

Animal welfare and production in conventional and cow-calf contact dairy systems in Ireland

A photograph of two cows grazing in a lush green field. The cow on the left is reddish-brown, and the cow on the right is black and white. Both cows have blue ear tags. In the background, there is a wooden fence and a line of trees under a bright blue sky with scattered white clouds.

Sarah E. McPherson

Propositions

1. Normal is a matter of perspective, for both cows and humans.
(this thesis)
2. Letting dairy cows rear their calves is a culture shock.
(this thesis)
3. Contrary to public perception, pre-formed opinions and biases are prevalent in science.
4. Using technology does not negate the need for in-field observations.
5. A label is only useful if its definition is agreed upon by all parties.
6. Following the COVID-19 pandemic, the art of conversation was lost between the Millennial and Gen Z generations.

Propositions belonging to the thesis, entitled

Animal welfare and production in conventional and cow-calf contact dairy systems in Ireland

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Wageningen,
31 January 2025

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This research was conducted under the auspices of the Graduate School of
Wageningen Institute of Animal Science (WIAS)

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr C. Kroeze,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 31 January 2025
at 1:00 p.m. in the Omnia Auditorium.

McPherson, Sarah E.

Animal welfare and production in conventional and cow-calf contact dairy systems in Ireland, 205 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2025)

With references, with summary in English

ISBN: 978-94-6510-415-7

DOI: <https://doi.org/10.18174/679735>

Abstract

Cow-calf contact (CCC) dairy systems, where physical contact is allowed between a calf and her dam or a foster cow, have increased in popularity with consumers and researchers in recent years. However, little research has been performed on CCC systems; the majority of recent research was performed in indoor housing systems with year-round calving. The overarching aim of this thesis was to assess and compare the welfare and production of cows and calves in conventional and CCC dairy rearing systems in Ireland, which has a pasture-based, seasonal-calving dairy system. Therefore, the objectives of this thesis were to: 1) estimate associations between cow and calf welfare and production performance indicators on conventional dairy farms in Ireland; 2) establish a behaviour baseline for group-housed, pre-weaned dairy calves reared under conventional management conditions during the pre-weaning period; 3) investigate the effects of two dam-calf CCC rearing systems on cow production performance, health, and udders, compared to the conventional, no-contact system, within the context of the Irish, spring-calving, pasture-based dairy system; and, 4) measure the physiological health, performance, and behaviour of cows and calves within three dairy systems (two CCC rearing systems and one conventional system) before and after weaning to estimate whether animals within the three investigated systems responded differently. To achieve the first goal, an on-farm welfare assessment survey was performed on farms in the south of Ireland. Different welfare-related variables pertaining to cows, calves, and farmer management were collected and later associated with farm production and health indicators. The second goal was achieved by conducting an experiment where calf behaviour was recorded using video cameras and scored to generate a behaviour baseline of group-housed dairy calves, reared under normal management conditions, during the pre-weaning period. To achieve the last two goals of this thesis, a prolonged CCC experiment was conducted within the Irish pasture-based, seasonal calving dairy system, where three different calf-rearing systems were investigated (the conventional system and two CCC systems). Various measurements regarding cow and calf welfare and production were collected and investigated. Cow machine milk yield was negatively affected by CCC, both during and after the CCC period, and the process of weaning and separating bonded cow-calf pairs also negatively affected cow and calf performance.

In conclusion, this thesis reflected upon animal welfare and production within the conventional dairy system in Ireland, and tried to estimate whether welfare and production could be improved if CCC was adopted.

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| CHAPTER 1 |

General Introduction

Dairy farming in Ireland

Ireland has a temperate climate, allowing grass to grow nearly year round; grass growth peaks in the summer months (April to August) and is lowest during the winter (November to February; Hurtado-Uria et al., 2014). As a result, grass provides an economic and nutritious feed source for dairy cattle (Shalloo and Hanrahan, 2020). To capitalise on this, Irish dairy farmers utilise compact, seasonal (spring) calving, where 90% of cows calve within a 6-week window, starting in late January/early February (Shalloo and Hanrahan, 2020), so that the cows' fluctuating nutritional demands throughout the lactation match the expected grass growth (Dillon et al., 2005; Horan et al., 2005). Compact calving is achieved in Ireland through selectively timed breeding (i.e. breeding late April to June for calving in late January to March; Butler et al., 2019). However, compact, seasonal calving also results in a highly labour-intensive period for Irish dairy farmers (O'Donovan et al., 2008; Deming et al., 2018; Hogan et al., 2022), which can have negative effects on both human and animal welfare. There are many different definitions of animal welfare, each of varying complexity. Within this thesis, we define welfare as the balance between, and accumulation of, positive/pleasant and negative/unpleasant experiences over time (Webb et al., 2019; Reimert et al., 2023).

During the winter months, dairy cattle in Ireland are typically not at pasture, due to both low grass growth and inclement weather conditions. Ireland therefore, has a 'hybrid' dairy system, where cows are at pasture for the majority of the year, but are housed indoors in sheds for a short period during the winter months, coinciding with their dry period. As a result, Irish dairy cows are exposed to the welfare benefits and also the welfare risks of both indoor and outdoor housing systems (Mee and Boyle, 2020; Crossley et al., 2022a; Crossley et al., 2022b). Ireland's hybrid dairy system has also meant that welfare assessments developed for indoor housing (i.e., Welfare Quality®) or outdoor housing (i.e., New Zealand; Sapkota et al., 2020) cannot be used directly. For a complete insight into dairy cattle welfare in Irish systems both the indoor and outdoor period should be considered (see Crossley, 2022).

Irish farmers are recommended to separate calves from their dam soon (<24 h) after birth, provide high quality colostrum, and then artificially rear the calves indoors (i.e. Conneely et al., 2014). Individual pens are often used for a few days after birth, after which calves are moved into group pens (Sinnott et al., 2023). Calf rearing may differ based on whether the farmer is keeping the calf (i.e., a replacement heifer calf) or if the calf is a non-replacement calf (i.e. male dairy calf,

beef calf, or crossbred dairy-beef calf). Replacement calves are typically weaned from 6 to 12 weeks of age while non-replacement calves are sold at 2 to 4 weeks of age (Barry et al., 2020), thus are not weaned on-farm. Depending on the farm facilities, some calves may be provided with outdoor access pre-weaning (Sinnott et al., 2023); however, the vast majority of calves have pasture access post-weaning.

Public perception of farm animal welfare

The vast majority of European Union citizens are concerned about farm animal welfare (Eurobarometer, 2016); however, there is misalignment with conventional dairy farming practices and societal perceptions of good animal welfare (Weary and von Keyserlingk, 2017), specifically with regards to the concept of natural living and thus the way farm animals are housed (Beaver et al., 2020). This may, in part, be due to different stakeholders opposing 'unnatural' husbandry practices (Beaver et al., 2020); some practices may be unnatural to the public, but to a farmer, may allow them to provide a higher level of individualised care, or may be more practical or economical (von Keyserlingk et al., 2009). This gap in both knowledge and ethics between farmers and the public can sometimes be mitigated by educating both the public and farmers on why specific practices are performed; however, for some practices (i.e., lack of outdoor access and early cow-calf separation) educating the public on why the practice takes place only increases their concerns (Ventura et al., 2016; Busch et al., 2017; Hötzel et al., 2017).

Cow-calf contact rearing systems

As stated above, current conventional calf rearing practice is to separate the cow and calf soon after birth (i.e., Teagasc, 2017a). This early cow-calf separation is undertaken for many reasons, including reducing the risk of calf disease exposure, ensuring proper colostrum consumption, increasing saleable milk yield, and preventing the formation of the cow-calf bond. In recent years, many of these explanations for early cow-calf separation have come under scrutiny (i.e., von Keyserlingk and Weary, 2007; Beaver et al., 2019; Meagher et al., 2019), with many questioning the research and opinions that led to the practice in the first place. As a result, an alternative calf rearing method – cow-calf contact (CCC) – has been rising in popularity in recent years, especially within the European Union (Aytemiz Danyer et al., 2024). In their article where they set definitions of CCC systems, Sirovnik et al. (2020) define CCC rearing as "...a system allowing physical contact between a dam and her own calf, or between a foster cow and her foster calf." The length of the duration of CCC rearing can vary, thus it is not

included in the definition; it is typically stated when describing the CCC system (i.e., an 8 week period of CCC or a 12 week period of CCC). Interestingly, there is no minimum contact duration specified in the Sirovnik et al. (2020) definition of CCC systems. Although farms that separate cow and calf <24 h post-birth are not considered to be practicing CCC, it is unclear whether farms that leave cow and calf together for several days stand within this definition.

Cow-calf contact systems have become so desirable to consumers and researchers alike that a recent scientific opinion paper from the European Food Safety Authority (EFSA) on Animal Health and Welfare (2023) has recommended dairy farmers keep their cows and calves together for at least 24 h, with the long-term goal of increasing the minimum duration of contact required. Despite the urgency with which CCC systems are being recommended for farmers, there is limited research regarding the effects CCC may have on the welfare and production of cows, calves, as well as the dairy farmers within these systems.

The majority of recent international CCC research has been performed on cows and calves in indoor housing systems with year-round calving (i.e., Barth, 2020; Wenker et al., 2022a; Neave et al., 2024a). Only one preliminary study has been performed on cows within a pasture-based dairy system (Opsina-Rios et al., 2023). Pasture-based dairy systems can differ from indoor systems in a myriad of ways: they often utilised a compact, seasonal calving method, the cows' diet is primarily grass-based, and cows spent little or no time indoors (Shalloo and Hanrahan, 2020). In addition, cows typically found in pasture-based, seasonal-calving dairy systems have been bred to be smaller and lower yielding, meaning that they can obtain the majority of their energy requirements from grass (Berry et al., 2005a). Therefore, it is plausible that CCC would have a different effect on pasture-based, seasonal-calving dairy cows compared to those in year-round calving, indoor housing systems. In addition, most CCC research appears to focus more on the calf's health and performance than the cow's (i.e., Beaver et al., 2019; Meagher et al., 2019); cow-centred variables in CCC research often are solely focused on milk yield and composition (Barth, 2020; Wenker et al., 2022a; Sørby et al., 2024), somatic cell count and/or mastitis incidence (Wenker et al., 2022a), and occasionally body weight and body condition score (Metz, 1987; Johanssen et al., 2024). Although these are important measures of cow health and performance, there is a lack of studies that investigate cow health on a physiological level, especially around weaning and separation, and particularly in seasonal-calving, pasture-based systems.

One of the primary welfare issues regarding CCC systems is the separation of the cow-calf pair, which often occurs simultaneously with weaning

(Newberry and Swanson, 2008). The combination of separation and weaning of a bonded cow-calf pair can cause distress to both the cow and the calf (Johnsen et al., 2021a; Wenker et al., 2022b; Bertelsen and Jensen, 2023), and has previously been reported to cause changes in behaviour (Stěhulová et al., 2008; Enríquez et al., 2010; Johnsen et al., 2015a) and performance (Metz, 1987; Bar-Peled et al., 1995; Sinnott et al., 2024) in both cows and calves. As a result, it is likely that cow and calf health may also be affected; however, there is a lack of studies investigating the cow and calf health impacts of weaning and separation in CCC systems.

Cow-calf contact rearing systems: farmer concerns

In response to the growing public and academic interest in CCC system, and in part due to their own values, farmers have started adopting CCC rearing practices on their own farms. For example, in Norway, 2.8% of farmers practice CCC, but up to 15.3% want to or have plans to practice CCC in the future (Hansen et al., 2023). However, the method with which farmers achieve CCC systems varies (Erikson et al., 2022); each CCC system is unique to that specific farm, as each farm has different pre-existing facilities and each farmer has different values (Hansen et al., 2023). This may mean that regardless of their personal values, a farmer's pre-existing farm infrastructure may not easily adapted to a CCC system.

Despite the growing application of CCC, many dairy farmers have concerns regarding the feasibility of CCC on their farms. In a survey of New Zealand dairy farmers, Neave et al. (2022) determined the farmers' three main areas of concern regarding CCC systems as being: poorer animal welfare of both cow and calf within the system, increased labour and stress for the farmers and their staff, and the system-level changes (i.e. infrastructure) required to adopt such a system. Other farmer feasibility concerns of CCC systems include economics (Neave et al., 2022; Bertelsen and Vaarst, 2023; Hansen et al., 2023), an increased fearfulness of CCC-reared calves towards humans (Waiblinger et al., 2020; Webb et al., 2022), separation distress (Hansen et al., 2023), and concerns about facilities, space, and other practical issues (Erikson et al., 2022; Bertelsen and Vaarst, 2023; Hansen et al., 2023).

Assessing cow and calf welfare

Models of animal welfare are commonly used as a framework for animal welfare assessment schemes, mostly within the research community. Common frameworks include the Five Freedoms (FAWC, 1993, 2009), the Circles Model (Fraser, 1997), and the Five Domains Model (Mellor and Reid, 1994; Mellor et al.,

2020). As a result of varying systems and values, there are a variety of different schemes found globally within different farm animal production systems. In Europe, the most prevalent welfare assessment scheme for dairy cattle is the Welfare Quality® Assessment (2009), which was developed by 40 institutions within 13 European countries and four Latin American countries. The Welfare Quality® assessment features animal-based (i.e., cow injury scores) and environment/resource-based (i.e., amount of pen space/calf) measures in four categories: good feed, good housing, good health, and appropriate behaviour. In North America, both Canada and the United States have their own national dairy welfare assessment schemes. In Canada, proAction® (Dairy Farmers of Canada, 2024) focuses on six areas: milk quality, food safety, animal care, traceability, biosecurity, and environment (Dairy Farmers of Canada, 2024); as part of the animal care portion, animal-based measures are assessed on-farm (BCS, injuries, and lameness). In the United States, the FARM Program focuses on five areas: animal care, antibiotic stewardship, biosecurity, environmental stewardship, and workforce development (National Milk Producers Federation, 2024). Within the animal care program area, on-farm evaluators score animal-based measures on cows and calves (animal hygiene/cleanliness, body condition score, locomotion injuries to the hock and knee, and injured and docked tails; National FARM Program, 2024). Although these assessment schemes pull from multiple animal welfare frameworks, the dairy industry appears to be moving towards the adoption of the Five Domains model (Grandin, 2022).

Understandably, these dairy cattle welfare assessment schemes are primarily based on indoor housing systems (i.e., open pack, cubicle, and tie-stall housing systems), as assessing welfare in indoor systems is more convenient than in outdoor systems. As a result, specific aspects of those assessment schemes, such as nutrition and health, may be transferable to countries with outdoor dairy systems, but aspects regarding the cows' physical environment are not necessarily transferable, and may not take into account intricate details of the system on each particular farm (i.e., evidence of keeping cow and calf together for an extended period of time). In addition, of those mentioned, only the Welfare Quality® assessment measures aspects of animal behaviour. Welfare Quality® also attempts to measure the mental states through quantitative behaviour assessment, but the reliability and validity of this method is currently under debate (Andreasen et al., 2013).

Within dairy welfare research, there is the capacity for on-farm welfare assessments to include more measurements, such as behaviour. Several recent studies on cow (de Vries et al., 2015; Sapkota et al., 2020; Crossley et al., 2021)

and calf (i.e., Barry et al., 2019a) welfare on commercial farms have previously incorporated aspects of behaviour into their assessments. However, behaviour scoring during welfare assessment requires a significant portion of time, which can be costly (de Vries et al., 2013). The usefulness of behaviour measurements captured within the time constraints of a farm visit may also be limited. Behaviour tests can be performed to determine the responsiveness of cows to human approach (i.e., Welfare Quality®, 2009; Crossley et al., 2021), thus inferring their mental domain (and the state of human-animal interactions on-farm; Mellor et al., 2020), but then the behaviour test is not determining whether the cow (or calf) is able to perform its natural behaviour. For behaviour tests to capture normal behaviour, a longer duration of time is required. Due to time constraints of their on-farm visit, Barry et al. (2019a, 2020) recorded calf behaviour for 1 h and then used 5-min scan sampling to measure behaviour. Although this was sufficient for their purposes, abnormal behaviours are typically uncommon, and thus less likely to be captured with scan sampling (Wilder et al., 2021). Other positive behaviours (i.e., bouts of play) that are well accepted indicators of positive mental states (Ahloy-Dallaire et al., 2018) are also less easily captured with scan sampling.

Animal behaviour as a measure of welfare

Understanding animal behaviour is key to understanding the motivational (and affective) states of animals, which in turn enables us to make inferences about their welfare (e.g. Mench and Mason, 1997; Dawkins, 2003; Wechsler, 2007). Excluding behavioural reflexes, and behaviour linked to illness, learning, and development, motivation is the causal, proximate, mechanistic explanation of behaviour (Mason and Bateson, 2009; Bateson and Laland, 2013) and controls the probability that an animal will engage in a particular behaviour (Jensen & Toates, 1993). In other words, motivation explains the moment-to-moment decisions that animals make when faced with a choice in terms of which behaviour to perform. This means that animal behaviour is governed by internal stimuli (e.g. disruptions to homeostasis such as hunger), external stimuli (e.g. the eliciting cue of food), and, more commonly, the interaction of internal and external stimuli (Darwin, 1873; Jensen and Toates, 1993; Fraser, 2009). An animal's physical environment, as well as other internal and external factors, can thus influence their behaviour to a significant extent, which may make comparison between groups difficult. To truly understand an animal's behaviour, we require a 'normal' baseline with which to compare.

Aim and outline of this thesis

The overarching aim of this thesis is assess and compare the welfare and production of cows and calves in conventional and cow-calf contact dairy rearing systems in Ireland. Therefore, the objectives of this thesis are to:

- To estimate associations between cow and calf welfare and production performance indicators on commercial dairy farms in Ireland
- To establish a behaviour baseline for group-housed, pre-weaned dairy calves reared under conventional management conditions during the pre-weaning period
- To investigate the effects of two dam-calf CCC rearing systems on cow production performance, health, and udders, compared to the conventional, no-contact system, within the context of the Irish, spring-calving, pasture-based dairy system
- To measure the physiological health, performance, and behaviour of cows and calves within three dairy systems (two CCC rearing systems and one conventional system) before and after weaning to estimate whether animals within the three investigated systems responded differently

To examine these objectives, three experiments were conducted. Chapter 2 describes an on-farm welfare assessment survey that was performed on farms in the south of Ireland. The goal of Chapter 2 were to explore how different welfare-related variables – pertaining to cows, calves, and farmer management – were associated with farm production and health indicators. Chapter 3 describes a calf behaviour experiment, where the aim was to generate a behaviour baseline of group-housed dairy calves, reared under normal management conditions, during the pre-weaning period. Chapter 4 and Chapter 5 describe a prolonged CCC experiment conducted within the Irish pasture-based, seasonal calving dairy system. In Chapter 4, the effects CCC had on cow production, health, and udders, both during the CCC period and for the rest of the lactation on cows within the investigated systems are estimated and discussed. In Chapter 5, two time-points, one before and one after the weaning and separation period, are investigated to determine whether CCC system affected the cow or calf's response to weaning through physiological and clinical markers of health, performance, and behaviour. Finally, in Chapter 6, all the results of this thesis are brought together and discussed with respect to their scientific and practical implications, and recommendations for future research.



| CHAPTER 2 |

Associations between cow and calf welfare and production performance indicators on Irish dairy farms

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Abstract

As the public becomes more concerned about the welfare of production animals, it has become increasingly important to both assess and identify improvements for the welfare of animals on commercial farms within the current system. This study had two objectives, both revolving around cow and calf welfare on Irish commercial dairy farms. The first objective was to estimate how cow and calf welfare-related variables were associated with measures of farm performance (305-d project milk yield (MY), milk solids yield (MSY), and average somatic cell score (SCS)) and cow and calf health (calf 28-d mortality rate, calf immunoglobulin G (IgG), and cow serum amyloid A (SAA)). The second objective was to record Irish farmers' perceptions of the advantages and disadvantages of early cow-calf separation. Farm-level welfare-related variables, pertaining to both cow and calf health, environment, and nutrition, were collected from 45 pasture-based, spring calving dairy farms in the southeast of Ireland at the end of the spring calving period (March to April). A questionnaire was completed with the farmer to obtain management-related information and their perceptions of the advantages and disadvantages of early cow-calf separation. Measures of production and health were analysed using linear mixed models. Farms that provided pain relief to calves during disbudding were associated with higher 305-d projected MY (6662 vs. 6190 ± 124.8 kg; $P = 0.007$) and MSY (554 vs. 489 ± 9.6 kg; $P < 0.001$) than farms that did not. Farms that had cubicles within or above the recommended length also had higher MY (6705 vs. 6147 ± 131.5 kg; $P = 0.007$) while farms that fed single source colostrum had higher 305-d projected MSY (542 vs. 501 ± 9.8 kg; $P = 0.006$). Somatic cell score was higher on farms with obstructions at the entrance and/or exit of their parlour disrupting cow flow (4.74 vs. 4.67 ± 0.017 ; $P = 0.001$) and more than 2 calf sheds (4.68 vs. 4.73 ± 0.017 ; $P = 0.021$), implying that farm infrastructure may affect SCS. Calf 28-d mortality rates were lower on farms that provided fresh rather than stored colostrum (4.9 vs. 11.1 ± 1.43 %; $P = 0.003$), and calf IgG was higher on farms that met the minimum calf shed air space recommendation (39.2 vs. 24.9 ± 3.10 mg/mL; $P = 0.007$). Cow SAA concentration was affected by the farm visit's week of year ($P = 0.016$) and decreased throughout the weeks; as SAA peaks shortly after calving, this likely reflected the higher average days in milk of the herd at the visit. The majority of Irish farmers surveyed appeared to consider early cow-calf separation advantageous for them, as it allowed for easier management (colostrum provision, facilities/space, and labour efficiency), ensured better cow and calf health, and minimised the amount of distress felt by both cow and calf. However, a few farmers objected to early cow-

calf separation, delayed separation for ≥ 24 h post-birth, and expressed that they were open to leaving cow and calf together for longer.

Keywords: milk yield, milk solids yield, somatic cell count, early cow-calf separation; on-farm welfare assessment

Introduction

Pasture-based dairy systems predominate in temperate countries, such as Ireland and New Zealand, where grass grows almost year-round and is used as an economic and nutritious feed source for dairy cattle (Shalloo and Hanrahan, 2020). In Ireland, grass growth peaks during the spring and summer months and is lowest during winter (Hurtado-Uria et al., 2014). To capitalise on the yearly grass growth pattern, Irish dairy farmers utilise compact, seasonal (spring) calving, where 90% of cows are targeted to calve within a 6-week window in the spring (Shalloo and Hanrahan, 2020), which is achieved through selectively timed breeding (Butler et al., 2019). However, compact calving also results in a highly labour-intensive period for Irish farmers, with their work week averaging around 60 h (Hogan et al., 2022). Due to the inclement weather and low grass growth in winter (November to January), cows cannot be kept outdoors at pasture and are housed indoors, in sheds. Calves are typically housed indoors in Ireland pre-weaning, but are moved outdoors to pasture post-weaning (Sinnott et al., 2023). As a result, cows and calves are susceptible to the welfare benefits and risks of both indoor and outdoor housing systems (Mee and Boyle, 2020).

Dairy cow welfare assessment schemes are typically based on indoor housing systems, as the majority of dairy systems worldwide are primarily based indoors. As a result, a recent Irish dairy cow welfare assessment (Crossley et al., 2021) had to take components of both indoor and outdoor assessments to create an assessment specific to the Irish dairy system. Although assessing cow and calf welfare separately can be useful to answer specific questions, when considering the overall welfare of dairy animals on a farm, all age categories of animals should be assessed. Aspects of calf welfare (i.e., calf health and nutrition pre-weaning) can have long-term negative effects on future production (Moallem et al., 2010; Soberon et al., 2012). Furthermore, farmers have a finite amount of labour they are able to expend; if they spend most of their time with the cows, calf welfare may suffer as a result, and vice versa.

In addition to their usefulness for research and highlighting any on-farm issues to farmers, on-farm welfare assessment can be adapted to inform consumers about the welfare of production animals. One current area of concern

for consumers is early cow-calf separation (Ventura et al., 2016; Sweeney et al., 2022). Currently on conventional dairy farms in Ireland, cows and calves are separated within 24 h after birth; however, the European Food Safety Authority Panel (EFSA) on Animal Health and Animal Welfare (2023) has recently recommended that cows and calves should be left together for at least 24 h after calving, and has stated that longer periods of contact should be implemented in the future. Keeping cow and calf together for a prolonged period of time after birth is commonly referred to as a cow-calf contact (CCC) system (Sirovnik et al., 2020). There has been increasing interest in CCC systems from the public and research community in the past 10 years (see reviews: Johnsen et al., 2016; Beaver et al., 2019; Meagher et al., 2019; Aytemiz Danyer et al., 2024), but limited research is available (i.e., Neave et al., 2022; Hansen et al., 2023; Johanssen et al., 2023) examining farmer perceptions and opinions about CCC systems and early cow-calf separation.

This study had two objectives, both revolving around cow and calf welfare on Irish commercial dairy farms. The first objective was to estimate how welfare-related variables, relating to cows, calves, and farmer management (e.g., cow body condition score (BCS), calf shed air space, whether farmer offers pain relief to calves during disbudding) are associated with measures of farm performance (milk yield (MY), milk solids yield (MSY), and somatic cell score (SCS)) and cow and calf health (cow serum amyloid A (SAA) concentration, calf serum immunoglobulin G (IgG) concentration, 28-d calf mortality rate). The second aim was to record Irish farmers' perceptions of the advantages and disadvantages of early cow-calf separation. With the recent EFSA recommendation of delaying cow-calf separation to at least 24 h after birth to improve cow and calf welfare, we wanted to capture a snapshot of farmers' perceptions of the current conventional method.

Materials and Methods

Ethics approval and consent forms

Before beginning this study, ethical approval was granted (TAEC2022-354) by the Teagasc Animal Ethics Committee (Cork, Ireland) and the project was licensed by the Irish Health Products Regulatory Authority (AE19132/P166). All animal measurements were carried out in compliance with the European Union (Protection of Animals Used for Scientific Purposes) Regulation 2012 (S.I. 543 no. of 2012) and the European Directive 2010/63/EU. The study involved one 4-5 hour visit for each farm during the end of the spring calving period (1st March 2023 – 13th April 2023). Farmer participants signed a General Data Protection Regulation

(GDPR) form to agreeing to participate, allowing the researchers to complete the on-farm assessment (including questionnaire, animal-based measurements, and facility-based measurements) and access with farm records through the Irish Cattle Breeding Federation (ICBF) database. Through the GDPR form, farmers also gave the researchers consent to use the results of the assessment in scientific publications.

Development and structure of the on-farm assessment

This on-farm welfare assessment was designed based on two previous on-farm welfare surveys conducted in previous years: a calf welfare survey by Barry et al. (2019a) and a cow welfare survey by Crossley et al. (2021, 2022a, 2022b). By combining the surveys, we captured the welfare of cows and calves on the farm directly, while heifer welfare was assessed indirectly during the farm visit by asking several heifer-related questions in the farmer questionnaire.

The on-farm assessment was designed to coincide with either the morning or afternoon milking, so that cows could be scored and sampled safely when they left the parlour; as a result, the procedure for the farm visit varied slightly depending on the timing of the visit. A morning visit typically followed the following procedure: brief explanation of assessment and consent form signing, cow-based measurements as cows left the milking parlour, farmer-led tour of the facilities after milking, and then simultaneous farmer questionnaire and environment-based measurements (one person always performed the questionnaire with the farmer while the other person always performed the environment-based measurements). Calf-based health measurements were undertaken by the same person performing the environment-based measurements. Any measurements that required both researchers (i.e. calf blood samples, one person safely restrained the calf while the other took the blood sample) were taken either before or after the farmer questionnaire was completed. An afternoon visit differed only in the order of events; the cow health measurements were performed at the end of the visit as cows exited the parlour. The tasks each observer had remained the same on each farm to limit any variation in measurements between the observers.

Farm recruitment and selection

This on-farm welfare assessment was never intended to report on the current welfare status of Irish dairy cows and calves, so we utilised a convenience sample of Irish dairy farms. Our recruitment criteria for farms were that they were located within 2 h drive of our research facility (Teagasc Moorepark, Co. Cork, Ireland) and that they were spring-calving (calving occurred January–April),

pasture-based dairy farms. Farms were located within 2 h of our research facility for feasibility reasons and to facilitate sample collection (blood samples needed to be kept on ice and refrigerated as soon as possible). Due to the funding body and sponsors of this project, we were only able to recruit farmers that supplied milk to two specific milk processors. As a result, farmers were recruited through their Teagasc advisor, as based on their database they could invite farmers that met our criteria. This meant that farmer selection was biased towards farms with relatively good welfare already (or that thought they had good welfare standards), as they had willingly signed up to participate in a welfare survey during their busy calving season.

Farmer questionnaire

With the farmer questionnaire, we wanted to obtain information that could not be easily measured efficiently during the farm visit (e.g., details about replacement heifers), verify some of the information measured, obtain management information that could only be provided by the farmer, and gather farmer perceptions about management practices and welfare. The questionnaire was completed face-to-face with the farm manager and covered aspects of farm management, herd health and welfare. It comprised of 110 questions devised into 14 sections and took approximately one hour to complete. Not all questions from the questionnaire are reported in this paper. The sections included in the questionnaire were:

- Section 1: farm background information such as farm size, number of farm workers, and working hours.
- Section 2: cow management covering contract rearing, herd size, breeding methods, and calving season length.
- Section 3: milking management, discussing topics such as milking frequencies, parlour specifications, concentrate feeding, and the way cows arrive/leave the milking parlour.
- Section 4: cow grazing management, including information on separate grazing groups for sick/lame cows.
- Section 5: farm infrastructure, such as roadways, average time from parlour to paddock, and herding methods.
- Section 6: cow drinking water supply and quality.
- Section 7: cow dry off methods, cow housing, and hygiene.
- Section 8: cow feeding during the housing period and feed quality.

- Section 9: cow and heifer health, discussing the herd health plan, frequency of ill health on farm, dosing for parasites, tuberculosis testing, calf disbudding, and health monitoring.
- Section 10: calving, colostrum management, and transition milk.
- Section 11: calf feeding information such as feeding methods, weaning, and outdoor access.
- Section 12: calf health, hygiene, and management.
- Section 13: participants' perceptions on welfare and what they considered to be the advantages and disadvantages of early cow-calf separation.
- Section 14: participant demographics and education level.

The questionnaire may be made available, upon reasonable request, by emailing E. Kennedy (emer.kennedy@teagasc.ie).

Environment-based measurements

Collecting yard, parlour, and main roadways

A variety of measurements and observations were taken of the parlour and collecting yard including: shape (square/rectangle or round); dimensions (length, width, and height of ceiling – if indoors); whether the yard was covered (yes, no, or partially covered); presence of obvious slope to parlour (up, down, or level); presence of backing gate (yes or no) and water troughs (yes or no); presence of cow brushes (yes or no, if yes what type and how many); and the yard flooring type (smooth concrete, grooved concrete, slats, rubber, or slatted rubber; approximate percentage of each type). Measurements relating to the parlour included: parlour slipperiness at the entrance and exit (slippery, somewhat slippery, or not slippery; scored using method from Crossley et al., 2022a, adapted from de Vries et al., 2015); parlour style (side-by-side, herringbone, rotary, robotic); cow divisions in parlour (open/none, head partition, headlocks, sequential bailing, rapid exit); flooring type (smooth concrete, grooved concrete, slats, rubber, or slatted rubber; approximate percentage of each type) in parlour and at parlour exit; number of milking units; lighting level (bright, dim, dark); distance from milking row exit to wall (m); and the presence or absence of steps up/down, slopes, 90° or 180° turns at the exit, human doors, and obstructions.

To efficiently assess the roadways on each farm, only the first 50 m leading away from the milking parlour were assessed. Measurements were taken on all roadways leading from the parlour, and included: surface material, surface condition, visible slope (when looking towards parlour), and whether or not the roadway had consistent width, sharp turns ($\geq 90^\circ$), or an obvious cow track. At the

50 m mark (determined using a trundle wheel; Forge Steel Measuring Wheel, ScrewFix, Co. Cork, Ireland), measurements included: the width of the roadway, width of both verges, whether there was a drainage ditch (yes, no), and how many loose stones (≥ 0.5 cm) there were present within a set area (quadrat method; see Browne et al., 2022).

Cow sheds, pens, and cubicles

Two different types of cow sheds are commonly found on Irish farms: cubicle sheds and loose-housing sheds. Slightly different measurements were taken based on the shed type, but common measurements across both shed types included: building type (indoor converted farm building, indoor specifically-built cow shed, outdoor shelter, outdoor with no shelter, other), shed roof type (duo-pitch, mono-pitch, round-top, round-top with lean-to), shed dimensions (height, length, width, ceiling height), ventilation type (natural, mechanical), whether there was access to an outdoor area, the presence and number of cow brushes, the number and type of water sources, and the number and total length of feed face sides. In each loose-yard shed, the following measurements were also taken: bedding type (none, straw, sawdust, woodchips, lime, sand, wood shavings), bedding cleanliness (clean, partly dirty, dirty), bedding depth (sparse, thin, thick, very thick), flooring type (smooth concrete, grooved concrete, slats, rubber, slatted rubber; approximate percentage of each type), and dimensions of cow pen. In each cubicle shed, we measured and/or observed: whether there were automatic floor scrapers (and if so, what type of scraper (cable, robot) and track (recessed or above ground)), the cubicle base and bedding type, cubicle condition (very good, good, poor, or bad), the total number of cubicle rows, type of cubicle rows (head-to-head, facing wall, or open facing), and how many cubicles there were per row. The number of cubicles measured depended on how many of each design type were present; 5% of the total number of each cubicle design type were scored, but a minimum of two cubicles of each design type needed to be scored (i.e. if less than 40 cubicles of a specific design were present, two cubicles had to be measured). Cubicles were measured using a digital laser distance measure (range: 0 to 200 m; Spectra Precision QM95, Celtic Surveys Ltd., Dunshaughlin, Co. Meath, Ireland).

Calf sheds and pens

On each farm, we first determined how many calf sheds were present; every calf shed present was measured and scored. For each calf shed, we measured and/or scored: shed dimensions (height, length, width, and ceiling height), shed roof type (duo-pitch, mono-pitch, round-top, or round-top with lean-

to), ventilation type (mechanical or natural), and how many calves were located in each shed. Within each calf shed, we measured the dimensions of each calf pen and recorded the number of calves per pen. The type and average depth (categories: <10 cm, 10-20 cm, >20 cm) of bedding material for each pen was recorded, as well as the pen cleanliness score (clean, partly dirty, or dirty). Cubic air space was calculated after the farm visits, using the shed dimension measurements. A sketch of each shed's shape was made to aid in later air space calculations; a sketch of the calf shed's floor plan was also made in some cases, where calf pens were irregular shapes.

Animal-based measurements

Sample size calculations

To make the on-farm welfare assessment time-efficient, not all cows or calves could be scored. To determine the required sample size for cow and calf health measurements, we used the formula detailed in Cochran (1977) with a precision level of 15% (also used by prominent welfare assessments, Welfare Quality® (2009) and proAction® (Dairy Farmers of Canada, 2024)). For the expected prevalence of each animal-based variable, we used the prevalence data reported in Barry et al. (2019b; 2020) and Crossley et al. (2022a, 2022b) for the calf and cow measurements, respectively. For each animal-based variable, we determined the necessary sample size for a range of herd sizes (10 – 500 cows or calves). For each herd size, we selected the maximum number of animals needed from the group of measurements (i.e. if all but one cow or calf metric required seven animals, but one required 10, the minimum number of animals needed for that herd size was 10). The full list of sampling rates based on the number of lactating cows and calves present on the farm on the day of the assessment, can be found in Supplemental Table S1.

On-farm measures of cow health and welfare

Cow-based health variables scored on-farm included ocular and nasal discharge, integument injuries, tail injuries, BCS, and mobility. Cow ocular and nasal discharge was scored on a 4-point scale, from 0 = normal and 3 = copious bilateral mucopurulent discharge (Crossley et al., 2021). If animals had an ocular or nasal discharge scores of ≥ 2 they were considered in poor health. Integument damage was scored at three separate body locations: knee (front carpal joint), hock (lateral tarsal joint), and hindquarters. Each area was assessed for the presence of hair loss, lesions, scabs, and/or swelling, following the procedure outlined by Crossley et al. (2021), which was based on the scoring protocols of

Welfare Quality® (2009) and Gibbons et al. (2012). Integument injury scores of ≥ 2 were considered injuries in the analysis. Cow tails were visually assessed and, if required, manually palpated for signs of breaks or docking (both short and long), in a modified version of the scoring method described by Crossley et al. (2021). If no issues were found, tails were recorded as 'ok'; otherwise, all issues were recorded (docked, bent, or broken; if all applied, all were recorded). Although a bent tail was likely a broken tail, bent was included as a separate category initially to try to capture the severity of the break. Cow BCS was scored on a 5-point scale, from 1 = emaciated to 5 = extremely fat, with 0.25 increments (Edmonson et al., 1989). Mobility was scored as cows left the race, crush, or headlocks (method differed based on what was available on each farm). The 4-point Agriculture and Horticulture Development Board (2020) method of mobility scoring was used, where: 0 = good mobility, where the cow walks with even weight-bearing and rhythm on all four feet, a flat back, and long fluid strides; 1 = imperfect mobility, where the cow walks with uneven (rhythm or weight-bearing) or shortened steps, but the affected limb(s) are not identifiable; 2 = impaired mobility, where the cow walks with uneven weight-bearing on a limb that is immediately identifiable and/or obviously shortened strides (typically accompanied with an arched back); and 3 = severely impaired mobility, where a cow cannot walk as fast as a brisk human pace, the lame leg is easily identifiable (may be unable to put weight on leg), and their back is arched when standing or walking. A cow with a score of ≥ 2 was considered lame. Cows were let out of the race (or other method) individually; the observer positions themselves behind the cow, so that they would not impede the cow exiting the race. Each cow was scored for mobility on a clean, level, open surface (no cows were scored walking on slats). If there was not such a surface immediately at the exit of the race, or if the cow was required to turn immediately upon exiting, the observer followed the cow until they had been observed walking on a clean, flat, level surface. Depending on the farm, cows were either going to the shed or outside to pasture. All cow health scoring was performed by a single observer (intra-observer reliability: weighted kappa = health, 0.8123, 0.9231, and 0.8958, for clinical health, integument and tail injuries, BCS, and mobility, respectively).

Blood samples were obtained from a subsample of cows (Supplemental Table S1) while they were restrained in headlocks or in the crush. Blood samples were taken by coccygeal venepuncture using a 20G needle BD Vacutainer PrecisionGlide Multiple Sample Blood Collection Needle – 20G x 1" (0.9 x 25 mm)); one 10 mL serum tube (BD Vacutainer Serum tube, no additive, silicone-coated interior) was collected per animal. The area was cleaned and sanitised with

methyated spirits before sampling. One farm did not have any appropriate handling facilities, so no blood samples from cows were taken. After collection, blood samples were stored in an insulated box with icepacks while on the road, then transferred to a refrigerator (4°C) after return to Moorepark.

On-farm measures of calf health and welfare

Clinical health scoring of calves consisted of assessing calf demeanour, eyes, ear position, nose, cough, dehydration, mobility, interest in surroundings, faecal cleanliness and naval characteristics. Visual assessment was completed using a 4 point scale of 0-3 where 0 is normal appearance and 3 is very poor appearance. Health scoring was completed using the scoring system described in full by Barry et al. (2019a).

All calves that were between 1 and 7 d old on the farm at the time of the visit were blood sampled for IgG. Calf blood samples were taken by jugular venepuncture using a 20G needle (BD Vacutainer PrecisionGlide Multiple Sample Blood Collection Needle – 20G x 1" (0.9 x 25 mm)); one 10 mL serum tube (BD Vacutainer Serum tube, no additive, silicone-coated interior) was collected per animal.

Blood sample processing and analysis

Blood samples were allowed to clot for 24 h in the refrigerator, then were centrifuged at 3000 rpm for 15 min at 4°C. Cow and calf serum samples were then decanted and frozen at -20°C for further analysis.

Calf serum IgG was analysed using commercially available bovine IgG Radial Immunodiffusion Test (RID) Kits (Triple J Farms, Kent Labs, Bellingham, WA, USA), which were stored at 4°C; each kit contained a 24-well test plate and three reference sera samples. Kits were taken out of the refrigerator and allowed to come to room temperature (20-24°C) about 30 min before use. Serum was defrosted at 4°C. Before serum was analysed using the RID kits, it was prepared at a 1:2 dilution using distilled water as a diluent (following the procedure detailed in Barry et al., 2022). All samples were analysed in duplicate. Otherwise, RID kits were analysed according to the manufacturer's instructions. If a sample produced a value that was beyond the range of the reference curve, the sample was rerun at a different dilution.

Serum SAA (g/mL) was analysed using commercially available bovine ELISA kits (Life Diagnostics, Inc., West Chester, PA, USA). Before serum could be used in these kits, it had to be diluted. Both 400x and 600x dilutions were tested;

the 400x dilution was used for all analyses. Otherwise, all tests were conducted according to the manufacturer's instructions.

Calf faecal sampling

A pooled calf faecal sample was collected to test for the presence of harmful pathogens such as coronavirus, rotavirus, cryptosporidium parvum, or escherichia coli. A small amount of naturally voided faeces (obtained from the floor of the pen) from each measured calf pen was collected using faecal containers (faeces tube with blade and screw cap, 55 x44mm; Sarstedt AG & Co., Nümbrecht, Germany). Samples were transported back to the laboratory where they were frozen at -20°C for later analysis. Calf faecal samples were later thawed and then analysed using vertical flow immunochromatography test kits (Bio-X Diagnostics SA, Belgium) following all manufacturer instructions.

Additional farm records

Additional production data from all visited farms were retrieved from the ICBF database. The specific records obtained for each visited farm included all available milk recordings data (individual cow MY, milk composition, and somatic cell count, along with projected 305 d MY and MSY) and calf mortality rates. The projected 305 d MY and 305 d MSY were available for each cow at each available milk recording, as well as individual cow somatic cell count. To calculate the average farm projected 305 d MY and MSY, each cows' 305 d MY and MSY were averaged, and then all cow average 305 d MY and MSY were averaged. To calculate average farm SCS, the somatic cell score from each cow's milk recording was converted to SCS (\log_{10} of SCC) then averaged for each cow, and then the cows on each farm were averaged. Each farm's 28-d calf mortality rate for 2023 was calculated using the method described by Barry et al. (2019b): first the number of calves that died in 2023 when they were ≤ 28 d was determined using the calf birth dates and death dates (where applicable), then the mortality rate was calculated out of the total number of cows that had calved that year. The 7-d, 3 month, and 6 month mortality rates were calculated in a similar manner, just using their respective age cut-offs.

Data editing

Three farms did not have milk records for the year (2023), so were excluded from the analysis. Another three farms were not solely spring-calving (<100% of cows calved between January and May) and thus were also excluded

from the final analysis (final analysis $n = 39$). However, all farms ($n=45$) were retained in the reporting of the farmer perceptions of early cow-calf separation.

Creation of categories from welfare-related variables

Categories for the welfare-related variables were created from the measurements obtained during the on-farm visit (environment- and animal-based measurements) as well as management-related measurements obtained from the questionnaire. Some animal-based measurements were also supplemented with data collected after the on-farm visit from ICBF. The welfare-related variables were made into categories based on the results from the previous cow (Crossley et al., 2022a; Crossley et al., 2022b) and calf (Barry et al., 2019a) welfare surveys, legal requirements for cow and calf housing and management, and current recommendations for Irish dairy farm buildings and practices. The final welfare variables used in the analysis are separated into overarching categories: farm demographics (Table 1); collecting yard, parlour, and roadways (Table 2); calf management practices (Table 3); cow management practices (Table 4); calf health (Table 5); cow health (Table 6); calf housing (Table 7); and cow housing (Table 8). For some variables, there were no set recommendations (i.e., grazing season length), so categories were created using the average values (i.e., top 50% and bottom 50%). The number and percentage of farms that fell in each category are also presented in Tables 1 to 8.

Farmer perceptions of early cow-calf separation

Farmer responses to the questionnaire regarding their perceptions of the advantages and disadvantages of early cow-calf separation were written down during the on-farm visit. After the visit, the answers were coded into categories based on the farmers' common responses. The categories were binary (yes/no) based on whether the farmer provided that response. For advantages of early cow-calf separation, the categories were: easier colostrum management, better cow and/or calf health, reduces overall labour, more saleable milk, prevention of the formation of the cow-calf bond/ prevent distress, safety (less safe to separate cow and calf if left together for a while), easier management, more space efficient, more humanised calves, no advantages, and other. For the disadvantages of early cow-calf separation, the categories were: prevention of the formation of the cow-calf bond/ prevent distress, negative public perception of the practice, calf 'missing out' on being licked by dam, reduced farmer safety when separating cow and calf later, no disadvantages, and other. In addition, the number of recorded advantages and

disadvantages given by the farmer was also recorded. All farmers (n=45) were included in the collation of their perceptions of early cow-calf separation.

Statistical analysis

All data was analysed using SAS (v9.4, SAS Institute). For the analysis of associations with farm-level performance (average farm projected 305-d MY, average farm projected 305-d MSY, and average farm SCS) and health (28-d calf mortality rate, average SAA concentration, and average IgG concentration) outcome variables, linear mixed models were used with the Kenwood-Rogers method of determining denominator degrees of freedom. We were interested in the farm, rather than the individual animal, so farm nested within milk supplier was used as the random effect. Final models for the six outcome variables were determined in a two-step process. In step one, all cow and calf welfare-related variables described in Tables 1 to 8 were tested individually as fixed effects, unless confounded with the outcome measure (i.e., 6 month mortality rate was not tested in the 28-d calf mortality rate model). In step 2, all cow and calf welfare-related variables that had a p-value of <0.10 when tested individually were added back into the model, and then removed one-by-one using backwards selection (the fixed effect with the highest p-value was removed and the model rerun) until all remaining effects had a p-value of <0.10 . In the final models, variables were considered significant if $P < 0.05$. Farmer responses to the question about early cow-calf separation were not statistically analysed; instead, percentage of total farmers that gave a specific response are reported.

Results

The welfare-related variables, and their associated categories, used in the analysis are presented in Tables 1 to 8. For each variable, the mean (and range) along with the current recommendation (or legal requirement) in Ireland is presented, where applicable.

Associations between welfare-related variables and farm production

Only the welfare-related variables that were included in the final models are reported below. All other variables were not significantly associated with farm milk production. We report all variables included in the final model and their associated p-values, but only go into detail if $P < 0.05$.

