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The impact of living space on dairy cow production, reproduction and welfare

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Abstract

The majority of dairy cows worldwide are housed for several months of the year, yet there is limited literature available on how the housed environment impacts on their productivity, health and wellbeing. The extent to which specific housing conditions affect individuals or herds of cows or how to improve housing to optimise productivity, health and welfare is unclear. Furthermore, the terminology used for housing requirements is often poorly defined or lacks a standard definition.

It is likely that the housed environment will affect dairy cows. Research on housing for humans has shown that space allowance is the single most important characteristic of the housed environment. Sustainable intensification has suggested that animal welfare and production are inter-linked meaning higher welfare standards should be factored into assessments around the suitability of a cow's environment, alongside health and production which have been used historically. The conditions within dairy cow housing are therefore fundamental to the sustainability of dairy farming and the wellbeing of farmed cattle.

The context of this research was underpinned by a UK stakeholder group convened by funders of the research, the Agricultural and Horticultural Development Board (AHDB) to identify areas related to cow housing which were deemed to have an inadequate scientific basis or limiting the industry. The stakeholder group comprised retailers, milk processors, dairy consultants, academics and farmers, and the group undertook a one-day workshop with detailed discussions on all aspects of dairy cow housing. There was unanimous agreement that "loafing space" was an essential component of housing for adult cows for which the scientific basis was poor. This insight provided the basis for this research; to provide important and relevant information to the industry to enhance the understanding of space provision for dairy cows.

The aims of this thesis were to investigate:

1. The quantity, types and variability of space allowances currently provided for dairy cattle in British dairy herds.
2. The extent to which an increase in living space influenced productivity, fertility, behaviour and economic returns of dairy cows.

Initially a field survey of 53 randomly selected dairy farms across Great Britain was undertaken to obtain representative data on current commercial housing systems, to measure space provision for cows and gained farmers opinions on the housed infrastructure. Living space, a novel metric to describe the additional space above that deemed to be a baseline requirement for housed dairy cows was defined. Statistical analysis showed that there was large variation among GB dairy farms in terms of total space allowance (range from 5.4m² to 12.7m², mean= 8.3m², median= 8.2m², IQR= 1.9m²) and living space allowance (range from 0.5m² to 6.4m², mean= 2.5m², median= 2.4m², IQR=1.6-3.2m²). Questionnaire responses regarding loafing space importance revealed that the majority of farmers felt it was essential for cow health, welfare and production.

To evaluate the extent to which an increase in living space could influence productivity, fertility, behaviour and economic returns of adult dairy cows a 1-year randomised controlled trial (RCT) was carried out. The RCT was a longitudinal, matched, parallel-group, cross-over trial to quantify the superiority/inferiority of specified living space allowances for housed dairy cows. Two groups of matched commercial Holstein cows were provided with 9m² (3m² living space; Control space group) and 14m² (6.5m² living space; High space group) respectively. Three main outcomes were investigated: milk production, reproductive performance and behaviour.

In terms of milk production, it was found that a primiparous cow given a greater living space produced 643L more milk over a 305-day lactation than a primiparous cow in the control space group (12,235 vs. 11,592L). Less of a difference was observed in the multiparous subpopulation, with cows in the high space group producing 14,746L compared

to the control space cow of 14,644L per 305-day lactation. Physiologically, the high space cows ruminated for a greater duration per day and produced more milk solids per lactation.

Analysis of reproductive performance showed a negative association between increased space allowance and time to conception. Primiparous cows in the high space group had median time to conception of 155 days compared to primiparous cows in the control space group of 83 days. Median time to conception for multiparous cows was 133 days when in the high space group compared to 109 days in the control space group. However, no physiological reproductive parameters were different between groups, thus evidence of an underlying mechanism for the difference in reproductive outcome was not identified.

