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## Investigating the impact of early life housing on play behaviour in dairy calves

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Submitted in fulfilment of the requirement for the Degree of Master of Veterinary Medicine

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March 2025

#### Abstract

Play behaviour is widely recognised as an indicator of positive welfare states in dairy calves but it's measurement has traditionally relied on direct behavioural observations which are labour intensive and not always suitable for studies conducted on farms. The growth of wearable accelerometer technology within the farming industry has given researchers a practical opportunity to measure play behaviour in real-time. By utilising accelerometer technology to measure calf play, the welfare impacts of different calf management systems can be easily compared. The early life housing experience of dairy calves is known to impact their development, but the immediate and long-term impacts on play behaviour are not well understood.

This research first validated IceTag accelerometer technology (Peacock Technology, UK) to measure play behaviour in weaned dairy calves. Eight female dairy calves aged three to five months old were monitored using leg-mounted accelerometers and closed circuit television cameras for a 48-hour recording period. The validation process evaluated the correlation between visual observations of weaned calf play and IceTag motion index (MI) data output, then used classification and regression tree analysis to establish a MI threshold value which would best indicate play. A MI value of  $\geq 69$  was established as the optimum threshold to detect play behaviour in weaned dairy calves (sensitivity = 94.4%; specificity = 93.6%; balanced accuracy = 94.0%).

The second part of this study utilised accelerometer technology to measure the immediate and long-term welfare impacts of different early life dairy calf housing conditions. A total of 96 female dairy calves were recruited from four Scottish dairy farms and assigned to individual, paired or group housing at birth. Play behaviour was measured using IceTags in the same cohort of calves for two 48-hour recording periods: neonatal (calves aged 24 to 72 hours old) and weaned (calves aged three to five months old). Mixed effect negative binomial regression models were used to assess the impact of early life social housing and early life playfulness on weaned calf play behaviour. Compared to calves housed individually, calves housed in pairs (IRR = 1.29; p = 0.002) and calves housed in groups (IRR = 1.43; p < 0.001) performed significantly more neonatal play. No difference in neonatal play was found between calves housed in pairs versus calves housed in groups (IRR = 1.11; p = 0.334). No significant effect of previous early life housing type nor early life playfulness was found on weaned calf play.

Collectively, the results presented in this study demonstrate that accelerometer technology can be utilised to measure welfare in dairy calves under various management systems. This study highlights the importance of validating accelerometer devices to measure animal play behaviour in a research setting and presents the potential for extension of this technology into a commercial environment for use as a farm-based welfare monitoring tool. The findings of this work contribute to the growing body of evidence indicating that social housing provides calves with a more positive early life experience than individual housing. Though no relationship between early life experience and play behaviour in weaned calves was found, this study highlights the need for further research to understand the management factors which influence weaned calf play.

## **Table of Contents**

Abstract	2
List of Tables	6
List of Figures	7
Acknowledgement	8
Author's Declaration	9
Chapter 1: Introduction	10
1.1 General introduction	10
1.2 The evolution of animal welfare research	10
1.2.1 What is animal welfare?	10
1.2.2 Traditional definitions of welfare	11
1.2.3 Drivers of changes	14
1.3 Calf play behaviour	15
1.3.1 Defining play behaviour	15
1.3.2 Factors affecting play behaviour in dairy calves	16
1.3.3 Measuring play behaviour	19
1.4 Early life housing for dairy calves	21
1.4.1 Dairy calf housing systems	21
1.4.2 Impact of social housing on dairy calf development	22
1.5 Aims of the study	25
Chapter 2: Detecting play behaviour in weaned dairy calves using accelerometer data	ı26
2.1 Abstract	26
2.2 Introduction	26
2.3 Materials & methods	28
2.3.1 Study population	28
2.3.2 Accelerometer device overview	29
2.3.3 Accelerometer data collection	29
2.3.4 Behavioural analysis	30
2.3.5 Statistical analysis	32
2.4 Results	33
2.4.1 Description of behavioural analysis and IceTag activity data	33
2.4.2 Calculation of optimal MI threshold to determine play	34
2.5 Discussion	36

Chapter 3: The impact of early life social housing on dairy calf play behaviour in the neonatal and post-weaning periods	40
3.1 Abstract	40
3.2 Introduction	40
3.3 Materials & methods	43
3.3.1 Recruitment	43
3.3.2 Calf management	44
3.3.3 Period 1: Measuring neonatal calf play behaviour	
3.3.4 Period 2: Measuring weaned calf play behaviour	
3.3.5: Statistical analysis	
3.4 Results	
3.4.1 Period 1: Factors influencing neonatal calf play behaviour	
3.4.2 Period 2: Factors influencing weaned calf play behaviour	
3.5 Discussion	
3.6 Conclusion	
Chapter 4: Discussion	
4.1 General discussion	
4.2 Using accelerometers to measure calf behaviour	
4.2.1 Measuring the behaviour of weaned calves	
4.2.2 Validation in other settings	
4.3 The future of precision livestock farming (PLF) technology in welfare assessme	
4.3.1 Development of PLF technology	
4.3.2 Potential concerns with PLF technology	
4.4 Responsibility for improving dairy calf housing and early life experience	
4.5 The impact of early life experience on development of calf play behaviour	
4.6 Study limitations	
4.0 Study miniations	
List of References	03

## **List of Tables**

<b>Table 1-1</b> : The Five Freedoms and Five Provisions in their most recently updated format (FAWC 2009).       12
Table 2-1: Ethogram of calf locomotor play behaviour (adapted from (Jensen et al., 1998))
Table 2-2: Summary statistics (range, mean and median) of visual one-zero behavioural analysis and IceTag activity data for all test calves (n=8).       34
Table 2-3: Results of manual sensitivity, specificity and balanced accuracy calculations for various MI threshold values
<b>Table 2-4</b> : Contingency table demonstrating the count and percentage of true positive, true negative, false positive and false negative play events at a MI threshold of $\geq 69$
Table 3-1: Summary of pre- and post-weaning calf housing and nutritional management 45
Table 3-2: Final multilevel mixed effects negative binomial regression model of count of neonatal calf play behaviour
Table 3-3: Final multilevel mixed effects negative binomial regression model of count of weaned calf play behaviour

List of Figures Figure 1-1: The 1994 Five Domains Model (Mellor et al. 2020)
Figure 1-2: Three overlapping areas of welfare concern (von Keyserlingk et al., 2009) 14
<b>Figure 2-1:</b> Demonstration of IceTag application to the lateral aspect of the hindlimb of one calf using cohesive bandage (Wrapz®, Millpledge Veterinary, UK) and elastic bandage (Tensoplast®, BSN Medical, UK)
<b>Figure 2-2:</b> Screenshot of video recording from CCTV cameras fixed to the back wall of the calf shed at a height of 2.8 m
<b>Figure 2-3:</b> Receiver operating characteristic curve demonstrating the sensitivity and specificity of MI threshold $\geq 68.5$ to determine play behaviour. The area under the curve for the training dataset (grey dashed line) was 0.94 and for the test dataset (black dashed line) was 0.93
<b>Figure 3-1:</b> Scatterplot and regression line demonstrating the correlation between count of neonatal and weaned calf play behaviour by housing type

## Acknowledgement

I would like to begin by expressing my warmest thanks to my supervisors Nicola Gladden, Marie Haskell and Kathyrn Ellis. I massively value the support and knowledge you have shared with me during this project. You have been kind and encouraging at all points and have made this an enjoyable and unforgettable learning experience.

A special thank you is extended to all those who worked hard to make this project possible. From the farmers who kindly volunteered their resources and time to the students who assisted with wrangling calves during the data collection periods. An additional thanks must go to the Hannah Dairy Research Foundation who provided additional funding for this project.

I am extremely grateful to all my colleagues and friends of the Scottish Centre for Production Animal Health & Food Safety. In particular I want to say thank you to my fellow residents: Richard, Elena, Giovanni, Sander, Jaka, Catriona and Hannah. I feel extremely lucky to have had your guidance and friendship to help me through this process.

Finally, a heartfelt thank you to my family who provide me endless love and support with all of my endeavours in life. Thomas, Mum, Dad, Katie and Declan- I couldn't have done it without you.

### **Author's Declaration**

"I declare that this thesis is the result of my own work, except where explicit reference is made to other people's contributions. It has not been submitted for any other degree at the University of Glasgow or any other institution."

Ciara McKay March 2025

# Chapter 1: Introduction 1.1 General introduction

The discussion of farmed animal welfare is gaining interest not only from stakeholders within the farming industry but also across wider society. There is a shared industry and societal drive for higher welfare standards which has increased the need for research to better understand the needs and nature of animals (von Keyserlingk and Weary, 2017). The concept of Positive Animal Welfare (PAW) is a more recent development within welfare research which focuses on promoting positive experience as an indicator of good welfare (Lawrence et al., 2019). Behavioural assessment is a key component of PAW and of particular interest is the measurement of play behaviour as it can be used to reflect positive mental and physical states in both human and animal species (Frost, 2012; Ahloy-Dallaire et al., 2018). Play is a behaviour observed in the absence of adverse conditions (such as hunger or predation) and, as such, reflects a positive welfare state. By measuring play, the welfare standards of different animal management practices can be assessed. For example, the benefits of different calf housing systems is currently a topic of debate within the dairy industry, so play could be used to assess the welfare of calves in different systems, with the findings used to guide best practice recommendations for on-farm calf management. Although a recent European Commission report has advocated for early life social (pair or group) housing (Nielsen et al., 2023), the move away from individual calf housing is a concern for some producers (Mahendran et al., 2022; Doyle et al., 2024). While some conflicting opinions over the short and long-term health, welfare and production impacts of social housing compared to individual housing exist, social housing is largely viewed as providing an improved early life experience for calves (Costa et al., 2016).

This chapter will first explore the different definitions of animal welfare and outline the importance this has within society. Next, the measurement of animal play behaviour and its use as a positive welfare indicator will be discussed. Finally, calf housing systems will be reviewed to demonstrate the effect different levels of early life socialisation may have on health, production and welfare.

# 1.2 The evolution of animal welfare research 1.2.1 What is animal welfare?

The definition of animal welfare has changed over time, but all definitions fundamentally refer to the feelings and physical wellbeing of animals (Weary and Robbins, 2019). The need for a concept of animal welfare arises from the fact that animals are sentient beings, capable

of experiencing positive and negative affective states (Reimert et al., 2023). The term "affective state" is often used when considering an animal's welfare status and is an overarching term encompassing the ability to consciously experience a range of positive or negative emotions, moods or sensations (Mendl and Paul, 2020). Assessing and protecting animal welfare is a societal concern which is influenced by many groups including the public, scientists and government bodies (von Keyserlingk and Weary, 2017). As we become more educated about the importance of understanding animal welfare, the body of research surrounding it has grown, particularly over the past four decades (von Keyserlingk and Weary, 2017). Welfare research is complicated by the many definitions and models of measuring animal welfare that exist (Weary and Robbins, 2019); therefore, as research evolves to better understand the biological needs and nature of animals, the definitions and approaches to studying welfare must also be revised (Mellor, 2016).

#### 1.2.2 Traditional definitions of welfare

The Five Freedoms (Table 1-1) are the most widely recognised animal welfare standards which incorporate subjective experience, health status and behaviour in the assessment of animal welfare (FAWC, 2009). They were introduced by the Farm Animal Welfare Council (FAWC) in 1979 as a response to a UK Government report from the Brambell Committee in 1965. They also highlight management actions, termed the Five Provisions, which have been used as the framework for animal welfare legislation, animal welfare accreditation schemes and practical animal welfare recommendations for many decades (McCulloch, 2012). However, the Five Freedoms, which focus solely on the elimination of negative experience, have received criticism as they do not encompass the range of biological processes which are fundamental to our current understanding of animal welfare (Mellor, 2016). It is argued that they should be thought of as an outcome-based tool to ensure the basic needs of animals are met, rather than used to assess mental state and overall welfare status (Webster, 2016).

Freedoms	Provisions
1. Freedom from thirst, hunger and malnutrition	By providing ready access to fresh water and a diet to maintain full health and vigour
2. Freedom from discomfort and exposure	By providing an appropriate environment including shelter and a comfortable resting area
3. Freedom from pain, injury, and disease	By prevention or rapid diagnosis and treatment
4. Freedom from fear and distress	By ensuring conditions and treatment which avoid mental suffering
5. Freedom to express normal behaviour	By providing sufficient space, proper facilities and company of the animal's own kind

**Table 1-1**: The Five Freedoms and Five Provisions in their most recently updated format (FAWC 2009).

After their development, the Five Freedoms were used as a basis for creating a research tool, termed the Five Domains Model. This model aimed to provide a more systematic method of evaluating the welfare impacts of a proposed animal experiment or usage (Mellor and Reid, 1994). Welfare impacts were divided into four functional or physical domains (nutrition, environment, health, and behaviour) and one mental domain (mental state as expressed in terms of negative experience). It was proposed that the impact of the four functional domains would influence the net affective outcome in the mental domain, which therefore could be used as a representation of the animals' overall welfare state (Figure 1-1). In its original form, the Five Domains Model aimed only to assess and minimise negative experiences but has been updated in recent years as animal welfare research grows to emphasise the promotion of positive welfare states (Mellor and Beausoleil, 2015). The most recent Five Domains Model has been extended to differentiate between evaluating welfare compromise versus enhancement of positive welfare and now incorporates a grading of positive affective states (Mellor et al., 2020).

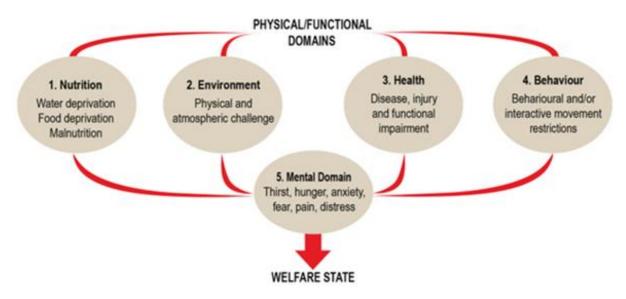


Figure 1-1: The 1994 Five Domains Model redrawn by Mellor et al. (2020).

