 days/week), the positive effect of the exercise yard (i.e., 1 score reduction in overall gait score) persisted for 8 weeks after the provision of outdoor access had ceased (Nejati, 2021). These discrepancies may imply that the provision of outdoor access in our study was not sufficient enough to provide long-term and clinical effects on gait, which confirms the absence of improvement in the clinical assessment of gait (i.e., visual locomotion scoring). Additionally, more measurements on cow fitness and muscle recovery and other kinematic variables related to cows' postures, like back and head position, would provide more information on kinematic evolution while cows are provided with increased movement opportunities.

5.1.3. Kinetic Assessment of Gait

In our study the kinetic assessment was performed on the rear legs of the cows, and revealed that the contact area between the hoof and pressure plate increased at the Post-trial, then fell at the Follow-up, in accordance with our clinical assessment of claw conformation in which the sole width and sole length showed the same pattern of increase followed by a decrease in Post-trial and Follow-up (section 4.2.3).

The provision of outdoor access, and the associated ability of the cows to walk on natural surfaces (sandy alley, soil exercise yard) while released from their stalls 1-3h per week, led to an increase in the contact area. This potentially indicates that greater movement opportunities result in changes to the weight-bearing surface of claws, due to physical alterations in the growth and wear rate of claw horn tissue (van der Tol et al., 2004; Telezhenko et al., 2008; Ouweltjes et al., 2009). When cows walk on more abrasive flooring, such as slatted concrete as opposed to rubber flooring, they displayed higher contact areas (Ouweltjes et al., 2009), which may be attributed to the more leveled sole, consequently enhancing grip with the floor. According to van der Tol et al. (2004), cows walking on natural surfaces may develop more protruding walls to bear most of the cow's weight. Telezhenko et al. (2008) suggested that the increase in contact area on hard flooring might be due to disruption of wear in the horn tissue and expansion of contact area on the sole, which is considered undesirable because soles are thinner and softer compared to walls and should not bear the cow's weight. It is important to note that our study did not investigate changes in contact area in different claw zones, therefore which area(s) this increase happened is unknown. Notably, after eight weeks of re-confinement (i.e., Follow-up point, once the treatment application phase was completed), the claws' contact areas reduced again, possibly due to an increase in the hoof growth rate in comparison to its wear rate, leading to longer claws with disruptions in the weight-bearing surface.

The kinetic assessment of gait also revealed a notable increase in the pressure applied by claws in the EX3 group during the Post-trial and Follow-up points, compared to the Pre-trial.

This rise in applied pressure could originate from two reasons. Firstly, it might be a result of observed changes in the weight-bearing surface of claws, causing alterations in pressure distribution within the claws and leading to a concentration of pressure on a specific point of the sole which is in corroboration with the increase in the contact area. Secondly, the increase in loaded pressure by claws could be linked to the potential improved locomotion comfort experienced by the cows, corroborating our results on gait and kinematic analysis. Alsaaod et al. (2017) suggested that cows tend to apply more pressure when walking on pasture during the toe-on phase in comparison to other artificial floorings. While increased pressure and force applied by claws during walking are generally considered unfavourable due to the potential risk of lesion development (Medina-González et al., 2022), it is essential to note that our study measured the applied pressure while cows were standing. Therefore, it is plausible that the observed increase in pressure is a result of cows becoming physically fitter and possessing stronger muscles after having more movement opportunities, particularly in the EX3 cows. As a complementary effect, they might be loading more pressure on their claws as they experience less pain and discomfort. For instance, Liu et al. (2011) found that cows with a locomotion score of 1 loaded more maximum and average force on the ground when compared to cows with a score of 3 or higher in a 5-point scaling system. Future research investigating changes in the contact area and pressure applied based on claw anatomical zones while cows are standing and walking would allow us to better understand the dynamic of outdoor access on the kinetics of cow gaits.

5.2. Hoof Health Assessment

5.2.1. Clinical Hoof Health Assessment

Claw lesion prevalence and severity showed no changes throughout the study, which is in accordance with our hypothesis, specifically as we chose to enrol cows with no obvious signs of lameness or ulcerative lesions at the start of the trial, aiming to study the impact of our treatments on sound tie-stall cows with no experience of stall release as part of their routine. As such, our findings corroborate those of Nejati (2021) who found no change in the prevalence or severity of claw lesions after 5 weeks of higher frequencies of outdoor access (1 h per day for 5 days per week) for cows with the same characteristics.

One of the main concerns brought up by producers when discussing the provision of outdoor access is the potential for arising health issues amongst their cows (Smid et al., 2021).

The cow-level prevalence of claw lesions in Canadian tie-stall dairy farms was 25.7% with a 7.1% prevalence of hemorrhages (Cramer et al., 2008). Our results corroborate those of Nejati (2021), who observed a prevalence of sole hemorrhages under 10%. Both white line and sole hemorrhages are generally considered low in severity compared to other types of claw lesions and are under- or unreported by hoof trimmers (Solano et al., 2016). Popescu et al. (2013), found more than 10% lower prevalence of non-infectious lesions in tie-stalled cows who had access to the outdoors compared to ones without any access. As reviewed by Shepley and Vasseur (2021b), lower risks of non-infectious hoof lesions are usually associated with the provision of outdoor access. The discrepancy with our results might be due to a combination of low lesion prevalence amongst enrolled cows at the start of the study and the effect of providing low frequencies of outdoor access being too small to be detected clinically. Therefore, we used IRT cameras to investigate more subclinical effects on the hoof health of cows.