Analysis of daily time budgets identified that lying times increased by 63 minutes (High space: 12.43 hours/day, 95% CI = 11.70-13.29; Control space: 11.35 hours/day, CI = 10.73-12.12) and time spent in passageways decreased by 87 minutes (High space: 7.10 hours/day, 95% CI: 6.48-7.53; Control space: 8.17 hours/day, 95% CI: 7.48-8.88) when an increase in living space was provided. Therefore, fundamental changes in behaviour were associated with changes in the living environment.

Using a theoretical simulation model, it was shown that an increase in living space is likely to lead to increased financial returns. Dairy herds with average culling rates for the UK were estimated to have an increased financial return of £33.75-53.34 per cow per year for a living space allowance of 6.5m² compared to 3m². This is likely to be economically viable in terms of payback for the fixed costs associated with providing 3.5m² more living space allowance, mainly due to the increased financial returns from additional milk yield of the cows given greater living space.

In conclusion, this research provides evidence that the quantity of living space offered to adult dairy cows affects milk production, reproductive performance and behaviour. Additional research is urgently needed to allow further exploration of this complex issue to optimise the housed environment for adult dairy cows and therefore enhance the sustainability of dairy farming.

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Abbreviations

AHDB	Agriculture and Horticulture Development Board
AI	Artificial insemination
AMH	Anti-Mullerian Hormone
ASPA	Animal Scientific Procedure Act
BHB	beta-Hydroxybutyrate
CI	Confidence interval
CL	Corpus luteum
CLA	Commencement of luteal activity
CONSORT	Consolidated standards of reporting trials
DIM	Days in milk
DOV	Delayed ovulation
DP	Days pregnant
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
GB	Great Britain
GBP	pound sterling
GHG	Greenhouse gas
IQR	Inter-quartile range
ISI	Inter-service interval
LOOCV	Leave one out cross validation
MAE	Mean absolute error
NEFA	Non-esterified fatty acid
RCT	Randomised controlled trial
RSPCA	Royal Society for the Prevention of Cruelty to Animals
SD	Standard deviation
STROBE	Strengthening the reporting of observational studies in epidemiology
THI	Temperature humidity index
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation

Publications

CHAPTER 2: The work in this Chapter was published in the Journal of Dairy Science. Thompson, J.S., Huxley, J.N., Hudson, C.D., Kaler, J., Gibbons, J., Green, M.J., 2020. Field survey to evaluate space allowances for dairy cows in Great Britain. *JDS*, Volume 103, Issue 4, April 2020, Pages 3745-3759. <https://doi.org/10.3168/jds.2019-17004>

Ethics approval

This project was approved by the University of Nottingham School of Veterinary Medicine and Science ethics committee, with work conducted under ASPA license number MG_P07992717.

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Chapter 1. Introduction and literature review

This chapter introduces the dairy industry, detailing the current knowledge base with regards to dairy cow housing, welfare, production and reproductive performance by reviewing past literature. An evaluation of current use of data in the industry is also undertaken. From this, gaps in the literature were identified to form the aims and objectives of the thesis.

1.1 Context of the research

Prior to the commencement of this research, a formal stakeholder meeting was held by the funding body Agriculture and Horticulture Development Board (AHDB) and the University of Nottingham. The purpose of this meeting was to identify a range of key areas in which industry stakeholders believed there were gaps or a lack of information regarding dairy cow housed environments. The stakeholders of this meeting included but were not limited to retailers, milk processors, dairy consultants, academics and farmers. Following brief introductory presentations, breakout groups were allocated to ensure a mix of backgrounds in each. All breakout groups stated that “loafing space” was a key term that lacked a sufficient definition and evidence regarding its use. This insight provided the basis for this research; to provide important and relevant information to the industry to enhance the understanding of space provision for dairy cows during the housed period.

1.2 Dairy cow housing in relation to sustainability

The sustainability of livestock agriculture, including the dairy industry, is of critical importance (Herrero and Thornton, 2013). The three pillars of sustainability that govern livestock agriculture are: to be socially acceptable for animal, farmer and society, to have a

neutral or positive environmental impact and to allow economic reparation for continuation of development (ten Napel et al., 2011). Economic profitability of dairy enterprises is of great importance to a sustainable business but these farms must also ensure high health and welfare status of their stock, social equity and limit environmental impact (Appleby and Mitchell, 2018).

