Analysis of claw health, performance and behavioural parameters of Simmental cows on commercial dairy farms for implementation of a lameness prediction model

By Isabella Lorenzini

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von Isabella Lorenzini

aus Bolzano (Italien)

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To my family

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List of abbreviations

AC	activity
ACR	activity during daytime
AFF	position "at feeding fence"
AFF	at feeding fence
ALSS	automatic locomotion scoring system
AMS	automatic milking system
AN	activator number
AUC	area under the curve
В	farm
BCS	body condition score
BS	Brier Score
BTN	animal identification with farm
C_FDM	feeding duration per feeding visit measured by the pedometers
C_MN	number of feeding visits measured by the pedometers
C_MNR	number of feeding visits during daytime measured by the pedometers
CAI	calving interval
CDF1	commercial dairy farm 1
CDF2	commercial dairy farm 2
CDF3	commercial dairy farm 3
CDF4	commercial dairy farm 4
CI	confidence interval
DD	dermatitis digitalis
DE	digital dermatitis
DIM	days in milk
DLMS	daily locomotion scoring
DO	direct observation
DS	double sole
ENBM	elastic net best model
ENET	elastic net
ENFM	elastic net full model
F	position "feeding"
F	feeding
F_LMS	type of locomotion score
FD	feeding duration measured at weighing troughs
FDM	feeding duration per meal measured by weighing troughs
FDO	feeding duration observed
FDP	feeding duration recorded by pedometers
FDR	feeding duration during daytime measured by pedometers
FDRW	feeding duration during daytime measured by weighing troughs
FDV	feeding duration per feeding visit measured by weighing troughs
FDW	feeding duration measured by weighing troughs
FI	feed intake
FIM	feed intake per visit

FIM	feed intake per meal
FIV	feed intake per visit
FLMS	fortnightly locomotion scoring
FP	feeding pace
FV	feeding visit
FVO	feeding visits observed
FVP	feeding visits recorded by pedometers
GLMM	generalised linear mixed model
GLMMRE	generalised linear mixed model with random effects
HF	horn fissure
HHE	heel horn erosion
HMS	herd management software
ICC	intraclass correlation coefficient
IFT	infrared technology
IH	interdigital hyperplasia
ILT	Institute for Agricultural Engineering and Animal Husbandry
IM	intake minutes
IP	interdigital phlegmon
IQ	interquartile range
K_LMS	locomotion score correction reason
LB	lying bout
LBN	number of lying bouts
LBNR	number of lying bouts during daytime
LBO	observed lying bouts
LBP	lying bouts recorded by pedometers
LD	lying duration
LDB	lying duration per bout
LDO	observed lying duration
LDP	lying duration recorded by pedometers
LDR	lying duration during daytime
	Bavarian State Research Centre for Agriculture
LKV	"Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern
	e.V." (state board of producer associations' trustees for animal breeding
LMS	locomotion score
LMS	locomotion scoring system
LMSSGL	locomotion scoring system according to Grimm & Lorenzini
LW	live weight
MAE	mean absolute error
MCIII	Third metacarpal bone
MCIV	fourth metacarpal bone
MI	milking interval
MLSS	manual locomotion scoring system
MN	number of meals measured by the weighing troughs
MNR	number of meals during daytime measured by the weighing troughs

MTIII	third metatarsal bone
MTIV	fourth metatarsal bone
MY	milk yield
MY305	milk yield for lactation
MYM	monthly milk yield average
NEL	net energy lactation
NFF	near feeding fence
OR	odds ratio
Р	parity
PA	percentage of agreement
РТ	pain test
RFID	radio-frequency identification
ROC	receiver operating characteristics
SD	standard deviation
SEN	sensitivity
SH	sole haemorrhage
SMOTE	synthetic minority oversampling technique
SPE	specificity
SU	sole ulcer
TMR	total mixed ration
TN	animal identification
VM	visit minutes
VN	number of visits measured by the weighing troughs
VNR	number of visits during daytime measured by the weighing troughs
VO	video observation
W	Kendall's coefficient of concordance
WLD	white line disease

XVIII

List of publications

Parts of this dissertation were published in the following studies:

Publications with practical relevance:

- Lorenzini I., Schindhelm K., Haidn B., Weingut F., "Lahmen Kühen auf der Spur", *Bayerisches Landwirtschaftliches Wochenblatt*, Deutscher Landwirtschaftsverlag, 22 Sep., pp. 48-49, 2017.
- Lorenzini I, "Lahme Kühe früh erkennen", *Bayerisches Landwirtschaftliches Wochenblatt*, Deutscher Landwirtschaftsverlag, 21 Sep., pp. 27-28, 2018.
- Lorenzini I, "Lahmheitserkennung: wie früh ist früh genug?", *Allgäuer Bauernblatt*, Agrar Verlag Allgäu, 29 Nov., pp. 24-27, 2018.
- Lorenzini I., Grimm K., "Die Kuh ist lahm Was bedeutet das eigentlich? Lösungsansätze der Landesanstalt der Landwirtschaft", In Proc. Info-Tag Klauengesundheit, Bayerische Landesanstalt für Landwirtschaft, 2019.

Publications with scientific relevance:

- Lorenzini I., Schindhelm K., Haidn B., Weingut F., Koßmann A., Reiter K., Misha E., "Validation and Comparison of Two Different Pedometers that Could be Used for Automatic Lameness Detection in Dairy Cows", *Chemical Engineering Transactions*, The Italian Association of Chemical Engineering, vol. 58, 2017, pp. 187-192.
- Lorenzini I., Schindhelm K., Haidn B., Misha E., "Using a three point lameness scoring system combined with a clinical examination to increase the reliability of visual locomotion scoring", In Proc. 19th International Symposium and 11th Conference on Lameness in Ruminants, Munich, Germany, 2017, pp. 240-242.
- Lorenzini I., Schindhelm K., Haidn B., Misha E., "Development of a prediction model automatic detection for lameness in dairy cows", In Proc. 21. Arbeitswissenschaftliches Kolloquium, HBLFA Francisco Josephinum, Wieselburg, Austria, 2018, pp. 196-208.
- Lorenzini I., Grimm K., Haidn B., "Advancements in the analysis of behavioural and performance data for early lameness detection in dairy cows", In Proc. Precision Livestock Farming Workshop Seminar, Wageningen University and Research, 2018, p. 8.
- Lorenzini I., Grimm K., Haidn B., Misha E., "Advancements in the analysis of behavioural and performance data for early lameness detection in dairy cows", In Proc. 20th International Symposium and 12th Conference on Lameness in Ruminants", 2019.
- Lorenzini I., Grimm K., Haidn B., Misha E., "Erhebung und Analyse von Aktivitätsund Leistungsdaten zur Früherkennung von Klauenerkrankungen bei Milchkühen in Praxisbetrieben", In Proc. "14. Tagung Bau, Technik und Umwelt in der landwirtschaftlichen Nutztierhaltung", Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), 2019 (not yet published).

1 Introduction

The term "lameness", derived from the Anglo-Saxon word *lam*, meaning weak [1], refers to, in animals, the manifestation of pain in one or more limbs during standing or locomotion [2]. The importance of production diseases such as lameness, defined by J.M. Payne as a "man-made problem" affecting the body as a consequence of the strain of modern intensive animal husbandry [3], has greatly increased over time. Lameness in dairy cows is one of the most common production diseases across Europe; according to a scientific report published by the European Food Safety Authority in 2009 [4], the incidence of lameness has increased in the last decades. Compared to the 20.6 % lameness prevalence in the United Kingdom found by Clarkson et al. (1996) [5] between 1989 and 1991 for example, more recent studies reveal the mean prevalence across farms to have increased to over 30 % [6] and even 36 % in Austria [7] in more recent years.

Pain being the cause for lameness [8], makes this disease an important welfare issue. Lameness prevents cows from exhibiting their natural behaviour and modifies social interactions in the herd [8–11]. Lameness is also an important economic issue; it affects dairy cows' productivity by reducing milk yield and longevity [12–17] and has to be treated resulting in work intensive care and treatment costs [12, 18].

Thus, recognizing lameness at an early stage is critical for both animal welfare and to reduce lameness-induced economic losses. Manual locomotion scoring, which is the standard method for estimating the level of lameness prevalence on a farm, is a time-consuming practice and is intrinsically subjective [19]. Also, studies show that farmers tend to underestimate the lameness prevalence in their herds [20]. For these reasons, the development of an automatic lameness detection system would be crucial in reducing pain and suffering caused by lameness in dairy cows and avoiding the high costs caused by lameness being recognised only when the underlying structural damage is already severe.

For this reason, in this study, behaviour and performance data from four commercial dairy farms and one research farm were collected and in combination with manually collected claw health data were used to test a predictive algorithm previously developed at the Institute for Agricultural Engineering and Animal Husbandry [21], which could be implemented in an automatic lameness detection system.

2 Literature review

2.1 Anatomy and biomechanics of the bovine claw

The commonly used term "claw" for bovines refers to all structures that are contained within the horn capsule [22]. The bone structures in the claw include the pedal bone, the distal section of the short pastern, and the distal sesamoides, which are connected by the pastern and coffin joint respectively (Articulatio interphalangeal proximalis and articulatio interphalangea distalis). The pastern joint is functionally supported by the ligaments around it [23, 24]. The tendons in the claw include the final tendons of the common digital extensor tendons, the medial and lateral digital extensors and the deep digital flexor, which inserts at the solar surface of the distal phalanx [23, 24]. The coffin joint capsule has a dorsal recess beneath the extensor tendons and a palmar and plantar recess beneath the deep digital flexor tendon for the fore and hind limbs respectively [23]. The podotrochlea is the functional unit that incorporates the distal sesamoid bone, the deep digital flexor, and the navicular bursa [25]. The skin of the bovine claw is divided into three layers; the subcutis, the corium and the keratinised epidermis. The subcutis contains the digital cushion, a layer of subcutaneous fat beneath the bulb of the claw which extends to the middle of the sole. On the wall, tip and part of the sole of the claw the corium is connected directly to the pedal bone with no subcutis. The papillary layer of the corium is densely innervated and vascularised and linked to the epidermis with villi, which enable both a stable mechanical connection of the two tissues and the nourishment of the horn capsule. The epidermis covers the corium on all sides and is divided into axial and abaxial claw wall horn, sole horn and bulb covering the digital cushion. The claw wall and the sole horn are connected by the white line [25].



Figure 1: Structures of the claw in a sagittal section through the phalanges (modified according to Shearer et al. (2005) and Mülling et al. (2014) [26, 27])

The structures in the claw support the transmission of forces from the skeletal system to the claw horn and subsequently to the ground. The pedal bone is suspended in the horn capsule through laminae that connect the corium to the abaxial and dorsal parts of the claw wall horn [25]. The subcutaneous fat cushion in the heel bulb of the claw is the first structure to absorb the impact of the foot placement during the cow's movement. Subsequently, the claws spread apart and the weight is borne by the wall and sole horn [28]. The tension and pressure that occur inside the horn capsule put a strain on the tissues and their microcirculation even in physiological conditions, making claws prone to lesions if the natural compression is exceeded [25].

2.2 Causes for lameness in dairy cows

Lameness in cattle is a clinical symptom with a multifactorial aetiology [29]. The main cause for lameness in dairy cows are lesions of the claw [30, 31], although cows can be lame for other less common reasons, such as neurologic disorders or traumatic injuries involving fractures of the proximal limbs.[32–34]. The factors that play a role in the development of claw diseases and lesions can be divided into intrinsic and extrinsic factors [28, 29].

2.2.1 Intrinsic factors influencing claw health

Intrinsic factors influencing claw health in dairy herds are fixed effects related to the animal that cannot be externally influenced, such as parity, milk yield (MY) and days in milk (DIM) [28].

2.2.1.1 Milk yield

The improvement of genetic selection and better management practices are factors that helped increase cows' productivity [35], at the same time making animals more prone to developing health problems [36, 37] such as claw diseases. The relationship between claw disease and milk yield for example, is complex [38]. According to some studies, animals with a higher milk yield are more likely to develop sole ulcers (SU) and white line disease (WLD) [39, 40], although Solano et al. (2015) [41] on the other hand, found the increase in daily milk production to be associated with a slight decrease in the odds of being lame.

2.2.1.2 Parity and stage of lactation

Increasing parity is associated with a higher risk of lameness [42–46], as is body condition; for instance the risk of lameness for high yielding cows increases with live weight loss in the first 50 DIM [47] or if cows have a low body condition score (BCS) [41, 48–50]. Some studies have shown the thickness of the digital cushion is negatively correlated to the occurrence of lameness [51]; the stage of lactation in fact influences the thickness of the digital cushion [49], which reduces around the time of calving and is also affected by metabolic disorders involving lipolysis that can arise around the time of calving [28]. In general, the high metabolic stress associated with the perinatal transit phase in particular the change of feeding ration, the negative energy balance and the reintroduction into the herd, are contributing factors to an increased risk of lameness [52, 53]. Other studies on the other hand, found no statistically significant relationship between lameness and DIM [44], or an influence of MY in combination with other factors, such as feeding. Grimm et al. (2019) [21] for example, found that cows were more likely to be lame if they had a high MY only if they also spent less time feeding. Additionally, the herd ranking of the cows and their social interactions also both have an influence on their behaviour, for example the time spent feeding or lying, and thus on the risk of developing lameness, which is increased in low ranking animals or in a socially competitive environment [54, 55]. Breed also influences claw health; Holstein-Friesian cows are more likely to be lame than cross-breed cows or cows of other breeds [56, 57].

2.2.1.3 Seasonality

Finally, seasonality also plays a role in the occurrence of lameness. Studies reporting on lameness prevalence in the summer and in the winter, showed lameness prevalence to be

lower in the summer months [5, 43, 58], even though Lawrence et al. (2011) [59] reported more than 40 % of lameness cases in pasture-based herds in New Zealand occurring in the summer months (October to December), although seasonal calving should be taken into account as an influencing factor in this study.

2.2.2 Extrinsic factors influencing claw health

Extrinsic factors that influence claw health in dairy herds are factors that can be externally influenced, such as type of housing, diet, herd management and claw trimming regime [28].

2.2.2.1 Housing

Cows' claws has evolved and adapted to locomotion on soft ground, for this reason housing cows in barns with concrete flooring negatively affects the structures of the claw [52, 60]. From an anatomical point of view, although the weight bearing is evenly distributed on both the inside and the outside claw, the slight asymmetry of the metacarpal and metatarsal bones is exasperated by the mechanical wear caused by hard flooring [28, 41], leading to an increased development of the outside claw on hind limbs. The difference between size and length of the outside and inside claws in cows kept in zero-grazing systems changes the way the claw is affected during locomotion and puts a strain on the underlying tissues by moving the load from the wall of the claw to the sole and heel horn [28]. Thus, management factors such as housing and flooring have a statistically significant effect on claw health [41, 53, 61].

2.2.2.2 Lying areas

Studies show that lying comfort is an important factor affecting cows' lying behaviour and claw health. Modern free stall barns allow cows more freedom of movement compared to tiestall barns, but findings regarding the effect of type of housing on lameness prevalence are nonetheless discordant. Some studies show a negative effect of tie-stall herds on claw health [62], whilst other found lameness prevalence to be lower in tie-stall herds with sand stall surfaces and only mildly higher for tie-stall herds with non-sand stall surfaces compared to free-stall herds with sand stall surfaces [58]. Free stalls with rubber mats and sparse quantity of bedding are connected to a higher risk of lameness [41, 45, 56, 61, 63], whilst deep bedding decreased the odds for lameness [61, 64, 65]. Compost barns are a form of housing which has a positive effect on claw health and on animal welfare [66, 67].

2.2.2.3 Flooring

The effect of the type of flooring on claw health has often been the focus of research, the presence of slippery floors for instance increases the risk of lameness, due to a higher risk of injury during movement and herding to and from milking [41, 44]. Concrete flooring increases the wear on the claw horn [60], leads to higher pressure on the claw's structures and to a less even load distribution between lateral and medial claw [68], thus increasing the risk

for lameness. Somers et al. (2003) [60] found a lower prevalence of digital dermatitis (DD) and heel horn erosion (HHE) in cows kept in barns with slatted flooring and a manure scraper, although other studies found automatic scrapers to be correlated with a higher number of claw disorders, possibly due to the higher risk of traumatic injury [56]. Studies indicate that pasture-based dairy herds have a lower lameness prevalence compared to housed cattle and that the odds for lameness to decrease when cows had access to pasture [64]. Sjöström et al. (2018) [69] found zero–grazing cattle to have significantly higher odds of becoming lame than cows that were allowed grazing periods, although results from the same study indicate that long grazing periods and wet weather could also influence claw health.

In addition to the type of housing, flooring and stalls, hygiene in herds plays an important role in maintaining claw health. Studies found a positive correlation between decreasing levels of cow cleanliness and increasing levels of lameness [44, 62, 70].

2.2.2.4 Feeding

Feeding also affects claw health, both regarding the content of the ration and the feeding management. A high rise in concentrate feed in the ration after calving is positively correlated with development of DD, as are by-products of the food industry, possibly because of the imbalanced protein intake [71, 72]. Feeding of high-energy concentrates interferes with ruminal flora and induces the release of endotoxins that can have an effect on the microcirculation in the claws and cause pain and stress for the animals [28, 73]. High roughage content in the feeding ration and adequate levels of carbohydrates positively influence claw health [74, 75]. Furthermore, low-roughage diets results in more liquid faecal matter which negatively affects the hygienic conditions in free stall housing and weakens the structure of the claw horn promoting HHE and the spread of DE in infected herds [28, 76–78].

2.2.2.5 Claw trimming

Finally, the frequency and timing of claw trimming also has a strong influence on claw health in dairy cows [6, 71, 79]. Overgrown claws, resulting from long intervals between herd trimmings, are associated with higher lameness prevalence levels [44, 79] and regular trimming reduces the number and duration of clinical cases of lameness [72], mitigates the effects of lameness on the cows' behaviour [80] and reduces economic losses [81].

2.3 Lameness prevalence

A scientific report by the European Food Safety Authority [4] listed a number of studies analysing the prevalence of lameness in Europe and in the rest of the world, and concluded, that there has been no improvement in the amount of lameness in dairy farms in the last decades. Table 1 summarises studies that relay the prevalence of lameness, i.e. the relative proportion of animals that are lame in the observed population at a given point in time. In
1983, Whitaker et al. (1983) [82] reported an average lameness case incidence of 25 %, in accordance with the 17.4 % incidence found by Esslemont and Kossaibati (1996) [83] ten years later. Studies by Wells et al. (1993) and Clarkson et al. (1996) [5, 43] reported 13.7 % and 18.6 % lameness prevalence in the summer and 16.7 % and 25 % in winter. A similar study by Cook et al. (2003) [58] found a lameness prevalence of 21.1 % in the summer and 26.9 % in the winter in dairy farms in Wisconsin. A more recent study by Griffiths et al. (2018) [6] found a mean within farm lameness prevalence of 31.6 % in UK, whilst Costa et al. (2018) found a mean lameness prevalence of 21.1 % on dairy farms in Brazil.

Study	No of herds/farms	Country	lameness in-max) (i	prevalence n %)	
			Summer	Winter	Overall
Wells et al., (1993) [43]	17 herds	USA (Minnesota and Wisconsin)	13.7	16.7	
Clarkson et al. (1996) [5]	37 farms	United Kingdom	18.6	25	
Manske et al., (2002) [79]	101 farms	Sweden			5.1 (0 - 33)
Cook, (2003) [58]	30 herds	USA (Wisconsin)	21.1	23.9	
Winckler and Brill (2004) [84]	17 herds	Germany			45 (25 - 58)
Rouha-Mülleder et al., (2010) [7]	80 herds	Austria			36 (0 - 77)
Espejo et al., (2006) [42]	50 farms	USA (Minnesota)			26.4 (3.3 - 57.3)
Barker et al. (2010) [56]	205 farms	United Kingdom			36.8 (0 - 79.2)
Fabian et al. (2014) [85]	59 herds	New Zealand			8.1 (1.2 - 36)
Griffiths et al., (2018) [6]	61 farms	United Kingdom			31.6 (5.8-65.4)
Sjöström et al. (2018) [69]	201 herds	Europe			18
Costa et al., (2018) [86]	50 farms	Brazil			21.2 (15.2-28.5)

 Table 1:
 List of studies describing lameness prevalence.

2.4 **Perception of lameness**

The structural changes taking place in the dairy industry all over the world have a high impact on the management of dairy farms and on animal welfare. In Germany, there has been a continuous reduction of the number of dairy farms since the introduction of new milk hygiene laws in the 1950s; from 1.5 million dairy farms in 1950 to just 396,920 farms in 1983 [87]. The decrease in the number of dairy farms was slowed by the introduction of milk quotas in 1984 [88, 89], but continues today. The number of dairy farms sank from November 2016 to May 2017 by a further 2.7 % to 67,319 farms [90]. A similar pattern can also be observed in the rest of Europe [88]. The decrease in the number of dairy farms is accompanied by a slight decrease in the number of cows, from over 5.5 million in 1950 to just above 4 million in 2018 [91]. But also, both the annual milk production and the average number of cows per farm in Germany has increased in the last decades [88, 91, 92] (see Figure 2). If in 1969 an average German dairy farm had seven cows, in 2018 this number has increased almost tenfold, with an average of 65 cows per farm.



Figure 2: Number of cows, farms, produced milk and average number of cows per farm in Germany from 1960 to 2018 [91–93].

The standard method for lameness detection in dairy cows is locomotion scoring, which is used to indicate the quality of locomotion by assigning scores as a function of different posture and movement traits on a scale [19]. The higher number of animals per farm combined with the subjectivity of locomotion scoring make individual animal observation and lameness detection problematic. Studies show different results on the effect of herd size on lameness; Chapinal et al. (2013) and Sjöström et al (2018) [64, 69] found that smaller farms have lower levels of lameness, while other studies found that cows in larger herds had lower odds of becoming lame [41, 45]. Another aspect of the individual animal observation is that farmers significantly underestimate lameness prevalence on their farms [43, 94]. Šárová et al. (2011) [20] found that farmers significantly underestimated the lameness prevalence (mean 6 $[\pm 6]$ %) compared to the trained observer (mean 31[\pm 15] %). Higginson Cutler et al. (2017)

[95] found farmer estimation to be 1.6 times lower in AMS barns, 1.8 times lower in freestall barns and 4.1 times lower in tiestall barns than that of trained researchers. Similarly, farmers in New Zealand only identified 27.3 % of cows with reduced mobility, regardless of herd size, in their pasture-based herds [85]. Another notable factor that plays a role in lameness detection is cows' ability to hide pain. Pain recognition in farm animals can be difficult, as the exhibition of pain or distress exposes them to possible predators. Pain in cattle is not always identifiable by visual observation; signs are often subtle and may consist in vocalization, teeth grinding, changes in facial expression, reluctance to move and decrease in production.

The lack of pain exhibition in dairy cattle should therefore not be interpreted as lack of pain [96] and although locomotion scoring is considered the standard method of reference for estimating claw health at herd-level, the stoic nature of cows can prevent the observer from detecting cows with claw lesions [97].

2.5 Consequences of lameness

2.5.1 Economic Consequences

Lameness generates both expenditures and costs on many different levels. Dependable estimates of total lameness costs are problematic to make due to the many influencing factors [18]; Bruijnis et al. (2010) [98], calculated the impact of clinical and subclinical lameness using a stochastic simulation model and identified the losses in milk production as the most influential cost factor at 44 % of the total cost, followed by increased risk of culling (22 %), prolonged calving interval (12 %) and produced labour (12 %). If the cost of a severe lesion (white line disease, sole ulcer or digital dermatitis) ranges from \$402 to \$622 [99] per affected cow per year, the cost of subclinical cases of lameness should also not be underestimated; Charfeddine and Pérez-Cabal (2016) [99] estimated the total cost of foot disorders for an average 65-cow farm at \$4899 per year, or \$75 per cow and the costs connected to subclinical cases of lameness made for 32 % of the total costs. Cha et al. (2010) [100] used dynamic programming to calculate the cost of lameness for different claw lesions and estimated an average cost of \$216, \$133 and \$121 for sole ulcer, digital dermatitis and foot rot respectively. The contributors to economic losses were different for every case, with the main losses due to decreased milk yield for sole ulcers, treatment for digital dermatitis and decreased fertility for foot rot [100].

2.5.2 Influence of lameness on the milk yield

Although many studies found a negative influence of lameness on milk yield [12–17], some authors found no relation between milk production and lameness [9, 101], while others in contrast found a positive association between lameness and milk production [102]. These contrasting findings highlight the complex nature of the interaction between milk production and claw diseases.

The decrease in milk yield connected to lameness is not always immediately evident; a study by Reader et al. (2011) [103] showed that daily milk yield dropped by 0.5 to 0.9 kg for cows that went from being mobility scored "sound" to "lame" six to eight weeks prior to detection. Green et al. (2010) [13] also found a decrease in milk yield a long time before treatment suggesting an impact on animal welfare. Cows with digital dermatitis on the other hand had higher milk yield than non-lame cows before treatment and all through lactation, and only showed a decrease one month before.

Archer et al. (2010) [14] calculated a potential reduction of 350 kg milk per lactation in cases of severe lameness in the early stages of lactation and of 620 kg in cases of persistent and recurrent sever lameness.

2.5.3 Influence of lameness on animal welfare

Lameness affects cows' natural behaviour and normal life cycle. Bruijnis et al. (2012) [104] estimated the impact of foot lesions on cow welfare by combining locomotion scoring and clinical examinations with the duration and incidence of lameness, indicating that a lame cow has an average welfare impact of 20 %, which corresponds to a score 12 (on a 0 to 60 welfare impact scale) and translates into, for example, having severe pain for three months. A score 60 was regarded to be the equivalent of a cow having severe pain for a whole year, highlighting the severity of the impact on animal welfare caused by lameness. Due to the prolonged duration of subclinical lameness compared to acute lameness and to a higher variation of locomotion scores for animals with chronic lesions, it is conceivable that these forms of lameness have a higher impact on animal welfare [97, 104].

2.5.4 Influence of lameness on dairy cow behaviour

An overview of the influence of lameness on cows' activity, lying and feeding behaviour and on their performance is given in Table 2.

2.5.4.1 Lying behaviour

Cows are diurnal animals whose main activities consist in feeding and resting. Lying is a very important behaviour for cows; in fact, cows lie when resting, sleeping or ruminating. If they are for some reason deprived of the possibility to lie down, cows will compensate by lying for longer periods when the impediment is no longer present [105, 106]. A dairy cow lies for about 12 to 14 hours a day in freestall housing [105, 107], but the expression of this behaviour can be influenced by the environment, in which the cow lives [52, 107, 108]. Cows in freestall housing are in fact more restless than at pasture; a study by O'Connell et al. (1989) [109], found that cows had significantly longer lying times when at pasture and that up to 90 % of cows in the herd were lying down at any one time between sunset and sunrise, which was never the case in freestall housing, where less than 45 % of cows were lying down at any one

time. These findings imply that confinement of cows in freestall housing affects their lying behaviour and highlight the importance of housing and management regarding the choice of cubicles and stocking policies [110, 111].

Lying behaviour is then influenced in turn by the presence of hoof lesions [8, 9]; the lying time of lame animals increases [9, 63, 112–114], with fewer, longer lying bouts, and a high variability in the duration of lying bouts [63, 113]. The differences in lying behaviour are particularly evident in the evening and at night [114], suggesting lame animals may modify their lying behaviour in order to avoid conflict situations. A study by Yunta et al. (2012) [115] found no statistically significant difference in the lying time and number of lying bouts of lame and non-lame cows, but a significant difference in the mean lying bout duration [112, 115].

2.5.4.2 **Feeding behaviour**

Cows spend around 4 to 14 hours a day grazing mostly during daylight, while cattle in loose housing spend about 5 hours a day feeding [106, 109]. Feeding space and social interactions connected to herd ranking influence feeding behaviour; if allowed to do so, cows will feed at the same time, but when if there is a lack of feeding places, dominant cows will displace lower-ranking animals, resulting in shorter feeding bouts for the lower-ranking animals [116].

Feeding behaviour has also been the object of studies investigating the effects of lameness on cows' behaviour and performance. Lameness negatively affects feeding time [9, 111, 117–120] and feeding frequency [9, 117, 119]. A study by Schindhelm et al. (2016) [9] measured feeding behaviour using automatic feeding troughs in relation to lameness and found no statistically significant difference in feed intake of lame cows, suggesting that feeding pace increased. Lameness does not seem to affect rumination time [120].

2.5.4.3 Activity

Studies analysing the influence of claw health on cows' activity show a reduction of activity connected to lameness [9, 103, 121] even with mild cases of lameness [120, 122]. Reader et al. (2011) [103] found levels of activity to be directly proportional to parity and lactation stage, O'Callaghan et al. (2003) [97] on the other hand, found the exact opposite to be true and also found a more pronounced difference in activity levels between lame and non-lame cows, suggesting activity may have a high individual and farm variance [103].

Parameter	Relation to lameness	Sources
Lying time	\uparrow/\rightarrow	[9, 21, 63, 112–114, 123,
		124]/[115]
Number of lying bouts	\downarrow/\rightarrow	[9, 21, 63, 111–113]/[115, 118,
		125]
Duration of lying bout	1	[9, 21, 112–115, 118, 125]
Variability of lying bouts	1	[63, 113]
Feeding duration	\downarrow	[9, 21, 111, 117–120, 125]
Visits to feeding table	\downarrow	[9, 21, 117, 119]
Feeding pace	1	[9, 21, 125, 126]
Feed intake	\downarrow/ \rightarrow	[126]/[9, 21]
Rumination	\rightarrow	[120]
Activity	\downarrow	[9, 21, 97, 103, 120–122, 124]
Standing bouts	\downarrow	[125]
Milk yield	$\downarrow/\rightarrow/\uparrow$	[12–17, 39, 114, 118, 127]/[9,
		21, 101]/[102]

 Table 2:
 Overview of studies investigating the influence of lameness on cows' behaviour and performance

2.6 Lameness detection

In a study by Horseman et al. (2013) [128] three quarters of interviewed farmers reported treating lame cows within two days of detection, but due to the subjectivity of mobility scoring, it is not possible to differentiate between cases which were treated when still mild and cases which were recognised only when already severe. Lack of time was reported to be one of the main barriers for immediate treatment. In a study by Leach et al. (2012) [129] only 13 % of the animals scored 2 (on a 0 to 3 scale) by trained observers were treated, suggesting farmers wait for lameness to become more evident before treating. A study by Alawneh et al. (2012) [130] showed that lame cows with a LMS > 3 (on a 1 to 5 scale) were more likely to be treated; animals with a LMS = 3 only had a 75 % chance of being treated at all, with over 50 % not being treated for at least 7 weeks. Animals who are treated earlier recovered more quickly and have less severe lesions [129, 131]. The implications of these findings on animal welfare and treatment effectiveness indicate that modern dairy farmers would benefit from automatic lameness detection systems, which would help them recognise their lame animals at an early stage.

2.6.1 Manual locomotion scoring systems

The most common gait traits used to evaluate the quality of the locomotion in locomotion scoring systems are asymmetric gait, reluctance to bear weight, short steps, abduction/adduction, step overlap and joint flexibility, while the most common posture traits are spine curvature and head bobbing [19]. The score given to the animal by an observer increases with mobility impairment. Manual locomotion scoring systems (MLSS) are performed by an observer scoring the animals either directly or through video recordings. Automatic locomotion scoring systems (ALSS) on the other hand rely on MLSS for

validation, but are based on mathematical algorithms which analyse data collected by sensors [19].

An overview of the most commonly used MLSS and of the MLSS developed for this study [19, 132] (see 4.2.2.1) and their level of reliability is given in Table 3. MLSS is performed by an observer, thus it is subject to human error and the observer's subjective judgement of the animal's locomotion. The inter-rater and intra-rater reliability are measures of the extent of the MLSS' validity (see 4.2.3.3). Some MLSS, such as the DairyCo. Mobility Score [133], only feature 4 possible scores (from 0 to 3), making it a suitable system for using on-farm. Other types of MLSS have more scores, such as the Flower and Weary (2006) [134] or the Manson and Leaver (1988) [75] have 9 possible scores from a minimum of 1 to a maximum of 5 in 0.5 steps, which makes them suitable for research purposes and to monitor clinical development of lameness, but also lower the level of inter-rater reliability (see Table 3).

 Table 3:
 Chosen manual locomotion scoring systems and their inter-rater and intra-rater reliability modified according to Schlageter-Tello et al.'s (2014) review of manual and automatic locomotion scoring systems [19].

MLSS	Min-Max		Inter-rater	reliability			Intra-rater	reliability		Source
		PA	к	r	Kw	РА	к	r	Kw	
Sprecher et al.	1-5	83	-	-	0.57-0.68	-	-	-	-	[136]
(1997) [135]		-	-	-	-	-	0.38-1.00	-	-	[9]
		-	-	-	0.30-0.40	-	-	-	-	[74]
DairyCo. (2007) [13	3] 0-3	61.3-83.3	-	-	-				-	[56]
		67.2			0.42-0.73					[137]
Manson and Leave	r 1-5	17.0-42.0	0.05-0.27	-	0.80-0.85	30.0	-	-	-	[138]
(1988) [75]		25.0-47.0		-	-	-	-	-	-	[139]
Flower and Weary	1-5	-	-	-	-	-	-	-	0.67	[140]
(2006) [134]		-	-	0.71-0.76	-	-	-	-	-	[141]
		-	-	0.88	-	-	-	-	-	[142]
		-	-	0.83	-	-	-	0.87-0.92	-	[134]
		-	-	-	-	-	-	0.88-0.99	-	[143]
Flower and Weary	0-	-	-	0.78	-	-	-	-	-	[142]
(2006) [134]	100	-	-	0.85	-	-	-	0.87-0.90	-	[134]
Winckler and Wille	en 1-5	46.0-95.0	-	-	0.41-0.87	-	-	-	-	[145]
(2001) [144]		63.0-74.0	-	-	-	-	-	-	-	[144]
		-	-	-	0.46-0.48	-	-	-	-	[146]
Lorenzini et al. (201 [132]	17) 1-3	77.9	-	-	0.60	82.3	-	-	0.60	[147]

Percentage of agreement (P_A), kappa coefficient (κ), weighted kappa coefficient (κ_w), Pearson correlation coefficient (r), Min: minimum score, Max: maximum score.