5.2.2. Subclinical Hoof Health Assessment

Infrared thermography stands as a relatively novel technology in veterinary medicine that has found application in detecting alterations in skin surface temperature. This is particularly significant as certain subclinical indicators like inflammation can be identified earlier than visible clinical symptoms (Alsaaod et al., 2015a). The increase in the coronary band temperature can be associated with inflammation due to the early stages of laminitis and incidences of claw lesion (Nikkhah et al., 2005; Bobić et al., 2017; Arican et al., 2018). Our results depicted a reduction in coronary band temperature of the overall hoof and both medial and lateral claws for both treatment cows. In contrast to our findings, Nejati (2021) found no effect of outdoor access on the coronary band temperature. This discrepancy with our findings might be due to some differences in the methodology of these studies. In our study, IRT images were taken in a room with controlled temperature to minimize the effect of ambient temperature on thermograms (Alsaaod et al., 2014; Landgraf et al., 2014), while in Nejati (2021) the thermograms were taken in the barn where the ambient temperature was not controlled. We also added each cow's eye maximum temperature as an indicator of the core body temperature of cows in our model as a co-variable to adjust for the individual variations between cows.

The coronary band is a well-vascularized part of the hoof and its temperature can be affected by the circulation and the metabolic activity of the underlying tissue (Alsaaod et al., 2015a). Although there is not enough evidence supporting the idea that some claw horn disruption lesions can be signs of subclinical laminitis (Randall et al., 2018), it is commonly acceptable that claw lesions such as sole and white line hemorrhages are the manifestation of subclinical laminitis (Greenough and Vermunt, 1991; Stone, 2004); Randall et al. (2018). Inflammatory agents such as histamine, interleukin-6, lipopolysaccharides, etc., have been shown to have an important role in subclinical laminitis, causing alterations in hoof blood circulation (Zhang et al., 2020). Furthermore, the increase in the locomotion scores of cows has been previously associated with an increase in the coronary band temperature (Rodríguez et al., 2016; Cramer et al., 2023). The reduction in coronary band temperature in our study might indicate that the provision of outdoor access as low as 1h/week could result in a reduction of inflammatory agents in blood circulation and subclinical laminitis and/or the pain associated with that. Further research on the serum concentration of these biomarkers before and after the provision of outdoor access and their relationship with coronary band temperature might be needed to corroborate our IRT results and better understand how outdoor access would affect subclinical laminitis.

The sole temperature in zone 4 (typical area for sole ulcers) exhibited a reduction at the Follow-up data collection point for both groups of treatment cows. Oikonomou et al. (2014) found that increasing the digital cushion thickness would reduce the temperature of the sole in zone 4. In addition, Oikonomou et al. (2014) and Rodríguez et al. (2016) found that an increase in the locomotion score of cows causes an increase in the temperature of the sole IRT. Increases in the volume and surface area of the digital cushion after the provision of exercise and more movement opportunities have been reported by Gard et al. (2015). Although the thickness of the digital cushion has not been investigated in our study, it can explain our results regarding zone 4 temperature which may suggest that the provision of outdoor access might positively affect digital cushion development in cows, therefore inducing more comfortable walking and standing, as well as reducing the risk of lameness (Griffiths et al., 2020). However, another explanation would be that a reduction in zone 4 temperature might be due to an increase in the sole thickness. Although the sole thickness was not measured in our study, the increase in toe length and reduction of sole width might suggest the increase in the sole thickness of claws in the Follow-up data collection point.

In contrast to the coronary band and zone 4, IRT images showed an increase in the maximum and average temperature of zone 10 (the skin above claws) and zone 0 (the skin between claws) for both treatment groups. Häggman and Juga (2015) and Moreira et al. (2019) reported that the provision of outdoor access can be related to an increased risk of infectious claw diseases such as digital dermatitis and interdigital dermatitis which occurs in zone 10 and 0, respectively. No infectious disease was detected during our study at the different clinical hoof health assessments; we are hypothesising that when cows walk on the soil, grass and natural substances in the exercise yard, minor injuries might happen on the skin of the sole. On the other hand, Bielfeldt et al. (2005) suggested that exercise could have positive effects on claws by increasing the blood circulation in the area, therefore more nutrients and oxygen would transfer to live tissues. Both hypotheses could explain our observed increase in sole temperature in zones 0 and 10. Overall, our results on the hoof and sole thermography showed that increasing movement opportunity could have promising effects on the hoof health of cows, however, more research is needed to better understand the effect of outdoor access on blood circulation and anatomy of the hoof.

5.2.3. Measuring Claw Conformation

Our findings concerning claw length revealed an increase throughout the study. The increase in the claw length can be attributed to the normal growth of the claw horn tissue (Shearer and van Amstel, 2001). These results corroborate the findings of Somers et al. (2005), who found a positive effect of time on the claw length of cows regardless of their walking surface.

Sole measurements exhibited increases in sole width in Post-trial. These results may indicate that increasing walking opportunities resulted in more wear on the plantar surface of soles (**Figure 5.2-1**). This also further corroborates our results for kinetic assessment and 3D motion analysis: an increase in stance time, hoof contact area and, pressure after the provision of outdoor access. The resulting increase in sole contact with the ground could have led to an increase in the wear rate of the sole width. However, after 8 weeks of re-confinement, a decrease in the sole width was observed at the Follow-up data collection point. This reduction in sole width may be explained by the lower opportunity of movement for cows housed in tie-stalls, leading to a reduction in their claws' contact with the ground. As a result, we hypothesise that this reduction in sole width could be the outcome of an increase in the growth rate of walls (**Figure 5.2-1**). It is important to note that this reduction in sole width was relatively small, less than 2 mm, and because this measurement was taken after sliver trimming, it could also be influenced by the trimming process. Similar to claw length, the increase in the sole length at Post-trial can also be the due to the normal growth of the claw horn tissue (Shearer and van Amstel, 2001).

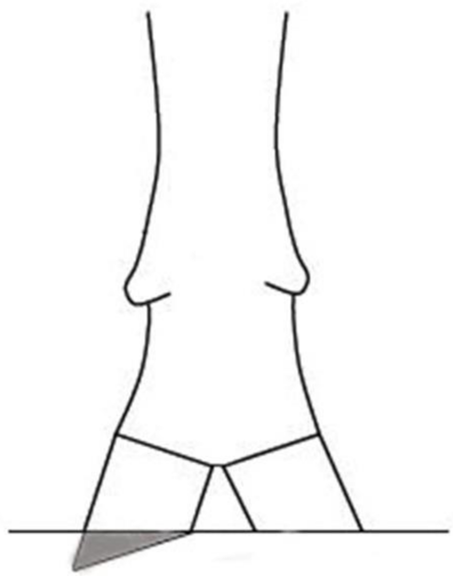


Figure 5.2-1 This figure is adapted from National Animal Disease Information Service (no date). The horizontal line shows the cut-off point to balance the sole's weight bearing surface at trimming and the gray spot represents how weight-bearing surface will change as a result of the growth of the wall.