Table 1: Farm information at the moment of the farm visit, including calving information, employees, and visit timing, as welfare-related variables. Variables obtained from the farmer questionnaire are denoted by * and variables obtained from ICBF records are denoted by †. All other variables were measured or scored during the on-farm visit.

Variable	unit	Mean (range)	Variable levels	Farms per level (n [%])
Visit time of day	-	-	AM	20 [51.3]
			PM	19 [48.7]
			9	3 [7.6]
			10	7 [17.9]
			11	6 [15.4]
Visit week of year (2023)	-	-	12	7 [17.9]
			13	7 [17.9]
			14	5 [12.8]
			15	3 [7.7]
			17	1 [2.6]
Number of full-time employees*	#	1.8 (1 to 3)	1	15 [38.5]
			2	15 [38.5]
			3	9 [23.1]
			<70%	2 [5.1]
Percent of herd calved at time of visit†	%	-	70-80%	9 [23.1]
			80-90%	9 [23.1]
			>90%	19 [48.7]
			1	4 [10.3]
Weeks calving at time of visit (category meanings – unknown)*			2	10 [25.6]
			3	9 [23.1]
			4	14 [35.9]
			No data	1 [2.6]
			1	1 [2.6]
Average cow lactation number†	-	3.5 (1 to 4)	2	1 [2.6]
			3	15 [38.5]
			4	22 [56.4]
Cow to stockperson ratio†	-	88.0 (29.3 to 175.0)	≤80	20 [51.3]
			>80	19 [48.7]
			0-100	7 [17.9]
Herd size*	#	150 (58 to 360)	>100 to 149	16 [41.0]
			150 to 200	8 [20.5]

Table 2: Welfare-related variables regarding the infrastructure and management of the collecting yard, parlour, and main roadways. Variables obtained from the farmer questionnaire are denoted by * and variables obtained from ICBF records are denoted by †. All other variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Condition of main roadway	-	Smooth ¹	-	smooth	31 [79.5]
				rough	6 [15.4]
				no data	2 [5.1]
Frequency main roads repaired*	years	Yearly ²	-	1 to 4	17 [43. 6]
				5 to 10	10 [25.6]
				>10	12 [30. 8]
Collecting yard space per cow	m ² /cow	≥1.4 ³	1.5 (0.64 to 3.36)	<1.2	14 [35. 9]
				>1.2	23 [59.0]
				no data	2 [5.1]
Use of backing gate present in collecting yard	-	Gentle use ³	-	yes	8 [20.5]
				no	31 [79.5]
Milking parlour – obstructions at entrance and/or exit	-	No obstructions ³	-	no	20 [51. 3]
				yes	19 [48.7]
Milking parlour – floor slipperiness	-	Non-slip floors ²	-	no	17 [43. 6]
				yes	22 [56.4]
Milking parlour – step(s) into and/or out of the parlour	-	No steps ²	-	no	28 [71.8]
				yes	11 [28.2]
Exit distance (distance from exit of milking row to wall)	m	≥2 ⁴	3.32 (1.13 to 6.31)	<2	1 [2.6]
				≥2	35 [89.7]
				no data	3 [7.7]
Average walk time to collecting yard*	min	-	-	<10	16 [41.0]
				10-20	15 [38. 5]
				>20	8 [20.5]
Whether cows walk to the parlour at their own pace*	-	At their own pace ¹	-	yes	31 [79. 5]
				sometimes	1 [2.6]
				no	5 [12.8]
				no data	2 [5.1]

¹DAFM, 2020; ²Crossley et al., 2022b; ³DAFM, 2024; ⁴Browne et al., 2022.

Table 3: Welfare-related variables regarding calf management. Variables obtained from the farmer questionnaire are denoted by * and variables obtained from ICBF records are denoted by †. All other variables were measured or scored during the on-farm visit.

Variable	Unit	Recommendation	Mean	Variable levels	Farms per level (n [%])
Number of faecal pathogens present	-	0	-	0	15 [38.5]
				≥1	22 [56.4]
				missing	2 [5.1]
Colostrum quality testing*	-	Yes ¹	-	yes	9 [23.1]
				no	30 [76.9]
Colostrum storage*	-	Fresh ²	-	fresh	32 [82.1]
				stored	7 [17.9]
Colostrum feeding method*	-	Bottle and teat or stomach tube if necessary ¹	-	bottle and teat	17 [43.6]
				stomach tube	14 [35.9]
				suckling	8 [20.5]
Colostrum feed timing*	-	≤2 ³	-	within 2h	30 [76.9]
				other	9 [23.1]
Colostrum source*	-	Single ⁴	-	single	29 [74.4]
				pooled	10 [25.6]
Colostrum volume*	L	≥3 ³	-	LR	11 [28.2]
				WR	25 [64.1]
				unknown	3 [7.7]
Timing of cow-calf separation post-birth*	h	Separate immediately ¹	-	≤2	20 [51.3]
				>2	19 [48.7]

Abbreviations: WR = within or above recommendation; LR = less than recommendation.

¹Animal Health Ireland, 2014; ²Cummins et al., 2017; ³Godden et al., 2019; ⁴King et al., 2020.

Table 4: Welfare-related variables regarding cow management. Variables obtained from the farmer questionnaire are denoted by * and variables obtained from ICBF records are denoted by †. All other variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Herd health plan*	-	Yes ¹	-	yes	33 [84.6]
				no	6 [15.4]
Offer pain relief to cows for lameness issues*	-	Yes	-	yes	30 [76.9]
				no	9 [23.1]
Whether farmer thought mastitis was an issue*	-	-	-	yes	13 [33.3]
				no	25 [64.1]
Use of selective dry cow therapy*	-	Yes ²	-	yes	27 [69.2]
				no	12 [30.8]
				varied	4 [10.3]
				quad	8 [20.54]
Cow herding method*	-	-	-	person	6 [15.4]
				jeep	11 [28.2]
				dog	5 [12.8]
				No data	5 [12.8]
				≤240	10 [25.6]
Grazing season length [†]	d	-	251 (217 to 287)	241 to 260	16 [41.0]
				>260	12 [30.8]
				No data	1 [2.6]
Days in milk: mean [†]	d	-	290 (255 to 312)	230 to 290	19 [48.7]
				290 to 312	18 [46.2]
				No data	2 [5.1]
Days in milk: minimum [†]	d	-	226 (187 to 260)	<220	15 [38.5]
				221 to 260	22 [56.4]
				No data	2 [5.1]
Days in milk: maximum [†]	d	-	336 (297 to 397)	235 to 320	11 [28.2]
				321 to 360	19 [48.7]
				>360	7 [17.9]
				No data	2 [5.1]

¹Field, 2021; ²Huey et al., 2021

Milk yield

Average projected 305-d MY was 6460 kg, with a range of 4385 to 7354 kg. The welfare-related variables that remained in the model for average farm projected 305-d MY were average cubicle length ($P = 0.007$), whether the farmer provided pain relief while disbudding calves ($P = 0.005$), and the percent of cows with elevated SAA ($P = 0.005$). Cows on farms with cubicles below current recommendation (6147 ± 131.5 kg) had lower 305-d MY than cows on farms with cubicles that met the current recommendation (6705 kg; $P = 0.007$). Farmers that provided their calves with pain relief during disbudding (6662 ± 124.8 kg) had higher 305-d MY than farmers that did not provide pain relief during disbudding to their calves (6190 kg; $P = 0.005$). Farms where $\leq 10\%$ of the cows sampled had elevated SAA (6185 ± 126.5 kg) had lower 305-d MY than farms where $>10\%$ of cows had elevated SAA (6667 kg; $P = 0.005$).

Milk solids yield

Average projected 305-d MSY was 518 kg, with a range of 359 to 635 kg. The welfare-related variables that remained in the model for average farm projected 305-d MY were colostrum source ($P = 0.006$), whether the farmer provided pain relief while disbudding calves ($P < 0.001$), and the percent of cows with elevated SAA ($P < 0.001$). Farms that fed colostrum from a single source (dam or other cow; 542 ± 9.8 kg) had higher projected 305-d MSY than farms that fed pooled colostrum (501 kg; $P = 0.006$). Farmers that provided their calves with pain relief during disbudding (554 ± 9.6 kg) had higher 305-d MSY than farmers that did not provide pain relief during disbudding to their calves (489 kg; $P < 0.001$). Farms where $\leq 10\%$ of the cows sampled had elevated SAA (495 ± 9.6 kg) had lower 305-d MSY than farms where $>10\%$ of cows had elevated SAA (548 kg; $P < 0.001$).

Somatic cell score

Average farm SCS was 4.73 with a range of 4.54 to 4.93. The welfare-related variables that remained in the model for average SCS were milking parlour obstructions ($P = 0.001$), frequency of main roadway repair ($P = 0.002$), timing of colostrum feeding ($P = 0.009$), and number of calf sheds ($P = 0.021$). Farms that had obstructions at the entrance and/or exit of their parlour had higher SCS than farms that did not (4.67 vs. 4.74 ± 0.017 ; $P = 0.001$). Farms that had repaired their main roadways within the past 4 years (4.76 ± 0.020) had higher SCS than farms that last repaired their main roadways 5 to 10 years ago (4.66; $P = 0.003$) and over 10 years ago 4.69; ($P = 0.043$); farms that repaired their roadways between 5 to 10 and over 10 years ago did not differ ($P = 0.504$). Farms that fed colostrum within

the first 2 h post-birth had higher SCS than farms that fed colostrum more than 2 h post-birth (4.74 vs. 4.67 ± 0.018 ; $P=0.009$). Farms that had one or two calf sheds had lower SCS than farms that had more than two calf sheds (4.68 vs. 4.73 ± 0.017 ; $P = 0.021$).

Associations between welfare-related variables and measures of animal health

Only the welfare-related variables that were included in the final models are reported below. All other variables were not significantly associated with farm milk production. We report all variables included in the final model and their associated p-values, but only go into detail if $P < 0.05$.

Serum amyloid A

Mean farm cow SAA concentration was $0.86 \mu\text{g/mL}$ with a range of 0.06 to $4.94 \mu\text{g/mL}$. Cow and calf welfare-related variables associated with average SAA concentration were first feed volume ($P = 0.086$) and visit week of year ($P = 0.016$). Farms visited during week 9 ($3.07 \pm 0.184 \mu\text{g/mL}$; the first week of farm visits) had higher SAA than farms visited during weeks 10 ($0.87 \mu\text{g/mL}$; $P = 0.020$), 11 ($0.74 \mu\text{g/mL}$; $P = 0.014$), 12 ($0.66 \mu\text{g/mL}$; $P=0.008$), 13 ($0.70 \mu\text{g/mL}$; $P = 0.007$), and 15 ($0.54 \mu\text{g/mL}$; $P = 0.022$), which were all similar. Farms visited on weeks 14 ($1.19 \mu\text{g/mL}$) and 17 ($1.05 \mu\text{g/mL}$) were similar to all other weeks. No farm was visited on week 16.

Calf IgG

Mean farm calf serum IgG concentration was 37.5 mg/mL with a range of 17.8 to 57.05 mg/mL . Cow and calf welfare-related variables associated with calf serum IgG concentration were calf shed air space ($P = 0.007$) and visit week of year ($P = 0.005$). Calf serum IgG was higher ($P=0.007$) on farms that met the calf shed air space recommendations ($39.2 \pm 3.10 \text{ mg/mL}$) compared to farms that did not (24.9 mg/mL). Farms that were visited on week 12 ($21.7 \pm 4.79 \text{ mg/mL}$) had lower IgG than farms in weeks 10 (39.2 mg/mL ; $P = 0.022$) and 13 (44.2 mg/mL ; $P = 0.009$); farms on weeks 10 and 13 did not differ ($P = 0.970$). All other farms were similar (29.8 mg/mL).

Table 5: Welfare-related variables regarding calf health. Variables obtained from the farmer questionnaire are denoted by * and variables obtained from ICBF records are denoted by +. All other variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Coughing – calves with a score ≥ 2	%	$\leq 3\%$	1.1 (0.0-20.0)	0	31 [79.8]
				>0	7 [17.9]
				no data	1 [2.6]
				0-3	18 [46.2]
Eye discharge – calves with a score ≥ 2	%	$\leq 3\%$	8.8 (0.0-44.8)	4-10	10 [25.6]
				>10	10 [25.6]
				no data	1 [2.3]
				0-3	16 [41.0]
Faecal cleanliness – calves with a score ≥ 2	%	$\leq 3\%$	8.1 (0.0-46.7)	4-10	12 [30.8]
				>10	10 [25.6]
				no data	1 [2.6]
				0-3	19 [48.7]
Naval swelling or tenderness – calves with a score ≥ 2	%	$\leq 3\%$	5.6 (0.0-42.9)	4-10	12 [30.8]
				>10	7 [17.9]
				no data	1 [2.6]
				0-3	13 [33.3]
Nose discharge – calves with a score ≥ 2	%	$\leq 3\%$	8.3 (0.0-35.7)	4-10	12 [30.8]
				>10	13 [33.3]
				no data	1 [2.6]
				0	26 [66.7]
Calves with failure of passive transfer	%	-	3.6 (0.0 to 33.3)	>0	6 [15.4]
				no data	7 [17.9]
				0	26 [66.7]
Calves provided pain relief during disbudding*	-	yes	-	no	22 [56.4]
				yes	17 [43.6]
Calf mortality rate: 7 days ⁺	%	$\leq 4\%$	2.4 (0.0-10.0)	0-4	35 [89.7]
				>4	4 [10.3]
				0-4	26 [66.7]
Calf mortality rate: 28 days ⁺	%	$\leq 4\%$	5.0 (0.0-29.0)	5-10	9 [23.1]
				>10	4 [10.3]
				0-4	18 [46.2]
Calf mortality rate: 3 months ⁺	%	$\leq 4\%$	7.0 (1.0-31.0)	5-10	15 [38.5]
				>10	6 [15.4]
				0-4	15 [38.5]
Calf mortality rate: 6 months ⁺	%	$\leq 4\%$	8.0 (1.0-33.0)	5-10	16 [41.0]
				>10	8 [20.5]

Table 6: Welfare-related variables regarding cow health. All variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Cows within BCS target	-	87% of cows within the recommended target grazing BCS of 2.75 to 3.25 ¹	-	yes	32 [82.1]
				no	7 [17.9]
Mobility score – cows with a score ≥ 2	%	Maximum 1-5% lame cows ²	6.2 (0.0 to 35.3)	0	16 [41.0]
				0 to 5	11 [28.2]
				>5	12 [30.8]
Eye discharge – cows with a score ≥ 2	%	3% warning threshold, 6% critical threshold ⁸	5.0 (0.0 to 99.0)	<3	21 [53.8]
				3 to 6	14 [35.9]
				>6	4 [10.3]
Nose discharge – cows with a score ≥ 2	%	5% warning threshold, 10% critical threshold ⁵	11.6 (0.0 to 35.3)	<5	6 [15.4]
				5 to 10	12 [30.8]
				>10	21 [53.8]
Cows with elevated SAA	%	->4.93 $\mu\text{g/mL}$ ⁴	9.4 (0.0 to 45.0)	≤ 10	25 [64.1]
				>10	12 [30.8]
Hock injuries – cows with a score ≥ 2	%	$\leq 2\%$	1.4 (0 to 20.7)	no data	2 [5.1]
				0	9 [23.1]
Knee (carpal joint) injuries – cows with a score ≥ 2	%	$\leq 4\%$ ²	2.4 (0.0 to 33.3)	>0	30 [76.9]
				0	29 [74.4]
				0 to 4	5 [12.8]
Hind quarter injuries – cows with a score ≥ 2	%	<14% ²	18.4 (0.0 to 99.0)	>4	5 [12.8]
				<4	10 [25.6]
				4 to 14	15 [38.5]
Tail injuries – cows without broken, bent, or docked tails	%	0% docked ⁵ <10% tail breaks ²	46.7 (7.4 to 96.4)	>14	14 [35.9]
				<50	19 [48.7]
				≥ 50	20 [51.3]

¹ Butler, 2016; ²Crossley et al., 2021; ³Welfare Quality®, 2009; ⁴Life Diagnostics, 2022; ⁵DAFM, 2014.

Table 7: Welfare-related variables regarding calf housing. All variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Air space per calf	m ³	New born calf: 7 8 week old calf: 10 ¹	-	WR	32 [82.1]
				LR	5 [12.8]
				no data	2 [5.1]
				clean	15 [38.5]
Calf pen cleanliness score	-	Clean ²	-	partly dirty	9 [23.1]
				dirty	10 [25.6]
				no data	5 [12.8]
				LR	15 [38.5]
Pen space per calf	m ² /calf	Legal requirement ³ : 1.5 Recommended ⁴ : 2	-	WR	23 [59.0]
				no data	1 [2.6]
				1-2	21 [53.8]
Number of calf sheds	#	-	2.4 (1 to 4)	>2	17 [43.6]
				no data	1 [2.6]
				<10	6 [15.4]
Average bedding depth in calf pens	cm	≥15 ³	-	10-20	19 [48.7]
				>20	11 [28.27]
				no data	3 [7.7]
Shared air space between cow and calf housing	-	No shared air space between cows and calves ²	-	no	22 [56.4]
				yes	17 [43.6]

Abbreviations: WR = within or above recommendation; LR = less than recommendation

¹Teagasc, 2017b; ²Jorgensen et al., 2017; ³DAFM, 2016; ⁴Animal Health Ireland, 2023.

Table 8: Welfare-related variables regarding cow housing. All variables were measured or scored during the on-farm visit.

Variable	unit	Recommendation	Mean (range)	Variable levels	Farms per level (n [%])
Calving pen type	-	individual ¹	-	individual	14 [35.9]
				shared	25 [64.1]
Feed space per cow	m/cow	0.6 to 0.75 ²	0.62 (0.13-1.18)	<0.6	21 [53.822]
				≥0.6	18 [46.2]
Cow brush on farm	-	-	-	yes	10 [25.6]
				no	29 [74.4]
Cubicle curb height	m	0.20 to 0.25 ³	0.22 (0.19 to 0.29)	LR	5 [12.8]
				WR	32 [82.1]
				NA	2 [5.1]
Cubicle diagonal length	m	2.15 ± 0.05 ⁴	1.98 (1.8 to 2.16)	LR	21 [53.8]
				WR	16 [41.0]
				NA	2 [5.1]
Cubicle total length	m	Wall-facing ³ : 2.3 to 2.6 Head-to-head or outward facing ³ : 2.2 to 2.5	2.21 (2.0 to 2.4)	LR	9 [23.1]
				WR	28 [71.8]
				NA	2 [5.1]
Cubicle lunge space	m	≥0.7 ⁴	0.57 (0 to 0.85)	LR	32 [82.1]
				WR	5 [12.8]
				NA	2 [5.1]
Cubicle width	m	1.15 ± 0.025 ³	1.10 (1.07 to 1.18)	LR	28 [71.8]
				WR	9 [23.1]
				NA	2 [5.1]
Cubicles per cow	-	1.1 ⁵	1.07 (0.59 to 1.63)	<1	12 [30.8]
				≥1	25 [64.12]
				NA	2 [5.12]

Abbreviations: LR = less than recommendation; WR = within or above recommendation; NA = not applicable, farms that had loose-housing rather than cubicles.

¹Governmnet of Ireland, 2023; ²DeVries, 2019; ³Clarke, 2016; ⁴Anderson, 2008; ⁵Grant and Ferraretto, 2018

Calf 28 d mortality rate

Mean 28 d calf mortality rate was 5.0%, with a range of 0 to 29.0%. Cow and calf welfare-related variables associated with 28 d calf mortality rate included: feeding fresh or stored colostrum ($P = 0.003$), cow walking time to the milking parlour ($P = 0.083$), herd lactation number ($P = 0.085$), whether the farmer considered mastitis an issue on their farm ($P = 0.072$), and whether cows fell within the correct BCS target ($P = 0.082$). Farms that only fed calves fresh colostrum ($4.9 \pm 1.43\%$) had lower 28 d mortality rates than farms that fed calves stored colostrum (11.1%; $P = 0.003$).

Perception of early cow-calf separation in Ireland

Perceived advantages of early cow-calf separation

Farmers were asked what they thought the advantages or benefits were of separating cows and calves soon after birth. The most commonly mentioned perceived advantage of early cow-calf separation was improved cow and/or calf health, which was mentioned by 49% of farmers (22/45). The second most common perceived advantage of separating cow and calf soon after birth, mentioned by 44% of farmers (20/45), was that it allowed for easier colostrum management (i.e., farmers are able to ensure that the calf received adequate, high quality colostrum within 2 h post-birth). The third most common perceived advantage, mentioned by 36% (16/45) of farmers, was the grouped together response that early cow-calf separation reduced the amount of distress experienced by the cow and/or calf or prevented the formation of the cow-calf bond. Several farmers (33%; 15/45) stated that early cow-calf separation allowed for easier management of both cow and calf (as opposed to keeping cow and calf together) and 22% of farmers (10/45) said that separating cow and calf soon after birth ensured the safety of the calf. Some farmers (13%; 6/45) considered early cow-calf separation advantageous as keeping cow and calf together after birth would require more housing, pen space, or facilities that they did not have available. Other advantages of early cow-calf separation mentioned included assumed less labour (22%; 10/45), a higher amount of saleable milk produced by cows (7%; 3/45), better calf temperament/attitude towards humans (4%; 2/45), and that they could achieve a higher standard of cleanliness of the calf's environment (9%; 4/45). Two farmers (4%) explicitly stated that there were no advantages to separating cow and calf immediately after birth; both of these farmers left cow and calf together for over 24 h. The total number of advantages provided by farmers varied, with 22% providing one advantage, 33% providing two (15/45), 27%

providing three (12/45), 2% providing four (1/45), 11% providing five (5/45), and 4% providing six (2/45).

Perceived disadvantages of early cow-calf separation

Farmers were asked what they thought were the disadvantages or drawbacks of separating cows and calves soon after birth. The most common farmer response (47%; 21/45) was that there were no disadvantages to separating cow and calf soon after birth. The next most common disadvantage of separation at birth, which was mentioned by 24% (11/45) of farmers, was the prevention of the formation of the cow-calf (or that it caused distress to the cow or calf). Two farmers said that the prevention of the formation of the cow-calf bond was both an advantage and a disadvantage of separating cow and calf soon after birth. Other disadvantages of separating cow and calf soon after birth mentioned by farmers were: that there was a negative public perception of the practice (11%; 5/45); that early separation meant it required more labour to feed colostrum manually to the calves (18%; 8/45), and that the calf 'missed out' on not being licked by the cow (9%; 4/45; the implication was that the calf was better off being cleaned off by their dam). Farmers provided fewer disadvantages to early cow-calf separation compared to advantages: 62% provided one disadvantage (28/45), 31% (14/45) provided two, and 7% (3/45) provided three.

Discussion

We had two aims with this study. The first was to estimate how both cow and calf welfare-related variables affected measures of farm performance and animal health on pasture-based, spring calving dairy farms in Ireland. In doing this, we wanted to consider dairy welfare as a whole instead of isolating each age group separately. The second aim was to record Irish dairy farmer's perceptions of the advantages and disadvantages of early cow-calf separation.

Associations between cow and calf welfare-related variables and farm productivity

On Irish farms, MY and MSY are the basis for how farmers are paid for their milk (Geary et al., 2010); therefore, economic losses due to poor infrastructure, management, and animal health may act as an additional driver towards positive on-farm welfare. In this study, we found that several welfare-related variables that were associated with MY, MSY, and SCS.

Farms where calves were provided with pain relief during disbudding were associated with higher MY and MSY. Without pain relief calf disbudding is a painful

procedure and a significant welfare concern (Stock et al., 2013; Winder et al., 2018); however, it is considered a necessary practice (unless polled cattle breeds are used) due to its benefits in reducing the risk of injury to both cows and handlers (Marquette et al., 2023). Providing local anaesthetic or systemic analgesia can reduce the pain experienced by calves during the procedure, and thus prevents disbudding from being a welfare concern (Winder et al., 2018). The increased MY and MSY on farms that provide pain relief for disbudding may suggest that calf health and management may have long-term effects on future cow performance. Previous research has shown that early life events, such as days of illness in the first 4 months of life or calving difficulty, can influence both first lactation and lifetime milk production (Heinrichs and Heinrichs, 2011). In addition, farmers that provide pain relief to calves for disbudding may be more aware of animal welfare issues, leading to higher production.

Interestingly, farms that had higher MY and MSY were associated with higher prevalence of cows with elevated SAA. This is counter-intuitive, as we expected farms with lower prevalence of cows with elevated SAA to yield more milk. Serum amyloid A is an indicator of inflammation in many species (Trela et al., 2022). Another marker of inflammation, haptoglobin, has previously been associated with lower MY (Huzzey et al., 2015). This requires further investigation.

Farms that had cubicles shorter in total length than the recommendation had significantly lower MY and MSY than farms with cubicles that fell within the recommended length (recommendation for wall-facing cubicles: 2.3 to 2.6 m; recommendation for head-to-head or passage-facing cubicles: 2.2 to 2.5 m; Clarke, 2016). Cubicles that are short in total length have been shown to reduce cow comfort; they provide less space for the cow to rest (McPherson and Vasseur 2020), may impede the normal rising and lying-down motion of the cow, and have been previously shown to reduce cow lying time (McPherson and Vasseur, 2021). Although longer tie-stalls have not previously shown a difference in cow milk production (McPherson and Vasseur, 2020), the stall lengths investigated were all within the current recommendations of their country. As other aspects of cubicle comfort (i.e., cubicle-to-cow ratio) have previously been shown to affect MY (Bach et al., 2008), it is possible that shorter than recommended cubicles may also reduce MY and MSY.

Feeding single source colostrum has been shown to result in higher passive immunity in calves (Barry et al., 2021). Successful passive transfer of immunity to calves have previously been associated with improved weight gains (Robison et al., 1988) and improved first and second lactation milk production (DeNise et al., 1989). As calves with higher weights at weaning have been shown

to have higher MY (Gelsinger et al., 2016), this may be why we observed that farms that fed single source colostrum had higher MSY.

Understanding the impact that cow and calf welfare have on elevated SCS is essential for assessing both the welfare and economic costs within dairy herds. The increase of SCS in milk is a reliable indicator of intramammary infection (Rearte et al., 2022), which presents a significant health issue. We found that average farm SCS was associated with frequency of roadway repair, parlour obstructions, timing of colostrum feeding, and the number of calf sheds on the farm. The frequency with which farm roadways were repaired is not an immediate link with SCS. Counterintuitively, the roadways that had been repaired most recently (within the last 4 years) had the highest SCS. Elevated SCS has previously been shown to be a risk factor for lameness on Irish dairy farms (O'Connor et al., 2020). As roadway condition has previously been linked with lameness prevalence (Doherty et al., 2014), we propose that farms that have recently had issues with lameness may have redone their roadways more recently as a result. Cleanliness of the parlour, roadway, and collecting yard have previously been associated with higher SCS on Irish farms (Kelly et al., 2009). Although we did not measure the cleanliness of the parlour, obstructions at the entrance and/or exit of the parlour may reduce cleanliness (by making it more difficult to clean thoroughly), thus causing an increase in SCS.

The efficacy of Ig transfer and absorption across the gut epithelium is optimal within the first 2 h post-birth; afterwards, there is a decline in the ability for the cells to absorb immunoglobulins (Godden et al., 2019). As mentioned previously, success of passive transfer of immunity pre-weaning may lead to increased milk production during the first or second lactation (Robison et al., 1988); however, this does not explain why we found that farms that fed colostrum within 2 h post-birth had higher SCS. In addition, even though these farms fed colostrum within the first 2 h post-birth, we do not know the quality of colostrum offered.

We observed that farms that had more calf sheds often had run out of room in their original calf shed and had put calves wherever they had space (personal observation). This may indicate that the farm had recently expanded, and had not yet prioritised calf housing. Cleaning multiple calf sheds can also be time consuming. To have time to deal with multiple calf sheds, farmers may be saving time by rushing through milking and subsequent cleaning of the parlour (i.e., not properly cleaning teats before milking or not thoroughly cleaning the parlour after milking); less clean milking parlours have been previously associated with higher SCS (Chassagne et al., 2005; Kelly et al., 2009).

Associations between cow and calf welfare-related variables and measures of health

Serum amyloid A is a marker of inflammation and physical stress (Trela et al., 2022), which has been shown to peak 48 h after calving in dairy cows (Alsemgeest et al., 1995). As such, we expected that cows lower in DIM would have higher SAA, meaning that farms we visited earlier in the year were likely to have higher SAA levels; this is what we observed. No other welfare-related variables were included in the final model; the volume of colostrum provided for the calf's first feed was included only as a tendency. These results indicate that SAA may not be the best measure of overall cow health for on-farm assessments; although it may be useful as a diagnostic tool on an individual cow basis.

Calf IgG was also affected by visit week of the year, but unlike SAA, the pattern of which weeks differed was not as distinctive; excluding the lowest and highest points at weeks 12 and 13, respectively, farm-level calf IgG did appear to decrease slightly over the weeks. This slight non-significant decrease over the calving season has been observed previously (Barry et al., 2019b), and may be attributed to the quality of colostrum (IgG concentration) decreasing towards the end of the calving season (Conneely et al., 2013), meaning that less IgG is available to be absorbed by the calf. However, all farms had an average calf IgG (mean = 37.5 mg/mL; range: 17.8 to 57.05 mg/mL) well over the limit of failure of passive transfer (10 mg/mL; Stilwell and Carvalho, 2011), indicating that regardless of the observed differences, the majority of calves received adequate colostrum.

Whether or not the farm met the minimum calf shed air space recommendation was also associated with calf IgG, with farms that met the calf shed air space recommendation having higher IgG than farms that did not meet the minimum air space recommendation. Calf shed air space recommendation are set to reduce the risk of respiratory infections in calves, as low amounts of air space can increase the concentration of bacteria in the air (Lago et al., 2006). Although this does not directly translate to IgG absorption, it may signal that overall, calf management and health on those farms was poorer.

Although 28 d calf mortality was associated with several cow and calf welfare-related variables in the model, most were tendencies; the only significant associated was with colostrum storage method. Farms that used stored colostrum (or transition milk) as their first feed (11.1%) had over double the 28 d calf mortality rate compared to farms that only used fresh colostrum (or transition milk; 4.9%). Although the colostrum storage method does not provide any indication of the quality of the colostrum provided to calves, farms that fed stored colostrum may be

storing it incorrectly. If stored for longer than recommended in the refrigerator (2 d at 4°C), bacteria can grow within the colostrum and IgG may degrade if stored for >2 d (Cummins et al., 2017). Since we did not inquire into the farmers' specific storage method, we cannot say for certain.

Farmer perceptions of early cow-calf separation

Overall, farmers provided many more advantages than disadvantages to early cow-calf separation. The most commonly given advantage of early cow-calf separation was that cows and/or calves were of better health when they were separated within 2 h post-birth. Although the question was slightly different, this contrasts with a previous study investigating European farmers' opinions regarding cow-calf contact (CCC) rearing systems (Eriksson et al., 2022), where cow and calf have prolonged contact after birth. Farmers that participated in the study of Eriksson et al. (2022) thought that calves in CCC systems had better general health and higher weight gains; however, this study did not include any farmers from Ireland. The farmer's perceptions here also contrast with the conclusions of recent systematic reviews on CCC (Beaver et al., 2019; Meagher et al., 2019), which concluded that calf health would not be worsened during CCC. However, more recent research has suggested that in Ireland, keeping calves and cows together for a prolonged period after birth outdoors at pasture leads to poorer calf health, due to weather and environmental conditions (Sinnott et al., 2024). The pasture-based, spring-calving dairy system likely has different restrictions and features compared to indoor systems, where much of the CCC research has taken place. Some advantages mentioned by the farmer for early cow-calf separation were not necessarily advantages, rather than current constraints of the pre-existing system: specifically, the concern about the amount of space, pens, or facilities required to keep cow and calf together for a period of time. Irish farmers often face space and facility constraints during calving season, and thus expanding their sheds to facilitate keeping cow and calf together for longer may not be possible for some farmers without financial aid.

The most common disadvantage of early cow-calf separation provided by farmers in our study was that there were no disadvantages. This may be symptomatic of system blindness, where farmers do not see problems on their own farm or with their own practices, or of compact calving. Compact calving results in a period of high labour requirements for Irish dairy farmers, with many of them working >60 h/week and checking on calving cows throughout the night (Hogan et al., 2022). The study was performed during the second half of the calving season, when farmers may have been sleep deprived and thus more pessimistic, which

may have altered their answers. However, we believe they are still important to note, as if cow and calf are to be kept together for at least 24 h post-birth (per the new EFSA recommendation; EFSA, 2023), this would represent their attitude towards the practice while performing said practice.

Some farmers mentioned that disadvantages of early cow-calf separation included that it causes distress to the cow and/or calf (or prevents the formation of the cow calf bond) and that there is a negative public perception of the practice. The combination of these two disadvantages of early cow-calf separation signals that Irish dairy farmers are not unaware of the potential welfare repercussions of the practice and that consumers are unhappy with the process. It is also interesting to note that two farmers mentioned the prevention of the cow-calf bond was both an advantage and disadvantage.

These results are taken from a convenience sample of dairy farmers, and may not be an accurate representation of the all the farmers in Ireland. However, as these are farmers that willingly signed up for and participated in a welfare assessment survey (this was how it was 'marketed' to farmers), we believe that these farmers are more welfare-conscious compared to the average farmer.

Conclusion

In this study, we found preliminary evidence that different cow and calf welfare-related variables impacted measures of farm productivity and cow and calf health. Farms that provided pain relief to calves during disbudding were associated with higher 305-d projected MY and MSY. Farms that had cubicles within or above the recommended length also had higher MY while farms that fed single source colostrum had higher 305-d projected MSY. Somatic cell score was affected by parlour obstructions, frequency of roadway repair, and number of calf sheds, potentially implying that the infrastructure on the farm may affect SCS. Farms that provided calves with fresh rather than stored colostrum had lower 28-d calf mortality rates, emphasising the importance of proper colostrum management. Farms that did not meet calf shed air space recommendations had lower calf IgG than those that did meet the recommendation. Both cow SAA concentration and calf IgG concentration were affected by the week of year during which the farm visit occurred; SAA decreased throughout the weeks while IgG did not follow a prescribed pattern. As both measures are affected by numerous individual animal factors, they may not be suitable for use as a measure of welfare. The majority of Irish farmers surveyed appeared to consider early cow-calf separation to be advantageous for them, as it allowed for easier management, ensured better cow and calf health, and minimised the amount of distress felt by both cow and calf.

However, a few farmers had some objections against early cow-calf separation, and made a point of allowing contact between cow and calf for at least 24 h.

Acknowledgements

The authors wish to thank all of the participating farmers that allowed us access to their farms, animals, and opinions. We would also like to thank and acknowledge Susan Moloney and Michaela O'Brien for assisting with some of the on-farm visits. This research is funded by Science Foundation Ireland and the Department of Agriculture, Food, and Marine on behalf of the Government of Ireland under Grant Number [16/RC/3835] – VistaMilk.

Supplemental Table S1: Sampling sizes for animal-based health measurements during the on-farm visit.

Current milking herd size	Number of cows to health score	Current milking herd size	Number of cows to blood sample	Current number of calves on the farm	Number of calves to health score
10-14	11	<20	7	10-14	11
15-19	14	20-29	9	15-19	14
20-24	16	30-39	11	20-24	16
25-29	18	40-49	12	25-29	18
30-34	20	50-59	13	30-34	20
35-39	21	60-69	13	35-39	21
40-44	22	70-100	14	40-44	22
45-49	23	101-200	15	45-49	23
50-59	25	201-500	16	50-54	24
60-64	26			55-59	25
65-69	27			60-64	26
70-79	28			65-69	27
80-89	29			70-74	28
90-99	30			75-79	28
100-109	31			80-89	29
110-124	32			90-99	30
125-144	33			100-109	31
145-164	34			110-124	32
165-194	35			125-144	33
195-229	36			145-169	34
230-274	37			170-194	35
275-324	38			195-234	36
≥325	39			235-289	37
				290-369	38
				370-496	39
				>494	40



| CHAPTER 3 |

Exploring baseline behaviour in group-housed, pre-weaned dairy calves

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Applied Animal Behaviour Science 281, 106446
<https://doi.org/10.1016/j.applanim.2024.106446>

Abstract

With increasing public concern for farm animal welfare, understanding their current welfare status is paramount. Animal welfare can be inferred from their behaviour, as behaviour represents the combination of internal and external cues. The aims of this study were to quantify a behavioural baseline for group-housed, pre-weaned dairy calves that were reared under conventional management conditions, and to determine how different internal (i.e. age) and external (i.e. temperature) factors affected this behaviour. Female dairy calves (n=47) were allocated to 1 of 3 pens based on birth date and reared under conventional Irish management conditions; after 3-4 d in individual pens, calves were moved into group pens where they had ad libitum access to water, concentrates, and forage (first barley straw, then hay). Milk replacer (6 L/d) was fed through an automatic milk feeder; calves were gradually weaned from day 42 to 84. A 24h period/week of video recording was used for behaviour scoring for 8 consecutive weeks (scan sampling at 10-min intervals). Behaviours included posture (lying or standing) and activity (17 behaviours). Calves were scored for clinical health twice weekly and only healthy calves were used in the analysis (n=39). Behaviour proportions were analysed using generalised linear mixed models. Proportion of time spent lying decreased as calves aged (week 1 vs. 9; percentage mean \pm standard deviation; 79.8 ± 4.04 vs. 72.1 ± 6.52 %; $P=0.004$), while time spent ruminating (2.0 ± 2.51 vs. 14.1 ± 8.72 %; $P<0.001$), eating bedding (0.8 ± 1.16 vs. 6.1 ± 4.66 %; $P<0.001$), eating forage (0.9 ± 1.20 vs. 1.8 ± 1.81 %; $P=0.007$), and eating concentrates (0.5 ± 1.15 vs. 2.2 ± 1.72 %; $P=0.018$) increased with age. On days when the minimum shed temperature was $<4^{\circ}\text{C}$ compared to $>6^{\circ}\text{C}$, calves spent more time lying (75.9 ± 5.27 vs. 72.3 ± 5.78 %; $P<0.001$) and less time eating concentrates (0.8 ± 1.11 vs. 1.4 ± 1.49 %; $P=0.035$), eating forage (0.8 ± 0.91 vs. 1.5 ± 1.57 %; $P=0.005$), eating bedding (2.7 ± 2.87 vs. 4.0 ± 4.78 %; $P=0.003$), and walking (1.5 ± 1.20 vs. 2.0 ± 1.40 %; $P=0.017$), independent of age. These findings provide a normal behaviour baseline for future calf behaviour studies and highlight potential areas of improvement in current, conventional calf rearing practices.

Keywords: lying time, rumen development, solid feed intake, temperature, welfare

Introduction

Consumers and the general public are increasingly concerned about the welfare of production animals (Busch et al., 2017; Hotzel et al., 2017; Sweeney et al., 2022), making understanding current welfare status of farm animals paramount. Welfare is defined here as the balance between, and accumulation of,

positive/pleasant and negative/unpleasant experiences over time (Webb et al., 2019; Reimert et al., 2023). Inferences about welfare can be made using animal behaviour, as it is key to understanding the motivational states of animals (e.g. Dawkins, 2003; Wechsler, 2007; Mason and Bateson, 2009). Animal behaviour is governed by internal (i.e. calf breed or age) and external (i.e. ambient temperature or space allowance) stimuli, as well as their interaction (Darwin, 1873; Jensen & Toates, 1993; Fraser, 2009). It therefore is important to consider, measure, and account for various internal and external factors when quantifying behaviour.

Many studies use behaviour to infer welfare states, and recently there has been a rise in studies using sensors to monitor behaviour, as they allow for long-term, continuous assessment with minimal labour input (Rutten et al., 2013; Steensels et al., 2017; Riaboff et al., 2022). Often these sensor-based systems are based on the principle of detecting deviations from 'normal' to identify periods of illness (Belaid et al., 2020; Duthie et al., 2021; Sun et al., 2021) or other low welfare states, such as social distress (Bus et al., 2021). Consequently, calf health status is also important to consider when defining 'normal ranges' of behaviour.

During the pre-weaning period, the dairy calf undergoes a substantial amount of development, both in terms of growth and development, in particular rumen development. New-born calves are considered monogastric, as they are born with a non-functional rumen and rely solely on milk for nutrient intake at the beginning of their life (Khan et al., 2016). Consumption of solid feeds (concentrates, hay, or straw) triggers the start of rumen development and rumination. Calves typically start ruminating at approximately 2-3 weeks of age (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022), but individual calf variability is high (Wang et al., 2022). High milk allowances can delay rumen development, as they discourage consumption of solid feeds (de Passillé et al., 2011; Eckert et al., 2015; Steele et al., 2016). There are a lack of studies describing normal feeding behaviours during the pre-weaning period in dairy calves under normal management conditions.

Lying behaviour is commonly used as a welfare indicator in dairy cattle (see review by Tucker et al., 2021) and calves (Webster et al., 1985; Færevik et al., 2008; Webb et al., 2017). It is known that young, pre-weaned calves will spend the majority of their day lying (Dwyer, 1960; Calvo-Lorenzo et al., 2016), and the amount of time they spend lying will decrease as they age (Hutchison et al., 1962; Vitale et al., 1986; Kerr and Wood-Gush, 1987). However, the majority of studies defining baselines of calf lying behaviour are dated and on a mixture of breeds (i.e. dairy, beef, and zebu), social groups (i.e. calves alone vs. calves with dams), and environments (i.e. indoors vs. outdoors on pasture). Additional factors, such as

temperature, may also affect lying and other behaviours (Hänninen et al., 2003; Tripon et al., 2014; Sawalhah et al., 2016).

The objective of this research was to observe calf behaviour during the pre-weaning period to establish a behaviour baseline for group-housed, pre-weaned dairy calves reared under conventional management conditions in Ireland. Specifically, we wanted to quantify the proportion of time per day (using scan sampling) calves spent performing specific behaviours related to feeding and lying, observe how the proportion of time spent performing these specific behaviours changed as calves aged, and determine whether the proportion of time calves spent performing specific behaviours was related to different internal (i.e. breed, colostrum amount and quality) and external (i.e. ambient temperature) factors. We hypothesised that as calves aged during the pre-weaning period, they would increase the proportion of time spent consuming solid feed, ruminating, and drinking water. Moreover, as they aged calves were expected to become more active.

Materials and Methods

Ethics statement

This study was conducted from 13 January to 16 April 2022 at Teagasc Moorepark Dairy Research Farm, County Cork, Ireland. Ethical approval for this study was received from the Teagasc Animal Ethics Committee (TAEC2021-319). Experiments were performed in accordance with European Union Regulations 2021 (Protection of Animals Used for Scientific Purpose; S.I. No. 543 of 2012).

Animals and experimental design

Forty-seven female dairy calves were enrolled in the experiment on a rolling basis, as they were born during the spring calving season. This experiment consisted of three pens of calves, or replicates. Calves were allocated into the pens (replicates) by (mean \pm standard deviation) date of birth, birthweight (kg), and breed (Holstein-Friesian, Jersey, or Holstein-Friesian x Jersey): replicate 1, 27 January 2022 \pm 10.0 d; 33 \pm 5.8 kg; 15 Holstein-Friesian and 2 Holstein-Friesian x Jersey; replicate 2, 30 January 2022 \pm 7.4 d; 33 \pm 5.2 kg; 12 Holstein-Friesian and 5 Holstein-Friesian x Jersey; and, replicate 3, 15 February 2022 \pm 3.2 d; 34 \pm 6 kg; 5 Holstein-Friesian, 3 Jersey, and 2 Holstein-Friesian x Jersey. Replicate 1 and 2 enrolled calves at the same time; replicate 3 enrolled calves after 1 and 2 had been filled to minimise the difference in ages between the youngest and oldest calf in each pen. Calves in replicate 3 were approximately 3 weeks younger than calves in replicates 1 and 2, thus replicates were running simultaneously for the majority of

the experiment. In the third replicate, one calf died of diarrhoea around 3 weeks old, thus no behaviour observations were used from that calf. There were two additional calves housed in the third replicate pen that were not used in this experiment (to maintain pen stocking density).

Animal management and housing

Birth and colostrum management

All calves were managed as per the conventional calf rearing system in Ireland (i.e. Barry et al., 2020), and all final treatment and management decisions were made by the farm manager. Calves were separated from their dam within 1 h post-birth, were weighed, ear-tagged, and had an iodine solution sprayed on their naval, and then were placed in an individual calf pen. Calves were fed a standardised amount (3 L) of high quality colostrum (>22% using a Brix refractometer; Biemann et al., 2010) within 2 h post-birth. Following colostrum feeding, calves received five feeds of transition milk (2.5 L fed twice/d) by a bucket fitted with a teat (Conneely et al., 2014). After the feedings of transition milk, calves were fed two feeds per day of 2.5 L of milk replacer (125 g/L; Heiferlac, Volac, Hertfordshire, United Kingdom; 26% crude protein) in the individual pens using a bucket with a teat attached until they were moved into the group pens, which occurred twice weekly. When in the individual pens calves were fed milk replacer twice daily at 08:00 h and 15:00 h.

Group housing and nutrition

The shed containing the calf pens was a converted shed with natural ventilation, and also contained the dry cows close to calving and individual calving pens, meaning calves shared airspace with mature animals. Ventilation within the shed could be manually altered by opening doors on opposing ends of the shed. Due to internal walls, calves could only see other calves, but could hear mature cows. Due to the calving pens, the lights were on in the shed 24 h/d, but the amount of light calves received differed day to night, due to sunlight. The calf pens had canopies at the back of the lying area (visible in Figure 1A and 1B) that could be manually lowered during cold temperatures, and also were each equipped with three heat lamps that automatically turned on when the ambient shed temperature near the sensor was $<4^{\circ}\text{C}$.

The calves were housed in group pens (Figure 1C), consisting of a grooved concrete standing area (12.6 m²) and a straw-bedded lying area (23.6 m²) separated by a wooden divider, giving a total space of 36.1 m² per pen. Replicates 1 and 2 (Figure 1A) housed 17 calves while replicate 3 housed 12 calves (10 on

the experiment plus two extras), giving space allowances of 2.13 m²/calf and 3.01 m²/calf, respectively. Once in the group pens, calves were fed milk replacer by an automatic milk feeder (Förster-Technik) at a rate of 125 g/L. Milk replacer allowance depended on each calf's number of days in the pen and corresponded to age. The plan was as follows: from d 1 to 7, calves were increased from 5 L/d to 6 L/d; from d 7 to 42, calves remained at 6 L/d; from d 42 to 56, calves were reduced from 6 L/d to 4 L/d; from d 56 to 89 calves were reduced from 4 L/d to 1 L/d. Daily milk allowance was split over four equal periods throughout the day (i.e. a quarter of their total allowance/d was offered every 6 h). Calves were fully weaned at 84 d of age (12 weeks) by removal from their group pen.