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The inter-rater reliability can be expressed in percentage of agreement between two raters (P_A), which only distinguishes between concordant and discordant scoring, while the weighted kappa statistics (κ_w) considers how large the measure of discordance is between two observers scoring the same animal. Both kappa statistics (κ_w and κ) correct the level of concordance for chance agreement [148]. Pearsons's correlation coefficient (*r*) measures correlation but fails to detect systematic error, i.e. deviation of the best fit line from the 45° line through the origin [149]. In their review of MLSS, Schlageter-Tello et al. (2014) evaluated the MLSS cited in literature and found a wide range of inter-rater reliability; from only 17-42 % (P_A) for the Manson and Leaver score (1988) [75] to 46-95 % (P_A) for the Winckler and Willen (2001) [144, 145] or 83 % (P_A) for the Sprecher et al. (1997) score [9, 135]. The difference in reliability highlights the complexity of locomotion scoring and the influence of subjectivity. Reducing the number of possible ratings could increase the interrater reliability [19, 138].

2.6.2 Automatic locomotion scoring systems (ALSS)

Research into automation of the locomotion scoring process aims at reducing the element of subjectivity involved in scoring by direct observation and offers a time-saving alternative to traditional locomotion scoring of the herd. Furthermore, ALSS could recognise signs of lameness earlier than a direct observer and improve the cows' welfare by allowing for earlier treatment. ALSS can be divided into direct and indirect systems [19].

2.6.2.1 Direct ALSS

Direct ALSS use either a kinetic, kinematic or thermographic approach. The kinetic approach uses load cells to measure forces exerted on a surface by the cow's hoofs or changes in weight distribution. Dunthorn et al. (2015) achieved a high sensitivity (90%) and specificity (93%) for their model using data from a force-plate system. Slightly lower levels of prediction accuracy were obtained by Pastell et al. (2010) [150], who used force plates to detect lameness employing only the vertical dimension and achieved a model with an AUC (area under the curve) of 0.88, while Chapinal et al. (2010) [151] achieved an AUC of 0.71 using the variability (standard deviation, SD) over time of the weight applied to the rear legs. Still, in a study by Bicalho et al. (2007) [146], visual and automated locomotion scoring were compared and the StepMetrix (BouMatic, Madison, WI, USA) lameness detection system had an AUC = 0.62, while the trained observers had an AUC = 0.80, demonstrating that the ALSS was less reliable than the MLSS and that research is still necessary to increase the accuracy of ALSS.

The kinematic approach on the other hand, involves the use of various locomotion variables to detect lameness [19]. Maertens et al. (2011) [152] used a pressure sensitive walkway and 20 basic kinematic gait variables (such as abduction, asymmetry and speed) in a commercial

dairy farm to detect lame animals and developed a model with a sensitivity of 85 %, 76 % and 90 % and a specificity of 86 %, 89 % and 100 % for the detection of non-lame, mildly lame and severely lame cows respectively [152, 153]. Some studies have found spine curvature to be a useful variable to use when detecting lameness using shape analysis. Studies using 2D image analysis [154, 155] found a high correlation between back posture and lameness. The need for a uniform background against which cows' spine curvature outline is analysed is problematic, especially due to changing light conditions [156] in on-farm settings, so Van Hertem et al. (2014) [157] used three-dimensional video analysis to detect lameness and achieved a correct classification rate of 60.2 % for a five-point model output. With a binary lame/non-lame classification the correct classification of cows increased to 81.2 [157]. Accelerometers can also be used to detect gait anomalies and thus lameness [158]. A study by Mangweth et al. (2011) [159] used accelerometers to distinguish between lame and non-lame cows and achieved a predictive accuracy of 91.7 % for binary classification and 61.7 % for lameness classification within score categories according to Sprecher et al. (1997) [135]. Finally, Alsaaod et al. (2017) [160] developed the first cow pedogram using pedometers with a high sampling rate (400 Hz) on both hind limbs and achieved 100 % specificity and sensitivity, but noted that accelerometers with a lower sampling rate would be more suitable for implementation in an on-farm lameness detection system.

Alsaood and Büscher (2012) [161] investigated the use of infrared technology (IFT) to detect claw diseases in dairy cows and found an increase in surface temperature of the coronary band area in cows with hoof lesions; the model used in the study had a sensitivity of 85.7 % and 80 % and specificity of 55.9 % and 82.9 % before and after claw trimming respectively. Harris-Bridge et al. (2018) [162] also used IFT to detect cows with DD and found the maximum temperature at an individual foot level to be the best predictor (AUC = 0.72).

2.6.2.2 Indirect ALSS

ALSS that use the indirect approach detect lameness based on behavioural and performance parameters. Lameness influences dairy cows' natural behaviour (see 2.5.4); these changes in the behavioural patterns of individual animals can be used for early lameness detection. In 2009 Kramer et al. (2009) [163] developed a classification model for lameness using milk yield, water intake, dry matter intake behaviour and activity as input data. Although the sensitivity (70 %) and specificity (75.9 %) were acceptable, the high error rate (98.9-99.5 %) made it not applicable to on-farm conditions due to the high number of false positives. The high false positive rate could be due to lameness being a rare event, the control cases thus vastly outnumbering the cases of lameness. Kamphuis et al. (2013) [164] used logistic regression to build a lameness detection model based on behavioural parameters recorded by accelerometers, as well as live weight and milk production data. The AUC for the univariate models were low (AUC = 0.66 for live weight, AUC = 0.60 for activity and AUC = 0.65 for

milking order), but the combination of the variables improved detection performance to an AUC of 0.74. Van Hertem et al. (2013) [165] also used multivariate logistic regression with a binary lame/not-lame outcome and ten-fold cross-validation with milk yield, neck activity and rumination time as input variables and obtained an AUC = 0.89. Lameness cases for Van Hertem et al.'s study (2013) [165] were chosen based on treatment data from the farms, meaning the reference date for lameness did not necessarily coincide with the date of actual lameness onset, also indicated by the fact that lame cows had an overall lower milk yield over the whole analysis period, suggesting lameness was already present before diagnosis. Garcia et al. (2014) [166] used partial least-squares discriminant analysis with milking and activity data collected by an AMS and developed a model with 320 variables, and although the classification error was too high for on-farm application (around 20 % for both tested models) the results were achieved using solely data from a single system which already present on the farm. A limitation of the study was the exclusion of cows scored 2 (on a 0 to 4 scale), which excluded animals from the analysis which may have been clinically lame but not recognised as such. Finally, the prediction model developed by Grimm et al. (2019) [21] using the regularized regression model Elastic Net (ENET) [167] and ten-fold cross-validation of the models produced a final model with an AUC = 0.94. The model is based on behaviour and performance parameters recorded using accelerometers, an AMS and automated weighing troughs and the interactions between these parameters. The choice of variables and interaction terms for the model provided insight in the complex relationships between behaviour and performance parameters used to predict lameness; the risk for lameness increased with a higher milk yield for instance only when in combination with decreased lying times. An overview of studies producing a lameness detection model using automatically generated data and their level of accuracy is shown in Table 4.

Overall, many studies have been conducted on the risk factors for lameness and the influence that lameness has on cows' behaviour and performance. The more recent studies involving the use statistical modelling to predict lameness show promising results, but more research has to be done to improve the accuracy of ALSS and investigate the complex interactions and relationships between behaviour and performance parameters.

Study Statistical model type Reference Sensitivity Specificity Mean Mean Input AUC Parameters (%) (%) Precision Accuracy [168] Support Vector Machine MLSS AC, LB 77 % 76 % 88.8 [169] Quadratic trend models, MLSS MY, AC, LB, 85.5 -_ dynamic linear model IN [163] Treatment AC, FB 72.7 75.9 / 75.3 Fuzzy logic model _ [170] AC, FB 74.2 / 73.3 81 / 80.1 Wavelet analysis, vector Treatment autoregressive model, multivariate cumulative sum charts [171] Treatment AC 40.4-48.3 Wavelet analysis, cumulative sum [...] charts [165] Kruskal-Wallis one-way ANOVA Treatment MY, AC, 89 85 0.89 -RU. by ranks, logistic regression [166] MLSS MY, MB, IN, ~80 ~80 Partial least squares discriminant --P, AC, DIM analysis, ROC analysis Logistic regression, ROC analysis [9] MLSS FB, LB, AC 81.8 80.6 0.85 -[21] Forward stepwise logistic MLSS MY, FB. 92 83 0.94 _ regression, ENET Beta, ROC LB,P analysis [125] T-test, Aspin-Welch-test, Wilcoxon MLSS FB, SB, WS 92.7 91.7 0.96 test, multivariable logistic regression, ROC analysis

Table 4: Overview of studies using behaviour and performance data for automatic lameness detection and level of prediction accuracy of the respective models.

AC: parameters connected to activity, LB: parameters connected to lying behaviour, MY: parameters connected to milk yield, IN: parameters connected to intake, FB: parameters connected to feeding behaviour, RU: parameters connected to rumination, MB: parameters connected to milking behaviour, SB: standing behaviour, WS: walking speed, P: parity, DIM: days in milk, MLSS: manual locomotion scoring, AUC: area under the curve

20

3 Study objective

The objective of this study was to test the model development method applied by Grimm et al. (2019) [21] in a previous study at the Bavarian State Research Centre for Agriculture's research farm. Due to the fact that the research farm was equipped with technology that isn't available on commercial dairy farms, it was necessary to test the algorithm in an on-farm environment outside the research facility and to further develop it for possible future implementation in a software environment connected to a herd management system. For this reason, four dairy farms were chosen, where claw health data was manually collected and limb-mounted activity sensors for continuous data recording were fitted on each animal. The data would then be used in a predictive algorithm for early lameness detection on Simmental cows.

Sub-objectives of this study were:

- To validate the pedometers used for data collection regarding the measurement of lying and feeding behaviour, in order to assess the accuracy of the system for data collection.
- To develop and test a new MLSS that could be applied both in research and in on-farm conditions as a reference method for claw health.
- To treat and document clinical cases of lameness and use pain tests to improve the accuracy of locomotion scoring and further understand pain manifestation in dairy cattle.
- To analyse video recordings in order to monitor the development of cases of lameness and allow estimation of ideal locomotion scoring frequency on dairy farms to minimize the impact on animal welfare.
- To combine behaviour and performance parameters in a predictive model and analyse the influence of the variables on the outcome lame/not-lame and the way different parameters interact.

The findings of this study were then compared to findings in current literature and suggestions were made towards possible more in-depth analysis of the collected data in future studies.

4 Animals, materials and methods

One of the aims of this study, conducted at the Institute for Agricultural Engineering and Animal Husbandry (ILT) of the Bavarian State Research Centre for Agriculture (LfL), was to test and further develop the algorithm developed by Grimm et al. (2019) [21] on four commercial dairy farms and on one research facility using commercially available pedometers for the automatic measurement of behaviour data.

The preparation for this study started in July 2016 with the choice of the project partners and the search for commercial farms in Bavaria which would be suitable to take part in the study. ENGS Dairy Solutions, a company that operates in the field of precision dairy farming, was chosen as a project partner following previous successful collaborations with the ILT. The pedometers for the project were produced by. ENGS Dairy Solutions, while the on-site technical assistance was provided by Bayern Genetik GmbH.

4.1 Husbandry systems and farm management

The "Track a Cow"-pedometer-system was installed on four dairy farms chosen to participate in the project. Additionally, the ILT's research farm in Grub, Upper Bavaria, was included in the project.

The farms were chosen based on size, general farmer compliance, willingness to take part in a project about claw health and to have activity sensors fitted on all milking animals. All farms had loose housing-type barns with free stalls and some farms had tie stalls for calving or sick animals. On all farms the animals were kept in a separate enclosure or building for the dry-off period.

4.1.1 Animals

Data for this study were collected from a total of n = 619 Simmental cows and n = 2 Brown Swiss cows. Due to the replacement and culling rates as well as the losses due to sale of the animals a median of 74.5 cows were milked at the same time, with the largest farm being CDF3 with a median of n = 102 cows milked and n = 180 cows in total and the smallest farm being the RFG with a median of n = 66 cows milked and a total of n = 87 cows. Table 5 shows an overview of the animals involved in the project and of the key reproductive figures per farm. 25.5 % of cows were heifers (n = 163), followed by cows in their first lactation (21.3 %).The median lactation number on all farms was one except for CDF4 and the RFG were the cows' median parity number was 2. A relative distribution of cows by number of lactations per farm is shown in Figure 3. The culling rate is defined as the number of animals sold, culled or died during the year divided by the total number of animals on the farm in percent [172]. CDF1 had the lowest calving interval at n = 365 days and also the lowest voluntary waiting period with an average of n = 53 days. The culling rate was highest for CDF2 at just under 37 % percent, but similar for CDF1 and CDF3 at 25.4 % and 23 % respectively.

	CDF1	CDF2	CDF3	CDF4	RFG	Total/Ø
Total no cows	152	114	176	92	87	621
Md cows milked	99	72	102	74.5	66	74.5
No Heifers (%)	44 (29)	36 (31)	64 (36)	6 (7)	13 (15)	163
No I L (%)	34 (22)	25 (22)	28 (16)	28 (30)	23 (26)	138
No II L (%)	26 (17)	19 (17)	31 (18)	18 (20)	12 (14)	106
No III L (%)	27 (18)	15 (13)	22 (13)	8 (9)	14 (16)	86
$N_0 > III L (\%)$	20 (13)	16 (15)	30 (17)	28 (30)	25 (28)	119
Lactation no NA	1 (1)	3 (3)	1 (1)	4 (4)	-	9
Med lactation no	1	1	1	2	2	1
Ø CAI [in days]	365	385	381	NA	396	382
Culling rate (%)	25.4	36.9	23	NA	27.9	28.3

 Table 5:
 Overview of animals and farms in data collection.

Med: median, CDF1 - CDF4 (commercial dairy farms 1 – 4), RFG (research farm), abs: absolute frequency, Ø: average, number of cows in their first (I L), second (II L), third (III L), or above third lactation (> III L), CAI: calving interval, NA: not available.



Figure 3: Relative proportion of cows in different lactations per farm. CDF1 – CDF4 (commercial dairy farms 1 – 4), RFG (research farm).

4.1.2 Commercial dairy farm 1 (CDF1)

Commercial dairy farm 1 (CDF1) was a family-run dairy farm that houses a herd of 162 Simmental cows, milking an average of 101.5 at a time. The cows were milked twice a day in a 14-unit herringbone milking parlour. The cows were kept in a loose housing system with deep bedded free stalls. Cows were kept in a separate building after drying-off, which occurs six weeks prior to the predicted calving date. Ten days before the calving date, the cows were moved into tie stalls and are kept there for between two (heifers) and five (cows) days after calving. Sick animals or animals that need to be kept under observation were also kept in the tie stalls. The solid rubber flooring in the main barn was cleaned by an automatic scraper (Figure 4). The yard that connected the main barn to the milking parlour (Figure 5) featured solid grooved concrete flooring and was cleaned manually. The free stalls were cleaned twice a day and new straw was added every two weeks. On CDF1, milking occurred twice a day between 06:00 and 07:30 and between 17:00 and 18:30; the cows were driven into the yard and from there they enter the milking parlour which was located in a separate building (Figure 5).



Figure 4: Solid rubber flooring and deep bedded free stalls on commercial dairy farm 1.



Figure 5: Sketch of floor plan of commercial dairy farm 1.

YS: young stock, TS: tie stalls, MP: milking parlour, Y: yard, C: video camera, CFS: concentrate feeding station, IL: induction loop, FT: feeding table

4.1.3 Commercial dairy farm 2 (CDF2)

Commercial dairy farm 2 (CDF2) was a family-run dairy farm milking 79.4 cows on average. The cows on CDF2 were kept in a free stall loose housing system with grooved, slatted concrete flooring which was cleaned by a scraper robot. The concrete-based raised free stalls were cleaned twice a day and are topped with rubber mattresses to which lime was added once a day (Figure 6). The barn had no yard or external area. Cows were moved to a separate area of the barn when drying-off occurred and about 50 days before the predicted calving date. The cows were then moved to tie stalls a few days before calving, coinciding with a drop in body temperature indicating imminent calving, which was measured daily a week before predicted calving date. After calving, the cows were integrated into the herd at the next milking, except when there were complications and they were kept in tie stalls up to 5 days postpartum. Milking occurred twice a day, respectively at 06:00 until 07:30 and at 17:00 until 18:30, in a 10-unit herringbone milking parlour inside the barn (Figure 7).



Figure 6: Slatted concrete flooring and concrete free stalls with rubber mattresses on commercial dairy farm 2.



Figure 7: Sketch of floorplan of commercial dairy farm 2.

YS: young stock, TS: tie stalls, MP: milking parlour, C: video camera, CFS: concentrate feeding station, IL: induction loop, FT: feeding table

4.1.4 Commercial dairy farm 3 (CDF3)

Commercial dairy farm 3 (CDF3) was a family-run dairy farm with an average of 114.5 milking cows, which were milked twice a day between 05:30 and 08:00 and 16:30 and 19:00. The cows on CDF3 were kept in a loose housing system with a yard (Figure 8) and deep bedded free stalls (Figure 9) which were cleaned twice a day and to which bedding was added once a day. The grooved, slatted concrete flooring in the barn was cleaned manually, whilst the solid grooved concrete in the yard was cleaned by an automatic scraper (Figure 9). The cows were dried off six weeks prior to the predicted calving interval, while heifers were put in with the dried-off cows eight weeks before calving. Dried-off animals were in a separate area of the barn and were moved into calving pens a week before calving. Sick animals were kept in tie stalls near the entrance of the barn. On CDF3 the cows were fed two different total mixed rations (TMR), according to the milk yield.



Figure 8: Sketch of floorplan of commercial dairy farm 3.

YS: young stock, TS: tie stalls, MP: milking parlour, C: video camera, IL: induction loop, FT: feeding table, Y: yard



Figure 9: Yard in commercial dairy farm 3 with deep bedded free stalls and grooved concrete flooring.

4.1.5 Commercial dairy farm 4 (CDF4)

Commercial dairy farm 4 (CDF4) was a family-run dairy farm with an average of 73 milking cows. CDF4 was the only commercial dairy farm chosen for the project that had an automatic milking system (AMS), produced by GEA (GEA Farm Technologies, Bönen, Germany), as opposed to a milking parlour. The cows were kept in a loose housing system with concrete based raised free stalls which were cleaned twice a day and to which bedding was added in the form of lime and straw. The slatted concrete flooring was cleaned by a scraper robot. A yard adjacent to the main barn (Figure 10) had deep bedded free stalls and a mixture of solid concrete and slatted concrete flooring which was cleaned manually. Cows were dried off eight weeks prior to the predicted calving date and were put in a separate area of the barn. Sick cows were either put into separate pens or in the separate lying area opposite the AMS (see Figure 11). Cows were integrated back into the herd one to seven days postpartum, according to their state of health. Sick cows were kept in tie stalls near the entrance of the barn.



Figure 10: Yard on commercial dairy farm 4 with deep bedded free stalls.





YS: young stock, TS: tie stalls, MP: milking parlour (not used), C: video camera, CFS: concentrate feeding station, IL: induction loop, FT: feeding table, Y: yard, SP: separation pen, AMS: automatic milking system.

4.1.6 Research farm

The fifth farm on which data was collected for this study was the research farm of the LfL. The research farm in Grub (RFG) was a state-run research facility with two separate herds and milking systems. The herd whose data was collected for this project consisted of an average of 68 milking cows. The specific nature of the RFG is due to the presence of various data collection systems such as automatic weighing troughs (Figure 12), automatic gates

registering animal movement between feeding and lying areas (Figure 13) and ultrasonic sensors over the free stalls. The herd in the RFG was kept in a loose housing system with slatted concrete flooring in the feeding area and slatted rubber flooring in the lying area. The flooring was scraped manually twice a day by employees. One of the features of the RFG was the presence of both deep bedded free stalls and raised concrete free stalls with rubber mattresses.



Figure 12: Automatic weighing troughs at the research farm.





WT: weighing troughs, C: video camera, CFS: concentrate feeding station, IL: induction loop, FT: feeding table, SP: separation pen, AMS: automatic milking system.

Table 6 represents an overview of animal husbandry on the project farms, including housing, flooring, and free stall type.

	CDF1	CDF2	CDF3	CDF4	RFG
Free stalls	DB	RC +RM	DB	RC +RM	DB/RC+RM
Flooring	SO/R	SL/C	SL+SO/C	SL/C	SL/R+C
MP / AMS	MP	MP	MP	AMS	AMS
FS maintenance	2x/day	2x/day	2x/day	2x/day	2x/day
Flooring scraping	А	А	M / A	А	М
No. feeding places	89	72	113	78	36
No. lying places	85	73	100	97	65
Md cows milked	99	72	102	74.5	66

 Table 6:
 Overview of type of housing, free stall and flooring, milking system.

MP: milking parlour or AMS: automatic milking system, FS: free stall, LHS: loose housing system, DB: deep bedded, RC: raised concrete, RM: rubber mattress, C: concrete, R: rubber, SL: slatted, SO: solid, M: manual, A: automatic, CDF1 – CDF4 (commercial dairy farms 1 – 4), RFG (research farm), Md: median.

4.1.7 Feeding management

Feeding routines were different on all farms, although all except CDF3 fed a partial mixed ration (PMR) once a day in the morning and additionally had concentrate feeders to which the animals had access to according to their milk yield. CDF3 fed a total mixed ration (TMR). An overview of feeding routine is given in Table 7.

 Table 7:
 Overview of feeding routine on the project farms for 2017.

	CDF1	CDF2	CDF3	CDF4	RFG
MJ NEL/kg DM	6.7	6.91	6.93 (EL) 6.61 (LL)	NA	6.8
Concentrate feed	>25 kg MY	>27 kg MY	-	NA	>25 kg MY
Concentrate feed/cow/day (kg)	2.47	5.10	3.8 (EL) 3.2 (LL)	NA	5.20 + 1
Push-up frequency/day	5x	5x	4x	NA	-

EL: early lactation, LL: late lactation, MY: milk yield, CDF1 – CDF4: commercial dairy farms 1 – 4, RFG: research farm. MJ NEL/kg DM: Megajoule net energy for lactation per kg dry matter, PMR: partial mixed ration, TMR: total mixed ration, NA: data not available

Feed was pushed up manually five times a day on CDF 1 and CDF2 and four times a day on CDF3. The cows on CDF4 were fed a total mixed ration once a day and a robotic feed pusher pushed the feed up regularly.

On the RFG, cows were fed a PMR once a day at about 05:30 in the morning. The feed was dropped into 36 weighing troughs, to which the animals had access to via radio frequency identification devices (RFID) in their ear tags. The cows on RFG were fed 1 kg concentrate feed in the AMS in addition to the 5.2 kg mixed in the PMR.

4.1.8 **Claw health management**

Hoof trimming on CFD1, CDF3, CDF4 and RFG was performed twice a year by a professional hoof trimmer. Hoof trimming on CDF2 was performed three times a year by a professional hoof trimmer, although only a part of the herd was trimmed every time, meaning cows' hooves were trimmed between once and twice a year. Acute cases of lameness occurring between the regular hoof-trimming sessions were treated either by the farmer or by a veterinarian on all farms.

4.2 **Data collection**

The data collection started in April 2017 and ended in June 2018. On CDF1, CDF2 and CDF3 data were collected over a period of 12 months between April 2017 and April 2018, while only three and six months of data were recorded on CDF4 and RFG respectively. An AMS was installed on CDF4 in March 2017, so the start of the project was postponed on this farm in order to allow the herd to adapt to the new conditions in the barn and avoid an influence of the new installation on the animals' behaviour. The activity monitoring system was removed from CDF4 in November 2017 due to the farmer's lack of compliance.

4.2.1 Automatically recorded data

Performance and behaviour data were collected automatically, while claw health data were collected manually during the data collection phase of the project (see Table 8). All dairy farms involved in the project were equipped with cow movement telemetry systems ("Track a Cow", ENGS Dairy Solutions, Rosh Pina, Israel). The systems were installed in July 2016 on the four commercial dairy farms and in December 2016 on the RFG. In February 2017 video cameras were installed on all project farms to perform LMS of the animals exiting the milking parlours or AMS. On CDF4 and RFG, additionally, data were collected automatically by the AMS as well as by automatic weighing troughs on the RFG.

Data	Type of collection	Collection system
Activity	automatic	pedometers
Lying behaviour	automatic	pedometers
Feeding behaviour	automatic	pedometers, WT
Milk yield	automatic	AMS, LKV
Reproductive data	automatic	HMS, LKV
Body Condition	automatic	AMS
Claw health	manual	-

 Table 8:
 Type of collection and collection systems on the farms included in the study.

WT: weighing troughs, AMS: automatic milking system, LKV: breeding refinement association, HMS: herd management software

4.2.1.1 **Pedometers**

The "Track a Cow" pedometers (Figure 14) measured feeding and lying behaviour. They consisted of a $6,88 \times 2,65 \times 5,07$ cm rigid plastic housing fitted to the cow's forelimb with a webbing strap, containing a position sensor, a three-dimensional accelerometer and an RFID coil. The pedometers measured acceleration at a frequency of 1000 Hz and could distinguish between three different states; standing, lying or moving. The lying, activity and feeding behaviour data of the cows were transmitted every 15 minutes to a receiver connected to an on-farm computer via a RS485 cable.

The position (standing or lying) of an animal was sampled every 8 seconds and the recorded states were then summarized into two-minute intervals by the pedometers. The number of lying bouts was defined as the number of changes of state from standing to lying. The cow's activity was measured by an algorithm which registered bouts of acceleration that corresponded to the cow moving and summarised them as an "activity index".



Figure 14: "Track a Cow" pedometer on the right front limb of a dairy cow

Feeding behaviour was measured via an induction loop that was installed in a groove along the feeding tables of all the project farms and set in concrete to record feeding behaviour. A magnetic field was induced once a minute for 300 ms, allowing all pedometers within the magnetic field to be activated. When a pedometer was activated, the so-called activator number (AN) increased by one unit. A feeding visit was defined as the time lapse between the first time the activator number increased by one unit, and the sixth consecutive minute in which the activator number had not increased again. A meal was thus a visit to the feeding table of at least six minutes, with interruptions of up to a maximum of six minutes. The data were summarized and collected in a database that was then displayed to the end-user in a software environment ("Eco Herd", ENGS Dairy Solutions, Rosh Pina, Israel). All data were summarized in an Access Database (Microsoft Corporation) into hourly intervals, as can be seen in Figure 15, as well as into a sum per day and average per day. Lying time and feeding time were both expressed in min/h while lying bouts and feeding visits were expressed in no of bouts or visits per hour. The activity was displayed in units per hour.

Cowld 👻	Date 🔹	• 00	01 -	02 -	03 -	040 -	05(-	06 -	07 -	081 +
331	31.08.2016	0	0	0	0	0	0	0	0	0
331	01.09.2016	0	0	0	38	58	58	60	58	60
331	02.09.2016	60	58	58	60	58	60	58	60	58
331	03.09.2016	60	58	60	58	60	58	58	60	59
331	04.09.2016	60	58	59	60	59	60	58	60	58
331	05.09.2016	60	59	60	59	60	59	58	60	58
331	06.09.2016	58	60	58	59	60	58	60	58	60
331	07.09.2016	58	60	58	60	58	60	58	59	60
331	08.09.2016	60	58	60	58	59	60	58	60	58
331	09.09.2016	58	59	59	60	59	60	59	58	60
331	10.09.2016	60	59	60	59	58	60	58	60	58
331	11.09.2016	24	60	23	45	56	35	30	0	18
331	12.09.2016	3	18	50	58	58	60	15	24	29
331	13.09.2016	18	44	38	60	60	47	20	60	35
331	14.09.2016	37	9	32	43	60	58	14	34	60

2000 +	2100 -	220 -	2300 -	sumForDay 😽	DailyAvgPer -
0	0	0	0	312	13
60	60	60	58	1218	50
58	60	58	58	1414	.58
60	59	60	58	1417	59
59	60	59	58	1421	59
58	60	58	60	1420	59
60	59	60	59	1420	59
59	58	60	58	1188	49
59	60	59	60	1420	-59
59	58	60	59	1420	59
24	15	57	0	962	40
12	19	47	17	618	25
20	52	60	40	760	31
4	28	18	0	632	26
50	0	54	44	653	27

Figure 15: Screenshot of the Access database.

Each row represents one animal on one day divided into hourly intervals. The last two variables represent the sum and average lying minutes per day respectively. The values are expressed in minutes per hour.

4.2.1.2 Cameras

Due to previous experience and following a locomotion score test on all farms in the project preparation phase, it was decided to install cameras on the farms in order to minimise the effect the presence of the observer has on the animals while scoring. In February 2017 Mobotix "D15 DualDome" (Mobotix AG, Langmeil, Germany) video cameras were installed on all project farms. The cameras were installed either facing the exit of the milking parlour or the exit of the AMS in order to be able to observe the animals exiting in single file. The video cameras have two lenses, which can be rotated separately both vertically and horizontally to allow for a wide-angle image. As on CDF1 the camera was installed outside of the barn, both a black and white and a colour lens were installed in order to have sufficient image quality in every light condition (Figure 16). To improve the image quality of the black and white lens in poor lighting conditions a LED spotlight was installed.



Figure 16: Freeze frames of video recordings on CDF1 with the black and white (1) and colour lenses (2). On CDF2 the video camera was installed on the inside of the barn, thus a black and white lens was not needed. The position of the camera was changed two weeks into the date collection phase of the project, as the original position did not allow the observer to see the line of the cow's back. The definitive position of the camera allowed for the animals to be observed briefly from the front, then from the side and finally briefly from the rear while walking away from the camera (Figure 17).



Figure 17: Freeze frames of video recordings form CDF2 (1) and CDF3 (2) respectively.

On CDF3 the original position of the video camera was also changed two weeks into the data collection phase. The final position allowed the observer to see the animals from the right-hand side and then from the back as they walk away from the camera.

On the project farms with an AMS, the video cameras recorded 24 hours a day, so the cameras were equipped with black and white lenses in order to have sufficient image quality even at night. On CDF4 the camera was opposite the exit of the two AMS boxes and allowed the observer to see the cows briefly from the right side, then from the front and then from the back as they walked away from the camera (Figure 18). On the RFG the camera was also placed at the AMS exit. Because the animals exiting the AMS on the RFG would walk

straight across to the feeding bins opposite the exit below the camera, a short barrier was installed (Figure 18) so that the cows had to walk a short distance along the side of the building, allowing the observer to see the animal from the left side and then from the rear.



Figure 18: Freeze frames form the video cameras on RFG (1) and CDF4 (2).

The video recordings from all the cameras were saved in network attached storages (NAS) on-site.

4.2.1.3 Automatic weighing troughs

On the RFG, in addition to the pedometers, the cows' feeding behaviour was also recorded by automatic weighing troughs (Figure 12). The weighing troughs have an automatic shutter which opens when the animals' ear tag is detected via RFID (radio frequency identification) technology at less than 30 cm distance. A process computer connected to each of the weighing troughs registers the initial and end weight of the trough with a time-stamp, thus calculating the feed intake as well as the time the animal spends feeding. The weighing trough data is stored in a database and accessible through queries. Differently from the feeding behaviour recorded by the pedometers, the weighing trough data is not summarised, so the exact time and length of each visit can be retraced.

4.2.1.4 Automatic milking systems (AMS)

The RFG had an AMS produced by De Laval (DeLaval, Sweden) with one box and one robot, while CDF4 had a GEA (GEA Farm Technologies, Bönen, Germany) robot with one robot and two boxes. The AMS collected data for every milking process, such as the amount of milk and time spent in the AMS. The data were then made available either in the Delpro software or through an interface in the Dairy Plan software (GEA, Farm Technologies, Bönen, Germany).

4.2.1.5 External data sources

For CDF1, CDF2 and CDF3, data regarding identity, breed, age, acquisition date, and culling date as well as parity and calving date were provided by the LKV (Bavarian breeding refinement association). None of the aforementioned farms have an automatic milk measurement system, so milk production data were also provided by LKV; these included days in milk, 100-day or 305-day milk production as well as the results of milk tests carried out eleven times a year that include amount of milk given on the test date, the amount of fat and of protein in the milk and the somatic-cell count.

For CDF4 and the RFG the data regarding identity, age, parity, days in milk, daily milk production, dry-off date, insemination date, calving date and culling date were all exported from the Dairy Plan software used on the farms.

4.2.2 Manually recorded data

All data regarding the cows' claw health were recorded manually. These data included locomotion scoring through video recordings of all animals after milking, recording of findings during professional hoof trimming, and recording of findings and treatment on the fortnightly farm visits.

4.2.2.1 Locomotion scoring

Locomotion scoring can be used as an indicator for hoof lesions in dairy cows [123]. In this study locomotion score was used as a reference for claw health and was supported by clinical examinations of the lame cows' claws (see 4.2). In a previous project at the LfL [9], the locomotion score according to Sprecher et al. (1997) [135] was used as a reference for cows' hoof health. The low reliability level of this manual locomotion scoring system, combined with the difficulty of detecting signs of pain in cows, was considered problematic and encouraged the development of a new score that was used in this study.

The locomotion score developed in the course of this project is a three-point scoring system meant for use in both a practical and research setting. The choice of the traits that should be considered during locomotion scoring of a cow was based both on experience and on existing literature on the subject. The three-point LMSS according to Grimm & Lorenzini (LMSSGL) (2017) [132] is illustrated in Figure 19 as a flowchart with dichotomous decision boundaries for each locomotion trait.



Figure 19: Locomotion scoring system according to Grimm & Lorenzini [132]

In the three-point LMSSGL, the observer begins with the assessment of the overall gait picture, considering whether the gait is regular or irregular, symmetric or asymmetric and smooth or uneven. In the case of an uneven, irregular or asymmetric gait, the cow is considered "lame" (LMS = 3). If the cow's movement is considered regular, smooth and symmetric, the observer continues by evaluating the presence or absence of an arched back, head bobbing or a compensatory posture, meaning the reluctance of the animal to bear weight on one or more feet while standing and avoiding weight bearing by either lifting the limb, or by positioning it further forward or further back compared to the other limbs (Figure 20).



Figure 20: Cow showing a compensatory posture by stretching the right hind limb out behind her to release weight off it.