The growth and wear rate of horn tissue at different anatomical parts of claws were not measured in our study. Although Somers et al. (2005) found that claw dimensions increase over time regardless of the flooring type, they did not find any impact of flooring type on the growth rate, wear rate, or shape of the claws. No difference in claw conformation was observed between our two treatment groups. Similar to our findings, Loberg et al. (2004) found no difference in the net growth of claws for cows with 1 and 2 days per week of outdoor access. However, they found the diagonal (i.e., the distance from the tip of the toe to the proximal end of the heel) net growth was smaller in cows with access to the outdoors (1, 2, and 7 days per week) compared to those without outdoor access with a lower growth rate of claw length for cows with 7 d/week of outdoor access compared to cows with no access to the outdoors. More studies on growth and wear rate and more measures of claw parameters, such as diagonal length or heel height, can provide better insight into how outdoor access and confinement would affect claw horn tissue.

CHAPTER 6 Conclusion

Consistent with our hypothesis, the lower frequency of outdoor access provided to confined tie-stall cows for this study (1-3 h/week) showed that no discernible effect was noted during the clinical assessments, including visual gait scoring and clinical hoof health assessment. However, the use of technologies (i.e., 3D motion gait analysis, pressure platforms and hoof thermography) revealed slight changes in the gait and hoof health of cows that were not visible in the clinical assessments. The subclinical assessment (i.e., technology-based techniques) showed that low frequency of outdoor access might have some positive effects on the gait and hoof health of cows. Cows confined at their stall have limited opportunity to move and walk, however, our results showed that 1-3h/week of outdoor access might improve cows' confidence while walking and increase their locomotor comfort, leading to cows having higher stance times and applying more pressure to their claws while standing. The reduction in the coronary band and sole temperature could be interoperated as a positive sign of a reduction in pain and discomfort from subclinical conditions like laminitis. This reduction might be the results of increase in the sole and digital cushion thickness; however, they were not directly measured in this study and more research in future to confirm this might be needed. The results of this study showed walking opportunities as low as 1h/week for confined cows can increase the blood circulation in hooves and increase claw horn tissue abrasion. Although positive effects were observed in lower frequencies of outdoor access, they were subclinical and some were only short term and smaller than what have been observed when cows were provided with higher frequencies of outdoor access (e.g., 5d/week in Nejati (2021) study). One limitation of our study was the necessity to adapt technologies developed for human research (such as 3D motion analysis and pressure platforms) for use in bovines – more research is needed to continue tailoring those technologies to be used to their full potential in bovine research. To strengthen the validity of certain findings—particularly those relating to kinetic assessment, hoof thermography, and claw conformation—future studies could consider incorporating additional time points both before and after the implementation of outdoor treatment access. This extended timeframe would provide deeper insights into the implications of increased movement opportunities and outdoor access frequencies on claw health and gait.

CHAPTER 7 SUPPLEMENTARY MATERIAL

Supplementary Table 5.2-1 Convergence of models for all the variables is shown here, the number 1 indicates the successful convergence and the number zero indicates that the model failed to converge. The astride (*) showed which model was chosen based on the Akaike Information Criteria (AIC) as the best-fitting model. (NA indicating that the model was not tested for the correlation structure)

	Statistical model			
Variable[1]	No correlation structure	General correlation structure	Autoregressive of order 1	Compound symmetry
NRS	1	0*	0	1
Swinging out	1*	1	1	1
Back arch	1	0	0*	1
Track-up	1	0	1*	1
Joint Flexion	1*	0	1	1
Asymmetric step	1*	0	1	1
Reluctance to bear weight	0*	0	1	0

Stride length	1	0*	1	1
Stride time	1	0*	1	1
Velocity	0	1	0*	1
Stance time	1*	1	1	1
Track-up X	1	0*	1	1
Track-up XY	1*	1	1	1
Hock angle (Min)	0	0	0*	0
Hock angle (Max)	1	1	1*	1
Hock angle range of motion (ROM)	0	0	0*	0
Hock angle (Med)	1	0	0*	0
Hock angle (Avr)	1	0	0*	1
Pressure Distribution	1*	1	1	1
MPressure_SC	1	0	1*	1
AVPressure_SC	1	0	1*	1
MPressure_30Srec	1*	0	0	1
MForce_30Srec	1	1	1*	1

AVForce_30Srec	1*	1	1	1
Contact_area_SC	1	1*	1	1
Contact_area_30Srec	1*	1	1	1
CB_Minimum Temperature	1	1	0*	1
CB_Maximum minus Minimum temperature	1	0	0*	0
CB_Maximum temperature	0	0*	0	0
CB_Average temperature	0	0*	0	0
CB_Standard deviation of temperature	1	1	0*	1
LCB_Minimum Temperature	0	1*	0	1
LCB_Maximum Temperature	0	0*	0	1
LCB_Maximum minus Minimum temperature	1	1	1*	1
LCB_Average temperature	0	0*	0	0
LCB_Standard deviation of temperature	1	1	1*	1

MCB_Minimum temperature	0	1	0	1*
MCB_Maximum temperature	0	0	0	1*
MCB_Maximum minus minimum temperature	1*	1	1	1
MCB_Average temperature	0	0	0	1*
MCB_Standard deviation of temperature	1	0	1	0*
Z10_Maximum temperature	1	1	1*	1
Z10_Minimum temperature	0*	1	0	1
Z10_Average temperature	1*	1	1	1
Z0_Maximum temp	1	1	1*	1
Z0_Minimum temp	1*	0	0	1
Z0_Average temp	1*	1	1	1
MZ4_Maximum temp	0*	1	0	1
MZ4_Minimum temp	0*	0	0	0
MZ4_Average temperature	1	0	1	0*