An alternative, integrated approach to defining and measuring animal welfare was outlined by Fraser et al. (1997) and has evolved in research since. It is proposed that three ethical concerns should be considered when assessing the quality of life of animals: biological function, affective state and natural living (Fraser et al., 1997). These three areas of concern arise from different views about what constitutes good animal welfare. For example, veterinarians and farmers have historically placed emphasis on the well understood link between welfare and physical health, while the general public may propose that good welfare is to enable an animal to live a natural life where they can engage in behaviours expressed in their non-managed (wild) environment (Englund and Cronin, 2023). Instead of considering welfare concerns in each of these concepts individually and giving rise to potentially conflicting conclusions, Fraser et al. (1997) suggested that an overlapping approach is required (Figure 1-2). It is now accepted that biological function and affective state are intrinsically linked to the body working as a whole, with natural living being the anticipated positive body response an animal may have in the absence of human-imposed environmental restrictions (Mellor, 2016). Animal care guidelines and welfare research should utilise this framework, to ensure that the animal's management and environment addresses all three components combined, in order to avoid implementing a solution to one welfare concern and inadvertently creating a new welfare compromise (von Keyserlingk and Weary, 2017).

# Biological Functioning Natural Living Affective State

**Figure 1-2:** Three overlapping areas of welfare concern developed by Fraser et al. (1997) and redrawn by von Keyserlingk et al. (2009).

It is now widely accepted that good welfare is not simply the absence of negative experiences but should also include the presence of positive experiences (Boissy et al., 2007; Yeates and Main, 2008). A positive welfare state is represented by the ability to experience positive emotions and respond appropriately to changes in both internal and external factors over time (Arndt et al., 2022). Positive animal welfare (PAW) is a concept that connects the wider literature on animal welfare and is characterised by four features: positive emotions, positive affective engagement, quality of life, and happiness (Lawrence et al., 2019). In farm animals, behavioural and physiological measurements are most commonly used to detect emotions and assess positive welfare (Désiré et al., 2002). Recently, Keeling et al. (2021) proposed a protocol for measuring positive animal welfare in cattle using animal-based measures and indicators of affective states, organised into categories of short term emotion, medium term mood and long term positive and negative experience. Ear position (Lambert and Carder, 2019), play (Held and Špinka, 2011), allogrooming (Sato et al., 1993), brush use (McConnachie et al., 2018) and assessment of the way in which animals behave (known as qualitative behaviour assessment) (Fleming et al., 2016) were identified as the most reliable and practical indicators of positive welfare in cattle (Keeling et al., 2021). Moving forward, welfare research in cattle should focus on the identification of these positive welfare indicators in addition to the traditional approach of measuring the absence of negative welfare.

#### 1.2.3 Drivers of change

Recent studies have identified animal welfare as a top priority of consumers, both in the UK and worldwide, when buying meat and dairy products (Ammann et al., 2024; Blair et al., 2024). The increased public concern about the welfare of farm animals has become a driver for change in research, legislative and market domains (Hårstad, 2024). There must be

sustained engagement and education between the agricultural industry and the general public to develop a common image of what constitutes a good quality of life for animals (Weary and von Keyserlingk, 2017). As consumers become increasingly aware of the importance of animal welfare, various stakeholders need to be accountable for prioritising the wellbeing of farm animals within management and policy decisions, in order to ensure the farming sector can continue to grow and thrive in modern society. In a survey of UK consumers, the majority of respondents felt that government has the most power to make difference to animal welfare (Ellis et al., 2009); however, in reality, changes in animal welfare legislation seem to be slow and public governance have been viewed as inactive in promoting positive welfare policies (Hårstad, 2024). As a result, in order to maintain consumer confidence and increase market access, many producers choose to be associated with private industry or retailer-based assurance schemes which promote higher standards in relation to areas including product quality, environmental sustainability, and animal welfare (More et al., 2017; Esbjerg et al., 2022).

An important link between animal welfare research and social sciences has been highlighted, demonstrating that the merging of approaches to change is required to develop optimal welfare practices (von Keyserlingk and Weary, 2017). Together with considerations of societal values which help identify issues and anticipate public objections to new practices, there is a movement of scientists, veterinarians and policy makers towards promoting positive animal welfare which, overall, brings a positive outlook on the future of animal welfare in the farming industry.

#### 1.3 Calf play behaviour 1.3.1 Defining play behaviour

Behavioural assessment is widely recognised as a sensitive method of measuring animal welfare. Behaviour can provide information about both the physical and emotional health status of animals and thus assessment of behaviour can answer two key questions in relation to animal welfare: is the animal physically healthy and does the animal have what it wants? (Dawkins, 2003). Of particular interest is play behaviour, which can be used to assess the affective state of both human and animal species (Held and Špinka, 2011; Ahloy-Dallaire et al., 2018). Animal play is a complex behaviour that must be defined and understood before being used to indicate welfare states. Burghardt (2012) outlines five key criteria for recognising and defining animal play:

- 1. Play is not completely functional in that it does not contribute to survival or reproduction
- 2. Play is spontaneous and self-rewarding
- 3. Play does not mimic serious behaviours in its presentation or timing
- 4. Play is repeated but not in a stereotypic manner
- 5. Play is initiated in the absence of stress

It can be summarised from the above definition that play is a luxury behaviour, occurring when an animal is free from stress and immediate threats to wellbeing, and as such reflects a good welfare state. It has been shown that high levels of play can be directly linked to optimal welfare, or conversely that a lack of, or reduction in play, may indicate poor welfare (Held and Špinka, 2011; Ahloy-Dallaire et al., 2018).

In dairy calves, attempts at play behaviour begin within hours of birth and develop over the first weeks of life (Brownlee, 1954). In wild cattle (*Bos taurus* and *Bos indicus*), it has been shown that calves engage with other calves during activities such as play, grazing, and resting (Whalin et al., 2021). The manifestations of play observed in both wild and domesticated calves can be broadly categorised into three subtypes: locomotor play (e.g. running, jumping, bucking), object play (e.g. interacting with environmental stimuli such as feed troughs or toys), and social play (e.g. interacting with other calves) (Ahloy-Dallaire et al., 2018). While the functional purpose of play is not fully understood, it is known that most of the patterns used in play are co-opted from other behaviour systems such as grazing, social or survival (Pellis et al., 2019) and therefore play can be seen as a form of practice to build the skills necessary for mature cows to succeed in herd settings (Whalin et al., 2021).

#### 1.3.2 Factors affecting play behaviour in dairy calves

Observations of play behaviour can provide valuable insights into the health, wellbeing and welfare of dairy calves. The effect of various management factors including food availability (Krachun et al., 2010), social contact (De Paula Vieira et al., 2012) and space allowance (Jensen et al., 1998) on calf playfulness has been widely studied over the past two decades (Ahloy-Dallaire et al., 2018).

Play is a contagious behaviour which tends to build and spread to other individuals within a group (Held and Špinka, 2011). Calves housed socially in pairs or groups have been widely reported to display more play and exploratory behaviours than individually housed calves

(De Paula Vieira et al., 2012; Valníčková et al., 2015; Lv et al., 2021; Zhang et al., 2021; Mahendran et al., 2023). The effects of pair versus group housing on play behaviour have not been studied; however, it has been shown that there is no difference in levels of social or locomotor play between calves housed in pens of three, six or twelve (Lv et al., 2021). Timing of socialisation may influence play, with calves housed in social group housing at a younger age (three days old) demonstrating increased play behaviour and social interactions compared to calves that are socially housed later in the pre-weaning period (Abdelfattah et al., 2018). Early social housing has also been shown to influence calf personality traits and calves pair housed from birth are typically bolder and more outgoing in unfamiliar situations than individually housed animals (Gingerich et al., 2023; Suchon et al., 2023).

In addition to increased social contact, group or paired pens often have increased space allowance which is fundamental to locomotor play. When healthy calves are housed with ample space, they perform vigorous locomotor play behaviours (Jensen et al., 1998). Elements of locomotor play that involve large movement (e.g. running or bucking) are rarely seen in calves in small single pens (Jensen et al., 1998), whereas in group pens, increasing space allowance above the minimum requirements of 1.5 m<sup>2</sup> per calf is associated with increased locomotor play (Jensen et al., 2000; Tapki et al., 2006). Interestingly, calves deprived of space and social contact while housed in individual pens show increased locomotor play when exposed to an open arena (Jensen, 1999; Bertelsen and Jensen, 2019). This is believed to be a rebound effect, with motivation to play building over time during confinement.

Multiple elements of a calf's physical environment have been shown to influence expression of play. For example, calves are less likely to express social and play behaviours when temperatures are outside their thermo-neutral zone (Größbacher et al., 2020a; Sinnott et al., 2022) and the duration and frequency of play is increased with higher artificial light intensity, likely due to increased visual ability (Dannenmann et al., 1985). Calf comfort and bedding type has been shown to influence locomotor play behaviour, with calves reared on stones playing less than those reared on more comfortable bedding types such as rubber or sawdust (Worth et al., 2015). Calves housed in enriched environments with items such as brushes, artificial teats and rubber chains spend more time playing than calves in standard hutch pens (Pempek et al., 2017). Calves fed a higher milk allowance have been shown to play more, with a peak of play at feeding times, suggesting that there is a connection between increased feed availability and positive affective states (Krachun et al., 2010; Duve et al., 2012; Jensen et al., 2015; Größbacher et al., 2020a). Weaning and reduction in milk allowance has been linked to a reduced drive to perform locomotor play and is likely related to a motivational shift towards conserving energy and searching for milk (Jensen et al., 2015). This decrease in play associated with weaning is seen with both abrupt (Jensen et al., 2015) and gradual (Krachun et al., 2010) weaning methods and has been shown to persist in the period following weaning (Rushen and de Passillé, 2012). When weaned from their dam, calves show a stress response characterised by increased vocalisation and reduced locomotor play which is believed to be related to reduced energy intake (Rushen et al., 2016). At the same time, during the weaning process, suckling calves are separated from their dam and the maternal bond is broken. While the impact of breaking the maternal bond may also cause a negative emotional response at weaning, it has been suggested that this can be reduced if nutritional dependency on the dam is reduced prior to weaning (Johnsen et al., 2015; Rushen et al., 2016). These findings suggest that the stress associated with weaning may be substantially mitigated through improved nutritional management and are a positive step towards reducing the negative welfare impact of weaning calves.

Over the past decade, public opinion has motivated the dairy industry to explore management systems which maintain cow-calf contact while at the same time optimising animal welfare and farm profitability (Cook and von Keyserlingk, 2024). Recent work by Waiblinger et al. (2020) and Bailly-Caumette et al. (2023) have found a positive link between rearing calves with increased maternal contact and increased locomotor play. However, in both studies increased dam contact is intrinsically linked to increased space allowance and the importance of space versus maternal presence needs further clarification in future work.

In addition to being used as an indicator of positive welfare under different management systems, play behaviour can be used as a potential indicator of pain. Dystocia and calving assistance with associated discomfort immediately postpartum has been associated with reduced play behaviour in newborn calves in the first 48 hours of life (Gladden et al., 2019). Play has also been shown to be reduced following routine disbudding of calves, though appropriate use of local anaesthesia and analgesia may minimise this reduction compared to untreated animals (Rushen and de Passillé, 2012; Mintline et al., 2013). Play behaviour may also be an indicator of calf health with studies finding that calves suffering from disease

including diarrhoea or bovine respiratory disease play less often than their healthy counterparts (Größbacher et al., 2020a; Vázquez-Diosdado et al., 2024).

#### 1.3.3 Measuring play behaviour

Studying animal behaviour has traditionally relied on direct observation (either manually or via video recording) of undisturbed behaviour within an animal's natural environment (Haskell and Langford, 2023). Continuous observational studies provide a complete record of animal behaviour over a given period; however, they are time consuming, rely on the observer's ability to process data and are not suitable for studies of long duration (Bateson and Martin, 2021). Time sampling, which involves sampling behaviour periodically over successive short periods, is a less labour intensive alternative to continuous observation. Instantaneous sampling, where an animal's behavioural patterns are measured at fixed time intervals across a recording period, is a commonly used type of time sampling. This recording method is useful for collecting information regarding an animal's time budget or natural pattern of activity but is not suitable for recording discrete events of short duration such as play (Bateson and Martin, 2021). One-zero sampling is a less commonly used type of time sampling which uses a binary (yes or no) scoring system to record whether or not a behaviour has occurred within a given period. One-zero sampling is more reliable than instantaneous sampling for capturing brief, spontaneous behaviours, but cannot provide detailed information about how often, or how long the behaviour occurs. For measuring spontaneous and short-lived behaviours such as play, continuous recording has the advantages of providing detailed information such as frequency and length of play bouts whereas time sampling, most commonly one-zero sampling, tends to be more reliable at recognising the presence of play because it is less demanding (Bateson and Martin, 2021).

All manual methods of behaviour assessment require a skilled observer and risk observer fatigue in studies of long duration (Altmann, 1974). Therefore, there is growing interest in developing alternative methods of monitoring animal behaviour. Precision livestock farming (PLF) is a concept combining a collection of technologies such as pedometers, accelerometers and computer vision systems, which monitor and manage farmed animals and their environment in real-time (Beaver and Rutter, 2023). Various PLF devices are commercially available and are commonly used on cattle farms to monitor animals and aid management decisions. More recently, the data generated by PLF devices have been used by researchers to study the behaviour, health and welfare of cattle. Many devices can provide continuous individual animal monitoring and therefore can be used to measure short-

duration, spontaneous and infrequent behaviours as a less labour intensive alternative to manual behavioural observations (Brown et al., 2013; Beaver and Rutter, 2023). Prior to use in research, PLF devices must be calibrated and validated; this process typically requires the comparison of direct behavioural observations or physiological variables to the accelerometer data (Brown et al., 2013).

Accelerometers measure three dimensional changes in body velocity over time and are of particular interest when measuring play because they have the ability to record fine scale animal movements and posture changes (Brown et al., 2013; Beaver and Rutter, 2023; Hlimi et al., 2024). Accelerometers measure animal behaviour with a high degree of accuracy when validated against visual observation (Rushen et al., 2012; Hlimi et al., 2024). Accelerometers are frequently integrated into wearable devices such as collars, leg straps or ear tags, which can be easily applied for remote monitoring of animals in their natural environment (Hlimi et al., 2024). Additionally, accelerometers are relatively inexpensive compared to other PLF tools and can be easily transported between farms and animals, making them very useful for welfare assessment at group level (Rushen et al., 2012).