Upon entering the group pen, calves were offered *ad libitum* access to concentrates (first four weeks: Prime Elite Krispi Kaf, DairyGold Agri Business Limited, Mitchelstown, Co. Cork, Ireland, 18% protein; five weeks old until weaning: Prime Elite Kaf Gro, DairyGold Agri Business Limited, Mitchelstown, Co. Cork, Ireland, 16% protein), water through a water bowl installed in each pen, and forage available from a rack installed on the wall over the lying area (Figure 1C). The forage provided to calves differed: during the first four weeks of the study, barley straw was provided in the forage feeder and after this, hay was provided.

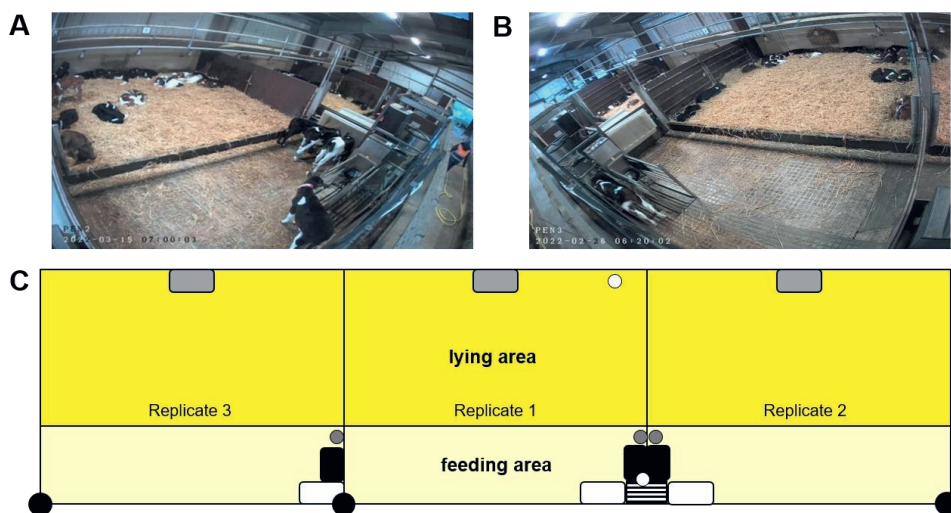


Figure 1: Diagram showing the camera views for replicates 1 (A) and 2 (B) and the set-up of the three replicates' pens (C). In the diagram (A), the black circles represent the camera locations in each pen, the grey circles represent the water bowls, the white circles represent the two temperature and humidity logger positions (hanging 1.5 m off the ground, out of calf reach), the white rectangle represents the automatic milk feeder, the black rectangle represents the concentrate trough feeder, and the grey rectangle represents the forage (barley straw or hay) feeder.

Health checks and treatments

All medical treatments (i.e. an injection of an antibiotics or an NSAID) were provided at the discretion of the farm manager and were recorded. All calves were checked twice a day by the farm manager. On five occasions (5 calves, 1 occasion per calf), calves were temporarily removed from the group pens and placed into an individual or group sick pen. Calves were returned to their original group pen once deemed recovered by the farm manager (i.e. able to drink independently from the automatic milk feeder and no longer requiring a higher level of attention). All instances of movements in and out of sick pens were noted by the researchers.

Dehorning and vaccinations

Following normal farm management procedure, calves were dehorned in three batches (midday on a Tuesday: 8 February 2022, 22 February 2022, and 29 March 2022), at an average age of 17 ± 10.7 d (7 to 13 d = 16 calves; 14 to 20 d = 22 calves; 21 to 27 d = 4 calves; 35 to 41 d = 2 calves). All calves were provided with a local nerve block (2 mL lidocaine/side), applied at least 10 minutes before dehorning commenced. Calves were restrained in a dehorning crate for the procedure, during which they were also vaccinated for Clostridium (Blackleg, Braxy, Black Disease, and Tetanus; Tribovax, MSD, Ireland; subcutaneous injection) and coccidiosis (Bovicox; oral suspension). One of the purebred Jersey calves was polled, and thus was vaccinated but not dehorned. Calves were carefully monitored by the farm manager in the days following this procedure.

Shed measurements

Temperature and relative humidity were recorded every 10 minutes using data loggers (Tinytag TGP 4017 Temperature Data Logger; Gemini Data Loggers, West Sussex, United Kingdom). Only two loggers were available, thus were both placed in the middle of the three adjacent pens (replicate 1; Figure 1). Two loggers were placed in one calf pen (replicate 1 pen; Figure 1) to get an average temperature across the pen. One logger was positioned under the canopy, overtop of the calves' lying area, but high enough so the calves could not reach them (1.5 m from bedding surface). The other logger was positioned in the feeding area of the pen, also out of calf reach (1.5 m from ground). The first logger's position under the canopy allowed it to capture the temperature under the canopy when the heating lamps were turned on.

Video recording and calf identification

Each pen of calves was continuously recorded by a video camera (8MP-4K Varifocal Dome CCTV Camera with 40 m night vision, Equicom Limited, Cobh, Co. Cork, Ireland) connected to a digital video recorder (PRIMA XR5 8MP 4K, Equicom Limited, Cobh, Co. Cork, Ireland). The camera in each pen was positioned approximately 2.7 m above the ground, so that the majority of the pen was within view of the camera (Figure 1A and 1B); only the entrance gate of the pen, directly under the camera, was not visible. For calf identification purposes, pictures were taken of all calves from several different angles (front, sides, back, and above). The majority of calves within each pen were wearing collars in three different colours (red, blue, or yellow) which also helped to identify the calves.

Calf health measurements

All calves were clinically health scored twice a week for the duration of the experiment, using a modified health scoring system by Barry et al. (2019a). Ten aspects of calf health (demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, faecal hygiene, and naval score) were scored on a 4-point scale, from 0 to 3, except interest in surroundings (2-point scale). A score of 0 indicated there were no issues while a score of 3 indicated the calf is severely affected (i.e., for nasal discharge, a score of 0 = no discharge, eyes are bright and pronounced, while a score of 3 = dull and sunken eyes with excessive non-clear discharge present in both eyes). Health scoring was performed by three individuals throughout the pre-weaning period (inter-observer reliability; weighted kappa = 0.939). A composite clinical health score was calculated for each health-scoring event by summing all individual scores. Calf body weight (kg) was recorded weekly from birth using a weighing scale (TrueTest XR 3000, Tru-test Limited, Auckland, New Zealand). Average daily gain (ADG) for each week was calculated by subtracting the previous week's weight from the current week's weight, then dividing by the number of days between the weight measurements.

Behaviour scoring

Ethogram

Calf behaviour was scored using scan sampling from the video recordings, using the ethogram in Table 1. Behaviours were separated into two categories that were scored at each time-point: posture and activity. Postural behaviours included standing and lying; the calf was always performing one or the other. Activity

behaviours included anything else the calf might be doing; if the calf was idle or sleeping, nothing was noted for that time-point.

Training and validation

Five independent observers performed the behaviour scoring for this experiment. Of the five observers, only one had had previous behaviour scoring experience. Before commencing scoring, all observers were trained and then completed a two-part validation process. To train all the observers, they were first given the ethogram (Table 1), practice videos (not videos from the scoring days), and calf identification materials (see section 2.5). Once they felt confident in their scoring ability, they completed the first round of validation (validation 1). The validation video was one hour long, and was scored using 5-minute scan samples (13 observations total). Each calf in the pen ($n = 17$) was scored. This validation was completed within 1 day and independently; the observers were not allowed to ask questions or compare answers. The results for each observer were then compared for validation 1, and a percent of agreement was calculated for posture (98.55% agreement) and activity (80.00% agreement). After validation 1, any major scoring issues (i.e. clarifying of behaviours, incorrect labelling, or times when a calf was scored differently by all) were viewed, discussed, and a common behaviour classification was agreed upon. Sometimes, the issue was not the behaviour but misidentification of the calves. After the issues had been discussed, validation 2 was performed. Validation 2 was performed using the same method and video as validation 1. The final percent agreements were 99.19% for posture and 81.99% for activity.

Scan sampling behaviour scoring

Behaviour scoring was performed on the videos extracted from the DVR for one day each week. Saturday was chosen as the scoring day, as no research measurements were taken on this day and the only disturbance to the calves were those considered normal management (i.e. health checks by the farm manager or farm staff adding more concentrates to the feeder). Each calf was followed for eight weeks. Calf behaviour was scored using 10-minute scan sampling, from 00:00 h to 23:50 h, so that 144 observations were recorded. On two Saturdays, the farm staff spread straw in the calf pens (additional straw was added, pens were not completely cleaned out), disrupting the calves and preventing the view of the majority of the calves in the pen. When this occurred, no behaviour was recorded for that observation and the total number of scans per day was reduced to 143.

Table 1: Ethogram describing the different calf behaviours scored using 10-minute scan sampling from a 24-h video recording once a week for 8 weeks. The behaviours were split into two categories: posture and activity. Posture behaviours were always classified (144 combined observations). Activity behaviours were only recorded if they were occurring; if the calf was idle or sleeping, no activity behaviour was recorded.

Behaviour	Description
Posture	
Lying	Calf is resting either sternally or laterally with all four legs hunched close to body either awake or asleep; brisket is in contact with the ground
Standing	Calf is in a static upright standing position with weight placed on all four legs
Activity behaviours	
Drinking milk	Calf is standing in the automatic milk feeder drinking milk, with their mouth on the nipple.
Defecation/ urination	Calf defecates or urinates
Drinking water	Calf is standing at the water bowl with their nose/mouth partially submerged in the water.
Eating bedding	Calf is lying down on the straw and is making repeated lateral motions of the jaw with straw sticking out of their mouth. / Calf is lying down on the straw and is rooting their nose around in the bedding. / Calf is standing with their head near the ground making lateral motions of the jaw.
Eating concentrates	Calf is standing with their head within the flaps of the concentrate feeder. / Calf is standing with their head just outside the concentrate feeder while making lateral motions of the jaw
Eating forage	Calf makes a lateral motion of the jaw while standing at the hay feeder.
Grooming	Calf uses tongue to repeatedly lick own back, side, leg, tail areas
Oral manipulation of the pen structure	Calf licks, nibbles, sucks, or bites at the pen structure (barriers, walls, buckets, troughs etc.)
Other	Calf performs any other activity not mentioned above.
Play	Calf runs, jumps, changes direction suddenly, bucks, kicks hind legs, twists or rotates body. / Calf mounts or attempts to mount another calf. / Calf is engaged in head-to-head pushing with another calf. / Calf plays with an object in the pen.
Pacing	Calf repeatedly walks back and forth the same area in an active manner
Rumination/ chewing	Calf is lying down and making repetitive motions of the lower jaw in the lateral plane; calf is standing, not near the hay feeder, and making repetitive motions of the lower jaw in the lateral plane with their head with their head in a lateral position (not with head near floor)
Social interaction	Calf licks another calf in the same area multiple times. / Calf nudges another calf with its nose. / Calf sniffs another calf's head
Sniffing	Calf sniffs at their surroundings, including the ground, any part of the pen structure, or other calves bodies (not the head)
Scratching / rubbing / stretching	Calf scratches itself with one of their legs (generally hind legs). / Calf rubs itself on pen structure. / Calf stretches itself.
Tongue-rolling	Calf makes repeated movements with its tongue inside or outside its mouth.
Urine drinking / orally manipulate prepuce / cross sucking	Calf drinks the urine of another calf. / Calf attempts to suck the naval area of another calf. / Calf attempts to suck any body part of another calf.
Walking	Calf is actively moving from one point in the pen to another in an active walking motion.
Other	Calf performs any other activity not mentioned above.
Out of frame	
Out of frame	The calf's head is out of frame of the camera (i.e. in the corner of the pen or hidden behind another calf).

Data processing

Calculation of week of age

Week of age was determined for each calf at each behaviour observation date based on their age in days (Table 2). As calves entered the group pen at 3-4 d old, the first behaviour observation that they were in the group pen might have occurred at either week 1 or week 2 of age. Therefore, some calves have behaviour observations from week 1 to 8, while others have observations from week 2 to 9 (Table 2).

Temperature and relative humidity

For each temperature and humidity logger (two total, one from the feeding area and one from under the canopy; Figure 1C), the average, standard deviation, minimum, and maximum daily temperature (°C) and average, standard deviation, minimum, and maximum daily relative humidity (%) for each day of the study were calculated in SAS (PROC MEANS). For each logger, the daily average, standard deviation, minimum, and maximum daily temperature-humidity index (THI) was also calculated using the formula from Kelly and Bond (1971):

$$THI = (1.8 * AT + 32) - (0.55 - 0.55 * RH) * ((1.8 * AT + 32) - 58)$$

where AT is the temperature (°C) and RH is relative humidity (expressed as a fraction). This formula is changed slightly from the original to use (1.8 * AT + 32) to change the formula to use Celsius (°C) rather than Fahrenheit (°F). The individual logger values for average, standard deviation, minimum, and maximum were then averaged to obtain pen-level average values for each variable (temperature, relative humidity, and THI; Figure 2).

For the purpose of analysis, average daily temperature (avgT) and minimum daily temperature (minT) of each behaviour-scoring day were categorised into three levels (Table 2). The maximum temperature category was not included in the behaviour analysis, as it was always within the range of thermo-neutral zone (TNZ; average maximum temperature = 14.5 ± 2.61°C). Relative humidity and THI were both initially categorised for the analysis, but were confounded with temperature and thus were not used as factors.

Animal-based factors

A number of animal-based variables were categorised into factors to be used in the behaviour analysis. Specific details on the animal-based categories can be found in Table 2, including the number of calves or days and the number of observations within each sub-category. Two breed categories were used due to the

low number of purebred Jersey calves; Holstein-Friesian (<25% Jersey) calves were compared with the combined category of Holstein-Friesian x Jersey ($\geq 25\%$ Jersey) and purebred Jersey calves (Table 2). The Economic Breeding Index (EBI; see Berry et al., 2007 for more details) for each calf was extracted from the Irish Cattle Breeding Federation database. As average EBI in Ireland at the time of analysis (February 2024) was €160, calves were split into three EBI categories: average, high, and elite (Table 2).

As Irish dairy calves are, on average, smaller than other strains in other countries (mature bodyweight approximately 550 kg; Murphy et al. 2023), instead of using calf birthweight, to make our results more transferable we instead calculated calf birthweight as a proportion of projected mature bodyweight. Each calf's projected mature bodyweight could be calculated from its value for the Maintenance sub-index of the EBI; a value of €0 for Maintenance gives a projected mature bodyweight of 641 kg, with each €5 increase or decrease resulting in a lower or higher projected mature bodyweight, respectively (Teagasc, 2024). Projected mature bodyweight (kg) was thus calculated using the formula: projected mature bodyweight = $(-5 * \text{Maintenance}) + 641$. Each calf's birthweight as a percentage of their projected mature bodyweight was then calculated $((\text{birthweight} / \text{projected mature bodyweight}) * 100)$ and categorised into three categories (Table 2).

Quality of the colostrum the calf received (all colostrum given to calves measured $\geq 22\%$ on a Brix refractometer; median = 24.2%) was categorised into three categories (Table 2). As calves were given a standardised amount of colostrum (3 L), we calculated how much colostrum was given as a proportion of their birthweight (colostrum amount divided by their birthweight = $(3 \text{ L} / \text{birthweight}) * 100$), which was then categorised into three categories (Table 2). Whether the calf received colostrum from its dam or another cow was included as a factor (colostrum source), as was dam parity (primiparous or multiparous).

A composite, 2-level calving difficulty score was created based on calving data recorded by the farm staff, where 0 = no assistance required, single birth, and normal presentation and 1 = assistance required and/or multiple-calf birth and/or backwards presentation. No calves on the experiment were born by Caesarean section. Weekly ADG (ADGw) and ADG from birth (ADGb) were calculated based on the values from the preceding and following weeks. Both ADGw and ADGb were categorised into three categories, based on their respective average values for each (Table 2).

Behaviour proportions

Behaviour proportions were independently calculated for each calf on each behaviour scoring date. The proportion of time spent lying was calculated by taking the number of observations the calf spent lying divided by the total number of scans on that scoring date. The number of scans each calf spent out of frame on each behaviour scoring date was calculated by summing the number of occurrences. Activity-based behaviours were calculated as the number of occurrences of that particular behaviour divided by the total number of scans where the calf was visible.

Exclusion criteria

Calves were excluded from the behaviour analysis based on their health status, as the aim of this study was not to investigate the behaviour of sick calves:

- If a calf received an antibiotic or NSAID injection, the behaviour observation immediately preceding and following the antibiotic treatment were removed. If a calf received the antibiotic treatment on the behaviour scoring date, only that behaviour observation was excluded.
- If a calf received an antibiotic or NSAID injection due to dehorning (1 calf), only the behaviour event following the treatment was excluded (not the behaviour observation preceding the injection). For all other calves, the behaviour observation following the dehorning event was included in the dataset on the basis that it was a normal management practice and occurred 4 d previously.
- If a calf was removed from their group pen (for any length of time) and placed into an individual or group sick pen, then the behaviour observations immediately preceding and following the movement were removed (these mostly coincided with antibiotic/NSAID injections).
- If a calf had a high health score (cumulative score >7 and/or a score of 3 in ≥ 1 category and/or a score of 2 in ≥ 3 categories), then the behaviour observation closest to the health scoring date was removed, regardless of whether it was before or after.
- After all of the exclusions were made, if an individual calf only had ≤ 3 behaviour observations remaining (out of 8 total) then that calf was removed from the analysis altogether (5 calves removed, leaving 39 calves included in the analysis). This left 15 calves with 8 observations, 15 calves with 7 observations, three calves with 6 observations, five calves with 5 observations, and one calf with 4 observations.

Statistical analysis

Behaviour prevalence data

Behaviour data were expressed as a proportion of total (visible) scans. The mean, minimum, maximum, and standard deviation of each of the 20 different behaviours for each week of the observation period were calculated in SAS (PROC MEANS). For ease of understanding, all proportions were converted to percentages (percentage of total scans) for the tables and graphs. Due to their low number of occurrences, three behaviours (pacing, tongue-rolling, and urine drinking/orally manipulating the prepuce/cross sucking) were combined to create an abnormal behaviour category.

Analysis

Analysis of each behaviour or behaviour category was done in SAS using generalised linear mixed models (PROC GLIMMIX) with a binomial distribution, a logit link function, and the Kenwood-Rogers method of determining denominator degrees of freedom. The model consisted of the fixed effect of week, and then animal-based factors were kept in the model based on significance in a two-step procedure. In step 1, for each behaviour, each animal-based factor (described above and in Table 3) was tested in an interaction effect with week and separately as a fixed effect. In step 2, all animal-based factors (whether alone or as an interaction) that had a p-value <0.10 were then added to a multi-factorial model for that behaviour. Fixed effects in these multi-factorial models with p-values >0.10 were then removed using backwards selection (the effect with the highest p-value was removed each time) until all remaining effects (other than week) had a p-value <0.10 (see Table 3 for the fixed effects included in the model for each behaviour). No interaction with week was included in the final model for any behaviour as none of the interactions were found to have a p-value <0.10 . The random effects were calf nested within pen and the residual. Week was also included as a random repeated measure, acting upon the subject of the calf, using a first-order autoregressive lag 1 or compound symmetry covariance structure (Table 3). In the final models, p-values <0.05 were considered significant. Due to the nature of the logit function used in the generalised linear mixed models, only raw statistical means (\pm standard deviation) are reported throughout.

Table 2: Week and animal-based factor categories used in the analysis, including the number and percentage of total days, calves, and/or observations in each category. *Birthweight is presented as percentage of projected mature bodyweight.

Animal-based factor	Mean (range)	Unit	Category levels	Category labels	Calves or days per level (n [%])	Observations per level (n [%])
Week	-	d	0 to 6	week 1	16 [5.9]	-
			7 to 13	week 2	35 [12.9]	-
			14 to 20	week 3	36 [13.2]	-
			21 to 27	week 4	35 [12.9]	-
			28 to 34	week 5	35 [12.9]	-
			35 to 41	week 6	35 [12.9]	-
			42 to 48	week 7	30 [11.0]	-
			49 to 55	week 8	37 [13.6]	-
			56 to 62	week 9	13 [4.8]	-
Breed	-	-	HF (<25% Jersey)	HF	28 [71.8]	196 [72.1]
			JE (100%) and HF x JE (>25% JE)	Purebred JE and HF x JE	11 [28.2]	76 [27.9]
Birth-weight	6.0 (4.2-11.7)	%	<5.5	<5.5	16 [25.6]	111 [40.8]
			5.5 to 6.5	5.5 to 6.5	13 [33.3]	93 [34.2]
			>6.5	>6.5	10 [25.6]	68 [25.0]
Colostrum amount	9.5 (6.1-13.0)	%	<8.5	<8.5	13 [33.3]	91 [33.5]
			8.5-10	8.5-10	14 [35.9]	95 [34.9]
			>10	>10	12 [30.8]	86 [31.6]
Colostrum quality	25.3 (22.1-34.7)	%	<23	Adequate	11 [28.2]	76 [27.9]
			23 to 25	Moderate	15 [38.5]	106 [39.0]
			>25	High	13 [33.3]	90 [33.1]
Colostrum source	-	-	Dam	Dam	4 [10.3]	31 [11.4]
			Not dam	Not dam	32 [82.1]	225 [82.7]
			No data	No data	3 [7.7]	16 [5.9]
Calving score	-	-	No assistance	No assistance	28 [71.8]	195 [71.7]
			Assistance required	Assistance required	11 [28.2]	77 [28.3]
Dam parity	-	-	Primiparous	Primiparous	23 [59.0]	167 [61.4]
			Multiparous	Multiparous	16 [41.0]	105 [38.6]
Economic breeding index	241 (129 - 307)	€	<200	Average	7 [17.9]	51 [18.8]
			200 to 275	High	24 [61.5]	162 [59.6]
			>275	Elite	8 [20.5]	59 [21.7]
Average daily gain from birth	0.51 (-0.57 - 1.23)	kg/d	<0.4	<0.4	-	41 [15.7]
			0.4 to 0.6	0.4 to 0.6	-	162 [59.6]
			>0.6	>0.6	-	69 [25.4]
Average daily gain per week	0.54 (-0.29 - 1.35)	kg/d	<0.4	<0.4	-	88 [32.4]
			0.4 to 0.6	0.4 to 0.6	-	80 [29.4]
			>0.6	>0.6	-	104 [38.2]
Average daily pen temp.	10.1 (6.7 - 13.3)	°C	<9	<9	5 [41.7]	106 [39.0]
			9 to 11	9 to 11	2 [16.7]	71 [26.1]
			>11	>11	5 [41.7]	95 [34.9]
Minimum daily pen temp.	6.0 (2.0 - 11.0)	°C	<4	<4	3 [25.0]	58 [21.3]
			4 to 6	4 to 6	5 [41.7]	117 [43.0]
			>6	>6	4 [33.3]	97 [35.7]

Abbreviations: BW = birthweight; HF = Holstein-Friesian; JE = Jersey; temp. = temperature

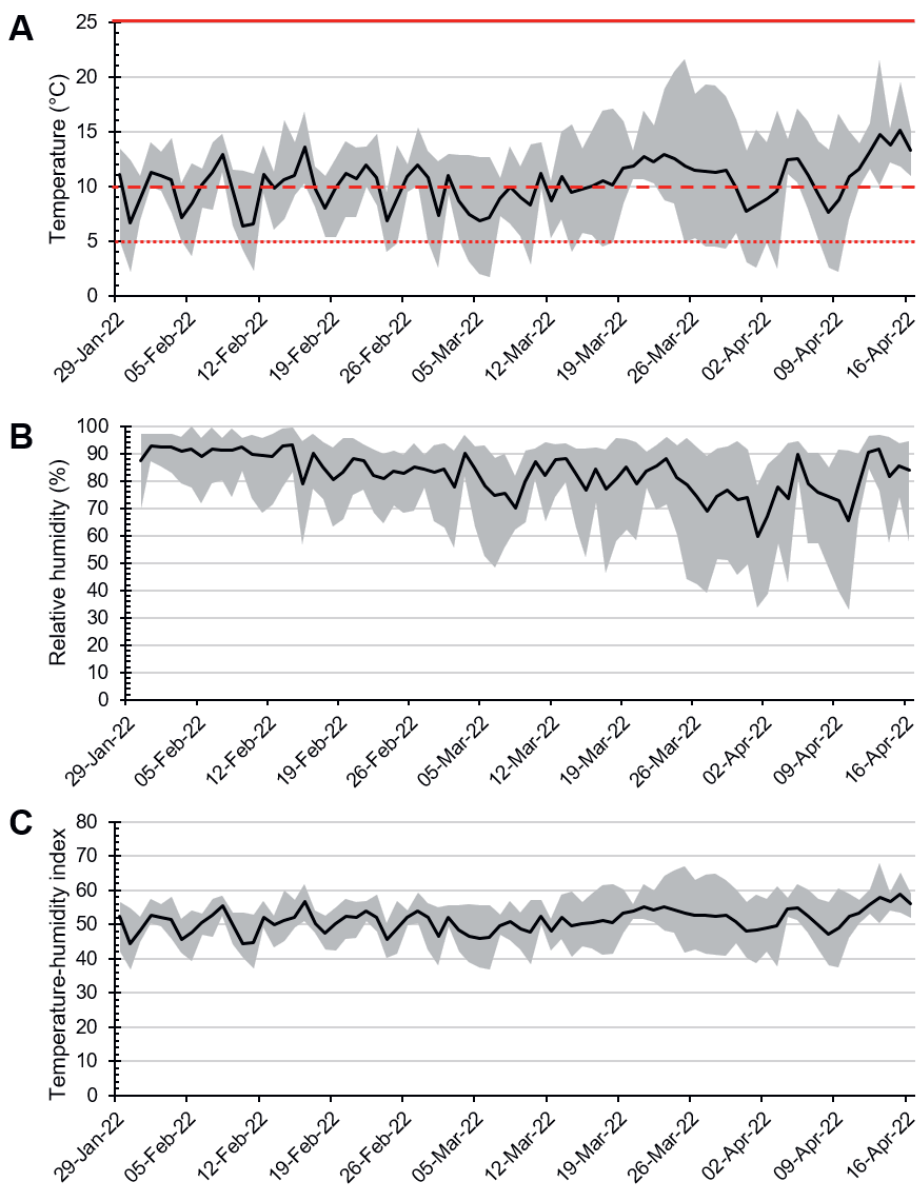


Figure 2: Temperature (A; °C), relative humidity (B: %), and temperature-humidity index (C) of the calf shed during the study period. The black line represents the average daily temperature, while the grey shaded area represents the temperature or relative humidity range for each day (top and bottom of the shaded area represent the minimum and maximum temperature or relative humidity that was recorded that day). The solid red line represents the high critical temperature threshold for a calf's thermo-neutral zone (Drackley, 2008). The dashed line represents the low critical temperature threshold for a calf's thermo-neutral zone, when the calf is under 21 d old (Drackley, 2008). The dotted line represents the low critical temperature threshold for a calf's thermo-neutral zone, when the calf is over 21 d old (Drackley, 2008). The average temperature and relative humidity between two different data loggers, one placed under the canopy in a calf group pen and one placed at the front of a pen in the feeding area, are presented. Behaviour observations were made each Saturday during the study period, which are the dates denoted at the bottom on the graphs.

Table 3: Covariance structure and fixed effects included in the generalised linear mixed models (SAS; PROC GLIMMIX) used to analyse the proportion of time calves spent performing specific behaviours. Week was always included in each behaviour model as a repeated measure, regardless of its significance. Each covariate was tested individually as a fixed effect and in an interaction with week for each behaviour model. Covariates where $P < 0.10$ were then included in the model, and were removed if $P > 0.10$, one at a time using backwards selection, until all included fixed effects had $P < 0.10$. No interactions were included in the final model. The covariance structure used for the repeated measure in each model is also included. '✓' denotes that the variable was included in the model ($P < 0.05$) and '✓*' denotes that the variable was included in the model but was not a significant effect ($P > 0.05$).

Behaviour model	Covariance parameter	Week	ADGb	ADGw	Breed	Calving score	Colos. quality	Colos. source	Dam parity	avgT	minT
lying	ar(1)	✓	-	-	✓	-	✓*	-	-	-	✓
abnormal	cs	✓*	-	-	-	-	-	-	✓	-	-
drinking milk	cs	✓*	-	-	-	-	-	-	-	-	-
defecate & urinate	cs	✓*	-	-	-	-	-	✓*	-	-	-
drinking water	ar(1)	✓	-	-	-	-	-	-	-	-	✓
eating bedding	ar(1)	✓	-	-	-	-	-	✓	✓	-	✓
eating concentrate	ar(1)	✓	-	✓*	✓*	-	-	✓	-	-	✓
eating forage	cs	✓	-	-	-	-	-	-	-	-	✓
grooming	ar(1)	✓*	-	-	✓*	-	-	-	-	-	-
oral manip. of pen structure	ar(1)	✓	-	-	-	-	-	-	-	-	✓
play	ar(1)	✓	-	✓	-	-	-	-	✓	-	-
ruminate / chewing	ar(1)	✓	-	-	-	-	-	✓	-	✓	-
social interaction	ar(1)	✓*	-	✓	-	✓	-	-	-	-	✓*
sniffing	ar(1)	✓*	-	-	-	-	-	✓*	-	✓	-
stretch/ rub/ scratch	ar(1)	✓*	-	-	-	-	-	-	-	-	✓
walking	ar(1)	✓*	✓*	-	-	-	-	-	-	-	✓

Abbreviations: colos. = colostrum; manip. = manipulation

Checking for confounding factors

For animal-based factors that were obviously confounded (i.e. ADGw and ADGb; minT and avgT), only one was chosen to be included in the final model for each behaviour using backwards selection; of the obviously confounded variables, whichever one had the lowest p-value was selected to remain in the model. To check the potential confounding of ADG and breed, the non-categorised ADG from birth and weekly ADG were analysed in a generalised linear mixed model (as described in section 2.9.2), using all of the other animal-based factors as fixed effects. The fixed effects were removed one-by-one using backwards selection; the fixed effect with the highest p-value was removed each time, until all remaining variables (other than week) had $P < 0.10$. Breed was removed from the weekly ADG models before all variables had $P < 0.10$, signalling that breed and ADGw were not confounded. Breed was significantly correlated to ADG from birth; therefore, only one of the two was selected to be in the final model for each behaviour, using the method described above.

Results***Temperature and relative humidity***

In the calf shed during the study period (29 January to 16 April, 2022), average temperature was $10.3 \pm 2.43^{\circ}\text{C}$, average relative humidity was $82.6 \pm 8.13\%$, and average THI was 51.1 ± 4.08 (average daily values can be found in Figure 2). On behaviour scoring days (Saturdays), the average temperature in the calf shed was always below the lower critical temperature of the TNZ for calves under 21 d of age (15°C ; Drackley, 2008) and was always above the lower critical temperature of the TNZ for calves over 21 d of age (5°C ; Drackley, 2008).

Prevalence of behaviours in healthy calves

The statistical prevalence (\pm standard deviation) of each behaviour per week is presented in Table 4 and Figure 3. .

Effect of age and other variables on normal pre-weaned calf behaviour

Due to the nature of the backwards selection used in the analysis, not all variables were included in each behaviour model (see Table 3 for which variables were included in each behaviour model); therefore, we only report on the variables that were included in each model for each behaviour.

Table 4: Average prevalence (%) of calf behaviours (mean \pm standard deviation) from scan sampling observations. Calves were scanned every 10 minutes over a 24 h period (144 total scans) once a week for 8 weeks. Activity behaviours were calculated out of the total number of scans – the number of out of frame observations for each individual calf at each scoring period (each week). Cross-suckling represents the behaviour category urine drinking/orally manipulate the prepuce/cross-suckling. Abnormal represents the combination of tongue-rolling, pacing, and urine drinking, cross suckling, and oral manipulation of prepuce. Dehorning occurred at an average age of 17 ± 10.7 d (0 to 6 d old = 0 calves; 7 to 13 d = 16 calves; 14 to 20 d = 22 calves; 21 to 27 d = 4 calves; 28 to 34 d = 0 calves; 35 to 41 d = 2 calves).

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	P-value
Out of Frame	3.9 \pm 3.50	3.1 \pm 3.23	4.2 \pm 3.79	6.8 \pm 5.86	6.6 \pm 5.31	6.6 \pm 5.12	7.3 \pm 5.02	7.4 \pm 5.49	7.3 \pm 6.16	NA
Behaviours										
Drinking milk	0.4 \pm 0.48	0.7 \pm 0.62	0.6 \pm 0.86	0.4 \pm 0.58	0.6 \pm 0.64	0.5 \pm 0.62	0.5 \pm 0.61	0.5 \pm 0.48	0.6 \pm 0.75	0.136
Abnormal	0.6 \pm 0.71	0.7 \pm 1.09	0.7 \pm 1.22	0.9 \pm 1.05	0.6 \pm 0.93	1.0 \pm 1.47	0.8 \pm 1.27	0.9 \pm 1.19	0.4 \pm 0.60	0.713
Cross-suckling	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.14	0.0 \pm 0.17	0.2 \pm 0.50	0.6 \pm 1.40	0.4 \pm 0.77	0.6 \pm 1.12	0.3 \pm 0.52	-
Pacing	0.0 \pm 0.18	0.0 \pm 0.16	0.0 \pm 0.12	0.0 \pm 0.12	0.0 \pm 0.00	0.0 \pm 0.16	0.0 \pm 0	0.0 \pm 0.16	0.1 \pm 0.19	-
Tongue-rolling	0.5 \pm 0.68	0.7 \pm 1.08	0.6 \pm 1.12	0.9 \pm 1.07	0.5 \pm 0.81	0.4 \pm 0.77	0.4 \pm 0.82	0.3 \pm 0.68	0.1 \pm 0.19	-
Social interaction	5.7 \pm 3.17	5.8 \pm 2.8	5.5 \pm 3.40	6.8 \pm 4.53	5.2 \pm 3.00	5.7 \pm 3.44	4.7 \pm 3.64	4.3 \pm 2.87	3.9 \pm 2.34	0.100
Sniffing	1.2 \pm 0.73	1.6 \pm 1.27	1.8 \pm 1.55	2.0 \pm 1.82	1.7 \pm 1.58	2.1 \pm 1.79	2.2 \pm 2.00	2.7 \pm 1.60	3.0 \pm 2.64	0.058
Defecation and urination	1.7 \pm 0.95	2.4 \pm 1.24	2.2 \pm 1.41	2.5 \pm 1.36	2.6 \pm 1.82	2.6 \pm 1.74	2.3 \pm 1.65	1.9 \pm 1.29	1.8 \pm 1.60	0.631
Grooming	3.6 \pm 1.48	3.2 \pm 1.86	2.5 \pm 1.48	2.8 \pm 1.56	2.4 \pm 1.57	2.3 \pm 1.29	2.9 \pm 1.97	3.2 \pm 1.62	2.8 \pm 2.24	0.085
Stretching, rubbing, scratching	0.4 \pm 0.68	0.5 \pm 0.62	0.4 \pm 0.59	0.3 \pm 0.46	0.4 \pm 0.69	0.3 \pm 0.62	0.6 \pm 0.64	0.7 \pm 0.94	0.3 \pm 0.39	0.421
Walking	1.8 \pm 1.10	1.6 \pm 1.14	1.6 \pm 1.21	1.6 \pm 1.14	1.5 \pm 1.05	2.0 \pm 1.54	1.5 \pm 1.26	1.8 \pm 1.13	2.4 \pm 1.68	0.657

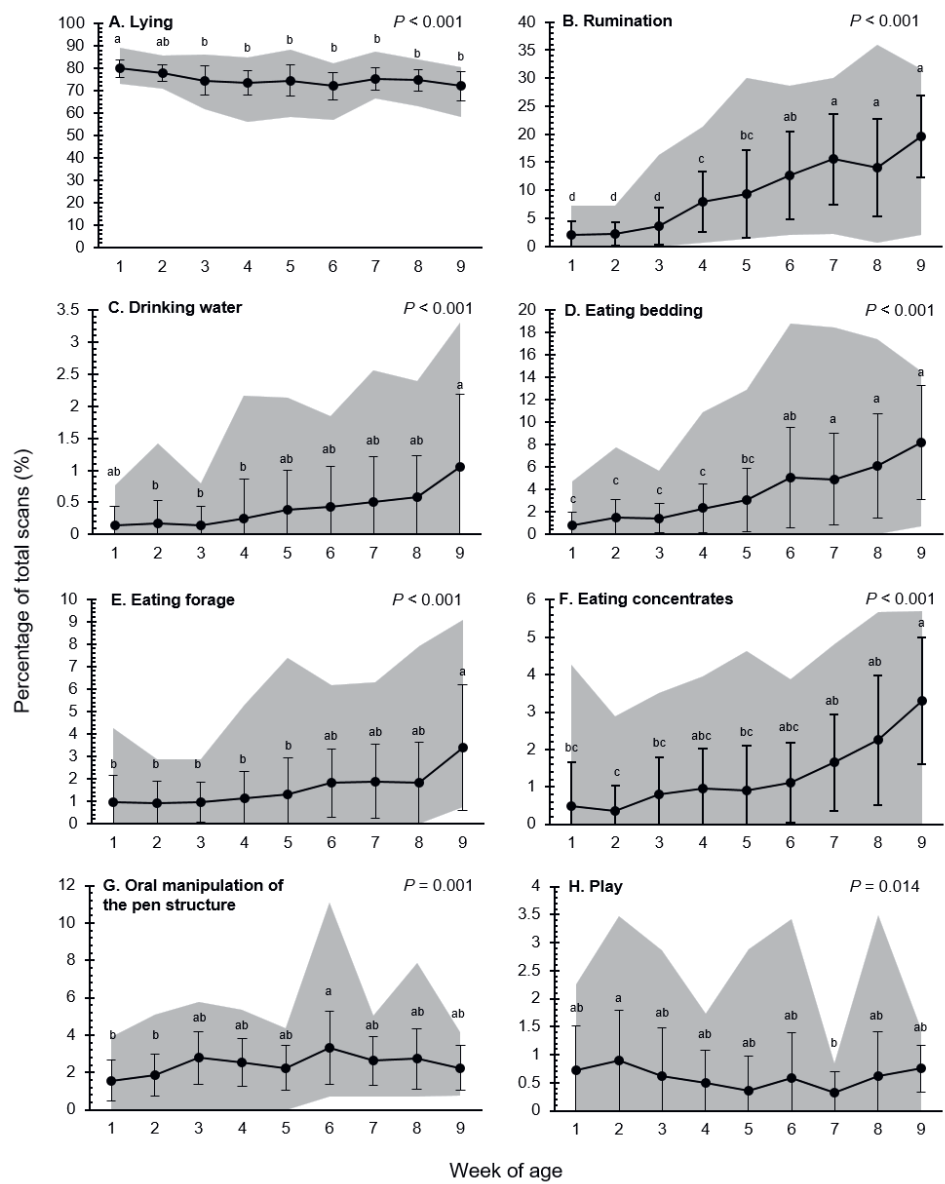


Figure 3: Average prevalence (%) of each calf behaviour (mean \pm standard deviation) from scan sampling observations made over time during the pre-weaning period in group-housed dairy calves. Calves were scanned every 10 minutes over a 24 h period (144 total scans) once a week for 8 weeks. Lying was calculated out of the total number of scans. Activity behaviours were calculated out of the total number of scans – the number of out of frame observations for each individual calf during each scoring period (each week). P-values were generated through generalised linear mixed models, using week as the fixed effect. The grey bands represent the range in observations for each week (minimum to maximum) and the error bars represent standard deviation. Dehorning occurred at an average age of 17 ± 10.7 d (0 to 6 d old = 0 calves; 7 to 13 d = 16 calves; 14 to 20 d = 22 calves; 21 to 27 d = 4 calves; 28 to 34 d = 0 calves; 35 to 41 d = 2 calves).

Posture

The proportion of time a calf spent lying was affected by age (Figure 3A), breed ($P = 0.056$), colostrum quality ($P = 0.018$), and minT ($P < 0.001$). Lying time was highest in week 1 and had decreased by week 3 where it remained similar from weeks 3 to 9 (Figure 3A). Calves fed colostrum of <23% quality ($73.1 \pm 6.46\%$) spent less time ($P = 0.022$) lying than calves given colostrum of >25% quality ($75.8 \pm 6.03\%$); calves given moderate quality (23-25%) colostrum did not differ from either ($74.9 \pm 5.04\%$). Calves spent less time ($P < 0.001$) lying when minT were $>6^{\circ}\text{C}$ ($72.3 \pm 5.78\%$), compared to when minT were $<4^{\circ}\text{C}$ and $4-6^{\circ}\text{C}$, which were similar ($76.0 \pm 5.45\%$).

Activity-based behaviours

The proportion of time calves spent ruminating was affected by age (week; Figure 3B), colostrum source ($P = 0.001$), and avgT ($P = 0.025$). Rumination time was consistently low for weeks 1 to 3, but had increased by week 4 (21-27 d of age); rumination time in weeks 7 to 9 was similar and higher than the rest of the weeks (except week 6; Figure 3B). Calves given colostrum from their dam ($14.3 \pm 10.51\%$) spent more time ruminating ($P < 0.001$) than calves given colostrum from other cows ($8.6 \pm 7.89\%$). Calves spent less time ruminating when avgT were $9-11^{\circ}\text{C}$ ($7.7 \pm 7.32\%$) compared to when avgT were $<9^{\circ}\text{C}$ ($9.8 \pm 7.81\%$; $P = 0.025$); avgT were $>11^{\circ}\text{C}$ ($9.8 \pm 9.43\%$) rumination time did not differ.

There was no effect of calf age ($P = 0.136$) on the proportion of time spent drinking milk (Table 4), and it was not affected by any other variable (Table 3). The proportion of time calves spent drinking water was affected by age (Figure 3C) and minT ($P = 0.019$). Calves spent more time drinking water in week 9 compared to weeks 2, 3, and 4 (Figure 3C). The minT categories did not differ significantly in the post-hoc analysis.

The proportion of time calves spent eating bedding was affected by age (Figure 3D), colostrum source ($P = 0.032$), dam parity ($P = 0.013$), and minT ($P < 0.001$). The proportion of time spent eating bedding increased over time (Figure 3D); calves spent less time eating bedding in weeks 1 to 4 and had increased by week 6; time spent eating bedding was highest during weeks 7 to 9. Calves fed colostrum from their dam ($5.5 \pm 4.75\%$) spent more time ($P = 0.004$) eating bedding than calves fed colostrum from another cow ($3.2 \pm 3.63\%$). Calves born from primiparous dams ($4.0 \pm 3.78\%$) spent more time ($P = 0.004$) eating bedding than calves from multiparous dams ($2.7 \pm 3.82\%$). Calves spent more time eating

bedding when minT were $>6^{\circ}\text{C}$ ($4.0 \pm 4.78\%$) compared to when minT were $<4^{\circ}\text{C}$ ($2.7 \pm 2.87\%$; $P = 0.003$) and $4\text{--}6^{\circ}\text{C}$ ($3.5 \pm 3.33\%$; $P = 0.045$), which were similar.

There were effects of age (Figure 3E) and minT ($P = 0.002$) on the proportion of time calves spent eating forage. Time eating forage increased over the weeks, with calves in week 9 spending more time eating forage than calves in weeks 1 to 5 (Figure 3E). During days when minT were $<4^{\circ}\text{C}$ ($0.8 \pm 0.91\%$), calves spent less time eating forage than on days when minT were $4\text{--}6^{\circ}\text{C}$; ($1.7 \pm 1.78\%$; $P = 0.002$) and $>6^{\circ}\text{C}$ ($1.5 \pm 1.57\%$; $P = 0.005$).

There were effects of age (Figure 3F), colostrum source ($P = 0.044$), and minT ($P = 0.045$) on the proportion of time calves spent eating concentrates. Calves spent more time eating concentrates as they aged (Figure 3F); time spent eating concentrates was highest in week 9 and lowest in week 2. Calves that received colostrum from their dam ($2.0 \pm 1.71\%$) spent more time ($P = 0.044$) eating concentrates than calves that received colostrum from another cow ($1.1 \pm 1.36\%$). Calves spent more time ($P = 0.028$) eating concentrates when minT were $>6^{\circ}\text{C}$ ($1.4 \pm 1.49\%$) compared to when they were $<4^{\circ}\text{C}$ ($0.8 \pm 1.11\%$); when minT were $4\text{--}6^{\circ}\text{C}$ ($1.3 \pm 1.43\%$) did not differ from either.

There was no effect of age on the proportion of time spent performing abnormal behaviours (Table 4), but there was an effect of dam parity ($P < 0.001$). Calves born from primiparous cows ($0.6 \pm 1.00\%$) spent less time ($P < 0.001$) performing abnormal behaviours compared to calves born from multiparous cows ($1.1 \pm 1.26\%$). The proportion of time calves spent orally manipulating the pen structure was affected by age (Figure 3G) and minT ($P = 0.037$). Calves spent more time orally manipulating the pen structure during week 6 compared to weeks 1 and 2 (Figure 3G). Calves spent more time orally manipulating the pen structure when minT were $>6^{\circ}\text{C}$ ($2.8 \pm 1.66\%$) compared to when minT were $4\text{--}6^{\circ}\text{C}$ ($2.3 \pm 1.34\%$; $P = 0.037$); minT $<4^{\circ}\text{C}$ ($2.5 \pm 1.29\%$) were similar to both.

There were effects of age (Figure 3H), dam parity ($P = 0.001$), and ADGw ($P = 0.014$) on the proportion of time calves spent playing. Calves spent more time playing in week 2 compared to week 7 (Figure 3H). Calves from primiparous dams ($0.7 \pm 0.81\%$) spent more time ($P = 0.005$) playing than calves from multiparous dams ($0.4 \pm 0.53\%$). Calves with ADGw <0.4 kg/d ($0.5 \pm 0.64\%$) spent less time playing than calves with ADGw >0.6 kg/d ($0.7 \pm 0.80\%$; $P = 0.015$), calves with ADGw $0.4\text{--}0.6$ kg/d ($0.6 \pm 0.75\%$) were similar ($P = 0.871$) to calves with ADGw >0.6 kg/d and tended ($P = 0.053$) to spend more time playing than calves with ADGw <0.4 kg/d. The proportion of time calves spent socially interacting was not affected by age (Table 4), but was affected by calving score ($P = 0.032$) and ADGw ($P = 0.035$). Calves that had a normal calving (no issues; $1.9 \pm 1.53\%$) spent less

time ($P = 0.038$) socially interacting than calves that had a difficult calving ($2.4 \pm 2.07\%$). Calves with ADGw >0.6 kg/d ($2.6 \pm 1.97\%$) spent more time socially interacting than calves with ADGw of 0.4 - 0.6 kg/d ($1.6 \pm 1.31\%$); calves with ADGw <0.4 kg/d ($1.8 \pm 1.58\%$) did not differ from either.