LZ4_Maximum temp	1*	1	1	1
LZ4_Minimum temp	0*	0	0	0
LZ4_Average temperature	0*	0	1	0
Sole width	0*	0	1	0
Sole length	1*	1	1	1
Claw length	1	0	0*	1
Toe angle	1*	0	1	0
Lesion score	1*	NA	NA	NA
Lesion prevalence	1*	NA	NA	NA

[1]Abbreviation:

Min = minimum, Max = Maximum, Med= Median Avr = average, SC= Screenshot, 30Srec= 30 seconds recording, MPressure = maximum pressure, AVPressure = Average pressure, MForce= Maximum force, AVForce = average force, CB = coronary band temperature, LCB= lateral claw coronary band temperature, MCB= medial claw coronary band temperature, Z10 = zone 10 temperature, Z0 = zone 0 temperature, MZ4 = zone 4 of medial claw temperature

Supplementary Table 5.2-2 Average ± standard errors of overall gait score (NRS) and gait attributes followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up).

	Treatment Group		Time			P-value		
Variable	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
NRS	1.97 ± 0.10	1.84 ± 0.10	2.09 ± 0.11	1.79 ± 0.11	1.83 ± 0.11	0.55	0.26	0.97
Swing out	1.17 ± 0.15	1.29 ± 0.15	1.37 ± 0.14	1.07 ± 1.14	1.24 ± 0.14	0.42	0.80	0.29
Back arch	0.69 ± 0.15	0.59 ± 0.15	0.63 ± 0.15	0.54 ± 0.15	0.75 ± 0.15	0.58	0.45	0.45
Tracking up	0.80 ± 0.18	0.78 ± 0.18	0.94 ± 0.15	0.73 ± 0.15	0.70 ± 0.15	0.93	0.20	0.11
Joint flexion	1.51 ± 0.13	1.41 ± 0.13	1.55 ± 0.11	1.53 ± 0.13	1.30 ± 0.18	0.07	0.27	0.19
Asymmetric step	0.93 ± 0.12	0.78 ± 0.12	0.88 ± 0.14	0.81 ± 0.14	0.86 ± 0.14	0.93	0.91	0.83

Reluctance to bear weight	0.73 ± 0.13	0.64 ± 0.13	0.82 ± 0.15	0.62 ± 0.15	0.61 ± 0.16	0.78	0.95	0.77

Supplementary Table 5.2-3 Average ± standard errors of overall gait (NRS) and gait attributes scores based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect.

Variable	Treatment	Pre-trial	Post-trial	Follow-up	P-value
NRS	EX1	2.16 ± 0.16	1.84 ± 0.16	1.91 ± 0.16	0.97
	EX3	2.02 ± 0.16	1.75 ± 0.16	1.75 ± 0.16	
Swing out	EX1	1.25 ± 0.20	1.16 ± 0.20	1.09 ± 0.21	0.29
	EX3	1.48 ± 0.20	0.98 ± 0.20	1.39 ± 0.20	
Back arch	EX1	0.55 ± 0.21	0.63 ± 0.21	0.89 ± 0.22	0.45
	EX3	0.71 ± 0.21	0.44 ± 0.21	0.62 ± 0.21	
Tracking up	EX1	0.96 ± 0.22	0.62± 0.21	0.83 ± 0.22	0.11
	EX3	0.93 ± 0.22	0.84 ± 0.21	0.57 ± 0.21	

Joint flexion	EX1	1.76 ± 0.16	1.43 ± 0.18	1.33 ± 0.25	0.19
	EX3	1.35 ± 0.16	1.62 ± 0.18	1.27 ± 0.25	
Asymmetric step	EX1	0.89 ± 0.19	0.89 ± 0.19	0.99 ± 0.21	0.83
	EX3	0.87 ± 0.19	0.73 ± 0.19	0.73 ± 0.20	
Reluctance to bear weight	EX1	0.78 ± 0.22	0.69 ± 0.22	0.72 ± 0.23	0.77
	EX3	0.87 ± 0.22	0.55 ± 0.22	0.50 ± 0.22	

Supplementary Table 5.2-4 Average ± standard errors of gait variables obtained through 3D motion followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up). Different letters in superscript indicate the significance.

	Treatment Group		**Time**			**P-value**		
Variable[1]	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
Stride length (cm)	162 ± 1.15	160 ± 1.07	160 ± 0.94	162 ± 0.96	161 ± 0.95	0.31	0.31	0.54
Stride time (s)	1.10 ± 0.01	1.11 ± 0.01	1.09 ± 0.01 [a]	1.13 ± 0.01 [b]	1.09 ± 0.01 [a]	0.22	0.007	0.37

Velocity (cm/s)	149 ± 1.52	145 ± 1.47	148 ± 1.47	145 ± 1.53	148 ± 1.63	0.032	0.07	0.36
Stance time (s)	0.71 ± 0.01	0.72 ± 0.01	0.70 ± 0.01 [a]	0.73 ± 0.01 [b]	0.70 ± 0.01 [a]	0.11	0.003	0.17
Track-up X (cm)	-0.47 ± 1.39	0.76 ± 1.30	-0.65 ± 1.07	0.46 ± 1.08	0.62 ± 1.08	0.55	0.54	0.02
Track-up XY (cm)	9.85 ± 0.59	9.43 ± 0.55	10.02 ± 0.57	9.50 ± 0.58	9.39 ± 0.62	0.48	0.13	0.08
Hock angle (Min)	127 ± 0.87	129 ± 0.81	128 ± 0.74	127 ± 0.76	129 ± 0.76	0.58	0.45	0.85
Hock angle (Max)	164 ± 0.86	164 ± 0.81	164 ± 0.71	162 ± 0.73	165 ± 0.81	0.44	0.13	0.48
Hock angle range of motion (ROM)	36.1 ± 0.61	34.9 ± 0.56	36.0 ± 0.63	34.9 ± 0.64	35.7 ± 0.64	0.82	0.79	0.30
Hock angle (Med)	147 ± 0.89	147 ± 0.83	147 ± 0.73	146 ± 0.75	147 ± 0.74	0.90	0.19	0.69
Hock angle (Avr)	149 ± 0.81	149 ± 0.75	149 ± 0.74	148 ± 0.76	149 ± 0.75	0.59	0.29	0.16

[1]Abbreviation:

Min = minimum, Max = Maximum, Med = Median Avr = average

Supplementary Table 5.2-5 Average ± standard errors of variables obtained from 3D motion analysis of gait based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect.