Over the past decade, various accelerometer devices have been validated and used to detect play behaviour in dairy calves under different experimental settings (Rushen and de Passillé, 2012; Luu et al., 2013; Gladden et al., 2020; Größbacher et al., 2020b). Early studies have found a strong correlation between accelerometer generated data and play events in dairy calves, measured both in an experimental arena test (Rushen and de Passillé, 2012; Luu et al., 2013) and in their home pen (Größbacher et al., 2020b). These studies required post-hoc manipulation of the accelerometer data to detect play and therefore are limited to a research setting and unsuitable for on-farm welfare assessment. The leg-mounted IceTag accelerometer (Peacock Technology Ltd, UK) has been validated to measure play behaviour in neonatal dairy calves (Gladden et al., 2020). Importantly this study eliminated the need for processing raw data and established a threshold to identify play based on a readily available output of the IceTag termed "motion index" (MI), which calculates overall animal activity based on the force and duration of movement. This direct output means IceTag devices have the potential to offer real-time measurement of play events and therefore have great potential for practical on-farm welfare assessment. However, validation of the IceTag to measure play has only been performed in neonatal dairy calves, and further work is required in order to utilise this technology in other age groups of cattle. The acceleration force and duration of movement in older calves is different to their younger counterparts

(Rushen and de Passillé, 2012) and therefore a different MI threshold to identify play is likely to be needed. Most studies utilising accelerometers to measure play have used these devices in a manner analogous to one-zero sampling where the data output can indicate the presence or absence of play within a given period; however, limitations including over reporting of the number of play events and inability to record the detailed nature of play have been consistently reported (Gladden et al., 2020; Größbacher et al., 2020b).

#### 1.4 Early life housing for dairy calves 1.4.1 Dairy calf housing systems

In the dairy industry, newborn calves are commonly separated from their dam within hours of birth and may be managed in multiple types of housing systems. Indoor purpose built pens are commonly used and can accommodate calves in individual, pair or group systems. Outdoor housing options include pasture based grazing systems that do not confine the calf, or hutches that combine a sheltered house with a confined outdoor run area and are available in individual, pair and group sizes. Dairy calf housing systems must comply with stringent regulations set by both national and European authorities (Council Directive 2008/199/EC) which determine standards for housing factors including space allowances, ventilation, bedding quality and social contact. However, these regulations outline the minimum requirements for calf housing and recently various stakeholders within the dairy industry have been advocating a change towards housing types which promote higher welfare standards. A recent European Food Safety Authority (EFSA) report, requested by the European Commission, advocated for group housing from birth, increased space allowance to permit unrestricted play behaviour and the provision of a comfortable lying surface in order to improve animal welfare (Nielsen et al., 2023). In the UK, milk buyers and farm assurance schemes have become industry leaders in establishing changes to calf housing with a particular drive towards reducing individual housing in order to promote positive welfare experience through social contact (Mahendran et al., 2022). Consumers show a preference for group housing of dairy calves as it allows a more natural living experience with increased space and improved social contact (Perttu et al., 2020). Producers that already implement social housing have a positive perception of it and believe that access to other calves is beneficial for both calf comfort and development (Mahendran et al., 2022; Doyle et al., 2024). In contrast, producers that have no experience with social housing seem reluctant to implement it over individual housing due to negative perceptions over health and feeding management (Marcé et al., 2010; Mahendran et al., 2022).

Despite the industry drive towards promoting social housing of calves, uptake on farms across the world has been variable. European Council Directive 2008/199/EC specifies that calves must not be housed individually beyond eight weeks old; however, similar legislation is not present outside of the European Union (EU) and United Kingdom. A recent survey of US dairy producers and calf managers found that 44% of farms still use individual housing, with only 35% of these allowing both visual and tactile contact between calves (Doyle et al., 2024). In the EU, individual housing from birth remains common with examples including 90% of Austrian farms (Klein-Jöbstl et al., 2015) and 97% of Czechian farms (Staněk et al., 2014) implementing individual housing from birth. In the UK, individual housing is the most prevalent type of housing for newborn dairy calves, with a 2022 survey reporting its use on 34.8% of UK farms (Mahendran et al., 2022). However, this has markedly decreased from 60% use of individual pens on UK farms reported in 2010 (Marcé et al., 2010) and is likely due to milk buyer changes stipulating pair or group housing from birth. Some countries are leading positive change, for example in the Republic of Ireland, farmers are moving away from individual housing and the majority (58.8%) of farms now use group housing from immediately post-birth (Sinnott et al., 2023).

#### 1.4.2 Impact of social housing on dairy calf development

Early life housing can greatly impact the health and welfare of dairy calves. There is strong evidence linking social housing, where calves are reared in pairs or groups, to improved early life experience due to its positive impact on factors including behaviour, cognitive development, feed intake, growth and health (Costa et al., 2016; Donadio et al., 2024).

In addition to increasing play behaviour, as discussed above, pair and group housing has been shown to positively impact calves' social behaviour and response to novel scenarios. Calves that experience early life social housing are bolder, more competitive, and more inquisitive in their interactions with novel objects (Gingerich et al., 2023; Suchon et al., 2023) than calves housed individually in early life. Calves raised in social groups with other calves and cows are also more accepting of new feed types (Costa et al., 2014). By contrast, individually housed calves are more reactive when exposed to a novel environment, as indicated by increased defecation and more frequent backing away, possibly due to a heightened anxiety or emotionality associated with early social deprivation (De Paula Vieira et al., 2012). Cross-suckling, defined as the act of a calf suckling on the body part of another calf, is often cited by farmers as a negative behavioural effect of social housing; however, this behaviour is motivated by hunger (Jensen, 2003) and various studies have shown that,

when fed sufficient milk allowances, there is no difference in cross suckling or other nonnutritive oral behaviours between individually and socially housed calves (Chua et al., 2002; Reipurth et al., 2020; Mahendran et al., 2023). Social housing from birth also allows calves to establish social bonds, with individually housed calves being more fearful of unfamiliar calves (De Paula Vieira et al., 2012; Jensen and Larsen, 2014) and pair housed calves showing a preference for spending time with their pen-mate as opposed to a less familiar calf (Duve and Jensen, 2011; Lindner et al., 2022).

Socially housed calves have consistently been shown to achieve higher growth rates in the pre-weaning period compared to individually housed calves (Costa et al., 2015; Jensen et al., 2015; Knauer et al., 2021; Ahmadi et al., 2022). Optimising pre-weaning daily liveweight gain is essential to drive future growth and milk production in the first lactation (Soberon et al., 2012). Increased pre-weaning growth rates in socially housed calves are likely related to social facilitation of feeding where one calf is more likely to approach the feed space while another calf is eating (Miller-Cushon and DeVries, 2016). Multiple authors have reported that socially housed calves consume more starter and solid feeds than individually reared calves in the pre-weaning period (Jensen et al., 2015; Mahendran et al., 2021; Ahmadi et al., 2022). This positive impact remains during and after the weaning process, with individually housed calves showing a growth check at weaning that is not exhibited by pair housed calves (Chua et al., 2002) and pair housed calves showing shorter latency to feeding and increased starter intake after weaning (De Paula Vieira et al., 2010). Increased solid feed intake in socially housed calves is extremely beneficial as it promotes rumen development, facilitates the transition from a milk-based pre-weaning diet to a forage based adult ruminant diet and ensures positive post-weaning outcomes such as increased growth rates and improved fertility performance (Diao et al., 2019; Costigan et al., 2022).

Housing is considered one of the major risk factors for developing disease in pre-weaned calves (Nordlund and Halbach, 2019). Housing calves in individual pens until weaning has traditionally been seen as the best option to promote health and control enteric and respiratory disease (Marcé et al., 2010), but recent evidence supporting the use of individual pens to control disease has been inconsistent. An increased risk of bovine respiratory disease and diarrhoea in group or pair housed calves has been reported by various authors (Cobb et al., 2014; Curtis et al., 2016; Breen et al., 2023). In contrast, other studies have reported either no difference (Jensen and Larsen, 2014; Bučková et al., 2021; Sinnott et al., 2022) or improvements (Mahendran et al., 2023) in health outcomes of group and pair housed calves.

Disease transmission is a complex process and variations in study design such as age at grouping or number of calves per group may influence the morbidity and mortality results of these studies. Given the inconsistency in the literature at the present time, it can be concluded that calves could be raised in social groups without increasing disease morbidity and mortality but other housing and management factors, such as colostrum provision, shed ventilation, and pen hygiene, must be considered in order to optimise calf health (Costa et al., 2016).

The impact of early social experience of dairy calves on their long-term behaviour and production has not been widely studied but is a topic of growing interest. Exploratory behaviour and personality traits such as boldness are not consistent in calves after weaning and change after the onset of puberty (Neave et al., 2020). Despite these age-related personality changes, the positive effects of early socialisation on adaptation to novel experiences appear to carry forward into adulthood. Socially reared calves become more socially dominant later in life and show more competitive interactions than calves reared in social isolation when tested at both eight and 20 months old (Broom and Leaver, 1978). Early life social housing prepares heifers for the transition into adulthood, with positive effects on activity, feeding and response to competition when transitioned into cubicle adult housing (Clein et al., 2024). A recent study by Mahendran et al. (2024) found that calves raised in social housing are less likely to leave the herd between weaning and the end of the first lactation, possibly due to learned behavioural differences which allow these calves to better adapt to competitive group environments. Furthermore, socially reared heifers show no long-term advantages or disadvantages in performance compared to individually reared heifers. The improvements in weight gain seen in pre-weaned socially reared animals do not persist immediately post-weaning (Valníčková et al., 2015) nor across the rearing period until first calving (Mahendran et al., 2024). No differences in fertility performance or first lactation milk production have been found between individually versus socially reared heifers (Valníčková et al., 2020; Mahendran et al., 2024). The lack of future performance differences between calves raised in different housing types may be viewed as a positive outcome, as producers with reservations about social housing can be assured that no longterm negative impacts will be seen from adopting this housing type. This is an area of increasing knowledge and understanding and where further studies will allow more informed management of calves and youngstock.

#### 1.5 Aims of the study

Play behaviour is well reported to be an indicator of positive welfare in dairy calves; however, studies measuring play often rely on traditional visual observational methods of assessing calf behaviour which can be labour intensive and impractical for studies of long duration. Accelerometer technology has been validated for practical and real-time measurement of calf play behaviour in neonatal calves (up to 48 h old) but not in any other age group of cattle. The acceleration force and duration of movement in older calves is different to their younger counterparts and therefore the accelerometer data thresholds established to detect play in neonatal calves are unlikely to be the same for weaned calves. Therefore, the first aim of this study was to assess the ability of IceTag accelerometers to detect play behaviour, and therefore assess welfare, in weaned (three to five months old) dairy calves. We hypothesised that IceTags would be able to reliably measure play in weaned calves at a higher MI threshold than established for neonatal calves.

There is extensive literature outlining the advantages of early life social housing for dairy calves with documented positive effects on growth, socialisation and welfare as measured via behavioural observations of positive welfare indicators such as play behaviour. No studies have implemented accelerometers as a welfare assessment tool to assess calf welfare under different on-farm early life housing conditions. Hence, the second aim of this study was to use accelerometer technology to investigate the impact of early life housing types, specifically individual, paired or group housing, on calf play behaviour in the first week of life. We hypothesised that calves in social housing types would play more than calves in individual pens.

While the long-term impact of early life socialisation on calf personality traits and response to novel situations has been described, no study has investigated the impact of differing social experiences and early life playfulness on future play behaviour. The final aim of this study was therefore to assess whether social housing and increased play early in life impacts a calf's playfulness post-weaning at three to five months old. We hypothesised that calves housed socially pre-weaning would play more post-weaning and that, regardless of preweaning housing type, calves categorised as more playful early in life would remain more playful after weaning.

# Chapter 2: Detecting play behaviour in weaned dairy calves using accelerometer data

Published in "Journal of Dairy Research"

Reference: McKay C, Ellis K, Haskell MJ, Cousar H, Gladden N. Detecting play behaviour in weaned dairy calves using accelerometer data. Journal of Dairy Research. 2024. DOI: 10.1017/S0022029924000542

#### 2.1 Abstract

This research paper describes a validation study evaluating the ability of IceTag accelerometers (Peacock Technology, UK) to detect play behaviour in weaned dairy calves. Play behaviour is commonly observed in young animals and is regarded as an indicator of positive welfare states. Eight Holstein Friesian calves aged three to five months old were monitored using leg-mounted accelerometers for 48 hours. Data generated by accelerometers to quantify calf activity included step count, lying times and a proprietary measure of overall activity termed "motion index" (MI). Calf behaviour was filmed continuously over the same 48-hour period using closed circuit television cameras and analysed using one-zero sampling to identify the presence (1) or absence (0) of play within each 15-minute time period. A positive correlation between MI and visually recorded play was found. Visual observations were compared with accelerometer-generated data and analysed using 2 x 2 contingency tables and classification and regression tree analysis. A MI value of  $\geq$  69 was established as the optimum threshold to detect play behaviour (sensitivity = 94.4%; specificity = 93.6%; balanced accuracy = 94.0%). The results of this study suggest that accelerometer-generated MI data have the potential to detect play behaviour in weaned dairy calves in a more time efficient manner than traditional visual observations.

#### 2.2 Introduction

Animal welfare, particularly that of calves, is a topic of growing discussion within the dairy sector and in wider society (von Keyserlingk and Weary, 2017). It is increasingly recognised that the measurement of animal welfare must not focus solely on the elimination of negative experiences and instead should be moving towards the identification and promotion of positive experiences (Lawrence et al., 2019). Behavioural assessment is becoming more widely used as a method of measuring positive animal welfare (Mattiello et al., 2019) and

of growing interest is play behaviour, which is a spontaneous and short duration behaviour commonly observed in young animals (Burghardt, 2012). Play behaviour is not required for survival and is exhibited by animals when they feel free from stress and immediate threats to wellbeing (Held and Špinka, 2011). Play may be adversely affected by negative experiences and, accordingly, is considered to be an indicator of positive welfare (Held and Špinka, 2018).