If the cow shows any one of these traits, but its gait remains smooth, symmetric and regular, it is considered "unsound" (LMS = 2). If none of these traits is present and the animal's gait is regular and symmetrical, the animal is considered "sound" (LMS = 1). As experienced pain and the presence of lameness symptoms are not always correlated, the differentiation between mild and severe lameness was deliberately avoided with the prospect of use on-farm in order to discourage postponing treatment by considering lameness as merely "mild".

4.2.2.2 Locomotionscoring including clinical examination

During the data collection phase of this study data regarding the claw health of the animals were collected on a fortnightly basis. As locomotion scoring was carried out through video recordings the farmers wrote down the order in which the cows exited the milking parlour, ensuring the identification of the animals which wouldn't otherwise have been possible.

After locomotion scoring, the lame animals scored LMS = 3 were separated from the herd and driven into the claw-trimming chute. The claws were then examined and if necessary trimmed and treated. The claws on the hind limbs were always examined, while the claws on the front limbs were examined depending on time availability and the animal's level of compliance. Not all lame animals were examined; sometimes the animals were treated by the farmers, other times they were left to heal if they had already been examined in the chute multiple times shortly beforehand. The animals who were scored LMS = 2 (see 4.2.2.1) for three times,

thus six weeks, in a row were also separated from the herd and examined for pain in the claws. These animals were always examined. The pain test was carried out in the claw trimming chute and consisted in observing signs of pain when pressure was exerted on the claws with hoof pincers (Figure 21). A positive reaction to the pain test manifests itself through twitching or jerking of the limb or defensive movements of the cow as a direct reaction to the hoof pincers. If a positive pain reaction was observed, the cause for the pain was further investigated and if necessary, the claw was trimmed and treated. If an open wound or pathological change was evident or if pain test was positive on any of the four limbs the cow's score was changed to LMS = 3 (lame) and a remark was made, to indicate that originally the animal did not show explicit signs of lameness during locomotion. If the pain test was negative and no evident cause was found for the gait anomalies, the cow remained recorded as LMS = 2.



Figure 21: Pain test with hoof pincers on the right hind limb of a cow.

All findings of the clinical examinations were documented according to the ICAR Claw Health Atlas [173] of which the codes and descriptions are summarised in Table 9.

Code	Clinical finding	Description
CC	Corkscrew claws	Any torsion of either the outer or inner claw. The dorsal edge of the wall deviates from a straight line
DD	Digital dermatitis	Infection of the digital and/or interdigital skin with erosion, mostly painful ulcerations and/or chronic hyperkeratosis/proliferation
ID	Interdigital/superficial dermatitis	All kind of mild dermatitis around the claws, that is not classified as digital dermatitis
DS	Double sole	Two or more layers of under-run sole horn
HHE	Heel horn erosion	Erosion of the bulbs, in severe cases typically V- shaped, possibly extending to the corium
HFA	Axial horn fissure	Vertical crack in the inner claw wall
IH	Interdigital hyperplasia	Interdigital growth of fibrous tissue
IP	Interdigital phlegmon	Symmetric painful swelling of the foot commonly accompanied with odorous smell with sudden onset of lameness
SHD	Sole haemorrhage diffused form	Diffused light red to yellowish discoloration of the sole and/or white line
SHC	Sole haemorrhage circumscribed form	Clear differentiation between discoloured and normal coloured horn
SU	Sole ulcer	Ulceration of the sole area with penetration through the sole horn exposing fresh or necrotic corium
TU	Toe ulcer	Ulceration of the sole area located at the toe
WLF	White line fissure	Separation of the white line which remains after balancing both soles
WLA	White line abscess	Necro-purulent inflammation of the corium

Table 9:	Codes used for	documenting	clinical	findings	and	description	of	findings,	as	found	in	the
	ICAR Claw Hea	alth Atlas [173]	•									

In addition to the codes summarised in Table 9, the severity of the lesion was also documented in a 1 to 3 scale (1 = mild, 2 = moderate, 3 = severe). In the case of digital dermatitis, both the stage and the size of the lesion were documented. The stages were recorded according to Döpfer at al. (1997) and Berry et al. (2012) [174] [175] as summarised in Table 10.

Stage	Description
M0	Normal skin, no clinical signs of digital dermatitis
M1	Circumscribed epithelial lesions, <2 cm in diameter, red-grey in colour
M2	Acute, ulcerative or granulomatous lesions on digital skin, ≥ 2 cm, bright red, mostly painful on manipulation
M3	Ulcerated lesion covered in brown scab-like material, not painful on manipulation
M4	Chronic lesions with proliferative dyskeratotic or growths
M4.1	Chronic lesion displaying a M1 stage

Table 10:Digital dermatitis staging as by Döpfer et al. (1997) [174] and modified by Berry et al. (2012)
[175]

Functional claw trimming was performed on the claws of lame animals which were examined in the chute and corrective trimming was additionally performed on claws with pathological changes. Moreover, a foot block was applied if the lesion could not be sufficiently relieved with corrective trimming. Lesions which were also suspected of being affected by digital dermatitis were treated topically with Oxytetracycline spray. For acute and chronic lesions (M2, M3, M4 and M4.1) a cream containing salicylic acid was applied topically to the lesion under a padded bandage. In the case of interdigital phlegmon the cows were treated with systemic wide-spectrum antibiotics and non-steroidal anti-inflammatory drugs.

4.2.2.3 Daily locomotion scoring via video analysis

If a cow was classified as lame (LMS = 3) in a fortnightly locomotion scoring (FLMS), or if the animal was classified as "suspected lame" (LMS = 2) three FLMS in a row and showed a pain reaction, the animal was locomotion scored in the daily (DLMS) video recordings retrospectively to find the lameness onset date.

Due to the lack of visible identification on the animals, a cow-identification index was created for every farm, including the animal's identification number and pictures of the cow from as many different angles as possible in order to document all markings. For the project farms with a milking parlour, the lame animal was searched for in the video recording of either the morning or the evening milking using the MxManagementCenter software (Mobotix AG, Langmeil, Germany) according to its patterns and/or markings. On the farms with an AMS, the animals were found in the video recordings using the time stamp of the individual milking events documented in the management system.

The use of video analysis and the availability of daily locomotion scores for lame animals allowed for a high resolution for claw health data. The assessment of the exact day of lameness onset provided insight on the time span connected to lameness development.
4.2.2.4 Interpolation of locomotion scores

The focus of the DLMS was the transition between different locomotion scores and the development of lameness over time. The day of the lameness discovery was taken as a starting point and the video recordings were analysed retrospectively before that. Not every day was evaluated, so in order to have one LMS per day, rules for interpolation were previously established. An adequate interval for interpolation was chosen according to previous experience and adapted to every individual lameness case.



Figure 22: Illustration of examples of interpolation between two fortnightly locomotion scores. The DLMS of animals which were not lame were interpolated between two FLMS. If the locomotion scores were the same for two FLMS in a row, the DLMS between the two FLMS were interpolated to the same score (Case 1); so for $x = LMS \in (1-3)$, day_1 being the

day where the starting FLMS was assigned and day_n being the day of the next FLMS:

Case 1:
$$FLMS_{day_1} = FLMS_{day_n} = x$$
 (1)

then

$$\mathrm{DLMS}_{\sum_{i=2}^{n-1} day_i} = x \tag{2}$$

If the FLMS were one point apart (FLMS_{*day*1} = x, and FLMS_{*day*2} = x + 1 or FLMS_{*day*1} = x + 11, and $FLMS_{day_n} = x$), half the number of days separating the two FLMS would be interpolated as one score, the other half as the other (Case 2.1), unless the number of days separating the two scores was uneven (Case 2.2), in which case the majority of days would be attributed the lower score.

Case 2.1:
$$FLMS_{day_1} = x$$
 and $FLMS_{day_n} = x + 1$ (3)

and

$$\frac{\sum_{i=2}^{n-1} day_i}{2} \in \mathbb{Z},\tag{4}$$

 $\text{DLMS}_{\sum_{i=\frac{n}{2}+1}^{n-1} day_i} = x+1$

(5)

(9)

then

then
$$DLMS_{\sum_{i=2}^{n} day_{i}}^{n} = x$$
 and $DLMS_{\sum_{i=\frac{n}{2}+1}^{n-1} day_{i}} = x+1$ (5)
Case 2.2: $\frac{\sum_{i=2}^{n-1} day_{i}}{2} \notin \mathbb{Z}$ (6)

2

and

then
$$DLMS_{\sum_{i=2}^{\left(\frac{n-1}{2}\right)+1} day_i} = x$$
 and $DLMS_{\sum_{i=\left(\frac{n-1}{2}\right)+2}^{n-1} day_i} = x+1$ (7)

If the two FLMS were two scores apart ($FLMS_{day_1} = x$, and $FLMS_{day_n} = x + 2$ or $FLMS_{day_1} = x + 2$, and $FLMS_{day_n} = x$), the days separating the two FLMS would be divided by three (Case 3.1). If the number of days is not dividable by 3, the middle score (x + 1) would be attributed to $\left[\frac{n-2}{3}\right] + 1$ days (Case 3.2) and the DLMS interpolated accordingly as shown in an example in Figure 22.

Case 3.1:
$$FLMS_{day_1} = x$$
 and $FLMS_{day_n} = x + 2$ (8)

 $\frac{\sum_{i=2}^{n-1} day_i}{2} \in \mathbb{Z}$

and

and

then
$$DLMS_{\sum_{i=2}^{\left(\frac{n-2}{3}\right)+1} day_i} = x,$$
 $DLMS_{\sum_{i=\left(\frac{n-2}{3}\right)+2}^{n-\left(\frac{n-2}{3}\right)-1} day_i} = x+1$ (10)

$$\text{DLMS}_{\sum_{i=n-\left(\frac{n-2}{3}\right)}^{n-1} day_i} = x+2 \tag{11}$$

Case 3.2:
$$\frac{\sum_{i=2}^{n-1} da y_i}{3} \notin \mathbb{Z}$$
(12)

then
$$DLMS_{\sum_{i=2}^{\left[\frac{n-2}{3}\right]} day_i} = x,$$
 $DLMS_{\sum_{i=\left[\frac{n-2}{3}\right]+1}^{n-\left[\frac{n-2}{3}\right]-1} day_i} = x+1$ (13)

and
$$DLMS_{\sum_{i=n-\left\lfloor \frac{n-2}{3}\right\rfloor}^{n-1} day_i} = x+2$$
(14)

A limit of 21 consecutive days was decided as the maximum number of days that should be interpolated between two FLMS for the same animal. The automatically recorded data from animals that were not milked for more than 21 days, for example when dried off or if excluded from milking due to illness, were not analysed for that period. To define this 21-day

limit for interpolation, the difference in days was calculated between all consecutive FLMS for all animals, and a probability density function was used on the calculated differences. The density function was then plotted using the ggplot2 package in RStudio as shown in Figure 23. The peaks with the highest density are between seven and 21 days difference between two FLMS, with a high peak at 28 days. The peak at 28 days was due to a visit on CDF3 which could not take place for organisational reasons. For this visit, the video recordings were analysed and the animals that were a score 2 or 3 were identified using the animal identification index. All other animals on CDF3 did not have a FLMS for this visit.





4.2.3 Technology validation

At the beginning of the project, the data collection methods and technology were tested and validated to ensure and accurate recording of behaviour and claw health data.

4.2.3.1 Validation of measurement of lying behaviour

The validation of the pedometers regarding the measurement of lying behaviour took place as part of a master thesis [176] between February and July 2017. The lying times measured by direct observation were compared to lying times measured by the ENGS "Track a Cow"

pedometers. 26 animals from the RFG were randomly selected and observed for 30 hours over five days. The selected animals were all fitted with pedometers on their right front limb and were marked with numbers on their haunches for visual recognition.

The observer recorded the lying times of the animals from a platform inside the barn and the time each animal took in the process of lying down and standing up using an HTML page on a tablet computer which converted the input data into a text file. The data were then analysed using Microsoft Excel 2010 [177] and R [178] in RStudio [179]. Due to the data collected by the pedometers being summarised into hourly values (minutes lying per hour and number of lying bouts per hour) in the Access database, the data collected by direct observation (DO) was summarised in the same way to allow for comparability. The values compared between DO and pedometers were the sum of the lying duration per hour, defined for the DO as the sum of all minutes the observed animal was in a recumbent position with bent tarsal and carpal joints, and the sum of lying bouts per hour, defined as the number of changes between the positions "lying" and "standing", where all four of the animal's limbs were stretched.

4.2.3.2 Validation of measurement of feeding behaviour

The validation of the pedometers regarding the measurement of feeding behaviour also took place as part of a master thesis [180] and the data were then further analysed after the data collection phase of this study. The accuracy of the recorded feeding behaviour was also measured by comparison to DO. The DO took place between June and July 2017 on all the commercial dairy farms in the project, as well as on the RFG. 21 cows were observed for a total of 120 hours. The cows on the farms were divided into groups according to the position that the pedometers were in on the animal's limb at the beginning of data collection as illustrated in Figure 24. The distinction between the different pedometer positions was made in order to account for possible inaccuracy of the individual animal recognition at the induction loop, as had been indicated by the manufacturer beforehand. One animal was randomly selected per group per farm. Each animal was observed singularly for a total of six hours each divided into two three-hour sessions.



Figure 24: Pedometer positions used to divide the animals in different observation groups during the validation of the measurement of feeding behaviour [180].

Similarly to the validation of the measured lying times (see 4.2.3.1), for the validation of the measured feeding behaviour the data collected by DO was also summarised into hourly values

(number of minutes feeding per hour and number of visits to the feeding table per hour) to make it comparable to the data collected by the pedometers.

To define how accurately the animals were detected by the induction loop installed along the feeding tables on all the project farms, possible positions of the animal in relation to the feeding table were defined before beginning the observation. The four different behavioural feeding parameters are summarised in Table 11.

Behavioural parameter	Definition
Feeding (F)	Cow's front limbs $0 - 0.2$ m behind feeding table with
	head through feeding fence, front limb inside the magnetic field of the induction loop
At feeding fence (AFF)	Cow's front limbs $0 - 0.2$ m behind feeding table with head not through feeding fence, feet still inside the magnetic field of the induction loop
Near feeding fence (NFF)	Cow's front limbs $0.2 - 1m$ behind feeding table (outside the magnetic field of the induction loop)
Outside induction field	Cow's front limbs over 1m distance from feeding table (outside the magnetic field of the induction loop)

 Table 11: Different behavioural parameters defined for direct observation and validation of measured feeding behaviour.

The observations took place either from the feeding table or from an elevated platform inside the barn. The data were entered in an app programmed for this purpose and used on a smartphone. The raw data were then analysed using Microsoft Excel 2010 [177] and R [178] in RStudio [179].

In the data analysis a comparison was made between DO and feeding behaviour measured by the pedometers by summarising different recorded positions of the animal (Table 11) as a reference method, in order to determine the possible sources of inaccuracy of the pedometers, i.e. if they were recording the animal feeding, when it was actually only standing near the induction loop. The comparison was made between feeding times measured by DO when the cow was in position F ("feeding") (case 1), and a second comparison was made by summarising the positions F, AFF ("at feeding fence") (case 2) and then F, AFF, NFF ("near feeding fence") were summarised (case 3). For the FV, the same system was applied as for the FD and additionally a fourth case was compared, where the visits recorded by DO were summarised according to the six minutes criterion used by the pedometers' algorithm (Case 4).

4.2.3.3 Validation of locomotion scoring system

As well as validating the technology used in the study, the LMSSGL was also tested for overall consistency. Both inter-observer agreement and reliability and the intraobserver

reliability tests as well as the comparison between live (DO) and video locomotion scoring (VO) were carried out for the three-point LMS.

The inter-rater agreement determines the extent to which two raters agree on the evaluation of different observations, while the inter-rater reliability refers to the consistency with which raters differentiate between observed items. A high level of agreement does not imply a high level of reliability and vice-versa [181, 182]. Intra-rater reliability is used to define the level of consistency of a rater evaluating the same observations over time [183].

For the inter-rater agreement 475 cows were scored by three observers respectively in pairs. The scoring occurred both live and through video recordings on separate days. For the intraobserver reliability, 430 locomotion scores were performed on 215 cows on multiple occasions, using both repeated viewings of the same video recording and viewing recordings of a locomotion score that was originally carried out by direct observation.

4.3 Data processing

The data analysis phase of this study started after finishing data collection in autumn 2018. The first step was the integration of data from different sources into one combined dataset and the creation of daily records for each animal and each recorded variable. The finished dataset was then checked for implausible and daily records were removed that had no value for LMS or no data from the pedometers.

4.3.1 **Development of day records**

In order to create a single dataset containing daily observations for each animal spanning over the data collection period, data from every source later used in the analysis were homogenised and combined using a PostgreSQL database management system. An overview of the data sources is shown in Figure 25.

Each observation refers to one day between 00:00 and 24:00 and contains values for each of the 42 variables listed in Table 12 for each cow present and being milked at that time on the farm. As data was collected over different time spans for each of the project farms, the animals have a different number of day records. For CDF1 and CDF2 an observation was created for each day between 01.02.2017 and 18.04.2018, for CDF3 between 01.02.2017 and 19.04.2018, for CDF4 observations were created for every day between 01.07.2017 and 27.10.2017 and for the RFG between 01.01.2018 and 18.06.2018.



Figure 25: Flowchart illustrating the external data sources that were included in daily records. AMS: automatic milking system, LKV: breeding refinement association.

Variable name	Explanation	Format	Unit	Source 1	Source 2	Definition
В	Farm	factor				Farm number
TN	Animal number	factor		ENGS		Animal's farm identification number
BTN	Farm and animal number	factor				Combination of animal and farm numbers
Date	Date	date	yyyy/mm/dd	ENGS		Date of data collection
Р	Parity	integer		ENGS	LKV	Current lactation number
DIM	Days in milk	integer		ENGS	LKV	Progressive number indicating number of days since calving
MY	Milk Yield	numeric	kg	AMS		Sum of each milking on this day
MI	Milking interval	numeric	hh:mm:ss	AMS		Longest time interval between two milkings on current day
MY305	Milk yield of last lactation	numeric	kg	LKV	AMS	Cumulative milk yield from beginning to end of last lactation
MYM	Monthly milk yield	numeric	kg	LKV		Result of monthly milk tests
MMY	Average monthly milk yield	numeric	kg	LKV	AMS	Average monthly milk yield calculated using MYM and MY
LW	Live weight	numeric	kg	AMS		Median value of all weighing results for current day
BCS	Body condition score	factor		AMS		Median value of all BCS measurements for current day
LD	Lying duration	numeric	min	ENGS		Sum of lying duration for current day
LDR	Lying duration (day/night)	numeric		ENGS		Sum of lying duration during daytime
LBN	Number of lying bouts	integer		ENGS		Number of lying bouts for current day
LBNR	Number of lying bouts (day/night)	numeric		ENGS		Number of lying bouts during daytime
LDB	Lying duration per bout	numeric	min	ENGS		Sum of lying duration divided by number of lying bouts for current day

 Table 12:
 List of variables of the finished data set with explanation of the variable, format, unit, and source.

Table 12 (continuation): List of variables of the finished data set with explanation of the variable, format, unit, and source.

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Variable name	Explanation	Format	Unit	Source 1	Source 2	Definition
AC	Activity index	numeric		ENGS		Activity index
ACR	Activity index (day/night)	numeric		ENGS		Activity index during daytime
FI	Feed intake	numeric	kg	Weighing troughs		Sum of roughage intake of all visits to the weighing troughs on current day.
FD	Feeding duration	numeric	min	ENGS		Sum of intake minutes
FDW	Feeding duration weighing troughs	numeric	min	Weighing troughs		Sum of feeding duration of all visits to the weighing troughs
FDR	Feeding duration (day/night)	numeric		ENGS		Sum of intake minutes during daytime
FDRW	Feeding duration weighing troughs (day/night)	numeric		Weighing troughs		Sum of feeding duration of all visits to the weighing troughs during daytime
FP	Feeding pace	numeric	kg/min	Weighing troughs		Roughage intake/feeding duration
MN	Number of meals	integer		Weighing troughs		Number of meals at the weighing troughs
MNR	Number of meals (day/night)	numeric		Weighing troughs		Number of meals during daytime
FDM	Feeding duration per meal	numeric	min	Weighing troughs		Sum of feeding duration at the weighing troughs/number of meals
FIM	Feed intake per meal	numeric	kg	Weighing troughs		Feed intake/number of meals
VN	Number of visits to the trough	integer		Weighing troughs		Number of visits to the trough
VNR	Number of visits to the trough (day/night)	numeric		Weighing troughs		Number of visits to the trough during daytime
FDV	Feeding duration per visit	numeric	min	Weighing troughs		Sum of feeding duration at the weighing troughs/number of registrations at the weighing troughs

 weighing troughs

 Table 12 (continuation): List of variables of the finished data set with explanation of the variable, format, unit, and source.

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Variable name	Explanation	Format	Unit	Source 1	Source 2	Definition
FIV	Feed intake per visit	numeric	kg	Weighing troughs		Feed intake/number of registrations at the weighing troughs
C_MN	Clustered number of meals	numeric		ENGS		Number of visits to the feeding table
C_MNR	Clustered number of meals (day/night)	numeric		ENGS		Number of visits to the feeding table during daytime
C_FDM	Clustered feeding duration per meal	numeric		ENGS		Feeding duration/number of visits
LMS	Locomotion score	factor		Excel table		Locomotion score
F_LMS	Frequency locomotion score	factor		Excel table		0 = daily (video observation) 1 = fortnightly (video observation) 2 = interpolated (video observation) 3 = score on farm visit (direct observation)
K_LMS	Correction reason locomotion score	factor		Excel table		0 = not corrected 1 = corrected following pain test 2 = corrected following new video analysis 3 = corrected following clinical examination 4 = corrected following notification from farmer and clinical examination
B_LMS	Locomotion score before correction	factor		Excel table		Locomotion score before correction
РТ	Pain test	factor		Excel table		99 = no pain test 0 = negative pain test 1 = positive paint test

AMS: automatic milking system, ENGS: pedometers, LKV: breeding refinement association.

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4.3.1.1 Master data and dates

The master data that flowed into the final dataset included the number of the farm (B), the identification number of the animal (TN) as used by the farmers, and the combination of these two variables (BTN). The date (Date) referred to the date of data collection.

4.3.1.2 Reproductive data, lactation data and condition

The reproductive data included parity (P), a consecutive number starting at the first calving and increasing at every new calving event, and the days in milk (DIM), a consecutive number starting on the first day of lactation (day of calving) and increasing once a day.

The lactation data provided by LKV included the monthly milk yield (MYM), a daily value extrapolated from the results of the milk test carried out eleven times a year for each lactating cow. For each date the MYM corresponded to the result of the most recent milk yield test, meaning the result of a milk test was applied in average 16.6 days prior and 16.6 days after the actual date of the milk test. The milk tests were carried out twice on the same date, so the result is the average between morning and evening milking. The milk yield of the last lactation (MY305) referred to the total milk yield at 305 days in milk. The milking interval (MI) and the daily milk yield (MY) were not available for CDF1, CDF2 and CDF3, while for CDF4 and for the RFG these data were both provided by the AMS and accessed through the Dairy Plan software. In order to have a performance variable in terms of milk yield comparable for all farms, the variable MMY (average monthly milk yield) was created by computing the average MY per month for RFG and CDF4 and using the value for MYM for CDF1, 2 and 3. The milking interval (MI) is defined as the longest interval between two consecutive milking events of the current day. If a milking event started before 24:00 and finishes after 00:00 the milk yield is distributed equally between the two days. The same principle was applied to the feed intake (FI), if the intake time spanned between two days.

The data for the variables live weight (LW) and body condition score (BCS) were only available for the RFG, where the animals were weighed every time they entered the AMS and their body condition was analysed by software connected to cameras inside the AMS (Body Condition Score Camera, DeLaval, Sweden). The daily value corresponded to the median of all measured values for that day.

4.3.1.3 Lying behaviour data

Lying behaviour data were collected continuously for all animals on all farms. The lying duration (LD) is the sum of minutes the animal spent lying per day, not counting interruption of < 8 seconds, which wouldn't be captured by the pedometers' algorithm. The number of lying bouts (LBN) corresponds to the number of changes from the position "standing" to the position "lying" made on the specific date (see 4.2.1.1). All variables containing a day/night

ratio (LDR, ACR, FDR, FDRW, MNR, C_MNR and VNR) refer to the total number of minutes for the respective behaviour on the respective date that occurred in during daytime. Daytime was defined differently on each farm, in order to take into account the different routines influencing the animals' day/night rhythm. To define daytime, the median values for each hour of the day for all animals present on the farm were calculated between 01.04.2017 and 01.04.2018 (CDF1, CDF2 and CDF3), between 01.04.2017 and 01.11.2017 (CDF4) and between 01.11.2017 and 01.07.2018 (RFG) for the behaviour parameter "lying", "activity" and "feed intake" respectively. The overall median for each behavioural parameter was then calculated for each farm. The beginning of "daytime" is defined as the first hour in which the hourly median activity index is higher as the overall median activity index (Med_{activity indexhourly} > Med_{activity was chosen as a defining parameter for daytime as it showed a higher variation (calculated as the SD in relation to the mean) compared to the lying duration. The period of daytime for each farm can be seen in Figure 26.}



Figure 26: Daytime periods for each farm

4.3.1.4 Feeding behaviour data

Some of the variables regarding feeding behaviour (FI, FDW, FDRW, FP, MN, MNR, FDM, FIM, VN, VNR, FDV, FIV) were only available for the RFG as the data were recorded by the weighing troughs. The number of meals (MN) is defined as the number of loggings into the weighing troughs spaced less than 20 minutes apart. The feeding duration per visit (FDV) is calculated by dividing the feeding duration at the weighing troughs (FDW) by the number of

loggings at the weighing troughs (MN). The clustered feeding duration per meal (C_FDM) is defined as the feeding duration (FD) divided by the clustered number of meals (C_MN) for the pedometers.

4.3.1.5 Claw health data

The manually collected claw health data was entered into Microsoft Excel 2010 tables and then integrated into the final dataset. The locomotion scores (LMS) were denoted according to the occasion in which they originated (F_LMS). LMS that originated as FLMS were recorded as $F_LMS = 1$, DLMS from the video recordings were marked $F_LMS = 0$ and LMS which were interpolated were recorded as $F_LMS = 2$. Finally, the LMS given to cows on the day of the farm visit, for example in cases in which the cow was too lame to be milked with the herd and did not appear in the video recordings, were marked $F_LMS = 3$. Some LMS were corrected, so both the reason for correction (K_LMS) and the LMS before correction (B_LMS) were recorded. The correction reason was either a positive pain test (K_LMS = 1, see 4.2), a subsequent viewing of the video recordings (K_LMS = 2), a clinical examination (K_LMS = 3), or the result of the cow being reported lame by the farmer and not being recognised as such in a first scoring and subsequently changed if confirmed lame in the video recordings of the previous days (K_LMS = 4). Finally, the occurrence of a pain test was recorded (PT) and whether it was positive (PT = 1) or negative (PT = 0).

4.3.2 Statistical analysis

4.3.2.1 Validation of methods

All data were analysed using RStudio [179]. For the validation of the pedometers, statistical summaries of the observed and measured values per hour, as well as of the differences between observed and measured values, were calculated for each observation hour. The distribution of the data was tested visually using histograms and then transformed using a log transformation to achieve near-normal distribution. A Bland-Altman-Plot of the data was the produced using the blandr package [184] and the limits of agreement computed by the function were used to define the outliers. The measured data was then plotted against the observed data and the correlation was calculated using the concordance correlation coefficient (ρ_c) [149] for the FD and LD and for Kendall's coefficient of concordance (W) for the FV and LB. ρ_c corresponds to Pearson's correlation coefficient with a bias correction factor that indicates the distance of the best fit line from a 45° angle line through the origin and has values of either 0 (no concordance), +1 (perfect agreement) or -1 (perfect disagreement). W on the other hand is a value between 0 and 1, where 0 corresponds to a level of agreement equivalent to chance, 1 represents perfect positive agreement. The Kruskal-Wallis test [185] was performed on the feeding data to calculate the statistical significance of the differences between data from pedometers in different positions.

To quantify the level of inter-rater and intra-rater reliability for the LMSSGL the percentage of agreement (PA) was calculated, as well as Cohen's Kappa (κ) with squared weighting, according to current literature [19] (see 2.6.1). κ is a measure of agreement for categorical data that takes into account the possibility of chance agreement and has values between -1 and 1, where 1 represent perfect agreement and values ≤ 0 represent poor agreement [186, 187].

4.3.2.2 Lameness development and clinical data

To analyse the LMS results and the data collected during farm visits and clinical examinations, a statistical summary with the number of observations, the mean, median, maximum and minimum values as well as the first (q25) and third quartile (q75) and the standard deviation (SD) was performed for all farms, months, seasons and for the different diagnoses and locomotion scores. The distribution of the data was analysed visually with the use of histograms and boxplots. The correlation between LMS and findings was tested using Spearman's rank correlation coefficient (ρ) corrected for ties.

A Poisson regression for dependant samples was performed to test for statistically significant interactions within farms and seasons for the LMS and the clinical findings. The Poisson regression is a form of regression analysis suitable for analysing count data.

4.3.2.3 Univariate analysis

For the analysis of the variables in the final dataset that was then used for the for the prediction models, statistical summaries with the number of observations, the mean, median, maximum and minimum values, the first (q25) and third quartile (q75) and the standard deviation (SD) was performed for each farm and across farms. The distribution of the variables and the presence of outliers was checked using histograms and boxplots. An example of the type of boxplot used is shown in Figure 27. The black dots represent the outliers, which lie 1.5 *IQ (interquartile range) above the third quartile or below the first quartile. The middle line of each box represents the median, the top whisker represents the third quartile plus 1.5*IQ, while the bottom whisker is the first quartile minus 1.5*IQ. The length of the box is the third quartile minus the first quartile and represents the IQ.

For variables which were not normally distributed, a transformation was attempted using the common logarithm, or the square root transformation. The variables were tested for homogeneity of variances using Levene's test [188].

In the univariate analysis, the relation between the single variables for the outcome lame was investigated; statistically significant differences between LMS groups were computed using the non-parametric Kruskal-Wallis test [185]. Additionally, a post-hoc analysis was then performed to investigate the significance of the differences between the individual LMS

groups using the Wilcoxon signed rank test, a non-parametric statistical hypothesis test that can be used when the tested samples are related [189].



Figure 27: Example of the type of boxplot used in this study IQ: interquartile range, q25: first quartile, q75: third quartile

Furthermore, binomial generalised logistic regression was performed for each variable with the outcome lame/not-lame and the coefficients extracted to compute the odds ratio (OR) and their significance. The OR is estimated by calculating the ratio between the probability of binary outcome (such as lameness) being positive, and the probability of the outcome being negative [190].

To check for collinearity between variables, a correlation matrix for all variables was calculated using Kendall's rank correlation coefficient (τ). Kendall's rank correlation coefficient is a non-parametric test used to compute the level of dependence between two variables based on ranks and has values between 0 (no correlation) and 1 (perfect correlation) [191].

4.3.2.4 Multivariate analysis: Model formulation

One of the aims of this study was the application of statistical modelling to the automatically and manually collected data to predict cases of lameness. After the data was summarised in the final dataset, it was cleaned and prepared for the application of a predictive model to all variables listed in Table 12.

Initially, all variables were checked for missing values and were excluded from the model if they had > 5 % NA (not available) values. Following transformation, the data was centred and scaled; the mean of each variable was subtracted from the variable itself, meaning the means of all variables were zero, and then each parameter was divided by its SD [192]. Centring is applied to data to distribute the variation of the variables around zero as opposed to around the means of the variables and also improves interpretability. Scaling data on the other hand is important to adjust the units of the data so that all variables are of equal relevance in the dataset. In the case of LW and LBN for example, where LW has mean values around 750 kg and LBN has a mean around 23 units, both parameters have the same level of relevance for the outcome lame and should be scaled to have values in the same range.

Two dummy variables were added to the dataset as the outcome to be predicted by the regression; one indicated the outcome lame/not-lame using LMS = 3 as a threshold for lameness (L3), meaning animals with LMS = 2 and LMS = 1 would be considered not lame, while the other variable had LMS = 2 and LMS = 3 coded as positive lameness outcome (L23).

The data was then split into a training set (60 % of the data) and a test set (40 % of the data). The frequency of the occurrence of a case of lameness (defined as L3 = 1 or L23 = 1) was calculated, and due to the infrequency of positive cases (lame) compared to control cases (sound), the data was balanced before applying the model. Unbalanced data is in fact a frequent problem, especially in epidemiological studies, and can bias predictive models and lead them to treat cases in the minority group as random noise and concentrate on the classification of the sample in the majority group thus causing misleading results in terms of predictive accuracy [193]. The technique applied for balancing the data was the Synthetic Minority Oversampling Technique (SMOTE) [194]. SMOTE was applied to the training dataset to randomly create new observations by using the k-nearest neighbours algorithm, thus oversampling the minority class, in this case the lame animals, by creating synthetic observations as opposed to creating new observations by replacement [194].

In a first step, the data was analysed across all farms accounting for the farm and the individual animals as random effects that could influence the variance within the respective groups, for example within farms. To improve the accuracy of the model, interaction terms between the variables were also added. Because a model with a high number of regressor can

reduce the accuracy of the coefficient estimates, a variable selection process was applied to find statistically significant relationships between the variables that could improve the model's accuracy. Forward stepwise logistic regression is a method of variable selection that chooses predictors based on the level of significance of their coefficient when added to the model [195]. Starting with a base model, predictors are added by the algorithm and the significance of all estimate coefficients in the model is revaluated. If the new predictor added to the model is not statistically significant or reduces the significance of other predictors, it is removed again. This method enables screening of the statistically significant variables that add to the accuracy of the model, while keeping the number of parameters at the minimum possible.

The data for all farms with the random effects and interaction terms was then modelled using a generalised linear mixed model (GLMM). Generalized linear models are an extension of linear models that allow for the response variable to not present with a normal distribution [196]. This is the case for example for count data, or for binomial data such as the response variable lame/not-lame in the case of this study. GLMM on the other hand incorporate random effects which can cause data grouped by a parameter, such as the animals in a herd, to be correlated and to have a variance different from the overall variance [197]. The intraclass correlation coefficient (ICC) of the random effects was calculated within the GLMM to interpret the level of interference in the overall variance of the data caused by the random effects. The ICC has a value between -1 and +1 and is calculated by dividing the between-group variance by the total variance and measures the extent to which observations within a cluster or group, defined by the random effect, are correlated with one another [198, 199].