Variable[1]	Treatment	Pre-trial	Post-trial	Follow-up	P-value

Stride length (cm)	EX1	161 ± 1.37	164 ± 1.40	162 ± 1.42	0.54
	EX3	159 ± 1.30	160 ± 1.30	160 ± 1.30	
Stride time (s)	EX1	1.07 ± 0.02	1.14 ± 0.02	1.09 ± 0.02	0.37
	EX3	1.10 ± 0.02	1.12 ± 0.02	1.09 ± 0.02	
Velocity (cm/s)	EX1	152 ± 2.29	145 ± 2.38	150 ± 2.41	0.36
	EX3	145 ± 2.17	144 ± 2.17	147 ± 2.17	
Stance time (cm)	EX1	0.69 ± 0.01	0.74 ± 0.01	0.70 ± 0.01	0.17
	EX3	0.71 ± 0.01	0.73 ± 0.01	0.71 ± 0.01	
Track-up X (cm)	EX1	-1.29 ± 1.56	0.70 ± 1.58	-0.82 ± 1.58	0.02
	EX3	0.01 ± 1.47	0.23 ± 1.47	2.05 ± 1.47	
Track-up XY (cm)	EX1	10.02 ± 0.83	10.61 ± 0.83	8.92 ± 0.92	0.08

	EX3	10.03 ± 0.79	8.39 ± 0.78	9.87 ± 0.83	
Hock angle (Min)	EX1	128 ± 1.08	126 ± 1.12	128 ± 1.12	0.48
	EX3	128 ± 1.02	128 ± 1.02	130 ± 1.02	
Hock angle (Max)	EX1	163 ± 1.04	162 ± 1.07	165 ± 1.19	0.48
	EX3	162 ± 0.98	162 ± 0.98	164 ± 1.09	
Hock angle range of motion (ROM)	EX1	35.8 ± 0.91	35.9 ± 0.95	36.6 ± 0.96	0.30
	EX3	36.1 ± 0.86	33.9 ± 0.86	34.6 ± 0.86	
Hock angle (Med)	EX1	147 ± 1.07	145 ± 1.10	147 ± 1.10	0.69
	EX3	147 ± 1.01	146 ± 1.01	147 ± 1.01	
Hock angle (Avr)	EX1	149 ± 1.07	148 ± 1.11	150 ± 1.12	0.16
	EX3	150 ± 1.02	148 ± 1.02	148 ± 1.02	

[1]Abbreviation:

Min = minimum, Max = Maximum, Med = Median Avr = average

Supplementary Table 5.2-6 Average ± standard errors of variables obtained from pressure plate while cows stood on them for 30 seconds, followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up). Different letters in superscript indicate the significance.

	Treatment Group		Time			P-value		
Variable[1]	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
Pressure Distribution	27.2 ± 1.16	28.1 ± 1.15	27.3 ± 1.13	28.8 ±1.11	26.8 ±1.21	0.70	0.07	0.11
MPressure_SC	256 ± 15.8	256 ± 15.5	234 ± 16.0	256 ± 13.8	271 ± 21.0	0.16	0.93	0.11
AVPressure_SC	41.5 ± 2.08	44.8 ± 2.05	38.3 ± 2.12	45.7 ± 1.84	45.4 ± 2.61	0.284	0.342	0.014
MPressure_30Srec	76.5 ± 4.31	78.5 ± 4.24	69.6 ± 4.36	79.2 ± 3.89	83.7 ± 4.45	0.113	0.995	0.042
MForce_30Srec	64.4 ± 3.73	68.0 ± 3.67	58.8 ± 3.69	67.9 ± 3.10	71.8 ± 4.35	0.32	0.88	0.12
AVForce_30Srec	2502 ± 144	2495 ± 141	2280 ± 180	2695 ± 131	2519 ± 147	0.16	0.33	0.083
Contact_area_SC	2054 ± 115	2167 ± 112	1873 ± 129	2301 ± 120	2157 ± 123	0.29	0.38	0.094

Contact_area_30Srec	25.8 ± 0.75	25.9 ± 0.75	26.0 ± 0.68 [ab]	26.8 ± 0.62 [a]	24.8 ± 0.67 [b]	0.78	0.017	0.80

[1]Abbreviation:

SC = Screenshot, 30Srec= 30 seconds recording, MPressure = maximum pressure, AVPressure = Average pressure, MForce= Maximum force, AVForce = average force

Supplementary Table 5.2- Average ± standard errors of variables obtained from pressure plate while cows stood on them for 30 seconds based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect. Different letters in superscript indicate the significance.