The study of animal behaviour has traditionally relied on manual observation or video recording to capture the undisturbed behaviour of animals in their "home" environment (Haskell and Langford, 2023). These observational methods of behavioural assessment can be labour intensive and are often not practical for studies of long duration, or for on-farm assessment (Bateson and Martin, 2021). Precision livestock farming (PLF) is a concept that uses technology to monitor farmed animals and their environment in real-time (Beaver and Rutter, 2023), and recent PLF developments in wearable sensors for animals have provided researchers with a variety of tools that can aid in the monitoring of calf health and behaviour (Brown et al., 2013; Costa et al., 2021). Tri-axial accelerometer devices, which measure three-dimensional changes in body velocity over time, have the ability to record fine scale animal movements and posture changes and are increasingly being used to monitor the behaviour of livestock (Brown et al., 2013; Costa et al., 2021). Accelerometers have been reported to measure a variety of daily behaviours (such as lying and rumination times) in dairy calves of ages ranging from three weeks to two months old (Trénel et al., 2009; Bonk et al., 2013; Hill et al., 2017; Roland et al., 2018). The change in pattern of behaviours detected by accelerometers has been shown to aid in the early detection of disease including neonatal diarrhoea (Goharshahi et al., 2021) and bovine respiratory disease (Gardaloud et al., 2022).

Accelerometer technologies also have potential for use in welfare assessment as they have the capability to detect behaviours, such as play, that reflect positive welfare (Rushen et al., 2012). Different accelerometer-based devices have been used in previous studies to identify play behaviour in dairy calves in differing experimental circumstances (Rushen and de Passillé, 2012; Luu et al., 2013; Gladden et al., 2020; Größbacher et al., 2020b). Using tests in experimental arenas, studies have shown a strong correlation between summed acceleration calculated by leg mounted accelerometers and duration of locomotor play events such as running, in pre- and post-weaned calves (Rushen and de Passillé, 2012; Luu et al., 2013). Similarly, Größbacher et al. (2020b) manipulated accelerometer-generated data to identify play behaviours in four and eight week old calves in their home pen. Recent work by Gladden et al. (2020) validated the leg-mounted IceTag accelerometer (Peacock Technology Ltd, UK) to measure play behaviour in neonatal dairy calves (up to 48 hours old) in their home pen. Importantly, this work analysed direct outputs generated by the IceTag, eliminating the need for additional processing of raw accelerometer data as was required in previous studies. Moreover, this study established a threshold to identify play based on a readily available output of the IceTag termed "motion index" (MI), which calculates overall animal activity based on the force and duration of movement. While previous studies have shown that accelerometer data can be used to correctly identify play behaviour in dairy calves, limitations including overestimation of the number of play events and inability to record the duration and detailed nature of play have been consistently reported (Trénel et al., 2009; Gladden et al., 2020; Größbacher et al., 2020b).

Despite there being a growing body of literature using accelerometer technology to measure calf behaviour, only a few studies have focused on measuring the behaviour of healthy, older calves in a typical commercial dairy context during the post-weaning period (Hänninen et al., 2005; Rushen and de Passillé, 2012). Lying and activity times have been reported for pre-weaned dairy calves (Wormsbecher et al., 2017; Gladden et al., 2019; Duthie et al., 2021) but not for weaned calves. The acceleration force and duration of movement in older calves is different to their younger counterparts (Rushen and de Passillé, 2012). Therefore, we hypothesised that accelerometer data thresholds established to detect play in young (preweaned) calves will not be appropriate for older (post-weaning) calves. The primary objective of this study was to assess the ability of IceTag accelerometers to detect play behaviour in three to five month old dairy calves, with a particular focus on MI. A secondary objective of this study was to establish, using IceTag accelerometers, a daily time budget for weaned dairy calves aged between three to five months old.

#### 2.3 Materials & methods 2.3.1 Study population

Ethical approval for the study was obtained from the University of Glasgow School of Veterinary Medicine Research Ethics Committee (ref EA05/22). Eight weaned, female Holstein-Friesian dairy calves aged three to five months old (mean 118 d, SD  $\pm$  16 d) were recruited from a 50-cow dairy herd in central Scotland. Calves were housed in individual straw bedded pens from birth until weaning at eight weeks old, following which they were moved to group housing. During the study, calves were housed in a straw-bedded group pen

measuring 7.3 x 13.6 m in groups of ten. Calves were fed concentrate pellets once daily and had *ad libitum* access to straw and water. Data were collected over a three-week period from February to March 2022.

#### 2.3.2 Accelerometer device overview

IceTags (Peacock Technology Ltd, UK) are small (66 x 55 x 27 mm), lightweight (117 g) devices previously validated for behavioural monitoring in adult cattle and calves (Trénel et al., 2009; Nielsen et al., 2010; Ungar et al., 2018; Gladden et al., 2020). The IceTag is a triaxial accelerometer with a high data recording frequency rate of 16 Hz (16 sample points recorded every second); data are collated to generate specified output resolutions that are dictated by the IceTag software (1 sec, 1 min, 15 min, 1 hour, 2 hours, 1 day and 1 week). Acceleration forces occurring during animal movement are measured in three dimensions and collated by the device to generate outputs reflective of animal activity such as standing time, lying time, step count and number of lying bouts. Additionally, a proprietary metric termed "motion index" (MI) is generated by the IceTag, by calculating the average vector sum of acceleration in three dimensions (the MI value is equivalent to the gravitational acceleration applied to the IceTag device x 10 (C.Malcolm, IceRobotics, personal communication)). Motion index combines the duration of animal movement and forces applied to the accelerometer during movement and, therefore, can be viewed as an indicator of overall animal activity (Gladden et al., 2020). Using this type of accelerometer, a strong positive correlation between MI and play behaviour (R = 0.922, P < 0.001) has previously been reported in newborn calves (Gladden et al., 2020).

#### 2.3.3 Accelerometer data collection

A single IceTag was attached to one hindlimb of each calf using a methodology similar to that described by Gladden et al. (2020) and demonstrated in Figure 2-1. Briefly, the IceTag was cushioned inside a small fabric sock and attached to the lateral aspect of one hindlimb of the calf, proximal to the metatarsophalangeal joint, using cohesive bandage (Wrapz®, Millpledge Veterinary, UK). The bandage was secured at the proximal and distal aspects using a layer of elastic bandage (Tensoplast®, BSN Medical, UK). IceTags were attached to each calf for a maximum of three days, including a minimum 12-hour period of adjustment to the device placement prior to commencing the 48-hour data collection period. To provide a daily time budget for each calf, daily lying times and individual lying bout data were extracted from the IceTag and analysed over a 24-hour time period from 08:00 on day one of the study until 08:00 the following day. To capture a representative sample of behaviour

amongst the group of calves, data collection periods were staggered across the calf group and were performed over four recording periods, where a new pair of calves was enrolled at each session. IceTags were attached to only two calves at any one time, with all other calves in the pen acting as companion animals. IceTag generated data were downloaded at the end of each recording period using an IceReader device (Peacock Technology Ltd, UK) in combination with IceManager software (Peacock Technology Ltd, UK). Calf activity data were summarised in 15-minute sampling intervals and exported as CSV-format files for further analysis. An output of 15-minute sampling intervals was selected based on the available preset IceTag output resolutions and previous work which has demonstrated that MI output at this resolution could predict the presence or absence of play behaviour in neonatal dairy calves (Gladden et al., 2020).



**Figure 2-1:** Demonstration of IceTag application to the lateral aspect of the hindlimb of one calf using cohesive bandage (Wrapz®, Millpledge Veterinary, UK) and elastic bandage (Tensoplast®, BSN Medical, UK).

#### 2.3.4 Behavioural analysis

Calf behaviour was continuously recorded over the three-week study period using two closed circuit television cameras (CCTV: Sony CCD, Vari-focal, 700 TV L, Sony, Japan) fixed to

the back wall of the calf shed at a height of 2.8 m, chosen to allow maximum visibility whilst not obstructing farm machinery. The cameras provided a mostly unobstructed view of the pen aside from two blind spots directly underneath each camera, as shown in Figure 2-2. Video footage was stored on digital video recorders (Guardian II+DVR 8 Channel, Digital Direct Security, UK) and extracted at the end of the study period. To allow identification from video recordings, calves were marked along their back with a unique colour of agricultural spray which was recorded alongside the calf ID and IceTag serial number.

#### Camera 1

Camera 2



**Figure 2-2:** Screenshot of video recording from CCTV cameras fixed to the back wall of the calf shed at a height of 2.8 m.

Extracts of video recordings were time matched to the 48-hour IceTag data collection period and analysed individually for each calf. Play behaviour was measured using one-zero sampling (Altmann, 1974) at 15-minute intervals corresponding with the IceTag output. Play was identified based on an ethogram of calf locomotor play behaviour (Table 2-1) based on previous work by Jensen et al. (1998). The ethogram was modified to include only locomotor play behaviours as these behaviours typically include leg movement, recorded here due to the positioning of the IceTag on the hindlimb. If any behaviour identified as play, regardless of count or duration, occurred during the given 15-minute interval, this entire time period was recorded as "Play (1)". If no play was observed during the selected 15-minute interval, this entire time period was recorded as "No Play (0)". Time periods where the calf was not observed due to positioning outwith the camera range were recorded as "Not Visible". Complete IceTag and video datasets were available for all eight calves, with 192 15-minute intervals per calf and a combined total of 1536 15-minute intervals available for the initial analysis. However, due to camera positioning, calves were not visible in 4.8% (73/1536) of the 15-minute intervals, and these time periods were excluded from any visual analysis of calf behaviour. Accordingly, IceTag data analysis was based on a cumulative total of 1536 sample intervals, while correlation of video derived calf behaviour data with MI data and the calculation of the optimal MI threshold to determine play was based on a cumulative total of 1463 sample intervals.

Locomotor play event	Description
Buck (low)	The calf raises both hindlimbs simultaneously with the hooves remaining below the tarsal joints.
Buck (high)	The calf raises both hindlimbs simultaneously with the hooves raised to at or above the tarsal joints.
Buck-kick	The calf raises both hindlimbs simultaneously with the hooves raised to at or above the tarsal joints. One or both hindlimbs are kicked out in a caudal or lateral direction.
Gallop	The calf moves with a fast four-beat gait that includes a period of suspension in air.
Jump	The calf lifts both forelimbs from the ground and elevates the front of the body to move upwards. The hindlimbs may also be lifted from the ground during the last phase of movement.
Leap	The calf lifts both forelimbs from the ground and stretches the front of the body to move forwards. The hindlimbs may also be lifted from the ground during the last phase of movement.
Turn	The calf lifts both forelimbs from the ground and elevates then turns the front of the body to one side to move sideways.

**Table 2-1:** Ethogram of calf locomotor play behaviour (adapted from (Jensen et al., 1998))

#### 2.3.5 Statistical analysis

Corresponding intervals of IceTag MI data and video behavioural analysis were combined and summarised in Microsoft Excel (Version 2308, Microsoft Corporation, USA) then exported for analysis in Minitab (Version 20, Minitab LLC, USA).

Descriptive statistics were calculated separately in Minitab for IceTag and visual observations. Lying times and lying bouts of individual calves were assessed using IceTag generated data, which has previously been validated by Trénel et al. (2009) to accurately estimate standing and lying activities in group housed dairy calves. To indicate whether more detailed analysis was appropriate, the Mann–Whitney U test was used to determine whether

MI was associated with the presence or absence of play. Statistical significance was considered at a P-value of < 0.05. Motion index values and one-zero sampling results were formatted in 2 x 2 contingency tables to determine the sensitivity (Se), specificity (Sp) and balanced accuracy of selected MI threshold values to detect play behaviour. These values were calculated for each MI threshold point using the following formulae:

Se = (true positive play events)/(true positive play events + false negative play events) Sp = (true negative play events)/(true negative play events + false positive play events) Balanced accuracy = (Se + Sp)/2

Different MI threshold values (a range of 25 to 300) were investigated with this method to determine the value which provided optimal sensitivity, specificity and balanced accuracy. The MI threshold value which would best indicate play was selected based on optimal balanced accuracy, as this figure represents the best balance between specificity and sensitivity. The optimal MI threshold to detect play behaviour was confirmed using classification and regression tree (CART) analysis performed using the Gini node splitting method. Data was subdivided into training and test datasets by the CART model using 10-fold cross validation. Performance of the model determined MI threshold in identifying play was summarised using a confusion matrix and receiver operating characteristic (ROC) curve.

#### 2.4 Results

#### 2.4.1 Description of behavioural analysis and IceTag activity data

Lying times and lying bout data output by the IceTags were assessed for all calves for a 24hour period (Table 2-2). Mean (SD) daily lying time was  $16.1 \pm 0.71$  hours/day. Calves spent an average 67.1% of their day lying compared to an average 32.9% of time standing or engaged in activity. The median (SD) number of lying bouts per day was  $42 \pm 13$ . The mean (SD) length of lying bout was  $21.9 \pm 5.7$  minutes.

Based on visual one-zero sampling, across all test calves, play was recorded in 13.5% (197/1463) of the visible 15-minute intervals; no play was recorded in 86.5% (1266/1463). For individual animals, the percentage of visible 15-minute intervals in which play was recorded ranged from 5.3% (9/169) to 21.0% (39/186: Table 2-2). Motion index values varied depending on the calves' activity state, as indicated by one-zero sampling (Table 2-2). Overall, regardless of activity state, MI ranged from 0 to 2343 (median 6). In intervals where one-zero sampling indicated calves to be engaged in play, MI ranged from 25 to 2343

and in intervals where no play was recorded, MI ranged from 0 to 280. A significant difference was found between the median MI for intervals with play recorded (median = 130) compared to intervals where no play was recorded (median = 1) (P < 0.001).