Modelling was also carried out on the data from the individual farms by applying the Elastic Net regression with the ENET Beta approach proposed by Liu and Li (2017) [167, 200]. Elastic Net regression is a form of variable selection and regularisation proposed by Zou and Hastie (2005) [167] which combines the penalties of the ridge regression and of the lasso technique. Ridge regression minimizes the residual sum of squares by continuously shrinking the coefficient estimates [201], while lasso also allows variable selection for a more sparse and therefore more interpretable final model [202]. The Elastic Net both shrinks coefficients and performs a selection of the variables in the model, and is suitable for datasets with correlated variables [167]. The Elastic Net regression features two tuning parameters, λ_1 and λ_2 , which regulate the shrinkage and choice of parameters included in the model [203]. The elastic net penalty is defined as $(1-\alpha)|\boldsymbol{\beta}|_1+\alpha|\boldsymbol{\beta}|^2$, where $\alpha \in [0,1]$ and when $\alpha = 1$, the regression becomes a ridge regression, while when $\alpha = 0$ the regression is a lasso regression [167].

First, a base model was created for each farm using forward stepwise regression on the balanced dataset and all possible interaction terms to determine relevant interactions. Then,

the cv.glmnet function from the glmnet package in RStudio was applied to the data [179, 204]. The cv.glmnet function supports two tuning parameters; α , which controls the penalty between ridge ($\alpha = 0$) and lasso ($\alpha = 1$), and λ , which regulates the overall strength of the penalty [204]. Ten-fold cross validation was then applied to the Elastic Net model to find the value of lambda with the lowest mean absolute error. The predictors of the cross validated model were added to the model one by one in descending order of strength of the corresponding coefficients. Subsequently, the Brier Score was computed for each model created. The Brier Score (BS) measures the accuracy of predictions using the mean squared difference between predicted probability for the outcome lame/not-lame for each observation and the actual outcome [205]. The lowest BS was then divided by the BS of each individual model and the model whose BS ratio was ≥ 0.9 was selected as final, thus sacrificing prediction accuracy for a sparser model with a higher level of interpretability [200]. The same model selection procedure carried out for the data on the individual farms was also carried out on the data across farms but without the random effects from the regression.

The model performance was assessed by the Receiver Operating Characteristics (ROC) Curve analysis. The ROC is a plot of the true positive rate against the false positive rate and measures test accuracy in terms of Area Under the Curve (AUC) [206]. A test with perfect prediction accuracy will have an AUC = 1, while a test whose prediction accuracy corresponds to chance will have the lowest possible AUC (0.5). The ROC also determines the threshold for optimal specificity (SPE) and sensitivity (SEN) levels, specificity being the true negative rate and sensitivity the true positive rate of a predictive algorithm.

5 **Results**

5.1.1 Data cleaning

The dataset with the daily performance and behavioural parameters contained 102372 daily values and 40 variables from n = 630 animals. All daily observations were filtered, that were considered implausible and in which at least one of the following conditions was true: LD < 30 minutes, LBN = 0, FD = 0 or FDW = 0 minutes, AC < 10, $C_MN = 0$, MN = 0, MY > 50 or MYM > 50 kg, and DIM > 742. The limit for the MY and MYM were chosen as plausible upper limits for daily MY or average MYM and the limit for DIM was set by multiplying the IQ by three and adding it to the upper quartile value. The limits for the feeding and lying behaviour and for the activity were chosen based on estimates of what value could be considered realistic for the given behaviour. The final dataset contained 73220 observations from n = 383 animals. Figure 28 shows the amount of missing data in the final dataset after exclusion of implausible data. Most missing values can be traced back to the data being collected from the weighing troughs and body condition (BCS and LW), which were only available on RFG, or the daily milk yield and MI, which were only available from CDF4 and RFG. Values for K_LMS and PT were only entered in the dataset if a pain test had been carried out or if the LMS had been corrected and were otherwise left empty.



Figure 28: Representation of missing data in the final dataset variables.

AC: activity, ACR: activity during daytime, BCS: body condition score, C_FDM: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feed intake, FP: feeding duration during daytime measured by weighing troughs, FIX: feed intake per visit, FIV: feed intake per visit, FIM: feed intake per meal, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MYR: number of meals during daytime measured by the weighing troughs, MY: monthly milk yield average, P: parity, PT: pain test, VN: number of visits measured by the weighing troughs, VNR: number of visits during daytime measured by the weighing troughs.

Of the n = 630 cows that had appeared with at least one LMS, n = 191 were excluded at the beginning of the analysis due to implausible or missing data from the pedometers (see 5.5). For the analysis of lameness development n = 10 cows were excluded due to their identification number recorded during LMS not corresponding to any animals present in the herd at that time, while n = 19 cows were excluded due to the absence of particular markings or coat patterns that allowed for visual identification in the video recordings. N = 8 cows were not included in the analysis as there were no Dairy Plan milking data to identify them in the video recordings of them exiting the AMS.

An overview of data collection time span, the number of visits carried out on the farms, the number of locomotion scores (LMS) performed at the fortnightly visits the farms and the number of animals clinically examined is shown in Table 13.

			Farm			
	CDF1	CDF2	CDF3	CDF4	RFG	Total
Duration of DC	04.2017-	04.2017-	04.2017-	08.2017-	01.2018-	-
	04.2018	04.2018	04.2018	11.2017	06.2018	
No visits	26	26	25	5	13	95
No LMS	33154	24569	30143	5076	10529	103,471
No FLMS	2594	1945	2549	445	853	8,386
No $F_LMS = 2$	28,970	22011	27328	4305	9104	91,718
No. DLMS	1582	599	261	290	572	3,304
No AS	155	117	180	92	87	631
No CE	136	62	22	47	46	313
No AE	68	39	19	28	35	189

Table 13: Overview of data collection

DC: data collection, LMS: locomotion score (total LMS); FLMS: fortnightly locomotion scores, $F_LMS = 2$: interpolated scores, DLMS: daily locomotion scores, AS: animals scored, CE: clinical examinations, AE: animals examined, CDF1 – CDF4: commercial dairy farm 1 – 4, RFG: research farm.

The number of visits on CDF3 was lower than on CDF1 and CDF2 despite the same data collection time span due to an impediment on 29.10.2017 which prevented the visit from taking place that week. Locomotion scoring was performed by analysing video footage of the milking on 29.10.2017 and by searching for the animals which were lame or suspected lame. These cows were then identified using the animal identification list (see 4.1.1) for CDF3. On CDF4 on two occasions (25.09.2017 and 09.10.2017) it was not possible to carry out a farm visit; the LMS for those dates were carried out nonetheless by means of video footage.

5.2 Validation of technology and methods

5.2.1 Validation of lying behaviour recording

In order to verify the accuracy of the pedometers regarding the measurement of lying behaviour, the recorded lying duration (LDP) was compared to data collected by DO (LDO) (see 4.2.3.1). Firstly, the lying duration in minutes per hour was compared between DO and pedometers. The results of the summary statistics of the n = 271 observations are shown in Table 28 in the Annexe. The mean difference between LDO and LDP was -1.5 minutes, indicating that the pedometers measured on average longer lying times than those recorded by DO. In Figure 29 the LDO and LDP observations are represented in a scatter plot in which the outliers are highlighted as blue dots. To assess whether the pedometers were consistent with the method of reference (DO) the concordance correlation coefficient (ρ_c) [149] was calculated. The ρ_c for the LD was 0.96 and the best fit line had a 6 % location shift and a 2 % scale shift. Koch and Spörl (2007) suggest a heuristic interpretation of the concordance correlation coefficient and indicate $0.81 \le \rho_c \le 1$ as an "almost perfect" level of agreement [207].



Figure 29: Scatter plot showing the lying duration measured by direct observation plotted against the lying duration measured by the pedometers. The blue dots represent the outliers. n = 271 observation hours

The number of lying bouts (LB) measured by DO (LBO) and by the pedometers (LBP) was also compared (see Table 29 in Annexe). On average, the pedometers measured more lying bouts than the observer. To quantify the level of agreement between the pedometers' and the observer's measurement of LB Kendall's coefficient of concordance (W) [208] was calculated and showed a good level of concordance (W = 0.80).

5.2.2 Validation of feeding behaviour recording

The results of the summary statistics for the analysis of the FD are shown in Table 30, while the summary statistics of the differences calculated between FD by DO (in cases 1, 2 and 3) and FD measured by pedometers are shown in Table 14. On average the pedometers measured almost two minutes less feeding time than the observer for Case 1, 2.49 minutes less for Case 2 and almost seven minutes less for Case 3. A visual representation of the comparison between FDP and FDO in Case 1 is shown in Figure 30. The red dots represent the outliers. The concordance correlation coefficient for the feeding duration was $\rho_c = 0.87$ for Case 1, $\rho_c = 0.86$ for Case 2 and $\rho_c = 0.78$ for Case 3.

	Differences (FDP – FDO) in min/h								
FDP - FDO	n	Min	q25	Median	q75	Max	Mean	SD	
Case 1	91	-39	-2	0	1	6	-1,84	6,94	
Case 2	91	-39	-2	0	1	5	-2,49	7,3	
Case 3	91	-40	-9,5	-3	0	2	-6,38	8,81	

 Table 14:
 Summary statistics of differences between feeding duration measured by direct observation (cases 1, 2 and 3) and feeding duration measured by pedometers (in minutes per hour).



Figure 30: Scatter plot showing the observed feeding duration plotted against the feeding duration measured by the pedometers. The blue dots represent the outliers. n = 91 observation hours.

The non-parametric Kruskal-Wallis test was performed for the differences between FDP and FDO for each case and showed a statistically significant (p < 0.05) influence of the pedometer position on the discrepancy between DO and pedometer measurements for Cases 1 and 2. The influence of the pedometer position can also be seen in Figure 31, where the absolute differences are summarised according to the pedometer position for each Case. The differences between FDP and FDO are particularly pronounced for the pedometers in the positions "11 o'clock" and "12 o'clock".



Figure 31: Barplot of the sum of the absolute differences between feeding duration measured by the pedometers and by direct observation (in minutes per hour) for each case. n = 91 observation hours

Case 1 (position "feeding", red), case 2 (positions "feeding" and "at feeding fence", green) and case 3 (positions "feeding", "at feeding fence" and "at feeding table", blue), P: pedometers, DO: direct observation.

The results of the summary statistics of the differences between number of FVP and FVO per hour are shown in Table 15. The difference between FVP and FVO for Case 4 was minimal, with only 0.04 cases' difference per hour, while Case 2 had the most pronounced difference, with an average of 4.53 visits per hour more measured by DO than by the pedometers. To quantify the level of agreement for the FV, Kendall's concordance coefficient (W) was calculated and was W = 0.65 in Case 1, 0.66 in Case 2, 0.71 in Case 3 and 0.79 in Case 4. The position of the pedometers did not have any statistically significant effect of the difference between FVP and FVO (p = 0.67 for Case 1, p = 0.75 for Case 2, p = 0.41 for Case 3 and p = 0.23 for Case 4).

				Difference	es (FVP	– FVO)	visits/h		
FVP - FVO	n	Min	q25	Median	q75	Max	Mean	SD	W
Case 1	91	-15	-7,5	-3	-1	0	-4.44	4.35	0.65
Case 2	91	-17	-7	-3	-1	0	-4.53	4.45	0.66
Case 3	91	-10	-3	-1	0	1	-1.89	2.4	0.71
Case 4	91	-1	0	0	0	2	0.04	0.61	0.79

 Table 15:
 Summary statistics of differences between feeding visits measured by pedometers and by direct observation (in visits per hour).

Case 1: position "feeding", case 2: positions "feeding" and "at feeding fence", and case 3 positions "feeding", "at feeding fence" and "near feeding fence", FVP: feeding visits measured by pedometers, FVO: feeding visits measured by direct observation, Min: minimum, Max: maximum, q25: first quartile, q75: third quartile, SD: standard deviation, W: Kendall' coefficient of concordance.

5.2.3 Validation of the locomotion scoring system

To quantify the level of inter-rater and intra-rater reliability for the LMSSGL the PA was calculated, as well as Cohen's Kappa (κ) with squared weighting, according to practice in current literature [19] (see Table 3). The results for the inter-rater reliability test are shown in Table 16. The methods DO and VO are considered separately; showing only a slight difference in level of agreement between observers. Cohen's Kappa was calculated using square weighting and resulted in $\kappa = 0.423$ for the video observation and $\kappa = 0.606$ for the LMS by DO.

 Table 16:
 Inter-rater reliability by method of observation.

Parameter	Direct observation	Video observation
n	244	231
PA	77.9 %	80.1 %
к	0.606	0.423

PA: percentage of agreement, κ: Cohen's Kappa, N: number of observations.

For the intra-rater reliability, κ was 0.6 and the PA was 82.3%. The jitter plot in Figure 32 illustrates the distribution of the scores in the different viewings for the purpose of illustrating the level of intra-rater reliability. Each dot represents an animal (n = 430) and its position in the graph represents the scores assigned respectively in the first and second viewing. The green dots represent concordant scores for the first and second viewing. The discordant scores, represented by the red and blue dots, are more concentrated around the score 1 with score 2 combinations.



Figure 32: Jitter plot illustrating the combinations of scores for one observer and two different viewings of the same group of animals (n = 430).

5.3 **Descriptive statistics**

5.3.1 Locomotion scores

The final dataset contained a total of 103,471 LMS, including the FLMS (n = 8386, 8.1 %), DLMS (n = 3304, 3.2 %), scores given on the day of the farm visit (n = 63, 0.1 %) and interpolated scores (n = 91718, 88.6 %) (see 4.2.2.4). In total n = 55 LMS were excluded from data analysis due to lack of identification and in n = 59 cases no FLMS was possible due to overcrowding in front of the milking parlour or poor lighting conditions.

Of the total number of scores n = 201 (0.2 %) were modified after a clinical examination or after a subsequent video analysis. A total of 631 animals was scored; with a median of 251 scores per animal but with a high SD (SD = 139.5).

The distribution of the scores across the farms is shown in Figure 33; 32 % of the total number of scores was carried out on CDF1, followed by CDF3 (29.1 %) and CDF2 (23.7%). The RFG and CDF4 had fewer scores (10.2 % and 4.9 % respectively). The LMS considered for the analysis of lameness development and those used as a reference for claw health in the

predictive model (see 0), are the LMS after correction (see 4.3.1.5). Of the total number of LMS after correction, 78.8 % were a score 1, 15.6 % were a score 2 and 5.6 % were a score 3.



Figure 33: Number of locomotion scores per farm by score. CDF1 – CDF4: commercial dairy farms 1 – 4, RFG: research farm.

The lameness prevalence per month, defined as the number of non-interpolated LMS = 3 per month for all farms as relative share of the overall number of non-interpolated scores, is shown in Table 31 in the Annexe. The lameness prevalence across all farms ranged between 3.5 % and 12.7 % (SD =2.3) and was lowest in March 2018 and highest in May 2018. When considering the farms individually, CDF2 had the lowest prevalence with no lame animals in January and April 2018. The RFG had the highest prevalence with 19.3 % LMS = 3 in February 2018.

A representation of lameness prevalence and mean LMS across all farms per month can be seen in Figure 34.



Figure 34: Mean lameness prevalence (blue) and mean fortnightly locomotion score (red) per month across all farms. CDF1 – CDF4: commercial dairy farms 1 - 4, RFG: research farm.

Even though the LMS data did not follow a normal distribution, the mean LMS was calculated in order to better analyse the fluctuation of the scores that was only minimal across farms and months. In Table 32 in the Annexe the mean LMS per month and per farm are listed alongside the mean across farms for the FLMS, and the mean across farms for all scores, including the interpolated ones. The month with the highest mean FLMS was May 2018 ($\overline{x} = 1.8$), the lowest were April and May 2017 ($\overline{x} = 1.1$ and $\overline{x} = 1.2$ respectively).

If analysed singularly, the farms show different trends for the mean LMS value over the course of the data collection period. In Figure 35 a negative trend is apparent for CDF4 and the RFG, meaning the mean LMS decreased over time, while a slight positive trend is visible for CDF1, meaning the average LMS increased slightly towards the end of the data collection period.



Figure 35: Development of mean locomotion score for all farms. CDF1 – CDF4: commercial dairy farms 1 – 4, RFG: research farm in Grub.

5.3.2 Lameness development

To analyse the development of lameness, the number of days it took from the animal last being scored sound (DLMS1) and lameness onset (DLMS3) was calculated. n = 243 lameness cases, defined as FLMS = 3, of n = 156 animals were analysed to investigate the development of cases of lameness during the study. The analysis involved cases of FLMS = 3 which had been followed back to lameness onset using video recordings of milking times. A median of five days passed between the cow last being scored sound and lameness onset across all farms (SD = 13.4), the values for the single farms can be found in Table 33.

The farm with the longest time until detection was CDF4 (Med = 10.5 days), while the least number of days was on CDF1 and CDF2 (Med = 3 days). A median of twelve days passed from the cow last being scored sound to discovery across all farms (SD = 13.8). In the density plot in Figure 36 the number of days between the animal being scored sound and becoming lame (red) are represented overlaid by the density of the number of days between the cow being sound and being discovered at FLMS.



Figure 36: Overlayed density plots of the number of days from sound to lame (pink) and sound to discovery (blue).

DLMS: daily locomotion score, FLMS: fortnightly locomotion score.

A median of five days passed between the animal becoming lame (DLMS = 3) and being discovered lame and treated at FLMS (FLMS = 3). The median values were similar for the single farms; CDF4 had the most pronounced difference with a median of 8 days, while CDF1 had a median of only 3 days between lameness onset and lameness discovery. A density plot of the difference in days between lameness onset and discovery is shown in Figure 37.



Figure 37: Density plot of the difference in days between lameness onset and lameness discovery (DLMS = 3 to FLMS = 3) across all farms. The blue line represents the median.

5.3.3 Clinical claw examinations

The results of the data collected during visits to the farms and clinical examination of lame animals' claws was analysed without the degree of lesion severity, as these annotations were made in order to assess improvement of the lesion.

In total, n = 313 clinical examinations were carried out by the author, and a further n = 202 were carried out either by the farmer or by a professional hoof trimmer. An overview of the relative share of diagnoses to the total number of findings per farm can be seen in Table 17. For reasons of clarity, the documented findings (see 4.2), were summarised by animal (i.e. if an animal had SH on two claws, SH would be counted once for the respective animal during the respective examination) were grouped into larger categories. Sole ulcers, toe ulcers and toe necrosis were grouped into the more general UL (ulcers), diffused and circumscribed sole haemorrhages were grouped into SH (sole haemorrhages), white line fissures and abscesses were grouped into WLD (white line disease), digital and interdigital dermatitis were analysed as DE (dermatitis) and horn fissures and axial horn fissures were combined into HF (horn

fissures). Interdigital hyperplasia (IH) and interdigital phlegmons (IP) as well as double soles (DS) were considered separately. The presence of HHE was not considered when analysing the findings, as it was not continuously documented due to its ubiquity.

A summary of the number of findings per visit for all farms is shown in Table 18. N corresponds to the total number of findings on the farm. The variation of values was especially high for the RFG (SD = 11.3) and CDF4 (SD = 13.7), who also had the highest mean number of findings ($\bar{x} = 11.81$ and $\bar{x} = 36.75$ respectively). CDF3 had the lowest number of mean findings per visit ($\bar{x} = 3.4$).

	Relative share of diagnosis (%)								
Farm	SU	SH	DE	HF	WLD	DS	IP	IH	
CDF1	5.2	27.4	19.6	3	31.5	7	0	6.3	
CDF2	15.2	33	16.1	3.6	28.6	3.6	0	0	
CDF3	19.5	29.3	12.2	0	34.1	2.4	0	2.4	
CDF4	11.5	29.5	18	0.8	23.8	9.8	0	6.6	
RFG	4.1	30.8	19.8	2.3	26.7	7.6	2.9	5.8	
Total	8.4	29.6	18/	2.4	28.7	6.8	0.7	5	

 Table 17:
 Relative proportion of clinical findings to total findings per farm for specific diagnosis.

SU: sole ulcer, SH: sole haemorrhage, DE: dermatitis, HF: horn fissure, WLD: white line disease, DS: double sole, IP: interdigital phlegmon, IH: interdigital hyperplasia, CDF1 – CDF4: commercial dairy farms 1 – 4, RFG: research farm in Grub.

	Number of findings per visit									
Farm	n	Min	q25	Median	q75	Max	Mean	SD		
CDF1	270	3	9	11	13	18	11.3	3.5		
CDF2	112	0	3	4	6.5	10	4.7	2.9		
CDF3	41	0	0	3	6	8	3.4	3.1		
CDF4	122	17	33.5	41	44.2	48	36.8	13.7		
RFG	172	0	5.5	10	14.8	48	11.8	11.3		

Table 18: Summary statistics of number of findings per farm.

N: total number of findings, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, CDF1 - CDF4: commercial dairy farms 1 - 4, RFG: research farm in Grub.

The results regarding the number of findings per month on the farms can be found in Table 32 in the Annexe, where the findings are also divided by diagnosis.

Most findings occurred in the autumn (September to November, n = 200), followed by summer (June to August, n = 187), spring (March to May, n = 177) and winter (December to February, n = 153).

The overall monthly incidence for DE was highest in September 2017 with 35 cases (for n = 313 animals scored), and lowest in May 2017 with only 3 cases (for n = 278 animals scored). For SU the incidence was lowest in December 2017 (for n = 275 animals scored) with no cases and highest in August (for n = 321 animals scored) and September of 2017. SH

were least present in June 2018 (for n = 72 animals scored) and had a very high incidence in September 2017 with 103 cases. WLD had a low incidence in April 2018 (for n = 316 animals scored) and December 2017 and a high incidence in September 2017 with 55 cases.

Of the clinical examinations considered for the analysis (n = 365), n = 37 resulted in no clinical findings or lesions. In n = 55 cases, only one lesion was found, while in n = 71 cases two lesions were found. In most cases (n = 202) at least three lesions were found per animal and examination.

Of the n = 80 cases of cows being tested for pain in the claws, 52 % (n = 41) either had clinical findings or had a positive pain reaction and were subsequently treated as LMS = 3.

5.4 **Comparative statistics**

5.4.1 Locomotion scores

The relationship between mean LMS and number of findings per month across farms was investigated by calculating Spearman's rank correlation coefficient and resulted in $\rho = 0.85$. The positive correlation indicates an increase in mean LMS when there is an increase in total number of findings. This trend is also evident in Figure 38, where a linear model (y = 0.0074096x + 1.1478304) was added to the graph as a blue line to illustrate the relationship between clinical findings and mean LMS. If the farms are considered singularly on the other, there is a statistically significant positive correlation between number of findings and mean LMS only for CDF2 and CDF3 ($\rho = 0.78$ and $\rho = 0.69$ respectively).



Figure 38: Correlation between mean locomotion score (LMS) and number of total findings per month. Trend, represented by the blue line, modelled using the linear model function in ggplot [209]

5.4.2 Clinical claw examinations

A Poisson regression was performed on the total number of findings per visit between farms and showed that the farms all had a statistically significant influence on the number of findings per visit. CDF1 was the farm with the most findings overall (n = 270, $\bar{x} = 20.8$) followed by the RFG with an average of $\bar{x} = 28.7$ findings per month and a total of n = 172 findings.

There was no evident trend in the total number of findings per season across all farms, although when considering the farms singularly, a trend can be recognised, for example on CDF1, CDF2 and CDF3 even if not statistically significant in the case of CDF1, where the number of total findings dropped in the summer months and was higher in winter and autumn, while it dropped in the autumn through to the spring for CDF3.

5.5 Application of predictive model to data

5.5.1 Variables

Statistical summaries were calculated for all variables in the dataset used for statistical modelling (see 4.3.2.4), and are shown in Table 19. The distribution of the variables was analysed visually using histograms and boxplots. Not considering the identifying variables (B, TN, BTN and Date), only nine variables were normally distributed.

_	Variable values								
Var	n	Min	q25	Med	q75	Max	Mean	SD	
AC	73,220	16.0	1,467	1,796	2,172	10,634	1,885.4	655.7	
ACR	73,220	-	1,010	1,248	1,517	6,729	1,305	455.5	
BCS	7,574	2.7	3.8	3.9	4.1	4.6	3.9	0.3	
C_FDM	73,220	1.0	18.4	25.4	33.9	348.0	27.4	13.4	
C_MN	73,220	1.0	8.0	10.0	13.0	140.0	10.8	6.6	
C_MNR	73,220	-	5.0	7.0	9.0	105.0	7.5	4.6	
DIM	73,220	1.0	103.0	179.0	255.0	741.0	190.1	115.4	
F.LMS	73,220	-	2.0	2.0	2.0	3.0	1.9	0.4	
FD	73,220	2.0	203.0	266.0	322.0	727.0	262.4	94.0	
FDM	7,576	1.3	16.7	21.3	26.8	88.6	22.5	8.3	
FDR	73,220	-	149.0	200.0	251.0	666.0	200.4	80.0	
FDRW	7,576	-	86.5	108.1	130.6	343.0	109.4	33.5	
FDV	7,576	0.4	2.8	3.9	5.1	21.0	4.1	2.0	
FDW	7,576	9.1	104.2	127.8	152.7	367.5	129.8	38.4	
FI	7,576	3.9	38.3	44.9	51.3	77.0	44.7	9.9	
FIM	7,576	0.6	5.9	7.5	9.4	27.0	7.8	2.7	
FIV	7,576	0.2	0.9	1.3	1.9	11.8	1.5	0.8	
FP	7,576	0.1	0.3	0.3	0.4	1.6	0.4	0.1	
K.LMS	148	1.0	2.0	2.0	2.0	4.0	2.0	0.8	
LBN	73,220	1.0	14.0	18.0	28.0	138.0	24.0	16.2	
LBNR	73,220	-	7.0	9.0	14.0	102.0	11.9	8.5	
LD	73,220	34.0	597.0	692.0	785.0	1,412	686.3	146.4	
LDB	73,220	2.5	23.1	37.3	52.0	296.3	39.7	22.7	
LDR	73,220	-	231.0	307.0	383.0	883.0	306.9	104.3	
LMS	73,220	1.0	1.0	1.0	1.0	3.0	1.3	0.5	
LW	7,526	535.5	684.2	748.9	804.0	986.0	745.3	75.6	

 Table 19:
 Statistical summary of each variable across all farms.
				Varia	ble values	5		
Var	n	Min	q25	Med	q75	Max	Mean	SD
MI	7,534	327.9	651.9	763.2	857.8	1,437	772.0	164.6
MMY	73,220	-	25.0	30.0	35.8	49.8	30.3	7.4
MN	7,576	1.0	5.0	6.0	7.0	14.0	6.2	1.9
MNR	7,576	-	4.0	5.0	6.0	12.0	4.9	1.6
MY	10,473	-	26.0	31.3	38.2	50.0	31.8	8.3
MY305	54,059	-	7,090	8,358	9,511	12,268	7,521.7	3,166.6
MYM	62,747	7.4	24.7	29.9	35.4	49.8	30.1	7.4
Р	73,220	1.0	1.0	3.0	4.0	10.0	2.8	1.6
РТ	59	-	-	1.0	1.0	1.0	0.6	0.5
VN	7,576	2.0	24.0	34.0	49.0	222.0	40.1	23.6
VNR	7,576	_	20.0	29.0	42.0	174.0	34.0	20.5

Table 19 (continuation): Statistical summary of each variable across all farms.

Var: variable, N: number of observations, Min: minimum, q25: first quartile, Med: median, q75: third quartile, Max: maximum, SD: standard deviation. AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDRW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration measured by weighing troughs, FDR: feeding duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of visits during daytime measured by the weighing troughs, VNR: number of visits during daytime measured by the weighing troughs, MY: milk yield.

5.5.2 Univariate analysis

Due to the absence of normal distribution and homoscedasticity in most of the variables, the Kruskal-Wallis test was chosen to test for statistically significant differences between LMS = 1, 2 and 3 respectively for each variable. All variables showed statistically significant differences between LMS groups (p < 0.05) except for MY (p = 0.311), MI (p = 0.07) and the FI (p = 0.409). The differences between groups were further investigated with a non-parametric post-hoc analysis. The results of the applied Wilcoxon signed-rank test are show in Table 20.

		p-value	
Variable	LMS = 2 vs. LMS = 1	LMS = 3 vs. LMS = 1	LMS = 3 vs. $LMS = 2$
Р	< 0.01	< 0.01	0.487
DIM	1.000	0.012	0.274
MY	0.390	1.000	0.917
MI	0.605	0.091	0.801
MY305	< 0.01	< 0.01	0.303
MYM	< 0.01	0.029	1.000
LW	0.229	0.013	0.486
BCS	0.041	0.026	1.000
LD	< 0.01	< 0.01	< 0.01
LDR	0.321	< 0.01	< 0.01
LBN	< 0.01	0.023	1.000
LBNR	< 0.01	1.000	0.051
LDB	< 0.01	< 0.01	1.000
AC	< 0.01	< 0.01	< 0.01
ACR	< 0.01	< 0.01	< 0.01
FI	0.766	1.000	0.767
FD	< 0.01	< 0.01	< 0.01
FDW	0.139	< 0.01	0.020
FDR	< 0.01	< 0.01	< 0.01
FDRW	0.068	< 0.01	0.064
FP	< 0.01	< 0.01	< 0.01
MN	< 0.01	< 0.01	< 0.01
MNR	< 0.01	< 0.01	< 0.01
FDM	0.012	< 0.01	0.053
FIM	< 0.01	< 0.01	< 0.01
VN	< 0.01	< 0.01	< 0.01
VNR	< 0.01	< 0.01	< 0.01
FDV	< 0.01	< 0.01	0.025
FIV	< 0.01	< 0.01	< 0.01
C_MN	< 0.01	< 0.01	< 0.01
C_MNR	< 0.01	< 0.01	0.068
LMS	< 0.01	< 0.01	< 0.01
C_FDM	< 0.01	< 0.01	0.217
MMY	< 0.01	< 0.01	1.000

 Table 20:
 Significance of difference between locomotion score (LMS) groups for each variable and each group combination.

AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDV: feeding duration per feeding visit measured by weighing troughs, FIRW: feed intake, FP: feeding pace, FIM: feed intake per visit, FIV: feed intake per visit, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MY:

milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, VN: number of visits measured by the weighing troughs, VNR: number of visits during daytime measured by the weighing troughs.

The statistical summary for all variables divided by LMS is shown in Table 41 in the Annexe. The post-hoc analysis revealed that The BCS did not have statistically significant differences between the LMS = 2 and LMS = 3 groups nor did the FI, P, DIM, MY, MI MY305, MYM, LW, LBN, LBNR, LDB, FDRW, FDM, C MNR, C FDM, and MMY. Between the groups LMS = 1 and LMS = 2 DIM, MY, MI, LW, FI, FDW, and FDRW had statistically significant differences. Finally, all variables showed a statistically significant difference between sound (LMS = 1) and lame (LMS = 3) animals except MY, MI LBNR and FI. These findings were confirmed by the boxplots plotted for each variable. For some of the variables, such as LD, there was a visible trend, with the lying duration increasing with increasing LMS (see Figure 39), while for others there was no obvious trend. The jitter plots combined with boxplots for each variable included in the multivariate analysis can be found in the Annexe (Figure 46-Figure 78). Notably, the mean AC decreased with increasing LMS (mean = 1,938.9 for LMS = 1, mean = 1,847.2 for LMS = 2 and mean = 1,679 for LMS = 3) as did the C FDM (mean = 27.3, 25.6 and 24.2 minutes for LMS = 1, 2 and 3 respectively) and the FD (mean = 266.7, 236.2 and 201.2 minutes for LMS = 1, 2 and 3 respectively). The mean FP increased with increasing LMS, while the FI sank from LMS = 1 (mean FI = 44.3 kg) to LMS = 3 (mean FI = 41.8 kg). The mean LW on the other hand increased with increasing LMS (741.3 for LMS = 1 to 779.2 for LMS = 3). The mean LD also increased with increasing LMS (from 676.6 minutes for LMS = 1 to 743.9 minutes for LMS = 3) as did the mean MI.

The differences between single farms were tested for significance for each variable with a Kruskal-Wallis test followed by a Wilcoxon signed rank test. The results are summarised in Table 21.



Figure 39: Jitter plot with boxplots of daily lying duration (LD) (in minutes per day) grouped by locomotion score.

Table 21: P values for each variable tested between different farms

Variable	CDF2v1	CDF3v1	CDF4v1	RFGvCDF1	CDF3v2	CDF4v2	RFGvCDF2	CDF4v3	RFGvCDF3	RFGvCDF4
В	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Р	1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.2248	< 0.001
DIM	< 0.001	1	< 0.001	< 0.001	< 0.001	1	0.8113	< 0.001	< 0.001	1
MY305	< 0.001	< 0.001	< 0.001	< 0.001	0.0056	< 0.001	< 0.001	< 0.001	< 0.001	0.0014
LD	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	1	< 0.001	< 0.001	< 0.001
LDR	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0047	0.0563	0.2871	< 0.001	< 0.001
LBN	0.2869	0.0338	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
LBNR	< 0.001	< 0.001	1	< 0.001	0.0062	< 0.001	< 0.001	0.0015	< 0.001	< 0.001
LDB	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	1	< 0.001	< 0.001
AC	< 0.001	< 0.001	< 0.001	0.7742	< 0.001	1	< 0.001	< 0.001	< 0.001	< 0.001
ACR	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	1	0.0247	< 0.001	< 0.001	0.9635
FD	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
FDR	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0022	< 0.001	1	< 0.001	< 0.001
C_MN	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
C_MNR	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	1	< 0.001	< 0.001
C_FDM	0.1374	1	1	< 0.001	< 0.001	0.0069	< 0.001	1	< 0.001	< 0.001

CDF 1-4: commercial dairy farms 1-4, RFG: research farm, AC: activity, ACR: activity during daytime, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, P: parity, MMY: average monthly milk yield.