Sustainability of the dairy industry means negating environmental impacts, whilst providing enough food for the population via methods considered to be socially acceptable (Muller et al., 2017). To produce a sufficient quantity of food, intensification processes have increased, however some of these have led to detrimental effects on the environment including deforestation and reduction in biodiversity, soil degradation, and chemical run-off (Pingali, 2012; Smith et al., 2013). Intensification, if performed with sustainability in mind, could limit environmental impacts by increasing or maintaining global milk production from fewer animals (Lillford and Hermansson, 2020; Thornton and Herrero, 2010; Willett et al., 2019).

Livestock intensification should also be achieved with reference to animal health, welfare, production, and land use as well as greenhouse gas (GHG) emissions (Herrero et al., 2013a). Animal wellbeing and production are generally related, as indicated by the OIE-World Organisation for Animal Health (OIE, 2019), and therefore should be as high on the priority agenda as production efficiency (Herrero et al., 2013b). The management of dairy cows usually includes periods of housing within their productive lifetime (Barkema et al., 2015; March et al., 2014; USDA, 2016), and these housed environments are known to influence the magnitude of GHG production through different housing infrastructure systems and configurations (Baldini et al., 2016). The housed environment will also effect cow welfare (Greger, 2006) and therefore influences the sustainability of dairy farming.

Despite the importance of housing to the industry, quantitative research to evaluate and optimise housed conditions to achieve sustainable intensification in dairy farming is virtually non-existent. Consequently, there is a void of critical evidence available to decision makers to support appropriate policies and regulations (Appleby and Mitchell, 2018).

An additional reason for research to be focused on dairy cow housing is that the management of dairy cows is an emotive subject; the general public have diverse opinions on the way dairy farmers should manage cows (Jackson et al., 2020). It is increasingly important for farming practices to fit with societal and environmental goals, as well as providing suitable conditions for animal well-being (Mandel et al., 2016; Mehrabi et al., 2020; Nannoni et al., 2019). These perceptions are particularly important to consider because the general population are becoming increasingly distant from frontline agriculture. Visits to dairy farms may mitigate some concerns of the public but can also reinforce or prompt new misgivings, particularly in the subjective area of animal welfare (Ventura et al., 2016).

Therefore, for a variety of reasons, dairy cow housing is likely to be an important contributor to the sustainability of dairy farming.

1.3 Background to the dairy industry

1.3.1 Dairy industry worldwide

A key aim of the dairy industry is to produce a commodity with a secure supply and high nutritious value; this is crucially important for people's diets worldwide (Muehlhoff et al., 2013). From Figure 1.1, it can be seen that dairy cattle are distributed worldwide, through a vast range of environments, in high population numbers. There are 133 million dairy farms globally, spread across all regions (FAO, 2019) and milk production worldwide in 2019 totalled 852 million tonnes (FAO, 2020). The dairy industry produces an array of products including liquid milk, cheese, butter and whey protein, with each product in each geographical location carrying different economic value.

Total milk production is currently increasing; between 2005-2015 the global dairy industry produced 30% more milk, mainly through increased yield per cow. Expansion of milk production is mainly occurring in regions such as South Asia, Sub-Saharan Africa and West Asia-North Africa. Despite this increase in cow population and production, GHG

emissions per unit of production have decreased by 11% in 10 years to 2.5kg CO₂ eq./kg FPCM (FAO, 2019).