		. ,		
Variable	Minimum	Maximum	Mean	Median
Visual Observations <sup>1</sup>				
Play (% of visible intervals)	5.3	21.0	13.5	
No play (% of visible intervals)	79.0	94.7	86.5	
IceTag Motion Index Output <sup>2</sup>				
Motion index overall	0	2343		6
Motion index during play	25	2343		130
Motion index during no play	0	280		1
Motion index during not visible	0	119		6
IceTag Lying Activity Output <sup>3</sup>				
Lying time (hours)	15.4	17.5	16.1	
Lying (%)	64.1	72.9	67.1	
Lying bouts	32	74		42
Lying bout length (mins)	12.9	29.5	21.9	

**Table 2-2:** Summary statistics (range, mean and median) of visual one-zero behavioural analysis and IceTag activity data for all test calves (n=8).

<sup>1</sup> Behavioural observations based on one-zero sampling of calf activity while visible over 1463 15-minute intervals.

<sup>2</sup> Motion index values based on IceTag output of 1536 15-minute intervals.

<sup>3</sup> Daily calf lying activity based on IceTag output over a 24-hour period.

#### 2.4.2 Calculation of optimal MI threshold to determine play

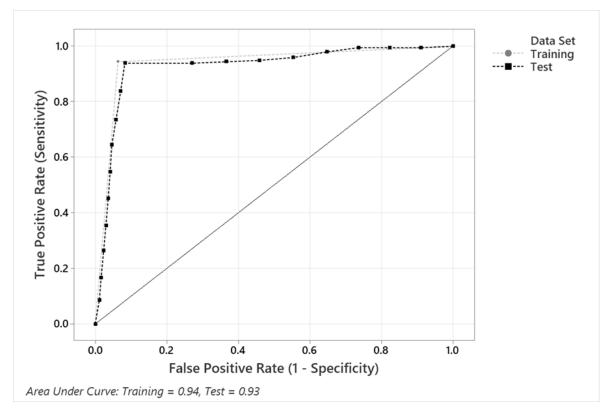
Results of manual threshold determination for a selection of MI cut off points are presented in Table 2-3. Maximum sensitivity (Se = 100%) was associated with a MI threshold of  $\geq$  25. However, specificity and balanced accuracy were low at 28.8% and 64.4%, respectively. Maximum specificity (Sp = 100%) was associated with a MI threshold of  $\geq$  281, but at this threshold sensitivity was poor at 14.2% and balanced accuracy was moderate at 57.1%. A threshold of  $\geq$  69 provided the most balanced overall performance, with 94.4% sensitivity, 93.6% specificity and 94.0% balanced accuracy. The count and percentage of true positive, true negative, false positive and false negative play events at this optimal MI threshold are presented in Table 2-4. Classification and regression tree analysis indicated that the optimum MI threshold for determining play was MI  $\geq$  68.5, with excellent overall performance in the model's training (Se = 94.4%; Se = 93.6%) and test (Se = 93.9%; Sp = 91.7%) datasets. The area under the ROC curve for the training and test datasets were 0.94 (95% CI: 0.48 - 1.0) and 0.93 (95% CI: 0.91 - 0.95) respectively (Figure 2-3).

Motion Index Threshold	Sensitivity	Specificity	Balanced Accuracy
≥25	100%	28.75%	64.38%
$\geq 60$	95.43%	91.00%	93.21%
$\geq 65$	94.92%	92.58%	93.75%
$\geq 66$	94.92%	92.65%	93.79%
$\geq 67$	94.92%	92.73%	93.83%
$\geq 68$	94.42%	93.13%	93.77%
≥69	94.42%	93.60%	94.01%
$\geq 70$	93.40%	93.92%	93.66%
$\geq 80$	89.34%	96.37%	92.85%
≥ 281	14.21%	100%	57.11%

**Table 2-3:** Results of manual sensitivity, specificity and balanced accuracy calculations for various MI threshold values

**Table 2-4**: Contingency table demonstrating the count and percentage of true positive, true negative, false positive and false negative play events at a MI threshold of  $\ge 69$ .

0			
Play (1)	No Play (0)	Total	
11 (0.75%)	1185 (81.00%)	1196 (81.75%)	
186 (12.71%)	81 (5.54%)	267 (18.25%)	
197 (13.47%)	1266 (86.53%)	1463 (100.00%)	
-	Play (1) 11 (0.75%)	Play (1)         No Play (0)           11 (0.75%)         1185 (81.00%)           186 (12.71%)         81 (5.54%)	



**Figure 2-3:** Receiver operating characteristic curve demonstrating the sensitivity and specificity of MI threshold  $\ge 68.5$  to determine play behaviour. The area under the curve for the training dataset (grey dashed line) was 0.94 and for the test dataset (black dashed line) was 0.93

#### 2.5 Discussion

Previous studies have demonstrated the ability of leg-mounted accelerometer devices to identify play behaviour in dairy calves of different ages (Rushen and de Passillé, 2012; Luu et al., 2013; Gladden et al., 2020; Größbacher et al., 2020b). Similarly, our study has shown that IceTag accelerometer generated data can identify play behaviour in weaned dairy calves (aged three to five months old) in their home pen. The results of our study suggest that MI values generated in 15-minute sample intervals can reliably detect play behaviour at a threshold of 69. By contrast, in a similar study Gladden et al. (2020) found play behaviour in younger calves was identified using an MI threshold of 24.5, which was lower than our findings. It should be considered that this finding was expected, given that MI is a measure of overall animal activity and weaned calves are known to move with greater acceleration than younger calves (Rushen and de Passillé, 2012), and supports our hypothesis that accelerometer generated data thresholds for detecting play differ with calf age.

Previous work investigating the use of accelerometers to monitor calf behaviour has highlighted the limitations of this technology in being able to accurately describe the nature of specific behaviours (Trénel et al., 2009; Gladden et al., 2020; Größbacher et al., 2020b). Similarly, in this study the technique used to identify play behaviour is comparable to onezero sampling, whereby within any given 15-minute interval a score of "Play (1)" or "No play (0)" was assigned but the exact duration or number of play events of individual calves could not be determined. Despite defining a threshold with high sensitivity and specificity (94.4% and 93.6%, respectively) to detect play behaviour, the use of 15-minute sampling intervals further limits accurate assessment of play as multiple play events could occur within this period but would not be counted individually. Therefore, future work to determine the performance of lower IceTag resolutions (e.g. 1-minute sampling intervals) may be warranted to provide a more accurate estimation of play behaviour in weaned dairy calves. Overall, the methods described in this study are beneficial for providing a proxy measurement of play events within a 15-minute sampling period, however, if a more detailed description of the nature of play is desired then shorter sampling intervals or continuous observational methods of behavioural assessment may be preferred (Haskell and Langford, 2023).

It is known that young dairy calves spend a large portion of their daily time budget engaged in lying behaviours (Whalin et al., 2021), but no studies have examined the daily time budget of dairy calves of the age studied here. Newborn calves housed in groups are reported to spend over 80% of their daily time budget lying (Gladden et al., 2019), whereas slightly older animals (three to four weeks old) have been reported to spend approximately 70% of their daily time budget lying with lying times of between 16.7 and 16.9 hours per day reported (Wormsbecher et al., 2017; Duthie et al., 2021). Similarly, calves in the present study were found to be engaged in lying behaviours for 67% of their day (16.1 hours/day). This finding is consistent with previous research which describes a decrease in resting behaviour with increasing age in calves studied up to five months old (Hänninen et al., 2005). Understanding the typical lying and activity patterns of calves is important, as deviations from normal lying times may be associated with stressful events such as onset of disease (Duthie et al., 2021; Goharshahi et al., 2021; Gardaloud et al., 2022), heat stress (Kovács et al., 2018) and introduction to social grouping (Horvath and Miller-Cushon, 2018).

While weaned calves were found to spend a similar amount of time per day lying to the times reported by other authors for younger calves, the pattern of lying behaviour was not similar. The calves in our study engaged in a higher number of shorter duration lying bouts than calves in previous studies, which report less frequent (mean 19.6 bouts/day), longer duration

(mean 56 minutes/bout) lying periods in younger calves housed and managed under similar conditions (Duthie et al., 2021). Increased frequency of lying has been related to discomfort or disrupted rest patterns in both calves and adult cattle (Vasseur et al., 2012; Kovács et al., 2018). The positioning of the calf shed in this study meant that calves had frequent visual contact with people and farm machinery, and it is possible that the lying patterns observed here are reflective of interrupted rest due to a busy environment rather than an impact of age or housing. A variation in individual lying bout frequency and duration was observed between calves in this study. This is believed to be a normal behavioural variation as similar differences in daily lying periods have been reported in adult cattle, with animal level factors such as parity or stage of lactation and farm level factors such as bedding type or overstocking reported to influence both overall lying times and the frequency and length of lying bouts (Tucker et al., 2021).

Given the complexity of a calf's interaction with their environment (Whalin et al., 2021), a limitation of this study is that the results may not be transferable to calves of different ages reared in different conditions, as alternative environments may alter the dynamics of how calves play. Furthermore, the amount of time calves engage in play will be influenced by housing and management related factors such as space allowance, housing group size and feeding or weaning management (Jensen et al., 1998; Krachun et al., 2010). Similar to the study reported here, existing studies typically analyse behaviours of dairy calves reared in a commercial, indoor housing setting. Further work is warranted to compare play behaviour of weaned dairy calves reared under different conditions.

While this study offers a practical approach to measuring play behaviour in weaned dairy calves, the devices and methodology described are currently limited to a research-based setting. PLF technologies have been developing in the dairy industry over the past four decades (Beaver and Rutter, 2023) and now many accelerometer devices are commercially available for measuring calf health and behaviour in an on-farm setting (Costa et al., 2021). These devices may measure at different sampling frequencies or be worn by the calf on areas other than the leg (Costa et al., 2021); therefore, the data output may differ to that reported here. In addition, these devices may have the ability to capture other forms of play behaviour not measured in this study such as social play, which typically involves head and neck movement (Held and Špinka, 2011). Given that there is a known relationship between play behaviour and positive welfare states (Held and Špinka, 2011; Ahloy-Dallaire et al., 2018), validation of such devices to measure play offers potential for real-time, on-farm assessment

of calf welfare. Further research investigating the ability of commercially available calf accelerometer technology to measure play behaviour is merited.

In conclusion, this study has shown that IceTag accelerometers can reliably detect play behaviour in weaned dairy calves and contributes to the growing body of literature highlighting the value of sensor technology for measuring calf behaviour. Developing technology to detect play behaviour is particularly important, given that play is considered to be an indicator of positive welfare but has historically been difficult to reliably measure given it's short-lived and spontaneous nature. Future work validating commercially available accelerometer devices in calves under different management conditions is warranted to facilitate the extension of this technology into a commercial environment for use as a practical farm-based welfare assessment tool.

# Chapter 3: The impact of early life social housing on dairy calf play behaviour in the neonatal and post-weaning periods

#### 3.1 Abstract

We aimed to assess the impact of early life housing and play experiences on neonatal and weaned calf play behaviour. A total of 96 female dairy calves were recruited from four Scottish dairy farms and assigned to individual (n = 48), paired (n = 24) or group (n = 24)housing at birth. Play behaviour was measured using IceTag accelerometers (Peacock Technology, UK) during two experimental periods in the same cohort of calves, at neonatal and weaned stages. Statistical analysis was performed using mixed effects negative binomial regression to evaluate the impact of early life social housing on neonatal calf play behaviour, and to evaluate the impact of both early life social housing and early life playfulness on weaned calf play behaviour. Calves housed in paired (IRR = 1.29; P = 0.002) or group (IRR = 1.43; P < 0.001) pens from birth performed significantly more neonatal play compared to calves housed individually from birth. No lasting effect of early life housing on weaned calf play behaviour was observed. There was no significant correlation between counts of neonatal and weaned calf play. When categorised by level of neonatal playfulness, compared to calves characterised as the most playful, calves characterised as less playful displayed numerically more play after weaning. These findings add to the growing body of literature indicating that early life social housing provides a more positive welfare experience for preweaned dairy calves. The study also highlights the need for future research to understand the impact of rearing experiences and different management systems on play behaviour in weaned calves.

#### **3.2 Introduction**

The early life housing experience of dairy calves impacts their development, health, and welfare. Research has demonstrated the importance of early life socialisation of calves and links social housing, where calves are reared in pairs or groups, to improvements in growth, feed intake and cognitive development (Costa et al., 2016). However, despite the growing body of evidence supporting social housing, individual housing remains the most prevalent type of housing for newborn dairy calves in the United Kingdom (Mahendran et al., 2022), USA (Doyle et al., 2024) and in several European countries (Marcé et al., 2010; Staněk et al., 2014; Klein-Jöbstl et al., 2015).

Newborn dairy calves have traditionally been housed in individual pens due to the perceived benefits of controlling respiratory and enteric pathogens (Marcé et al., 2010) with health concerns commonly found to be the main limiting factor for producers adopting social housing (Mahendran et al., 2022). However, data supporting this hypothesis are conflicting, with some authors reporting an increased risk of diseases including bovine respiratory disease (BRD) and neonatal calf diarrhoea (Cobb et al., 2014; Curtis et al., 2016) in socially housed calves; whilst others report no difference (Abdelfattah et al., 2024) or even improvements in health parameters in socially housed calves (Mahendran et al., 2023). Cross-suckling, defined as one calf suckling on the body part of another, is commonly viewed by farmers as a negative behaviour associated with paired and group housing (Mahendran et al., 2022; Doyle et al., 2024). However, this behaviour is driven by hunger and a motivation to suckle (Jensen, 2003), so may be reduced in social housing if calves are fed higher milk volumes, fed via teats as opposed to open buckets, and are provided environmental enrichment such as dummy teats or scented hay (Reipurth et al., 2020; Zhang et al., 2021).

Another suggested advantage of individual housing is to allow individualised feeding regimes (Hulbert and Moisá, 2016). Some producers cite concern over monitoring of individual calf feeding habits as a drawback to social housing (Mahendran et al., 2022). The growing popularity of automated calf feeders overcome these concerns as they allow producers to create individualised feed plans and monitoring systems in group housed calves (Costa et al., 2021). Moreover, social housing facilitates learning of feeding behaviours and can lead to increased starter feed intakes compared to individually housed calves (Costa et al., 2015; Mahendran et al., 2021; Ahmadi et al., 2022). Improved feed consumption in socially housed calves can have a positive impact on growth, and multiple studies have shown increased daily liveweight gain over the pre-weaning period in calves housed in pairs or groups compared to individually housed calves (Costa et al., 2015; Jensen et al., 2015).