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For the behavioural parameters, there was a statistically significant difference for the LD on all farms except between CDF2 and the RFG, while the LBN had statistically significant differences between all farms except CDF1 and CDF2. The LDB was also significantly different for all farms except CDF3 and CDF4, so was the AC, except between CDF1 and the RFG and CDF4 and CDF2.

The LD varied significantly on all farms except CDF2 and the RFG and ranged from a Med = 631 minutes (10.4 hours) on CDF1, to Med = 758 on CDF3 (12.6 hours). For the LBN, the SD = 16 indicates a high level of variation for daily values, as well as between farms; whereas CDF2 had a Med = 21 LBN, RFG had a Med = 14 LBN per day. The FD was significantly different on all farms, with a minimum Med = 110 minutes on RFG and more than double the feeding time on CDF1 (Med = 311 minutes).

The differences between farms with an AMS and farms with a milking parlour were also statistically significant for all variables, as were the differences between farms with deep bedded stalls and raised stalls.

The OR for the outcome lame/not-lame as well as its significance was computed for each variable except LMS, K_LMS, F_LMS and PT as these were explanatory variables connected to the outcome lame/not-lame. The OR are shown in Table 22 with both the outcome lame as LMS = 2 and LMS = 3 and the outcome lame as LMS = 3 only.

L3 L23 2.5 % 97.5 % Var OR p value OR 2.5 % 97.5 % p value BCS 0.567 0.332 0.973 0.5227 0.3428 0.7915 0.0023 0.038 **FDM** 1.033 1.012 1.054 1.0277 1.0447 0.0009 0.002 1.0114 **FDRW** 0.990 0.984 0.995 < 0.001 < 0.0010.992 0.988 0.996 **FDV** < 0.001 1.275 1.174 1.387 < 0.001 1.3526 1.2565 1.4605 **FDW** 0.990 0.985 0.995 < 0.001 0.9934 0.9899 0.9968 < 0.001 FI 0.995 0.978 1.012 0.536 1.0042 0.9917 1.0169 0.5132 < 0.001 **FIM** 1.236 1.166 1.312 < 0.001 1.1882 1.1329 1.2479 **FIV** 2.345 1.898 2.7299 < 0.001 2.916 < 0.001 2.24 3.3559 FP < 0.001 15.082 3.881 58.617 < 0.001 8.081 2.5101 27.234 LW 1.003 1.001 1.005 0.007 1.0023 1.0007 1.0039 0.0042 MI 1.000 1.001 1.002 0.031 1.0008 1 1.0016 0.0427 **MN** 0.694 0.624 0.768 < 0.0010.7697 0.7167 0.8248 < 0.001**MNR** 0.684 0.605 0.769 < 0.001 0.7353 0.6767 0.7969 < 0.001 MY 0.0203 1.007 0.987 1.028 0.481 1.018 1.0028 1.0334 VN 0.98 0.91 1.04 > 0.05 0.9632 0.9553 0.9707 < 0.001 VNR 0.950 0.936 0.963 < 0.001 0.9996 0.9995 0.9996 < 0.001 AC 1.000 1.000 1.000 < 0.001 0.9993 0.9992 0.9994 < 0.001 ACR 0.999 0.999 0.999 < 0.0010.992 0.9886 0.9953 < 0.001C FDM 0.992 0.989 0.995 0.003 0.9958 0.9898 1.0014 0.1502 C MN 0.996 0.990 1.001 0.533 0.9974 0.9893 1.0052 0.5185 C MNR 0.997 0.989 1.005 0.290 0.9996 0.9993 1 0.0666 DIM 0.999 0.9962 0.9958 0.9967 < 0.001 1.000 1.000 0.003 0.996 0.996 FD 0.997 < 0.001 0.9961 0.9956 0.9967 < 0.001 FDR 0.996 0.996 0.997 < 0.001 0.9954 0.9927 0.9982 0.0012 LBN 0.995 0.993 0.998 0.024 0.9935 0.0137 0.9883 0.9986 **LBNR** 0.994 0.988 0.999 0.553 1.0015 < 0.0011.0012 1.0009 LD 1.001 1.001 < 0.001 < 0.001 1.002 1.0069 1.005 1.0088 < 0.001 LDB 1.007 1.005 1.009 < 0.001 1.0014 1.001 1.0018 1.001 1.001 LDR < 0.001 1.002 < 0.001 1.0185 1.0125 1.0245 MMY 1.019 < 0.001 1.013 1.025 0.011 1.0277 1.0114 1.0447 **MY305** 1.000 < 0.001 0.9999 0.9999 0.9999 < 0.001 1.000 1.000 Р 1.189 1.157 1.222 < 0.001

Table 22: Odds ratio with 95% confidence intervals for the outcome lame/not-lame for each variable and with LMS = 2 + LMS = 3 as lame outcome (L23) and only LMS = 3 as lame outcome (IL=3).

Var: variable, OR: odds ratio, 2.5%: 2.5% quantile, 97.5%: 97.5% quantile. AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDRW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration measured by weighing troughs, FI; feed intake, FP: feeding pace, FIM: feed intake per visit, FIV: feed intake per visit, FIM: feed intake per meal, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MY: milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, VN: number of visits measured by the

1.189

1.1569

1.2222

< 0.001

weighing troughs, VNR: number of visits during daytime measured by the weighing troughs, MMY: average monthly milk yield.

For the outcome lame including both lame and unsound (LMS = 2 and LMS = 3) animals, an increasing FIM, FIV and FP increase the odds of becoming lame by 1.19, 2.73 and 8 units respectively. P had an OR = 1.18 for both L3 and L23, indicating that animals with a higher parity are at an increased risk of becoming lame. MNR, MNR and BCS had ORs < 0.90 indicating a positive effect on claw health connected to an increase in the respective variable. The rest of the variables all had an OR of approximately 1. For the outcome lame considering only LMS = 3 on the other hand, some variables had a high OR compared to the outcome lame for LMS = 2 and LMS = 3, notably FP (OR: 15.1, CI: 3.88-58.61) and FIV (OR: 2.35, CI:1.90-2.92). BCS, MN and MNR all had an OR < 0.7 indicating a protective effect of an increase in the respective variable on the odds for developing lameness.

To investigate the level of correlation between the single variables, a correlation matrix was computed using Kendall's rank correlation coefficient τ (see Table 23). There was a mild correlation between all variables that had a corresponding parameter for the day/night ratio, for example the LBN and LBNR, AC and ACR and FD and FDR, but also for related variables such as LBN and LDB ($\tau = -0.78$) and FD and C_FDM ($\tau = 0.50$). Some unrelated variables such as C_MN and AC were also moderately correlated ($\tau = 0.34$), meaning that an increased number of visits to the feeding table was accompanied by increased activity.

	AC	ACR	C_FDM	C_MN	C_MNR	DIM	FD	FDR	LBN	LBNR	LD	LDB	LDR	MMY	Р
AC	1.00	0.74	-0.18	0.34	0.28	-0.13	0.10	0.07	0.07	0.04	-0.36	-0.18	-0.32	0.07	-0.25
ACR	0.74	1.00	-0.12	0.31	0.34	-0.09	0.13	0.15	0.08	0.03	-0.29	-0.16	-0.38	0.02	-0.28
C_FDM	-0.18	-0.12	1.00	-0.42	-0.37	0.08	0.50	0.49	0.02	0.00	0.04	0.00	-0.02	-0.05	-0.02
C_MN	0.34	0.31	-0.42	1.00	0.73	-0.07	0.11	0.06	0.06	0.03	-0.26	-0.14	-0.21	0.04	-0.14
C_MNR	0.28	0.34	-0.37	0.73	1.00	-0.03	0.08	0.10	0.07	0.02	-0.19	-0.13	-0.26	-0.02	-0.15
DIM	-0.13	-0.09	0.08	-0.07	-0.03	1.00	0.02	0.05	-0.01	-0.02	0.07	0.03	0.01	-0.34	-0.03
FD	0.10	0.13	0.50	0.11	0.08	0.02	1.00	0.76	0.04	0.00	-0.20	-0.10	-0.24	-0.03	-0.18
FDR	0.07	0.15	0.49	0.06	0.10	0.05	0.76	1.00	0.05	-0.02	-0.16	-0.09	-0.29	-0.07	-0.16
LBN	0.07	0.08	0.02	0.06	0.07	-0.01	0.04	0.05	1.00	0.74	-0.08	-0.78	-0.04	-0.04	-0.11
LBNR	0.04	0.03	0.00	0.03	0.02	-0.02	0.00	-0.02	0.74	1.00	0.00	-0.61	0.07	-0.01	-0.08
LD.	-0.36	-0.29	0.04	-0.26	-0.19	0.07	-0.20	-0.16	-0.08	0.00	1.00	0.30	0.60	-0.05	0.12
LDB	-0.18	-0.16	0.00	-0.14	-0.13	0.03	-0.10	-0.09	-0.78	-0.61	0.30	1.00	0.22	0.01	0.14
LDR	-0.32	-0.38	-0.02	-0.21	-0.26	0.01	-0.24	-0.29	-0.04	0.07	0.60	0.22	1.00	0.03	0.17
MMY	0.07	0.02	-0.05	0.04	-0.02	-0.34	-0.03	-0.07	-0.04	-0.01	-0.05	0.01	0.03	1.00	0.18
Р	-0.25	-0.28	-0.02	-0.14	-0.15	-0.03	-0.18	-0.16	-0.11	-0.08	0.12	0.14	0.17	0.18	1.00

Table 23: Correlation matrix of all variables used in the modelling.

AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits during daytime measured by the pedometers, C_MN: Number of feeding duration measured by the pedometers, C_MN: number of feeding duration measured by the pedometers, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, P: parity, MMY: average monthly milk yield.

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5.5.3 Multivariate analysis

The dataset was prepared for the modelling by transforming variables using either the natural logarithm, or the square root transformation. All variables except for LMS, K_LMS, F_LMS, PT, FI, FIM, MY305, MYM and MMY were transformed.

Following data pre-processing, the number of missing values for each variable was computed and parameters were excluded that contained more than 5 % NA (not available) values (see Figure 28). The following variables were excluded from the across-farms analysis: MY305, MYM, FI, BCS, FP, FDM, FDV, FIV, FDRW, LW, VN, VNR, FDW, MN, MNR, MI and MY. The excluded variables were then included in the regressions performed on the data from the RFG only.

For the regression, only observations were included that had an LMS either carried out on one the FLMS (F_LMS = 1), on the day of the farm visit (F_LMS = 3) or using the video recordings (F_LMS = 0). After excluding observations that had missing values, the final dataset that was used for the Elastic Net regression comprised 8,716 observations and 19 variables. The observations where the animal was scored lame (for L23) were 3112 (1248 for L3), meaning only 35 % (for L23) or 14 % (for L3) of observations included a lame animal. Using the SMOTE technique [194] for both L3 and L23 the data was balanced and included 50 % observations with the outcome lame and 50 % with the outcome not lame.

5.5.3.1 Generalised linear mixed model

For the data spanning across farms a generalised linear mixed model was built to account for the random effects represented by the farms (B) and individual animals (BTN). The first model included both random effects and interaction terms which were included in the forward stepwise regression performed for the Elastic Net regression (see 5.5.3.2). All variables in the regression were continuous except for the variable Season, which was categorical and the L3/L23 dummy variables. The first model (GLMMRE) included the following predictors for the outcome lame with:

lame ~ $(1 | B) + (1 | BTN) + AC.sqr + ACR.sqr + C_FDM.log + C_MN.log$

- + C_MNR. sqrt + DIM. sqr + FD. sqr + FDR. sqr + LBN. log
- + LBNR.sqrt + LD.sqr + LDB.log + LDR.sqr + MMY + P.log

+ Season + FDR. sqr: LBN. log + C_FDM. log: FD. sqr

+ C_MN. log: FD. sqr + FD. sqr: FDR. sqr + DIM. sqr: FD. sqr

- + C_MNR. sqrt: FDR. sqr + LBNR. sqrt: LDB. log + DIM. sqr: MMY
- + C_MNR. sqrt: LBN. log + C_FDM. log: FDR. sqr + C_MN. log: FDR. sqr
- + FD. sqr: Season + ACR. sqr: P. log + C_MN. log: Season

The model had a sensitivity (SEN) = 0.86 with a 95 % confidence interval (CI) of 0.85 to 0.87, and a specificity (SPE) = 0.91 (CI: 0.90, 0.92) on the training data, and a SEN = 0.94 (CI: 0.91, 0.96) and SPE = 0.74 (CI: 0.72, 0.76) on the test data. Additionally to the SEN and SPE the prediction accuracy was also measured using ROC curve analysis and resulted in an AUC = 0.96 (CI: 0.96, 0.97) (Figure 40) for the training data and AUC = 0.91 (CI: 0.89, 0.92) on the unsmoted test data.

Due to high number of predictors in the GLMMRE model, a new reduced model (GLMMRE2) was created excluding variables that exhibited > 0.7 correlation (see 4.3.2.3) and parameters whose coefficient estimates were not statistically significant in the GLMMRE and their interaction terms. The resulting model took the following form:

The reduced number of predictors allow for a higher level of interpretability and only marginally reduced the predictive accuracy compared to GLMMRE with an AUC = 0.93 (CI: 0.93, 0.94) on training data and 0.91 (CI: 0.89, 0.92) on test data. The SEN of the model for training data was 0.88 (CI: 0.87, 0.89) and SPE = 0.83 (CI: 0.81, 0.84) and SPE = 0.76 (CI: 0.75, 0.78) and SEN = 0.91 (CI: 0.88, 0.93) on the test data.

The ICC was calculated for the random effects, which was 0.1 and 0.3 for B and BTN respectively. According to Koo and Li (2016) and Cicchetti et al. (1994) [210, 211], an ICC < 0.4 (or 0.5) is considered poor, so a model was built excluding the random effects (GLMM) and resulted in SEN = 0.73 (CI: 0.71, 0.74) and SPE = 0.90 (CI: 0.89, 0.91) with an AUC = 0.88 (CI: 0.87, 0.88) (Figure 40) for the training dataset and SEN = 0.59 (CI: 0.55, 0.63), SPE = 0.73 (CI: 0.71, 0.74) and AUC = 0.70 (CI: 0.67, 0.72) for the test data. In Figure 40 and the ROC curves are represented for the GLMMRE (right) and GLMM (left) with three threshold values indicated and the SPE and SEN for the respective thresholds in brackets.



Figure 40: Receiver Operating Characteristics curve (ROC) for the generalised linear model (GLMM) (left) and the generalised linear model with random effects (GLMMRE) (right), with thresholds and respective (specificity, sensitivity).

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5.5.3.2 Elastic net

After excluding the random effects from the dataset, it was possible to perform the Elastic Net regularization on the data. First, a forward stepwise logistic regression was performed on all possible interaction terms between variables to filter out statistically significant ones for both L3 and L23. The Elastic Net model was fitted to the data from each farm individually with both L3 and L23 as outcomes. The coefficients of the Elastic Net models for each farm are listed in Table 42-Table 46 in the Annexe. The direction of the relationship between variables and the outcome lame, was different for most farms; the predictor C_MN for example, was negatively related to the outcome lame for CDF2 and RFG, while for CDF1 the coefficient had been shrunk to 0. For CDF3 on the other hand, C_MN had a positive relationship with the outcome lame, as also with CDF4. Similarly, MMY was positively related to lameness on CDF1 and RFG, negatively so on the rest of the farms. The only coefficients that had the same direction of relationship on all farms were AC (negative relationship) and C_MNR (positive relationship). A visual comparison of the coefficient estimates of the individual farm models before ENET Beta variable selection process can be seen in Figure 41





ENET.CDF1: elastic net model for commercial dairy farm 1, ENET.CDF2: elastic net model for commercial dairy farm 2, ENET.CDF3: elastic net model for commercial dairy farm 3, ENET.CDF4: elastic net model for commercial dairy farm 4, ENET.RFG: elastic net model for research farm. MMY: average monthly milk yield, LDR.sqr: square root of lying duration

during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of meals during daytime of feeding duration per meal, ACR.sqr: square root of activity during daytime, AC.sqr: square root of activity, P.log: natural logarithm of parity.

The number of predictors for each model varied between 17 for CDF4, where the forward step regression didn't include any interaction terms, and 41 for CDF3, where 23 interaction terms were included. The results of the models on the balanced training data can be seen in Table 25. The classification performance was better for L3 for all farms, where the mean AUC was 0.88 (0.83, 0.95), compared to AUC(mean) = 0.81 (CI: 0.77, 0.86) for L23. For this reason, only LMS = 3 was considered for the outcome "lame" in further calculations.

The best model was that of CDF3 with an AUC = 0.95 for L3, while the lowest AUC for L3 was on CDF4 (0.84). The overall predictive accuracy for the Elastic Net models for individual farm data was lower on the unsmoted test data than on the training data, as can be seen in Table 26. The model for RFG was different to the other individual farm models, as it included data from the weighing troughs and different predictors such as FI, FP and LW (see Table 46), and showed the best predictive accuracy for L23, but not for L3.

For the data across all farms forward stepwise regression was applied to the model without random effects and resulted in the following statistically significant interaction terms for L23: LBNR:LDB, DIM:FD, ACR:MMY, LDR:P, LD:MMY, DIM:MMY, FD:LDR, C_MN:P, LD:LDR, LDB:P, C_FDM:C_MN, C_MN:MMY. For L3 the statistically significant interaction terms were C_FDM:FD, FD:C_MN, FD:FDR, C_FDM:FDR, C_MN:FDR, FD:DIM, LBN:C_MNR, DIM:MMY, P:ACR, FDR:LBN, LDB:LBNR,FDR:C_MNR, C:MN:Season and FD:Season. The Elastic Net regression with ten-fold cross validation was applied to both models (L23 and L3) to determine the value for α with the lowest mean absolute error.

The full model (ENFM) for L3 was:

lame ~ factor(Season) + AC. FDM. log + FD. sqr + C. MN. log + C. FDM. log: FD. sqr + C. MN. log: FD. sqr + FD. sqr: FDR. sqr + FDR. sqr + C. FDM. log: FDR. sqr + C. MN. log: FDR. sqr + LDB. log + LBN. log + LD. sqr + C. MNR. sqrt + DIM. sqr + P. log + LBNR. sqrt + DIM. sqr: FD. sqr + DIM. sqr + C. MNR. sqrt: LBN. log + ACR. sqr + DIM. sqr: MMY + MMY + LDR. sqr + ACR. sqr: P. log + FDR. sqr: LBN. log + LBNR. sqrt: LDB. log + FD. sqr: Season + C. MN. log: Season + C. MNR. sqrt: FDR. sqr ENFM had a SEN of 0.59 (CI: 0.54, 0.63) and a SPE of 0.75 (CI: 0.73, 0.77) on the training data. The AUC was 0.72 (CI: 0.69, 0.74). In Figure 42 the MAE for ENFM is represented as a function of the λ value and corresponding to the number of non-zero coefficients. The MAE increases steadily when the number of non-zero coefficients sinks below 32. The left dotted line represents the minimum λ value with the lowest MAE for the model with the corresponding number of non-zero predictors indicated on the top x-axis, while the right dotted line represents the largest lambda value within one standard error of the minimum lambda. The λ with the lowest MAE for ENFM was low, meaning that all predictors were kept in the model and no coefficient was shrunk to zero.



32 32 32 32 32 32 31 31 29 28 25 24 19 17 12 6 3 2

Figure 42: Plot of mean absolute error (MAE) with error estimates for the Elastic Net model computed with data from all farms on the training dataset and ten-fold cross validation.

On the top x-axis the number of predictors is displayed in function of the natural logarithm of lambda (bottom x-axis) and the MAE (y-axis).

The ENET Beta approach was then used to improve the interpretability of the model by further reducing the number of predictors (see 4.3.2.4). Starting with the predictor with the highest absolute values for its estimated coefficient, the parameters were added to the model one at time and the Brier Score (BS) was computed for each model. A summary of the BS for each model (EN1-EN25) is shown in Table 24. According to the approach suggested by Liu and Li (2017) [200], the model was chosen where $\frac{BS_{min}}{BS} \ge 0.9$. The best model (ENBM) presented the following predictors for the outcome lame:

The SEN of ENBM was 0.70 (CI: 0.68, 0.72) and the SPE 0.81 (CI: 0.79, 0.82) while the AUC was 0.81 (CI: 0.80, 0.83) on the training and test data respectively. The ENBM had a higher predictive accuracy as the ENFM, meaning the reduction of regressors improved both accuracy and interpretability. Overall the GLMMRE model had a better predictive accuracy than the ENFM and ENBM.

The ENET Beta approach was also applied to the Elastic Net models on the individual farm data on smoted training data and on test data. The average AUC was lower for the models following the ENET Beta variable selection, especially for CDF1 and CDF2, where the AUC was only 0.73 and 0.74 respectively on the test data. A summary of the results of the final models for the individual farms after variable selection process can be seen in Table 26. As in the case of the ENET model before the ENET Beta method was applied, CDF3 had the model with the highest predictive accuracy (AUC = 0.93, CI: 0.92, 0.94) on the training and test data, followed by CDF2 (AUC = 0.88, CI: 0.88, 0.90).

Model	Brier Score	BSmin/BS
EN1	0.24	0.67
EN2	0.24	0.67
EN3	0.24	0.68
EN4	0.20	0.82
EN5	0.20	0.82
EN6	0.20	0.82
EN7	0.20	0.82
EN8	0.20	0.82
EN9	0.19	0.86
EN10	0.19	0.87
EN11	0.18	0.87
EN12	0.18	0.92
EN13	0.18	0.92
EN14	0.17	0.94
EN15	0.17	0.94
EN16	0.17	0.95
EN17	0.17	0.95
EN18	0.17	0.95
EN19	0.17	0.97
EN20	0.16	0.98
EN21	0.16	0.98
EN22	0.16	0.98
EN23	0.16	0.98
EN24	0.16	1.00
EN25	0.16	1.00

 Table 24:
 Brier Score (BS) and its ratio with lowest Brier Score (BS_{min}) for each Elastic Net regression model computed according to the ENET Beta approach [200] for data across all farms.

 Table 25:
 Results of the application of the Elastic Net models to the data from the single farms.

Farm		L23			L3	
	SEN	SPE	AUC	SEN	SPE	AUC
CDF1	0.70 (0.67, 0.72)	0.72 (0.70, 0.74)	0.76 (0.75-0.78)	0.72 (0.69, 0.74)	0.84 (0.82, 0.87)	0.85 (0.83,0.86)
CDF2	0.69 (0.65, 0.72)	0.79 (0.76, 0.81)	0.81 (0.79-0.82)	0.85 (0.82, 0.88)	0.83 (0.80, 0.86)	0.91 (0.90-0.92)
CDF3	0.72 (0.68, 0.76)	0.80 (0.77, 0.83)	0.83 (0.81-0.85)	0.93 (0.91, 0.94)	0.85 (0.82, 0.87)	0.95 (0.94-0.96)
CDF4	0.82 (0.78, 0.86)	0.66 (0.60, 0.71)	0.80 (0.77 -0.83)	0.80 (0.75, 0.85)	0.79 (0.75, 0.84)	0.84 (0.81-0.87)
RFG	0.81 (0.77, 0.85)	0.81 (0.77, 0.85)	0.86 (0.84-0.89)	0.77 (0.72, 0.82)	0.85 (0.79, 0.89)	0.89 (0.87-0.92)
Mean	0.75	0.76	0.81	0.81	0.83	0.88

SEN: sensitivity, SPE: specificity, AUC: area under the curve, L3: outcome "lame" for LMS = 3 only, L23: outcome "lame" for LMS = 2 and LMS = 3, CDF 1-4: commercial dairy farms 1-4, RFG: research farm. 95% confidence intervals are shown in brackets.

Table 26:	Predictive accuracy	for the Elastic	Net model for individual	farms after ENET Beta	variable selection process
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Farm		Training data			Test data	
	SEN	SPE	AUC	SEN	SPE	AUC
CDF1	0.72 (0.69, 0.75)	0.80 (0.78, 0.82)	0.83 (0.81,0.85)	0.67 (0.63, 0.71)	0.68(0.66, 0.70)	0.73 (0.71,0.75)
CDF2	0.80 (0.77, 0.83)	0.81 (0.78, 0.84)	0.88 (0.87,0.90)	0.60 (0.54, 0.66)	0.78 (0.76, 0.80)	0.76 (0.70,0.77)
CDF3	0.90 (0.88, 0.92)	0.83 (0.81, 0.86)	0.93(0.91,0.94)	0.79 (0.71, 0.87)	0.70 (0.68, 0.72)	0.80 (0.76,0.84)
CDF4	0.79 (0.74, 0.83)	0.76 (0.71, 0.81)	0.82 (0.79,0.86)	0.75 (0.68, 0.81)	0.72 (0.67, 0.77)	0.78 (0.74,0.82)
RFG	0.65 (0.59, 0.71)	0.88 (0.83, 0.92)	0.83 (0.80,0.87)	0.75 (0.68, 0.82)	0.68 (0.65, 0.72)	0.76 (0.72,0.81)
Mean	0.77	0.81	0.86	0.71	0.71	0.77

CDF 1-4: commercial dairy farms 1-4, RFG: research farm, SEN: sensitivity, SPE: specificity, AUC: area under the curve, 95% confidence intervals shown in brackets.

5.5.3.3 Interaction terms

Due to the multitude of interaction terms that were selected for both the GLMM, GLMMRE and the ENET Beta models (see Table 42-Table 46), the interactions between the untransformed variables for performance (MY, MMY, DIM) and behaviour were further investigated by visualizing the data with the the ggplot package [209]. The geom_smooth argument in ggplot was applied to illustrate the relationship between variables. Additionally, the relationship between each interaction term and the outcome lame was tested for significance using a generalised linear model.

The interaction between MY, MMY and DIM and the behaviour parameters of interest (LD, LDR, FD, FDR, LBN, LBNR, C_MN, C_MNR, FP, FI and FIM) was tested in order to expand on the understanding of the relationship between lameness, performance and behaviour.

The statistically significant (p < 0.05) interaction terms between LMS = 1 and LMS = 2 were LDR*DIM, FP*DIM, C_MNR*DIM, and MY*FI. Between the groups LMS = 2 and LMS = 3 the statistically significant interaction terms were: MMY*LBNR, DIM*FD, DIM*FDR, DIM*LBN, DIM*FI, MMY*LDR, MMY*LBN, DIM*LDR, MY*FI, DIM*C_MN and DIM*C_MNR. Finally, between LMS = 1 and LMS = 3 the statistically significant interaction terms were DIM*C_MN, MMY*LDR, MY*FI, MMY*LBN, MMY*C_MN, MMY*C_MNR, DIM*FD, DIM*FDR. A summary of the p value for the tested interaction terms can be seen in Table 27.

The interaction between LDR and MMY is visually represented in Figure 43. The observations are divided by LMS and show the 95 % CI for the generalised linear model (grey area around coloured lines). For LMS = 1 there is a slight positive relationship between MMY and LDR, meaning a high average MMY leads to an increase in lying time during daytime. For LMS = 3 and LMS = 2 on the other hand, an increase in MMY leads to a decrease in lying time during daytime. A similar pattern can be seen in the interaction between DIM and FDR, where for lame animals the FDR decreases with increasing DIM.

		p-value	
Interaction term	LMS = 1 and 2	LMS = 2 and 3	LMS = 1 and 3
MMY*LD	0.35	0.42	0.08
MMY*FDR	0.55	0.12	0.26
MMY*FD	0.20	0.08	0.44
MMY*LDR	0.23	0.04	< 0.005
MY*FP	0.59	0.37	0.17
MY*FI	< 0.005	0.01	< 0.005
MY*FIM	0.23	0.58	0.17
MMY*LBNR	0.10	0.04	0.27
MMY*LBN	0.60	0.03	0.02
MMY*C_MN	0.09	0.06	< 0.005
MMY*C_MNR	0.19	0.05	< 0.005
MY*BCS	0.66	0.38	0.81
DIM*FD	0.67	< 0.005	< 0.005
DIM*FDR	0.31	< 0.005	< 0.005
DIM*LBN	0.13	0.03	0.17
DIM*LBNR	0.51	0.06	0.10
DIM*LDR	< 0.005	0.04	0.86
DIM*LD	0.08	0.08	0.46
DIM*FP	< 0.005	0.42	0.11
DIM*FI	0.59	0.03	0.15
DIM*FIM	0.98	0.61	0.88
DIM*C_MN	0.78	< 0.005	< 0.005
DIM*C_MNR	< 0.005	< 0.005	0.17

 Table 27:
 P values for interaction terms with the locomotion score as outcome.

BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, FI; feed intake, FP: feeding pace, FIM: feed intake per visit, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LMS: locomotion score, MY: milk yield, MMY: average monthly milk yield.



Figure 43: Interaction between lying duration during daytime (in minutes) and average monthly milk yield (in kg) for each locomotion score group (LMS).

The grey areas around the lines represent the 95% confidence interval for the generalised linear model.



Figure 44: Interaction between feeding duration per day (in minutes) and days in milk for each locomotion score group (LMS).

The grey areas around the lines represent the 95% confidence interval for the generalised linear model.

Finally, a statistically significant difference can be seen in the slope of the interaction between FI and MY (see Figure 45) for LMS = 1, where FI increases more sharply with an increasing MY than for LMS = 2 and LMS = 3.



Figure 45: Interaction between milk yield (in kg) and feed intake (in kg) per day divided by locomotion scores (LMS).

The grey areas around the lines represent the 95% confidence interval for the generalised linear model.

6 Discussion

6.1 Validation of pedometers

The aim of this study was to test and further develop a predictive algorithm which only uses data collected by pedometers and data available on all commercial dairy farms, such as average monthly milk yield. The aim of the validation of the technology used in this study was to assess the accuracy of activity sensors available on the market in order to assess the feasibility of lameness detection using equipment widely in use on commercial dairy farms.

6.1.1 Validation of measured lying behaviour

The validation of the lying times showed a very good level of correlation and agreement between LD measured by the pedometers and LD measured by an observer. The concordance correlation coefficient for the LD was high at $\rho_c = 0.96$. The best fit line's 6 % location shift indicates the possibility of a systematic error. The scale shift on the other hand was of only 2 % and suggests the measured values are slightly lower in lower value ranges, and slightly higher in high value ranges the values measured by DO. These results are in line with Borchers et al.'s (2016) [212] who compared the "Track a Cow" pedometers to direct observation and calculated $\rho_c = 0.99$, an 11 % location shift and a 3 % scale shift. Studies that validated pedometers using other previously validated pedometers attached to different limbs achieved lower levels of correlation than the ones found in this study ($\rho = 0.82$ for the pedometers validated in Higginson et al's study [213]), indicating that the use of DO as a gold standard is more reliable than using other sensors, especially if fixed to two different limbs. In the study by Borchers et al. [212], 48 animals were observed for 4 hours while in this study the animals were observed during daytime and for a varying number of hours. Also, the agreement between LBO and LBP was not reported. It is conceivable, that a more accurate statistical estimate of the pedometers' accuracy regarding lying time could be achieved with longer observation sessions distributed over both night and daytime and the same number of observation hours per animal. The comparison of lying bouts showed more discrepancies than the lying duration; in 2.6% of the total observation hours, the LBO > LBP and the LDO > LDP and in 37 % of these cases, the difference between LDO and LDP was ≤ 2 minutes. Alsaaod et al. (2015) [214] hypothesised grooming behaviour to be a possible reason for pedometers recording more LB but only a slightly longer LD than an observer. This behaviour could not be observed in this study, so only assumptions can be made regarding the origin of this inaccuracy. In 5.9 % of cases, the LBO < LBP and the LDO > LDP; this could be due to cows resting with their leg with the pedometer attached to it, in an angled position, meaning the pedometers registered the cow as "standing" when she is actually lying, thus measuring more LB ad less lying time. In 29 % of cases, LBO = LBP but LDO < LDP, this could be due to incongruences in the time stamp or the observer reacting more quickly than

the pedometers in registering the animal standing. The two-minute sampling interval for the lying duration proved suitable for accurately estimating cows' lying time. Mattacchini et al. (2013) [215] also came to a similar conclusion in their study comparing two types of pedometers and found that sampling intervals ≤ 2 minutes are required to accurately measure LD and LBN per day.

6.1.2 Validation of measured feeding behaviour

The validation of the measured FD showed the highest level concordance [207] for Case 1, where the FDP were compared to the FDO only when the animal was in the position "feeding", and decreasing degrees of concordance for Cases 2 and 3. This means that the pedometers effectively recognised the animal when it was in the "feeding" position and less when the animal was "at feeding fence" or "near feeding fence". The outliers and the extreme values of up to 39 minutes difference show that there was a strong influence of the position of the pedometers on the difference in minutes per hour between FDP and FDO. The pedometers that were parallel (12 o'clock) to the magnetic field induced by the induction loop were therefore not recognised as well as the pedometers which were at an angle. The position of the pedometer did not on the other hand, have any influence on the difference between FVP and FVO. The case with the least number of discrepancies for the FV was Case 4, in which the visits were counted using a six-minute meal criterion similar to the pedometer's algorithm (see 4.2.3.2). In n = 13 cases, the FVP < FVO in spite of the counting of visits being clustered in the same way as the pedometers. In n = 7 of these cases, the cow was already near the feeding fence when the observation started and the event was counted as one visit. The pedometers on the other hand would have counted the visit in the previous hour. This would result in a discrepancy between the two methods. In another n = 3 cases, the animal was in the position "near feeding fence" and then moved away again, accounting for one visit in the DO but not for the pedometers. Borchers et al. (2016) [212] also evaluated the precision of measured FD for the pedometers used in this study and found a concordance correlation coefficient $\rho_c = 0.79$ between DO and measured values. These results are similar to the results found in this study for Case 3 (where the positions F, AFF and NFF were summarised in the DO) but lower than the results for Case 1, where only the position "feeding" was compared to the values measured by the pedometers. The positioning of the animals at the feeding table recorded by the observer as feeding behaviour were not reported in Borcher et al.'s study (2106) [212], so a comparison of the different Cases was not possible. The number of visits to the feeding table was not evaluated so could not be compared to the results of this study. The results of this study are also similar to those obtained by Mattachini et al. (2016) [216], who validated automatically recorded feeding behaviour with visual observation as the gold standard. In their study, Mattachini et al. concluded that there was a strong linear relationship between automatically recorded feeding time and visual observation ($R^2 = 0.90$, n = 12, p < 0.001), but the FV were not accurately recorded by the accelerometers used in the study ($R^2 = 0.31$, n = 12, p < 0.06). Their study was not directly comparable to this study, as the accelerometers used were attached to the neck as opposed to the limb and used three-dimensional acceleration to detect feeding behaviourNonetheless, the conclusion that feeding visits are more problematic in their classification than actual feeding times confirms the results of this study.