There was an effect of avgT ($P = 0.026$) on the proportion of time calves spent sniffing (Table 4). Calves spent more time ($P = 0.013$) sniffing when avgT were 9 - 11°C ($6.8 \pm 3.38\%$) compared to when avgT were $>11^{\circ}\text{C}$ ($4.5 \pm 2.76\%$); avgT $<9^{\circ}\text{C}$ ($5.2 \pm 3.67\%$) did not differ from either. The proportion of time calves spent defecating and urinating was not significantly affected by age (Table 4) or any animal-based factor (Table 3; animal-based factors that were included in these respective models were $P < 0.10$, but not $P < 0.05$). There was a tendency for the proportion of time calves spent grooming to be affected by age (Table 3) and was not affected by any other animal-related factors (Table 3). There was no effect of age on the proportion of time calves spent scratching, rubbing, or stretching (Table 4), but there was an effect of minT ($P = 0.022$). The proportion of time spent scratching, rubbing, and stretching was less when minT were $<4^{\circ}\text{C}$ ($0.3 \pm 0.49\%$) compared to minT between 4 - 6°C ($0.6 \pm 0.76\%$; $P = 0.045$); calves did not differ from either when minT $>6^{\circ}\text{C}$ ($0.4 \pm 0.58\%$). The proportion of time a calf spent walking was not affected by age (Table 4) but was affected by ADGb ($P = 0.038$) and minT ($P = 0.001$). Calves with ADGb <0.4 kg/d ($1.2 \pm 0.91\%$) spent more time walking than calves with ADGb from 0.4 - 0.6 kg/d (1.8 ± 1.28 kg/d; $P = 0.030$); calves with ADGb >0.6 kg/d (1.8 ± 1.24 kg/d) tended ($P = 0.071$) to differ from calves with ADGb <0.4 kg/d. Calves spent more time walking when minT were $>6^{\circ}\text{C}$ ($2.0 \pm 1.40\%$) compared to when minT were $<4^{\circ}\text{C}$ ($1.5 \pm 1.20\%$; $P = 0.012$) or between 4 - 6°C ($1.5 \pm 1.05\%$; $P = 0.010$).

Discussion

The observation and quantification of normal calf behaviour was explored in this experiment, and led to our establishment of a behaviour baseline for group-housed, pre-weaned dairy calves, reared under conventional management conditions in Ireland. Our results can be used as a comparison in future studies and can help guide areas where calf welfare and management can be improved.

Effect of age on calf behaviour

In this study, calves spent the most time lying in week 1 ($\sim 80\%$) and had reduced their lying time by week 3; from then they remained relatively stable for the rest of the study ($\sim 74\%$). This was expected, as it is generally accepted that young calves spend the majority of their time lying (Vitale et al., 1986; Hutchison et al,

1962), and lie down for a larger proportion of their day compared to 6-month-old calves (38-47%, season-dependent; Tripon et al., 2014) or mature cattle (38-50%, system-dependent; Tucker et al., 2021). The difference observed in lying time between pre-weaned calves (<12 weeks) and older calves/cows can primarily be attributed to the difference in sleep required; calves spend the majority of their time lying down sleeping (calves aged 2-3 d slept around 20 h/d; Hänninen et al., 2008a), but this has been shown to decrease with age (calves aged 97 d were asleep 25% of all observations, approximately 6 h/d; Hänninen et al., 2008b).

Calves increased the proportion of time they spent ruminating, eating concentrates, drinking water, eating bedding, and eating forage as they aged. The observed changes in feeding behaviour were characteristic of rumen development, a critical process in calf development. Calves are born with a non-functional rumen and thus initially rely on milk digestion via the abomasum to meet their nutrient needs (Khan et al., 2016); the first rumination event typically occurs from 2 to 3 weeks old and rumination time increases from there (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022). In this study, the proportion of time calves spent ruminating and consuming solid feed (eating bedding, forage, and concentrates) were low in weeks 1 to 3, similar to what has been found previously (Swanson and Harris, 1958; Noller et al., 1959; Wang et al., 2022). From weeks 4 to 6, calves gradually increased the amount of time they spent ruminating and consuming solid feed, likely because calves were still nutritionally reliant on milk (at maximum amount of 6 L/d). However, gradual weaning started after 6 weeks (42 d; reduction in milk ~0.14 L/d from 42-54 d), which coincided with the observed increase in rumination and solid feed consumption, thus emphasising the critical importance of weaning gradually.

Calves likely have an innate need to consume solid feed to initiate rumen development. The solid feeds provided in this study were concentrates and barley straw/hay, while barley straw was used as the bedding material. Calves in this study spent more time eating bedding than they did eating forage. For their behaviour to be classified as eating forage, calves had to stand (unless they were eating it while laying directly under the feeder) and often were observed clustered around the forage feeder, in line with social facilitation. In contrast, calves could eat bedding while standing or lying down, from almost anywhere in the pen. Calves were also frequently observed eating bedding while sniffing and slowly walking across the lying area of the pen, in a motion that mimicked grazing (Werner et al., 2018). This suggests that their motivation to eat bedding may be spurred by their curiosity, by an innate need to consume roughage, or by a combination thereof. As such, calves may benefit from additional or alternative methods of forage provision,

such as outdoor access to pasture, which may also help to meet these and other behavioural needs. In addition, if calves are consuming bedding, it should be kept clean and dry.

The two other behaviours that changed as the calves aged were oral manipulation of the pen structure and play. Oral manipulation of the pen structure is considered a normal, exploratory behaviour (Bertelsen and Jensen, 2019), but also may be considered an abnormal behaviour when expressed beyond normal levels of exploration (i.e., an indication of frustration or lack of satiety; Webb et al., 2015). The proportion of the other measured abnormal behaviour was very low, as was expected with the selected group of calves. Oral manipulation of the pen structure peaked during week 6, which coincided with the start of gradual weaning and likely reflected the calves' frustration with their reduced milk intake. Previous studies have shown that calves on lower milk allowances perform fewer play behaviours (Das et al., 2000; Krachun et al., 2010) and calves on more restricted diets play more after eating (Vitale et al., 1986; Das et al., 2000). Conversely, the increase in oral manipulation of the pen structure may be due to calves switching from locomotor to object play. In calves, play can be classified into three sub-types: social, object, and locomotor (Ahloy-Dallaire et al., 2018). Locomotor play (i.e. running, jumping, mounting, and head butting) can be negatively affected by low space allowances (Jensen et al., 1998; Jensen and Kyhn, 2000; Færevik et al., 2008). Although all replicates (replicates 1 and 2 = 2.13 m²/calf; replicate 3 = 3.01 m²/calf) had a space allowance over the minimum required (1.5 m² required for calves <150 kg, 1.7 m² recommended; European Union, 2009), 20 m²/calf is required for them to express their full extent of locomotor play in group-housing systems (EFSA, 2023); this is unattainable in most indoor systems. The amount of space required to exhibit locomotor play may also increase with growth. As locomotor play may have been restricted by space as calves grew, in order to express their play behaviour urge, they may have switched from locomotor to object play and this may have been classified as oral manipulation of the pen structure in our ethogram (i.e., a calf excessively orally manipulating a loose chain on a gate). This suggests calves may benefit from environmental enrichment in their pens (i.e., designated objects to play with; Zhang et al., 2021).

Effect of environmental temperature on calf behaviour

Environmental temperature influenced calf behaviour to almost the same extent as age (and thus expected rumen development); the cold minT (<4°C) was linked to a generic decrease in activity (i.e. feeding behaviours and walking). Low temperatures often result in dairy cows and calves modifying their behaviour by

seeking out shelter or warmer areas (Hänninen et al., 2003; Borderas et al., 2009; Sawalhah et al., 2016), increasing their lying time (Tripon et al., 2014; Tucker et al., 2021), huddling together while lying (Bøe and Havrevoll, 1993), and adopting a more tucked or nestled lying posture to reduce their surface area (Hänninen et al., 2003; Lago et al., 2006), all of which help to reduce the lower critical TNZ threshold (Webster, 1984). It appears that during cold temperatures, the calves' need to moderate their own body temperature might outweigh their needs to be active and perform social or feeding behaviours. Colder temperatures may also reduce appetites (Arnold, 2020). Therefore, the risk low temperatures may have on calf behaviour and rumen development, and thus growth, should be emphasised to farmers. This is especially relevant in areas with year-round calving and areas with seasonal-calving during colder weather. During extended periods of cold temperatures, farmers may need to wean calves later to promote solid feed intake pre-weaning or improve their temperature management (i.e., providing more bedding material or installing heaters).

Effect of animal-based factors on calf behaviour

As mentioned previously, some animal-based factors seemed to have caused some variation in the proportion of time calves spent performing specific behaviours. Calves with higher ADG_w performed more play and social interactions; this may indicate that they had more energy available to play and interact, which allowed them to be in more positive affective states (Altmann and Gotlib, 1988; Ahloy-Dallaire et al., 2018). Colostrum source affected behaviour, as calves provided with colostrum from their own dam spent more time ruminating, eating concentrates, and eating forage. Calves given maternal colostrum are provided with maternal leukocytes and cytokines (Godden et al., 2019), which have been suggested to be better at priming the mucosal innate immune system in piglets (Bandrick et al., 2011). This may promote faster rumen development, by decreasing the time required to reach a stable commensal population within the gastrointestinal tract (Amin and Seifert, 2021). Calves with a more difficult calving spent more time socially interacting with other calves. This was not expected, as calving dystocia can cause injuries to the calf (Murray and Leslie, 2013), causing pain and deterring social interactions. Perhaps these calves used tactile social interactions as a method of self-soothing, which has previously been found in calves after disbudding (Adcock and Tucker, 2021). Calves with multiparous dams spent a higher proportion of time performing abnormal behaviours and a lower proportion of time eating bedding and playing than calves born from primiparous dams. Dam parity can influence foetal calf growth and metabolism (Duncan et al.,

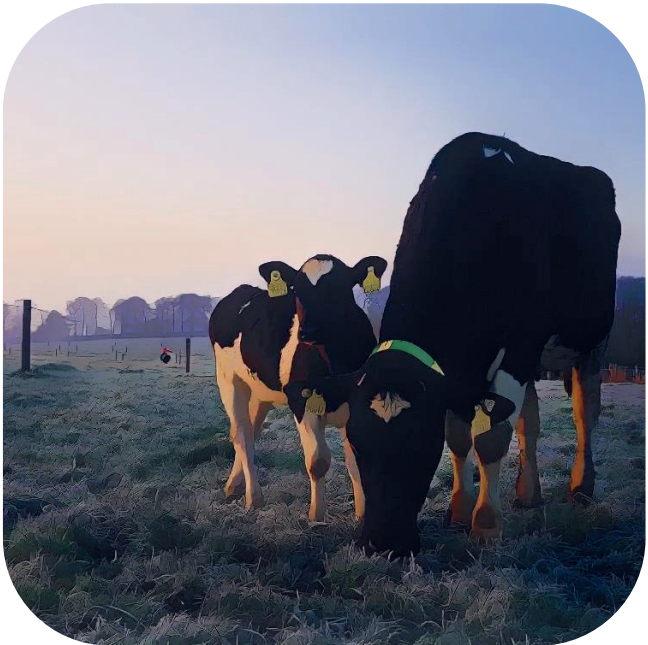
2023), which might influence future growth and performance. Although this experiment was not set up to explicitly test each of these fixed effects (it was designed to observe and quantify), our results suggest that they did have an effect on behaviour, and thus should be explored further.

Conclusion

We have observed and quantified how the behaviour of group-housed dairy calves changed during the pre-weaning period, to establish a baseline of normal calf behaviour under conventional management conditions. Calves started exploring solid feeds (forage, bedding, and concentrates) immediately after entering the group pens, emphasising that calves should be provided access to solid feeds as soon as possible after birth, as solid feeds help meet calves' behavioural needs and their consumption promotes rumen development and growth. Time spent ruminating and consuming solid feeds increased at the start of weaning, accentuating the importance of a gradual weaning process to allow calves to slowly shift their reliance on milk to solid feed. Calves appeared to have an innate drive to consume forage, and consumed both the provided forage in the feeder and their bedding. Farmers should ensure that forage feeders remain full; if calves are consuming their bedding, it should be kept clean and dry to prevent health issues. Environmental enrichment (i.e., objects for calves to play with) may also improve calf welfare, by increasing play opportunities in group pens and decreasing the performance of negative oral behaviours. Low ambient temperatures ($<4^{\circ}\text{C}$, below TNZ) caused calves to modify their behaviour by increasing the proportion of time spent lying and decreasing all other activities, highlighting the importance of temperature management in calf housing (i.e., using ventilation, heaters, or sheltered areas to keep the ambient temperature within the calves' TNZ). The behaviour baseline of dairy calves during the pre-weaning period will be useful for future studies to use as a comparison.

Acknowledgements

The authors wish to thank and acknowledge the Moorepark farm staff for their care of the animals in this experiment. The authors also wish to thank the others who assisted in the behaviour scoring – Margaret Mulvihill (University College Cork), Louise Boisgontier (L'Institut Agro Rennes-Angers), and Cecelia McInnes (Munster Technological University). This research is funded by Science Foundation Ireland and the Department of Agriculture, Food, and Marine on behalf of the Government of Ireland under Grant Number [16/RC/3835] – VistaMilk.



| CHAPTER 4 |

A preliminary study on the feasibility of two different cow-calf contact systems in a pasture-based, seasonal calving dairy system: effects on cow production and health

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animal (2024) 18(8): 101222

<https://doi.org/10.1016/j.animal.2024.101222>

Abstract

Internationally, consumer dissatisfaction with cow-calf separation at birth has led to increased interest in alternative calf rearing methods, specifically cow-calf contact (CCC) systems. The objectives of this preliminary study were to estimate whether CCC could be incorporated into an Irish spring-calving, pasture-based system, and to investigate the effects on cow milk production and health. Three systems were compared: the conventional Irish system (CONV; 18 cows), cow and calf were separated <1 h post-birth, cows were pasture-based and milked twice-a-day; a full-time access system (FT; 14 cows), cow and calf were allowed constant, unrestricted access, were pasture-based, and cows were milked twice-a-day; and a part-time access system (PT; 18 cows), cow and calf had unrestricted access when indoors at night, cows grazed outdoors by day while calves remained indoors, and cows were milked once-a-day in the morning. Cows were blocked and balanced across the three systems by previous lactation machine milk yield (MMY), bodyweight (BW), and body condition score (BCS). Following an 8-week CCC period, all calves were weaned (FT and PT underwent a 7-d gradual weaning and separation process) and all cows were milked twice-a-day. Cow MMY was recorded daily and milk composition was recorded weekly; milk data were analysed from weeks 1-8 (CCC period), weeks 9-35 (post-CCC period), and weeks 1-35 (cumulative lactation). Cow BW and BCS were taken weekly for weeks 1-12, and at the end of the lactation. During the CCC period, all systems differed ($P < 0.001$) in MMY (mean \pm SEM; 24.0, 13.6, and 10.3 ± 0.50 kg/d for CONV, FT, and PT cows, respectively). After the CCC period, CONV MMY (20.2 ± 0.48 kg/d) remained higher ($P < 0.001$) than the FT (16.6 kg/d) and PT cows (15.7 kg/d). The FT and PT cows yielded 24 and 31% less in cumulative lactation MMY and 26 and 35% less in cumulative lactation milk solids yield, respectively, compared to CONV (5072 ± 97.0 kg and 450 ± 8.7 kg). During the CCC period, somatic cell score was higher ($P = 0.030$) in PT cows (5.15 ± 0.118) compared to FT cows (4.70 ± 0.118), while CONV (4.94 ± 0.118) were inconclusive to both. The PT cows (523 ± 4.9 and 520 ± 6.8 kg) were heavier than the CONV (474 ± 4.9 and 479 ± 6.8 kg) and FT (488 ± 4.9 and 487 ± 6.8 kg) cows at week 4 and 8 (both $P < 0.001$). The PT cows had higher BCS than CONV and FT at all observed times. This preliminary research suggests that although CCC was incorporated without impacting cow health, the two CCC systems investigated negatively affected cow production.

Keywords: dam-calf contact, milk yield, somatic cell score, milking frequency, cow-calf separation

Introduction

Pasture-based dairy systems are predominant in certain temperate countries, such as Ireland and New Zealand, where grass grows nearly year round (Hurtado-Uria et al., 2014) and thus provides a nutritious, economic feed source for dairy cattle (Shalloo and Hanrahan, 2020). To ensure that the cows' nutritional demands match the seasonal fluctuation of grass growth (Dillon et al., 2005; Horan et al., 2005), the majority of dairy farmers in these countries utilise seasonal (i.e. spring) calving. In Ireland a compact, spring-calving dairy system is generally applied, meaning 90% of the cows calve within a 6-week window, centred on mid-February (Shalloo and Hanrahan, 2020). Although consumers consider pasture-based dairy systems more desirable than indoor systems (Schuppli et al., 2014; Ventura et al., 2016), consumers still have welfare concerns about cow-calf separation soon after birth (Sweeney et al., 2022). Separating cow and calf soon (<2 h) after birth is a common management practice, and has been used for many reasons, including increasing saleable milk and reducing calf health risk. However, consumers, researchers, and farmers are conflicted on whether the practice is beneficial or harmful for animal welfare. In contrast to this, cow-calf contact (**CCC**) systems, are a type of management system that allow calves to have some contact with either their dam, or a foster cow, for a period of time (Sirovnik et al., 2020). A recent scientific opinion paper from the European Food Safety Authority Panel on Animal Health and Animal Welfare (2023) has recommended that dairy farmers keep cows and calves together for at least 24 h, and has stated that longer periods of contact between cow and calf should be implemented in the future. However, most international CCC research has been conducted on indoor housing systems with year-round calving (i.e. Barth, 2020; Wenker, et al., 2022; Neave et al., 2024a). For CCC to be implemented in Ireland, it is preferred to integrate it into the pre-existing pasture-based, compact calving system, and it should increase animal welfare without decreasing human welfare or majorly affecting productivity.

Cows in CCC systems have a decreased machine milk yield (MMY) during the nursing period (Barth, 2020; Johnsen et al., 2021; Ospina-Rios et al., 2023). After this time period, research varies on whether or not the cow's cumulative lactation MMY and daily milk yield after weaning and separation are impacted. Although a review (Meagher et al., 2019) concluded that CCC did not have a negative impact on cumulative lactation MMY, the research described within their review was variable, both in the CCC systems investigated and the results they provided, and the majority of included studies were indoor systems. The impact of CCC on milk components (fat, protein, and lactose concentration), and thus milk

solids yield (MSY), should also be considered as calf nursing has been shown to reduce milk fat concentration of machine-harvested milk (Bar-Peled et al., 1995; Barth, 2020; Wenker et al., 2022a). Dairy farmers are also concerned with the risk of mastitis in CCC cows (Neave et al., 2022). Although two reviews (Johnsen et al., 2016; Beaver et al., 2019) concluded that suckling was beneficial in reducing the risk of mastitis, the majority of studies were not performed on pasture-based dairy farms with spring-calving. Monitoring the udder and teats is another important aspect, as the risk of udder damage during CCC was noted as a concern for dairy farmers (Neave et al., 2022), teat damage has been observed previously (Ospina-Rios et al., 2023), and udder conformation can impact the calf's ability to nurse (Edwards and Broom, 1979; Edwards, 1982; Ventorp and Michanek, 1992). Other than udder-based concerns, the health of cows has often been considered to not be impacted by the CCC system. However, regular health-related scoring, including measurements of bodyweight (BW), body condition score (BCS), injury status, locomotion score (lameness), and clinical health scoring, should be used to monitor cow health status when comparing systems.

This preliminary study aimed to investigate whether dam-calf CCC rearing could be incorporated into the pre-existing Irish spring-calving, pasture-based dairy system and estimate the effects on cow production and health. We implemented two different CCC rearing systems that varied in housing environment and milking frequency and compared them to the conventional dairy and rearing system in Ireland (cow and calf separated at birth and milked twice a day). We were specifically interested in the differences between the systems regarding cow performance (cumulative and average MMY, MSY, and milk composition) and cow health, both in terms of overall health (BW, body condition score, clinical health, and locomotion) and udder health (mastitis incidence, somatic cell score (SCS), and udder conformation).

Material and methods

Animals, Management, and Study Design

This preliminary study was conducted from 20 January to 14 November 2021 at Teagasc Moorepark Research Farm, County Cork, Ireland. Calf-associated measurements can be found in Sinnott et al. (2024). The three systems compared were: the conventional dairy and rearing system in Ireland (CONV), where dam and calf are separated <1 h post-birth, cows were pasture-based (24 h/d) and milked twice a day; a full-time access (FT) system, where dam and calf had constant, unrestricted access to each other, the pairs were pasture-based, and

cows were milked twice a day; and a part-time access (PT) system, where cows went outdoors during the day to graze while their calves remained indoors, and to ensure cows had sufficient milk for calves when they returned indoors at 1500 h PT cows were not milked in the afternoon (milking occurred once-a-day (OAD) in the morning at 0800 h). All multiparous cows enrolled in the study had no previous experience raising a calf in their previous lactations; thus cow-calf contact was novel to all experimental cows. As the research farm was 100% spring-calving (January to March), all trial cows were selected before the start of the calving season (early January). Fifty-four cows of the following characteristics were blocked and balanced equally across the three different systems (CONV, FT, and PT) by (mean \pm standard deviation, where applicable): cow breed (70% Holstein-Friesian and 30% Holstein-Friesian \times Jersey (>25% Jersey)), parity (mean = 2.4; range 1 to 5; 16 parity 1, 19 parity 2, 19 parity 3+), previous 35-week lactation cumulative milk yield (4677 ± 1047.4 kg; in the case of primiparous animals this was based on their dams first lactation milk yield), previous lactation SCS (4.9 ± 0.44 ; in the case of primiparous animals this was based on their dam's first lactation SCS), pre-calving BW (599 ± 65.8 kg), pre-calving BCS (3.22 ± 0.173 ; 5-point scale; Edmonson et al., 1989), expected calving date (16 February 2021 \pm 15 d), expected calf sex and breed (sexed semen artificial insemination – dairy bull, conventional artificial insemination – dairy bull, natural insemination – beef bull), and Economic Breeding Index ($\text{€}176 \pm \text{€}33.9$; see Berry et al., (2005b) for more details). Trial cows entered the systems once they had calved, which occurred over an 8 week period (a typical distribution for the Irish system). The randomised complete block design was completed by an individual independent to the study using Microsoft Excel. Sample size calculations were completed, based on previous experimental results, using cow daily MMY and calf plasma immunoglobulin G, and gave a group size of 18. Eighteen cow-calf pairs were enrolled per system, but 4 pairs from the FT group had to be removed early in their lactation and were not replaced due to lack of additional cow availability. One FT cow (parity 1) was removed at 3 days in milk due to failure to bond with her calf. Three FT cows (one parity 3 and two parity 4) were removed due to their calves becoming sick and requiring intervention (i.e. removal from dam to be placed into a hospital pen in the calf shed for treatment and monitoring; done at the discretion of the farm manager and veterinarian) at 10, 11, and 14 days in milk. One PT pair (parity 2) was removed from the system at 11 days in milk due to calf illness, but this occurred early enough in the calving season that we were able to replace the pair with a similar cow yet to calve. One PT cow (parity 3+) was culled from the herd at week 11 of lactation for reasons unrelated to this experiment, so was

retained in all analyses where appropriate (included in all CCC period analyses but not included milk data analysis of weeks 13-35 or weeks 1-35). Final cow numbers per system for analysis were: CONV system, 18 cow-calf pairs (13 female dairy calves and five male beef calves); FT system, 14 cow-calf pairs (10 female dairy calves, two female beef calves, and two male beef calves); PT system, 18 cow-calf pairs (10 female dairy calves, one male dairy calf, two female beef calves, and four male beef calves). Caretakers and researchers were not blind to the systems due to the highly differentiable attributes of each system. Measurements were performed using each individual cow as the experimental unit. This trial consisted of two phases: the active experimental phase (calving to week 12 of lactation; included CCC period and weaning and separation) and the rest of the lactation (weeks 13-35).

Management of different systems

Conventional cows and calves were separated within 1 h post-birth. After separation, colostrum was collected from the CONV cows; following which they joined a grazing herd where they were managed independently following typical Moorepark grazing management practices (see below for more details). Conventional cows were offered a predominantly grazed grass diet (>85 %), did not come into contact with any calves, and were milked twice-a-day (0700 h and 1500 h).

The FT cows were allowed continuous (24 h/d), unrestricted access to their calves, apart from milking times, until weaning and separation, which occurred during their 9th week of lactation (more information on weaning and separation can be found below). The FT pairs were kept together primarily outside at grass, but were housed indoors depending on weather and grass availability (see grazing management section for more details). When the FT pairs were housed, they had access to identical but adjacent facilities to the PT pairs, as described below (and in Figure 1). The FT cows were milked twice-a-day (0800 h and 1600 h); during milking times calves were left at pasture, separated outside the collecting yard, or, in the straw-bedded pen, if indoors, and were reunited immediately after milking.

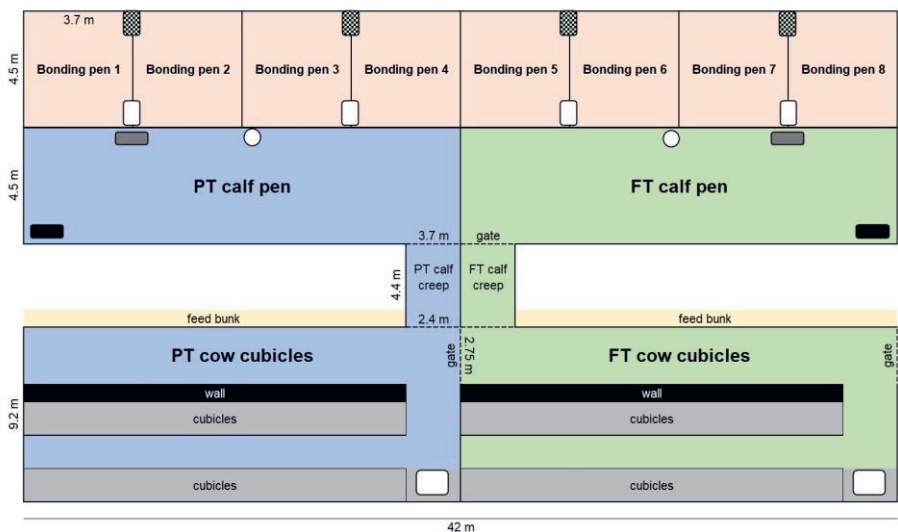


Figure 1. Indoor housing and pens, including the bonding pens, for cow-calf pairs in the full-time access (FT) and part-time access (PT) cow-calf contact systems. White-filled boxes and circles represent water troughs, black-filled boxes represent concentrate feeders, grey-filled boxes represent forage (hay) feeders, and black and white boxes represent grass silage feeders. The gates separating the FT and PT calf pens from the bonding pens were solid and did not enable any contact between groups. The gate between the FT and PT calf pens, creep areas, and cubicle areas did allow for physical contact between groups. The diagram represents a portion of a shed.

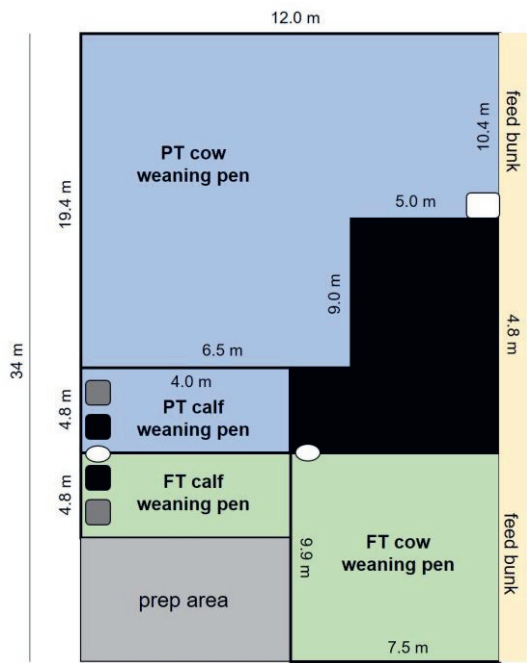


Figure 2. Indoor weaning and separation areas for cow-calf pairs in the full-time access (FT) and part-time access (PT) cow-calf contact systems. All areas were separated by gates that allowed for some degree of physical contact. The black area represents other pens not used for the weaning and separation process. The prep area (grey area) was not an animal-housing area. White-filled boxes and ovals represent water troughs, black-filled boxes represent concentrate feeders, and grey-filled boxes represent forage (hay) feeders.

The PT cows were allowed part-time (17 h/d), unrestricted access to their calves until weaning and separation, which occurred during their 9th week of lactation. During the night (1500 h to 0800 h), the PT cows were housed indoors in the cubicle area (Figure 1) and the gates that connected the calf pen and cubicle area (creep area) were open, allowing calves to enter the cubicle area. Cows could not access the straw bedded calf pen. When indoors, cows were provided with *ad libitum* grass silage; PT calves were also able to access the grass silage during contact times once they were tall enough to reach over the feed bunk. The PT cows were milked OAD in the morning (0800 h). Part-time cows were not milked in the afternoon to ensure that they would have milk when they returned to their calves in the afternoon and to reduce the labour associated with the system. The PT calves were not supplemented with any milk. If calves were in the cubicle area at the time of milking, they were moved to the calf pen and the gates were closed, preventing calf access to the cubicle area during the day. After morning milking, cows went outside to grass (a separate paddock to the FT pairs) where they stayed until 1500 h, when they returned directly inside to be reunited with their calves. The PT cows returned to twice-a-day milking as soon as weaning commenced (start of week 9 of lactation). During periods of inclement weather when it was deemed unsuitable to let cows out to grass by day, PT cows remained housed indoors, where they could see and hear their calves but had no access (gates separating calf pen from the cubicle area were closed).

Selection of different systems

As the success of pasture-based dairy production systems relies on maximizing the number of grazing days and amount of grass that can be utilized (Kennedy et al., 2005), it was necessary for this preliminary study to choose CCC systems that would work in tandem with the pre-existing system (CONV). The FT system was chosen as it mimicked the conventional, pasture-based system in Ireland, with the addition of keeping calves with their dam at grass (as well as indoors). However, as we thought that the FT system may not be feasible in non-experimental settings for various reasons (i.e. concerns about calf health during inclement weather and cold temperatures at pasture, additional labour requirements, and health and safety issues associated with the systems, such as separating cows from calves at milking times), we also chose to include the PT system. We thought that the PT system, which allowed calves to be kept indoors, would eliminate the pasture-related calf health concerns. To ensure that cows had milk when returning to their calves (lowering the risk of teat damage), we decided that the PT cows would only be milked OAD in the morning. Early lactation OAD

milking is a common strategy on seasonal calving dairy farms to reduce labour (Deming et al., 2018; Edwards et al., 2020; Kennedy et al., 2021). We acknowledge that there were several other iterations of the CCC systems that we could have implemented, however, we chose these two CCC systems as we felt they would be the most feasible within an Irish context.

Pre-calving (dry period) management

During the dry period, prior to the experiment, all cows were managed similarly. Target dry-off BCS was 2.75 (5-point scale; Edmonson et al., 1989), to allow for a target calving BCS of 3.25. Cows were housed indoors in a cubicle shed, provided with access to *ad libitum* grass silage and water, and supplemented with concentrates (1 kg/d) and pre-partum minerals. Cows were closely monitored and when calving was imminent (within 3-5 days) cows were moved from a cubicle shed to a straw-bedded pen located adjacent to the calf house. None of the trial cows calved in the cubicle shed.

Calving management and the bonding period

Immediately before calving, cows were brought into individual maternity pens. Conventional system cows and calves were separated within 1-h post-birth and treated as described in the above section. For CONV cows, cow-calf separation was performed by farm staff or a researcher during the day (0630h to 1830h) and by a night-watchman during the night (1830 h to 0630 h). Full-time and PT pairs were not separated at birth but moved to an individual bonding pen (Figure 1; approx. 17 m²) in a separate shed post-calving, where they stayed for a minimum of 48 h to allow for bonding. The pairs were not disturbed or removed during this period, except for the calf being removed for <5 min for a blood sample taken 24 h post-birth (to test for immunoglobulin G levels, data not included here). The pen had a water bowl and the cow was provided with *ad libitum* grass silage. The calf was not artificially fed colostrum, but rather nursed colostrum from its mother. Calves were not assisted in suckling, but the pairs were frequently monitored by researchers and farm staff to ensure bonding and nursing was occurring (approximately once every 1-2 h during the day; if lack of bonding was suspected, the night-watchperson was asked to check the pair 1-2 times during the night). Only on one occasion (described above) did a cow reject her calf (cow and calf stayed on separate ends of pen, farm staff intervened to provide colostrum) and thus was removed from the trial. Failure to bond was suspected in another pair (disinterest from cow, but no violence detected towards the calf), but after being given an extra bonding day, were determined to have bonded sufficiently (cow

appeared interested in calf and multiple nursing events were observed). Both instances occurred in parity 1 cows. Cows were milked for the first time in the milking parlour at the next scheduled milking after the 48 h bonding period was complete, and cows and calves joined the rest of the pairs in their respective system at this time.

Milking management

All cows were milked using a mid-line, 30 unit side-by-side parlour (Dairymaster, Ireland). The automatic cluster removers had a milk flowrate cut-off point of 0.2 kg/min with a 3 s time delay. Unless the specific system required it, the standard farm milking times were 0700 h to 0900 h (morning milking) and 1430 h to 1630 h (afternoon milking).

Grazing management

The experimental paddocks consisted of a permanent grassland site, which was approximately 7 years old at the time of the experiment; the pasture primarily consisted of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*). Pastures were rotationally grazed, with a target post-height of 3.5 to 4 cm during the first grazing rotation and 4 to 4.5 cm from the second rotation onwards. Fresh pasture was allocated, using a single strand temporary electric fence, after every milking during the first grazing rotation (start 30 January 2021; 62 d long); from the second rotation onwards pasture was allocated on a 24 h basis. Calves in the FT system were able to walk under the temporary electric fence and access the fresh pasture. Each system was allocated an individual farmlet that was grazed rotationally. Pastures were of similar age, composition and soil type for each system. While all systems grazed individually, they were located in adjacent paddocks to ensure pastures of similar composition were offered simultaneously.

In the CONV and FT systems, a total daily allowance (pasture and concentrate) of 18 kg DM/d was offered to each cow. The rate of concentrate supplementation ranged from 1 to 4 kg/d and was dependent on grass availability (i.e. if 16 kg DM/d of grass was available, 2 kg/d of concentrates was provided). Concentrate supplementation was common across all systems and was provided in the parlour during milking by an automatic concentrate feeder (CONV and FT cows split evenly between the morning and afternoon milkings; PT cows received all at morning milking). If the feed deficit (i.e. low grass availability) was so large that more than 4 kg concentrate/cow per d was required, grass silage was offered to meet the remainder of the deficit. Conventional and FT cows grazed fulltime (day

and night) from 26 February to the end of the study period (14 November), except during periods of inclement weather or low grass availability, and during weaning and separation for the FT cows.

In the PT system, pasture was allocated to achieve a post-grazing height of 3.5 to 4 cm during their 6 h grazing window; they were offered *ad libitum* grass silage when indoors at night. Part-time cows grazed by day until after weaning and separation (described in more detail below). After weaning and separation, PT cows grazed fulltime until the end of the study.

During periods of moderate inclement weather, restricted access to pasture was practiced (on/off grazing), to minimize the risk of poaching/pugging damage (Kennedy et al., 2009; Kennedy et al., 2011). During on/off grazing periods, the cows grazed for 3 h periods after each milking (6 h/d total). When removed from the paddock they returned to their respective housing (see above for more details). The same management strategy for the CONV and FT cows was used during periods of low grass availability (30 January to 26 February 2021) during the first rotation. As the PT cows only ever grazed by day (6-7 h/d) during the CCC period and could not graze after the afternoon milking due to their system requirements (indoors by night with calf), they never practiced on-off grazing. During periods of severe inclement weather, when cows needed to be housed fulltime (8 d total; 13-15 February, 19-21 February, and 23-24 February), they were housed by system and kept fully indoors and were provided with grass silage (14 kg DM/cow silage and 4 kg concentrates).

Weaning and separation

Conventional cows were separated from their calf at birth, thus did not go through the same weaning and separation process as the PT and FT pairs. Full-time and PT pairs were weaned based on calf age (mean \pm standard deviation; 58 ± 3.9 d) over a 7 d period using a gradual, three-stage process initiated by moving the FT and PT pairs to a different, separate shed. This was done on a system basis; the FT and PT pairs were kept separately until the end of the weaning and separation process (see Figure 2). To prevent unnecessary distress, pairs were not individually weaned; a minimum of two pairs underwent the process each time. The weaning and separation process was also only initiated twice a week (Monday or Thursday), to better allow small groups to be created based on calf age. As a result, two weaning and separation groups might have been housed together at once.

To initiate the weaning process, cows and calves were separated and placed in adjacent straw-bedded pens equipped with gates that allowed for the

exchange of visual, auditory, and tactile cues, but prevented suckling (Figure 2). Both cow and calf pens were equipped with water bowls. Cows had access to *ad libitum* grass silage in the pen and were fed concentrates (3 kg/d) in the milking parlour. Calves had *ad libitum* access to concentrates, grass silage, and hay. During the first stage (3 d period), cows and calves were allowed 1 h of unrestricted contact around 1 h after the morning milking (milking finished from 0900 h to 0930 h, contact was allowed from approximately 1030 h to 1130 h) and calves could suckle. For the rest of the day, the pairs could interact through the gate (restricted access) but no suckling could occur. The pairs were temporarily separated for the morning and afternoon milkings, where cows were removed from the shed and then were returned to their pens immediately after milking. During the second stage (2 d period), pairs were not allowed direct contact: pairs could interact through the gate but no suckling could occur. At the start of the third stage (2 d period), the pairs were fully separated. Calves remained in their pens during this period. Cows did not return to the shed after morning milking; they joined the general herd of cows at pasture and remained there for the rest of their lactation, where they were managed similarly to the CONV cows.

Measurements

Cow Production Measurements

Machine milk yield (kg/d) was recorded daily for each cow for their entire lactation (Dairymaster). Milk samples (1 composite sample/week) were obtained from each cow weekly on a consecutive evening and morning milking, meaning that the PT cows' samples during the CCC period were only collected during the morning milking. Milk composition (milk fat, protein, and lactose concentrations) and somatic cell count were determined using a Milkoscan FT6000 (Foss Electric DK). Each week, daily MMY was averaged across all 7 d to give an average daily MMY for each cow for that week. Milk fat and protein concentrations were used for calculating average and cumulative MSY (average daily MSY = (daily MMY * milk fat concentration of corresponding week) + (daily MMY * milk protein concentration of corresponding week); cumulative = average daily MSY * 7) for each week of lactation.

Cow Health and Welfare Measurements

Body weight and BCS were recorded following morning milking weekly for the first 12 weeks of the lactation and for 2 consecutive weeks at approximately 35-weeks of lactation (i.e. weeks 35 and 36). After exiting the parlour, cows entered a race that ended in a crush with a sliding backing gate. An electronic portable

weigh-scale with Winweigh software package (Tru-test Limited) was placed at the end of the crush, and body condition was scored by a single observer (intra-observer reliability; weighted kappa = 0.9589) when the cow was on the scales (BCS scale from 1 = emaciated to 5 = extremely fat, with 0.25 increments; Edmonson et al., 1989).

Weekly somatic cell counts (obtained using the method described above) were converted to SCS (log₁₀ of somatic cell count) further analysis. Incidence of mastitis was considered to have occurred when a teat was treated with an antibiotic; all incidences of mastitis were recorded throughout the entire lactation by the farm staff.

Cow clinical health scoring was performed twice a week (Tuesdays and Fridays) over the first 12 weeks of lactation. Nine aspects of cow health were scored (demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, and faecal hygiene) using a health scoring method (Supplementary Table S1), adapted from Barry et al. (2019a) and used in cows by Crossley et al. (2022a; 2022b). Health scoring was performed by two observers (inter-observer reliability: 89% agreement first attempt, 97% agreement second attempt). A total clinical health score was calculated by summing the nine aspects of clinical health (see Supplemental Table S1 for more details), where a higher score indicates a less healthy cow.

Locomotion was scored by a single independent observer (intra-observer reliability; weighted kappa = 0.9367) over the 11-week scoring period. After morning milking, cows individually exited a race and walked past the observer on a clean, level, open concrete surface heading towards pasture. Five aspects of cow locomotion (spine curvature, tracking, ab/adduction, speed, and head bob) were scored from 1 to 5 (where 1 = perfect; 5 = most impaired) using a system described and adapted by O'Driscoll et al. (2010). A total score for each cow was calculated by summing the five aspects of locomotion, where a higher score indicates poorer locomotion.

Udder Measurements

Milk leakage and udder firmness were assessed immediately prior to morning milking by the same observer on a weekly basis during the first 12 weeks of lactation. General monitoring of the state of the teats (i.e. if there was any damage to the teat) was observed weekly at this time but was not scored; however, no damage, from nursing was observed in any cows. Observations were performed once cows had entered the milking parlour and were standing in the milking stall, but before teat preparation, as this is the time when intramammary pressure is

assumed to be highest (Gleeson et al., 2007). Milk leakage was scored as positive (i.e. milk leakage from one or more teats) or negative (i.e. no milk leakage), further described in Kennedy et al. (2021). Udder firmness was assessed by manually palpating both rear quarters of the udder between the cows' hind legs and assigning a score on a 3-point scoring system described by Gleeson et al. (2007), where: score 1 = soft, udder yields significantly to gentle pressure from the fingers; score 2 = firm, udder yields slightly to gentle pressure from the fingers; score 3 = hard, the udder tissue does not yield to gentle pressure from the finger tips. If there was a significant difference between quarters (e.g. score of 3 on left rear quarter and score of 1 on right rear quarter), the score on both sides were recorded. For the purpose of analysis, only the higher score was used in the statistical analysis. Milk leakage was scored before udder firmness to prevent potential milk let-down caused by palpation of the udder. Both milk leakage and udder firmness were scored by a single observer.

Udder characteristics were scored during each cow's first appearance in the milking parlour (CONV cows: first milking post-calving; FT and PT cows: first milking post 48 h bonding period). During this time, the following measurements were taken: udder clearance (distance between the ground and the medial suspensory ligament; cm), relative teat placement (front and rear; score and distance; cm), teat length (all teats; cm), and teat-end hyperkeratosis (score). Udder clearance (cm), relative teat placement (front and rear teats; score), and teat-end hyperkeratosis (score) were also assessed at weeks 4, 8, and 12 of the lactation.

Statistical analysis of data

All data was analysed using SAS (v9.4; SAS Institute). The procedures PROC UNIVARIATE and PROC MEANS were used to test normality against the residuals of all variables; where data sets were considered normal if $P > 0.01$ using the Shapiro-Wilk test. The shape of the histogram of the residuals was also examined. If a data set had $P > 0.01$, then the dataset was checked for outliers. Two variables had binomial distributions (udder firmness and milk leakage) and were analysed using PROC LOGISTIC. Cow was used as the subject in all mixed models. The Kenwood-Rogers method of determining denominator degrees of freedom was used for all ANOVAs. For all analyses with more than 2 weeks included, the covariance structure that gave the lowest BIC was used (compound symmetry, autoregressive lag 1, heterogeneous autoregressive lag 1, and unstructured were all tested; only compound symmetry and autoregressive lag 1 were used; see Supplemental Table S2 for variance components). The Tukey-

Kramer test was used for all post-hoc pairwise comparison tests between fixed effects. The threshold for significance was $P < 0.05$ and tendencies were $P < 0.10$. Descriptive statistics (mean and standard deviation) were calculated in SAS for the health variables (total clinical health and total locomotion scores). Mean, variance, SEM, and coefficients of variation for all measured study variables are provided in Supplemental Table S3.

For the purpose of analysis, a three-tier parity structure was used: parity 1, $n = 15$; parity 2, $n = 19$; parity 3+, $n = 16$). Although the initial systems were blocked by parity, it was included in the models to help account for the difference in group-size. The FT cows that were removed from trial (due to calf health issues – see above for more details) were disproportionately of parity 3+ (one parity 1 cow, one parity 3, and two parity 4); when model fit was tested with and without parity, BIC was lower with parity included as a fixed effect. However, we do not report it as we did not set out to estimate the effects of parity.

All variables (except udder firmness and milk leakage) were analysed using a linear mixed model (PROC MIXED). The model contained the fixed effects of system (CONV, FT, PT), parity (primiparous, second lactation, third lactation or higher), and cow breed (Holstein-Friesian, Holstein-Friesian x Jersey). Week was included as a fixed effect and a repeated measure where appropriate. Days in milk on 1 June 2021 was used as a covariate to account for the difference in calving date. For milk variable covariates, previous lactation cumulative or average values (for primiparous cows, their dam's first lactation data was used) that had been centred within parity (individual animal values were subtracted from the average value of each parity) were used as covariates: previous lactation cumulative MMY for MMY data, previous lactation average fat percentage for fat concentration data, and so on. Sub-indices of the Economic Breeding Index were used as a covariates where appropriate (i.e. health sub-index for health-related variables, milk sub-index for milk-related variables, etc.; Kennedy et al., 2021; Murphy et al., 2023). The initial (week 1) BW or BCS was used as a covariate in those models to account for any initial differences.

For milk-associated measurements, only the first 35-weeks of lactation are used, as after that time point some cows were dried off, depending on their subsequent spring calving date. Machine milk yield, MSY, SCS, and milk components (fat, protein, and lactose concentrations) were analysed as an average during three time periods: i) week 1 to 8 of lactation (CCC period), ii) weeks 9 to 35 of lactation (post-CCC period), and iii) weeks 1 to 35 (full 35-week lactation). Cumulative lactation MMY and MSY (weeks 1 to 35) were also calculated and analysed. Body weight and BCS were analysed at the end of weeks

4, 8, 12, and 35 by taking the average weight or score from the last two weeks of the respective time period. In addition, for weeks 4, 8, and 12, the average change in BW (average daily change in BW over the 28 d period) and BCS (change in score from previous period) within that period was analysed. The average change from weeks 1 to 12 was also calculated and analysed for BW and BCS.

Logistic regression (PROC LOGISTIC) was used to analyse udder firmness and milk leakage. The model included the fixed effects of system (CONV, FT, PT), week of lactation (1 to 12, udder scores), parity (primiparous, second lactation, third lactation or higher), and cow breed (Holstein-Friesian, Holstein-Friesian x Jersey). Covariates included days in milk and the appropriate sub-indices (health, management, or milk) of the Economic Breeding Index (centred within parity). The CONV cows, primiparous cows, Holstein-Friesian cows, and week 1 were designated as the reference categories (odds ratio (OR) = 1).