Variable[1]	Treatment	Pre-trial	Post-trial	Follow-up	P-value
Pressure distribution	EX1	27.8 ± 1.61	29.2 ± 1.58	24.6 ± 1.78	0.11
	EX3	26.9 ± 1.61	28.3 ± 1.58	29.0 ± 1.69	
MPressure_SC	EX1	257 ± 22.8	254 ± 19.7	244 ± 30.3	0.11
	EX3	211 ± 22.7	258 ± 19.7	298 ± 29.5	
AVPressure_SC	EX1	40.6 ± 3.02	44.8 ± 2.63	39.0 ± 3.77	0.014
	EX3	36.0 ± 3.02[a]	46.7 ± 2.62[b]	51.8 ± 3.67[b]	
MPressur_30Srec	EX1	76.6 ± 6.25	76.8 ± 5.57	76.2 ± 6.51	0.042

	EX3	62.7 ± 6.17[a]	81.6 ± 5.56[b]	91.2 ± 6.21[b]	
AVPressure_30Srec	EX1	62.4 ± 5.29	65.3 ± 4.44	65.5 ± 6.33	0.12
	EX3	55.1 ± 5.22	70.6 ± 4.42	78.2 ± 6.05	
MForce_30Srec	EX1	2537 ± 258	2694 ± 188	2274 ± 216	0.12
	EX3	2023 ± 255	2697 ± 187	2764 ± 205	
AVForce_30Srec	EX1	2011 ± 185	2230 ± 171	1920 ± 180	0.083
	EX3	1735 ± 183	2372 ± 170	2394 ± 171	
Contact area_SC	EX1	25.8 ± 0.98	27.0 ± 0.89	24.7 ± 0.97	0.094
	EX3	26.2 ± 0.98	26.6 ± 0.88	24.9 ± 0.93	
Contact area_30Srec	EX1	28.9 ± 0.98	30.3 ± 0.94	27.8 ± 0.99	0.80
	EX3	28.0 ± 0.98	29.8 ± 0.94	27.2 ± 0.95	

[1]Abbreviation:

SC = Screenshot, 30Srec= 30 seconds recording, MPressure = maximum pressure, AVPressure = Average pressure, MForce= Maximum force, AVForce = average force

Supplementary Table 5.2-7 Average ± standard errors of variables obtained from Infrared thermography from the dorsal view of the hoof (coronary band) followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up). Different letters in superscript indicate the significance.

	Treatment Group		Time			P-value		
Variable[1]	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
CB_Minimum Temperature	21.1 ± 0.20	21.7 ± 0.20	22.1 ± 0.28 [a]	21.1 ± 0.27 [b]	21.0 ± 0.26 [b]	0.72	0.028	0.69
CB_Maximum temperature	31.6 ± 0.23	32.1 ± 0.23	32.5 ± 0.31	31.4 ± 0.29	31.8 ± 0.29	0.40	0.30	0.50
CB_Maximum minus Minimum temperature	10.6 ± 0.24	10.5 ± 0.24	10.4 ± 0.31	10.4 ± 0.29	10.9 ± 0.28	0.86	0.77	0.64
CB_Average temperature	27.9 ± 0.17	28.3 ± 0.17	29.1 ± 0.26 [a]	27.6 ± 0.25 [b]	27.7 ± 0.24 [b]	0.68	0.005	0.45
CB_Standard deviation of temperature	1.88 ± 0.05	1.88 ± 0.05	1.89 ± 0.05	1.78 ± 0.05	1.91 ± 0.05	0.85	0.75	0.61
LCB_Minimum Temperature	26.1 ± 0.19	26.4 ± 0.19	27.4 ± 0.32 [a]	26.1 ± 0.27 [b]	25.4 ± 0.25 [b]	0.78	0.001	0.41
LCB_Maximum Temperature	30.4 ± 0.22	30.8 ± 0.22	31.7 ± 0.29 [a]	30.0 ± 0.27 [b]	30.1 ± 0.26 [b]	0.81	0.001	0.26
LCB_Maximum minus Minimum temperature	4.35 ± 0.14	4.47 ± 0.15	4.42 ± 0.22 [ab]	3.84 ± 0.16 [a]	4.97 ± 0.19 [b]	0.35	0.002	0.25
LCB_Average temperature	29.0 ± 0.20	29.2 ± 0.20	30.3 ± 0.28 [a]	28.7 ± 0.26 [b]	28.4 ± 0.25 [b]	0.81	0.0002	0.29
LCB_Standard deviation of temperature	0.88 ± 0.03	0.87 ± 0.04	0.88 ± 0.05 [ab]	0.78 ± 0.03 [a]	0.97 ± 0.04 [b]	0.18	0.0008	0.08

MCB_Minimum temperature	25.7 ± 0.21	26.3 ± 0.22	27.1 ± 0.33 ^{a}	25.5 ± 0.29 ^{b}	25.4 ± 0.30 ^{b}	0.30	0.03	0.95
MCB_Maximum temperature	30.3 ± 0.21	30.8 ± 0.22	31.4 ± 0.35 ^{a}	29.9 ± 0.28 ^{b}	30.4 ± 0.25 ^{ab}	0.9	0.02	0.23
MCB_Maximum minus minimum temperature	4.59 ± 0.15	4.53 ± 0.15	4.28 ± 0.19	4.38 ± 0.18	5.03 ± 0.18	0.098	0.99	0.006
MCB_Average temperature	28.7 ± 0.21	29.1 ± 0.22	30.0 ± 0.34 ^{a}	28.3 ± 0.27 ^{b}	28.4 ± 0.25 ^{b}	0.92	0.003	0.57
MCB_Standard deviation of temperature	0.94 ± 0.03	0.88 ± 0.35	0.88 ± 0.04	0.91 ± 0.04	0.94 ± 0.39	0.04	0.49	0.02

[1]Abbreviation:

CB = coronary band temperature, LCB= lateral claw coronary band temperature, MCB= medial claw coronary band temperature

Supplementary Table 5.2-8 Average ± standard errors of variables obtained from Infrared thermography from the dorsal view of the hoof (coronary band) based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect. Different letters in superscript indicate the significance.