The validation of the pedometers regarding measurement of lying and feeding behaviour demonstrated that the pedometers have a high level of accuracy and are suitable for both research purposes and for use in the field, although loss of information regarding the number of actual visits to the feeding table, where a visit is counted every time the animal enters the induction loop's magnetic field, is conceivable, due to the clustering performed by the pedometer's algorithm. The use for research is also limited by the summarising of the raw data into hourly values in the data bank, which offer a low data resolution not suitable for a more in-depth analysis of dairy cattle behaviour.

6.2 Locomotion scores and claw health

6.2.1 Lameness prevalence

Due to the different number of data collection months on the farms in this study, some farms had a higher number of scores than others. The SD of the number of scores per animal was also high, indicating that some animals only had one or two scores during the data collection and were then either culled or sold. Especially on CDF3, many heifers only briefly entered the herd before being sold on.

The results of this study confirm the level of lameness prevalence already reported by previous studies. The lameness prevalence in this study, calculated as the relative share of number of LMS = 3 (excluding interpolated scores) in the total number of scores per month and per farm, ranged between 3.5 and 12.7 % and was considerably lower than the 25-58 % reported by Winckler and Willen [84] for German dairy herds in 2004, but was in the range of the more recently reported results by Griffiths et al. in 2018 (5.8-65.4%) [6] for the United Kingdom. Although the cows with LMS = 2 were unsound (or suspected lame), they were not included in the calculation of prevalence. The cows which had been examined for pain in the claws with a positive outcome had been changed to LMS = 3 so were included in the lame group for the estimation of lameness prevalence on the farms. Unfortunately, only one study was found regarding the prevalence for the farms in their study investigating risk factors in cubicle housed cows, of 31 %. The results of this study are still close to those reported by Wells et al. (1993) [43] (13.7 % prevalence in the summer and 16.7 % in the winter) almost

25 years ago. However, the results of studies which provide an amount of lameness prevalence or incidence are not always directly comparable as the authors all give a different definition of lameness. Some authors, such as Costa et al (2018) [86] calculated the lameness prevalence using only severely lame animals (scores 4 and 5 on a five-point scale). Others, such as Espejo et al. also used a five-point MLSS [135], but considered all animals with a LMS \geq 3 to calculate lameness prevalence. Griffiths et al.(2018) [6] on the other hand, considered animals to be "clinically lame" with a LMS \geq 2 on a 0 to 3 point scale, similarly to Borrero et al. (2017) who found a very high lameness prevalence (98.66%) on a farm in Spain when considering all animals with LMS \geq 2 [217]. Using only lame and severely lame animals and excluding animals which show moderate signs of lameness, leads to underestimation of the actual lameness prevalence. In studies which present a relative share of lameness incidence on the other hand, the relative share corresponds to the number of new cases of lameness that arise over a certain period of time, meaning that the incidence could be underestimated if only severely lame animals were noticed and subsequently examined and treated.

Although studies report the beneficial effect of claw trimming and early treatment of lame animals on hoof health [129, 218, 219], due to the lack of an overall decreasing trend in the relative share of lame animals on the farms, it cannot be assumed that the lower lameness prevalence in March 2018 (3.5 %) was due to the regular claw health assessments carried out in this study. It is more likely, that due to the limited number of lame animals on the farms at the beginning of the data collection phase, there was no considerable reduction in lameness prevalence during the course of this study. Also, no changes were made to the farm routine, so any management aspects that could have negatively influenced claw health were not addressed. On CDF1, there was a slight increase in lameness prevalence towards the end of the data collection period. As it is widely accepted, that hoof trimming has a beneficial effect on claw health [218, 219], it is possible, that the increase in lame animals could have been due to the time lapse between the summer claw trimming, which took place in June 2017, and the winter trimming, which took place seven months later in January 2018. As CDF1 was the only farm to have solid rubber flooring, regular hoof trimming of the whole herd should have been carried out more often. Although studies suggest that rubber flooring has a beneficial effect on claw health [52, 220], claws on solid rubber flooring tend to be longer at trimming than those of cows kept on concrete flooring [52, 221] as the claws are not subject to the abrasive effect of concrete and thus need to be trimmed more regularly. A study by Fjeldaas et al. (2011) [221] found more SU in animals kept on rubber flooring but less SH and WLD, which could be due to the non-physiological load exerted on the centre of the claw sole when interdigital cleft is overgrown. Furthermore, compared to slatted floors, solid flooring leads to increased accumulation of slurry in the alleys [222], and poor hygiene increases the risk of claw lesions such as HHE [223] and DD [60].

The higher lameness prevalence in May 2018 can be traced back to the fact that only the animals from the RFG were scored in that month, which also had the highest mean LMS and the highest number of findings per visit after CDF4. Not considering the months where data were only collected from cows on the RFG, the month with the highest overall lameness prevalence was February 2018. Although claw trimming has a beneficial effect on claw health [129, 218], the highest level of lameness prevalence in this study unexpectedly coincided with the month after which routine claw trimming took place on three of the five farms. A study by Chapinal et al. (2010) [142] reported increased LMS and lying times for up to five weeks after claw trimming, so it is possible that a deterioration of the mean LMS followed claw trimming and increased the lameness prevalence. In the last week of February 2018, the outside temperatures dropped to -18°C and due to the frozen ground, it was sometimes problematic distinguishing lame animals in the video recordings, from animals that had an uneven gait due to the slippery ground conditions. Although the animals were then clinically examined, the high prevalence of SH and WLD on CDF1, CDF2, CDF4 and RFG made it difficult to distinguish clinical findings that were causing lameness from those which were not.

The months with the lowest mean LMS (April and May 2017) did not coincide with the month with the lowest lameness prevalence; this indicates that in the months with a low lameness prevalence, there was still a high number of animals with LMS = 2, which would increase the mean LMS but not the prevalence.

As it is widely recognised that lameness is a painful condition [8, 94, 97, 224] and that farmers tend to underestimate lameness prevalence [20] on their farms, research aimed at detecting lameness should consider all lame animals, regardless of the degree of locomotion impairment. When using the score according to Sprecher et al. (1997) [135] for instance, all animals with a LMS \geq 3 should be considered lame, in order to raise awareness about the high average lameness prevalence on dairy farms and to sensitise farmers, claw trimmers and veterinarians to recognise early signs of lameness and to treat dairy cattle before locomotion deteriorates further, causing unnecessary pain for the animal. The LMSSGL developed in the course of this study was conceived for use in an on-farm environment and is connected to a recommendation for action (LMS = 1: no action required, LMS = 2: observe and LMS = 3: treat without delay) which should help the scorer decide what to do with both mildly and severely lame animals.

6.2.2 Lameness detection

Cattle are a stoical species that evolved as animals of prey, so they avoid overtly exhibiting pain [225], making early lameness detection problematic. Nonetheless, early recognition and treatment of lame animals reduces lameness prevalence within the herd as well as the number of animals whose lesions go on to become severe and influence long term animal welfare and

milk yield. In a study by Leach et al. (2012), [129] lame animals treated early had a significantly lower lameness prevalence than the control group, and presented with less severe foot lesions and were less likely to be selected for further treatment, thus reducing the amount of labour involved and benefitting animal welfare. The short development period (mean n = 9.3 days) for cases of lameness analysed in this study was presumably a consequence of the frequent farm visits and trimming of lame animals. Unfortunately, no similar research was found to compare the time span of lameness development found in this study.

90% of animals who were scored lame and were subsequently examined had clinical findings. Although this confirms the validity of MLSS for recognising lame animals, it does not account for the presence of false negatives. The use of video recordings allowed for retrospective evaluation of lame animals and in some cases (in 0.2 % of scores) implied the modification of a previously assigned FLMS (indicating the animal was at the time falsely scored) thereby reducing the number of false negatives. It is on the other hand possible, that a case of lameness which was overlooked at the FLMS resolved itself within a fortnight and thus went unrecognised at the following FLMS. The presence of false positives can also not be excluded; only 6 cows that were scored lame were subsequently changed to sound or unsound if they had no pain, no clinical findings and were not scored lame in the video recordings. Especially in the winter when the ground was frozen and slippery, cows sometimes had an uncertain and irregular gait when exiting the milking parlour. If these cows were then examined and had a mild form of SH or WLD they could have been false positives as this type of lesion is not always painful enough to cause a modification of the gait [126] (although their gait would have been unbalanced due to slippery flooring). Future studies investigating the relationship between LMS and claw lesions should consider examining all animals, in order to investigate the presence of highly prevalent lesions such as SH and WLD and their effect on both sound and lame cows.

An insight into the manifestation of pain in dairy cattle in this study was given by the results of the pain test performed on repeatedly "unsound" (LMS = 2) cows; over half of the animals tested for pain either had clinical findings or reacted to the pain test, demonstrating that absence of lameness does not necessarily imply absence of pain. Cows manifest pain in different ways [226], and their tendency to hide pain combined with the subjectivity of pain assessment [227] limits the effectiveness of detection through locomotion scoring. A solution to the problem of subjective pain assessment could be automatic lameness detection; studies investigating the use of algorithms for lameness detection systems report promising results [125, 160, 228]. It is conceivable, that algorithms that include behaviour parameters, have the potential of detecting lameness before methods that only use locomotion parameters, as they do not rely on changes in the animals' locomotion for detection of lameness, which may only occur when the underlying clinical lesion is already at an advanced stage.

6.2.3 Manual locomotion scoring systems

The LMSSGL proved to be an effective tool for recognising lame animals in this study. The reliability of the LMSS was close to the levels of reliability mentioned in literature; the PA for the inter-rater agreement (PA = 77.9-80.1 %) was similar to the 61.3-83.3 % reported by Barker et al. (2010) [56] for the DairyCo [133] LMSS and the 83 % PA reported by Hoffmann et al. (2013) [229] for video LMS with the score according to Sprecher et al. (1997) [135] (see Table 3). The Kappa statistics ($\kappa = 0.423-0.606$) correspond to the results obtained by Rutherford et al. (2009) [137] for the DairyCo score. The PA for the intra-rater agreement (PA = 82.3 %) was higher than the results reported by Channon et al. (2009) [138] for the Manson and Leaver score [75] (PA = 30 %), but the Kappa statistics for the intra-rater agreement ($\kappa = 0.6$) were very similar to the results reported by Yamamoto et al. (2014) [140] for the score according to Flower and Weary (2006) [134] ($\kappa = 0.67$). Although the inter-rater agreement for the LMSSGL was higher for the DO than the VO, video observation was used in this study in order to have a consistent reference method. Furthermore, doing the FLMS by DO could also have influenced the animals more in their behaviour. The video recordings also allowed for greater flexibility during locomotion scoring, giving the viewer time to pause and rewind recordings and analyse different gait parameters such as head bobbing or stride length at different moments. Also, the viewing of the recordings at double speed increased the fluency of the animals' movement and made the gait parameters easier to analyse. The VO for locomotion scoring was, on the other hand, problematic due to the potential inaccuracy connected to adverse lighting or weather conditions. Although the level of reliability and agreement can be increased by reducing the number of available scores [56, 138], reducing the number of scores also implies a loss of information regarding the severity of lameness, which could negatively impact studies investigating the link between lameness and claw lesions [230] or the development of lameness over time. In a commercial dairy farm setting however, this seems to be less of an issue and is compensated by the simplicity and clearness, and therefore the applicability, of a three-point score.

Not many studies have been made of the association between LMS and clinical lesions; although it could potentially increase the accuracy of MLSS as a reference method for claw health. The results in this study highlight the need for further research regarding the relationship between lameness and claw lesions, especially the presence of lesions in non-lame animals. In this study it was not possible to check sound animals for lesions, but testing unsound animals for pain in the claws provided information about how the perception of pain varies in individual animals, and also depends on how long they have been affected by the claw lesion causing the abnormalities in locomotion. The results of the claw examinations and of the LMS across all farms showed a positive correlation between number of claw lesions and increasing LMS. Similarly, Thomsen et al. (2012) [123] found higher odds of cows having claw lesions with a higher LMS using logistic regression. Tadich et al. (2010) [231] on

the other hand, found a relationship between SU and increasing LMS, but no statistically significant association between HHE, SH and WLD and increasing LMS, implying that different claw lesions affect dairy cows' locomotion in different ways.

6.2.4 Results of clinical examinations

In Thomsen et al.'s (2012) [123] study on claw lesions and LMS, of the 1340 animals included in the analysis, 452 had hoof lesions; 22 % of animals with lesions had SU, 2,5 % had severe SH, 1 % had WLD, 22% of cows had IH and 65 % had DE [123]. The incidence of lesions in this study was higher, especially for SH (44.2 %, intended as the number of animals with SH divided by the total number of animals scored during data collection), although this may be due to the severity of the haemorrhage being taken into account for the evaluation in Thomsen et al.'s study, while even mild cases of SH were documented in this study. The incidence of WLD was also much higher in this study with 42.9 %, while DE was a lower at only 27.3 %. The incidence for SU (11.7 %) was also lower than the incidence reported by Amory et al. (2008) [232] with approximately 29 % SU, 22 % WLD and 18 % DE.

Unsurprisingly, there were statistically significant differences between farms in the number of clinical findings; CDF3 had fewer findings than the other farms which could be a consequence of the fact that the farmer treated many lesions himself and did not always reported them. The claw trimming of the whole herd by a professional claw trimmer took place at least once on each farm during data collection except on CDF4 and findings were documented and included in the analysis, which accounts for the high SD in the number of findings per month. Even though animals with findings in these cases were then locomotion scored in the video recordings to check for lameness, some had findings that would not have been documented during regular farm visits as they did not present with an LMS = 3.

Studies report a correlation between different lesion types found on lame animals' claws [79, 231], and although no in-depth analysis of the correlation between occurrences of different findings was carried out for this study, the presence of more than three lesions per examined animal in 55 % (n = 202) of examined cases may indicate a relationship between different diagnoses, due for example to the weakening of the claw horn structure and the subsequent increased risk of further damage to the claw, as suggested by a study by Winkler and Margerison (2012) [233] who found the mechanical resistance of claws with SH to be lower and thus more prone to trauma or secondary infection.

6.2.5 Lameness development

The results of the analysis of the development of lameness cases in this study demonstrated how most cases of lameness developed in under two weeks, with a peak between one and three days. The high number of cases which developed in under five days (n = 110, 45%) could in part be due to the choice to compute the number of days between the last day the

animal was scored sound and the lameness onset. This did not account for the possibility that the last LMS = 1 could have been a temporary state, or have been wrongly scored, and that the actual lameness onset could in fact have been earlier. Cases of chronic lameness or suspected lameness were only followed back to the beginning of video recordings (February 2017), and only cases whose development could be fully analysed were included in this study. Still, there was a high SD (13.4) for the length of the development period, with a maximum of 103 days. The frequency of visits was probably the main reason for lameness cases being recognised and treated promptly and there only being a median difference of five days between lameness onset and lameness discovery. Also, the animals that were tested and reacted to the pain test, were changed to a LMS = 3 on the days of the test, but remained LMS = 2 for the days prior to the FLMS when the pain test took place. Of the n = 38 animals that had a difference of zero days between lameness onset and lameness discovery, only five were animals that had been scored "lame" following a pain test. These findings confirm the need for regular locomotion scoring, ideally every fortnight, in order to manage lame animals at an early stage and prevent chronic cases of lameness, which cause high levels of pain [234] and decrease the nociceptive threshold in dairy cattle [230].

It should be noted, that data was not collected in all seasons on all farms, so it was not possible to calculate the influence of seasonality across all farms for the number of clinical findings.

6.3 Behavioural and performance parameters

6.3.1 Lying and feeding behaviour across farms

The median lying duration per day of 11.5 hours found in this study is in agreement with the findings of Westin et al. (2016) [112], who reported a median lying duration of 11.4 hours a day for dairy cows in AMS farms. For free stall farms with milking parlours, where cows may have to stand for longer hours when waiting to be milked, the results reported by Keyserlingk et al (2012) [235] were in the same range (mean lying time between 10.6 and 11 hours a day depending on the geographic location in the study) as were Charlton et al.'s (2014) [236] (10.6 hours a day). No pronounced difference could be found between the overall LD and the LDB between AMS and milking parlour farms in this study. In the study by Keyserlingk et al (2012), the lying times had a high individual variation (2.8 to 20.5 hours a day). The findings by Charlton et al. (2016) on the other hand report less individual variation (Min = 8.7, max = 13.2 hours a day). The LD recorded by the pedometers in this study was also subject to a high level of variation in the daily values, with lying times ranging between 0.5 and 23.5 hours per day (SD = 148 minutes). The median LDB found in this study (37 minutes) is lower than that reported by Charlton et al. (2014) [236] and Westin et al (2016) [112] of respectively 1.2 hours and 71 minutes. The median and mean number of lying bouts across farms

(med = 14, mean = 24), was higher than the results reported by Gomez and Cook (2010) [111] for both herds with sand and with rubber mats used in the stalls (mean = 14.4 and 10.2 bouts per day respectively). The results were also higher than the results reported by Charlton et al. (2014) [236] (med = 10.5 lying bouts per day) and Westin et al. (2016) [112] (med = 9.5 lying bouts per day), which reflects the lower median duration per bout and indicates more frequent standing to lying events. The results from the validation of the sensors showed the pedometers measured on average more lying bouts than the observer, which could be a possible explanation for the higher median LBN than the results reported in literature.

The differences between the single farms were statistically significant for most behavioural parameters; notably the median FD on the RFG (med = 110 minutes per day) was less than half the median FD on all other farms, with the most extreme comparison being CDF1 (med = 311 minutes per day). The mean FD for the RFG (mean = 110 minutes per day) was lower than the mean found by Schindhelm (2016) [9] (mean = 177.3 minutes per day) in a study which was carried out at the same research facility. The FDW was slightly higher with a mean of 129.8 minutes per day, but still below that reported by Schindhelm (2016) [9]. A possible explanation could be that the maximum number of cows milked at one time in the study by Schindhelm (2016) [9] was 65, while the median number of cows being milked on RFG in this study was 66, meaning there were more animals per feeding trough and possibly more competition within the herd. The reduced FD on the RFG compared to the commercial dairy farms could be due to the structure of the weighing troughs (see Figure 12) that forces the animals to feed with their head in an unnaturally high position. Additionally, there are only 36 feeding troughs at the RFG for a median of n = 66 cows milked, and although there are no legal regulations regarding the maximum number of animals per feeding place in a barn, for Bavaria the guidelines for constructional requirements for the raising of dairy cattle [237] indicate a maximum cow to feeding place ratio of 1.5:1. The ratio for the RFG was 1.8:1, meaning the cows are restricted in their feeding behaviour, leading to lower feeding times.

The LD differed significantly between farms; the mean LD on CDF1 was 623 minutes, while on CDF3 it was 745 minutes. The limited number of farms in this study makes statistical analyses comparing housing systems and management choices problematic, but assumptions can nonetheless be made about the influence that these have on cows' behaviour. CDF1 had a stall to cow ratio of 1:1.2, while all other farms had a 1:1 ratio or lower, so overstocking could be a reason for reduced lying times, as suggested in a study by Fregonesi et al. (2007) [110]. Although some studies [63, 238, 239] report that cows have longer lying times on deep bedded stalls, no such assumptions could be made in this study due to the limited number of farms with different types of stalls. Furthermore, the two farms with the highest and lowest mean LD both had deep bedded stalls. A study by Fregonesi et al. (2007) [110] on the quality of 'bedding in stalls reported that cows preferred dry bedding to wet bedding, and spent more time standing if the bedding in stalls was wet. The fact that on CDF1 fresh bedding was only added every two weeks (compared to every day on CDF3) could have an influence on the animals' lying behaviour.

6.3.2 The influence of lameness on behavioural and performance parameters

The findings of this study regarding the influence of lameness on behavioural parameters agree with the results reported in current literature. The mean LD across farms in this study increased significantly for animals with LMS = 2 and LMS = 3 compared to animals with LMS = 1, as did the mean LDB. The LBN decreased with increasing LMS, but the variability of the length of lying bouts increased in lame animals, confirming the findings of Ito et al. (2010) [63] and Thomsen et al. (2012) [123]. Studies by Grimm et al. (2019), Ito et al. (2010) and Westin et al. (2016) [63, 112, 228], also came to the same conclusions and found longer lying times for lame cows. Lame cows' feeding behaviour was also influenced by lameness; all parameters associated with feeding behaviour (FD, FDR, FDM, FDV, C MN, C FDM, C MNR, FP, FIV, FIM, VN, VNR, MN, MNR, FDW and FDRW) showed statistically significant differences between at least two LMS groups, except for FI. The mean FD decreased with increasing LMS as did the C FDM and the C MN. So overall, lame cows fed for less time, in less frequent and shorter visits to the feeding table. It can be assumed that this change in behaviour is probably due to cows being reluctant to spend a long time standing if they have pain in their claws. The variables which were exclusive to the RFG and were measured by the automatic weighing troughs behaved in part differently. While FDW, VN, MN and FI decreased with increasing LMS, both the FIV, FIM and the FDV and FDM increased. This indicates that lame cows on the RFG spent less time feeding overall and had an overall reduced FI, but the meals and visits became longer. This could be a consequence of the C FDM for the RFG being distinctly below the average (14 minutes per meal vs. mean 27 minutes per meal across farms) to start with, so lame cows had to increase their time per visit or meal at the weighing troughs in order to satisfy their intake needs. The discrepancy between C FDM and FDM could be due to the fact that if a cow moved its front limb further back (outside the induction loop) while eating at the weighing troughs, the visit would be interrupted, while the visit would still be registered by the weighing trough. These findings are partly in accordance with the results in current literature; Grimm et al (2019) [228] found that cows feed for less time, in fewer, shorter meals, which agrees with the data recorded by the pedometers but not with the data recorded by the weighing troughs. These findings are unexpected, as the data collection took place in the same research facility with the same weighing troughs. It is possible, that the feeding trial that took place during Grimm et al.'s (2019) [228] study influenced animals' feeding behaviour at the time, and that the herd at the RFG had different feeding habits during the data collection phase of this study. González et al. (2008) [126] also found a decrease in FI, FD and VN in lame cows, as did Thorup et al.

(2015) [11]. In both these studies FP increased in lame animals; in this study only the median feeding pace increased by 0.1 kg/min from LMS = 1 to LMS = 2.

The influence of lameness on cows' lying [9, 63, 114, 117] and feeding behaviour [9, 114] could in turn influence performance [114], particularly milk yield. Many studies have investigated the influence of lameness on milk yield [13, 14, 17, 103, 127, 232] and came to different conclusions; although most studies indicate a reduction in milk yield for lame cows [12–17, 39, 114, 118, 127], some studies found no statistically significant differences in milk yield for lame and non-lame cows [9, 21, 101, 240] and some even report an increase in MY [102]. The results of this study show that there was a significant difference in average monthly milk yield between LMS = 1 and LMS = 2 and LMS = 3, but no statistically significant differences for daily milk yield values. Although the daily MY was slightly lower for lame cows than for sound cows, the MYM and MMY both showed a slight increase (average MMY for LMS = 1 30.4 kg and 30.8 kg for LMS = 3)These findings confirm that milk yield is not a reliable indicator for lameness, and that the decrease in milk production occurs at early stages of lameness [13].

Finally, the OR for lameness were close to 1 for most parameters analysed in this study. The parameters, which had an OR > 1.5 or OR < 0.9 for the outcome lame, were all statistically significant. The feeding pace had a very high OR for both L3 and L23, meaning animals that increased their feed intake per minute were more likely to be lame. Lameness also increased with longer visits and increased feed intake These findings agree with the results reported in literature [10, 126, 228]. The OR of FIV and FIM on the other hand were > 1 indicating a positive relationship with LMS and did not correspond to the findings reported in literature; in Grimm et al.'s (2019) as well Norring et al's (2014) [10, 228] studies, lame cows tended to decrease their feed intake.

6.3.3 Intrinsic factors

Studies show that the OR for lameness is higher for cows who are in their third lactation or above compared to cows below their third lactation[45], and that parity and stage of lactation are significantly associated with lameness [41, 43, 112], although cows with lower parity have higher odds of developing specific diseases, such as DE [71]. In agreement with current literature, the mean parity for lame cows in this study was 3.4, and was significantly higher than the mean parity of sound cows (2.7), as was the difference in average number of DIM for lame cows compared to sound cows.

Many studies reported a positive influence of low BCS on lameness [35, 112, 241], in this study there was no statistically significant difference between LMS = 2 and LMS = 3 groups for BCS, but the OR (in this case for both L3 and L23) was < 0.5, meaning an increase in

BCS had a protective effect. It should be noted, that BCS measurements were only available on RFG, so the findings may not be representative of other farms in the study.

6.4 **Results of data modelling**

Statistical modelling has been widely used in studies to attempt to predict lameness based on behavioural and performance parameters [113, 163, 165, 166, 228, 242] and has achieved promising levels of predictive accuracy with, for instance, an AUC = 0.94 for the model developed by Grimm et al. (2019) [228] and AUC = 0.96 for Beer et al. (2016) [125].

6.4.1 Generalized mixed linear model

The predictive accuracy of the GLMMRE model used in this study was comparable to the results of models applied in other studies. With an AUC = 0.96 (CI: 0.96, 0.97), the GLMMRE model had the highest accuracy on training data, but both GLMMRE2 and GLMMRE had an AUC = 0.91 on the unbalanced test data. The results are similar in terms of accuracy, to those reported by Grimm et al. (2019), Beer et al. (2016) [125, 228], and also to those of van Hertem et al. (2013) (AUC = 0.89 for their logistic regression model using MY, AC and ruminating time) [165]. Although the ICC of the random effects was < 0.4, meaning the within-farm variance differed only marginally from the overall variance, the model calculated without random effects (GLMM), had a lower predictive accuracy for the training data (AUC = 0.86, CI: 0.85, 0.87) and a much lower accuracy for the test data (AUC = 0.70, CI: 0.67, 0.72), indicating that farm and individual variance had a stronger influence on the overall strength of the model than initially assumed. This agrees with the results by Solano et al.(2016) [113] who, for example, found herd-level factors to significantly affect measures of lying behaviour, which would in turn represent a bias factor in a predictive model if the herdlevel variance was not accounted for. Although the GLMMRE had good level of accuracy, the high number of predictors limits the interpretability of the model. While the model computed by Grimm et al. (2019) [228] had 18 predictors, Van Hertem et al.'s (2013) [165] had seven predictors, and Beer et al's (2016) only featured two predictors; number of standing bouts and walking speed. The GLMMRE2 had a reduced number of predictors (23) compared to GLMMRE and only still retained a good level of predictive accuracy with an AUC = 0.93 on training data and 0.91 on the test data.

6.4.2 Elastic Net models

On individual farm data the ENET Beta approach used by Grimm et al. (2019) [21] in their study was also applied. The advantage of the ENET Beta approach is the reduction of predictors for the sake of model sparsity [200]. The Elastic Net model to which the ENET Beta approach was applied, had a good level of predictive accuracy for the training data (see Table 25), with an AUC ranging between 0.84 (CDF1 and CDF4) to 0.94 for CDF3, but after the application of hypothesis testing and the exclusion of predictors the accuracy of the
models decreased significantly and ranged between AUC = 0.73 (CDF1 and CDF2) and 0.80(CDF3) for the test data. CDF3's ENET Beta model was the one that retained the highest number of predictors after hypothesis testing, which probably contributes to the higher predictive accuracy. The strength and direction of the coefficient estimates for the different predictors varied significantly between Elastic Net models (see Figure 41). Only in the case of C MNR, LDR FDR and AC did the estimates have the same direction (negative or positive) relationship with the outcome. Some predictors, such as C MN, C FDM and FD, not only had a different direction but were also in different positions when ordered according to the strength of the relationship with the outcome on different farms. The ENET models on the single farms had a higher level of accuracy for the outcome L3 (only using LMS = 3 as positive cases) than for the outcome L23 (using both unsound and lame animals as positives). This indicates that although about half of the animals who were tested for pain in the claws were found to have clinical findings or a positive pain reaction, the threshold, for which the outcome lame is easier to predict, remains L3. Both RFG's and CDF4's ENET models had a higher SEN with the outcome L23 than they did for the outcome L3; this could be due to there being a higher relative proportion of LMS = 2 cows relative to the total number of scores on the farm, indicating that there is a greater chance that some of the animals scored "unsound" were actually lame, so using LMS = 2 as a threshold may have led to more of those animals being predicted as lame as opposed to being false negatives with the L3 threshold.

The ENET Beta approach applied to the data across farms did not achieve the same level of accuracy as the generalised linear mixed model. The training model before ENET Beta approach application (ENFM), achieved an AUC = 0.72 (CI: 0.69, 0.74), while the ENBM after hypothesis testing had an AUC = 0.81 (CI: 0.80, 0.83). In this case, variable selection achieved higher predictive accuracy, which was not the case for the Elastic Net models for the individual farm data. It is conceivable, that the factor "farm" (variable B in the dataset) had a strong influence on the predictors, which would explain the higher predictive accuracy of the GLMMRE model compared to the GLMM and Elastic Net models. A solution would have been to include random effects in the Elastic Net regression, but although studies exist on the implementation of regularization penalties on mixed effect models [243, 244], the approaches described in these studies are based on the assumption of normality in the data in this study.

Due to the factors that play a role in the detection of lameness, such as subjectivity [20, 85, 245] and dairy cattle's reluctance to exhibit pain [8, 97], ALSS may detect claw lesions before lameness is apparent to the observer [18]. The value of a lameness detection system depends greatly on its accuracy; a lameness detection system that detects false positives adds the cost of labour for examining the cow, without the effect of early detection. The balance between high SEN and SPE is a challenging aspect in the development of predictive algorithms;

ideally the two parameters have an equal value, but for many predictive models, as was the case for some of the models in this study, one of the two parameters is lower than the other. In the case of the GLMMRE2 for example, although the SPE for the test data was high (91 %) the SEN was low (76 %), meaning the model was not accurate in detecting lame animals. As the dataset was balanced using the SMOTE, it cannot be presumed that the low SPE was due to a high number of control cases and a low number of positive outcomes [246, 247].

The accuracy of the GLMMRE is comparable to the results reported in other studies which made use of behavioural parameters using sensors to detect lameness. Studies using the direct approach, so either measuring changes in dairy cows' locomotion (kinematic) or measuring the forces exerted by cows claws on a surface (kinetic) also achieved comparably high levels of accuracy. Dunthorn et al. (2015) [248] achieved a SEN of 90 % and a SPE of 93 % by using force plates to measure cows' weight bearing, while Pastell et al. (2010) [150] achieved an AUC = 0.88 using a similar system. The studies involving the measurement of variation of acceleration in the limbs report a particularly high predictive accuracy; Alsaaod et al. (2017) [160] achieved perfect SEN and SPE (both 100 %) for lameness detection using pedometers fixed to both hind limbs and measuring acceleration at high sampling rate, while Beer et al. (2106) [125] combined both behavioural parameters (standing bouts) and the kinematic approach (walking speed) and reported a SEN of 90.2 % and SPE 91.7 %. Although loggers with such a high sampling rate as those used by Alsaaod et al. (2017) [160] are, to the author's knowledge, not widely available on the market for on-farm use, the potentially very high detection rate of this type of kinematic approach is very promising. A combination of kinematic approach and continuous measurement of behavioural parameters may allow for an earlier detection of lameness, compared to the sole use of gait cycle variables.

Although the LMSSGL had a high level of reliability, the reference method for assessing claw health still remains fallible and subject to the observer's bias. Algorithms that are developed based on a LMSS for reference, such as the model applied to the data in this study, face the challenge of ordering a continuous state, such as lameness, into categories. In further research in predictive algorithms for lameness detection, the possibility should be considered of expressing the prediction in probabilities as opposed to a lame/non-lame binary outcome. On the one hand this could lead to subjectivity and personal preference playing a role in the interpretation of the results, but on the other hand, if implemented in herd management software, the probability outcome would lead to fewer false positives and possibly increase the validity of the prediction for the user.

6.4.3 Interactions between parameters

A moderate level of correlation was found between some of the predictors included in the models, such as between LBN and LDB ($\tau = -0.78$) and C_MN and AC ($\tau = 0.34$).

Multicollinearity is an issue for predictive models based on behavioural parameters. The increase of one type of behaviour can lead to the decrease in another, a higher level of AC for example, will lead to a decrease in the daily value of other parameters such as FD and LD. In the GLMMRE2 model, the correlated variables ($\tau > 0.7$) were excluded from the model and did not significantly affect the predictive strength of the model, indicating that they were not contributing to the pattern which was recognised by the model in the training data.

Some variable combinations showed different interaction mechanisms with lame and nonlame or unsound cows. This relationship is evident for example in Figure 45, where the FD decreases with increasing DIM for animals with LMS = 3 and increases for LMS = 1 and LMS = 2. Similarly, the interaction between MMY and LDR (Figure 44) for different LMS, suggests that animals whose LDR decreases with increasing MY, are more at risk of being lame. Grimm et al. (2019) [228] came to similar conclusions in their study in which they found an increased milk yield to be associated with an increased risk of lameness only if the LD decreased to below average. Bach et al. (2007) [118] also found a negative effect of MY on claw health connected to the decrease of dry matter intake and visits to the AMS.

The interactions between different variables in this study and their influence on the outcome for lameness highlight the complexity of the subject surrounding risk factors for lameness and consequences of lameness. Higher parity for example, is associated with higher odds of becoming lame [41, 43, 112] and with lower BCS [249]. Low BCS is in turn associated with a higher risk for lameness, as is a decrease in LW [35, 47, 112, 241].

At the same time loss of condition in early lactation is associated with higher MY, although this trend is reversed when cows lose condition too rapidly after calving [250]. Studies show that risk factors for lameness are often connected and influence one another, and that lameness on the other hand influences the same parameters in turn. Gráff et al (2016) [38] found animals to be at higher risk of being lame if they had a high MY in early lactation, as did Amory et al. (2008) [39], who found cows who developed an SU or a WLD to be higher yielding prior to diagnosis, although that was not the case for DD. Their milk yield then dropped to below the average of the unaffected cows before diagnosis.