Results

Cow Production

Machine milk yield and milk solids yield

An overview of MMY per system per week can be found in Fig. 3. During the CCC period (weeks 1-8), there were effects of system on daily MMY and MSY (Table 1). All systems differed, with the CONV cows having the highest MMY and MSY, followed by the FT, and then the PT cows. After the CCC period (weeks 9-35), the effect of system continued; CONV cows remained higher in MMY and MSY compared to the FT and PT cows. Across the entire lactation (weeks 1-35), average and cumulative MMY and MSY were affected by system. All systems differed, with the CONV cows yielding the most, followed by the FT, and then the PT cows (Table 1).

Milk composition

During the CCC period (weeks 1-8) there was an effect of system on milk fat concentration; the CONV cows produced milk with a higher fat concentration than the FT and PT cows (Table 1). There was no effect of system on milk fat concentration for the rest of the lactation (weeks 9-35) and overall (weeks 1-35). An overview of milk fat concentration per system per week can be found in Supplemental Fig. S1.

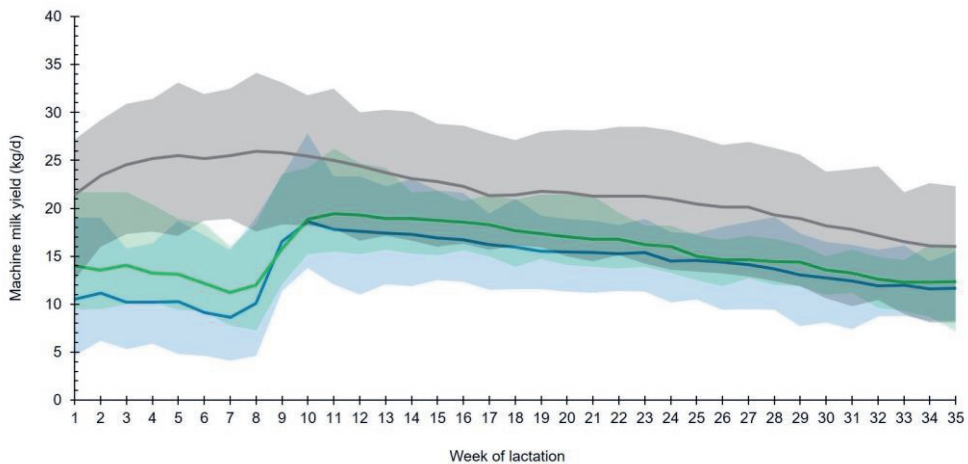


Figure 3. Statistical means and range for average daily machine milk yield (kg/d) for dairy cows in three different cow-calf contact rearing systems (conventional (CONV; grey): no access to calf and milked twice-a-day; Full-time access (FT; green): full-time access to calf and milked twice-a-day; Part-time access (PT; blue): part-time access to calf and milked once-a-day) by week across the 35-week lactation. The shaded bars represent the range of machine milk yields observed in each system during each week of lactation.

There was a tendency for an effect of system on milk protein concentration during the CCC period (Table 1). For the rest of the lactation (weeks 9-35), there was an effect of system, but the system means were inconclusive in the post-hoc comparison test. When averaged across all weeks of lactation (weeks 1-35), there was an effect of system, with the CONV cows producing milk with a higher milk protein concentration than the FT cows, while the PT cows were inconclusive (Table 1). An overview of milk protein concentration per system per week can be found in Supplemental Fig. S2.

There was an effect of system on milk lactose concentration during the CCC period, with the CONV cows producing milk with a higher lactose concentration than the PT cows, while the FT were inconclusive (Table 1). For the rest of the lactation (weeks 9-35), there was a tendency for milk lactose concentration to differ (Table 1); however, there was no effect of system (Table 1) when milk lactose concentration was averaged across all 35 weeks. An overview of milk lactose concentration per system per week can be found in Supplemental Fig. S3.

Somatic cell score and mastitis incidence

There was an effect of system on SCS during the CCC period (Table 1); the PT cows had a higher SCS compared to the FT cows, while the CONV cows were inconclusive. An overview of milk lactose concentration per system per week can be found in Supplemental Fig. S4. Eleven incidences of clinical mastitis (three CONV cases, four FT cases, four PT cases) occurred in seven trial cows (two CONV cows; two FT cows; three PT cows) over the 35-week lactation. Four out of the 11 incidences occurred during the first eight weeks of lactation (one CONV cow, one PT cow, and one FT cow with two occurrences in the same quarter).

Table 1 Effect of three different calf rearing systems (Conventional (CONV): cow and calf separated at birth and cow milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day) on average machine milk yield, milk solids yield, somatic cell score, and milk composition (fat, protein, and lactose concentration) during different periods across the first 35-weeks of lactation.

Variable	Systems			SEM	P-value
	CONV	FT	PT		
Machine milk yield (kg/d)					
Weeks 1 to 8	24.0 ^a	13.6 ^b	10.3 ^c	0.50	<0.001
Weeks 9 to 35	20.2 ^a	16.6 ^b	15.7 ^b	0.48	<0.001
Weeks 1 to 35	21.1 ^a	16.0 ^b	14.4 ^c	0.42	<0.001
Cumulative weeks 1 to 35 (kg)	5072 ^a	3872 ^b	3499 ^c	97.0	<0.001
Milk solids yield, kg/d					
Weeks 1 to 8	2.14 ^a	1.10 ^b	0.82 ^c	0.50	<0.001
Weeks 9 to 35	1.79 ^a	1.45 ^b	1.34 ^b	0.48	<0.001
Weeks 1 to 35	1.87 ^a	1.37 ^b	1.22 ^c	0.42	<0.001
Cumulative weeks 1 to 35 (kg)	450 ^a	332 ^b	294 ^c	8.7	<0.001
Milk fat concentration (%)					
Weeks 1 to 8	5.36 ^a	4.56 ^b	4.70 ^b	0.157	0.001
Weeks 9 to 35	5.04	5.05	4.88	0.117	0.527
Weeks 1 to 35	5.12	4.94	4.85	0.117	0.253
Milk protein concentration (%)					
Weeks 1 to 8	3.57	3.44	3.48	0.043	0.085
Weeks 9 to 35	3.86	3.68	3.70	0.054	0.034 ¹
Weeks 1 to 35	3.79 ^a	3.62 ^b	3.65 ^{ab}	0.049	0.036
Milk lactose concentration (%)					
Weeks 1 to 8	4.80 ^a	4.74 ^{ab}	4.67 ^b	0.030	0.008
Weeks 9 to 35	4.72 ^b	4.80 ^a	4.76 ^{ab}	0.021	0.050
Weeks 1 to 35	4.74	4.79	4.74	0.021	0.208
Somatic cell score					
Weeks 1 to 8	4.94 ^{ab}	4.70 ^b	5.15 ^a	0.118	0.039
Weeks 9 to 35	4.98	4.85	5.12	0.133	0.388
Weeks 1 to 35	5.01	4.86	5.15	0.126	0.305

¹ System means did not differ significantly at $P < 0.05$. Means were compared with Tukey's adjustment. Values within a row with different superscripts differ significantly at $P < 0.05$.

Health

Body weight and body condition score

An overview of BW per system per week can be found in Supplemental Fig. S5. There was an effect of system on cow BW at the end of weeks 4 and 8 of lactation (Table 2), where the PT cows were heavier than the CONV and FT cows. However, there was no difference between the three systems at the end of week 12 of lactation. At the end of week 35 of lactation, there was an effect of system on BW, with the PT cows heavier than the FT cows, and the CONV cows were inconclusive (Table 2). There was an effect of system on average daily change in BW from weeks 1 to 4 and week 8 to 12. From weeks 1 to 4, the PT cows were gaining weight, while the CONV and FT cows were losing weight, and from weeks 8 to 12, the PT cows were losing weight while the CONV and FT cows were gaining weight (Table 2). There was no effect of system on daily change in BW for the other time periods.

An overview of body condition score per system per week can be found in Supplemental Fig. S6. There was an effect of system on BCS during weeks 4, 8, 12, and 35 of lactation (Table 2). The PT cows had a higher BCS score than the CONV cows at all observed time points. The FT cows were different to the PT cows at weeks 8 and 35, and all systems were inconclusive at weeks 4 and 12. There was an effect of system on average change in BCS from weeks 1 to 4 ($P = 0.024$; Table 2); the CONV lost condition and the PT cows maintained condition, while the FT cows were inconclusive. There was also an effect of system on average change in BCS from weeks 1 to 12 (Table 2); the PT cows maintained condition while the CONV and FT cows lost condition.

Health parameters

Basic descriptive statistics for total clinical health score (sum of all components: demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, and faecal hygiene) and total locomotion score (sum of all components: spine curvature, tracking, ab/adduction, speed, and head bob) can be found in Table 3. There were effects of system ($P = 0.015$) and week ($P < 0.001$) on total clinical health score. The CONV cows (mean \pm standard error; 1.3 ± 0.08) had higher total clinical health scores than the PT cows (0.9 ; $P = 0.012$), and tended ($P = 0.057$) to have higher total clinical health scores than the FT cows (1.0). Cows had the lowest total clinical health scores in week 1 (0.6 ± 0.09) and had increased in score by week 4 (0.9 ; $P = 0.010$). Cows in weeks 10 (1.4) and 11 (1.4) had the highest total clinical health scores, and

differed ($P < 0.05$) from weeks 1 through 4 (but not 5 to 9). There was no effect of system ($P = 0.849$) on total locomotion score (sum of the five scored aspects of locomotion), but there was an effect of week ($P < 0.001$). Locomotion scores were lowest in weeks 2 (8.4 ± 0.23) and 3 (8.4) compared ($P < 0.05$) to the higher scores in weeks 10 (9.2) and 11 (9.1); all other weeks were inconclusive ($P > 0.05$) when compared to week 1. When the individual components of the locomotion score were analysed there were no significant effects of system.

Table 2 Effect of cow-calf contact systems (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day) on BW, average daily change in BW, body condition score, and average change in body condition score. The values reported for week 1 BW and body condition score were included in the statistical analysis as covariates, thus were not analysed.

Variable	Systems			SEM	P-value
	CONV	FT	PT		
BW (kg)					
Week 1	522	509	515	58.8*	-
Week 4	474 ^b	488 ^b	523 ^a	4.9	<0.001
Week 8	479 ^b	487 ^b	520 ^a	6.8	<0.001
Week 12	490	489	504	7.2	0.242
Week 35	525 ^{ab}	505 ^b	535 ^a	8.1	0.049
Average daily change in BW (kg/d)					
Week 1 to week 4	-1.50 ^b	-1.01 ^b	0.25 ^a	0.175	<0.001
Week 4 to week 8	0.19	-0.02	-0.10	0.210	0.586
Week 8 to week 12	0.41 ^b	0.05 ^b	-0.56 ^a	0.145	<0.001
Week 1 to week 12	-0.30	-0.32	-0.14	0.086	0.246
Body condition score					
Week 1	3.14	3.25	3.15	0.168*	-
Week 4	3.01 ^b	3.07 ^{ab}	3.14 ^a	0.034	0.024
Week 8	2.94 ^b	3.02 ^b	3.16 ^a	0.035	<0.001
Week 12	2.93 ^b	3.07 ^{ab}	3.22 ^a	0.044	<0.001
Week 35	3.03 ^b	3.01 ^b	3.19 ^a	0.043	0.009
Average change in body condition score					
Week 1 to week 4	-0.16 ^b	-0.10 ^{ab}	-0.03 ^a	0.034	0.024
Week 4 to week 8	-0.07	-0.05	0.02	0.034	0.133
Week 1 to week 12	-0.01	0.04	0.06	0.035	0.354
Week 1 to week 12	-0.25 ^b	-0.11 ^b	0.04 ^a	0.044	<0.001

Abbreviations: SEM = pooled standard error of the mean.

* standard deviation is reported rather than SEM.

Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 3 Basic descriptive statistics (mean and standard deviation) for the total scores (sum of all component scores) for locomotion and clinical health in cows in three different cow-calf contact rearing systems (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day).

Variable	Systems					
	CONV		FT		PT	
	Mean	SD	Mean	SD	Mean	SD
<i>Total locomotion score</i>						
Week 1	8.8	1.88	8.7	1.33	8.1	1.17
Week 2	8.8	1.93	8.4	1.15	8.2	0.94
Week 3	8.4	1.50	8.4	1.08	8.6	1.33
Week 4	8.8	1.70	8.6	1.55	8.8	1.52
Week 5	8.8	1.62	8.2	1.37	8.8	1.48
Week 6	9.6	1.82	8.6	1.34	8.8	1.44
Week 7	9.3	1.53	8.7	1.64	8.8	1.55
Week 8	9.2	1.42	8.7	1.37	8.8	1.56
Week 9	9.2	1.69	8.9	1.14	8.8	1.52
Week 10	9.3	1.64	9.1	0.99	9.5	1.61
Week 11	9.4	1.85	9.3	1.35	8.9	1.24
<i>Total clinical health score</i>						
Week 1	0.6	0.65	0.7	0.66	0.5	0.61
Week 2	0.8	0.80	0.9	0.74	0.8	0.64
Week 3	1.1	0.93	0.8	0.89	0.7	0.77
Week 4	1.2	0.89	0.8	0.76	0.9	0.83
Week 5	1.3	0.79	1.1	1.03	0.9	0.84
Week 6	1.3	0.79	1.0	0.88	0.9	0.89
Week 7	1.5	0.85	1.1	0.94	0.9	0.75
Week 8	1.3	1.06	0.9	1.02	1.0	0.87
Week 9	1.8	0.92	0.8	0.65	0.9	0.69
Week 10	1.3	0.79	1.4	0.92	1.5	0.75
Week 11	1.7	0.78	1.3	0.87	1.3	0.96

Table 4 Udder composition measurements taken from the first parlour milking of cows in three different cow-calf contact systems (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day).

Variable	Systems			SEM	P-value
	CONV	FT	PT		
Teat length (cm)					
Front right	5.1	5.0	5.2	0.24	0.915
Front left	5.0	4.9	5.1	0.27	0.891
Rear left	4.4	4.9	4.3	0.22	0.168
Rear right	4.5	4.5	4.5	0.19	0.975
Front teat position (cm)	13.0	15.3	14.5	0.81	0.136
Rear teat position (cm)	6.8	7.9	7.0	0.83	0.602

Abbreviations: SEM = pooled standard error of the mean.

Values within a row with different superscripts differ significantly at $P < 0.05$.

Udder Conformation and Scoring

At the first milking post-calving, there was no effect of system (Table 4) on teat lengths and front and rear teat placements (distance in cm, not score). None of the monthly udder scores (udder clearance, front and rear teat placement score, and teat-end hyperkeratosis) were affected by system (Table 5). There were effects of system ($P = 0.002$) and week ($P < 0.001$) on udder firmness. The FT (OR = 0.399, CI = 0.224 – 0.711, $P = 0.002$) and PT (OR = 0.424, CI = 0.224 – 0.737, $P = 0.002$) cows were less likely to have a firm udder across the first 12 weeks of lactation compared to the CONV cows. All cows, regardless of system, were less likely to have a firm udder from week 5 (OR = 0.040, CI = 0.013 – 0.120, $P < 0.001$) onwards, compared to week 0. There were no effects of system ($P = 0.405$) or week ($P = 0.090$) on milk leakage.

Discussion

Implementing cow-calf contact in a seasonal calving, pasture-based dairy system

The first aim of this preliminary study was to investigate whether our two different CCC systems could feasibly be incorporated into the Irish spring-calving, pasture-based system. To do this, we compared three different calf rearing systems within the context of the Irish spring-calving, pasture-based dairy system: CONV, where the cow had no access to her calf and was milked twice-a-day; FT, where the dam-calf pair had constant, unrestricted access to each other and the cow was milked twice-a-day; and PT, where the dam-calf pair had unrestricted access to each other by night and the cow was milked OAD in the morning. In this paper, we specifically report our results on the cows' production (MMY, MSY, milk composition, and SCS) and health (BW, BCS, clinical health, locomotion, and various aspects of udder health and conformation) within the three different systems. Calf-associated measurements, as well as information regarding the labour requirements of each system, can be found in Sinnott et al. (2024). Throughout this manuscript we have tried to emphasize that we compared three different systems to investigate their potential viability, rather than experimental treatments; therefore, variation from several different sources existed between the three different systems. As a result, we investigated not only the impact of novel exposure to CCC, but also the impact of milking frequency, housing, and diet.

We faced several practical implementation challenges over the course of this study, mainly centred on issues with calves, which led to a limitation of this study: group size. The PT pair removed due to calf illness occurred early enough in

the calving season to be replaced. However, the three multiparous FT cows (3+ lactations) that were removed from the trial at the beginning of their lactation due to calf illness could not be replaced as no cows were available to replace them, leading to unequal group sizes and a reduction in statistical power. As this was a preliminary study, investigating first whether it was feasible to implement CCC on a seasonal calving, pasture-based system, it was not possible for us to run multiple replicates of the systems within the same year or across multiple years. However, our rolling intake of cows onto the experiment (as they calved) led to an 8-week enrolment window, causing cows to have slightly different experiences within the same system, increasing the independence and variation between individuals. The experimental farm that this research was performed on is also set up to be a model of a typical Irish dairy farm, with similar housing, managements, and calving rates; as such, we believe that our results are reasonably transferable to other dairy farms in Ireland. However, in the future, a multiple year, multiple farm trial of CCC will be needed to fully understand the feasibility and effects of CCC within seasonal calving, pasture-based systems.

In our PT system, the OAD milking did appear to have an effect on MMY, as well as BW and BCS, which has been typical of non-CCC cows milked OAD (Clark et al., 2006; Kennedy et al., 2021; Murphy et al., 2023). The PT cows also had a slightly different diet (*ad libitum* silage by night) and were housed indoor at night, which may have also contributed to the observed differences between the systems. We believe that the PT system was chosen for valid feasibility reasons (see Selection of management systems section in Materials and Methods); however, we acknowledge that in choosing this PT system, we made the comparison between the systems more difficult. In addition, slightly different management choices (i.e. PT access by day rather than night or milking the PT cows twice-a-day) may have significantly affected the results. Therefore, achieving successful CCC within a seasonal calving, pasture-based system likely will depend on finding the correct combination of factors (i.e. milking frequency, housing, timing of access, labour required, etc.) for each individual farm and farmer.

Cow-calf contact was a novel experience for both cows and humans involved in this study. The cows had never experienced CCC, which may have influenced their mothering ability and their response to weaning and separation. Although some personnel at the research farm (staff and researchers) had experience in keeping beef cows and calves together, all were new to keeping dairy cows and calves together. In addition, all facilities used on the farm were not specifically designed for CCC.

When implementing CCC systems, human health and safety should also be considered. Both of the CCC systems implemented here required the cow-calf pairs to be routinely separated once or twice a day, either for two 1-h periods during milking (FT system) or for several hours while the cows grazed (PT system). For the FT pairs, this separation occurred either at pasture (cows were removed from the field while the calves remained) or in a holding pen beside the parlour. For the PT cows this temporary separation occurred in the cubicle shed. Although no incidences of cow aggression towards humans occurred during this trial, the human safety risks associated with the constant temporary separation of cow-calf pairs required for both systems to operate should be considered.

Effects of cow-calf contact on cow production within our systems

Reduced MMY during the CCC period was expected and observed from the FT and PT cows during the CCC period (weeks 1 to 8). Calves in this study had unrestricted access to their dam, as well as other cows within the same system, enabling the calves to nurse during allowed contact times, and thus decreasing the FT and PT cows' MMY. This result was comparable to other recent CCC studies (Barth, 2020; Wenker et al., 2022a; Neave et al., 2024a), which found a reduction in MMY when calves were able to nurse. Here, calf nursing was also confirmed by the udder firmness scores, which showed that the FT and PT cows had less firm udders than the CONV cows, likely due to calf nursing in between milking events. In addition to the decrease in MMY experienced by the FT and PT cows due to CCC, the PT cows also had a lower MMY than the FT cows during the CCC, yielding around 25% less milk in the parlour. This magnitude of decrease was typical of non-CCC cows experiencing OAD compared to twice-a-day milking frequency (Rémond et al., 2004; Murphy et al., 2023), especially during early lactation (Kennedy et al., 2021).

In addition to their milkings in the parlour, both FT and PT cows were being stimulated to produce milk by their calves multiple times a day. More frequent milking should increase milk synthesis, and thus total milk yield, by increasing the proliferation of mammary cells (Murney et al., 2015); therefore, we expected that although MMY might be decreased during the CCC period, it would only be reduced by the amount that the calves were consuming. Then, after weaning and separation, we expected that the FT and PT cows' MMY would increase to the level of the CONV cows. However, that is not what we observed in this study. Although MMY of the FT and PT cows increased after weaning and separation, they never reached the level of the CONV cows. This effect persisted for the rest of the lactation and, in combination with the lower MMY produced during the CCC

period, resulted in the FT and PT cows producing 24 and 31% lower cumulative MMY (weeks 1-35) than the CONV cows, who produced 5072 kg (a typical cumulative yield for cows in Ireland milked twice-a-day, Kennedy et al., 2021).

The FT and PT cows' MMY may have not reached the level of the CONV cows after weaning and separation because they may have been producing less milk than the CONV cows during the CCC period. Here, the results for milk fat concentration, where the FT and PT cows had lower fat concentrations in the milk yielded at the parlour than the CONV cows during the CCC period, suggest that the FT and PT cows had impaired milk ejection in the parlour. Secretion of low levels of oxytocin can inhibit the milk ejection response in dairy cows, causing a larger amount of milk to be left in the udder post-milking (residual milk; Bruckmaier, 2005). Residual milk has the highest concentration of milk fat, compared to milk produced during the rest of the milking event (Ontsouka et al., 2003). Higher amounts of residual milk in the udder can decrease milk synthesis (Kuehn et al., 2019) and lower MMY, especially in cows experiencing CCC (Metz, 1987; de Passillé et al., 2008). Kuehn et al. (2019) found that the reduction in MMY due to incomplete milking persisted even with an increased milking frequency, which matches what we observed here; even though the FT and PT cows were stimulated more to produce milk via calf nursing, they were likely producing less total milk than the CONV cows.

Although calf intake was likely to have increased over the weeks until weaning and separation, it was unlikely that calf intake alone would account for the entire observed differences in MMY (i.e. during week 8, the FT and PT cows yielded -14.3 and -16.9 kg/d less, respectively, than the CONV cows (25.5 kg/d); raw statistical means; see Fig. 3). The FT and PT calves were of similar weight at 8 weeks old (82 kg), with average daily gains of 0.95 kg/d (averaged from weeks 5 to 8; Sinnott et al., in press). The required intake for an 80 kg large breed calf (i.e. Holstein-Friesian from North America) to gain 1.0 kg/d is 1.63 kg DM/d (NRC, 2021). If the estimated DM of whole milk is 12.5% (NRC, 2021), then the differences in MMY compared to the CONV of the FT and PT cows would yield 1.79 and 2.11 kg DM/d, respectively. If the reduction in MMY was due to calf intake alone, then we would have expected higher growth rates in the calves. Rather, we suggest that the FT and PT cows' milk synthesis had decreased during the CCC period and did not recover for the rest of the lactation.

Weaning and separation of a bonded cow-calf pair is an event known to cause distress in dairy cows and calves (Flower and Weary, 2003), and has previously been shown to temporarily reduce MMY (Everitt and Phillips, 1971; Walsh, 1974; Metz, 1987). Metz (1987) also showed that the change in

environment (moving cows from one shed to another) temporarily decreased MMY in their cows, regardless of whether or not they had just been separated from their calf. In humans, psychological distress is hypothesized to impair oxytocin release (Nagel et al., 2022), thus reducing milk yield by impairing milk ejection, so it is possible that similar mechanisms were occurring in the FT and PT cows in this study. Although the decreases in MMY around weaning and separation found in previous studies were temporary, the timing of weaning and separation here may have further impacted the MMY of the FT and PT cows, causing the temporary decrease in MMY to become a long-term decrease. The CONV cows reached peak MMY around weeks 8-9 of lactation (Fig. 3), which coincided with the weaning and separation process for the FT and PT cows. Milk yield persistency has been shown to be negatively correlated with peak milk yield (Sorenson et al., 2008), so if the FT and PT cows' peak milk yield was reduced by various factors around weaning and separation, then this reduction may have persisted for the rest of the lactation.

Effect of cow-calf contact on cow health and udders within our systems

Previous CCC studies have shown that BW reduces temporarily after weaning and separation from the calf, for around 1-2 weeks (Everitt and Phillips, 1971, Metz, 1987, Bar-Peled et al., 1995). This has been attributed to a difference in feed intake during the distressful period (Metz et al., 1987). The data we presented in Table 2 show the average BW of cows in the different systems at week 12, 3-4 weeks after weaning and separation, so we are not able to determine if there was an immediate response in cow BW to weaning and separation. During week 8 of lactation, the PT cows were heavier than the similar CONV and FT cows, and during week 12, there was no difference in BW between the systems. Over the entire lactation, the PT cows remained in better condition and were numerically heavier than the CONV and FT cows. Although the differing diet of the PT cows may have also contributed to their increased BW, a similar carryover effect of BW and BCS was observed at the end of lactation in a comparison of pasture-based cows milked either OAD or TAD in early lactation with identical diets (Kennedy et al., 2021; Murphy et al., 2023). Although cows in all systems lost BW during the first 12 weeks of lactation, they did so in a different pattern.

Suckling is thought to be beneficial in reducing the risk of mastitis and decreasing SCS (Johnsen et al., 2016; Beaver et al., 2019), despite it being a matter of concern for dairy farmers regarding the implementation of CCC systems (Neave et al., 2022). Here, the FT cows had lower SCS during the CCC period compared to the PT cows, while the CONV were similar to both. There appears to be some effect of suckling on SCS, and it is likely that the difference in SCS

between the FT and PT cows was due to the combination of their differences in milking frequency and housing. Somatic cell score is known to be higher in cows milked OAD (Stelwagen et al., 2013, Kennedy et al., 2021, Murphy et al., 2023); however, this increase in SCS is not typically associated with a change in the risk of mastitis (Stelwagen et al., 2013). Cows housed indoors have more udder health problems (Goldberg et al., 1992), more incidences of mastitis (Washburn et al., 2002), and higher somatic cell counts (Kristensen et al., 2007) compared to cows kept at pasture. However, cows milked OAD and kept at pasture still were shown to have greater SCS during and at the end of the lactation compared to those milked twice-a-day (Murphy et al., 2023).

An increased risk of milk leakage has been a common explanation for why OAD cows often have increased SCS, as open teat sphincters can result in bacteria and other pathogens having easier access to the mammary gland (Gleeson et al., 2007). We did not observe any difference in milk leakage in the PT cows compared to the CONV and FT cows during the CCC period, but this was likely due to the fact that their calves were able to nurse from the PT cows until they went to the milking parlour in the morning. We noted that many calves would routinely nurse right before the cows would leave for the day (personal observation). We did not expect to find differences in udder characteristics and conformation between cows, and that is indeed what we found. This was especially important in this study, as the FT and PT calves were left to suckle naturally, and large pendulous udders have been correlated with slower suckling times post-birth (Edwards, 1982) and thus are thought to potentially lead to a reduction in calf immunity (as assessed by calf serum immunoglobulin G levels). We also did not observe any teat damage in the FT or PT cows. Teat damage has been observed previously in part-time CCC systems where the cow returns to the calf immediately after milking with an empty udder (Ospina-Rios et al., 2023); however, this was not an issue in this study, as the PT cows were not milked before they returned to their calves.

Future directions of cow-calf contact in Ireland

In this preliminary study, we compared three different dairy calf rearing systems, which varied in amount of CCC (with the dam) and milking frequency, within the context of the Irish spring-calving, pasture-based dairy system. We found that MMY was reduced in both of the CCC systems (FT and PT) during the CCC period, and although MMY did recover after weaning and separation, the MMY of the FT and PT cows never reached the level of the CONV cows, leading to a lower cumulative lactation MMY and MSY. The PT cows had a lower MMY during the

CCC compared to the FT cows due to their OAD milking. Cow health and udders remained largely unaffected by CCC. Although we managed to incorporate CCC into the existing Irish spring-calving, pasture-based dairy system, our two CCC systems had a negative impact on MMY, which would be an implementation deterrent for the dairy farmer. In our opinion, a modified version of the PT system, where calves are kept indoors, the cow and calf have contact by night, and the cows graze during the day, but the cows are milked twice-a-day, might be the most successful within the current Irish dairy system. However, future work is needed to understand why MMY was so affected by CCC and to develop strategies to ameliorate this effect.

Acknowledgements

The authors wish to thank and acknowledge the Moorepark farm staff for their care of the animals and assistance with this experiment.

Ethics approval

Ethical approval for this study was received from the Teagasc Animal Ethics Committee (TAEC2020-290) and procedure authorization was granted by the Irish Health Products Regulatory Committee (AE19132/P124). Experiments were performed in accordance with European Union (Protection of Animals Used for Scientific Purpose) Regulations 2012 (S.I. No. 543 of 2012).

Financial support statement

This research is funded by Science Foundation Ireland (SFI) and the Department of Agriculture, Food, and Marine on behalf of the Government of Ireland under Grant Number [16/RC/3835] – VistaMilk.

Supplemental Table S1: Dairy cow clinical health scoring chart used in the study, which was adapted from Barry et al. (2019a) and Crossley et al. (2021).

Indicator	Definition	Scoring scale
Demeanour	Combined evaluation of behaviour and responsiveness	4-point scale (0 to 3) where: 0 = Bright, alert, responsive 1 = Dull, possible depressed, less responsive 2 = Dull, markedly depressed, markedly unresponsive 3 = Unresponsive to any stimuli
Ocular discharge	Presence of any ocular discharge	4-point scale (0 to 3) where: 0 = Bright, pronounced 1 = Slightly dull, presence of a small amount of non-clear discharge in one eye 2 = Dull, sunken, small amount of non-clear discharge present in both eyes 3 = Dull, sunken, excessive non-clear discharge present in both eyes
Ear position	Position and activity of ears	4-point scale (0 to 3) where: 0 = Alert and mobile 1 = Slightly drooped 2 = Drooped 3 = Drooped and limp
Nasal discharge	Presence of any mucous discharge from the nasal passage	4-point scale (0 to 3) where: 0 = Clear, discharge free 1 = Small amount of cloudy mucous visible 2 = Medium amount of bilateral mucous discharge 3 = Excessive bilateral mucous discharge
Cough	Presence of a cough or an increased respiratory rate	4-point scale (0 to 3) where: 0 = Normal breathing 1 = Spontaneous coughing 2 = Intermittent coughing 3 = Continuous cough, increased respiration
Dehydration	Appearance of cow eyes in relation to hydration levels	4-point scale (0 to 3) where: 0 = Clear, bright eyes 1 = Eyes slightly sunken 2 = Eyes sunken 3 = Eyes markedly sunken
Mobility	Ability to stand unassisted and move freely	4-point scale (0 to 3) where: 0 = Stands unassisted, actively mobile 1 = Slow to stand, limited mobility 2 = Struggles to stand, limited mobility 3 = Assistance required to stand, no mobility
Interest in Surroundings	Willingness to interact with observer	2-point scale (0 or 1) where: 0 = Interactive when approached 1 = Uninterested when approached
Faecal hygiene	Cleanliness of cow tail area and hindquarters	4-point scale (0 to 3) where: 0 = Completely clean with no faecal matter 1 = Slight faecal matter present 2 = Heavier faecal matter present 3 = Extremely dirty with faecal matter

Supplemental Table S2 Model selection (Bayesian Information Criterion (BIC)), random effects variances (σ^2_{cow} , σ^2_e), covariance parameter estimates (compound symmetry (CS) or first-order autoregressive lag 1 (AR(1))), phenotypic variance (σ^2_p), variable means (\bar{x}), and CV (%) for all variables analysed using generalised linear mixed models (PROC MIXED) in SAS. The study population consisted of dairy cows in three different calf rearing systems: the conventional Irish system, where cow and calf were separated <1 h post-birth, cows were pasture-based and milked twice-a-day; a full-time access system, dam and calf were allowed constant, unrestricted access, were pasture-based, and cows were milked twice-a-day; and a part-time access system, dam and calf had unrestricted access when indoors at night, cows grazed outdoors by day while calves remained indoors, and cows were milked once-a-day in the morning.

Variable		BIC	σ^2_{cow}	CS	AR(1)	σ^2_e	σ^2_p	\bar{x}	CV%
Machine milk yield (kg/d)	Average weeks 1 to 8	222.6	3.69	-	-	0.00	3.69	16.0	12.02
	Average weeks 9 to 35	213.3	2.51	-	-	0.77	3.28	17.5	10.34
	Average weeks 1 to 35	203.3	0.00	-	-	2.56	2.56	17.2	9.32
	Cumulative weeks 1 to 35 (kg)	645.0	0.00	-	-	134973	134973	4148	8.86
Milk solids yield (kg/d)	Average weeks 1 to 8	107.2	0.00	-	-	0.36	0.36	4.87	12.37
	Average weeks 9 to 35	84.4	0.03	-	-	0.17	0.20	4.99	8.94
	Average weeks 1 to 35	84.1	0.20	-	-	0.00	0.20	4.97	8.94
	Cumulative weeks 1 to 35 (kg)	-1.7	0.00	-	-	0.03	0.03	3.50	4.62
Milk fat concentration (%)	Average weeks 1 to 8	17.3	0.00	-	-	0.04	0.04	3.75	5.39
	Average weeks 9 to 35	9.8	0.00	-	-	0.03	0.03	3.69	4.99
	Average weeks 1 to 35	-30.4	0.01	-	-	0.00	0.01	4.74	2.45
Milk fat concentration (%)	Average weeks 1 to 8	-58.5	0.01	-	-	0.00	0.01	4.76	1.70
	Average weeks 9 to 35	-59.8	0.00	-	-	0.01	0.01	4.75	1.68
	Average weeks 1 to 35	22.0	0.00	-	-	0.03	0.04	1.35	13.87
Milk fat concentration (%)	Average weeks 1 to 8	1.8	0.00	-	-	0.02	0.02	1.53	9.58
	Average weeks 9 to 35	-2.9	0.00	-	-	0.02	0.02	1.48	9.29
	Average weeks 1 to 35	442.2	0.00	-	-	1086	1086	359	9.18
Somatic cell score	Average weeks 1 to 8	99.4	0.21	-	-	0.00	0.21	4.93	9.21
	Average weeks 9 to 35	100.3	0.26	-	-	0.00	0.26	4.98	10.20
	Average weeks 1 to 35	95.3	0.04	-	-	0.19	0.23	5.01	9.55
BW (kg)	Week 4	408.1	352.53	-	-	1.00	353.53	495	3.80
	Week 8	434.9	680.11	-	-	1.00	681.11	496	5.27
	Week 12	440.1	770.88	-	-	1.00	771.88	494	5.62
	Week 35	413.7	0.00	-	-	922	922	522	5.82
Average daily change in BW (kg/d)	Week 1 to 4	134.9	0.14	-	-	0.31	0.45	-	-89.33
	Week 4 to 8	149.7	0.26	-	-	0.39	0.65	0.02	3585
	Week 8 to 12	119.4	0.07	-	-	0.24	0.31	-	-1564

Body condition score	Week 1 to 12	77.0	0.11	-	-	0.00	0.11	-	-130.53
	Week 4	-12.1	0.00	-	-	0.02	0.02	3.07	4.18
	Week 8	-9.2	0.00	-	-	0.02	0.02	3.04	4.38
	Week 12	9.3	0.00	-	-	0.03	0.03	3.07	5.44
	Week 35	6.5	0.00	-	-	0.02	0.02	3.08	5.12
Change in body condition score	Week 1 to 4	-12.1	0.00	-	-	0.02	0.02	-	-128.33
	Week 4 to 8	-11.4	0.00	-	-	0.02	0.02	-	-368.69
	Week 8 to 12	-9.0	0.00	-	-	0.02	0.02	0.03	449.34
	Week 1 to 12	9.3	0.00	-	-	0.03	0.03	-	-158.17
Clinical health score	Summed score	2681.2	0.07	0.00	-	0.64	0.71	1.10	79.69
Locomotion	Head	-392.9	0.00	-	0.51	0.02	0.03	1.00	15.80
	Spine	888.4	0.18	-	0.09	0.23	0.41	1.60	39.08
	Speed	912.3	0.18	-	0.27	0.27	0.44	1.40	48.42
	Tracking	771.9	0.09	-	0.12	0.19	0.28	3.70	14.45
	Ab/adduction	426.8	0.03	-	0.03	0.09	0.12	1.10	31.53
	Summed score	1605	1.14	-	0.11	1.01	2.15	8.80	16.65
	Udder scoring								
Udder scoring	Udder clearance (cm)	959.8	14.95	-	-0.05	3.61	18.56	57.7	7.47
	Front teat placement (score)	465.5	0.26	-	0.18	0.34	0.61	4.60	17.03
	Front teat placement (cm)	547.9	0.27	-	0.53	0.82	1.09	5.30	19.86
	Rear teat placement (score)	305.2	0.05	-	-0.08	0.15	0.19	1.40	31.66
	Rear teat placement (cm)	174.0	0.36	-	-	0.44	0.80	5.10	17.51
	Teat-end hyperkeratosis score	184.6	0.54	-	-	0.51	1.05	5.00	20.52
	Teat length, front left (cm)	167.9	0.28	-	-	0.41	0.68	4.50	18.30
	Teat length, front right (cm)	158.4	0.19	-	-	0.35	0.54	4.50	16.32
	Teat length, rear left (cm)	269.5	8.36	-	-	0.90	9.26	14.30	21.34
	Teat length, rear right (cm)	271.4	8.83	-	-	0.91	9.73	7.20	43.21

$$^1\sigma_p^2 = \sigma_{cow}^2 + \sigma_e^2$$

$^2\bar{x}$ = average between the system LSMEANS

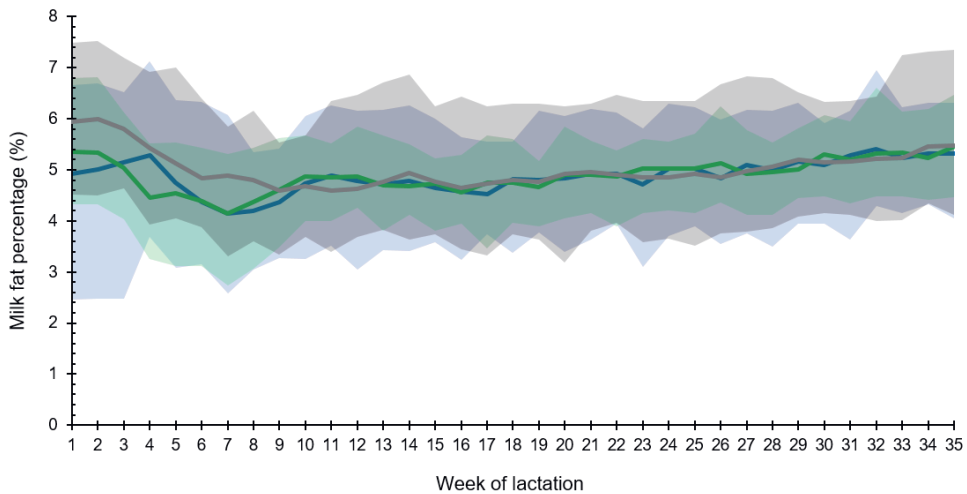
$$^3CV = (\text{sqrt}(\sigma_p^2)/\bar{x}) * 100$$

Supplemental Table S3: Mean (\bar{x}), SEM, variance (σ), and CV (\bar{x}/σ) for all measured study variables.

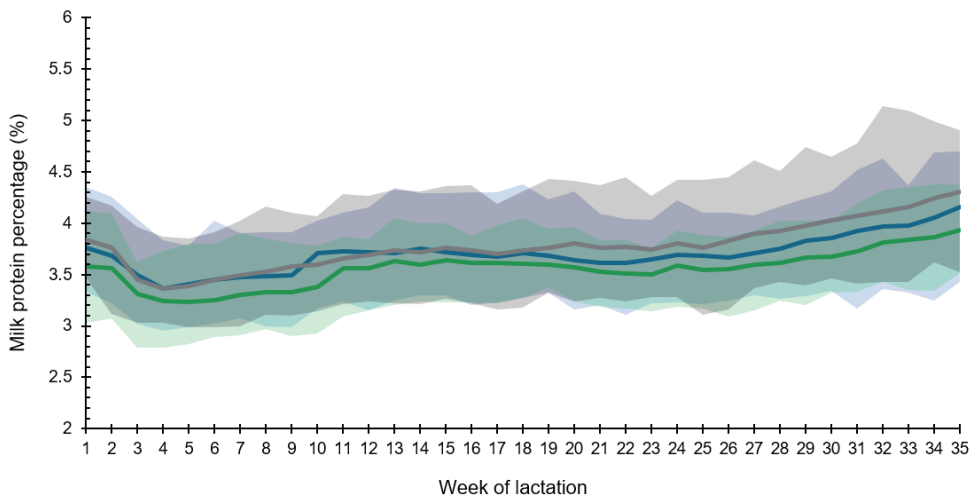
The study population consisted of dairy cows in three different calf rearing systems: the conventional Irish system, where cow and calf were separated <1 h post-birth, cows were pasture-based and milked twice-a-day; a full-time access system, dam and calf were allowed constant, unrestricted access, were pasture-based, and cows were milked twice-a-day; and a part-time access system), dam and calf had unrestricted access when indoors at night, cows grazed outdoors by day while calves remained indoors, and cows were milked once-a-day in the morning.

Category	Variable	mean (\bar{x})	variance (σ)	SEM	CV
Milk	Milk yield (kg/d)	17.1	5.527	0.133	0.322
	Milk fat (%)	4.93	0.772	0.019	0.157
	Milk protein (%)	3.69	0.355	0.009	0.096
	Milk lactose (%)	4.76	0.172	0.004	0.036
	Somatic cell score	4.80	0.589	0.015	0.123
BW and body condition score	BW (kg)	509	61.3	2.109	0.120
	Body condition score	3.09	0.219	0.008	0.071
	Change in BW (kg/d)	-0.22	0.978	0.080	-4.419
	Change in body condition score	-0.03	0.151	0.012	-4.408
Health score	Summed health score	1.0	0.762	0.023	0.789
Locomotion	Head	1.0	0.157	0.007	0.153
	Spine	1.6	0.665	0.029	0.405
	Speed	1.4	0.670	0.029	0.479
	Tracking	3.6	0.536	0.024	0.147
	Ab/adduction	1.1	0.345	0.015	0.310
	Summed locomotion score	8.8	1.501	0.066	0.170
Udder and teats	Teat length (cm)	4.8	1.034	0.073	0.217
	Udder clearance (cm)	58.4	5.793	0.412	0.099
	Teat-end hyperkeratosis (score)	1.43	0.574	0.021	0.402
	Front teat placement (score)	4.44	0.862	0.061	0.194
	Front teat placement (cm)	14.2	3.068	0.434	0.216
	Rear teat placement (score)	5.19	1.063	0.076	0.205
	Rear teat placement (cm)	7.3	2.864	0.405	0.393
	Milk leakage (binary score)	0.20	0.403	0.016	1.979
	Udder firmness (score)	2.2	0.456	0.018	0.205

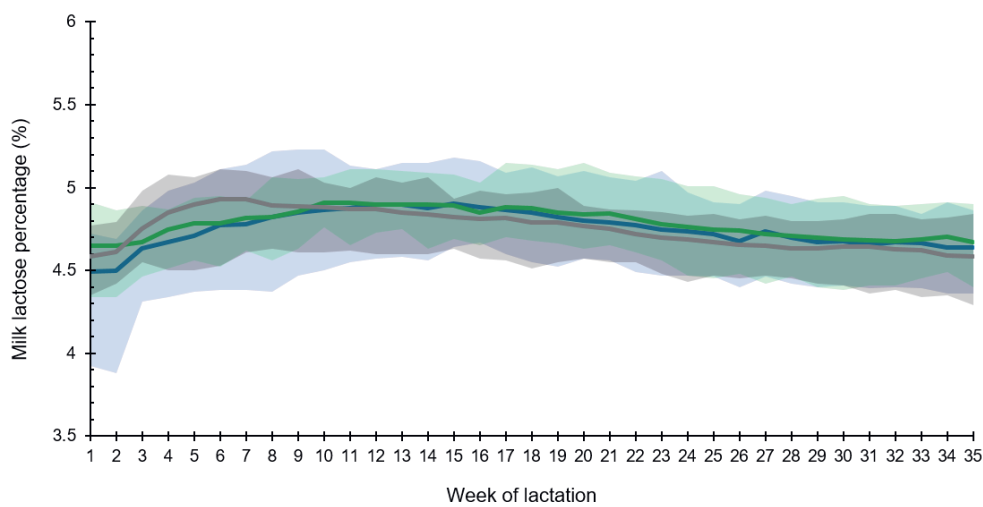
Supplemental Figure S1: Statistical means and range for average daily milk fat concentration (%) for dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the 35-week lactation. The shaded bars represent the range of milk fat concentration observed in each system during each week of lactation.



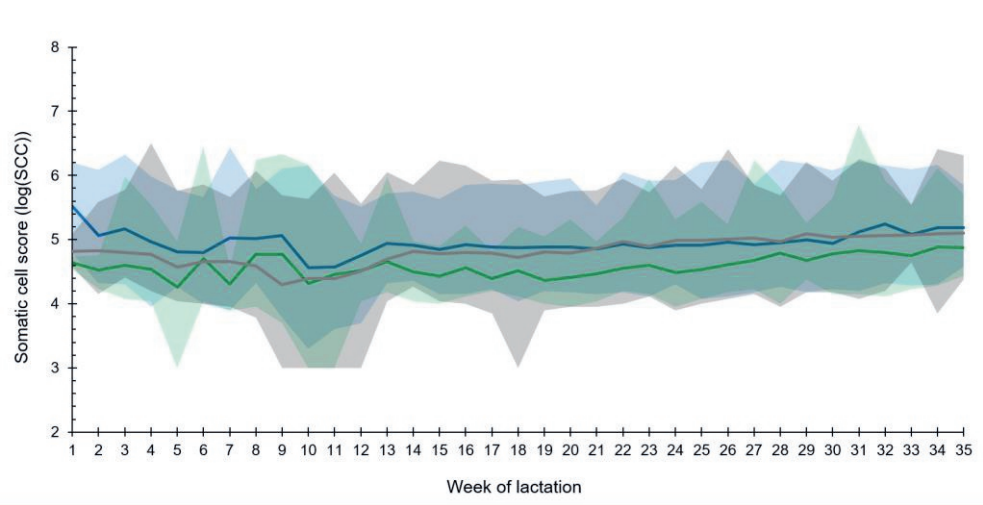
Supplemental Figure S2: Statistical means and range for average daily milk protein concentration (%) for dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the 35-week lactation. The shaded bars represent the range of milk protein concentration observed in each system during each week of lactation.