Variable[1]	Treatment	Pre-trial	Post-trial	Follow-up	P-value
CB_Minimum Temperature	EX1	22.0 ± 0.39	20.7 ± 0.39	20.6 ± 0.37	0.69
	EX3	22.2 ± 0.40	21.4 ± 0.37	21.4 ± 0.36	
CB_Maximum temperature	EX1	32.2 ± 0.43	31.4 ± 0.42	31.2 ± 0.40	0.50
	EX3	32.7 ± 0.44	31.6 ± 0.40	32.1 ± 0.40	

CB_Maximum minus Minimum temperature	EX1	10.4 ± 0.43	10.6 ± 0.42	10.8 ± 0.40	0.64
	EX3	10.5 ± 0.44	10.1 ± 0.40	11.0 ± 0.40	
CB_Average temperature	EX1	28.9 ± 0.36	27.6 ± 0.35	27.1 ± 0.34	0.45
	EX3	29.1 ± 0.37	27.8 ± 0.34	28.1 ± 0.33	
CB_Standard deviation of temperature	EX1	1.89 ± 0.08	1.84 ± 0.07	1.90 ± 0.06	0.61
	EX3	1.87 ± 0.08	1.74 ± 0.07	1.92 ± 0.06	
LCB_Minimum temperature	EX1	27.3 ± 0.43	26.2 ± 0.49	25.0 ± 0.36	0.41
	EX3	27.5 ± 0.45	25.9 ± 0.37	25.8 ± 0.35	
LCB_Maximum Temperature	EX1	31.8 ± 0.40	29.9 ± 0.39	29.6 ± 0.38	0.26
	EX3	31.6 ± 0.42	30.0 ± 0.38	30.6 ± 0.37	
LCB_Maximum minus Minimum temperature	EX1	4.62 ± 0.30	3.59 ± 0.24	4.84 ± 0.28	0.25
	EX3	4.22 ± 0.31	4.08 ± 0.22	5.11 ± 0.27	
LCB_Average temperature	EX1	30.4 ± 0.38	28.7 ± 0.37	28.0 ± 0.36	0.29
	EX3	30.2 ± 0.39	28.6 ± 0.36	28.7 ± 0.35	
LCB_Standard deviation of temperature	EX1	0.94 ± 0.07	0.73 ± 0.05	0.97 ± 0.06	0.08
	EX3	0.82 ± 0.07	0.83 ± 0.05	0.97 ± 0.06	
MCB_Minimum temperature	EX1	26.7 ± 0.46	25.2 ± 0.42	25.1 ± 0.44	0.95
	EX3	27.4 ± 0.47	25.9 ± 0.39	25.6 ± 0.43	
MCB_Maximum temperature	EX1	31.4 ± 0.49	29.8 ± 0.40	29.7 ± 0.37	0.23
	EX3	31.3 ± 0.50	30.0 ± 0.38	31.1 ± 0.35	
MCB_Maximum minus minimum temperature	EX1	4.59 ± 0.26	4.59 ± 0.27	4.60 ± 0.26	0.006
	EX3	3.96 ± 0.27[a]	4.17 ± 0.25[a]	5.45 ± 0.25[b]	
MCB_Average temperature	EX1	30.0 ± 0.47	28.2 ± 0.39	27.9 ± 0.36	0.57
	EX3	30.1 ± 0.49	28.5 ± 0.37	28.8 ± 0.35	

MCB_Standard deviation of temperature	EX1	0.97 ± 0.06	0.97 ± 0.06	0.89 ± 0.06	0.02
	EX3	0.80 ± 0.06 [a]	0.85 ± 0.06[ab]	0.99 ± 0.88 [b]	

[1]Abbreviation:

CB = coronary band temperature, LCB= lateral claw coronary band temperature, MCB= medial claw coronary band temperature

Supplementary Table 5.2-9 Average ± standard errors of variables obtained from Infrared thermography form the plantar view of the hoof (sole) followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up). Different letters in superscript indicate the significance.

	Treatment Group		Time			P-value		
Variable[1]	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
Z10_Maximum temperature	27.8 ± 0.36	27.6 ± 0.36	26.0 ± 0.36 [a]	28.2 ± 0.35 [b]	29.0 ± 0.36 [b]	0.60	6.95e-10	0.12
Z10_Minimum temperature	19.3 ± 0.30	19.6 ± 0.29	17.7 ± 0.32 [a]	20.3 ± 0.31 [b]	20.4 ± 0.32 [b]	0.60	3.23e-08	0.12
Z10_Average temperature	24.4 ± 0.33	24.4 ± 0.32	22.4 ± 0.33 [a]	25.2 ± 0.32 [b]	25.6 ± 0.33 [b]	0.74	5.74e-10	0.13
Z0_Maximum temp	29.3 ± 0.43	29.4± 0.43	28.1 ±0.41 [a]	29.3 ± 0.4 [a]	30.8 ± 0.41 [b]	0.58	7.69e-05	0.13

Z0_Minimum temp	17.4 ± 0.28	17.3 ± 0.27	17.3 ± 0.37 ^{a}	18.8 ± 0.31 ^{b}	16.0 ± 0.33 ^{c}	0.89	8.83e-07	0.62
Z0_Average temp	24.6 ± 0.35	24.7 ± 0.35	23.8 ± 0.35	24.9 ± 0.34	25.2 ± 0.35	0.85	0.017	0.17
MZ4_Maximum temp	23.4 ± 0.30	23.5 ± 0.30	23.7 ± 0.32 ^{b}	24.3 ± 0.31 ^{b}	22.4 ± 0.32 ^{a}	0.88	3.42e06	0.091
MZ4_Minimum temp	18.7 ± 0.23	18.7 ± 0.23	18.8 ± 0.25 ^{a}	19.8 ± 0.24 ^{b}	17.6 ± 0.25 ^{c}	0.96	1.1e09	0.22
MZ4_Average temperature	21.4 ± 0.27	21.5 ± 0.26	21.8 ± .028 ^{a}	22.5 ± 0.27 ^{a}	20.0 ± 0.28 ^{b}	0.75	1.79e-10	0.12
LZ4_Maximum temp	23.4 ± 0.28	23.2 ± 0.28	23.8 ± 0.30 ^{a}	24.2 ± 0.29 ^{a}	22.0 ± 30 ^{b}	0.98	2.27e-06	0.50
LZ4_Minimum temp	19.0 ± 0.21	18.9 ± 0.21	19.3 ± 0.23 ^{a}	20.0 ± 0.22 ^{a}	17.6 ± 0.23 ^{c}	0.69	2.41e-12	0.19
LZ4_Average temperature	21.5 ± 0.24	21.4 ± 0.24	22.1 ± 0.26 ^{a}	22.4 ± 0.25 ^{a}	19.9 ± 0.26 ^{b}	0.94	3.62e-11	0.30

[1]Abbreviation:

Z10 = zone 10 temperature, Z0 = zone 0 temperature, MZ4 = zone 4 of medial claw temperature,

Supplementary Table 5.2-10 Average ± standard errors of variables obtained from Infrared thermography from the plantar view of the hoof (sole) based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect.