It is difficult to establish a temporal relationship between behaviour and performance parameters and lameness, and whether the changes in these parameters led to lameness or lameness itself caused the changes. When considering LD for example, lame cows tend to lie for longer periods of time, and the number of lying bouts decreases [9, 63, 112–114]. In turn, LD is also influenced by BCS, increased parity and lactation stage [112, 251]. Lim et al. (2015) [241] attempted to investigate the temporal association between BCS and lameness and found that cows with a lower BCS at calving were at an increased risk of becoming lame, and if they were lame and had a low BCS at calving, they were less likely to recover from the

lameness. The reverse was also true, so animals with higher BCS at calving were less likely to become lame and more likely to recover if they already were lame. The findings support the results of Bicalho et al. (2009) [51] who found that lameness was associated with a decrease in the thickness of the digital cushion. Newsome et al. (2017) also found a positive association between decreased thickness of the digital cushion post-partum and back fat thickness [49].

Dry matter intake and feeding time are affected by lameness [118, 126, 228], probably due to reluctance of animals to spend long periods of time standing if they are experiencing pain in the claws due to lesions. This change in behaviour leads to a further decrease in BCS, indicating that BCS could be both cause and effect of lameness, as could be the case for other factors such as lying time, feeding behaviour and milk yield. Lim et al.'s (2015) [241] study on BCS in relation to lameness was a multilevel multistate discrete time event history model. Similarly, González et al. (2008) [126] conducted a study on the feeding behaviour of lame vs. non-lame animals 30 days before and 30 days after trimming and found a change in feeding behaviour in lame cows that reversed after trimming. Chapinal et al. (2010) [142] also monitored cows' lying behaviour before and after trimming and found cows increased their daily lying times for up to five weeks after trimming. This type of study involving analysis of continuously recorded daily data and its comparison with the individual pattern for each parameter and animal could be effective in recognising changes correlated with a lameness event. A study by Mazrier et al. (2006) [121] for example, compared the pedometric activity of lame cows to their previous ten-day activity average and found lame cows had a reduced pedometric activity seven to ten days before the appearance of clinical signs. Furthermore, timeline analysis could provide insight into the causality of factors that influence claw health and which are in turn influenced by lameness. This information could be used to improve the predictive accuracy of models aimed at early lameness detection.

6.5 **Outlook**

The LMS used for the GLMM and Elastic Net models were only a sample of the dataset. The use of repeated measurement at daily intervals for the same animal could have led to bias of the model, so the possibilities for analysis of the data collected in this project were not exhausted in this study. A first step for improving the models would be to experiment with different exclusion criteria for the data, excluding outliers that may have influenced the models in this study. In order to better understand the development of lameness over time, time series analysis should be applied to the DLMS data, to see how dairy cows' individual behaviour patterns change before and after a lameness event. The understanding of these patterns could lead to earlier lameness detection than with the application of algorithms which only use single observations of different animals to detect a pattern for lameness.

The use in this study of sensors that are already available on the market makes the implementation of the predictive algorithm in herd management software a realistic prospect.

In particular, the pedometers in this study recorded activity and lying as well as feeding behaviour, making the installation of further technology superfluous. The influence of the random effects for the individual farms on the models suggests that aspects of farm management should be included in the prediction algorithm to account for different environments influencing the cows' behaviour. Future studies should involve a larger number of farms and include with different housing systems and management choices as predictors in the model.

The further use of the LMSSGL for model development should be explored and the possibility of having a three-way outcome as opposed to just a binomial lame/not-lame outcome would be a useful feature in a software environment.

6.6 **Conclusion**

Not many studies have been made investigating the prevalence of lameness on farms with Simmental cows [228, 252]; the findings in this study offer an important insight into the level of lameness prevalence and incidence of claw lesions in Bavarian Simmental herds.

The pedometers that were used for data collection were validated and had a high level of accuracy for the recording of both feeding and lying behaviour, making them suitable for onfarm use and for behavioural studies that do not need high data resolution. At the same time the three-point LMSSGL was accurate as a reference method for claw health and could be used by farmers to simplify lameness detection by direct observation.

The fortnightly interval for locomotion scoring and treatment of the lame animals proved to be a suitable frequency for identifying animals at an early stage of lameness. The findings in this study demonstrate how locomotion scoring carried out only before claw trimming is not frequent enough to detect lame animals before the degree of lameness increases, affecting the dairy cattle's welfare and performance, as most cases of clinical lameness in this study took less than two weeks to develop.

The pain tests carried out on repeatedly unsound animals showed that over half of the animals that had only slight gait anomalies either had clinical lesions or reacted to pain. These results suggest that cows do not exhibit pain until the underlying claw lesion causing the lameness is so advanced that they cannot avoid showing signs of distress. For this reason, ALSS based on behavioural and performance parameters could help farmers recognise animals who have modified their behaviour due to lameness, but who would not be detected by visual locomotion scoring.

The behaviour and performance data, to which the models developed in this study were applied, were all collected by a single device and by using the average monthly milk yield.

The sparseness of technology involved in this study renders the accuracy of the GLMMRE regression more promising by increasing its potential for on-farm application.

Finally, the behaviour and performance parameters recorded in this study showed a high variation of the data at both herd and individual level. This indicates that management choices and structural differences on the single farms in this study play a statistically significant role in the expression of behaviour in dairy cows.

7 Zusammenfassung

Titel: Analyse von Klauengesundheits-, Verhaltens- und Leistungsdaten von Fleckviehkühen auf Praxisbetrieben zur Implementierung eines Lahmheitserkennungsmodells.

Lahmheit bei Milchkühen ist eine verbreitete Produktionskrankheit, die das Wohlbefinden beeinflusst und wirtschaftliche Verluste durch Leistungseinbußen verursacht.

Am häufigsten wird Lahmheit durch Klauenkrankheiten hervorgerufen. Die Klauengesundheit bei Milchkühen wird von extrinsischen Faktoren, wie zum Beispiel Haltungsbedingungen, sowie Managementfaktoren, wie Fütterung und Häufigkeit der Betriebsklauenpflege, beeinflusst. Zusätzlich hängen Klauenkrankheiten von intrinsischen Faktoren wie Laktationszahl, Gewicht, Body Condition, Rasse, Laktationsphase und Milchleistung ab. Lahmheit beeinflusst die Aktivität, das Liege- und Futteraufnahmeverhalten, die sozialen Interaktionen, sowie letztlich die Leistung von Milchkühen, insbesondere die Milchleistung, die Fruchtbarkeit und die Nutzungsdauer.

Eine frühe Lahmheitserkennung maßgeblich das Voranschreiten ist um von Klauenerkrankungen und somit Schmerzen und Leiden für die Tiere zu verhindern. Darüber hinaus, können durch Lahmheit erhebliche Kosten für die Landwirte entstehen. Die durch Lahmheit entstehenden Kosten lassen sich in direkte, zum Beispiel durch die Behandlung entstehenden und indirekte, z. B. durch Einbußen in der Milchleistung und verlängerte Zwischenkalbezeiten verursacht, unterteilen. Der Strukturwandel, der in den letzten Jahrzehnten auf Milchviehbetrieben stattgefunden hat, führte zu einem Anstieg der durchschnittlichen Tierzahl pro Betrieb. Diese Veränderung hat einen negativen Einfluss auf die für die Einzeltierbeobachtung zur Verfügung stehende Zeit. Außerdem unterschätzen Landwirte die Lahmheitsprävalenz auf ihren eigenen Betrieben signifikant; dies könnte unter Anderem auf die stoische Natur von Milchkühen und deren zurückhaltendes Schmerzverhalten, zurückzuführen sein.

Manuelles Lokomotionsscoring ist das Standardreferenzsystem zur Ermittlung der Klauengesundheit bei Milchkühen, die Reliabilität dieser Systeme wird allerdings von der individuellen Wahrnehmung von Lokomotionsparameter reduziert.

Automatische Lahmheitserkennungssysteme dagegen, nutzen Vorhersagemodelle, um die Klauengesundheit von Tieren zu beurteilen und könnten für eine frühzeitige Lahmheitserkennung eingesetzt werden. Automatische Lahmheitserkennungssysteme können direkt oder indirekt sein. Direkte automatische Lahmheitserkennungssysteme basieren auf kinetischen, kinematischen oder thermographischen Verfahren, während indirekte auf die Analyse von Verhaltens- und Leistungsparametern beruhen.

Das Ziel dieser Arbeit war es, ein in einem Vorgängerprojekt an der Bayerischen Landesanstalt für Landwirtschaft (LfL) entwickeltes Berechnungsmodell auf Praxisbetrieben zu überprüfen und weiterzuentwickeln. Teilziele dieser Arbeit waren, ein neues Lokomotionsscoringsystem zu entwickeln, das sowohl für die Forschungs- als auch für die Praxisanwendung geeignet ist, sowie die Bewertung des Systems hinsichtlich seiner Genauigkeit und Wiederholbarkeit zu überprüfen. Ferner sollten die für die Datenerfassung eingesetzten Pedometer validiert werden, um damit die Genauigkeit der Messung des Liege- und Futteraufnahmeverhaltens bestimmen zu können.

Über einen Zeitraum von 14 Monaten wurden Verhaltens- und Leistungsdaten von Milchkühen auf vier Praxisbetrieben in Niederbayern und vom Versuchsbetrieb der Bayerischen Landesanstalt für Landwirtschaft gesammelt. Alle am Versuch teilnehmenden Tiere wurden mit einem Pedometer an ihrem rechten Vorderbein ausgestattet. Die Pedometer enthielten einen drei-dimensionalen Beschleunigungssensor, der kontinuierlich die Aktivität und das Liegeverhalten maß und zwischen den Positionen "Liegen" und "Stehen" unterscheiden konnte. Zusätzlich enthielten die Pedometer eine RFID (radio frequency identification) Spule, die die Anwesenheit der Kühe am Futtertisch registrierte und somit in der Lage war, das Futteraufnahmeverhalten zu erfassen. Zusätzlich zu den von den Pedometern gemessenen Daten zum Futteraufnahmeverhalten wurden auch durch am Versuchsbetrieb vorhandene automatische Wiegetröge, Daten zu der Menge und Geschwindigkeit der Futteraufnahme erhoben. Daten zur Leistung der Tiere wurden von den automatischen Melksystemen auf zwei der vier Versuchsbetriebe sowie über die durch das LKV (Landeskuratorium der Erzeugerring für tierische Veredelung in Bayern e.V.) erhobenen Melkdaten erfasst. Als Referenzsystem zur Ermittlung der Klauengesundheit wurde alle zwei Wochen über Videoaufnahmen am Ausgang der Melkstände ein Lokomotionsscoring aller Projekts Tiere durchgeführt. Das im Rahmen des entwickelte Drei-Punkte-Lokomotionsscoringsystem, teilt die Tiere in drei Kategorien ein: lahm (unsymmetrischer, ungleichmäßiger Gang), Verdacht auf Lahmheit (bei der Anwesenheit mindestens einer der folgenden Merkmale: Entlastungshaltung, Rückenkrümmung oder Kopfnicken) und gesund (regelmäßiger, symmetrischer Gang). Im Anschluss an das Lokomotionsscoring wurden klinische Untersuchungen und gegebenenfalls Behandlungen der Klauen von den als lahm befundenen Tieren durchgeführt. Zusätzlich, wurden alle Tiere, die für drei aufeinanderfolgende Lokomotionsscores (oder sechs Wochen) als Verdacht auf Lahmheit eingestuft wurden, mithilfe einer Klauenuntersuchungszange auf Schmerzhaftigkeit der Klauen untersucht. Bei einem positiven Schmerztest oder bei der Anwesenheit von klinischen Befunden wurden die Tiere als lahm eingestuft. Die täglichen Videoaufnahmen ermöglichten eine retrospektive Lahmheitsentwicklungsanalyse, bei der der genaue Zeitpunkt des Einsetzens der Lahmheit bestimmt werden konnte. Die Identifikationsdaten, die Verhaltensund Leistungsdaten sowie die manuell erhobenen Daten zur Klauengesundheit wurden anschließend in einem SQL Datenbanksystem zu einem Tagesdatensatz pro Tier zusammengefasst.

Sowohl die Validierung des Liegeverhalten als auch die Validierung des Futteraufnahmeverhaltens ergaben einen hohen Übereinstimmungswert mit der Direktbeobachtung, mit $\rho_c = 0.96$ (Konkordanz-Korrelationskoeffizient) für die Liegedauer, W = 0,80 (Kendalls Konkordanzkoeffizient) für die Anzahl an Liegeereignisse und $\rho_c = 0.87$ für die Futteraufnahmedauer sowie W = 0.79 für die Anzahl an Mahlzeiten. Das drei-Punkte Lokomotionsscoringsystem wurde ebenso validiert und das Maß an Übereinstimmung zwischen zwei Beobachtern (Interrater-Reliabilität) und für einen Beobachter, der an zwei unterschiedlichen Zeitpunkten ein Lokomotionsscoring der gleichen Videoaufnahme durchführt (Intrarater-Reliabilität), berechnet. Die relative Übereinstimmung entsprach 80,0 % für die Interrater-Reliabilität und 82,3 % für die Intrarater-Reliabilität.

Die Lahmheitsprävalenz pro Monat für alle Betriebe betrug zwischen 3,5 und 12,7 % und durchschnittlich hatten 6,3 % der Tiere pro Lokomotionsscoring klinische Befunde an den Klauen. Die häufigsten Befunde waren Weiße-Linie-Defekte und Sohlenblutungen. Die Lahmheitsfälle, die im Rahmen dieser Arbeit über Videoaufnahmen rückwirkend analysiert wurden, entwickelten sich durchschnittlich in neun Tagen und wurden innerhalb von durchschnittlich fünfzehn Tagen im Rahmen eines zweiwöchigen Lokomotionsscorings entdeckt.

Die Häufigkeit der Betriebsbesuche und des Lokomotionsscorings war angemessen, um Lahmheitsfälle früh zu entdecken und weist darauf hin, dass Lokomotionsscoring mindestens alle zwei Wochen durchgeführt werden sollte, um zu vermeiden, dass Klauenläsionen sich weiterentwickeln und somit das Tierwohl und die Leistung der Tiere beeinflussen. Mehr als die Hälfte der Kühe, die wiederholt als *Verdacht auf Lahmheit* eingestuft worden waren und auf Schmerzhaftigkeit in den Klauen untersucht wurden, reagierten positiv auf den Schmerztest oder hatten klinische Befunde. Diese Ergebnisse zeigen, dass Kühe ihre Schmerzen so lange kaschieren, bis die zugrundeliegende Klauenerkrankung fortgeschritten ist. Die durch Klauenläsionen verursachten Schmerzen beeinflussen die Aktivität, sowie das Liege- und Futteraufnahmeverhalten der Tiere, und diese Verhaltensveränderungen können für die automatische Lahmheitserkennung eingesetzt werden.

Für jeden Parameter wurde eine Analyse der Tagesdatensätze durchgeführt und die Ergebnisse wurden dann zwischen Betrieben und für verschiedene Lokomotionsscoregruppen verglichen. Das Verhalten von lahmen Tieren unterschied sich signifikant von dem gesunder Tiere; insbesondere veränderten sich das Liege- und das Futteraufnahmeverhalten. Die Analyse der Wechselwirkungen zwischen den verschiedenen Leistungs- und Verhaltensparametern ergab komplexe Verhältnisse zwischen den Variablen, die sich innerhalb der Lokomotionsscoregruppen unterschiedlich verhielten. Aufgrund dessen, dass das Verhalten und die Leistung einen Einfluss auf die Klauengesundheit haben, aber selbst von Lahmheit beeinflusst werden, ist es teilweise problematisch, eine Kausalität zwischen den Parametern festzustellen.

Schließlich wurden die Daten noch mithilfe eines verallgemeinerten, gemischten linearen Modells sowie eines Elastic Net Modells mit dem Ziel analysiert, Lahmheitsfälle vorherzusagen. Die Genauigkeit des Modells wurde mittels ROC (receiver operating characteristics) Kurvenanalyse bewertet. Das gemischte lineare Modell wies eine AUC (Fläche unter der Kurve) von 0,91 (CI: 0,89 – 0,92) für die kombinierten Daten aller Betriebe mit den Betrieben und die individuellen Tiere als Zufallseffekte auf. Das Elastic Net Modell wies dagegen eine AUC zwischen 0,73 und 0,80 für die Daten der einzelnen Betriebe auf.

Die Ergebnisse dieser Arbeit geben einen Einblick in die Lahmheitsprävalenz und die Inzidenz an Klauenerkrankungen von Fleckviehkühen auf bayerischen Milchviehbetrieben. Darüber hinaus zeigen die Ergebnisse der Untersuchungen auf Schmerzhaftigkeit der Klauen, dass Milchkühe ihren Schmerz kaschieren bis die zugrundeliegende Klauenerkrankung schon fortgeschritten ist. Außerdem war ein wichtiges Ergebnis, dass sich die meisten Lahmheitsfälle innerhalb von zwei Wochen entwickelten. Die Genauigkeit der Pedometer und des Referenzsystems für die Klauengesundheit sind für die Erfassung von Daten für die Implementierung in einem Vorhersagemodell gut geeignet.

Abschließend lässt sich die eindeutige Aussage treffen, dass automatische Lahmheitserkennungssysteme Landwirten dabei helfen können, lahme Tiere früher zu erkennen und somit unnötige Schmerzen für die Tiere und wirtschaftliche Verluste zu vermeiden. Es ist möglich, dass die automatische Lahmheitsdetektion anhand von Zeitreihenanalysen verbessert werden könnte. Durch das Erkennen von Veränderungen im Verhaltensmuster und Leistung der Tiere in Kombination mit Daten zur Klauengesundheit, könnte die Vorhersagekraft gesteigert werden. Dennoch ist die Vorhersagegenauigkeit der in dieser Arbeit entwickelten Modelle vielversprechend und hat aufgrund des geringen Kostenund Technikeinsatzes, eine hohe Anwendbarkeit auch außerhalb des Forschungsbereiches.

8 Summary

Title: Analysis of claw health, performance and behavioural parameters of Simmental cows on commercial dairy farms for implementation of a lameness prediction model

Lameness in dairy cows is a common production disease that affects cows' welfare and causes economic loss for farmers. Most cases of lameness are caused by claw lesions, which are predominantly a result of extrinsic factors such as the animals' housing conditions, as well as management factors such as feeding and the frequency of claw trimming. Additionally, intrinsic factors such as parity, live weight, body condition, breed, stage of lactation and milk yield all have an effect on claw health. Lameness affects cows' activity, lying and feeding behaviour as well as their social interactions and ultimately their performance, in particular their milk yield, fertility and longevity.

Early lameness detection is fundamental to prevent claw lesions from becoming severe and causing the animals unnecessary pain and discomfort. Furthermore, the costs associated with a case of lameness can be considerable and include both direct costs, such as the cost of treatment, and indirect costs, such as reduced potential milk yield and longer calving intervals. Due to the structural changes that have occurred in the dairy industry over the past decades, the increased number of animals per farm has had a negative effect on the amount of time spent by farmers on individual animal observation. Furthermore, farmers tend to underestimate the lameness prevalence on their farms, also as a consequence of cows' stoic nature and reluctance to manifest signs of pain.

Manual locomotion scoring systems are the standard reference system for claw health in dairy cows, but they are subject to the influence of individual perception of locomotion traits. Automatic locomotion scoring systems on the other hand, use predictive algorithms to assess claw health and could be used to help farmers detect lame animals early. Automatic locomotion scoring systems can be direct, based on either the kinetic, kinematic or thermographic approach, or indirect, based on the use of behavioural and performance traits.

The aim of this study was to test a method of predictive statistical modelling developed in a previous study at the Bavarian State Research Centre for Agriculture's (LfL) research farm, on commercial dairy farms. Sub-objectives of the study included the development of a new manual locomotion system that could be used both in research and in an on-farm environment, the assessment of the score's reliability and the validation of the pedometers with regard to the accuracy of the measurement of feeding and lying behaviour.

Over a 14-month period, behavioural and performance data was collected on four commercial dairy farms in Lower Bavaria and one research farm of the LfL. All animals involved in the study were fitted with a pedometer on their right front limb. The pedometers contained a

three-dimensional accelerometer that distinguished between the positions "lying" and "standing" and continuously measured activity and lying behaviour. Furthermore, the pedometers were equipped with a radio frequency identification (RFID) coil that registered when the animal was standing at the feeding table, enabling the recording of feeding behaviour as well. In addition to the behavioural data collected by the pedometers, automatic weighing troughs on the research farm also recorded feeding behaviour using RFID technology to identify the animal's presence and its feed intake. Performance data was collected from the automatic milking system on two of the farms and from the milk reports issued eleven times a year. As a reference for claw health, locomotion scoring of the herds was carried out fortnightly on all farms using video recordings of the animals exiting the milking parlour or the automatic milking system. For the locomotion scoring, the three-point system which was developed categorised the animals into lame (unsymmetrical, irregular gait), unsound (characterised by the presence of at least one of the following: head bobbing, back arch and compensatory posture) and sound (regular, symmetrical gait). A clinical examination and treatment of the animals scored lame were carried out and the findings documented. Additionally, animals which were scored unsound for three successive locomotion scores (i.e. six successive weeks) were tested for pain in the claws using hoof pincers. If they reacted to the pain test or had clinical findings, the unsound cows were then documented as *lame*. The daily video recordings of the animals exiting the milking parlour or the automatic milking systems allowed lameness cases to be analysed retrospectively, in order to determine the exact onset of lameness. The cows' identification data, the behavioural and performance data and the claw health data were summarised into daily values per animal and entered into a SQL database system.

Before the beginning of data collection, the pedometers were validated in order to assess their accuracy when measuring lying and feeding behaviour. Direct observation was used as the gold standard, and both lying duration and number of lying bouts, and feeding duration and number of visits to the feeding table were recorded by an observer and compared to the data recorded by the pedometers. Both the validation of the measured lying behaviour and of the feeding behaviour resulted in a high level of accuracy, with an concordance correlation coefficient of $\rho_c = 0.96$ for the lying duration, W = 0.80 (Kendall's coefficient of concordance) for the number of lying bouts, and $\rho_c = 0.87$ for the feeding duration and W = 0.79 for the number of visits to the feeding table. The three-point locomotion scoring system was also validated using the level of inter-rater and intra-rater agreement as measurements for reliability, and resulted in a good level of agreement for both parameters, with a percentage of agreement of 80.1 % and 82.3 % for the inter-rater agreement using video observation and the intra-rater agreement respectively.

The lameness prevalence per month across farms ranged between 3.5 % and 12.7 % and on average, 6.3 % of scored animals per month had clinical findings, white line defects and sole

haemorrhages being the most common claw lesions. The cases of lameness analysed with video recordings in this study took on average nine days to develop and fifteen days to be discovered. The frequency of claw health assessment visits to the farms thus proved to be suitable for detecting animals soon after lameness onset and indicated that locomotion scoring should be carried out at least twice a month to prevent claw lesions from deteriorating and affecting the animal's welfare and performance. More than half the *unsound* animals tested for pain in the claws either reacted positively to the test or had clinical findings.

An analysis of the daily values was carried out and data for each behavioural and performance parameter were compared between farms and between locomotion score groups. Lame animals' behaviour differed significantly from that of sound animals, in particular the lying and feeding behaviour were affected by lameness. An analysis of the interaction terms between performance and behaviour parameters revealed complex relationships that differed according to the locomotion score group. Due to performance and behavioural parameters often affecting claw health and at the same time being influenced by lameness, assessing causality for risk factors can be problematic.

Finally, a generalised linear mixed model and an Elastic Net model were applied the data collected in this study. The accuracy of the tests was assessed using the ROC (receiver operating characteristics) curve analysis and resulted in an AUC (area under the curve) of 0.91 (CI: 0.89, 0.92) for the generalised linear mixed model using data across farms, with the individual animal and farms as random effects, and between 0.73 and 0.80 for the Elastic Net models applied to the data from the individual farms.

The results of this study provide insight into the level of lameness and the incidence of claw lesions of Simmental cow herds in Bavaria. Furthermore, the results of the pain tests indicate that cows only tend to exhibit pain when the underlying claw lesions are already in an advanced state and that most cases of lameness develop in under two weeks. The accuracy of both the pedometers and of the reference system for claw health were confirmed and proved suitable for the collection of data for implementation in a predictive algorithm for claw health.

In conclusion, automatic lameness detection could help farmers recognise lameness at an early stage thus reducing both pain for the animal and economic loss for the breeder. The predictive accuracy of algorithms for lameness detection might be improved if timeline analysis was applied to behavioural and performance parameters in relation to claw health data, in order to detect changes in the behavioural pattern of lame animals. The predictive accuracy of the models developed in this study are nonetheless promising and have a high level of applicability outside the field of research due to the sparse amount of technology required for data collection.

9 Literature

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10 Image source

Figure 24: Pedometer positions used to divide the animals in different observation groups during the validation of the measurement of feeding behaviour (page 47): Quirin Greil [180]
11 Annexe

		LD (min/h)	
	LDO	LDR	LDO-LDR
n	271	271	271
Mean	23.45	24.95	-1.51
SD	23.58	23.04	6.58
Min	0	0	-44.92
q25	0	0	-2
Median	18.40	21	0
q75	47.76	47	0
Max	60	60	23.58

 Table 28:
 Summary of comparison between observed lying duration (LDO) and lying duration measured by the pedometers (LDR) in minutes per hour. LD: lying duration

N: number of observations, Min: minimum, SD: standard deviation, Max: maximum, q25: first quartile, q75: third quartile.

 Table 29:
 Summary of comparison between observed lying bouts (LBO) and lying bouts recorded by the pedometers (LBR) in number of bouts per hour. LB: lying bouts

		LB (bouts/h)	
	LBO (LBO/h)	LBR (LBR/h)	LBO-LBR (LB/h)
n	272	272	272
Mean	0.4	0.5	-0.2
SD	0.6	0.7	0.6
Min	0.0	0.0	-2.0
q25	0.0	0.0	0.0
Median	0.0	0.0	0.0
q75	1.0	1.0	0.0
Max	3.0	3.0	2.0

N: number of observations, Min: minimum, SD: standard deviation, Max: maximum, q25: first quartile, q75: third quartile.

Table 30:Summary statistics of feeding duration (FD) measured by direct observation (DO) in Cases 1,
2 and 3), and FD measured by pedometers per hour

	FD (min/h)								
Case	n	Min	q25	Med	q75	Max	Mean	SD	
FD Pedometers	91	0	2,5	16	27.5	54	16.6	13.8	
Case 1 (DO)	91	0	2	18	29.5	55	18.5	15.2	
Case 2 (DO)	91	0	2	18	30.5	55	19.1	15.4	
Case 3 (DO)	91	0	4	22	36	58	23.0	17.8	

N: number of observations, Min: minimum, SD: standard deviation, Max: maximum, q25: first quartile, q75: third quartile.

		L	ameness pr	evalence (%	/ 0)	
Month	CDF1	CDF2	CDF3	CDF4	RFG	Overall
04.2017	8	2.9	3	-	-	4.9
05.2017	9.5	1.5	3	-	-	5
06.2017	5.9	4.4	6.8	-	-	5.9
07.2017	5.3	6.6	2	-	-	4.6
08.2017	4.1	4.6	1.5	19	-	6.3
09.2017	7.1	4.6	0.7	12.9	-	5.2
10.2017	7.6	8.4	1.9	9.6	-	7.4
11.2017	8.4	6.5	1.5	-	-	5.2
12.2017	8.9	1.4	2.5	-	-	4.5
01.2018	7	0	2	-	18	5.7
02.2018	10	4.1	0.9	-	19.3	7.7
03.2018	5.2	1.3	1	-	6.9	3.5
04.2018	12.7	0	0.9	-	4.7	4.9
05.2018	-	-	-	-	12.7	12.7
06.2018	-	-	-	-	8.9	8.9

 Table 31:
 Lameness prevalence (number of locomotion scores LMS = 3) in percent per month and per farm.

CDF1 - CDF4: commercial dairy farms 1 - 4, RFG: research farm.

 Table 32:
 Mean fortnightly locomotion score per farm and month, across farms per month and across farms per month including the interpolated scores.

			Mean I	LMS per m	onth		
Month	CDF1	CDF2	CDF3	CDF4	RFG	AF	AF (IN)
04.2017	1.2	1.1	1.1	-	-	1.1	1.3
05.2017	1.3	1.1	1.1	-	-	1.2	1.2
06.2017	1.2	1.2	1.2	-	-	1.2	1.2
07.2017	1.9	1.2	1.1	-	-	1.6	1.2
08.2017	1.8	1.2	1.1	1.5	-	1.4	1.3
09.2017	1.3	1.2	1.1	2.2	-	1.6	1.3
10.2017	1.9	1.3	1.6	1.7	-	1.7	1.3
11.2017	1.3	1.8	1.1		-	1.5	1.3
12.2017	1.3	1.2	1.7		-	1.5	1.2
01.2018	1.3	1.1	1.1	-	1.5	1.3	1.3
02.2018	1.4	1.2	1.1	-	1.9	1.6	1.3
03.2018	1.3	1.1	1.1	-	1.5	1.3	1.2
04.2018	1.4	1.2	1.1	-	1.4	1.3	1.3
05.2018	-	-	-	-	1.8	1.8	1.4
06.2018	-	-	-	-	1.5	1.5	1.5

AF: all farms, IN: interpolated, CDF1 - CDF4: commercial dairy farms 1 - 4, RFG: research farm. LMS: locomotion score

	Days from sound to lame										
	n	Min	q25	Median	q75	Max	Mean	SD			
CDF1	109	1	2	3	7	103	7.92	14,9			
CDF2	47	1	2	3	7	49	7.70	10,6			
CDF3	34	1	5	6.5	9.75	28	8.26	6,43			
CDF4	14	2	4.5	10.5	16.75	47	13.43	12,3			
RFG	39	2	6	9	17	69	14.59	15,5			

 Table 33:
 Summary statistics of number of days from sound to lame for each case of lameness and each farm

CDF1 – CDF4: commercial dairy farms 1 – 4, RFG: research farm. N: number of observations, Min: minimum, SD: stand0rad deviation, Max: maximum, q25: first quartile, Med: median, q75: third quartile

				Cli	nical findir	igs per	month		
Farm	Diagnosis	n	Min	q25	Median	q75	Max	Mean	SD
CDF1	D	53	2	2	3	6	7	4.08	2.06
CDF1	DS	19	0	1	1	2	3	1.46	0.78
CDF1	HF	8	0	0	0	1	3	0.62	1.04
CDF1	IH	17	0	1	1	1	3	1.31	0.85
CDF1	IP	0	0	0	0	0	0	0	0
CDF1	SH	74	3	4	5	6	15	5.69	3.01
CDF1	SU	14	0	0	1	2	4	1.08	1.32
CDF1	WLD	85	2	5	6	8	13	6.54	2.85
CDF2	D	18	0	0	1	2	4	1.38	1.39
CDF2	DS	4	0	0	0	0	2	0.31	0.63
CDF2	HF	4	0	0	0	0	2	0.31	0.63
CDF2	IH	0	0	0	0	0	0	0	0
CDF2	IP	0	0	0	0	0	0	0	0
CDF2	SH	37	0	1	3	4	6	2.85	2.03
CDF2	SU	17	0	0	1	2	5	1.31	1.6
CDF2	WLD	32	0	1	2	4	7	2.46	2.47
CDF3	D	5	0	0	1	1	1	0.56	0.53
CDF3	DS	1	0			1	0		0.33
CDF3	HF	0	0			0	0		0
CDF3	IH	1	0			1	0		0.33
CDF3	IP	0	0			0	0		0
CDF3	SH	12	0			5	0		1.94
CDF3	SU	8	0			1	1		0.53
CDF3	WLD	14	0			6	1		2.3
CDF4	D	22	1			13	8		6.03
CDF4	DS	12	1			8	3		3.61
CDF4	HF	1	0			1	0		0.58
CDF4	IH	8	2			3	3		0.58
CDF4	IP	0	0			0	0		0
CDF4	SH	36	3			23	10		10.15
CDF4	SU	14	3			6	5		1.53
CDF4	WLD	29	4			20	5		8.96

 Table 34:
 Statistical summary of the findings per farm and per month.

		Clinical findings per month								
Farm	Diagnosis	n	Min	q25	Median	q75	Max	Mean	SD	
RFG	D	34	3	3.75	6	6.75	9	5.67	2.34	
RFG	DS	13	0	0.25	1	1.75	9	2.17	3.43	
RFG	HF	4	0	0	0.5	1	2	0.67	0.82	
RFG	IH	10	0	1.25	2	2	3	1.67	1.03	
RFG	IP	5	0	0	0	0.75	4	0.83	1.6	
RFG	SH	53	4	5.5	8	12	15	8.83	4.4	
RFG	SU	7	0	1	1	1.75	2	1.17	0.75	
RFG	WLD	46	2	5.5	7	9.25	15	7.67	4.46	

Table 34: (continuation): Statistical summary of the findings per farm and per month.

SU: sole ulcer, SH: sole haemorrhage, D: dermatitis, HF: horn fissure, WLD: white line disease, DS: double sole, IP: interdigital phlegmon, IH: interdigital hyperplasia, N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, CDF1 – CDF4: commercial dairy farms 1 - 4, RFG: research farm.

Table 35:	Summary statistics	of fortnightly locomotion	scores per month for all farms.

FLMS per month									
Farm	n	Min	q25	Median	q75	Max	Mean	SD	
CDF1	4184	1	1	1	2	3	1.7	0.8	
CDF2	2558	1	1	1	2	3	1.5	0.7	
CDF3	2815	1	1	1	1	3	1.2	0.5	
CDF4	771	1	1	2	3	3	1.8	0.9	
RFG	1425	1	1	2	2	3	1.7	0.8	

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, CDF1 - CDF4: commercial dairy farms 1 - 4, RFG: research farm.