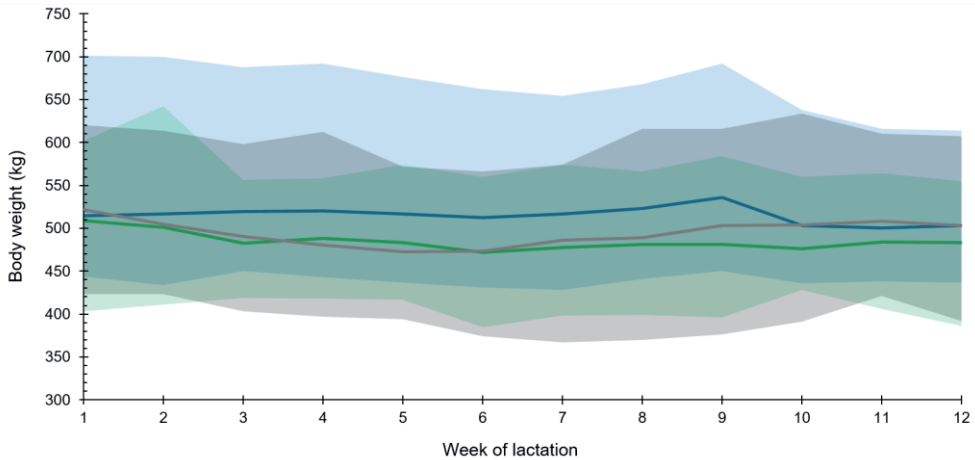
Supplemental Figure S3: Statistical means and range for average daily milk lactose concentration (%) for dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the 35-week lactation. The shaded bars represent the range of milk lactose concentration observed in each system during each week of lactation.



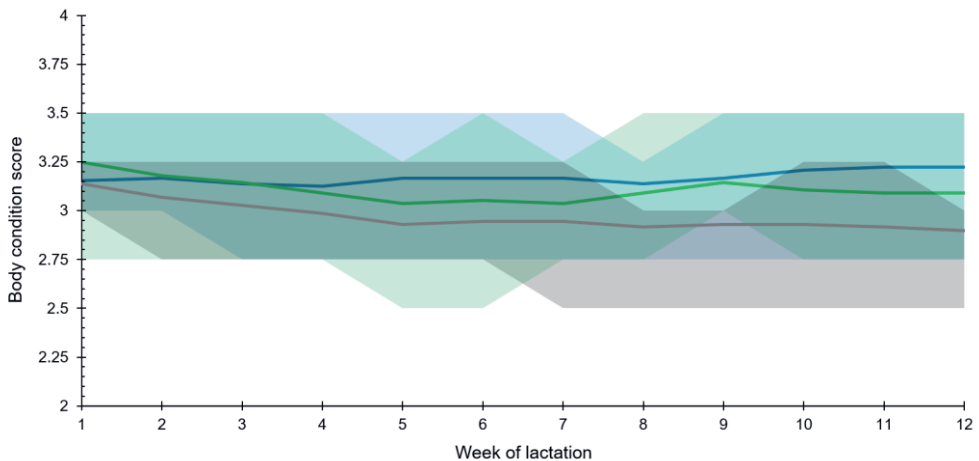
Supplemental Figure S4: Statistical means and range for average somatic cell score (log10(somatic cell count)) in dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the 35-week lactation. The shaded bars represent the range of somatic cell score observed in each system during each week of lactation.



Supplemental Figure S5: Statistical means and range for BW (kg) for dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the first 12 weeks of lactation. The shaded bars represent the range of BW observed in each system during each week of lactation.



Supplemental Figure S6: Statistical means and range for body condition score for dairy cows in three different cow-calf contact rearing systems (conventional (grey): no access to calf and milked twice-a-day; full-time access (green): full-time access to calf and milked twice-a-day; part-time access (blue): part-time access to calf and milked once-a-day) by week across the first 12 weeks of lactation. The shaded bars represent the range of body condition score observed in each respective system during each week of lactation.





| CHAPTER 5 |

Effect of weaning and cow-calf contact on the physiological and clinical health, performance, and behaviour of dairy cows and their calves

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Submitted to animal

Abstract

Cow-calf contact (CCC) systems have become increasingly popular calf rearing systems to promote positive welfare; however, weaning and separation may cause distress. This preliminary study aimed to investigate the interaction between weaning and CCC on the physiological health, performance, and behaviour of dairy cows and calves. Three systems were compared: conventional, pasture-based Irish system (CONV; 18 pairs), cow and calf separated ≤ 2 h post-birth, cows milked twice-a-day, calves artificially reared indoors; full-time access system (FT; 14 pairs), dam and calf allowed constant, pasture-based, unrestricted access and cows milked twice-a-day; and part-time access system (PT; 18 pairs), unrestricted access at night indoors, cows grazed outdoors by day while calves remained indoors, cows milked once-a-day (0800 h). Following an 8-week CCC period, all calves were weaned; FT and PT pairs underwent a 7 d gradual weaning and separation process (PT cows switched to twice-a-day milking). Clinical health scores (2x/week), blood samples (1x/week; analysed for physiological markers of health and performance), bodyweight (BW; 1x/week), body condition score (BCS), milk samples (1x/week; cows only), and behaviour (1 d/week; scan sampling 3x/d; 24 total observations) were taken the week before (preWS) and after (postWS) the weaning and separation process. The PT cows had higher BCS (3.18 ± 0.034) than CONV (2.95; FT cows were similar to both, 3.05) and lower non-esterified fatty acids (NEFA; 0.40 ± 0.038 mmol/L) than the FT cows (0.58 mmol/L; CONV cows were similar to both, 0.48 mmol/L) across both time-points. The FT (11.7 kg) and PT cows (9.4 kg) had lower ($P < 0.001$) daily machine milk yield (MMY) preWS compared to CONV cows (24.5 ± 0.60 kg). Although the FT (18.4 kg) and PT (19.1 kg) cows increased MMY postWS, they remained lower than CONV cows postWS (24.3 kg; $P < 0.001$). All calves preWS had lower summed clinical health scores (0.91 vs 1.25 ± 0.131 ; $P = 0.017$), beta-hydroxybutyrate (0.07 vs. 0.39 ± 0.023 mmol/L; $P < 0.001$), and globulin (12.0 vs. 14.5 ± 0.929 g/L; $P = 0.010$) than postWS. Despite no difference preWS (0.30 ± 0.037 mmol/L), postWS the FT (0.36 mmol/L; $P < 0.001$) and PT (0.34 mmol/L; $P = 0.001$) calves had higher NEFA than CONV calves (0.13 mmol/L). Calf weekly average daily gain (ADGw) was similar preWS (0.9 ± 0.142 kg/d), but CONV calves had higher ADGw postWS than FT (0.42 kg/d) and PT calves (0.40 kg/d). All calves performed more ($P = 0.009$) positive behaviours preWS ($4.6 \pm 6.37\%$) compared to postWS ($2.3 \pm 3.38\%$). Our results suggest the applied CCC provided no advantages to cow or calf health, performance, or behaviour around weaning and separation.

Keywords: dam-calf contact, cow-calf separation, energy balance, calf growth

Introduction

Currently in conventional dairy calf rearing systems, farmers separate the cow and calf soon after birth. This practice has been adopted for many reasons, including increasing the amount of saleable milk produced by the cow, reducing the risks to calf health, and preventing the formation of the maternal bond (thereby preventing future distress when they need to be separated); however, this separation soon after birth is viewed negatively by the public (Ventura et al., 2016; Hotzel et al., 2017; Meagher et al., 2019). As a result, there is an increased interest from consumers, farmers, and researchers for alternative calf rearing methods, such as cow-calf contact (CCC) rearing systems, where the cow and calf can stay in contact for a prolonged period of time (Sirovnik et al., 2020). However, CCC systems come with challenges that need to be overcome for CCC to be viable on commercial farms (Neave et al., 2022). One important challenge with CCC systems is the separation of the bonded cow-calf pair, especially as separation often occurs simultaneously with weaning (Newberry and Swanson, 2008).

The combination of weaning and separation can cause distress to both cow and calf (Johnsen et al., 2021a; Wenker et al., 2022a; Bertelsen and Jensen, 2023), which can result in changes in behaviour (e.g., increase in vocalisations, Stěhulová et al., 2008; Enríquez et al., 2010; Johnsen et al., 2015b; searching behaviour, Neave et al., 2024b; pacing or standing with their head out of the pen, Wenker et al., 2022a, Bertelsen and Jensen, 2023) and performance (e.g., reduction in calf weight gain, Sinnott et al., 2024 and cow body weight, Metz, 1987; Bar-Peled et al., 1995). Behaviour and performance are intrinsically linked with health, as cows and calves in poor health have altered behaviour (Dittrich et al., 2019; Belaid et al., 2020; Duthie et al., 2021) and lower production/performance (Bareille et al., 2003) compared to healthy individuals. Although a review concluded that immediate separation after birth was not advantageous to calf health (Beaver et al., 2019), more recent research has found that full-time access CCC with weaning and separation around 8-9 weeks of age can lead to poorer calf health (i.e. increased use of antibiotics; Wenker et al., 2022b; Sinnott et al., 2024) and potentially lead to more difficulty transitioning onto their post-weaning diet (Wenker et al., 2022b; Sinnott et al., 2024). Although clinical health was assessed in these cases, looking at physiological markers of health and performance in these animals may provide more information into sub-clinical health and physiological changes in performance that do not lead to alterations in growth and weight. Blood serum biomarkers of inflammation (e.g., serum amyloid A (SAA), albumin, globulin, and total protein) may indicate underlying infections or inflammation that may not be

obvious during clinical health scoring (Bobbo et al., 2017a; Trela et al., 2022), while serum mineral concentrations may identify underlying health issues or differences in diet intake (e.g., calcium, magnesium, and phosphorus; Suttle, 2022). Serum biomarkers of energy balance, such as beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA), in conjunction with changes in body weight and body condition score, can help monitor energy requirements and feed intake of cows (LeBlanc, 2010; Benedet et al., 2019) and calves (Quigley III, 1996; Deelen et al., 2016; Steele et al., 2017).

This explorative study aimed to measure the physiological health, performance, and behaviour of cows and calves within three systems (two CCC rearing systems and one conventional system) before and after weaning to estimate whether animals within the three investigated systems responded differently. More specifically, we wanted to see if there were any differences between the three systems in terms of cow and calf physiological health (i.e., serum markers of inflammation and minerals), clinical health, performance (i.e., energy balance, weight gain, and cow production), and behaviour (i.e., proportion of time spent lying, ruminating, and eating solid feeds). This study was specifically focused on two time-points: immediately before weaning and separation (preWS) and immediately after the 7 d gradual weaning and separation process (postWS).

Material and methods

Animals, Management, and Experimental Design

This study was conducted from 20 January to 14 November 2021 at Teagasc Moorepark Research Farm, County Cork, Ireland, as part of a larger research project investigating cow-calf contact systems and discussed in more detail in McPherson et al. (2024) and Sinnott et al (2024). The aim of the overall project was to investigate how CCC could feasibly be incorporated into an Irish spring-calving, pasture-based system and to determine its effects on cow and calf health, behaviour, production, and welfare. Three different dairy calf rearing systems were implemented and compared: the conventional, no contact system (CONV); a full-time access CCC system (FT); and, a part-time access by night CCC system (PT). The CCC period lasted for 8 weeks, until the gradual weaning and separation process began. Before calving (January 2021), 54 cows were balanced between the three different rearing systems (18 cows/system) in a randomised complete block design by an individual independent to the study. Cows were balanced by (mean \pm standard deviation): breed (70% Holstein-Friesian and 30% Holstein-Friesian x Jersey crosses (>25% Jersey)), parity (1.76 ± 0.46),

previous 35-week cumulative milk yield (4677 ± 1047.4 kg; the dam's first lactation cumulative 35-week milk yield was used for primiparous cows), previous lactation somatic cell score (SCS; \log_{10} of somatic cell count; 4.9 ± 0.44 ; the dam's first lactation SCS was used for primiparous cows), expected calving date (11 February 2021 ± 16 d), Economic Breeding Index (EBI; Berry et al., 2007), and expected calf breed (breed of sire). Sample size calculations were completed based on findings from previous experimental studies, using cow daily machine milk yield and calf plasma immunoglobulin G levels from 24 h post-birth, and gave a group size of 18 cow-calf pairs per system. Multiparous cows had all previously been separated from their calves immediately after (≤ 2 h) birth, thus CCC was novel to all experimental cows. Four pairs from the FT system had to be removed from trial soon after calving (one pair due to failure of cow to bond with calf; three pairs due to calf illness requiring intervention) and were not replaced due to lack of cow availability at the end of the calving season. One early calving PT pair was removed from the system due to calf illness, but was able to be replaced with a cow that had yet to calve. The final system groups used in this analysis were: 18 CONV cow-calf pairs (13 female dairy calves and five male dairy-beef calves), 14 FT cow-calf pairs (10 female dairy calves, two female dairy-beef calves, and two male dairy-beef calves), and 18 PT cow-calf pairs (10 female dairy calves, one male dairy calf, two female dairy-beef calves, and four male dairy-beef calves). Dairy-beef calves refer to a calf with a dairy dam and a beef sire. Measurements were performed using each individual cow or calf as the experimental unit.

Management of different systems

For a comprehensive overview of the management of the three systems during the CCC period, please refer to McPherson et al. (2024) and Sinnott et al. (2024).

Conventional system management

After calving, the CONV cows joined a grazing herd where they were managed following normal grazing practices at the Teagasc Moorepark facilities (i.e., Kennedy et al., 2021; Murphy et al., 2023). The CONV cows were offered a predominately grazed grass diet, and were milked twice-a-day (0700 h and 1500 h). Concentrates (1-4 kg/d) were provided during milking in the parlour, and the amount provided depended on grass availability (see McPherson et al., 2024).

The CONV calves were artificially reared following conventional Irish practices (Conneely et al., 2014). At 3 days old, calves were moved into a group pen where they were offered milk replacer (Heiferlac, Volac; 26% crude protein)

through an automatic milk feeder (Volac Förster Technik Vario, Germany). Calves received 6-9 L/d of milk replacer, depending on their age (see Sinnott et al., 2024 for more details); gradual weaning was started at 39 d on the automatic milk feeder. In the group pen, calves were also offered *ad libitum* water (via a water bowl), forage (hay), and concentrates (18% crude protein, Kaf Gro, Prime Elite, Dairygold, Cork, Ireland).

Full-time access system management: pre-weaning

Until the start of the gradual weaning and separation process, the FT pairs had continuous, unrestricted access to each other (22 h/d), except during milking times (twice-a-day at 0800 h and 1600 h). Calves always had access to *ad libitum* water (water trough) and concentrates. The FT pairs were primarily kept outdoors at pasture, but depending on weather or grass availability (see McPherson et al., 2024), they were also occasionally housed indoors in a housing identical and adjacent to the PT pairs (described below), and were also fed identically to the PT pairs.

Part-time access system management: pre-weaning

During the CCC period, PT pairs had unrestricted access to each other by night (17 h/d; 1500 h to 0800 h). During the nightly contact period, the PT cows were housed in an indoor cubicle area, which was connected to an adjacent straw pen (PT calf pen) via a creep with gates. The PT pairs were separated in the morning (0800 h), when the PT cows were brought to the parlour for milking; the PT calves remained in their straw pen, where they were provided with *ad libitum* access to water (via a water bowl), forage (hay), and concentrates. The PT cows were turned out to pasture after the morning milking, and remained there until the afternoon milking time (1500 h), when they were not milked and were brought back indoors to be reunited with their calves. In the cubicle area, PT cows had access to *ad libitum* grass silage and water via water troughs. The PT cows were fed concentrates (1-4 kg/d, dependent on weather and grass availability) in the parlour.

Weaning management: Conventional system

The CONV pairs were separated at birth, therefore did not undergo the same weaning and separation process as the FT and PT pairs. Instead, CONV cows remained at grass under normal management conditions. The CONV calves were weaned gradually by the automatic feeder over the course of 21 days. After weaning, CONV calves were moved and regrouped in an indoor straw-bedded pen with calves from all systems.

Weaning, separation, and post-weaning management: CCC pairs

The FT and PT cow-calf pairs were weaned based on calf age (57 ± 1.9 d) over a seven day period using a gradual, three-stage process. The weaning process was initiated after the morning milking. Cows went to the parlour for milking as normal, but during milking the calves were moved into a different, separate shed (Supplemental Figure S1). After morning milking, cows were brought back to the shed into which the calves had been moved. In the weaning shed, the pairs were housed in adjacent, straw bedded pens, with gates that prevented suckling but pairs could still hear, see, and touch each other through the gaps in the gates. The FT and PT cows and calves were kept separately (Supplemental Figure S1). All pens had access to *ad libitum* water through water bowls. In their respective pens, calves had access to *ad libitum* concentrates, grass silage, and forage (hay), while cows had access to *ad libitum* grass silage. Cows were fed concentrates in the parlour (3 kg/d). Single FT and PT pairs did not undergo the weaning and separation process by themselves, a minimum of two pairs were weaned together to prevent unnecessary distress.

In the first stage of weaning and separation (days 1 to 3; start of weaning), cows and calves were allowed 1 h of unrestricted access to each other around 1 h after morning milking (approximately from 1030 h to 1130 h) where calves could suckle freely. This 1 h period was chosen to be shortly after the morning milking so that the cows would have some milk for their calf, but would not have enough milk that the calf would be satiated. During the second stage (Days 4 to 5; calves were weaned), the pairs were not allowed access to one another; however, pairs were still able to see, hear, and touch each other through the gates separating the pens. At the start of the third stage (days 6 to 7; start of separation), calves remained in the weaning pen while the FT and PT cows did not return to the shed. Instead, the FT and PT cows joined a general herd of cows at grass, which were managed identically to the CONV cows. The FT and PT cows remained with this herd for the rest of their lactation and had no further contact with any calves. After the end of the weaning and separation process, all calves, regardless of system, were grouped in an indoor straw bedded pen (40 m^2 ; $2 \text{ m}^2/\text{calf}$) for 8 ± 1.7 d until they were moved to an outdoor paddock (at 71 ± 4.5 d old). Calves could not see mature cows in the indoor straw bedded pen, but may have been able to see mature cows when in the outdoor paddock.

Measurements

Physiological markers of health and performance

To be able to assess physiological markers, blood samples were taken from the cows and calves the week before weaning (preWS; calves: 52 ± 2.6 d; cows: 52 ± 2.6 d) and the week after weaning (postWS; calves: 65 ± 2.6 d; cows: 66 ± 2.6 d). Two tubes were collected at each time point: one 10 mL serum tube (BD Vacutainer Serum tube, no additive, silicone-coated interior) and one 10 mL plasma tube (BD Vacutainer Plasma tube, 158 USP units of sodium heparin (spray coated)). Cow and calf blood samples were taken using a 20G needle (BD Vacutainer PrecisionGlide Multiple Sample Blood Collection Needle – 20G x 1" (0.9 x 25 mm)). Jugular venepuncture was used to obtain the calf blood samples, while cow blood samples were taken by coccygeal venepuncture. After blood sample collection, plasma samples were immediately centrifuged at 3000 rpm for 15 min at 4°C, the plasma decanted and frozen in duplicate at -20°C. Serum samples were refrigerated immediately after collection for 24 h to allow for the blood to clot, then centrifuged at 3000 rpm for 15 min at 4°C, after which the serum was decanted and frozen in duplicate at -20°C.

One duplicate of each of the serum samples was sent to a commercial laboratory for analysis (FarmLab Diagnostics, Emlagh, Elphin, Co. Roscommon, Ireland), where serum levels of albumin (g/L), BHB (mmol/L), calcium (mmol/L), cortisol (µg/mL), globulin (g/L), magnesium (mmol/L), NEFA (mmol/L), phosphorus (mmol/L), and total protein (g/L) were determined using a BIOLIS 30i instrument. The samples were analysed according to Farmlab Diagnostic's standard operating procedures and the manufacturer specifications; there were no deviations from these procedures. The other serum duplicate was analysed for serum SAA (g/mL) using commercially available bovine SAA ELISA kits (Life Diagnostics, Inc., West Chester, PA, USA) in the cow and calf serum samples, at the Teagasc lab. For these kits, serum had to first be diluted. Cow and calf serum samples were tested at different dilutions: 400x and 600x for cows and 400x, 600x, and 1200x for calves. After testing the dilutions, all cows were tested at 400x and the majority of calves were tested at 600x; some calves were tested at 1200x if their concentration was too high at 600x (5 calves). All tests were conducted according to the manufacturer's instructions.

Clinical health scoring

Clinical health scoring was performed on cows and calves twice a week (Tuesdays and Fridays), starting from when cows and calves had entered their

respective system herds, and ended once calves were 12 weeks of age and cows were in their 12th week of lactation. Ten aspects of calf health (demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, faecal hygiene, and naval score) and nine aspects of cow health (demeanour, ocular discharge, ear position, nasal discharge, cough, dehydration, mobility, interest in surroundings, faecal hygiene) were scored using the calf health scoring method from Barry et al. (2019a) which had previously been used in cows by Crossley et al. (2021). Each aspect of health was scored on a scale, most from 0 to 3, based on severity (clinical health scoring scale can be found in Supplemental Table S1). Health scoring was performed by two observers (inter-observer reliability: 89% agreement first attempt, 97% agreement second attempt). A summed clinical health score was calculated by summing all aspects of clinical health (10 for calves and nine for cows), where a higher score indicates a less healthy animal.

Performance variables

Machine milk yield was recorded daily and milk samples were obtained weekly (one composite sample from a consecutive afternoon and morning milking) for each cow over their entire lactation. The weekly milk sample was analysed for composition (milk fat, protein, and lactose percentages) and somatic cell count (Milkoscan FT6000, Foss Electric DK). For each week, each cow's daily machine milk yield was averaged across the 7 d to give an average weekly machine milk yield (kg/d). Milk solids yield was calculated using the appropriate week's daily machine milk yield and average fat and protein concentrations (average daily milk solids yield = (daily machine milk yield * milk fat concentration) + (daily machine milk yield * milk protein concentration)). Somatic cell count was converted to somatic cell score (SCS) by taking the log₁₀ of the somatic cell count. Cow bodyweight (BW; kg) and body condition score (BCS; 5 point scale with 0.25 increments, where 1 = emaciated and 5 = extremely fat; Edmonson et al., 1989) were recorded weekly for the first 12 weeks of the lactation. Average daily gain (ADG) for cows at each time-point was calculated by taking the cow's BW at the time-point, subtracting their week 1 BW, then dividing the product by the number of days between the two measurements. Average change in BCS was calculated for each time-point by subtracting each cow's week 1 BCS from their BCS at each time-point.

Calf BW (kg) was recorded once a week using a weighing scale (TrueTest XR 3000, Tru-test Limited, Auckland, New Zealand). Weekly average daily gain (ADG_w; kg/d) was calculated by subtracting the previous week's weight from the

current week's weight, then dividing by the number of days between the weight measurements. Average daily gain from birth (ADGb; kg/d) was calculated by subtracting the birthweight from the current week's weight, then dividing by the calf's age in days.

Time-budget behaviour scoring

Live behaviour scoring was performed on cows and calves in all systems one day per week (Monday) by two independent observers for 11 weeks (inter-observer reliability: first attempt = 85% agreement, second attempt = 98%), starting once they had entered their respective systems. Cows and calves were scored during three different time periods in a day: before the morning milking, when cows had not yet been moved to the milking parlour (range from 0600 h to 0800 h); midday (range from 1030 h to 1230 h), after cows had settled following the morning milking (while the PT pairs were separated); and, after the afternoon milking (range from 1600 h to 1800 h). The 2 h window for each scoring time period was due to the number of system groups, which were often located in different sheds and paddocks across the farm. At each time period during the day, behaviour was scored for 16 minutes using scan sampling at a 2-min intervals (a total of eight observations were recorded for each animal during each time period). All cows and calves present (system dependent, i.e. all FT cows and calves were observed at the same time, while the CONV cows and calves never were) were scored at the same time. The ethogram used to score behaviour can be found in Table 1.

Table 1: Ethogram describing the different behaviours scored using live, in-person scan sampling. Cows and calves were behaviour scan sampled (2 minutes between each scan) during three sessions each day (15 minutes per session; before morning milking, midday, and after afternoon milking), one day per week. Behaviours with low prevalence were combined into the behaviour categories during the analysis process. Variables that were specific to cows or calves are also noted.

Category	Behaviour	Animal	Description of behaviour
Abnormal	Head out of pen	Cow and calf	Animal put the tip of its nose/ head through openings in a fence or over the top of the fence. Animal puts its head through or over a gate/fence.
	Oral Manipulation of Pen Structure	Cow and calf	Animal licks, nibbles, sucks, or bites at the pen structure (barriers, walls, buckets, troughs etc.)
	Pacing	Cow and calf	Animal repeatedly walks back and forth the same area (i.e. in front of a gate) in an active manner
	Tongue Rolling	Cow and calf	Animal makes repeated movements with its tongue inside or outside its mouth
	Urine drinking / oral manipulate prepuce / cross sucking	Calf only	Animal drinks the urine of another calf / Animal attempts to suck the naval area of another animal / Animal attempts to suck any body part of another animal.
Drink milk	Vocalisation	Cow and calf	Bellowing from animal
	Calf nursing	Cow only	A calf nurses from a cow
Drink water	Drinking Milk	Calf only	Calf is standing in the automatic milk feeder drinking milk, with their mouth on the nipple.
	Drinking Water	Cow and calf	Animal is stood at the water bowl or trough with their nose/mouth partially submerged in the water.
Eat solids	Eating concentrates	Calf only	Calf is standing with their head within the flaps of the concentrate feeder. / Calf is standing with their head just outside the concentrate feeder while making lateral motions of the jaw
	Eating forage	Calf only	Calf makes a lateral motion of the jaw while standing at the hay feeder. Calf is lying down on the straw and is making repeated lateral motions of the jaw with straw sticking out of their mouth. / Calf is lying down on the straw and is rooting their nose around in the bedding. / Calf is standing with their head near the ground making lateral motions of the jaw (inside on straw bed only).
	Eating grass silage	Cow and calf	Animal is stood at the feed bunk making a lateral motion of the jaw.
	Grazing	Cow and calf	Animal is standing with their head near the ground making lateral motions of the jaw. Active eating of grass is observed.
	Lying	Cow and calf	Animal is resting either sternally or laterally with all four legs hunched close to body either awake or asleep.
Maintenance	Defecation/ Urination	Cow and calf	Animal defecates or urinates
	Grooming	Cow and calf	Animal uses tongue to repeatedly lick own back, side, leg, tail areas
	Scratching / Rubbing / Stretching	Cow and calf	Animal scratches itself with one of their legs (generally hind legs)/ Animal rubs itself on pen structure/ Animal stretches itself
Other	Aggression	Cow and calf	Cow expresses aggressive behaviour towards (an)other cow(s)
	Bulling	Cow only	Cow only – cow is bulling
	Failed nursing	Cow and calf	Calf attempts to nurse a cow but fails at the attempt
Positive	Head-butt	Cow and calf	Cows aggressively head-butt each other (different from calf play head-butting).
	Sniff	Cow and calf	An animal sniffs at the pen structure, fence, ground, or other surrounding structure.
	Play Behaviour/ Mounting / Head butting/ Play with object	Cow and calf	Animal runs, jumps, changes direction suddenly, bucks, kicks hind legs, twists or rotates body / Animal mounts, or attempts to mount, a pen mate (calf only)/ Animal is engaged in head to head pushing with another animal (calf only) / Animal plays with an object
	Social Interaction	Cow and calf	Animal licks another animal in the same area multiple times / Animal nudges another animal with its nose
	Rumination	Cow and calf	Ruminating / chewing
Standing	Standing	Cow and calf	Animal is in a static upright standing position with weight placed on all four legs
Walking	Walking	Cow and calf	Animal is actively moving from one point in the pen to another in an active walking motion

Table 2: Age (calves) or days in milk (cows) at the two different time-points (preWS = before weaning and separation; postWS = after 7 d weaning and separation process) for different measurements.

	Calves			Cows		
	CONV	FT	PT	CONV	FT	PT
Weaning age				Weaning day of lactation		
	57 ± 1.7	57 ± 2.4	57 ± 1.6	56 ± 0.0	57 ± 2.4	57 ± 1.6
Time-budget behaviour				Time-budget behaviour		
preWS	51 ± 2.5	53 ± 2.3	51 ± 2.5	50 ± 1.7	53 ± 2.3	51 ± 2.5
postWS	66 ± 2.1	67 ± 2.3	65 ± 2.5	64 ± 1.7	67 ± 2.3	65 ± 2.5
Blood samples				Blood samples		
preWS	52 ± 2.1	51 ± 3.4	52 ± 2.1	53 ± 1.7	51 ± 3.4	52 ± 2.1
postWS	66 ± 2.1	65 ± 3.4	66 ± 2.1	67 ± 1.7	65 ± 3.4	66 ± 2.1
Body weights				Body weight/BCS and milk samples		
preWS	50 ± 1.9	51 ± 3.5	52 ± 2.2	51 ± 2.6	54 ± 2.4	52 ± 2.5
postWS	67 ± 2.4	68 ± 5.9	68 ± 2.5	71 ± 2.2	69 ± 3.5	71 ± 2.2

Data processing

Pre- and post-weaning date selection

Since we did not allow individual cow-calf pairs to undergo the weaning and separation process by themselves (discussed in more detail above), there was some variation between systems in the day of lactation/age that cows and calves were weaned (Table 2). Measurements were also only taken on specific days of the week. Therefore, to enable the comparison of before and after weaning, we selected the last measurement that was taken before weaning and separation for the before sample (preWS) and the next measurement that was taken after the 7-d weaning and separation process (postWS) as the after measurement. The average age (calf) or day in lactation (cow) of the preWS and postWS samples for each measurement can also be found in Table 2.

Time-budget behaviour proportions

Behaviour proportions were calculated independently for each cow and calf on each day of observation (preWS and postWS) and were calculated by summing the number of scans (occurrences) where that behaviour was observed and dividing by the total number of scans per day (24). Some behaviours were observed too rarely for analysis and hence were combined into behavioural categories (more details can be found in Table 1). All animals were retained in the

analysis. Although proportions were used in the analysis, for ease of understanding all proportions were converted to percentages for the results section.

Statistical analysis

All data were analysed using SAS (v9.4, SAS Institute). All data (other than behaviour proportion data) were first checked for normality using the Shapiro-Wilk test (PROC UNIVARIATE), where data were considered approximately normal if the W-statistic was > 0.85 ; the shape of the histogram was also checked. Mean, variance, SEM, and coefficients of variation for all measured study variables can be found in Supplementary Table S2. The Tukey-Kramer test was used for all post-hoc pairwise comparison tests. The threshold for significance was $P < 0.05$ and tendencies were $P < 0.10$.

All physiological markers of health and performance, clinical health scores, and other performance variables were analysed using linear mixed models in SAS (PROC MIXED). Each cow and calf variable was analysed separately. For the cows, the model consisted of the fixed effects of system (CONV, FT, and PT), time-point (preWS or postWS), and parity (1, 2, or 3+). For calves, the model consisted of the fixed effects of system (CONV, FT, and PT), time-point (preWS or postWS), calf breed (dairy or dairy-beef), and sex (male or female). The interaction between system and time-point was tested in all cow and calf models, and removed if not significant ($P \geq 0.05$). Time-point was used as a repeated measure, acting on the individual cow or calf, and several covariance structures were tested (compound symmetry, first-order autoregressive lag 1, and unstructured); whichever covariance structure gave the lowest Bayesian Information Criterion was used (only compound symmetry and first-order autoregressive lag 1 were used; for full details on which variance components were used in each model, please refer to Supplementary Table S3). Various covariates were used in the models, dependent on the variable being tested. In all analyses, each cow's days in milk or calf age on 1 June 2021 was used as a covariate to account for differences in calving date. Additional cow covariates included: the health sub-index of their EBI (centred within parity) was used for all health variables (physiological and clinical), week 1 BW and BCS for BW and BCS, respectively, and previous lactation cumulative milk yield or average fat, protein, or lactose concentration that had been centred within parity (for primiparous cows, their dam's first lactation values were used instead).

The analysis of cow and calf behaviour was performed separately. Analysis of each behaviour or behaviour category (Table 1) was done in SAS using generalised linear mixed models (PROC GLIMMIX) with a binomial distribution, a logit link function, and the Kenwood-Rogers method of determining degrees of

freedom. For the cows, the model consisted of the fixed effects of system (CONV, FT, or PT), time-point (preWS or postWS), and parity (1, 2, or 3+). Each cow's days in milk on 1 June 2021 was used as a covariate to account for differences in calving date. For the calves, the model consisted of the fixed effects of system (CONV, FT, or PT), time-point (preWS or postWS), calf breed (dairy or dairy-beef), and sex (male or female). Each calf's age on 1 June 2021 was used as a covariate to account for differences in birth date throughout the spring. For both cow and calf models the interaction between system and time-point was tested for each behaviour or behaviour category, and removed from the model if it was not significant ($P > 0.05$). The random effects for both cow and calf models were the individual animal (cow or calf) and the residual. Due to the nature of the logit function, only raw statistical means (\pm standard deviation) are reported throughout for behaviour data.

Results

Cow clinical and physiological markers of health and performance

The interaction between system and time-point was not significant for summed clinical health scores or cortisol. System ($P=0.065$) and time-point ($P=0.052$) tended to influence cow health scores. Slightly higher summed clinical health scores were found postWS (1.02 and 1.23 ± 0.077 ; $P=0.052$). The PT cows (0.98 ± 0.096) tended to have lower summed health scores than the CONV cows (1.30; $P=0.055$), while the FT cows (1.10) did not differ from either. Cortisol (Fig. 1A) was not affected by system ($P = 0.693$) or time-point ($P = 0.324$).

There were significant interactions between system and time-point for albumin, globulin, and total protein, but not SAA. During preWS, the FT cows had higher albumin than the PT cows, while CONV were similar to both (Fig. 1B); postWS all systems were similar. The FT cows had higher globulin preWS compared to postWS, while the CONV and PT cows had similar values preWS and postWS (Fig. 1C); all systems were similar preWS and postWS. Serum amyloid A (Fig. 1D) was not affected by system ($P = 0.627$) or time-point ($P = 0.398$). Despite the interaction between system and time-point for total protein (Fig. 1E), no post-hoc comparisons differed.

There were significant interactions between system and time-point for serum calcium and magnesium, but not phosphorus. Serum calcium (Fig. 1F) was higher in the FT cows preWS compared to postWS, while the CONV and PT cows had similar calcium preWS compared to postWS. The FT cows also had higher calcium than the PT cows preWS, while the CONV cows were similar to both. The

CONV and PT cows had similar serum magnesium levels preWS and postWS, while the FT cows had higher magnesium preWS compared to postWS (Fig. 1G). Although all systems were similar preWS, postWS the CONV cows had higher magnesium than the PT cows, while the FT cows were similar to both. Phosphorus (Fig. 1H) was not affected by time-point ($P=0.116$), but it was affected by system ($P=0.002$). The CONV cows had lower phosphorus (1.31 ± 0.064 mmol/L) compared to FT (1.56 mmol/L; $P=0.024$) and PT (1.63 mmol/L; $P=0.002$) cows (FT and PT cows, $P=0.740$).

Neither BHB nor NEFA had a significant interaction between system and time-point (Fig. 1). Serum BHB (Fig. 1I) was not affected by the fixed effects of system ($P=0.126$) or time-point ($P=0.350$). Serum NEFA (Fig. 1J) was affected by both system ($P = 0.013$) and time-point ($P = 0.003$). The FT cows (0.58 ± 0.038 mmol/L) had higher ($P = 0.009$) NEFA than the PT cows (0.40 mmol/L), while the CONV cows (0.48 mmol/L) were similar to both. All cows had lower NEFA preWS (0.41 ± 0.032 mmol/L) compared to postWS (0.56 mmol/L; $P = 0.003$).

Calf clinical and physiological markers of health and performance

For the calf clinical and physiological markers of health and performance, the only significant interaction was NEFA; therefore, for the rest of the variables in this section, we will only present the fixed effects of system and time-point.

Calf health scores were higher postWS (1.25 ± 0.131) compared to preWS (0.91 ; $P=0.017$), and there was no effect of system (1.01 , 1.03 , and 1.20 ± 0.164 for CONV, FT, and PT calves, respectively; $P=0.572$). Cortisol (Fig. 2A) was not affected by system ($P=0.547$) but tended to be affected by time-point ($P=0.056$). Calves preWS (0.61 ± 0.104 $\mu\text{g/dL}$) tended to have lower cortisol than calves postWS (0.82 $\mu\text{g/dL}$).

Albumin (Fig. 2B) was not affected by system ($P=0.623$) or time-point ($P=0.233$). Globulin (Fig. 2C) was not affected by system ($P=0.127$) but was affected by time-point ($P=0.010$); calves had lower globulin levels preWS (12.00 ± 0.929 g/L) compared to postWS (14.51 g/L). Calf SAA (Fig. 2D) was affected by time-point ($P<0.001$), but not system ($P=0.363$). Calves had higher SAA preWS (18.60 ± 0.174 $\mu\text{g/mL}$) compared to postWS (7.16 $\mu\text{g/mL}$). Total protein (Fig. 2E) was not affected by system ($P=0.896$) but tended to be affected by time-point ($P=0.061$); calves tended ($P = 0.061$) to have lower total protein preWS (53.6 ± 2.30 g/L) compared to postWS (58.5 g/L).

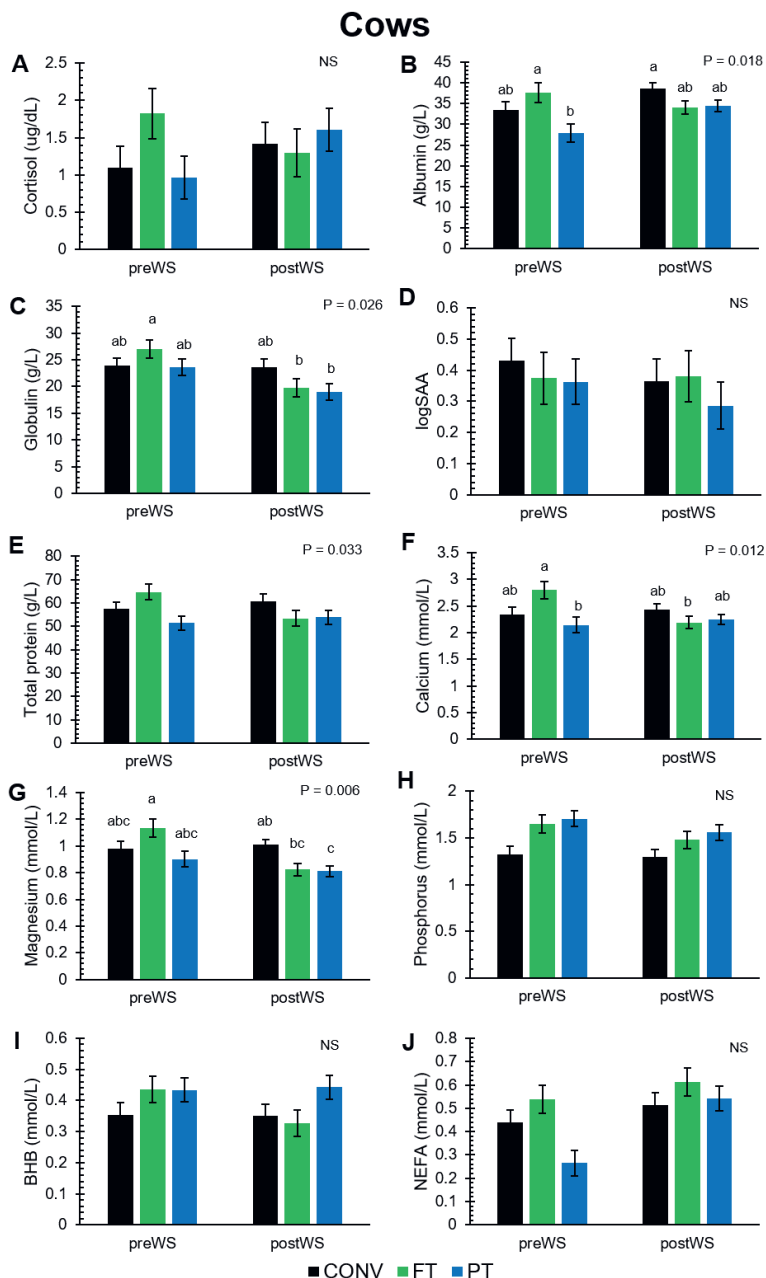


Figure 1. Effect of calf rearing system (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day) on cow physiological markers of health and performance before (preWS) and after weaning and separation (postWS). The interaction between system and time-point was tested in all models, but was removed if $P \geq 0.05$. The p-value for the interaction between system and time-point for each variable is placed in the top right-hand corner of each graph ('NS' denotes a non-significant interaction).

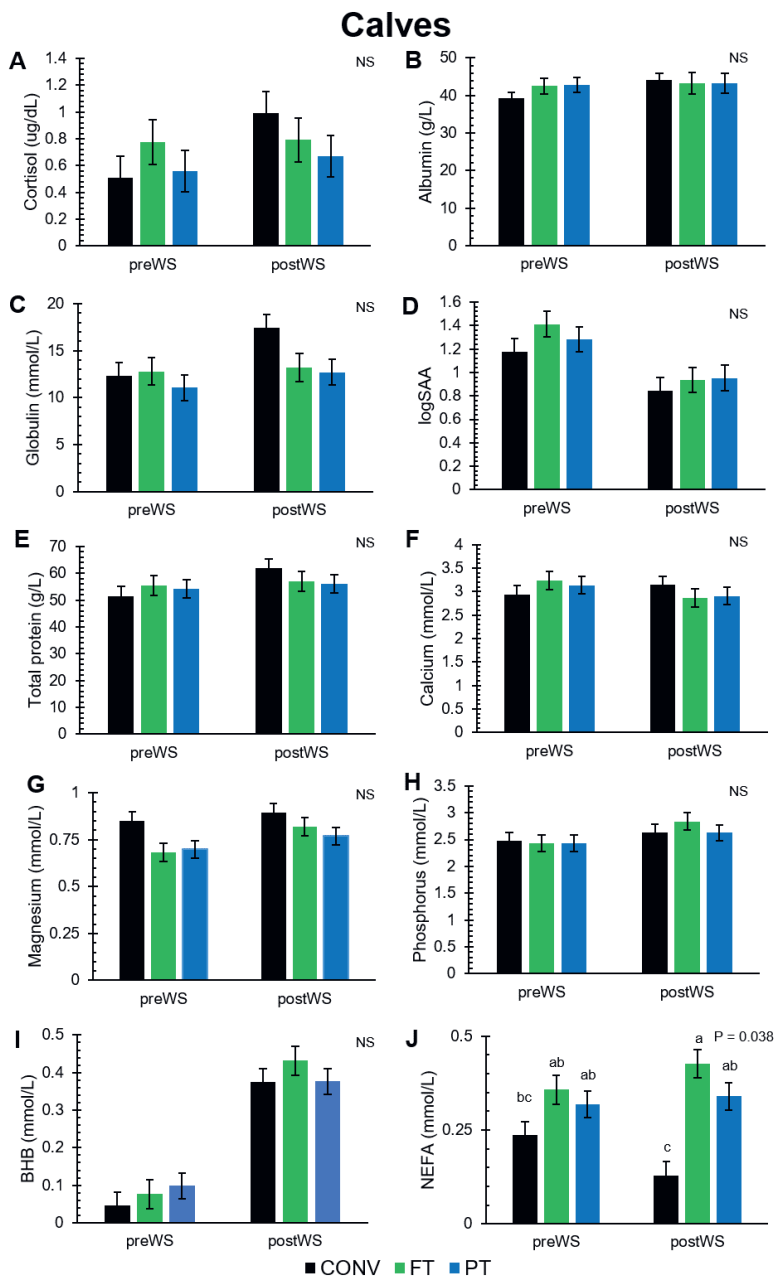


Figure 2. Effect of calf rearing system (Conventional (CONV): no access to dam and kept indoors; Full-time access (FT): full-time access to dam and kept outdoors at pasture; Part-time access (PT): part-time access to dam and kept indoors) on calf physiological markers of health and performance before (preWS) and after weaning and separation (postWS). The interaction between system and time-point was tested in all models, but was removed if $P \geq 0.05$. The p-value for the interaction between system and time-point for each variable is placed in the top right-hand corner of each graph ('NS' denotes a non-significant interaction).

Calcium (Fig. 2F) was not affected by system ($P=0.989$) or time-point ($P=0.389$). Magnesium (Fig. 2G) was affected by system ($P=0.012$) and time-point ($P=0.016$). The CONV calves (0.87 ± 0.039 mmol/L) had higher magnesium levels than PT calves (0.73 mmol/L; $P=0.015$) and tended to have higher magnesium than the FT (0.75 mmol/L; $P=0.050$). All calves had lower levels of magnesium preWS (0.75 ± 0.031 mmol/L) compared to postWS (0.83 mmol/L; $P=0.016$). Phosphorus (Fig. 2H) was not affected by system ($P=0.794$) but was affected by time-point ($P=0.024$); calves had lower levels of phosphorus preWS (2.45 ± 0.099 mmol/L) compared to postWS (2.69 mmol/L).

Calf serum BHB (Fig. 2I) was affected by time-point ($P < 0.001$), there was no effect of system ($P = 0.451$); preWS (0.07 ± 0.023 mmol/L) BHB was lower than postWS (0.39 mmol/L; $P < 0.001$). Within each system, NEFA did not differ between preWS and postWS (Fig. 2J); however, although all systems were similar preWS, postWS the FT and PT calves had higher NEFA than the CONV calves.

Cow performance variables

There was an interaction between system and time-point for daily machine milk yield (Table 3). The CONV cows had higher milk yields than the FT and PT cows preWS and postWS. The FT and PT cow's milk yield increased postWS compared to preWS, while the CONV cows remained similar between the time-points (Table 3). There was an interaction between system and time-point for milk fat concentration (Table 3). The FT and PT cows had higher milk fat postWS compared to preWS; the CONV cows did not differ between the time-points. There was an interaction between system and time-point for milk protein concentration (Table 3). There was no difference between the systems preWS, but postWS the CONV cows had higher protein concentrations than the FT cows, while the PT cows were similar to both. Neither FT nor PT cows differed between the time-points, but the CONV cows had higher protein concentrations postWS compared to preWS (Table 3). There was an interaction between system and time-point for milk lactose concentration (Table 3). The CONV cows had higher lactose than the PT cows preWS, while the FT cows were similar to both; postWS the systems were similar. The PT cows had higher lactose postWS compared to preWS, while the CONV and FT cows did not differ between time-points. There was an interaction between system and time-point for milk solids yield (Table 3). The FT and PT cows had similar milk solids yields at both time-points, but their milk solids yields significantly increased postWS compared to preWS. The CONV cows had higher milk solids yields than the FT and PT cows both preWS and postWS, and their milk solids yields did not change between the time-points. The interaction between

system and time-point was not significant for SCS and neither were their fixed effects (Table 3).