Variable[1]	Treatment	Pre-trial	Post-trial	Follow-up	P-value

Z10_Maximum temperature	EX1	25.8 ± 0.48	28.8 ± 0.48	28.9 ± 0.48	0.12
	EX3	26.2 ± 0.50	27.6 ± 0.48	27.6 ± 0.48	
Z10_Minimum temperature	EX1	17.5 ± 0.42	20.6 ± 0.42	19.9 ± 0.45	0.12
	EX3	17.8 ± 0.44	20.1 ± 0.42	21.0 ± 0.44	
Z10_Average temperature	EX1	22.3 ± 0.44	25.5 ± 0.44	25.2 ± 0.47	0.13
	EX3	22.5 ± 0.47	24.8 ± 0.44	25.9 ± 0.46	
Z0_Maximum temp	EX1	27.9 ± 0.56	29.8 ± 0.55	30.4 ±0.58	0.13
	EX3	28.3 ± 0.58	28.8 ± 0.55	31.2 ±0.57	
Z0_Minimum temp	EX1	17.2 ± 0.49	19.1 ± 0.46	15.9 ± 0.47	0.62
	EX3	17.3 ± 0.51	18.5 ± 0.43	16.1 ± 0.45	
Z0_Average temp	EX1	23.7 ± 0.47	25.2 ± 0.50	24.8 ± 0.50	0.17
	EX3	23.8 ± 0.50	24.6 ± 0.47	25.6 ± 0.50	
MZ4_Maximum temp	EX1	23.7 ± 0.43	24.7 ± 0.43	22.0 ± 0.45	0.091
	EX3	23.8 ± 0.45	23.9 ± 0.43	22.9 ± 0.44	
MZ4_Minimum temp	EX1	18.9 ± 0.33	20.0 ± 0.33	17.3 ± 0.35	0.22
	EX3	18.8 ± 0.35	19.5 ± 0.33	17.9 ± 0.34	
MZ4_Average temperature	EX1	21.8 ± 0.37	22.8 ± 0.37	19.6 ± 0.39	0.12
	EX3	21.9 ± 0.39	22.2 ± 0.37	20.4 ± 0.39	
LZ4_Maximum temp	EX1	23.8 ± 0.40	24.5 ± 0.40	22.0 ± 0.42	0.50
	EX3	23.8 ± 0.42	23.9 ± 0.40	22.1 ± 0.42	
LZ4_Minimum temp	EX1	19.3 ± 0.31	20.3 ± 0.31	17.4 ± 0.33	0.19
	EX3	19.2 ± 0.32	19.7 ± 0.31	17.8 ± 0.32	
LZ4_Average temperature	EX1	22.1 ± 0.35	22.7 ± 0.35	19.7 ± 0.37	0.30
	EX3	22.1 ± 0.37	22.1 ± 0.35	20.0 ± 0.36	

[1]Abbreviation:

Z10 = zone 10 temperature, Z0 = zone 0 temperature, MZ4 = zone 4 of medial claw temperature,

Supplementary Table 5.2-11 Average ± standard errors of claw and hoof dimensions followed by the statistical significance of treatments (EX1 and EX3) and time points (i.e., Pre-trial, Post-trial and Follow-up). Different letters in superscript indicate the significance.

	Treatment Group		Time			P-value		
Variable	**EX1**	**EX3**	**Pre-trial**	**Post-trial**	**Follow-up**	**Treatment**	**Time**	**Treatment × Time**
Sole width	5.35 ± 0.04	5.31 ± 0.04	5.23 ± 0.04 [a]	5.47 ± 0.04 [b]	5.29 ± 0.04 [a]	0.163	0.0003	0.23
Sole length	9.16 ± 0.08	9.33 ± 0.08	8.92 ± 0.08 [a]	9.46 ± 0.08 [b]	9.35 ± 0.10 [b]	0.68	0.006	0.78
Claw length	8.56 ± 0.07	8.35 ± 0.70	8.03 ± 0.06 [a]	8.43 ± 0.06 [b]	8.90 ± 0.06 [c]	0.028	3.33e-15	0.19
Toe angle	42.6 ± 0.56	42.2 ± 0.54	42.8 ± 0.61	42.3 ± 0.47	42.0 ± 0.36	0.45	0.19	0.47

Supplementary Table 5.2-12 Average ± standard errors of claw and hoof dimensions based on the interaction between the two treatment groups (i.e., EX1 and EX3) and each data collection point (i.e., Pre-trial, Post-trial and Follow-up). The P-value shows the interaction effect.

Variable	**Treatment**	**Pre-trial**	**Post-trial**	**Follow-up**	**P-value**

Sole width	EX1	5.20 ± 0.06	5.50 ± 0.60	5.36 ± 0.07	0.23
	EX3	5.26 ± 0.60	5.44 ± 0.62	5.23 ± 0.06	
Sole length	EX1	8.88 ± 0.12	9.37 ± 0.11	9.23 ± 0.14	0.78
	EX3	8.95 ± 0.12	9.55 ± 0.11	9.48 ± 0.13	
Claw length	EX1	8.17 ± 0.09	8.56 ± 0.09	8.94 ± 0.09	0.19
	EX3	7.89 ± 0.09	8.31 ± 0.08	8.86 ± 0.08	
Toe angle	EX1	43.3 ± 0.87	42.7 ± 0.69	41.8 ± 0.54	0.47
	EX3	42.3 ± 0.88	42.0 ± 0.65	42.2 ± 0.51	

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