	Value of variable										
Var	n	Min	q25	Med	q75	Max	Mean	SD			
AC	9,639	16.0	1,564	1,947	2,401	8,997	2,057.9	786.1			
ACR	9,639	-	973	1,223	1,519	6,240	1,288.1	514.7			
BCS	9,634	2.6	3.8	3.9	4.1	4.6	3.9	0.3			
C_FDM	9,639	1.0	9.6	13.7	18.5	84.0	14.6	7.0			
C_MN	9,639	1.0	6.0	8.0	10.0	25.0	7.9	3.0			
C_MNR	9,639	-	4.0	5.0	7.0	17.0	5.2	2.3			
DIM	7,670	1.0	82.0	153.0	230.0	488.0	160.2	94.3			
F.LMS	9,639	-	2.0	2.0	2.0	2.0	1.8	0.5			
FD	9,639	2.0	77.0	106.0	133.0	285.0	105.5	42.9			
FDM	9,639	1.3	16.4	20.9	26.3	108.7	22.0	8.2			
FDR	9,639	-	48.0	71.0	94.0	211.0	72.2	33.7			
FDRW	9,639	-	82.9	104.3	127.5	394.2	105.7	34.2			
FDV	9,639	0.4	2.8	3.9	5.2	21.0	4.2	2.0			
FDW	9,639	9.1	99.2	123.3	148.5	435.0	125.2	39.0			
FI	9,639	3.9	37.5	44.2	50.7	77.0	43.9	10.1			
FIM	9,639	0.6	5.9	7.5	9.4	27.0	7.8	2.7			
FIV	9,639	0.2	1.0	1.4	2.0	12.8	1.5	0.9			

 Table 36:
 Statistical summary of each variable for the research farm.

	Value of variable										
Var	n	Min	q25	Med	q75	Max	Mean	SD			
FP	9,639	0.1	0.3	0.4	0.4	1.6	0.4	0.1			
K.LMS	35	1.0	1.5	2.0	2.0	4.0	1.9	0.8			
LBN	9,639	1.0	11.0	14.0	17.0	111.0	14.7	7.1			
LBNR	9,639	-	5.0	7.0	9.0	77.0	7.4	3.8			
LD	9,639	34.0	619.5	723.0	817.0	1,253	712.2	164.8			
LDB	9,639	2.9	39.6	52.1	68.8	305.0	56.5	24.8			
LDR	9,639	-	263.0	329.0	388.0	706.0	324.0	101.0			
LMS	9,639	1.0	1.0	1.0	2.0	3.0	1.5	0.7			
LW	9,580	535.5	688.6	759.2	810.5	1,039.6	751.9	77.0			
MI	9,507	327.9	661.8	769.5	865.2	1,437	779.6	166.1			
MMY	9,319	2.7	25.3	29.2	35.9	49.6	30.3	7.8			
MN	9,639	1.0	5.0	6.0	7.0	16.0	6.1	1.9			
MNR	9,639	-	4.0	5.0	6.0	14.0	4.9	1.7			
MY	9,579	-	24.4	29.4	35.6	56.3	30.1	8.2			
MY305	5,186	-	-	-	-	6,462	562.9	1,506			
Р	7,670	1.0	1.0	2.0	4.0	8.0	2.9	1.8			
РТ	13	-	-	1.0	1.0	1.0	0.7	0.5			
VN	9,639	2.0	22.0	32.0	46.0	222.0	37.8	23.0			
VNR	9,639	-	19.0	27.0	39.0	174.0	32.1	20.0			

Table 36 (continuation): Statistical summary of each variable for the research farm.

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feeding duration during daytime measured by weighing troughs, FDR: feed intake, FP: feeding pace, FIV: feed intake per visit, FIM: feed intake per meal, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MY: milk yield, MY305: milk yield for lactation, P: parity, PT: pain test, VN: number of visits measured by the weighing troughs, VNR: number of visits during daytime measured by the weighing troughs, WNR: number of visits measured by the weighing troughs, WNR: number of visits during daytime measured by the weighing troughs, MY: milk yield

Table 37:	Summary s	tatistics	of the	variables on	commercial	dairy	farm	1

		Value of variables										
Var	n	Min	q25	Med	q75	Max	Mean	SD				
AC	23,973	28	1,689	1,976	2,318	10,634	2,077	641.83				
ACR	23,973	0	1,164	1,377	1,622	6,729	1,434	426.38				
C_FDM	23,973	1	18.67	27.65	37.86	175	29.93	15.62				
C_MN	23,973	1	9	11	14	35	11.84	4.01				
C_MNR	23,973	0	6	8	10	23	8.32	2.99				
DIM	23,973	1	109	185	262	741	203.92	130.16				
F.LMS	23,973	0	2	2	2	3	1.83	0.48				
FD	23,973	2	249	311	371	689	309.06	92.67				
FDR	23,973	0	195	250	301	564	247.66	80.3				
K.LMS	68	1	2	2	2	4	1.97	0.69				

Value of variables											
Var	n	Min	q25	Med	q75	Max	Mean	SD			
LBN	23,973	4	15	19	27	136	23.6	13.44			
LBNR	23,973	0	6	9	13	62	10.4	6.05			
LD	23,973	92	557	631	699	1357	623.1	116.97			
LDB	23,973	3.09	21.91	32.5	43.19	137.2	33.53	16.05			
LDR	23,973	0	178	226	272	731	225.23	71.16			
LMS	23,973	1	1	1	1	3	1.31	0.59			
MMY	23,973	12.8	24	28.6	34.2	49.8	29.58	7.3			
MY305	15,779	5,384	7,397	8,326	9,145	11,201	8,310.49	1,278.18			
MYM	23,973	12.8	24	28.6	34.2	49.8	29.58	7.3			
Р	23,973	1	1	2	3	7	2.43	1.39			
РТ	29	0	0	0	1	1	0.45	0.51			

Table 37 (continuation): Summary statistics of the variables on commercial dairy farm 1

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, AC: activity, ACR: activity during daytime, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MY: milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, MMY: average monthly milk yield

		Value of variables										
Var	n	Min	q25	Med	q75	Max	Mean	SD				
AC	19,813	325	1,520	1,832	2,200	8,200	1,928.29	644.71				
ACR	19,813	0	1,090	1,314	1,578	6,717	1,382	469.39				
C_FDM	19,813	4.32	19.88	25	31.78	181.75	26.66	9.88				
C_MN	19,813	1	9	11	13	35	11.21	3.5				
C_MNR	19,813	0	6	8	9	25	7.83	2.56				
DIM	19,813	1	96	168	245	567	178.41	105.17				
F.LMS	19,813	0	2	2	2	3	1.88	0.38				
FD	19,813	12	232	273	315	727	274.42	62.89				
FDR	19,813	0	168	201	236	666	202.48	50.72				
K.LMS	26	1	1.25	2	2	4	1.96	0.82				
LBN	19,813	3	14	21	36	138	27.81	20.15				
LBNR	19,813	0	7	11	18	82	14.26	10.48				
LD	19,813	86	613	716	806	1231	702.87	152.35				
LDB	19,813	2.52	19.03	34.46	52.69	296.33	39.05	26.68				
LDR	19,813	0	271	336	396	698	332.25	93.11				
LMS	19,813	1	1	1	1	3	1.18	0.45				
MMY	19,813	9.5	25.5	29.8	34.5	47.4	29.92	6.44				
MY305	12,856	6,641	7,935	8,651	9,635	11,282	8,818	1,283				
MYM	19,813	9.5	25.5	29.8	34.5	47.4	29.92	6.44				
Р	19,813	1	1	2	3	9	2.41	1.51				
РТ	13	-	-	1.0	1.0	1.0	0.5	0.5				

 Table 38:
 Summary statistics of the variables on commercial dairy farm 2

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, AC: activity, ACR: activity during daytime, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MY: milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, MMY: average monthly milk yield

	Value of variables											
Var	n	Min	q25	Med	q75	Max	Mean	SD				
AC	18,961	120	1,226	1,454	1,726	7,757	1,521.96	495.82				
ACR	18,961	0	842	1,006	1,201	6,403	1,053.47	357.8				
C_FDM	18,961	1	21.44	27.73	35.6	348	29.55	12.91				
C_MN	18,961	1	7	9	11	140	10.55	11.18				
C_MNR	18,961	0	5	6	8	105	7.21	7.66				
DIM	18,961	1	116	195	266	694	200.66	112.77				
F.LMS	18,961	0	2	2	2	3	1.9	0.34				
FD	18,961	2	208	253	297	696	253.78	68.76				
FDR	18,961	0	151	187	223	538	188.19	55.29				
K.LMS	7	1	1	2	2	4	1.86	1.07				
LBN	18,961	1	14	18	30	127	23.94	15.21				
LBNR	18,961	0	7	10	16	73	12.62	8.28				
LD	18,961	64	669	758	836	1216	745.23	131.63				
LDB	18,961	3.61	24.53	42.16	56.13	206	42.19	21.43				
LDR	18,961	0	322	378	429	663	371.75	82.58				
LMS	18,961	1	1	1	1	3	1.13	0.39				
MMY	18,961	7.4	25.3	31.5	37.1	49.3	30.77	8.2				
MY305	17,567	5,871	8,038	8,937	9,912	12,268	8,978.86	1,331.06				
MYM	18,961	7.4	25.3	31.5	37.1	49.3	30.77	8.2				
Р	18,961	1	2	3	4	10	3.37	1.69				
PT	5	0	0	1	1	1	0.6	0.55				

 Table 39:
 Summary statistics of the variables on commercial dairy farm 3

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, AC: activity, ACR: activity during daytime, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MN: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MY: milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, MMY: average monthly milk yield

		Value of variables									
Var	n	Min	q25	Med	q75	Max	Mean	SD			
AC	2,897	24	1,523	1,875	2,204	4,413	1,891.32	528.07			
ACR	2,897	15	1,076	1,358	1,636	3,490	1,379.87	426.28			
C_FDM	2,897	1	21.33	27.33	35	134	29.04	11.88			
C MN	2,897	1	7	9	11	24	9.15	2.97			

Table 40: Summary statistics of the variables on commercial dairy farm 4

	Value of variables										
Var	n	Min	q25	Med	q75	Max	Mean	SD			
C_MNR	2,897	0	5	7	8	22	6.8	2.52			
DIM	2,897	1	81	177	233	367	165.77	92.17			
F.LMS	2,897	0	2	2	2	3	1.74	0.63			
FD	2,897	2	193	248	307	634	251.19	91			
FDR	2,897	0	150	199	252	518	203.25	81.39			
C_MNR	2,897	0	5	7	8	22	6.8	2.52			
K.LMS	20	1	2	2	2	4	2.1	0.72			
LBN	2,897	3	13	18	28	134	24.69	19.14			
LBNR	2,897	2	7	10	16	102	14.32	12.53			
LD	2,897	92	568	670	772	1412	666.15	171.79			
LDB	2,897	2.53	22.13	38.73	52.12	156.56	39.4	22.56			
LDR	2,897	21	276	351	428	883	352.04	120.4			
LMS	2,897	1	1	1	2	3	1.63	0.81			
MMY	2,897	0	29.72	35.43	39.11	49.75	34.04	6.74			
MY	2,897	0	28.06	34.71	40.87	49.99	34.04	8.8			
MY305	2,746	0	0	0	995	5,005	546.04	992.94			
Р	2,897	1	2	4	5	8	3.89	1.9			
PT	2	1	1	1	1	1	1	0			

Table 40 (continuation): Summary statistics of the variables on commercial dairy farm 4

N: number of observations, Min: minimum, q25: first quartile, q75: third quartile, Max: maximum, SD: standard deviation, AC: activity, ACR: activity during daytime, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MNR: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, F.LMS: type of locomotion score, FD: feeding duration measured at weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, K.LMS: locomotion score correction reason, LBN: number of lying bouts, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MY: milk yield, MY305: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, MMY: average monthly milk yield

Table 41:	Summary	statistics	of all	variables	divided	by	locomotion scores.
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		Summary statistics of variables										
LMS	Var	n	Min	q25	Med	q75	Max	Mean	SD			
1	AC	63,622	21	1,500	1,841.5	2,229	10,634	1,938.89	689.84			
2	AC	12,724	16	1,443	1,751	2,113	8,997	1,847.15	664.4			
3	AC	4,543	34	1,302	1,589	1,942	6,671	1,679.04	609.88			
1	ACR	63,622	-	1,036	1,279	1,554	6,729	1,340.71	474.03			
2	ACR	12,724	0	976	1,201	1463	6,403.0	1,262.28	458.31			
3	ACR	4,543	0	860	1,078	1326.5	4,900.0	1,137.64	426.19			
1	BCS	5,896	2.7	3.8	3.9	4.1	4.6	3.91	0.25			
2	BCS	2,649	2.7	3.8	4	4.1	4.6	3.9	0.28			
3	BCS	1,089	2.6	3.8	4	4.1	4.6	3.88	0.35			
1	C_FDM	63,622	1	18.41	25.38	33.75	348	27.31	13.37			
2	C_FDM	12,724	1	16.12	23.6	32.78	131.75	25.64	13.34			
3	C_FDM	4,543	1	14.71	21.67	30.56	127.75	24.17	13.56			

	Summary statistics of variables										
LMS	Var	n	Min	q25	Med	q75	Max	Mean	SD		
1	C_MN	63,622	1	8	10	13	140	10.93	5.51		
2	C_MN	12,724	1	7	9	12	138	10.54	8.58		
3	C_MN	4,543	1	6	8	11	139	9.78	9.99		
1	C_MNR	63,622	0	6	7	9	105	7.58	3.91		
2	C_MNR	12,724	0	5	7	9	100	7.36	6.02		
3	C_MNR	4,543	0	4	6	8	103	6.78	6.72		
1	DIM	61,163	1	103	181	260	1022	196.96	131.01		
2	DIM	11,948	1	104	189	273	875	208.3	142.54		
3	DIM	4,089	1	86	177	254	610	181.29	115.87		
1	F.LMS	63,622	0	2	2	2	3	1.91	0.31		
2	F.LMS	12,724	0	2	2	2	2	1.73	0.63		
3	F.LMS	4,543	0	1	2	2	3	1.46	0.85		
1	FD	63,622	2	211	270	324	760	266.66	91.68		
2	FD	12,724	2	153	239	309	634	236.2	105.14		
3	FD	4,543	2	122	194	265	583	201.22	103.84		
1	FDM	5,897	1.25	15.97	20.3	25.77	88.62	21.51	8.2		
2	FDM	2,653	5.14	16.78	21.79	26.73	108.74	22.6	8.26		
3	FDM	1,089	4.13	17.78	22.13	27.78	71.76	23.24	8.23		
1	FDR	63,622	0	154	203	252	666	203.36	78.11		
2	FDR	12,724	0	110	182	242	508	181.88	89.47		
3	FDR	4,543	0	85	148	208	507	153.67	87.52		
1	FDRW	5,897	0	87.95	108.53	131.78	342.98	110.33	34.19		
2	FDRW	2,653	8.44	80.51	101.14	122.43	394.17	102.46	32.07		
3	FDRW	1,089	4.63	64.12	87.03	111.48	208.71	88.91	33.48		
1	FDV	5,897	0.39	2.54	3.64	4.76	17.74	3.79	1.84		
2	FDV	2,653	0.94	3.2	4.3	5.67	19.23	4.55	2		
3	FDV	1,089	1.23	3.78	4.91	6.55	21.01	5.42	2.49		
1	FDW	5,897	9.08	104.8	128.13	153.33	393.57	130.19	39.2		
2	FDW	2,653	20.8	96.27	119.75	143.57	434.95	121.32	35.73		
3	FDW	1,089	12.75	81.52	104.63	131.48	287.05	107.62	39		
1	FI	5,897	3.91	38.04	44.7	50.98	77.04	44.29	10.2		
2	FI	2,653	11.81	37.16	43.79	50.71	75.69	43.81	9.83		
3	FI	1,089	6.41	35.29	41.78	48.45	73.91	41.86	10.06		
1	FIM	5,897	0.56	5.55	7.07	8.84	19.46	7.38	2.57		
2	FIM	2,653	2.36	6.32	7.98	9.85	22.23	8.23	2.68		
3	FIM	1,089	1.88	7.38	8.99	10.79	26.98	9.25	2.82		
1	FIV	5,897	0.18	0.82	1.19	1.64	7.64	1.33	0.74		
2	FIV	2,653	0.25	1.14	1.6	2.15	6.29	1.69	0.79		
3	FIV	1,089	0.51	1.45	1.99	2.64	12.79	2.28	1.33		

 Table 41 (continuation):
 Summary statistics of all variables divided by locomotion scores.

	Summary statistics of variables										
LMS	Var	n	Min	q25	Med	q75	Max	Mean	SD		
1	FP	5,897	0.07	0.29	0.34	0.4	1.56	0.36	0.12		
2	FP	2,653	0.11	0.32	0.37	0.43	0.85	0.38	0.09		
3	FP	1,089	0.14	0.33	0.4	0.49	1.09	0.42	0.13		
1	K.LMS	12	2	2	2	2	2	2	0		
2	K.LMS	65	1	2	2	2	3	2.03	0.25		
3	K.LMS	89	1	1	2	2	4	1.96	1.02		
1	LBN	63,622	1	14	19	29	137	24.26	16.28		
2	LBN	12,724	1	13	18	27	138	23.2	16.36		
3	LBN	4,543	2	12	17	26	127	22.04	15.66		
1	LBNR	63,622	0	7	9	14	84	11.99	8.46		
2	LBNR	12,724	0	6	9	13	102	11.45	8.7		
3	LBNR	4,543	0	6	9	13	83	11.4	8.65		
1	LD	63,622	34	589	683	775	1412	676.55	143.63		
2	LD	12,724	42	611	706	808	1396	703.99	153.18		
3	LD	4,543	74	630	741	858	1245	743.91	173.12		
1	LDB	63,622	2.53	22.52	36.5	50.94	296.33	38.75	22.1		
2	LDB	12,724	2.52	24.58	39.71	55.81	305	42.52	24.28		
3	LDB	4,543	2.69	27	42.56	61.26	249.67	47.34	28.15		
1	LDR	63,622	0	226	302	377	880	301.22	102.6		
2	LDR	12,724	0	237	312	390	883	313.36	107.44		
3	LDR	4,543	0	265	345	431	714	347.69	117.95		
1	LW	5,885	535.5	681.45	748.3	800.2	963.5	741.28	72.94		
2	LW	2,621	555	704	771.6	822.95	986	764.45	78.46		
3	LW	1,074	575.2	722.36	774.2	851.14	1039.6	779.2	83.58		
1	MI	5,820	327.92	645.22	758.33	853.17	1437.02	766.38	164.26		
2	MI	2,619	400.72	676.86	776.12	870.4	1431.13	788.46	162.08		
3	MI	1,068	451.43	712.14	808.9	921.8	1436.47	830	174.6		
1	MMY	61,024	0	25.2	30.1	35.8	82.06	30.44	7.59		
2	MMY	12,038	11.22	24.3	29.65	36.2	52.2	30.34	7.84		
3	MMY	4,176	10.6	24.9	29.79	36.35	51.1	30.83	8.01		
1	MN	5,897	1	5	6	8	16	6.49	1.94		
2	MN	2,653	1	5	6	7	14	5.73	1.76		
3	MN	1,089	1	4	5	6	11	4.86	1.62		
1	MNR	5,897	0	4	5	6	14	5.18	1.66		
2	MNR	2,653	1	3	4	5	12	4.53	1.55		
3	MNR	1,089	0	3	4	5	10	3.82	1.44		
1	MY	7,699	0	26.18	31	37.52	99.23	31.98	9.19		
2	MY	3,258	0	23.71	29.93	37.54	61.48	30.7	9.18		
3	MY	1,714	0	23.9	29.57	38.32	57.63	30.68	9.4		

 Table 41 (continuation):
 Summary statistics of all variables divided by locomotion scores.

		Summary statistics of variables										
LMS	Var	n	Min	q25	Med	q75	Max	Mean	SD			
1	MY305	44,040	0	7343	8,535	9,631	12,268	7,904.63	2,849.95			
2	MY305	9,650	0	5550	7,603	9,109	12,268	6,511.91	3,615.67			
3	MY305	3,489	0	0	7,144	8,707	12,268	5,629.34	3,968.80			
1	MYM	53,442	7.4	25	29.9	35.4	56.3	30.21	7.44			
2	MYM	8,904	11.8	24.4	29.6	35.8	52.2	30.18	7.61			
3	MYM	2,514	11.8	24.9	29.7	35.4	51.1	30.7	7.87			
1	Р	61,163	1	1	2	3	10	2.69	1.62			
2	Р	11,948	1	2	3	4	9	2.97	1.55			
3	Р	4,089	1	2	3	4	9	3.4	1.86			
1	РТ	-	-	-	-	-	-	-	-			
2	PT	35	0	0	0	0	1	0.03	0.17			
3	РТ	35	1	1	1	1	1	1	0			
1	VN	5,897	4	26	37	52	222	42.89	24.67			
2	VN	2,653	5	20	28	39	139	32.31	18.23			
3	VN	1,089	2	15	21	29	76	23.37	12.39			

Table 41 (continuation): Summary statistics of all variables divided by locomotion scores.

AC: activity, ACR: activity during daytime, BCS: body condition score, C.FMN: feeding duration per feeding visit measured by the pedometers, C_MNR: number of feeding visits measured by the pedometers, C_MNR: Number of feeding visits during daytime measured by the pedometers, DIM: days in milk, FD: feeding duration measured at weighing troughs, FDM: feeding duration per meal measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDR: feeding duration during daytime measured by pedometers, FDV: feeding duration per feeding visit measured by weighing troughs, FDRW: feeding duration during daytime measured by weighing troughs, FDW: feeding duration measured by weighing troughs, FI; feed intake, FP: feeding pace, FIM: feed intake per visit, FIV: feed intake per visit, LBNR: number of lying bouts during daytime, LD: lying duration, LDR: lying duration during daytime, LDB: lying duration per bout, LMS: locomotion score, LW: live weight, MI: milking interval, MN: number of meals measured by the weighing troughs, MNR: number of meals during daytime measured by the weighing troughs, MY: milk yield for lactation, MYM: monthly milk yield average, P: parity, PT: pain test, VN: number of visits measured by the weighing troughs.



Figure 46: Jitter plot with boxplot of activity (AC) (in units of activity index) across all farms divided by locomotion score groups (LMS).



Figure 47: Jitter plot with boxplot of activity during daytime (ACR) (in units of activity index) across all farms divided by locomotion score groups (LMS).



Figure 48: Jitter plot with boxplot of body condition score (BCS) across all farms divided by locomotion score groups (LMS).





Figure 49: Jitter plot with boxplot of feeding duration per meal measured by the pedometers in minutes (C_FDM) across all farms divided by locomotion score groups (LMS).



Figure 50: Jitter plot with boxplot of number of meals measured by the pedometers (C_MN) across all farms divided by locomotion score groups (LMS).



Figure 51: Jitter plot with boxplot of number of meals during daytime measured by the pedometers (C_MNR) across all farms divided by locomotion score groups (LMS).



Figure 52: Jitter plot with boxplot of number of days in milk (DIM) across all farms divided by locomotion score groups (LMS).



Figure 53: Jitter plot with boxplot of feeding duration measured by the pedometers (FD) in minutes across all farms divided by locomotion score groups (LMS).



Figure 54: Jitter plot with boxplot of feeding duration per meal measured by the weighing troughs (FDM) in minutes for the research farm divided by locomotion score groups (LMS).





Figure 55: Jitter plot with boxplot of feeding duration during daytime measured by the pedometers (FDR) in minutes across all farms divided by locomotion score groups (LMS).



Figure 56: Jitter plot with boxplot of feeding duration during daytime measured by the weighing troughs (FDRW) in minutes for the research farm divided by locomotion score groups (LMS).





Figure 57: Jitter plot with boxplot of feeding duration during per visit to the feeding troughs measured by the weighing troughs (FDV) in minutes for the research farm divided by locomotion score groups (LMS).



Figure 58: Jitter plot with boxplot of feeding duration measured by the weighing troughs (FDW) in minutes for the research farm divided by locomotion score groups (LMS).





Figure 59: Jitter plot with boxplot of feed intake measured by the weighing troughs (FI) in minutes for the research farm divided by locomotion score groups (LMS).



Figure 60: Jitter plot with boxplot of feed intake per meal measured by the weighing troughs (FIM) in kg/meal for the research farm divided by locomotion score groups (LMS).



Figure 61: Jitter plot with boxplot of feed intake per visit measured by the weighing troughs (FIV) in kg/visit for the research farm divided by locomotion score groups (LMS).



Figure 62: Jitter plot with boxplot of feeding pace measured by the weighing troughs (FP) in kg/minute for the research farm divided by locomotion score groups (LMS).





Figure 63: Jitter plot with boxplot of number of lying bouts per day (LBN) measured by the pedometers across all farms and divided by locomotion score groups (LMS).



Figure 64: Jitter plot with boxplot of number of lying bouts per day during daytime (LBNR) measured by the pedometers across all farms and divided by locomotion score groups (LMS).



Figure 65: Jitter plot with boxplot of lying duration in minutes per day (LD) measured by the pedometers on all farms and divided by locomotion score groups (LMS).



Figure 66: Jitter plot with boxplot of lying duration per bout in minutes (LDB) measured by the pedometers on all farms and divided by locomotion score groups (LMS).





Figure 67: Jitter plot with boxplot of lying duration during daytime in minutes per day (LDR) measured by the pedometers on all farms and divided by locomotion score groups (LMS).



Figure 68: Jitter plot with boxplot of live weight (LW) in kg measured on the research farm and divided by locomotion score groups (LMS).





Figure 69: Jitter plot with boxplot of milking interval in minutes (MI) measured on commercial dairy farm 4 and the research farm and divided by locomotion score groups (LMS).



Figure 70: Jitter plot with boxplot of average monthly milk yield (MMY) measured on all farms and divided by locomotion score groups (LMS).



Figure 71: Jitter plot with boxplot of number of meals measured by the weighing troughs (MN) on the research farm and divided by locomotion score groups (LMS).



Figure 72: Jitter plot with boxplot of number of meals during daytime measured by the weighing troughs (MNR) on the research farm and divided by locomotion score groups (LMS).

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Figure 73.: Jitter plot with boxplot of daily milk yield (MY) in kg measured on the research farm and on commercial dairy farm4 and divided by locomotion score groups (LMS).



Figure 74: Jitter plot with boxplot of milk yield for the whole lactation (MY305) measured on all farms and divided by locomotion score groups (LMS).





Figure 75: Jitter plot with boxplot of average monthly milk yield in kg (MYM) measured on commercial dairy farms 1-3 and divided by locomotion score groups (LMS).



Figure 76: Jitter plot with boxplot of parity number (P) on all farms and divided by locomotion score groups (LMS).

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Figure 77: Jitter plot with boxplot of number of visits measured by the weighing troughs (VN) on the research farm and divided by locomotion score groups (LMS).



Figure 78: Jitter plot with boxplot of number of visits during daytime measured by the weighing troughs (VNR) on the research farms and divided by locomotion score groups (LMS).



Predictor	Coefficient
FD.sqr:Season3	-1.128
(Intercept)	-1.031
C_MN.log:Season3	-0.995
Season3	0.819
DIM.sqr:Season3	-0.810
Season4	0.656
FD.sqr:Season2	-0.606
ACR.sqr	-0.588
Season2	0.541
FD.sqr:LBNR.sqrt	-0.458
LBNR.sqrt	0.431
DIM.sqr:MMY	0.427
C_MN.log:Season4	-0.420
C_MN.log:Season2	-0.291
DIM.sqr:Season4	-0.274
DIM.sqr	-0.236
P.log	0.234
LBN.log	-0.180
DIM.sqr:Season2	-0.165
FDR.sqr	-0.157
C_FDM.log	-0.122
FD.sqr	-0.081
LDB.log	0.072
MMY	0.052
AC.sqr	-0.049
LDR.sqr	0.047
FD.sqr:Season4	0.028
LD.sqr	-0.012
C_MNR.sqrt	0.001
C_MN.log	0.000

 Table 42:
 List of predictors and the coefficients estimated in the Elastic Net model for commercial dairy farm 1.

MMY: average monthly milk yield, LDR.sqr: square root of lying duration during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration during daytime, FD.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of number of number of number of natural logarithm of number of number of number of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of natural logarithm of number of number of natural logarithm of number of number of natural logarithm of number of number of number of natural logarithm of number of number of natural logarithm of number of natural logarithm of number of number of number of natural logarithm of number of number of natural logarithm of natural logarithm of number of natural logarithm of number of natural logarithm of nattributed natural logarithm of natural logarithm of natural loga

Predictor	Coefficient
C_FDM.log	-14.095
C_MN.log	-12.377
FD.sqr	8.648
LBN.log	-4.360
LDB.log	-3.901
Season3	3.486
(Intercept)	-3.088
P.log:Season2	2.979
P.log:Season4	2.895
Season2	2.763
AC.sqr:Season2	2.291
P.log	-2.182
P.log:Season3	1.957
FDR.sqr:Season4	1.823
AC.sqr:Season3	1.643
AC.sqr	-1.477
FDR.sqr:Season2	1.462
LBNR.sqrt	1.303
LD.sqr	1.085
FDR.sqr:Season3	1.044
Season4	0.910
FDR.sqr	-0.884
FDR.sqr:P.log	-0.831
FD.sqr:FDR.sqr	-0.733
ACR.sqr	-0.530
C_FDM.log:LBN.log	-0.511
LBNR.sqrt:LDB.log	0.445
AC.sqr:Season4	0.409
DIM.sqr	-0.207
LDR.sqr	0.168
MMY	-0.095
C_MNR.sqrt	0.070

 Table 43:
 List of predictors and the coefficients estimated in the Elastic Net model for commercial dairy farm 2.

MMY: average monthly milk yield, LDR.sqr: square root of lying duration during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration during daytime, FD.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of number of number of natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of natural logarithm of number of number of number of natural logarithm of number of number of number of number of number of natural logarithm of number of number of number of number of number of natural logarithm of number of number of number of number of number of natural logarithm of number of number of number of number of natural logarithm of number of number of number of number of natural logarithm of feeding duration per meal, ACR.sqr: square root of activity during daytime, AC.sqr: square root of activity, P.log: natural logarithm of parity, Season 1: winter, Season 2: spring, Season 3: summer, Season 4: autumn.

Predictor	Coefficient
C_FDM.log:Season2	-11.232
C_MN.log:Season2	-9.889
C_FDM.log:Season3	-6.735
C_MN.log:Season3	-6.076
C_MN.log	-6.010
C_FDM.log	-5.954
FD.sqr	5.128
FD.sqr:Season2	5.124
Season4	-3.572
LDB.log:Season4	3.544
LBN.log	2.735
LDB.log:Season3	2.474
FD.sqr:Season4	-2.254
LBNR.sqrt:Season4	-2.221
LDB.log:Season2	2.189
FD.sqr:Season3	2.007
Season2	1.891
LBNR.sqrt:Season2	1.800
C_MNR.sqrt	1.553
AC.sqr:P.log	1.464
FDR.sqr	-1.438
LBNR.sqrt:Season3	-1.403
P.log	1.274
DIM.sqr	-1.191
LD.sqr	-1.188
LDB.log	1.179
Season3	-1.163
DIM.sqr:MMY	1.028
ACR.sqr	1.020
LDR.sqr	0.998
(Intercept)	-0.960
LBN.log:P.log	0.948
FD.sqr:FDR.sqr	-0.798
LBNR.sqrt	-0.732
FD.sqr:P.log	0.651
DIM.sqr:LDB.log	-0.585
LBN.log:LDB.log	0.542
C_MN.log:Season4	-0.461
	-0.398
AC.sqr:ACR.sqr	-0.372
C_FDM.log:Season4	0.181
AC.sqr	-0.167

 Table 44:
 List of predictors and the coefficients estimated in the Elastic Net model for commercial dairy farm 3.

MMY: average monthly milk yield, LDR.sqr: square root of lying duration during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration during daytime, FD.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of number of number of number of natural logarithm of number of number of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of activity during daytime, AC.sqr: square root of activity, P.log: natural logarithm of parity, Season 1: winter, Season 2: spring, Season 3: summer, Season 4: autumn.

 Table 45:
 List of predictors and the coefficients estimated in the Elastic Net model for commercial dairy farm 4.

Predictor	Coefficient
(Intercept)	-4.028
FD.sqr	-3.740
Season3	3.100
LBN.log	-3.089
LDB.log	-3.007
C_FDM.log	2.836
Season4	2.669
LDR.sqr	1.515
C_MN.log	1.432
AC.sqr	-0.888
ACR.sqr	0.586
P.log	-0.550
LD.sqr	-0.252
C_MNR.sqrt	0.182
LBNR.sqrt	-0.161
DIM.sqr	-0.152
FDR.sqr	0.147
MMY	-0.073

MMY: average monthly milk yield, LDR.sqr: square root of lying duration during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration during daytime, FD.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of natural logarithm of number of

Predictor	Coefficient
AC.sqr	-1.767
VN.log	1.665
ACR.sqr	1.627
VNR.sqr	-1.610
LDB.log	-1.556
FI	-1.492
P.log:FDV.log	1.442
(Intercept)	-1.344
LBN.log	-1.227
DIM.sqr	1.198
Season4	1.182
FP.log	1.168
FD.sqr	1.075
MN.sqr	-0.988
LDR.sqr	0.796
FDW.sqr	0.708
MMY	0.637
C_FDM.log	-0.637
LBNR.sqrt	-0.563
FDR.sqr	-0.490
FDRW.sqr	0.483
P.log	0.463
LW.sqr	0.453
MY.sqr	0.435
FDM.sqr	-0.421
BCS.sqr	-0.239
FIV.log	0.198
Season2	0.196
C_MN.log	-0.154
MNR.sqr	-0.102
MI.log	0.088
FDV.log	-0.060
C_MNR.sqrt	0.044
MY305	-0.005
LD.sqr	-0.002
Season3	0.000

 Table 46:
 List of predictors and the coefficients estimated in the Elastic Net model for the research farm.

MMY: average monthly milk yield, LDR.sqr: square root of lying duration during daytime, LDB.log: natural logarithm of lying duration per bout, LD.sqr: square root of lying duration, LBNR.log: natural logarithm of number of lying bouts during daytime, LBN.log: natural logarithm of number of lying bouts, FDR.sqr: square root of feeding duration during daytime, FD.sqr: square root of feeding duration, DIM: days in milk, C_MNR.log: natural logarithm of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of number of number of number of number of number of natural logarithm of number of number of number of meals during daytime measured by pedometers, C_MN.log: natural logarithm of number of activity during daytime, AC.sqr: square root of activity, P.log: natural logarithm of parity, Season 1: winter, Season 2: spring, Season 3: summer, Season 4: autumn.

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