There was an interaction between system and time-point on cow body weight (Table 3); the PT cows were heavier than the CONV and FT cows preWS. There was no difference between the system postWS, and no system was different between the two time-points. There was no interaction between system and time-point on cow BCS or effect of time-point, but there was an effect of system (Table 3). The PT cows (3.18 ± 0.036) were in better condition than the CONV (2.95; $P < 0.001$) and tended to be in better condition than the FT cows (3.05, $P = 0.052$; CONV and FT, $P = 0.126$). There was an interaction between system and time-point for ADG (Table 3). The CONV and PT cows differed in ADG preWS; the CONV cows were losing weight while the PT cows were gaining weight; although the FT cows also had negative ADG preWS, they did not differ from the CONV or PT cows (Table 3). Cows in all systems had similar ADG postWS. The FT and PT cows had similar ADG preWS compared to postWS, while the CONV cows had lower ADG postWS compared to preWS. The interaction of system and time-point was not significant for average change in BCS; there was a fixed effect of system but not time-point (Table 3). The PT cows (0.02 ± 0.042) maintained condition compared to week 1, while the similar CONV (-0.21 ; $P = 0.001$) and FT (-0.16 ; $P = 0.013$) cows lost condition.

Calf performance variables

There was no interaction between system and time-point on calf body weight (kg), but there was an effect of system and time-point (Table 3). The CONV calves (74.1 ± 2.19 kg) weighed less than the FT (84.4 kg; $P = 0.003$) and PT (85.0 kg; $P = 0.001$) calves (FT vs. PT, $P = 0.979$). Calves weighed more postWS (86.6 ± 1.51 kg) compared to preWS (75.7 kg; $P < 0.001$). There was an interaction between system and time-point for ADGw (Table 3). There was no difference between the systems preWS, but postWS the CONV calves had higher ADGw than the FT and PT calves (Table 5). The FT and PT calves had higher ADGw preWS compared to postWS, while the CONV calves were similar between the time-points (Table 3). There was also an interaction between system and time-point for ADGb (Table 3). Although no system differed between the time-points, the FT and PT calves had similar and higher ADGb than the CONV both preWS and postWS (Table 3).

Table 3: Effect calf rearing system (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf/dam, cow milked twice-a-day, and calf kept indoors; Part-time access (PT): part-time access to calf/dam, cow milked once-a-day, and calf kept indoors) on various cow and calf performance variables before (preWS) and after weaning and separation (postWS). The interaction between system and time-point was tested in all models, but was removed if $P \geq 0.05$.

	preWS			postWS			P-values		
	CONV	FT	PT	CONV	FT	PT	SEM	S	TP
Cow performance variables									
Machine milk yield (kg/d)	24.5 ^a	11.7 ^c	9.4 ^c	24.3 ^a	18.4 ^b	19.1 ^b	0.60	<0.001	<0.001
Milk fat (%)	4.83 ^{abc}	3.98 ^c	4.24 ^{bc}	4.73 ^{abc}	4.78 ^{ab}	4.93 ^a	0.204	0.198	0.002
Milk protein (%)	3.44 ^{bc}	3.41 ^{abc}	3.39 ^{bc}	3.61 ^a	3.37 ^{bc}	3.52 ^{ab}	0.055	0.145	0.004
Milk lactose (%)	4.90 ^a	4.82 ^{ab}	4.72 ^b	4.86 ^{ab}	4.91 ^a	4.88 ^a	0.038	0.145	0.008
Milk solids yield (kg)	2.02 ^a	0.86 ^c	0.71 ^c	2.02 ^a	1.50 ^b	1.59 ^b	0.058	<0.001	<0.001
Somatic cell score	4.65	4.37	4.98	4.43	4.55	4.68	0.200	0.292	0.184
Body weight (kg)	476 ^c	486 ^{bc}	520 ^a	494 ^{abc}	477 ^{bc}	508 ^{ab}	7.3	0.001	0.819
BCS	2.95	3.02	3.17	2.95	3.09	3.19	0.042	<0.001	0.247
ADG (kg/d)	-0.75 ^c	-0.56 ^{bc}	0.08 ^a	-0.30 ^{ab}	-0.57 ^{bc}	-0.11 ^{ab}	0.137	0.004	0.233
Change in BCS	-0.21	-0.20	0.00	-0.21	-0.13	0.03	0.048	0.001	0.243
Calf performance variables									
Body weight (kg)	68.1	78.8	80.2	80.1	90.0	89.7	2.24	<0.001	<0.001
ADG from birth (kg/d)	0.61 ^c	0.83 ^a	0.84 ^{ab}	0.64 ^c	0.79 ^{ab}	0.78 ^b	0.035	<0.001	0.005
ADG week (kg/d)	0.95 ^{ab}	1.01 ^a	0.94 ^{ab}	1.04 ^a	0.42 ^{bc}	0.40 ^c	0.142	0.043	0.002

Abbreviations: BCS = Body condition score; ADG = Average daily gain; SEM = Standard error of the mean; NS = not significant
^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.
Abbreviations: S = system; TP = time-point

Table 4: Average prevalence (%) of cow behaviours (mean \pm standard deviation) from live scan sampling observations before (preWS) and after weaning and separation (postWS) for cows within three different calf rearing systems (Conventional (CONV): no access to calf and milked twice-a-day; Full-time access (FT): full-time access to calf and milked twice-a-day; Part-time access (PT): part-time access to calf and milked once-a-day). The interaction between system and time-point was tested in all models, but was removed if $P \geq 0.05$. Behaviour categories with low prevalence were not statistically analysed.

Behaviour	preWS			postWS			P-value		
	CONV	FT	PT	CONV	FT	PT	S	TP	S*TP
Lie	27.1 \pm 19.24	23.2 \pm 20.20	16.2 \pm 16.47	30.6 \pm 20.36	9.2 \pm 16.68	13.7 \pm 19.00	0.017	0.354	NS
Ruminate	19.7 \pm 21.71 ^{ab}	33.3 \pm 29.73 ^a	36.8 \pm 18.65 ^a	22.9 \pm 17.40 ^{ab}	19.0 \pm 15.57 ^{ab}	11.3 \pm 16.16 ^b	0.660	0.003	0.008
Eat solids	43.8 \pm 15.67	54.5 \pm 34.96	51.9 \pm 19.76	41.9 \pm 18.93	49.4 \pm 15.92	58.3 \pm 24.79	0.088	0.954	NS
Abnormal	0.2 \pm 0.98	0.0 \pm 0.00	0.7 \pm 2.14	0.5 \pm 1.96	1.8 \pm 4.54	0.7 \pm 2.14	0.636	0.001	NS
Positive	0.0 \pm 0.00	1.5 \pm 4.51	0.7 \pm 2.14	1.16 \pm 3.13	0.0 \pm 0.00	0.0 \pm 0.00	-	-	-
Maintenance	1.2 \pm 1.92	2.1 \pm 4.25	1.2 \pm 1.92	1.6 \pm 2.53	1.5 \pm 2.07	0.9 \pm 1.78	-	-	-

*No comparisons were significant in post-hoc comparison

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

Abbreviations: S = system; TP = time-point

Table 5: Average prevalence (%) of calf behaviours (mean \pm standard deviation) from live scan sampling observations before (preWS) and after weaning and separation (postWS) for cows within three different calf rearing systems (Conventional (CONV): no access to dam and kept indoors; Full-time access (FT): full-time access to dam and kept outdoors at pasture; Part-time access (PT): part-time access to dam and kept indoors). The interaction between system and time-point was tested in all models, but was removed if $P \geq 0.05$. Behaviour categories with low prevalence were not statistically analysed. * Denotes that no comparisons were significant in post-hoc comparison tests.

	preWS			postWS			P-value		
	CONV	FT	PT	CONV	FT	PT	S	TP	S*TP
Lying	50.9 \pm 25.35	71.1 \pm 21.40	66.4 \pm 29.38	65.3 \pm 30.69	62.2 \pm 28.61	44.2 \pm 21.82	0.348	0.301	0.023*
Rumination	28.9 \pm 20.93	34.5 \pm 36.56	11.3 \pm 16.35	36.3 \pm 22.77	32.4 \pm 14.45	30.1 \pm 20.29	0.052	0.051	NS
Eating solids	9.0 \pm 9.07 ^{bc}	8.6 \pm 11.49 ^{bc}	0.5 \pm 1.35 ^c	13.0 \pm 14.84 ^b	16.7 \pm 15.16 ^{ab}	33.1 \pm 14.68 ^a	0.200	<0.001	0.002
Maintenance	7.2 \pm 8.06	4.8 \pm 5.86	10.4 \pm 10.43	5.3 \pm 8.06	5.1 \pm 5.70	5.6 \pm 6.06	0.422	0.055	NS
Positive behaviours	2.5 \pm 3.82	5.1 \pm 6.77	6.3 \pm 7.73	1.9 \pm 3.27	2.4 \pm 3.55	2.8 \pm 3.50	0.198	0.009	NS
Abnormal behaviours	5.6 \pm 6.85	0.6 \pm 2.23	7.2 \pm 13.00	3.7 \pm 6.53	4.8 \pm 6.30	3.5 \pm 5.94	0.294	0.440	0.034*
Abnormal oral behaviours	3.5 \pm 5.58	0.6 \pm 2.23	7.5 \pm 13.00	2.8 \pm 5.54	3.0 \pm 5.76	3.0 \pm 5.50	-	-	-
Drinking milk	0.0 \pm 0.00	2.4 \pm 8.91	1.9 \pm 7.86	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	-	-	-
Drinking water	0.9 \pm 2.69	0.0 \pm 0.00	0.5 \pm 1.35	1.4 \pm 2.86	0.3 \pm 1.11	1.4 \pm 3.50	-	-	-
Walking	0.2 \pm 0.98	0.6 \pm 1.51	1.6 \pm 3.24	0.7 \pm 2.95	0.0 \pm 0.00	0.0 \pm 0.00	-	-	-

*No comparisons were significant in post-hoc comparison
a,b Values within a row with different superscripts differ significantly at $P < 0.05$.
Abbreviations: S = system; TP = time-point

Cow and calf time-budget behaviour

The average prevalence (\pm standard deviation) of each behaviour or behaviour category for each system preWS and postWS can be found in Table 4 (cows) and Table 5 (calves).

There was an interaction between system and time-point for the proportion of time cows spent ruminating (Table 4); the PT cows spent more time ruminating preWS compared to postWS, while the CONV and FT cows did not differ between the time-points. There were no other significant interactions between system and time-point found in the cow behaviours. The proportion of time cows spent lying was affected by system (Table 4); CONV cows ($28.8 \pm 19.60\%$) spent more time lying than PT cows ($14.9 \pm 17.58\%$; $P=0.034$) and the CONV cows tended to spend less time lying than FT cows ($16.2 \pm 19.52\%$; $P=0.063$). There was no effect of time-point on the proportion of time cows spent eating solids; however, there was a tendency for the effect of system (Table 4). The CONV cows ($42.8 \pm 17.15\%$) tended ($P=0.088$) to spend less time eating solids than the PT cows ($55.1 \pm 22.34\%$), while the FT cows ($51.9 \pm 26.78\%$) were similar to both. The proportion of time cows spent performing abnormal behaviours (oral manipulation of the pen structure, head out of pen, pacing, and tongue rolling; see Table 1) was not affected by system, but was affected by time-point (Table 4); across all systems cows performed more abnormal behaviours postWS ($0.9 \pm 2.95\%$; $P=0.001$) compared to preWS ($0.3 \pm 1.42\%$).

There were interactions between system and time-point for the proportion of time calves spent lying, eating solids, and performing abnormal behaviours (Table 5); for all other behaviours, only the fixed effects of system and time-point are reported. There was an interaction between system and time-point in the proportion of time calves spent lying (Table 5); however, no post-hoc comparisons differed. The proportion of time calves spent ruminating tended to be affected by both system and time-point (Table 5). The CONV calves ($32.6 \pm 21.88\%$) tended ($P = 0.061$) to spend more time ruminating than the PT calves ($20.7 \pm 20.50\%$), while the FT calves ($33.4 \pm 27.30\%$) were similar to both. Calves tended ($P = 0.051$) to spend more time ruminating postWS ($33.0 \pm 19.63\%$) compared to preWS ($24.2 \pm 26.43\%$). There was an interaction between system and time-point in proportion of time calves spent eating solid feed (Table 5). There was no difference between the systems preWS, but postWS the PT calves spent longer eating solid feed than the CONV calves, while the FT calves were similar to both. The PT calves spent less time eating solid feed preWS compared to postWS, while the CONV and FT calves did not differ between time-points. The time calves spent performing maintenance

behaviours was not affected by system, but tended to be affected by time-point (Table 5); calves tended to spend more time ($P = 0.055$) performing maintenance behaviours preWS ($7.7 \pm 8.64\%$) compared to postWS ($5.3 \pm 6.63\%$). The proportion of time calves spent performing positive behaviours was affected by time-point but not system (Table 5); calves spent more time ($P=0.009$) performing positive behaviours preWS ($4.6 \pm 6.37\%$) compared to postWS ($2.3 \pm 3.38\%$). There was an interaction between system and time-point in the proportion of time calves spent performing abnormal behaviour (Table 5); however, no post-hoc comparisons differed.

Discussion

The aim of this exploratory paper was to investigate the effect of cow-calf contact rearing systems on cow and calf health, performance, and behaviour before (preWS) and after weaning (postWS). We compared three systems: the conventional, no contact system (CONV), a full-time access CCC system (FT), and a part-time access by night CCC system (PT). The two CCC systems were chosen to work in tandem with the pre-existing system (CONV). The FT system was chosen as it mimicked CONV, only with the addition of calves. The PT system was chosen as it allowed to calves to remain indoors (easing any potential health concerns) while the cows could still graze part-time, and it mimicked a common early-lactation labour reduction strategy often employed on Irish farms (OAD milking). Please refer to McPherson et al. (2024) for a more detailed description on why these specific CCC were chosen. This paper only discusses a portion of a larger research project; please refer to McPherson et al. (2024) and Sinnott et al. (2024) for more details on the long-term outcomes of this experiment on the cows and calves, respectively.

Cows and calves in the FT and PT systems experienced separation at the same time as weaning, while the CONV cows and calves experienced separation soon after birth and the CONV calves were weaned at the same age as the FT and PT calves. After the first stage of the weaning and separation process where calves were still nursed for 1 hour a day, FT and PT calves were prevented from nursing from their dam in the second stage onwards and were not provided with another source of milk. As a result, the cows were no longer being nursed, causing a decrease in their udder stimulation/milking frequency; for the PT cows, there was also an increase in parlour milking as they were switched from once-a-day to twice-a-day milking. All FT and PT pairs also underwent a temporary change in environment for the duration of the weaning and separation process. The PT pairs were moved from their original shed into a different shed and the FT pairs were

moved indoors. FT and PT pairs were in the same shed for the weaning and separation process. The FT and PT cows did not go outside, except for the walk to and from the parlour, for the duration of the weaning and separation process (6 d total), causing a change in diet; they were provided with *ad libitum* silage and water during this time, with their concentrates matching what was provided to the CONV cows. In contrast, the CONV cows experienced no changes to their udder stimulation, milking frequency, diet, contact with their calf, or location during this period; they were milked twice-a-day and were kept outdoors at grass. The CONV calves had begun their gradual weaning process at 39 d, thus their preWS samples were taken after weaning had already started. The CONV calves were kept in their group pen until the cessation of the weaning process, when they were regrouped indoors (calves from all systems were regrouped together).

Effect of calf rearing system on cow physiological health, performance, and behaviour before and after weaning

Overall, all cows in this experiment appeared to be in good health, as indicated by their summed clinical health scores. However, we were not just interested in their clinical health, but also in any underlying (sub-clinical) physiological differences in their health and performance, potentially caused by the cows' experience on the different systems and/or by weaning and separation from their calves. We were particularly interested in exploring this, as we had previously observed that CCC had a negative effect on these cows' machine milk yield over their entire lactation, due in part to the fact that their machine milk yield did not fully recover after weaning and separation (McPherson et al., 2024). As cow health and production are intrinsically linked (Bareille et al., 2003), we wanted to know whether cows experienced any changes in physiological markers of health and performance around weaning and separation, which might, in part, explain why we observed such differences in production between the systems.

Two of the most important cow performance variables, machine milk yield and milk solids yield, were both affected by the combination of system and weaning. As expected, machine milk yields were lower in the CCC cows nursing calves compared to the CONV cows preWS (Webb et al., 2022; Wenker et al., 2022b; Neave et al., 2024a). However, although the FT and PT cows did increase in machine milk yield postWS, they remained lower than the CONV cows. A temporary decrease in machine milk yield after weaning and separation has been observed in some past CCC studies (Everitt and Phillips, 1971; Metz, 1987; Webb et al, 2022), but not in others (Wenker et al., 2022b), and has been attributed to the distress from being separated from their calf as well as a change in their

environment (i.e., Metz, 1987). Although differences in milk solids yield may also be driven by differences in milk fat and protein concentrations, it is likely that machine milk yield was the larger factor contributing to the observed differences in milk solids yield in this experiment.

Weaning and separation from their calf combined with the change in milking frequency (from once-a-day to twice-a-day parlour milking) appears to have influenced the PT cows' energy balance. The PT cows appeared to be in a more positive energy balance preWS compared to the CONV and FT cows, as suggested by their higher BW and BCS and lower serum NEFA levels. Both higher BW and BCS scores (McNamara et al., 2008; Kennedy et al., 2021) as well as lower NEFA (Patton et al., 2006) have been observed previously in pasture-based dairy cows milked once-a-day in early lactation compared to cows milked twice- or thrice-a-day on the same diet. After weaning and separation and their switch to twice-a-day milking, the PT cows numerically increased in NEFA (0.26 mmol/L preWS to 0.54 mmol/L postWS; Fig. 1J) and numerically decreased in BW (-12 kg) and ADG (+0.08 kg/d preWS to -0.11 kg/d postWS). This likely indicates that the PT cows were in a more negative energy balance postWS compared to preWS, but we could not demonstrate this through statistical significance here due to large individual variation.

Cows that are milked once-a-day often have higher somatic cell counts or SCS compared to cows milked twice-a-day (Clark et al., 2006; Kennedy et al., 2021; Murphy et al., 2023). Although we did not observe a difference in the direct comparison of SCS preWS and postWS here, we did in our long-term study on the same cows (McPherson et al., 2024). When investigating SCS over larger periods of time, we found that the PT cows had higher SCS than the FT cows during the CCC period (week 1-8), but there was no difference after weaning and separation, once the PT cows had switched to twice-a-day milking (weeks 9-35, CONV cows did not differ to either FT or PT cows during any time-period; McPherson et al., 2024). The PT cows also had lower serum albumin than the FT cows preWS (CONV were similar to both), and this difference disappeared postWS. Albumin levels have been shown to decrease with increasing SCS (Bobbo et al., 2017a, 2017b). Higher SCS has also been associated with lower milk lactose concentration (Alessio et al., 2021), which may explain why the PT cows had lower lactose concentrations preWS compared to the CONV cows.

In contrast to Wenker et al. (2022b), who found no difference in serum calcium between the full-time CCC system they applied and their control, we found that preWS the FT cows had higher serum calcium than the PT cows (CONV were similar to both) and postWS there was no difference between the cows on the

different systems. The reason for this is unclear; it may have to do with CCC, but we would expect to see a similar response in the PT cows preWS if this were true. The difference is also likely not caused by the difference in milking frequency, as cows milked once-a-day have been reported to have higher serum calcium than cows milked twice-a-day at the start of lactation (Loiselle et al., 2009). We suggest that the difference observed in serum calcium may be due to the smaller sample size of the FT cows (n=14) compared to the CONV (n=18) and PT (n=18) cows. As a result of dropping four multiparous cows from the experiment due to issues with their calves' health, the FT cows were of younger parity than the CONV and PT cows. Younger lactating cows have been shown to have higher concentration of calcium and magnesium in their blood serum (McAdam and O'Dell, 1972), as more mature cows have a higher metabolic demand for calcium due to their increased milk production and decreased ability to mobilise bone calcium and absorb calcium from their intestines (Horst et al., 2005). However, we did not observe the same difference postWS, which suggests that some other mechanism is occurring, potentially related to their CCC system.

Serum magnesium was higher in the FT cows preWS compared to postWS, while the CONV and PT did not differ between time-points. Serum magnesium is typically considered to be highly reliant on cows' diets. Cows do not have the capacity to store magnesium in their bodies; the majority of magnesium absorption occurs in the rumen, where high levels of potassium can impair magnesium absorption (Suttle, 2022; Khiaosa-Ard et al., 2023). However, the FT cows were being provided an identical diet to the CONV cows preWS, so we should not have observed a difference in magnesium due to diet. It is possible that the FT cows did not consume the extent of their offered feed, potentially because they had a different daily routine than the CONV due to having their calf with them. We did not measure intakes of the cow, or the calves, but this should be done in the future to try and provide more insight into why we saw these differences. A potential difference in intake may also explain why we observed a system difference in serum phosphorus (FT and PT cows had higher phosphorus than the CONV cows). As serum phosphorus has been shown to be lower in dairy cows at peak lactation and during drought conditions (Betteridge, 1986), which may indicate that the FT and PT cows were not as metabolically stressed due to producing milk as the CONV cows both preWS and postWS. We observed that the CONV cows spent more time lying down than the PT cows (and tended to spend more time lying than the FT cows), suggesting that they had a different daily routine than the CCC cows. This opposes previous research on lying time in CCC cows. Although Wegner and Ternman (2023) found that CCC did

not affect cow lying time, Johnsen et al. (2021a) found that cow lying time increased when cows progressed from their CCC phase to being separated from their calf. The proportion of time cows spent eating solids also tended to be affected by system, with the CONV cows spending less time eating solids compared to the PT cows. However, our behaviour observations only encapsulated part of the day; these results may have differed if alternative or longer time periods were observed.

Effect of calf rearing system on calf physiological health, performance, and behaviour before and after weaning

We did not find many differences in calf physiological health linked to their rearing system; instead, many health-related variables were only affected by the weaning process. All calves, regardless of system, had higher summed clinical health scores postWS, indicating that weaning left them in worse health. Serum globulin, a marker of inflammation, was also higher postWS, which matches what has previously been found in post-weaned calves (Kim et al., 2011). However, we found that SAA, an α -globulin and thus another marker of inflammation (Eckersall and Bell, 2010; Bobbo et al., 2017a; Trela et al., 2017), was higher preWS. This warrants further investigation. In addition, preWS calves expressed more positive behaviours (play and social interactions) and tended to have lower serum cortisol, indicating that weaning may have had a negative impact on their affective states (Ahloy-Dallaire et al., 2018), regardless of their system. The large observed response to weaning may indicate that weaning occurred too early in our calves. Although all calves were weaned at 8 weeks, a typical age in Ireland (Barry et al., 2020) and elsewhere in Europe (Johnsen et al., 2021c; Mahendran et al., 2022; European Food Security Authority Panel on Animal Health and Animal Welfare, 2023), 8 weeks may still be too young for calves to have completely adapted to a reliance on solid feed.

Most of the differences we observed between the systems had to do with calf performance and behaviour, especially around weaning. Although all calves had higher BW postWS compared to preWS, the FT and PT calves had higher BW than the CONV calves across both time-points. This was most likely due to the FT and PT calves consuming a greater milk allowance during the pre-weaning period (CONV calves had a maximum allocation of 9 L/d of milk replacer while the FT and PT calves had essentially *ad libitum* access to milk) and is a common result in CCC research (Johnsen et al., 2021b; Wenker et al., 2022b; Sinnott et al., 2024). We also observed that the FT and PT calves had a decrease in ADGw postWS, while the CONV calves had a consistent ADGw, which was also an expected result of

weaning and separation in CCC systems (Johnsen et al., 2021a; Wenker et al., 2022b; Sinnott et al., 2024). This may have been due to the combination of their more abrupt weaning process (resulting in a larger change in diet composition compared to the CONV calves) and potentially the distress caused by the separation from their dam. The proportion of time calves spent eating solids also increased postWS, with the PT calves increasing their time eating solids from 0.5% preWS to 33.1% postWS. Both the CONV and FT calves were observed eating solids around 9% preWS and did not have as big of a jump postWS.

The serum NEFA for the FT and PT calves also reflect their difficulties adapting postWS. Serum NEFA typically decreases in calves after weaning (Ferronato et al., 2022), which appears to be what we observed numerically in the CONV calves. The FT and PT calves had numerically higher serum NEFA than CONV calves postWS, which may be an indication that they struggled more nutritionally post-weaning and likely reflects their lower ADGw. Previous work has found higher NEFA in abruptly weaned beef calves compared to more gradually weaned calves (González et al., 2023). All calves increased BHB postWS, which was expected as higher BHB is often associated with larger solid feed intakes (Quigley III et al., 1996; Deelen et al., 2016), particularly concentrate intake, and thus BHB is often used as a proxy for rumination. Calves on all systems tended to spend more time ruminating postWS compared to preWS, which matches our BHB results.

Conclusion

In this explorative study, in which we compared three different dairy calf rearing systems, we found evidence to suggest that the combination of weaning and separation in two different CCC systems influenced some cow and calf physiological health, performance, and behaviour indicators. Despite having no difference in their summed clinical health scores, the FT and PT cows differed from the CONV cows in several physiological health parameters. Differences in cow performance stemmed from both the different systems and the weaning process. Diet and management of CCC cows thus may play a large role in the transition from being suckled by their calf to no contact with their calf; future CCC research should focus on designing a management routine to minimise the impact that the transition appears to have had on the cows. Overall, weaning appeared to have more of an effect on our calves' health and performance than the system that they were reared in. However, calves in the CCC systems appeared to struggle more in the post-weaning transition, as suggested by their lower ADGw and higher NEFA, despite consuming more solid feeds than the CONV calves postWS. As cows and

calves in CCC systems need to adapt to the conventional dairy systems after separation, future work should focus on which factors are most important on easing this transitional period. Overall, management of CCC systems may be the key to their success.

Acknowledgements

The authors wish to thank and acknowledge the Moorepark farm staff for their care of the animals and their assistance and patience with this experiment. We would also like to thank Aoife Jones for her assistance with running the serum amyloid A analysis.

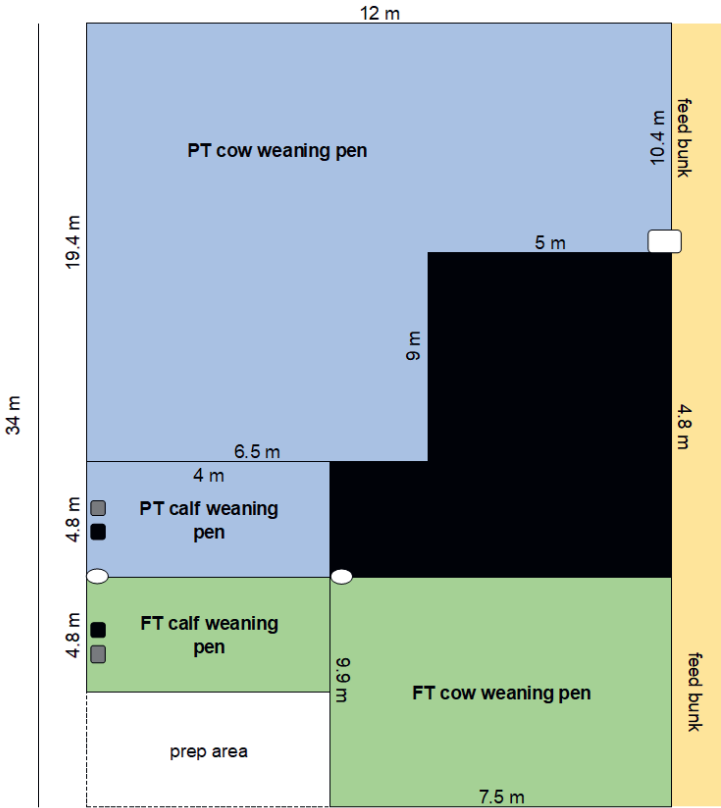
Ethics approval

Ethical approval for this study was received from the Teagasc Animal Ethics Committee (TAEC2020-290) and procedure authorization was granted by the Irish Health Products Regulatory Committee (AE19132/P124). Experiments were performed in accordance with European Union (Protection of Animals Used for Scientific Purpose) Regulations 2012 (S.I. No. 543 of 2012).

Financial support statement

This research is funded by Science Foundation Ireland (SFI) and the Department of Agriculture, Food, and Marine on behalf of the Government of Ireland under Grant Number [16/RC/3835] – VistaMilk.

Supplemental Figure S1: Indoor weaning and separation areas for cow-calf pairs in the full-time access (FT) and part-time access (PT) cow-calf contact systems. All gates allowed for some degree of physical contact between groups. The black area represents pens not used for the weaning and separation process. The white prep area was not used for animal housing. White-filled boxes and circles represent water troughs, black-filled boxes represent concentrate feeders, and grey-filled boxes represent forage (hay) feeders. In the calf pens, buckets of water were also provided (some calves were too short to use the water bowls).



Supplemental Table S1: Dairy cow and calf clinical health scoring chart used in the study, which was adapted from Barry et al. (2019a) and Crossley et al. (2021).

Indicator	Definition	Scoring scale
Demeanour	Combined evaluation of behaviour and responsiveness	4-point scale (0 to 3) where: 0 = Bright, alert, responsive 1 = Dull, possible depressed, less responsive 2 = Dull, markedly depressed, markedly unresponsive
Ocular discharge	Presence of any ocular discharge	4-point scale (0 to 3) where: 0 = Bright, pronounced 1 = Slightly dull, presence of a small amount of non-clear discharge in one eye 2 = Dull, sunken, small amount of non-clear discharge present in both eyes 3 = Dull, sunken, excessive non-clear discharge present in both eyes
Ear position	Position and activity of ears	4-point scale (0 to 3) where: 0 = Alert and mobile 1 = Slightly drooped 2 = Drooped 3 = Drooped and limp
Nasal discharge	Presence of any mucous discharge from the nasal passage	4-point scale (0 to 3) where: 0 = Clear, discharge free 1 = Small amount of cloudy mucous visible 2 = Medium amount of bilateral mucous discharge 3 = Excessive bilateral mucous discharge
Cough	Presence of a cough or an increased respiratory rate	4-point scale (0 to 3) where: 0 = Normal breathing 1 = Spontaneous coughing 2 = Intermittent coughing 3 = Continuous cough, increased respiration
Dehydration	Appearance of cow eyes in relation to hydration levels	4-point scale (0 to 3) where: 0 = Clear, bright eyes 1 = Eyes slightly sunken 2 = Eyes sunken 3 = Eyes markedly sunken
Mobility	Ability to stand unassisted and move freely	4-point scale (0 to 3) where: 0 = Stands unassisted, actively mobile 1 = Slow to stand, limited mobility 2 = Struggles to stand, limited mobility 3 = Assistance required to stand, no mobility
Interest in Surroundings	Willingness to interact with observer	2-point scale (0 or 1) where: 0 = Interactive when approached 1 = Uninterested when approached
Faecal hygiene	Cleanliness of cow tail area and hindquarters	4-point scale (0 to 3) where: 0 = Completely clean with no faecal matter 1 = Slight faecal matter present 2 = Heavier faecal matter present 3 = Extremely dirty with faecal matter
Naval	Swelling and/or tenderness of the naval	4-point scale (0 to 3) where: 0 = 1 = 2 = 3 =

Supplemental Table S2: Mean (\bar{x}), SEM, variance (σ), and CV (\bar{x}/σ) for all measured study variables. The study population consisted of dairy cows and calves in three different calf rearing systems: the conventional Irish system (CONV), where cow and calf were separated <1 h post-birth, cows were pasture-based and milked twice-a-day; a full-time access (FT) system, dam and calf were allowed constant, unrestricted access, were pasture-based, and cows were milked twice-a-day; and a part-time access (PT) system, dam and calf had unrestricted access when indoors at night, cows grazed outdoors by day while calves remained indoors, and cows were milked once-a-day in the morning.

Animal	Category	Variable	mean (\bar{x})	variance (σ)	SEM	CV
calf	health	Albumin, g/L	41.50	9.570	0.957	0.231
		BHB, mmol/L	0.23	0.122	0.012	0.530
		Calcium, mmol/L	2.99	0.734	0.073	0.246
		Cortisol, μ g/dL	0.73	0.577	0.058	0.790
		Globulin, g/L	13.12	5.428	0.543	0.414
		Magnesium, mmol/L	0.77	0.181	0.018	0.234
		NEFA, mmol/L	0.31	0.162	0.016	0.523
		Phosphorus, mmol/L	2.58	0.578	0.058	0.224
		logSAA*	1.12	0.382	0.040	0.342
		Summed health score	1.04	1.000	0.071	0.961
		Total protein, g/L	54.97	14.176	1.418	0.258
cow	performance	Average daily gain from birth, kg/d	0.75	0.123	0.012	0.164
		Body weight, kg	78.92	9.486	0.949	0.120
		Weekly average daily gain, kg/d	0.75	0.524	0.052	0.697
	health	Albumin, g/L	34.30	7.357	0.736	0.214
		BHB, mmol/L	0.39	0.154	0.015	0.396
		Calcium, mmol/L	2.35	0.502	0.050	0.213
		Cortisol, μ g/dL	1.37	1.104	0.111	0.807
		Globulin, g/L	22.76	6.537	0.654	0.287
		Magnesium, mmol/L	0.94	0.208	0.021	0.220
		NEFA, mmol/L	0.48	0.213	0.021	0.441
		Phosphorus, mmol/L	1.50	0.356	0.036	0.236
		logSAA	0.37	0.305	0.031	0.834
		Summed health score	1.13	0.772	0.055	0.685
		Total protein, g/L	56.79	12.494	1.249	0.220
	performance	Body condition score	3.06	0.194	0.019	0.063
		Body weight, kg	493.46	57.909	5.791	0.117
		Average daily gain, kg/d	-0.36	0.529	0.053	-1.456
		Average change in body condition score	-0.12	0.200	0.020	-1.668
		Machine milk yield, kg/d	17.93	3.670	0.367	0.205
		Milk fat concentration, %	4.59	0.865	0.086	0.189
		Milk lactose concentration, %	4.85	0.159	0.016	0.033

Milk protein concentration, %	3.45	0.287	0.029	0.083
Milk solids yield, kg	1.45	0.303	0.030	0.208
Somatic cell score	4.60	0.694	0.075	0.151

Mean (\bar{x}), S.E.M., variance (σ), and coefficient of variation ($CV = \bar{x}/\sigma$) for all measured health and performance variables for both cows and calves.

*LogSAA = $\log_{10}(SAA+1)$, original unit = g/mL

Supplemental Table S3: Model selection (Bayesian Information Criterion (BIC)), random effects variances (σ^2_{cow} , σ^2_e), covariance parameter estimates (compound symmetry (CS) or first-order autoregressive lag 1 (AR(1))), phenotypic variance (σ^2_p), variable means (\bar{x}), and CV (%) for all variables analysed using generalised linear mixed models (PROC MIXED) in SAS. The study population consisted of dairy cows and calves in three different calf rearing systems: the conventional Irish system (CONV), where cow and calf were separated <1 h post-birth, cows were pasture-based and milked twice-a-day; a full-time access (FT) system, dam and calf were allowed constant, unrestricted access, were pasture-based, and cows were milked twice-a-day; and a part-time access (PT) system, dam and calf had unrestricted access when indoors at night, cows grazed outdoors by day while calves remained indoors, and cows were milked once-a-day in the morning.

animal	Cate-gory	Variable	BIC	σ^2_{cow}	CS	AR(1)	σ^2_e	σ^2_p	\bar{x}	CV%
calf	health	Albumin, g/L	713.3	11.13	0.00	-	72.58	83.71	42.57	21.49
		BHB, mmol/L	-65.9	0.00	0.00	-	0.02	0.02	0.30	46.83
		Calcium, mmol/L	238.1	0.04	0.00	-	0.46	0.50	3.04	23.30
		Cortisol, $\mu\text{g/dL}$	207.3	0.07	0.00	-	0.30	0.37	0.72	84.65
		Globulin, g/L	611.0	7.18	0.00	-	21.53	28.71	13.25	40.43
		Magnesium, mmol/L	-19.0	0.01	0.00	-	0.03	0.03	0.79	22.87
		NEFA, mmol/L	-53.8	0.00	0.00	-	0.02	0.02	0.30	47.47
		Phosphorus, mmol/L	197.4	0.06	0.00	-	0.27	0.33	2.57	22.33
		logSAA	115.8	0.03	0.00	-	0.12	0.15	1.10	35.61
		Summed health score	602.0	0.09	0.00	-	0.98	1.07	1.08	95.74
		Total protein, g/L	785.7	20.11	0.00	-	161.54	181.65	56.01	24.07
	performance	Average daily gain from birth, kg/d	-	0.01	0.00	-	0.00	0.02	0.75	16.66
		Weekly average daily gain, kg/d	157.5	0.01	0.00	-	0.27	0.28	0.79	67.38
		Body weight, kg	599.7	56.79	0.00	-	6.58	63.37	81.15	9.81
cow	health	Albumin, g/L	672.6	6.49	0.00	-	51.65	58.14	34.34	22.20
		BHB, mmol/L	-26.8	0.00	0.00	-	0.02	0.03	0.39	41.26
		Calcium, mmol/L	191.3	0.06	-	-0.23	0.21	0.28	2.36	22.24
		Cortisol, $\mu\text{g/dL}$	337.6	0.42	0.00	-	1.06	1.48	1.37	89.09
		Globulin, g/L	634.8	14.53	0.00	-	26.55	41.08	22.83	28.08
		Magnesium, mmol/L	30.5	0.01	0.00	-	0.04	0.05	0.94	22.90
		NEFA, mmol/L	38.3	0.00	-0.01	-	0.06	0.06	0.49	49.72
		Phosphorus, mmol/L	118.9	0.00	0.00	-	0.12	0.13	1.50	23.53
		logSAA	90.0	0.01	0.00	-	0.08	0.09	0.37	82.73
		Somatic cell score	204.3	0.00	-	0.61	0.57	0.57	4.61	16.31
		Summed health score	486.4	0.00	0.00	-	0.59	0.59	1.13	67.93
		Total protein, g/L	761.8	16.01	0.00	-	140.54	156.55	56.91	21.98
	Performance	Body condition score	-33.3	0.00	-	0.42	0.03	0.03	3.06	5.45
		Body weight, kg	906.5	348.89	0.00	-	495.27	844.16	493.56	5.89

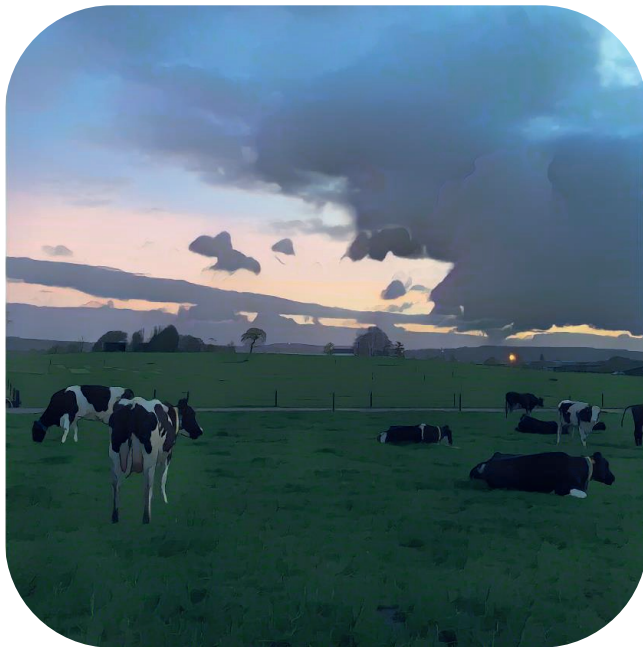
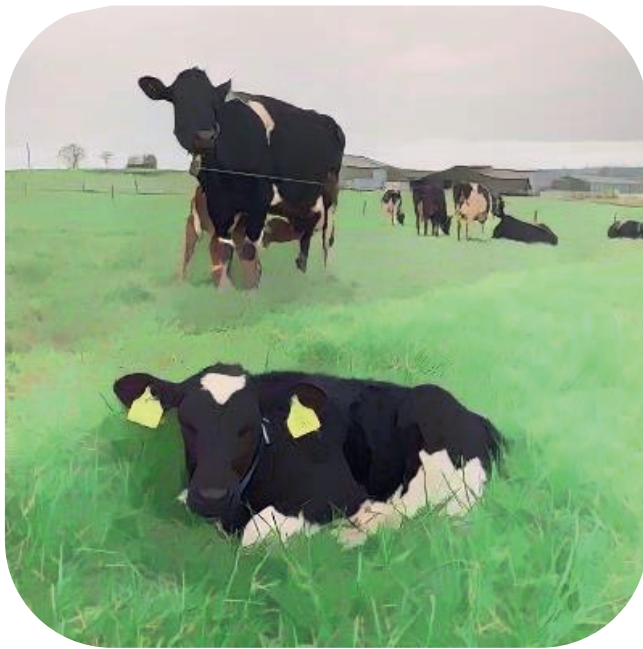
Average daily gain, kg/d	174.3	0.00	-	0.58	0.30	0.30	-0.37	-
Average change in body condition score	-14.9	0.02	-	0.00	0.02	0.04	-0.12	-
Machine milk yield, kg/d	456.6	0.00	-	0.50	5.82	5.82	17.90	13.48
Milk fat concentration, %	261.9	0.27	-	-0.19	0.40	0.67	4.58	17.88
Milk lactose concentration, %	-44.8	0.01	0.00	-	0.02	0.02	4.85	3.19
Milk protein concentration, %	5.7	0.03	0.00	-	0.02	0.05	3.46	6.30
Milk solids yield, kg	40.7	0.02	0.00	-	0.03	0.06	1.45	16.10

Model selection (Bayesian Information Criterion (BIC)), random effects variances (σ^2_{cow} , σ^2_{e}), covariance parameter estimates (compound symmetry (CS) or first-order autoregressive lag 1 (AR(1))), phenotypic variance (σ^2_{p})¹, the variable means (\bar{x}), and coefficient of variation (CV) for all cow and calf variables analysed using generalised linear mixed models (PROC MIXED) in SAS.

$$^1\sigma^2_{\text{p}} = \sigma^2_{\text{cow}} + \sigma^2_{\text{e}}$$

² \bar{x} = average between the system LSMEANS

$$^3\text{CV} = (\text{sqrt}(\sigma^2_{\text{p}})/\bar{x}) * 100$$



| CHAPTER 6 |

General Discussion

Introduction

The vast majority of European Union citizens are concerned about farm animal welfare (Eurobarometer, 2016). Specifically, the public seems to be focused on the concept of natural living, that is, that farm animals live in environments with naturalistic features and are able to express highly-driven natural, species-specific behaviours (Beaver et al., 2020). In relation to this, an aspect of dairy farming that has come under scrutiny is early cow-calf separation, which thwarts maternal care of the calf, suckling, and dam-calf bonding (von Keyserlingk and Weary, 2007; Beaver et al., 2019; Meagher et al., 2019). This has led to an increase in popularity of cow-calf contact (CCC) systems; calf rearing systems that allow for physical contact between a dam and her own calf or between a foster cow and her foster calf/calves (Sirovnik et al., 2020). Despite the increase in popularity of CCC systems, little research has been performed on CCC systems, with the majority of recent CCC research being performed on dairy cows in indoor housing systems, with year-round calving (i.e., Barth, 2020; Wenker et al., 2022a; Neave et al., 2024a). Only one preliminary study has been performed on cows within a pasture-based dairy system (Opsina-Rios et al., 2023). In addition, most CCC research appears to be focused more on calf health and performance than cow health and performance (see the reviews from Beaver et al., 2019 and Meagher et al., 2019), and few investigate health and performance from a physiological perspective. Ultimately, the bond between cow-calf pairs must also be broken in CCC systems. Although this can occur simultaneously with weaning (Newberry and Swanson, 2008), it is not recommended as the combination of these events often cause distress to both the cow and calf. In addition, dairy farmers have concerns about the feasibility of CCC systems (Neave et al., 2022; Johanssen et al., 2023).

To capitalise on the nearly year-round grass growth (Hurtado-Uria et al., 2014), Irish dairy farmers utilise compact, seasonal (spring) calving, where 90% of cows calve within a 6-week window, starting in late January/early February (Shalloo and Hanrahan, 2020), so that the cows' fluctuating nutritional demands throughout the lactation match the expected grass growth (Dillon et al., 2005; Horan et al., 2005). However, compact, seasonal calving also results in a highly labour-intensive period for Irish dairy farmers (O'Donovan et al., 2008; Deming et al., 2018; Hogan et al., 2022), which can have negative effects on both human and animal welfare. For CCC to be implemented in Ireland, it needs to be integrated into the pre-existing pasture-based, compact spring-calving system, and it should increase animal welfare without decreasing human welfare or majorly affecting productivity.

The aim of this thesis was to assess and compare the production performance, health, behaviour, and welfare of cows and calves in the current conventional dairy rearing system in Ireland, and see what the consequences were of implementing a prolonged cow-calf contact rearing system. To achieve this aim, three studies were conducted: an on-farm welfare assessment survey to identify the current state of welfare on conventional Irish dairy farms, a calf behaviour experiment to establish a baseline of normal, group-housed dairy calf behaviour during the pre-weaning period, and prolonged CCC experiment to investigate whether CCC could be successfully incorporated into the Irish pasture-based, spring-calving dairy system. Within this general discussion, I will reflect upon the current state of welfare on conventional dairy farms in Ireland, and discuss where potential improvements could be made based on their behaviour and production. I will also discuss the advantages, disadvantages, and feasibility of CCC systems within the Irish dairy system, along with my future research recommendations regarding CCC systems in Ireland and globally.

Using behaviour to assess welfare in calves

Pre-weaned calf behaviour can be a useful tool to inform us about the calf's development, health, and affective state. Calves that perform more negative behaviours (i.e., vocalisations, cross-suckling) may be in a more negative affective state than calves that perform more positive behaviours (i.e., play), and hence have lower welfare. Currently in Ireland, conventionally reared calves are kept separate from their dams and are typically housed in group pens once they have passed the colostrum/transition milk stage (Sinnott et al., 2023). Due to compact calving, calves are also usually very similar in age to their pen-mates, but may also be grouped based on breed and sex (Sinnott et al., 2023). Although calves are kept in a social environment, which has been shown to be beneficial to their behaviour and welfare when compared to individually-housed calves (Costa et al., 2016), calves may be provided additional benefits by being reared by their dams in CCC systems.