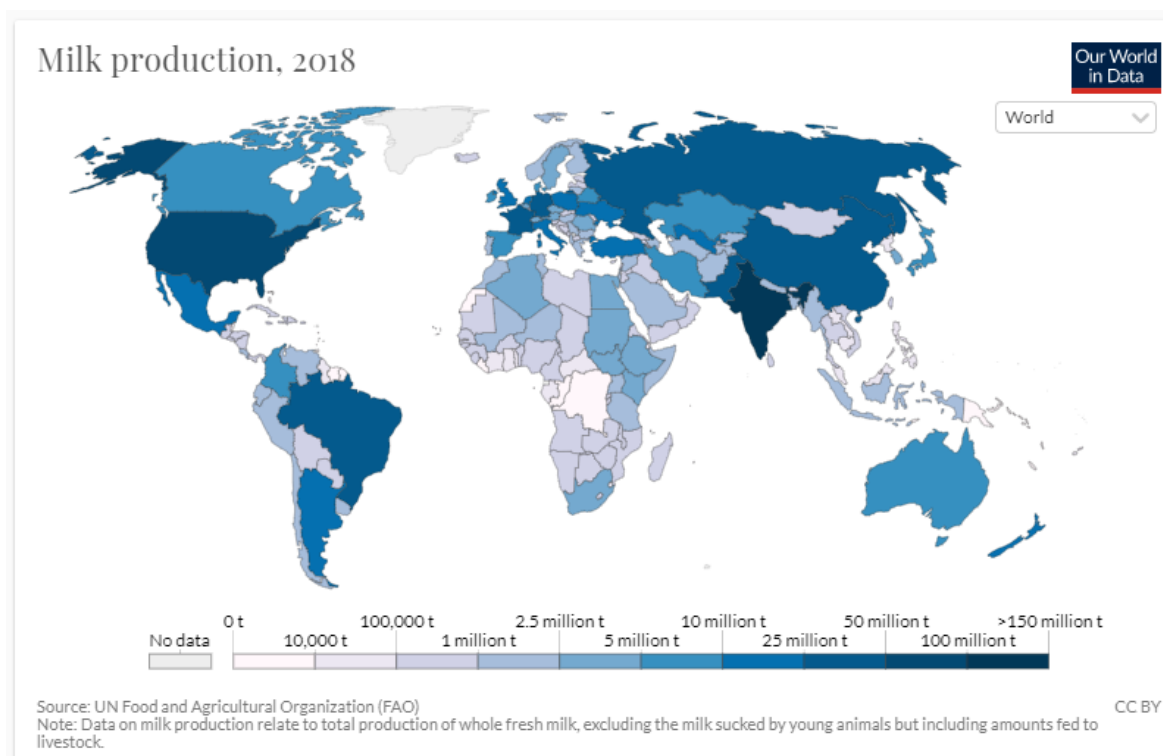


Figure 1.1 Cow milk production in millions of tonnes by country worldwide in 2018 (Clark and Tilman, 2017). <https://ourworldindata.org/meat-production#dairy-production-consumption>

1.3.2 Dairy production in the UK

The UK is estimated to be the 11th largest producer of milk worldwide, approaching 15 billion litres of production from 1.9 million head of stock (AHDB, 2020). This equates to an average cow yield of around 7,900 litres/cow/year, which is comparable to leading nations such as the USA and Canada and vastly higher than the global average cow producing 2,500 litres/cow/year.

The current trend being observed in the UK is for fewer dairy livestock with an increase of production per cow. The number of dairy livestock holdings is decreasing, with

the remaining farms appearing to increase their herd size. UK dairy farms are mainly located along the west of England, Wales and the south-west of England due to availability of land better suited to grass growth than cereals (DEFRA, 2011).

1.3.3 Outline of the structure of the UK dairy industry

The UK's agricultural sector, split between cereal and livestock income, contributes around one percent of the UK economy, with dairy livestock income calculated at £4,487 million in terms of milk value in 2019, equating to around 30% of the overall income from livestock of £14,700 million (DEFRA, 2020, 2019). Of all milk production in the UK, around 70% of annual milk output is processed by 9 major companies (AHDB, 2019a). This results in milk buyers maintaining a strong control over milk price and production requirements. Income to dairy farms is defined largely by the price per litre of milk set by the milk buyer.