Social housing has been shown to positively influence the cognitive and behavioural development of dairy calves. Pair housed calves are reported to have more bold and competitive personality traits than individually housed calves (Suchon et al., 2023). Compared with calves reared individually from birth, calves raised in group and pair settings are more accepting of changes to their management including the introduction of new feedstuffs (Costa et al., 2014), new environments (Duve and Jensen, 2012) and meeting unfamiliar calves (De Paula Vieira et al., 2012). Additionally, calves reared in social settings

form bonds with their pen-mates and show a preference to interact with familiar calves over other pen-mates (Duve and Jensen, 2011; Lindner et al., 2022). Social bonds formed early in life provide emotional support that may allow calves to better cope with stressful events such as weaning or mixing of social groups (De Paula Vieira et al., 2010).

The long-term benefits of early life housing on calf development and the influence of early social experience on adult cattle is a topic of growing interest. When subjected to behavioural testing at six months old, calves reared in social settings, either with their dam or with other calves, display more social behaviours towards other calves and have a shorter latency to enter an open arena compared to individually reared calves (Jensen et al., 1999). It has been shown that the social behaviours learned early in life aid calves with the transition into the mature dairy herd, where socially reared calves are more dominant later in life, adapt better to competition in cubicle housing and are more likely to remain in the herd past the first lactation than individually reared calves (Clein et al., 2024; Mahendran et al., 2024). However, even with these improved adult social skills, studies have failed to find a link between early life socialisation and improved production parameters such as fertility or milk yield (Valníčková et al., 2020; Mahendran et al., 2024). Despite knowing the short-term welfare benefits and long-term developmental benefits of early life social housing, the lasting impact of early life housing on calf welfare, specifically the impact on play behaviour, has not been investigated.

The impact on welfare of individual calf housing can be measured using behavioural monitoring. Play behaviour is performed by calves when they feel free from stress or threats to wellbeing and is widely recognised as an indicator of positive animal welfare (Held and Špinka, 2011). The role of play in calf development is not fully understood, however it has been suggested that play may be important for building social skills and preparing calves to cope within a mature herd setting (Whalin et al., 2021). The welfare impact of different calf management systems can be assessed using play, where increased play behaviour would indicate that an environment provides a more positive welfare experience. It is widely reported that calves housed socially from birth are more active and display more play behaviour than calves housed individually throughout the whole pre-weaning period (Babu et al., 2004; Duve et al., 2012; Lv et al., 2021; Mahendran et al., 2023). Though various authors have shown the association between social housing and increased play behaviour, most studies record play using direct visual behavioural observations which can be labour intensive and is often not practical in an on-farm setting (Bateson and Martin, 2021). Play

can also be monitored using wearable accelerometer devices, and over the past decade, various devices have been validated to measure play in dairy calves of varying ages in the pre- and post-weaning periods (Rushen and de Passillé, 2012; Luu et al., 2013; Gladden et al., 2020; Größbacher et al., 2020b; McKay et al., 2024). Despite technological advances to monitor calf play behaviour, no studies to date have used direct accelerometer outputs to measure calf play behaviour in order to compare the welfare states across multiple different on-farm early life housing conditions.

Across two sampling periods, we examined the impact of early life housing on neonatal and weaned dairy calf play behaviour. In Period 1, leg mounted accelerometer technology was used to measure play behaviour in calves across individual, pair and group housing from birth. We hypothesised that calves in social housing types would play more than calves in individual pens. In Period 2, the same leg mounted accelerometer technology was used to measure play behaviour in the same cohort of calves post-weaning, to determine if early life housing and early life play experiences influence play in older calves. We hypothesised that calves of housing type, calves categorised as more playful early in life would remain more playful after weaning.

# 3.3 Materials & methods 3.3.1 Recruitment

Ethical approval for the study was obtained from the University of Glasgow School of Veterinary Medicine Research Ethics Committee (ref EA15/22).

A total of 96 Holstein, Friesian and Holstein-Friesian cross calves were recruited at birth from four commercial year-round calving dairy herds in Central and Southwest Scotland. Only female calves subject to unassisted birth were eligible for recruitment. Data were collected over a 26-month period from March 2022 to May 2024. Farms were recruited based on their current calf housing practices and a willingness to adopt an additional method of housing for a proportion of calves for the duration of the study. Herd size ranged from 170 to 850 milking cows. A priori a sample size of 24 calves per farm was selected. This sample size was chosen based on similar previous work comparing play between housing types (Babu et al., 2004; Duve et al., 2012; Lv et al., 2021) together with practical consideration. For Period 1, 12 neonatal calves per farm were assigned to housing in individual pens (Farms 1,2,3 & 4) and 12 neonatal calves per farm were assigned to housing in paired (Farms 1 & 4) or group pens (Farms 2 & 3).

#### 3.3.2 Calf management

Calf management practices varied between farms and are summarised in Table 3-1. All calves had their umbilicus dipped with an iodine solution and were fitted with an identification ear tag immediately after birth. On all farms, calves received colostrum within four to six hours of birth and were removed from the dam soon after (< 8 hours after birth). Two farms housed calves both individually and in pairs, with one farm using straw bedded calf pens and the other using straw bedded hutches. The remaining two farms housed calves both individually and in groups in straw bedded calf pens accommodating four to six calves per group. All housing types met, or exceeded, the minimum standards for housing calves according to the European Council Directive 2008/119/EC. Milk feeding was provided twice daily from a teat or open bucket. Three farms provided a commercial milk replacer product immediately after colostrum feeding, while one farm provided cow's transition milk for three days prior to feeding commercial milk replacer (Table 3-1). Calves on all farms had *ad libitum* access to water, roughage and concentrates starting from birth. Age of weaning and weaning protocols varied between farms (Table 3-1). All calves were housed in straw bedded group pens following weaning.

	Farm 1	Farm 2	Farm 3	Farm 4
Milking herd size	430	170	850	240
Calf breed	Holstein	Friesian	Holstein	Holstein Friesian
Calf birthweight (mean; range)	39kg (28-49kg)	43kg (37-51kg)	39kg (29-53kg)	50kg (38-58kg)
Colostrum management	4L via stomach tube	3L via stomach tube	4L via bottle	4L via bottle
Pre-weaning housing	Straw bedded individual or paired hutches with an outside run from birth to 6 days old	Straw bedded individual or group pens of 4-5 calves from birth to weaning	Straw bedded individual or group pens of 5-6 calves from birth to 10 days old	Straw bedded individual or and paired pens from birth to 14 days old
Pre-weaning pen dimensions	Individual: 1.5m x 1.1m x 1.2m hutch & 1.4m x 1.1m run	Individual: 1.6m x 0.95m	Individual: 1.5m x 0.92m	Individual: 1.8m x 1.2m
	Paired: 2.2m x 1.2m x 1.5m hutch & 1.6m x 1.2m run	Group: 3.1m x 2.1m	Group: 3.0m x 3.0m	Paired: 1.8m x 1.8m
Pre-weaning space allowance	Individual: 3.19m <sup>2</sup> /calf (total)	Individual: 1.52m <sup>2</sup> /calf	Individual: 1.38m <sup>2</sup> /calf	Individual: 2.16m <sup>2</sup> /calf
	Paired: 2.28m <sup>2</sup> /calf (total)	Group: 1.30-1.62m <sup>2</sup> /calf	Group: 1.50-1.80m <sup>2</sup> /calf	Paired: 1.62m <sup>2</sup> /calf
Milk feeding management	3L milk replacer twice daily via teat bucket	3L milk replacer twice daily via open bucket	3L transition milk twice daily via teat bottle until 3 days old, then 3L milk replacer twice daily via teat bucket	3L milk replacer twice daily via open bucket
Milk replacer composition	<ul> <li>24% crude protein</li> <li>(whey),</li> <li>20% crude fat,</li> <li>0% crude fibre,</li> <li>7.5% crude ash</li> </ul>	<ul> <li>24% crude protein</li> <li>(whey),</li> <li>20% crude fat,</li> <li>0% crude fibre,</li> <li>7.5% crude ash</li> </ul>	<ul> <li>23% crude protein</li> <li>(skim),</li> <li>25.5% crude fat,</li> <li>&lt;0.05% crude fibre,</li> <li>7% crude ash</li> </ul>	23% crude protein (whey), 18% crude fat, 0% crude fibre, 7.5% crude ash
Weaning management	7-day milk volume reduction to wean by 56 days old	5-day milk volume reduction to wean by 70 days old	5-day milk volume reduction to wean by 65 days old	5-day milk volume reduction to wean at 60 days old
Post-weaning housing	Igloo with straw bedded group pen of up to 10 calves	Straw bedded group pen of up to 10 calves	Straw bedded group pen of up to 30 calves	Straw bedded group pen of up to 20 calves
Post-weaning pen dimensions	3.9m x 4.4m x 2.2m igloo & 5.1m x 5.1m pen	8.4m x 4.9m	5.4 x 18.1m	14.0 x 10.5m
Post-weaning space allowance	4.32m <sup>2</sup> /calf (total)	4.12m <sup>2</sup> /calf	3.26m <sup>2</sup> /calf	7.35m <sup>2</sup> /calf

 Table 3-1: Summary of pre- and post-weaning calf housing and nutritional management

#### 3.3.3 Period 1: Measuring neonatal calf play behaviour

Following birth, calves were assigned to a housing group depending on pen availability on the farm. Within 24 hours of birth, calves were fitted with a tri-axial accelerometer (IceTag, Peacock Technology Ltd, UK) on the lateral hindlimb following the protocol described by Gladden et al. (2020). The data output from this accelerometer, specifically the metric termed "motion index" (MI), which is a measure of overall animal activity, has been validated for measuring play behaviour in neonatal dairy calves (Gladden et al., 2020). At the time of IceTag application, calves were weighed to establish birthweight using either an electronic scale or weigh band placed around the girth behind the forelimb. Additionally, calves were visually examined by a veterinarian from the Scottish Centre for Production Animal Health & Food Safety (SCPAHFS) to assess health status prior to IceTag application. IceTags were attached to measure neonatal calf play behaviour over a 48-hour recording period from 24 to 72 hours old. In paired pens, IceTags were attached to one calf only, with the other calf in the pair acting as a companion animal. In group pens, IceTags were attached to a maximum of two calves at any one time, with all other calves in the pen acting as companion animals. IceTags were removed at the end of the recording period and data were downloaded using an IceReader device and IceManager software (Peacock Technology Ltd, UK). Data were output as 1-minute sampling intervals and the presence or absence of play in each interval was recorded using a MI threshold of  $\geq$  3 (Gladden et al., 2020). Complete IceTag datasets were available for 85 out of the 96 recruited calves as 11 calves had missing data due to accelerometer malfunction.

#### 3.3.4 Period 2: Measuring weaned calf play behaviour

Play behaviour was measured in the same cohort of calves following weaning when calves were aged three to five months old (mean 139 d, SD  $\pm$  16 d). IceTags were fitted to the lateral hindlimb of each calf for a 48-hour recording period following the protocol described by McKay et al. (2024). Data collection was performed over a two-week period on each farm individually, with staggered recording periods that meant IceTags were fitted to a maximum of six calves per pen at any one time. Farm medicine records were consulted at the time of IceTag application to record any treatment or disease events, including diarrhoea, bovine respiratory disease, bloat and omphalitis, occurring between birth and the second data collection period. IceTags were removed at the end of the recording period and data were downloaded as for the neonatal calves. Data were output as 15-minute sampling intervals and the presence or absence of play in each interval was recorded using a MI threshold of  $\geq$ 

69 (McKay et al., 2024). Complete IceTag datasets were available for 74 out of the 85 calves with full neonatal datasets either due to accelerometer malfunction (n=6) or mortality (n=5).

#### 3.3.5: Statistical analysis

Data were organised and summarised in Microsoft Excel (Version 2411, Microsoft Corporation, USA) and exported for analysis to Stata 18 (Release 18, StataCorp LLC, USA). Count data for neonatal and weaned play events were examined for normality by the visual appraisal of histograms and Shapiro Wilks tests of normality. Descriptive statistics were calculated for each recording period (neonatal and weaned) to establish the percentage of IceTag output intervals in which calves were engaged in play. Boxplots were constructed to visually assess the factors influencing counts of neonatal and weaned play.

Cumulative incidence of disease was 33.8% (27.0% [20/74] pneumonia; 1.4% [1/74] diarrhoea; 2.7% [2/74] bloat; 2.7% [2/74] omphalitis). Due to a low incidence of individual diseases within the dataset, any recording of disease was dichotomised within a "health event" (yes/no) variable. To establish if calves remain more playful over time, calves were categorised into a "play quartile" based, regardless of housing type, on their count of neonatal play with Q1 calves being least playful and Q4 calves being most playful. All other data including housing type, dam parity and farm were analysed as categorical variables.

To determine if calves housed socially play more than calves housed individually in Period 1, a multilevel mixed effects negative binomial regression model was used to analyse the impact of early life housing on count of neonatal play behaviour, using neonatal housing type and dam parity as fixed effects. To determine if early life housing and early life playfulness influence play in older calves during Period 2, a second multilevel mixed effects negative binomial regression model was used to analyse the impact of early life experience on count of weaned play behaviour, using neonatal housing type, dam parity, health event and neonatal play quartile as fixed effects. For both models, farm and calf ID were included as random effects, with calf ID nested within farm. Stepwise regression using Akaike Information Criterion (AIC) was conducted to find the best model significance and fit. Posthoc pairwise comparison was used to assess differences in incidence rate ratio (IRR) for categorical variables with three or more levels. Results were considered statistically significant at  $p \leq 0.05$ .

#### 3.4 Results

#### 3.4.1 Period 1: Factors influencing neonatal calf play behaviour

Regardless of housing type, across the 48-hour recording period the mean percentage of all 1-minute IceTag intervals in which neonatal calves were found to be playing was 10% (292/2880) and ranged from 4% (110/2880) for the least playful calf, to 22% (635/2880) for the most playful calf. On average, individually housed calves played in 9% (247/2880) of intervals, pair housed calves played in 11% (322/2880) of intervals and group housed calves played in 12% (353/2880) of intervals.