In Chapter 3, I observed and discussed that the calves appeared to have an innate need to consume solid feed to initiate rumen development; the calves seemingly preferred to eat their bedding rather than forage from the feeder. Calves were also frequently observed eating bedding while sniffing and slowly walking across the lying area of the pen in a motion that mimicked grazing (Werner et al., 2018) This suggests that, if provided with the opportunity, these calves would have been grazing pre-weaning. As such, calves may benefit from additional or alternative methods of forage provision, such as outdoor access to pasture, which

may also help meet these and other behavioural needs. Some of the farmers provided their calves with outdoor access pre-weaning in Chapter 2, but the ability to provide outdoor access to calves pre-weaning depended on the position of the calf shed (i.e., surrounded by yard or other buildings vs. beside suitable pasture); this is also why calves were not provided outdoor access at our research facility. However, in the CCC study described in Chapters 4 and 5, the full-time access (FT) calves were primarily housed outdoors at pasture, and were frequently observed grazing pre-weaning (Sinnott et al., 2024). In addition, both the FT and part-time access (PT) calves were frequently observed consuming grass silage (the FT calves only when they were housed due to inclement weather) alongside their dams and other cows at the feed bunk. I actually observed the FT calves eating grass silage with their dam when they were about a week old. At a week of age, it was too early for their rumen to have started to develop (Chapter 3; Wang et al., 2022), but this does suggest that the social learning component of CCC systems may have interesting implications on calves solid feed intake and subsequent rumen development.

Cows and calves in conventional systems likely have different daily activity and behaviour patterns than cows and calves in CCC systems. This was suggested in Chapter 5, but I was only able to compare two time-points (days). More frequent scan sampling on a weekly basis over many weeks may be useful to attempt to understand more about how social behaviours develop and to see if there are any differences in behaviour expression between calves and cows in conventional vs. CCC systems.

Cow-calf contact systems in Ireland: current barriers and potential solutions

While CCC systems are touted as promoting more positive experiences for both cow and calf while they are together (i.e., Beaver et al., 2019; Meagher et al., 2019), the feasibility of these systems is less commented on in the literature. Although promoting and maintaining animal welfare is important, if a dairy system is not feasible for the farmer, whether through labour requirements, economics, or more, then the likelihood of a practice becoming widespread on commercial farms is reduced. A balance must be struck. In the rest of this section I will outline several (perceived) barriers to CCC systems, specifically within the context of the Irish, pasture-based dairy system, and I will discuss what is currently known about them, how this thesis adds to the knowledge surrounding each barrier, and discuss future steps towards potentially eliminating the barriers.

Facilities and space

Typical housing infrastructure on dairy farms in Ireland consists of cow sheds (mainly cubicles although some have loose straw pens) and calf sheds (individual pens for approximately 7 d then group pens until weaning). Most farms will also have a calving pen, which typically is a loose straw pen, in a separate shed or within the cow or calf sheds (Chapter 2). If these farms were to change to a CCC system, modifications would need to be made to make the facilities suitable for calves. In particular, gaps in the feed barriers or elsewhere around the surrounds of the enclosure may be big enough for calves to slip through and escape, while gaps between slats in the floor may cause the calves to fall and injure themselves. If calves were to accompany cows to pasture, then additional fencing, both in the pastures and along the roadways, may also be required (additional fencing was required for study detailed in Chapters 4 and 5). However, these issues arise in converting pre-existing facilities; after conversion, the maintenance of such facilities would be similar regardless of rearing system. Less calf housing would be required in CCC systems, so calf pens could be converted to bonding pens or additional calving pen space. Theoretically, maintaining the housing required for CCC should not be different to maintaining current, conventional cow or calf housing.

The additional space available to calves in CCC system, whether inside in a pen or outdoors in a pasture, may actually be beneficial for them to express their normal play behaviours. Although cubicles (or other infrastructure) and other cows or calves may interfere or block their path, larger areas would enable calves to potentially express the full extent of their locomotor play (20 m²/calf is required for full extent of locomotor play; EFSA, 2023). We observed this in the CCC study described in Chapters 4 and 5; calves in the FT and PT systems would often find an open area (i.e., leave the cubicle cow area and enter the calf-only straw pen through the race) to run around, then would return to their dams. Most conventionally reared calves do not have access to this kind of space; the calves in Chapter 3 had an average pen space of 2.13 m²/calf (two pens) and 3.01 m²/calf (1 pen), while 38.2% of farms in Chapter 2 did not meet the average pen space recommendation of 2 m²/calf. In Chapter 3 I also discuss how the calves' inability to fully express their locomotor play may have led to more oral manipulation of the pen structure (an abnormal behaviour), but this may have been ameliorated if the calves had been provided with enrichment. This may not be an issue in CCC systems, as their dam, other cows, and other calves in their herd may serve as a type of enrichment.

Farmer concerns regarding the facilities and space required to switch to CCC have been recorded in a few previous surveys. Johanssen et al. (2023) found that farmers in Norway that had applied CCC on their farms had to modify their pre-existing facilities to accommodate calves, with some farmers specifying that they had to 'childproof' the pens to prevent calves from escaping. Farmers in New Zealand that did not already practice CCC on their farms also believed that significant infrastructure changes would be required if they were to switch to CCC (Neave et al., 2022). This is also what farmers mentioned in Chapter 2 when describing an advantage of early cow-calf separation; they believed that early cow-calf separation was more space efficient and they expressed that they did not have the additional space or facilities to facilitate keeping cow and calf together for a longer period of time. Interestingly, the second most commonly provided reason that farmers gave for why they discontinued their CCC systems was the space requirements in the calf shed (Hansen et al., 2023). Although facilities not designed for CCC systems can be modified, they may be less labour efficient than a system built specifically for CCC. This was our experience in running the experiment detailed in Chapters 4 and 5, especially with the added fences required to prevent calves from escaping from the allocated pasture.

Labour

The labour associated with CCC systems is also an extremely important aspect to consider, and the amount of labour associated with CCC systems may differ depending on the original dairy system. Farmers within pasture-based systems have been shown to be concerned with the amount of labour that would be required of them if they switched to a CCC system (Chapter 2; Neave et al., 2022). This is interesting, as surveys from Norwegian CCC farms reported that farmers had widely variable perceptions about the amount of labour required (Johanssen et al., 2023), but overall they agreed that the work was more flexible (Berge and Langeth, 2022). However, the Norwegian farms were primarily indoor, and many involved an automatic milking system (Berge and Langeth, 2022; Johanssen et al., 2023). Vaarst et al (2020) reported that more time was required by farmers to observe and evaluate their animals in CCC systems compared to non-CCC systems. In Ireland, compact spring calving results in a highly labour-intensive period for Irish farmers (O'Donovan et al., 2008; Deming et al., 2018; Hogan et al., 2022), so CCC systems may not be feasible unless the labour required to achieve them equals or is less than the labour associated with the current, conventional system.

Although the labour implications of CCC were not the focus of this thesis, when discussing the feasibility of CCC systems it is important to also understand the impact that CCC may have on farmer well-being in Ireland. Labour, both during calving and during normal management, were recorded for the experiment described in Chapters 4 and 5, and were fully reported and discussed in Sinnott et al. (2024). In brief, labour associated with calving (i.e., colostrum feeding) was lower in the two CCC systems than the conventional (CONV) system, but daily labour for each system (i.e., health inspections, cleaning and bedding pens, separating and reuniting cows and calves in CCC systems) was higher in the FT (outdoor, full-time access) CCC system than CONV. These findings contrasted previous assumptions (Asheim et al., 2016; Knierim et al., 2020) that CCC systems would involve a reduction in labour. Most of these differences likely have to do with the pre-existing dairy system to which CCC was applied. Although in this study, the FT system had higher daily labour than the CONV system (Sinnott et al., 2024), the labour efficiency of CCC applied in other dairy systems, such as an indoor system with a robotic milking system, will likely differ.

Management of CCC systems

In Chapter 2, several farmers were concerned about the management associated with keeping cows and calves together for longer after birth. Neave et al. (2022) also reported that farmers were concerned that adding CCC would make farm management more complicated. As CCC systems are fundamentally different to the current conventional dairy system, if CCC was to be implemented there may be a management learning curve for the farmer. The quality of stockmanship a farmer displays can have a large impact on animal welfare, through the knowledge and technical competence they display and the care they show towards the animals (Rushen and Passillé, 2010). A farmer's motivation will also contribute to the overall success of any system or change on their farm. In addition, the way farmers (or other people) handle animals can have large impacts on their welfare and productivity (Breuer et al., 2000; Rushen and Passillé, 2010).

As mentioned in Chapter 4, during the CCC study all farm staff and researchers were new to keeping dairy cows and calves together (although some had experience with beef systems). At the beginning, there was a large learning curve for researchers and farm staff in the adaption of the two systems. It all felt very new and we were often unsure about what to do in specific situations, but by the end of the study our confidence with the two CCC systems had grown. This suggests that what at first seemed crazy or impossible might be possible with more experience and some adaptations. I have since heard of other research centres

sending farm staff and researcher to farms that already practice CCC to learn about the system before their experiments began. Unfortunately, we were not able to do this, but I would highly recommend it to others.

Saleable milk yield

Saleable milk yield is a common concern farmers have regarding the adoption of CCC systems (Neave et al., 2022), and has been mentioned as a reason that farmers have discontinued CCC systems (Hansen et al., 2023). Farmers also mention issues with milk let down in the parlour (Johanssen et al., 2023), which may be annoying to farmers (not knowing if the cow is milked out), but also can cause a reduction in milk fat of the milk harvested in the parlour (i.e., Bar-Peled et al., 1995; Barth, 2020). Although only 7% (3/45) of the farmers in Chapter 2 mentioned saleable milk yield, I was asking about early cow-calf separation and not explicitly CCC systems, which may have been why we did not capture the same response as previous studies.

It is known and accepted that during the CCC period, when the calf is nursing the cow, there will be a reduction in the milk yielded in the parlour; however, the issue arises in the cows' milk yield after the end of the CCC period. In the review by Meagher et al. (2019), they concluded that CCC did not affect long-term milk yield; however, the results from the individual studies were variable, and came from a mixture of CCC systems (foster and dam-calf), contact length, contact durations, and were primarily from indoor-housing systems. In addition, not all studies follow cows until the end of their lactation). More recent studies (i.e. Barth, 2020; Chapter 4) have shown that CCC cows yielded lower amounts after the CCC period, so this area requires further attention. With countless factors influencing milk yield, a large amount of research in a variety of systems will be required to fully understand the effects of CCC on milk production.

Ultimately, the issue with saleable milk yield lies in economics. During the CCC period, the short-term losses are expected and accepted from an economic perspective (a short-term decrease in contribution margins of between 1 to 5.4% was found in CCC systems as compared to the conventional system; Alvåsen et al., 2023), as the farmer does not have to feed calves from the bulk tank or buy milk replacer (a 'redirection' of costs; Meagher et al., 2019). Whether or not milk yield is comparable after the CCC period is where the issue lies. In Chapter 4, I found that cows in the FT and PT systems had lower milk yields than cows in the CONV system during the CCC period. After weaning and separation, although milk yields of the FT and PT cows recovered and increased, the yields never reached the level of the CONV cows, and this persisted for the rest of the lactation. This

was also observed in the direct comparison of the week before and week after the weaning and separation process in Chapter 5. It is suggested that heifer dairy calves in CCC systems may have higher milk production potential in their first lactation, due to their (presumed) higher intakes and thus bodyweight during their pre-weaning period. However, this has previously been shown in conventionally reared dairy calves (Bar-Peled et al., 1997; Shamay et al., 2005; Moallem et al., 2010) fed larger amount of milk or milk replacer, which suggests it is primarily due to increased intake rather a benefit of CCC. More studies are required to fully determine the long-term economic costs or benefits of CCC systems, and even then, due the high amount of variability between farms, there may be a lot of inconsistency between individual farms. However, if farmers were able to sell the milk produced in CCC systems to consumers for a premium price, then lower milk yields may not be an issue. There is a brand in the Netherlands, KalverLiefde (2024), that sells milk produced on farms practicing CCC at a higher cost than conventionally-produced milk.

One potential cause of the lower observed milk yields in CCC cows revolves around impaired milk ejection. In Chapter 4 and 5, the FT and PT cows' milk fat concentration was lower than the CONV cows during the CCC period, but immediately recovered after weaning and separation. This is a common result of CCC studies investigating cow production (i.e., Bar-Peled et al., 1995; Barth, 2020; Wenker et al., 2022a). Secretion of low levels of oxytocin in the parlour can inhibit milk ejection, or milk let down, leading to higher amounts of residual milk left in the udder after the milking event. Residual milk has the highest concentration of milk fat compared to milk produced throughout the rest of the milking event (Ontsouka et al., 2003). Higher amounts of residual milk left in the udder can decrease milk synthesis (Kuehnl et al., 2019) and eventually lower the cow's milk yield, which has been shown in cows in CCC systems (Metz, 1987; de Passillé et al., 2008). This effect has also been shown when cows were milked (in a parlour) at a higher frequency (Kuehnl et al., 2019), which matches what we observed in Chapter 4; although the FT and PT cows were being stimulated more to produce milk (parlour and calf nursing), they were likely producing less total milk than the CONV cows. This also may have interesting implications on calf nutrition; if calves are able to nurse the residual milk from their dam's udder, they would be consuming milk with a higher than normal fat content, which may reduce their need to consume solid feed (Hill et al., 2009).

Another potential cause of the lower observed milk yields in the CCC cows was diet and intake. Excluding the PT cows (who had a slightly different diet, environment, and milking frequency), the CONV and FT cows were offered a

comparable grass-based diet (PT cows were indoors by night, which necessitated a slightly different diet) and an identical amount of concentrates. However, intake data from these cows was not collected, thus I cannot say for certain that these cows were consuming the same amount of feed. In Chapter 5, I also found that the systems had differences in blood serum mineral concentrations that have been associated with diet and intake (i.e., magnesium, Suttle, 2022a; phosphorus, Suttle, 2022b).

Future pasture-based CCC studies should investigate the intakes of the cows, both during and after the CCC period. In addition, grass and grass silage samples of the paddocks should be taken to be analysed for dry matter and composition. Obtaining feed intake of calves, both of milk and of solid feed, would also provide valuable information into their growth and rumen development, and may also provide insight into the discussion of whether the CCC cows' total milk yield was less than the CONV cows during the CCC period (Chapter 4).

Cow and calf health concerns

One of the main concerns that conventional systems farmers have regarding CCC systems is cow and calf health within these systems (Chapter 2; Neave et al., 2022; Beaver et al., 2019). For cows, the main health concern in CCC has been mastitis (Beaver et al., 2019; Neave et al., 2022, while a myriad of other health issues have been proposed for calves (Beaver et al., 2019). Mastitis is a major concern for farmers when considering CCC systems (Chapter 2, Neave et al., 2022). Despite this perception that CCC increases cows' risk of mastitis, two reviews concluded that suckling was beneficial (or did not affect) mastitis risk and somatic cell score (Johnsen et al., 2016; Beaver et al., 2019). In Chapters 4 and 5, I provide more evidence that CCC does not negatively affect somatic cell score, and may slightly decrease SCS (Chapter 4). The mechanism for this decrease is not known, but would be interesting to determine.

Teat damage is another concern farmers have about cows in CCC systems (Neave et al., 2022). I did not observe any damaged teats (Chapter 4); however, if the PT cows included in this thesis (Chapters 4 and 5) had been milked in the afternoon, immediately before reuniting with their calves, we may have observed some teat issues. Teat damage has been observed anecdotally in a pasture-based, part-time CCC system (Ospina-Rios et al., 2023), despite having provided a buffer period of 30 minutes after milking before reuniting cow and calf, teat damage was observed in these cows, presumably due to calf frustration. In Chapter 4, both the FT and PT calves were observed nursing immediately before they were separated from their dam for milking, indicating that the calves had

learned the daily routine and were anticipating the temporary separation. When the FT pairs were reunited, the calves had nursed around 1 to 2 h beforehand, so they presumably were not hungry enough to cause such damage to teats. Teat damage was however also noted in the study described in Chapter 3; the teats on the automatic milk feeder needed to be replaced several times a week in most pens during the middle to end of the study (once gradual weaning started). Although there were likely other motivational factors at play in Chapter 3 (i.e., boredom), I observed that it was only a few calves that were aggressively chewing on the automatic feeder teat in frustration that they had finished their allotment of milk. Some pens of calves rarely caused damage to their automatic feeder teat. This is likely also true in CCC systems; only specific calves may be causing teat damage. It would be interesting to determine whether these calves could be identified early and alter their management to prevent teat damage (i.e., ensure their dam has sufficient milk in the udder when they are reunited).

Udder conformation has also been noted as a potential issue noted for CCC systems (Beaver et al., 2019), as calves have been shown to have a reduced ability to nurse from cows with larger, more pendulous udders (Edwards and Broom, 1982). Cows on the CCC study in this thesis (Chapter 4 and 5) did not have any udder issues (no too short teats, narrow teat placement, or low udder clearance) that may have prevented or reduce the calf's ability to nurse. However, the Irish Holstein-Friesian cow (and Holstein-Friesian x Jersey cow) typically have more compact udders than a North American Holstein-Friesian cow, which may provide an advantage for calf nursing.

Specific health concerns for calves regarding CCC include scour, Johnes, respiratory disease, and mortality rates (Beaver et al., 2019). Overall calf health is typically considered in CCC system research (i.e., Wenker et al., 2022a; Sinnott et al., 2024) and calf bodyweight and average daily gain (ADG) are commonly reported. An issue with calf bodyweight and ADG in CCC systems is that calf ADG typically decreases immediately after the CCC period (Johnsen et al., 2021a; Wenker et al., 2022b; Sinnott et al., 2024; Chapter 5), but this depends on the method(s) of weaning and separation. An abrupt transition away from milk can negatively impact calf bodyweight and ADG post-weaning (Steele et al., 2017), so calves need to be weaning gradually. Calf intake was not measured in the study described in Chapters 4 and 5, so obtaining feed intake of calves, both of milk and of solid feed, would provide valuable information into their growth and rumen development. It may also provide insight into the discussion of whether the CCC cows' total milk yield was less than the CONV cows during the CCC period (Chapter 4).

Distress from separation

One of the most important challenges of CCC systems is the separation of the bonded cow-calf pair. This separation often occurs in conjunction with weaning, which can compound the amount of distress experienced by calves. The distress felt by the cows and calves is one of the main issues provided by farmers with CCC systems (Chapter 2; Neave et al., 2022). Weaning and separation distress was also the most commonly provided main reason for discontinuing CCC on their farms (54% of participants [114/213]; Hansen et al., 2023). The combination of weaning and separation can result in changes in behaviour (e.g., increase in vocalisations, Stěhulová et al., 2008; Enríquez et al., 2010; Johnsen et al., 2015b; searching behaviour, Neave et al., 2024b; pacing or standing with their head out of the pen, Wenker et al., 2022a, Bertelsen and Jensen, 2023) and performance (e.g., reduction in calf weight gain, Sinnott et al., 2024 and cow body weight, Metz, 1987; Bar-Peled et al., 1995). We presume that these changes in behaviour and performance are caused by weaning and separation distress, either directly (i.e., increase in vocalisations) or indirectly (i.e., cow body weight decrease due to lower intake caused by distress). The consensus currently is that both weaning and separation need to be done gradually and not at the same time. Within this view, more detailed studies are required to determine the optimal duration, timing, method, and more. This is especially true for pasture-based CCC systems; to avoid a large change in environment (i.e., moving pairs indoors to wean and separate them), strategies to wean and separate cow-calf pairs at pasture should be investigated. The length of the CCC period should also be investigated; in Chapter 5, I speculate that 8 weeks may be too short based on the effect that weaning caused to all calves, regardless of their system. In addition, future research should also consider the diet and environment management of cows and calves during this time (Chapter 5); there's the potential that cows and calves need to be additionally supported nutritionally (i.e., additional concentrates to the cows or provision of milk/milk replacer to calves) during the transition from CCC.

Colostrum management

Colostrum management of new-born calves is considered one of the most important management factors for determining and ensuring calf health and survival (Godden et al., 2019), and thus has been the focus of much dairy farmer outreach and education in the past few decades. This is likely why in Chapter 2, a significant portion of dairy farmers thought that an advantage of early cow-calf separation was that they could ensure the calf received adequate colostrum. This

sentiment has also been reflected in other CCC perception studies (Neave et al., 2022; Johanssen et al., 2023). An assumption of CCC systems is that when dairy cows and calves are left together after birth, the calf will stand and nurse colostrum themselves. However, the calf's ability to stand and nurse will depend on several factors, including their vitality (Murray and Leslie, 2013). Calves born following dystocia have been shown to be less vital, and thus less likely to get up and nurse (Murray and Leslie, 2013) and have also been shown to have lower apparent efficiency of immunoglobulin G absorption even when artificially fed colostrum (Murray et al., 2015). Perhaps in CCC systems, an emphasis should be put on calving ease.

Nonetheless, in CCC systems farmers can feed colostrum artificially, either to all calves or only to those that they have observed not nursing. Dairy farmers practicing CCC in Norway have been shown to practice a mix: some farmers always fed colostrum artificially and some only intervened when they noticed a calf not nursing (Johanssen et al., 2023). Feeding colostrum artificially to all calves ensures that they are provided with a sufficient proportion of colostrum; although this would be the same amount of labour as the current conventional system, it negates a potential farmer benefit of CCC systems (lower calving labour, mentioned in Chapter 2). However, only intervening if there is an issue with calf nursing also requires more labour, just in a different form (observation).

Although colostrum management was not the focus of the study described in Chapters 4 and 5, it was carefully monitored. When calves were born, the cow's teats were cleaned and a colostrum sample was obtained and tested on a Brix refractometer. If the cow did not have colostrum of sufficient quality, then the cow-calf pair would have been excluded from the study (this did not occur). All cow-calf pairs were carefully monitored to ensure multiple observed instances of calf nursing had occurred during the bonding period (minimum of 48 h period post-birth). In addition, a calf blood sample was taken at 24 h post-birth and tested for failure of passive transfer (immunoglobulin G) on a Brix refractometer. Although these results are not reported elsewhere in this thesis or statistically analysed, the systems were similar (mean \pm standard deviation; CONV calves = $10.4 \pm 1.49\%$; FT calves = $10.8 \pm 1.31\%$, and PT = $10.5 \pm 1.22\%$) and above the threshold of failure of passive transfer ($\leq 8.5\%$ on Brix; Hernandez et al., 2016).

Human safety

The human safety element of CCC systems should also be considered. The reduced safety/increased risk of accidents associated with CCC systems have been why some farmers have discontinued them (Hansen et al., 2023). Although

we did not experience any safety issues regarding the daily temporary separation (for milking) of the cow-calf pairs in the experiment outlined in Chapters 4 and 5, human health and safety should be taken into consideration when considering the feasibility of CCC systems. Different technologies (i.e., one way gates) may be utilised to reduce safety risks, but their use depends on the farmer, pre-existing infrastructure, and type of original system. Dairy cows have an innate instinct to protect their calf (von Keyserlingk and Weary, 2007) and the routine separation of cow and calf at birth still poses risks to farmers (i.e., regardless of the timing of cow-calf separation, there's always a risk that a cow will become aggressive). Although cows and calves in CCC systems likely adapt to the daily routine of temporary separations, it still poses a risk. In New Zealand, Neave et al. (2022) found that around a third of surveyed conventional farmers thought that implementing CCC systems would compromise staff well-being or have negative implications on staff safety. However, Hansen et al. (2023) reported that on Norwegian CCC farms, only a few had experienced more accidents or decreased work safety for them or their staff.

Calf temperament

A benefit of early cow-calf separation provided by farmers in Chapter 2 was better calf temperament. While I did not directly measure calf temperament in the study described in Chapter 4 and 5, anecdotally, I found that the FT calves were, as expected, quite wild and difficult to handle. Although this may have been caused by the FT calves being happier and more playful in general, handling and approaching the FT calves during the CCC period was stressful at times for both researchers and calves alike. However, I believe that because the FT calves had such a large flight zone, it made herding them easier (which was important during the twice-daily temporary separation of cow-calf pairs for milking). After weaning and separation, the FT calves became much easier to handle and approach. On an actual farm, with less routine handling than a research farm, this may be less of an issue. Previous work by Waiblinger et al. (2020) has also found that gentle human contact in the first five days of life for CCC calves helped to reduce the calves' fear of humans later in life, so a different approach to handling calves may be needed in CCC systems.

Cow-calf contact is a type of dairy system

Implementing CCC rearing on a farm means changing the cow and calf management and rearing systems on that farm, which when considered in this

way, means that CCC is not solely about the contact time per day or weaning and separation date. Instead, CCC systems need to be considered as combinations of type of CCC (dam-calf or foster cow, duration of CCC period, timing and duration of contact time), housing, environment, nutrition, and so on. As a result, there are several ways of achieving CCC systems, and like any dairy system, some combinations of factors are more likely to be successful than others.

This varying success with small changes in CCC system was what I observed in the experiment described in Chapters 4 and 5. To achieve the two chosen CCC systems (a full-time access (FT) system, where cow and calf had constant, unrestricted access to each other at pasture and cows were milked twice a day and a part-time access (PT) system, where cow and calf had access by night indoors, the cows grazed outdoors during the day while the calves remained indoors and the cows were milked once-a-day in the morning) the cows varied in milking frequency, housing, and diet. Although the systems were chosen for valid feasibility reasons (labour efficiency, calf health and cow teat injury concerns; described in further detail in Chapter 4), the differences between the two CCC systems made it more difficult to explicitly compare them. As both CCC systems were valid options for farmers in Ireland, it was worth implementing both. However, if the CCC systems had been slightly different (i.e., milking the PT cows twice-a-day instead of once-a-day), the results may have been very different.

Future research suggestions

Large scale investigation into Irish farmer's perceptions on cow-calf contact systems

In Chapter 2, we describe our findings regarding Irish farmer's opinions regarding early cow-calf separation; we did not explicitly ask about cow-calf contact systems. To fully understand Irish farmers' opinions and perceptions about CCC systems, a survey asking specifically about CCC systems (including questions about their opinions on CCC systems feasibility, benefits, disadvantages, and more) that includes more dairy farmers across Ireland should be performed.

Repeat of spring-calving, pasture-based CCC system experiment

The CCC study detailed in Chapters 4 and 5 was a feasibility study. Although as many different measurements were taken as physically possible, there was a large learning curve for researchers and farm staff in the adaption of the two systems (discussed above). Additional measurements could also be taken that were not feasible in the first study: intake data from cows and calves, sampling of grass and grass silage, and more long-term behaviour measurements.

Perform a multiple year, multiple farm trial of CCC systems on spring-calving, pasture-based dairy farms in Ireland

To fully understand the long-term feasibility of CCC systems in Ireland, a multi-year and/or multiple farm experiment is required. Several farms should implemented CCC in a way that best suits them and all aspects of their effect on cow and calf production, health, behaviour, labour, and welfare should be captured. Cows that are born into CCC systems also had different early-life experiences, and thus might have a different mothering ability. However, the actual feasibility of such a study is currently unknown.

Main Conclusions

- Commercial farms that had higher standards of welfare or more welfare-positive management practices had higher production performance
- Pre-weaned calves in both conventional and CCC systems prioritise a significant portion of their time and energy towards consuming solid feed
- Regardless of rearing system, weaning calves gradually and at an appropriate age is hugely important for their welfare
- Ambient temperature can alter calf feeding behaviour, so temperature management is vitally important for pre-weaned calves
- Cows' machine milk yield was negatively affected by CCC, both during and after the CCC period
- Cow health was similar in the conventional and CCC dairy system.
- The process of weaning and separating bonded cow-calf pairs negatively affected cow and calf performance
- Weaning appeared to have more of an effect on calf health and behaviour than separation from their dam

Summary

The vast majority of European Union citizens are concerned about farm animal welfare. Specifically, the public seems to be focused on the concept of natural living, that is, that farm animals live in environments with naturalistic features and are able to express highly-driven natural, species-specific behaviours. As such, an aspect of dairy farming that has come under scrutiny is early cow-calf separation, which thwarts maternal care of the calf, suckling, and dam-calf bonding. This has led to an increase in popularity of cow-calf contact (CCC) systems; calf rearing systems that allow for physical contact between a dam and her own calf or between a foster cow and her foster calf/calves. Despite the increase in popularity of CCC systems, little research has been performed on them, with the majority of recent research being performed on dairy cows in indoor housing systems, with year-round calving. Only one preliminary study has been performed on cows within a pasture-based dairy system, as is found in Ireland. In addition, most CCC research appears to be focused more on calf health and performance than cow health and performance, and few investigate health and performance from a physiological perspective. Ultimately, the bond between cow-calf pairs must also be broken in CCC systems. The separation of the bonded cow-calf pair is a necessary aspect of CCC systems and although this can occur simultaneously with weaning, it is not recommended as the combination of these events often cause distress to both the cow and calf. For CCC to be implemented in Ireland, it needs to be integrated into the pre-existing pasture-based, spring-calving system, and it should increase animal welfare without decreasing human welfare or majorly affecting productivity.

The overarching aim of this thesis was to assess and compare the welfare and production of cows and calves in conventional and CCC dairy rearing systems in Ireland. Therefore, the objectives of this thesis were to: 1) estimate associations between cow and calf welfare and production performance indicators on conventional dairy farms in Ireland; 2) establish a behaviour baseline for group-housed, pre-weaned dairy calves reared under conventional management conditions during the pre-weaning period; 3) investigate the effects of two dam-calf CCC rearing systems on cow production performance, health, and udders, compared to the conventional, no-contact system, within the context of the Irish, spring-calving, pasture-based dairy system; and, 4) measure the physiological health, performance, and behaviour of cows and calves within three dairy systems (two CCC rearing systems and one conventional system) before and after weaning

to estimate whether animals within the three investigated systems responded differently.

As the public becomes more concerned about the welfare of production animals, it has become increasingly important to both assess and identify improvements for the welfare of animals on commercial farms within the current, conventional system. It is also important to assess farmer's perceptions of current management practices to see how willing or able they would be to modify or improve their current practices. Through an on-farm welfare assessment and farmer questionnaire, **Chapter 2** explores how different cow and calf welfare-related variables influenced measures of milk production and cow and calf health. The welfare assessment was novel in that it combined aspects of cow, calf, and heifer welfare to obtain a more complete picture of welfare on commercial dairy farms in Ireland. Farms that provided pain relief to calves during disbudding were associated with higher milk yield and milk solids yield. Farms that had cubicles within or above the recommended length also had higher milk yield while farms that fed single source colostrum had higher milk solids yield. Somatic cell score was affected by parlour obstructions, frequency of roadway repair, and number of calf sheds, potentially implying that the infrastructure on the farm may affect somatic cell score. Farms that provided calves with fresh rather than stored colostrum had lower 28-d calf mortality rates, emphasising the importance of proper colostrum management. Farms that did not meet calf shed air space recommendations had lower calf immunoglobulin G than those that did meet the recommendation. Both cow serum amyloid A concentration and calf immunoglobulin G concentration were affected by the week of year during which the farm visit occurred; serum amyloid A decreased throughout the weeks while immunoglobulin G did not follow a prescribed pattern. As both measures are affected by numerous individual animal factors, they may not be suitable for use as a farm-level measure of welfare. The majority of Irish farmers surveyed appeared to consider early cow-calf separation to be advantageous for them, as they felt it allowed for easier management, ensured better cow and calf health, and minimised the amount of distress felt by both cow and calf. However, a few farmers had some objections against early cow-calf separation, and made a point of allowing contact between cow and calf for at least 24 h.

Animal welfare can be inferred from their behaviour, as behaviour reflects the integration of internal and external cues. In **Chapter 3**, I observed and quantified how the behaviour of group-housed dairy calves changed during the pre-weaning period, to establish a baseline of normal calf behaviour under conventional management conditions. Calves started exploring solid feeds (forage,

bedding, and concentrates) immediately after entering the group pens, which emphasised that calves should be provided access to solid feeds as soon as possible after birth, as solid feeds help meet calves' behavioural needs and their consumption promotes rumen development and growth. Time spent ruminating and consuming solid feeds increased at the start of weaning, accentuating the importance of a gradual weaning process to allow calves to slowly shift their reliance on milk to solid feed. Calves appeared to have an innate drive to consume forage, and consumed both the provided forage in the feeder and their bedding. Farmers should ensure that forage feeders remain full; if calves are consuming their bedding, it should be kept clean and dry to minimise health issues. Environmental enrichment (i.e., objects for calves to play with, brushes, or additional space) may also improve calf welfare, by increasing play opportunities in group pens and decreasing the performance of negative oral behaviours. Low ambient temperatures ($<4^{\circ}\text{C}$, below calves' thermo-neutral zone) caused calves to modify their behaviour by increasing the proportion of time spent lying and decreasing all other activities, highlighting the importance of temperature management in calf housing (i.e., using ventilation, heaters, or sheltered areas to keep the ambient temperature within the calves' thermo-neutral zone).

Data collection for **Chapters 4 and 5** were derived from a preliminary study to investigate whether CCC systems could successfully be incorporated into the Irish, pasture-based, spring-calving dairy system. To do this, I compared three different calf-rearing systems within the context of the Irish spring-calving, pasture-based dairy system: the conventional (CONV) system, where the cow had no access to her calf and was milked twice-a-day; a full-time access (FT) system, where the dam-calf pair had constant, unrestricted access to each other and the cow was milked twice-a-day; and a part-time access (PT) system, where the dam-calf pair had unrestricted access to each other by night and the cow was milked once-a-day in the morning.

Cows in CCC systems have been shown decreased machine milk yield during the CCC period because their calf is nursing; however, after the CCC period, previous research varies on whether the cow's milk yield recovers after weaning and separation. In **Chapter 4**, I report on our results of the cows' production, health, and udders within the three different systems. I found that the cows' machine milk yield was reduced in both of the CCC systems (FT and PT) during the CCC period, and although machine milk yield did recover after weaning and separation, the machine milk yield of the FT and PT cows never reached the level of the CONV cows, leading to a lower cumulative lactation machine milk yield and milk solids yield. Due to a reduced milking frequency (once-a-day) the PT

cows had a lower machine milk yield during the CCC compared to the FT cows. Cow health (clinical health scores, lameness, and body condition score) and udders remained largely unaffected by CCC, in line with previous research. Although we managed to incorporate CCC into the existing Irish spring-calving, pasture-based dairy system, our two CCC systems had a negative impact on machine milk yield, which would be an implementation deterrent for the dairy farmer unless costs were made up elsewhere, e.g. via a better growth and future milk yield of replacement heifer calves.

Although CCC systems are viewed as better for animal welfare than the current conventional dairy system, CCC systems come with challenges that need to be overcome for CCC to be viable on commercial farms. One important challenge with CCC systems is the separation of the bonded cow-calf pair, especially as separation often occurs simultaneously with weaning. For CCC to be viable on commercial farms, it should increase animal welfare without decreasing human welfare or majorly affecting productivity. In **Chapter 5**, I assessed the physiological health, performance, and behaviour of cows and calves within the three systems (two CCC rearing systems and one conventional system) before and after weaning to estimate whether animals within the three investigated systems responded differently. I found evidence that suggested the combination of weaning and separation in my two investigated CCC systems influenced some cow and calf physiological health, performance, and behaviour indicators. Despite there being no visual differences in cow health (through clinical health scoring), the FT and PT cows differed from the CONV cows in several physiological health parameters. Differences in cow performance stemmed from both the different systems and the weaning process. Diet and management of CCC cows thus may play a large role in the transition from being suckled by their calf to no contact with their calf. Overall, weaning appeared to have more of an effect on the calves' health and performance than the system that they were reared in. However, calves in the CCC systems appeared to struggle more in the post-weaning transition, as suggested by their lower weekly average daily gains and their higher levels of serum non-esterified fatty acid, despite consuming more solid feeds than the CONV calves after weaning and separation. As cows and calves in CCC systems need to adapt to the conventional dairy systems after separation, future work should focus on which factors are most important on easing this transitional period. Overall, management of CCC systems may be the key to their success.

In **Chapter 6**, I reflect on the current state of welfare on conventional dairy farms in Ireland, and discuss where potential improvements could be made based on their behaviour and production. The advantages, disadvantages, and feasibility

of CCC systems within the Irish dairy system are also discussed, along with future research recommendations regarding CCC systems in Ireland and globally.

In conclusion, this thesis reflected upon animal welfare and production within the conventional dairy system in Ireland, and tried to estimate whether welfare and production could be improved if CCC was adopted. In most cases, conventional farms that had higher standards of welfare, or more welfare-positive management practices, had higher production and better animal health. Calf behaviour within the conventional system was observed to create a behaviour baseline for normal, group-housed dairy calves during the pre-weaning period, which helped to identify areas where calf welfare may be improved. For CCC to be viable within the current, conventional Irish dairy system, it needs to be feasible. Cow machine milk yield was negatively affected by CCC, both during and after the CCC period, and the process of weaning and separating bonded cow-calf pairs also negatively affected cow and calf performance. Future work should investigate strategies to ameliorate these effects, both for welfare and production purposes. Altering management around the weaning and separation transition period, such as weaning after a longer CCC period and splitting the weaning and separation processes, may ease the animal's transition.

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Acknowledgements

When I started my PhD, in the depths of COVID-19 restrictions and lockdowns, I had no idea what the next four years of my life would look like. This experience has been a rollercoaster, and although I'm not sure I would recommend it to others (mainly moving countries during a global pandemic), I do not regret my decision.

First and foremost, I would like to thank my supervisors. I am grateful for all of your support and could not have done this without you. To Emer, thank you for all your help and support throughout my PhD journey. Thank you for knowing when to push and when not to, for asking me the difficult questions, for being a listening ear when I needed one, and for always wanting the best for me. To Laura, thank you for providing me with a different perspective, a listening ear when needed, and for your support throughout this process. To Eddie, thank you for your steadfast support and for being an excellent sounding board. To Riona, you were only my supervisor for a few months at the beginning, but thank you for welcoming me to Ireland, giving me somewhere to stay during my mandatory two-week, COVID-19 required quarantine when I arrived, and helping me feel welcome in a new country. I would also like to thank Katie, Niamh, and John Paul for stepping in and helping me when needed during my first experimental trial when Emer was on maternity leave and Laura and Eddie were in the Netherlands.

I am so incredibly grateful to everyone that helped me with my research – whether it was at Moorepark, on the road, or in the office. To Anna, thank you for joining me in taking over the calf shed in 2022 – I think we made an excellent team and I couldn't have done it without you! To Marie, you're amazing – I would have been much worse off without your help, your support, and your behind-the-scenes organisation. To Aoife, thank you for going on the crazy adventure that was the on-farm welfare survey – I don't even want to know how much time we collectively spent in a car over those 2 months! Thank you to all the farm staff in Moorepark that helped me with my trial work, but especially John Paul and Des, thank you for putting up with me, my research, my cameras, and my weird tendency to put colourful collars on calves – I could not have done it without all of your help and chats!

Thank you to my house-mates, office-mates, and colleagues throughout my years in Fermoy that helped me to settle into a new work-place, town, and country! This has been an amazing journey, and you all were such a large part of it. To Rachel and Tasha, thank you so much for taking a chance on a random girl from Canada and giving me more than just a place to live when I first got here – I will always appreciate your friendship and kindness. To Anna, thank you for being my PhD buddy, in-office sounding board, and friend – the Friday night takeaway while we chat over a movie tradition must live on, just maybe with less trial work-

related exhaustion. An additional thank you to the girls of Grange LGFA – thank you for welcoming me in, despite my utter lack of experience playing, and giving me the chance to meet people outside of Moorepark. I didn't realize how much I appreciated being able to walk around Fermoy and recognise people not from Moorepark until it happened.

Finally, last but certainly not least, thank you to my family – my biggest cheerleaders and supporters. Thank you for helping me to make the ginormous decision to move across the ocean in the middle of a global pandemic – you were right, I would have regretted it if I hadn't of done it. Thank you to my parents, Hilary and Greg, my sister, Lydia, and my brother, Luke, my aunt, Margo, for you visits, calls, texts, and general support that brightened my day, allowed me to rant, and helped make the ocean between us seem just a bit smaller. Without you, none of this would have been possible.

About the author

Sarah Elizabeth McPherson grew up on a dairy farm in Teeswater, Ontario, Canada, where she realised her love of cows and asking her parents increasingly complicated questions about the world around her. Sarah completed her Honours Bachelor of Science degree in Integrated Science (with a Concentration in Biology and a Minor in Psychology) at McMaster University in 2015, where she fell in love with scientific research but could not find a research area that she wanted to pursue. Deciding to combine her love of science with her love of cows, Sarah completed her MSc in Animal Science at McGill University (2020), where her research focused on dairy cow comfort and welfare.



In 2020, Sarah was offered the opportunity to move to Ireland to pursue this PhD on cow-calf contact systems within the Irish, pasture-based dairy system. Sarah was a VistaMilk-funded Teagasc Walsh Scholar, and her research was a joint project between Teagasc Moorepark (Fermoy, Co. Cork, Ireland) and the Animal Production Systems group at Wageningen University & Research (Wageningen, the Netherlands). During her PhD, Sarah has had the opportunity to present her research at international conferences in Ireland, the Netherlands, Canada, Portugal, and Italy.

Publications

Referred Scientific Journals

- McPherson, S.E.**, Webb, L.E., Murphy, J.P., Sinnott, A.M., Sugrue, K., Bokkers, E.A.M., Kennedy, E., 2024. A preliminary study on the feasibility of two different cow-calf contact systems in a pasture-based, seasonal calving dairy system: effects on cow production and health. *animal* 18, 101222.
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Conferences Proceedings and Abstracts

- McPherson, S.E.**, Webb, L.E., Murphy, J.P., Sinnott, A.M., Sugrue, K., Bokkers, E.A.M., Kennedy, E., 2023. Cow-calf contact rearing systems in a pasture-based dairy system: effects on cow health. In: Abstracts of the 2023 American Dairy Science Association Annual Meeting, Ottawa, Canada, 25-28 June 2023. *Journal of Dairy Science* 106(1), p. 116.
- McPherson, S.E., Riaboff, L., Dissanayake, O., Sinnott, A., Cunningham, P., and Kennedy, E., 2022. Effect of separation at weaning on the activity of cows and calves reared in a cow-calf contact system measured with accelerometer sensors. In: Proceedings of the 10th European Conference on Precision Livestock Farming, Vienna, Austria.
- McPherson, S.E.**, Webb, L.E., Bokkers, E.A.M., and Kennedy, E., 2024. Effects of age and temperature on the behaviour of healthy, group-housed dairy calves during the pre-weaning period. In: WIAS Annual Conference, Ede, Netherlands, 25 April 2024. p. 35.
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Assessment of Animal Welfare at Farm Level (WAFL), Florence, Italy, 30-31 August 2024, p. 70.

Dissanayake, O., **McPherson, S.E.**, Kennedy, E., Sugrue, K., Conneely, M., Shalloo, L., Cunningham, P. and Riaboff, L., 2022. Identification of the Best Accelerometer Features and Time-Scale to Detect Disturbances in Calves. In: International Workshop on Advanced Analytics and Learning on Temporal Data, Grenoble, France, 19-23 September 2022. Springer International Publishing, p. 167-180.

McPherson, S.E., Webb, L.E., Sinnott, A., Bokkers, E.A.M., and Kennedy, E., 2022. Impact of prolonged cow-calf contact on dairy cow production in a pasture-based system. In: Proceedings of the 73rd Annual Meeting of the European Federation of Animal Science, Porto, Portugal, 5-9 September 2022, p. 241.

Sinnott, A.M., Kennedy, E., **McPherson, S.E.**, and Bokkers, E.A.M., 2022. Pasture-based cow-calf contact: effects of full-time, part-time, and no contact on calf growth. In: Proceedings of the 73rd Annual Meeting of the European Federation of Animal Science, Porto, Portugal, 5-9 September 2022, p. 387.

Dissanayake, O., **McPherson, S.E.**, Allyndree, J., Kennedy, E., Cunningham, P. and Riaboff, L., 2023. Personalized Weighted AdaBoost for Animal Behavior Recognition from Sensor Data. 31st Irish Conference on Artificial Intelligence and Cognitive Science (AICS), Letterkenny, Ireland, p.1-8.

Technical Articles

McPherson, S.E., Sinnott, A., Murphy, J.P., Sugrue, K., and Kennedy, E., 2023. Cow-calf contact systems: an Irish perspective. In: Proceedings of Moorepark Open Day – Irish Dairying: Securing a sustainable future. Fermoy, Cork, Ireland, 4 July 2023, p. 204-205.

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Education Certificate

Training and Supervision Plan¹

A. The Basic Package	1.70
<ul style="list-style-type: none"> • WIAS Introduction Day (2022) • WGS Scientific Integrity course (2022) • WGS Ethics in Animal Sciences course (2022) 	
B. Disciplinary Competences	12.90
<ul style="list-style-type: none"> • WIAS Proposal (2021) • WIAS Course: The fundamentals of animal emotion (2021) • Laboratory and Animal Science Training (LAST) course (2021) • Introduction to Systematic Review and Meta-Analysis of Animal Studies (2023) 	
C. Professional Competences	3.25
<ul style="list-style-type: none"> • Project and Time Management (2022) • Research data management course (2022) • Effective behaviour in your professional surroundings (2023) • Last Stretch of the PhD Programme (2024) • Writing propositions for your PhD (2024) 	
D. Societal Relevance	2.50
<ul style="list-style-type: none"> • I'm a Scientist, Get me out of here - outreach activity (2022) • WIAS course: Societal impact of your research (2024) 	
E. Presentation Skills	4.00
<ul style="list-style-type: none"> • EAAP 2022, Porto, Portugal – Oral • ADSA 2023, Ottawa, Canada – Oral • WAFL 2024, Florence, Italy – Poster • WIAS Science Day 2024, Ede, Netherlands – Oral 	
F. Teaching competences	6.00
<ul style="list-style-type: none"> • Supervising 5 BSc Internship students (2021-2023) • Supervise MSc Internship student (2022) 	
Education and Training Total	30.40

¹With the educational activities listed, the PhD candidate has complied with the educational requirements set by the graduate school Wageningen Institute of Animal Sciences (WIAS) of Wageningen University & Research, which comprises a minimum of 30 ECTS (European Credit Transfer and accumulation System). One ECTS equals a study load of 28 hours.

Colophon

The research described in this thesis was supported by VistaMilk and the Teagasc Walsh Scholarship Programme.

Cover designs by S.E. McPherson and L.G. McPherson.

Printed by ProefschriftMaken, De Bilt, the Netherlands,
<https://www.proefschriftmaken.nl/>