Independent to the processors, most retailers who sell milk have set standards that their farms must attain to continue to keep their milk contracts, this is also used as a means to distinguish themselves from competitors. This means, to some extent, the dairy industry self-regulates dairy farmers. Some regulations include aspects of cow management believed to be important to consumers' perceptions of cow management, historically accepted values, and others of perceived positive outcome such as "loafing spaces" and lying times. Some of the regulations do not have an adequate scientific basis for implementation on farm and their consequential effects are unknown, with many requiring a financial input which may have been better placed elsewhere on farm.

Housing represents a key overhead cost for dairy enterprises. New housing is a large investment but offers the ability to design sheds with modern characteristics, whereas adapting existing buildings often requires significant investment without providing the optimal design. Housing is often necessary for management of dairy cows during certain periods of the year (i.e. winter in the UK) and also when animal genetics dictate that nutrient

dense diets which cannot be easily fulfilled at grass. Housing is being increasingly used as a year-round management tool to provide a consistent environment which can be maintained by the farmer and to ensure cows have access to necessary requirements such as feed, water and shelter. Given the significance of housing to the dairy industry the following sections describe the importance of the housed environment in greater detail.

1.4 The housed environment

1.4.1 General effects of housing; a background in human literature

Literature that has reported on housing conditions for humans has described direct impacts on the health of inhabitants through exposure to hazards that are biological, chemical or physical, with the consequence of increased disease likelihood (Bonney, 2007; Jacobs, 2011; Krieger and Higgins, 2002). The environment in which people spend the majority of their daily life is known to directly impact their health (Hancock, 2002). Building design in terms of layout and air quality determines environment quality which is known to be associated with disease incidence (Hood, 2005). Important links between the physical and mental health of people and their built environment have been reported (Gan et al., 2017) and for humans, as the number of poor housing characteristics increases, the odds of poorer health and hospitalisations increase (Boch et al., 2020). Interestingly, space allowance within human housing is considered the most important factor when assessing housing poverty (Blake et al., 2007; WHO, 2016).

Furthermore, stressors within an environment are known to have wide effects on human populations, for example, pregnancy rates in humans have been shown to increase or decrease dependent on speed of reclamation of the housed environments following a stressful event (Cohan and Cole, 2002; Seltzer and Nobles, 2017).

Despite strong evidence that living environment strongly impacts the health, fertility and wellbeing of humans, relatively little science has been conducted into the precise living conditions required by farmed animals. As a global society, we have an ethical duty to

provide suitable care and conditions for our animals yet in many circumstances, robust evidence on which to base this is minimal or absent. The following sections outline the history and derivation of current recommendations for adult dairy cow housing.

1.4.2 Brief history of dairy cow housing worldwide

Efficient use of land has been a major contributor towards the trend of housing dairy cows for increased periods (VandeHaar et al., 2016), alongside increasing land prices (Jaevious et al., 2015) and pasture access (Robbins et al., 2016). In addition, breed (specifically Holstein type cattle) and genetic selection for higher milk yields means dietary requirements of modern cattle are often best met when housed (Huyghe et al., 2017).

Over recent decades, intensification of dairy farming has led to more dairy cattle being housed indoors for longer time periods (March et al., 2014). Zero grazing systems were first seen in North America in the 1960's and have become more common since (Albright and Alliston, 1971). With this increase in popularity of year round housing (or zero grazing) systems, rather than grazing, the use of automated milking systems has had a continuous rise in uptake since the 1990's with few signs of slowing down (Svennersten-Sjaunja and Pettersson, 2008).

Before the 1900's, dairy cows in the UK were housed in a variety of building types such as longhouses, ox-houses and open-fronted sheds (Heritage Policy Department and Square, n.d.). Farms in the USA initially used stanchion barns from the 1850's, which were designed to house a variety of different livestock species in the same shed, for either seasonal housing or year-round cover if milking (Bewley et al., 2017). Dairy cows started to be moved into specifically designed buildings from the early 1900's with the advent of tiestall barn designs (housing where animals are tethered within an individual cubicle) seen from the 1930's. In the 1950's, straw yards (bedded packs) allowed loose housing of cows (Bickert and Light, 1982), however, this required a much larger lying/resting area to prevent disturbance of the herd whilst other animals were moving around. In comparison to other housing types yarded systems usually require more intensive management and greater

bedding material usage. Cubicle (freestall) accommodation for dairy cows was first introduced in the 1960's following an installation in Washington State in 1959. This was a crude version of the current form with individual cubicles installed in existing loose bedded housing.