Eighty-five calves were included in the final analysis of neonatal play behaviour (Table 3-2). Compared to calves housed individually, pair housed calves had a 1.29 times higher rate of play (P = 0.002) while group housed calves had a 1.43 times higher rate of play (P < 0.001). Post hoc pairwise comparison of group housed calves versus pair housed calves found no significant difference in play between the two housing types (IRR = 1.11; P = 0.334). There was no difference in neonatal play between calves born to multiparous compared to primiparous dams (IRR = 1.10; P = 0.170).

	Incidence Rate Ratio	Standard Error	Z	P >  z	95% Confidence Interval
Early life housing					
Individual	Reference				
Paired	1.29	0.11	3.04	0.002	1.10 - 1.53
Group	1.43	0.11	4.77	< 0.001	1.24 - 1.66
Dam parity					
Primiparous	Reference				
Multiparous	1.10	0.08	1.37	0.170	0.96 - 1.27

**Table 3-2:** Final multilevel mixed effects negative binomial regression model of count of neonatal calf play behaviour

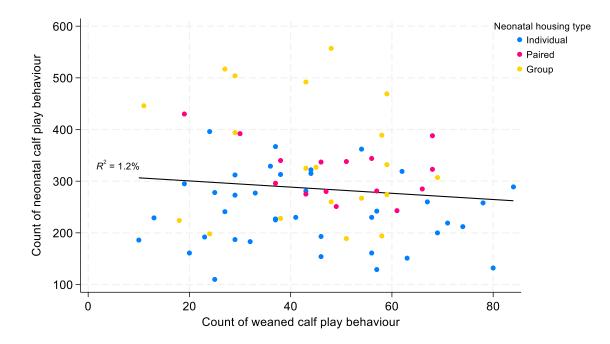
#### 3.4.2 Period 2: Factors influencing weaned calf play behaviour

Regardless of housing type, across the 48-hour recording period the mean percentage of 15minute IceTag intervals in which weaned calves were found to be playing was 23% (45/192) and ranged from 5% (10/192) for the least playful calf to 44% (84/192) for the most playful calf. On average, previously individually housed calves played in 23% (44/192) of intervals, previously pair housed calves played in 26% (49/192) of intervals and previously group housed calves played in 23% (44/192) of intervals. Seventy-four calves were included in the final analysis of weaned play behaviour (Table 3-3). No effect of early life housing on weaned calf play behaviour was detected, with no significant difference in weaned play between paired versus individually reared calves (IRR = 1.21; P = 0.381); group versus individually reared calves (IRR = 1.03; P = 0.789) or group versus pair reared calves IRR = 0.91; P = 0.599). Dam parity (IRR = 1.03; P = 0.801) had no significant impact on weaned calf play. Presence of a health event between the two experimental periods (IRR = 1.06; P = 0.534) had no significant impact on weaned calf play.

No correlation between count of neonatal play and count of weaned play was identified (Figure 3-1). There was no difference in weaned play between calves in different neonatal play quartiles, though compared to calves in the highest neonatal play quartile (Q4), all calves in the lower quartile of play displayed numerically more play when weaned (Q4 versus Q3 IRR = 1.22; P = 0.143; Q4 versus Q2 IRR =1.15; P = 0.260; Q4 versus Q1 IRR = 1.09; P = 0.555).

1 7	Incidence Rate Ratio	Standard	Z	P >  z	95% Confidence
	Rate Ratio	Error			Interval
Early life housing					
Individual	Reference				
Paired	1.12	0.15	0.88	0.381	0.87 - 1.45
Group	1.03	0.12	0.27	0.789	0.82 - 1.30
Dam parity					
Primiparous	Reference				
Multiparous	1.03	0.11	0.25	0.801	0.84 - 1.25
Health event					
No	Reference				
Yes	1.06	0.10	0.62	0.534	0.88 - 1.28
Play quartile					
Q4	Reference				
Q3	1.22	0.16	1.46	0.143	0.94 - 1.58
Q2	1.15	0.15	1.13	0.260	0.90 - 1.48
Q1	1.09	0.16	0.59	0.555	0.82 - 1.45

**Table 3-3:** Final multilevel mixed effects negative binomial regression model of count of weaned calf play behaviour



**Figure 3-1:** Scatterplot and regression line demonstrating the correlation between count of neonatal and weaned calf play behaviour by housing type

#### 3.5 Discussion

We found that calves housed in either paired or group pens from birth engaged in more neonatal play behaviour than calves housed individually. This finding agrees with other studies reporting increased play in calves housed in pairs (Duve et al., 2012) or groups (Babu et al., 2004; Lv et al., 2021) from birth. Given that play is an indicator of positive animal welfare, our results suggest that social housing from birth provides calves with a better early life experience. Interestingly, we found no difference in neonatal play between pair and group housed calves. Given the known relationship between increased space allowance and increased calf play behaviour (Jensen et al., 1998), it is possible that the lower space allowance per calf in the group pens reduced their ability to perform unrestricted play. Alternatively, it is possible these findings indicate that the presence of social companionship, regardless of group size, promotes play and improved calf welfare. Though no previous studies have compared pair and group housing, Lv et al. (2021) similarly found no difference in play between calves housed in groups of three, six or twelve. The contagious nature of play, where it becomes more likely for pen-mates to start playing after an individual play bout is started (Held and Špinka, 2011), likely contributes to an increase in this behaviour and an improvement in affective state in socially housed calves. This finding is important for producers looking to implement social housing, as housing type can be selected based on

factors such as ease of management or space availability since all social housing types, regardless of group size, increases play and improves early life welfare.

Recent work assessing calf vigour in beef suckler herds has shown that calves born from primiparous cows are less vigorous and take more time to stand following birth than calves born from multiparous cows (Brereton et al., 2024). We therefore hypothesised that calves born from primiparous dams may be less active and display less play behaviour in the neonatal recording period; however, we found no dam parity effect on neonatal play. This finding may be explained by our calf recruitment protocol where only calves born from unassisted calvings were eligible for inclusion in the study. Primiparous cows are three times more likely to experience dystocia compared to multiparous cows (Tsaousioti et al., 2024) and assisted calving has been associated with reduced play behaviour in newborn dairy calves in the first 48 hours of life (Gladden et al., 2019). Additionally, primiparous cows display reduced motivation to attend to their offspring immediately after calving which may reduce calf vigour and latency to stand (Campler et al., 2015) though in our study, calves were separated from the dam immediately after birth so poorer mothering by primiparous animals was less likely to impact calf activity.

The impact of early life housing on calf development is an area of growing interest, and multiple previous studies have found early social housing to have a positive long-term impact on calf behaviour. Studies have indicated that personality traits such as boldness or social behaviours such as exploration of novel environments and interaction with new penmates are influenced by early life housing, with individually housed calves being characterised as less bold and more reactive to social novelty (De Paula Vieira et al., 2012; Neave et al., 2020). The negative impact of early life individual housing on calf behaviour appears to carry forward and has been shown to negatively impact calves' adaptation to new environments and social groups at key management stages such as after weaning (Lyu et al., 2023), at six months old (Jensen et al., 1999) and when introduced to the adult herd prior to first calving (Clein et al., 2024). We found no lasting impact of early life housing on play behaviour in weaned calves, suggesting that play may not be a learned behaviour or personality type which is influenced by early life housing. Whilst early life play experience is believed to strengthen an animal's physical and mental capabilities to allow them to perform more play later in life (Held and Špinka, 2011), we found no difference in weaned play between calves characterised as more or less playful early in life – a contrast to our original hypothesis. If play is a behavioural response to positive welfare conditions rather

than a developed personality trait, these findings suggest that a reduced early life play experience can be reversed by providing sufficient space and social companions to facilitate play after weaning. We found that compared to the most playful neonatal calves (Q4), calves characterised as less playful in the neonatal period displayed numerically more play events in the weaned play recording period. Although no significant relationship was found, this difference could be linked to a long-term rebound effect of restricted early life play. While no studies have shown that motivation to play builds over a prolonged period, previous work has described a short-term impact of restricted play: pre-weaned calves deprived of the ability to play in individual pens will build motivation to play and will display increased play behaviour compared to socially reared calves when exposed to an environment with ample space allowance (Jensen, 1999; Bertelsen and Jensen, 2019).

A limitation of our study is the variation in weaned calf housing, particularly space allowance, across the four farms in Period 2. While the effect of space allowance on weaned calf play behaviour has not been studied, it is well understood that increased space allowance increases play in the home pen of pre-weaned dairy calves (Jensen et al., 1998; Tapki et al., 2006). Low space allowance does not prevent play behaviour but restricts the capacity of multiple calves to perform it simultaneously (Jensen et al., 2000). Given that accelerometers were used to measure play in up to six weaned calves during each recording period, it is possible that some calves on the farms with lower space allowance were unable to express play at all times. Findings regarding the impact of early life experience on weaned calf play are potentially confounded by management and may vary across different farms with different space allowances. To investigate this relationship further, future work studying a greater number of farms and husbandry systems is warranted.

When assessed using visual observations, calf play behaviour has been reported to decline with increasing age (Jensen et al., 2000; Krachun et al., 2010). Similar effects (comparing play in the neonatal and post-weaning periods) could not be assessed in this study due to the nature of accelerometer data recording which defines only the presence or absence of play within a given period but does not describe the exact count, duration and nature of play. Several authors have reported restrictions in the ability of accelerometer technology to accurately describe the nature of specific calf behaviours including play (Trénel et al., 2009; Gladden et al., 2020; Größbacher et al., 2020b; McKay et al., 2024). Recent work by Vázquez-Diosdado et al. (2024) used location data provided by ultra-wide band sensors to monitor calf play behaviour over an 18 week period. Unlike accelerometers, this technology

allowed constant and detailed reporting of play behaviour and may be the future of both research-based and on-farm sensor driven measurement of calf behaviour.

#### 3.6 Conclusion

This study provides valuable insights into the impact of early life housing and play experiences on the behaviour of dairy calves. Our findings show that calves housed in paired or group pens from birth perform more neonatal play than calves housed individually and confirms that social housing from birth should be promoted on farms in order to improve early life welfare. Contrary to our hypothesis, we found no lasting impact of early life housing or early life playfulness on post-weaning play behaviour. These findings suggest that the welfare impacts of early life housing, both positive and negative, do not carry forward into later stages of development and that negative early life experiences can potentially be compensated for with improved management conditions after weaning. Despite early play experience being believed to strengthen an animal's future capability to play, our findings suggest that play is more of a dynamic positive behavioural response influenced by current management and environmental factors. The results of our study indicate that future work is required to understand the balance between behavioural development and management factors which influence play behaviour in weaned calves.

# Chapter 4: Discussion 4.1 General discussion

Over the past four decades, the use of precision livestock farming (PLF) methods has advanced throughout the dairy industry and multiple types of technology are now available to measure the health, behaviour and welfare of farmed animals in real-time (Beaver and Rutter, 2023). At the same time, the focus of animal welfare research has moved away from measuring negative welfare states and instead is driven by the promotion and measurement of positive welfare states (Lawrence et al., 2019). Taken together, these changes within the farming industry have great potential to advance the way farmers, veterinarians and researchers approach animal welfare measurement.

Play behaviour is a widely recognised indicator of positive welfare in dairy calves (Held and Špinka, 2011; Ahloy-Dallaire et al., 2018). Despite a growing body of research validating PLF devices to measure play behaviour, no studies have implemented this technology as a tool to assess welfare across different calf management systems. The first part of the study described in this thesis demonstrates the ability of accelerometer devices to measure play behaviour in weaned dairy calves and contributes to the growing body of literature advocating the use of this technology as a practical tool for monitoring calf behaviour. The second study reported in this thesis utilises accelerometer technology to measure dairy calf play behaviour in order to assess the immediate and longer-term welfare impacts of early life housing.

## 4.2 Using accelerometers to measure calf behaviour 4.2.1 Measuring the behaviour of weaned calves

It is crucial to optimise management of replacement dairy heifers in the post-weaning period in order to efficiently and economically produce high performing adult cattle for the milking herd. Research on replacement animal management has traditionally focused on performance-based measures such as achieving growth or reproductive targets (Le Cozler et al., 2008). The role of management in allowing natural behaviour to occur and optimising weaned calf welfare is starting to receive more attention from researchers (Miller-Cushon and Van Os, 2021). Despite this promising drive towards understanding the importance of welfare in the post-weaning period, little research focusing on measuring normal behaviours of healthy, weaned calves in a typical commercial dairy context has been published. Our study presents typical lying times and lying bout patterns for weaned (three to five months old) dairy calves. While no previous studies have reported lying times for weaned dairy calves, our results are consistent with literature reporting a decrease in resting behaviour with increased age in calves (Hänninen et al., 2005). As newborns, calves spend over 80% of their daily time budget lying (Gladden et al., 2019) and this decreases to approximately 70% of their daily time budget by three to four weeks old (Wormsbecher et al., 2017; Duthie et al., 2021). We observed a small further decrease in lying times following weaning, with calves aged three to five months old found to be engaged in lying behaviours for 67% of their day (16.1 h/d). Understanding the typical behavioural patterns of calves is important, as deviations from normal lying times may be associated with stress and negative welfare effects caused by events such as disease or social regrouping (Horvath and Miller-Cushon, 2018; Goharshahi et al., 2021; Gardaloud et al., 2022). It should be noted that the behavioural patterns reported in our study are specific for weaned calves housed in a straw bedded group pen and, given the complex relationship between environment and calf behaviour (Whalin et al., 2021), may be different for calves managed in different systems such as pasture based or cubicle housing.

Researchers are increasingly implementing a range of PLF technologies when studying animal behaviour as they are able to record fine scale animal movements and are a less labour intensive, more practical alternative to traditional observational methods of behavioural recording (Brown et al., 2013). Several studies have found that accelerometer devices have the ability to measure behaviours including standing, lying and play in dairy calves before weaning (Bonk et al., 2013; Luu et al., 2013; Hill et al., 2017; Gladden et al., 2020; Größbacher et al., 2020b). Despite the growing use of these devices in monitoring the behaviour of young dairy calves, few studies have implemented accelerometer technologies to assess calves after weaning. Rushen and de Passillé (2012) demonstrated that accelerometers can be used in an experimental arena setting to measure the acceleration forces associated with running in weaned calves; however, the additional processing of raw accelerometer data in this study make it unsuitable for use in real-time behavioural assessment. The results of our work are the first to demonstrate that direct accelerometer data output can identify play behaviour in weaned dairy calves in their home environment. Our study offers a practical approach to measuring play behaviour in weaned dairy calves and has the potential to be used as a welfare assessment tool given that play behaviour is an indicator of positive animal welfare (Held and Špinka, 2011).

#### 4.2.2 Validation in other settings

Validation is a critically important process which must be carried out prior to using accelerometers as an alternative to direct behavioural observation (Brown et al., 2013). An unavoidable limitation of validation work is that it is specific to the management system studied and care should be taken when extrapolating the results into different management systems. The present study validated IceTag accelerometers to detect play behaviour in weaned dairy calves (aged three to five months old) when housed in a straw bedded group pen. The expression of play varies with housing environment and therefore the motion index (MI) threshold to determine play may also be different depending on housing conditions. For example, factors such as space allowance (Jensen et al., 1998), bedding surface (Worth et al., 2015) and maternal contact (Waiblinger et al., 2020) have all been shown to affect the count, duration and type of play exhibited by dairy calves. The MI threshold determined to identify locomotor play in our study (MI  $\ge$  69) is applicable to weaned calves aged three to five months old and may be different for calves of differing ages as the acceleration force measured by the IceTag during play is known to increase as calves age (Rushen and de Passilléde Passillé, 2012). While the MI threshold determined to identify play in the current study may not be the same for assessing play in calves of different ages or reared under different settings, the validation methods presented here can be used to validate accelerometer devices in future work in other calf management systems.

# 4.3 The future of precision livestock farming (PLF) technology in welfare assessment 4.3.1 Development of PLF technology

The study described here used IceTag accelerometer technology as they are lightweight, small devices with a high data recording frequency rate (16 Hz) so were well suited to measuring sporadic behaviours such as play in calves. Additionally, IceTags have previously been validated for measuring behaviours including leg movement and postural changes in adult cattle (Nielsen et al., 2010; Ungar et al., 2018) and lying, standing and play in pre-weaned dairy calves (Trénel et al., 2009; Gladden et al., 2020). Unfortunately, at this time the use of IceTags to measure play behaviour is limited to a research setting as these devices are not commercially available as wearable sensors for calves. Other PLF devices, some of which are commercially available, are showing promising advancements for use in welfare monitoring and should be considered as key areas for future research.

Wearable accelerometer devices for calves are available as leg mounted devices, ear tags and collars. Due to the positioning of the leg mounted IceTag used in this study, play recording was restricted to locomotor play only as this typically involves movement of the hindlimbs such as running, kicking or bucking. Ear tags and neck collars may have the potential to capture other types of play such as social play or object play which typically involve head and neck movements (Held and Špinka, 2011). Various ear tag devices are commercially available for monitoring calf behaviours including lying, rumination and running. Using changes in the pattern of behaviours measured, these devices have been validated as a tool to aid the early detection of disease including neonatal diarrhoea and bovine respiratory disease (Goharshahi et al., 2021; Gardaloud et al., 2022). Despite their growing popularity on farms and evidence for the use of other accelerometer devices to measure calf play behaviour, no study has explored the possibility of measuring different types of play behaviour with ear tag accelerometers. Further work in this area has the potential to allow widespread, on-farm assessment of positive welfare states in calves and should be the focus of future research.

Computer vision (CV) is a growing sector of PLF which uses cameras and machine learning techniques to monitor the behaviour of farmed animals in their home environment (Beaver and Rutter, 2023). It is thought that by tracking changes in animal posture, activity and behavioural patterns, CV can provide useful insights into animal health and welfare (Bhujel et al., 2025). Work using CV to monitor animal-based indicators of welfare has traditionally focused on adult cattle, where cameras are being used in the assessment of behavioural patterns, lameness, and body condition (Silva et al., 2021). There are limited studies utilising CV in calves, with most studies focusing on using this technology to monitor body weight and growth (Silva et al., 2023) and no current studies utilising this technology to measure behaviour and welfare. While limitations such as inconsistency with individual animal tracking and identification have been difficult to overcome, in the future CV may potentially offer a fully automated, non-invasive solution to calf welfare monitoring on farms.

Commercially available ultra-wideband (UWB) systems are able to provide real-time animal identification and location tracking information to provide insights into the expression of behaviours such as feeding, resting and rumination (D'Urso et al., 2023). Recent work by Vázquez-Diosdado et al. (2024) is the first study to utilise UWB location data to measure locomotor play in housed dairy calves. This form of behavioural monitoring is a promising development in the assessment of positive welfare states in calves as it allows for continuous,

long-term measurement of calf behaviour and unlike accelerometers, which output a measure analogous to one-zero sampling, provides detailed analysis of play events.

#### 4.3.2 Potential concerns with PLF technology

While PLF technology has the potential to advance practical welfare assessment over the next decade, the farming industry must also be aware that technological advances may also pose threats to the natural living and welfare of farmed animals. Regardless of their purpose, consideration of the direct and indirect threats to welfare a PLF device may pose must be made a priority before implementation. Direct welfare concerns may include increased stress and discomfort or potential injury from wearable sensors, or poor validation leading to unreliable alerts or inaccurate predictions (Tuyttens et al., 2022). Rigorous testing, training and quality checking of any technology implemented on a commercial basis is required to limit these possible issues. The potential for over-reliance on technology and reduced time spent with animals is an indirect welfare impact, though could be address by training to ensure keepers must view PLF as a management tool which can assist with health and welfare monitoring and day-to-day decision making (Tuyttens et al., 2022). The volume of data received through devices may overwhelm animal keepers and they must be appropriately trained to interpret and act upon alerts (Papakonstantinou et al., 2024). In response, the role of the veterinarian is changing with an increasing focus on data analysis and advisory skills. Veterinary schools must place more emphasis on non-technical skills such as teaching and data management to ensure the next generation of farm vets are equipped to work within this evolving farm environment (Woodward et al., 2019).

Despite the potential benefits of using technology in welfare monitoring discussed throughout this thesis, no PLF device is currently being used outside a research setting as a welfare management tool. When designing such a tool in a commercial setting, Dawkins (2021) suggested that three key questions must be answered: what definition of "welfare" is used; how does a computer recognise welfare and is the welfare of farmed animals actually improved by the application of smart farming technology? It is also important to consider that cost, hardware investments and ease of use of PLF devices may be barriers to their use on farms for welfare monitoring. While there are many potential benefits to implementing PLF technology to monitor welfare, there is still much work to be done in this area and future research must be understanding of the challenges and potential welfare threats any new technology may bring.

## 4.4 Responsibility for improving dairy calf housing and early life experience

Our study found that calves housed in paired and group pens from birth displayed a higher count of neonatal play behaviour than calves housed individually from birth. As play is an indicator of positive animal welfare (Held and Špinka, 2011) this result indicates that social housing from birth provides calves with a better early life experience compared to individual housing. This finding is consistent with the results of multiple other studies showing increased play behaviour in socially housed neonatal calves (Babu et al., 2004; Duve et al., 2012; Lv et al., 2021; Mahendran et al., 2023). Early life social housing has also been linked to the development of improved cognitive and social skills in dairy calves both early in life (Duve and Jensen, 2011, 2012; De Paula Vieira et al., 2012) and as they mature and move into the adult herd (Valníčková et al., 2020; Clein et al., 2024; Mahendran et al., 2024). Despite this growing body of evidence indicating that social housing is beneficial for calf welfare and development, individual calf housing remains the most prevalent housing type for newborn dairy calves in many countries worldwide (Marcé et al., 2010; Staněk et al., 2014; Klein-Jöbstl et al., 2015; Mahendran et al., 2022; Doyle et al., 2024). Any drive to change routine calf management processes must be a collaborative effort between various stakeholders including animal keepers, consumers and policy makers.

The general public plays a large role in influencing change within the agricultural sector. Recent surveys have indicated that consumers identify high animal welfare standards as a main priority when buying meat and dairy products (Ammann et al., 2024; Blair et al., 2024). Specifically in relation to calf housing, the general public associates pair or group housing of calves with higher welfare due to benefits including increased space allowance and increased socialisation (Perttu et al., 2020). Despite animal welfare growing as a concern for consumers there are still many barriers to purchasing higher welfare products, such as affordability, poor availability and lack of understanding labels (Cornish et al., 2019). Addressing these challenges and educating the general public about farming practices may aid in pushing the farming industry to provide higher welfare products. As the consumer becomes more educated, management standards followed by farmers and policies made by government bodies must advance to promote higher welfare in order to maintain consumer trust (Hårstad, 2024). Despite some producers understanding that social housing is beneficial for calf comfort and expression of natural behaviours (Doyle et al., 2024), many still have outdated negative perceptions of this housing type. Concern over spread of enteric or respiratory diseases and an increased incidence of cross-suckling are commonly cited by producers as barriers to introducing social housing (Marcé et al., 2010; Mahendran et al., 2022). Evidence linking pair and group housing to poor health outcomes is inconsistent and it is hypothesized that if other key management factors (e.g. pen hygiene and colostrum provision) are optimised then no increase in disease morbidity or mortality should be seen with social housing (Costa et al., 2016). Increased farmer education and dissemination of research, such as the results of our study, which promotes the benefits of early life social housing is a key driver to changing farmer's views and management policies in order to improve calf welfare.

Though farmers have control over their day to day management, ultimately the housing type they provide for calves must adhere to strict regulations set by the European Council, specifically Council Directive 2008/199/EC which lays down the minimum standards for protection of calves. This directive outlines the minimum space requirements, feeding standards and housing guidelines for calves up to six months old. The European Commission has recently published a European Food Safety Authority (EFSA) report on the welfare of calves following criticism that current legislative regulations are based on scientific opinions that are outdated (Nielsen et al., 2023). For example, despite evidence promoting early life social housing, current recommendations state that calves may be kept in individual pens until eight weeks old (provided they have visual and tactile contact with other calves). Based on the results of work similar to our study which promote the benefits of early life social housing, the EFSA report concluded that calves should be kept in pairs or small groups (2-7) in the first week of life to promote social behaviour, improved learning ability and positive affective states (Nielsen et al., 2023). The findings of this report will support the ongoing revision of the European Union's animal welfare legislation and is a promising move towards improved welfare and early life experience for calves.

## 4.5 The impact of early life experience on development of calf play behaviour

The impact of early life experiences on calf development is a topic of growing interest. Multiple studies have shown that early life social housing is associated with reduced stress at key points later in the rearing period such as social regrouping at two months old (Lyu et al., 2023) or introduction to a novel environment at six months old (Jensen et al., 1999). In contrast to these studies, weaned calf behaviour was measured with calves in a familiar environment with no changes or stressors applied during our study. It is possible that the previously socially reared calves in our study may have been better able to cope and may display more play behaviour when subject to stressors such as management changes, however this was not tested as the study was performed on commercial farms where it was not possible to make changes to calf management.

Our study found no lasting impact of early life housing or play early in life on the count of play behaviour displayed by calves in the post-weaning period. As neonates, individually reared calves displayed less play than socially reared calves, but this did not carry forward into the post-weaning period when management (i.e. group housing) was the same for all calves. Even though early play experience is believed to strengthen an animal's ability to perform play later in life (Held and Špinka, 2011), our findings suggest that a negative early play experience can be overcome. This finding is particularly important for systems such as calf finishing units or "flying herds" where neonatal calf management is not controlled by the final producer. As play behaviour is an indicator of positive welfare that is highly influenced by environmental factors such as space allowance, bedding types and feed allowance (Jensen et al., 1998; Krachun et al., 2010; Worth et al., 2015), producers can potentially reverse previous poor welfare experience and improve calf welfare states by providing enriched, comfortable environments.

#### 4.6 Study limitations

Similar to previous studies which highlight limitations in the data provided by accelerometers when measuring calf behaviour (Trénel et al., 2009; Gladden et al., 2020; Größbacher et al., 2020b), our study found the IceTag device was unable to provide details regarding the specific nature of play performed by weaned dairy calves. The IceTag provides a proxy measurement of play events within a given sampling period but cannot be used to describe the exact duration or nature of play bouts. For weaned calves, a 15-minute sampling interval was chosen based on the preset IceTag output resolutions and prior research indicating that IceTag data at this resolution could effectively predict the presence or absence of play behaviour in neonatal dairy calves (Gladden et al., 2020). However, using this output resolution restricted the ability to accurately count the number of play events as multiple play events could occur within each 15-minute interval but would not be counted separately.

Additionally, using accelerometers to measure play in this fashion limited the ability to compare changes in the play behaviour of calves between the pre- and post-weaning periods. Should a more accurate description of play be needed in future work, validation of lower output resolutions from the IceTag or alternative PLF devices that allow continuous behavioural recording are required.

The use of commercial dairy farms in this study led to some unavoidable limitations in study design. To comply with current farm management processes, calves were assigned to housing types based on pen availability instead of using a randomisation process as would be gold standard for a study of this type (Kendall, 2003). Some potentially confounding management variables existed between farms and while these effects were accounted for in the statistical analysis by including farm as a random effect, they still need to be considered when interpreting the findings of our study. Most notably, given the known relationship between space allowance and calf play behaviour (Jensen et al., 1998), the differences in space allowances may have been unable to express play at all times. Additionally, the results presented here are specific to dairy calves managed indoors as is typical in the UK (Mahendran et al., 2022). Future work across a greater number of farms and husbandry systems is recommended in order to explore the relationship between early life housing and calf play behaviour further.

#### 4.7 Conclusion

These studies demonstrate that accelerometer technology can be used to measure calf play behaviour as an indicator of welfare states across different management systems. The results add to the growing body of evidence supporting social housing for promoting more positive early life experience. Our results did not find any association between early life housing and early play experience on play behaviour post-weaning, suggesting that poorer early life experience does not carry forward as long as subsequent management promotes positive welfare. This study highlights the need for continued development of PLF technology to measure calf behaviour and demonstrates its great potential for use as a practical farm-based welfare assessment tool.

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