The period in which freestall buildings were built had an impact on the overall space allowance which was given to cows. For example, early designs from 1960's combined the two key areas of indoor dairy cow management, feeding and bedded areas, within a single built unit compared to the traditional "U" or "L" designs which separated feeding and bedded areas. By combining the areas into a single unit, a smaller amount of housing space was required by design. This is important because the change to minimise building space was made to reduce building costs, arguably without sufficient consideration about its impact on cow wellbeing. This is illustrated by the recommendation for space allowances being 7.4m² in the 1980's which was a reduction from the 1960's standards (where it consisted of 9.3 m² of floor space area or approximately 13-15 m² of total area per cow (Bewley et al., 2017). During the 1980's, philosophies around cow comfort and cleanliness fell out of favour with the new focus being cubicle design and thus the reduction in total space allowance recommendations (Bickert and Light, 1982).

A more recent widespread addition to dairy cow housing used commercially is robotic milking systems (Hulsen and Rodenburg, 2008). The first prototype robotic milking unit was installed in the Netherlands in 1992 and robotic installations reached 8000 farms worldwide by 2009 (De Koning, 2010; Jacobs and Siegford, 2012; Svennersten-Sjaunja and Pettersson, 2008). Barn design has developed to align with this advancement as it requires good cow flow to allow cows to voluntarily enter the milking robot (Jacobs and Siegford, 2012). For example, it is suggested that robot exits point towards the feed alley to account for the cow's desire to eat following milking (Halachmi et al., 2000). Simulation models have shown that cow flow is improved by robotic milking because resource use is more evenly spread (Halachmi et al., 2000).

Therefore, at present, farmers choose from a variety of possible housing systems including pasture, conventional bedded packs (straw yards), tiestall housing, cubicle (freestall) housing and compost bedded packs, all of which remain in use today.

In an attempt to increase housing space allowances, the industry has adopted the term “loafing space” to indicate regions where cows could spend part of their day in areas not associated with feeding or lying down. Ambiguity within the definition of loafing space is evidenced from the summaries described below and this has created confusion within the industry; there are polarised opinions when the definition of this term is discussed. Passageways are necessary for cow flow but their inclusion as loafing areas is disputed. Typically, “loafing areas” are considered as additions to existing buildings to increase overall space allowance, usually as outdoor areas of concrete or composted pads (Haskell et al., 2013). It is hypothesised that loafing areas will provide space for cow to inhabit away from herd-mates and perform behaviours such as oestrous activity which could be more of a risk within the confines of passageways which are usually narrower and contain more obstacles (such as cubicle infrastructure) (Haskell et al., 2013; Mandel et al., 2016). Currently, how cows use this space and the fundamental biological understanding in terms of how these areas would promote improvements to wellbeing and change behaviours remain hypotheses.

More recent research in the Netherlands has explored novel approaches to the dairy cow housed environment such as artificial floor surfaces, which separates urine and faeces to reduce gaseous emissions (Blanco-Penedo et al., 2020) and translucent roofing material that lets in light but not heat (Driessen, 2015). Such novel approaches have not yet become widely used.

1.4.3 Dairy cow cubicle (freestall) housing space allowance recommendations

The majority of UK dairy farms have a cubicle (freestall) housing system with the main differences being the style of the cubicle and layout of the living areas. This section will

review space allowance recommendations provided by policy makers and assurance schemes worldwide, including some common definitions.

1.4.3.1 United Kingdom definitions and recommendations

For modern cubicle housing, few guidelines declare specific values for the total space required by adult dairy cows when housed. The British Standards (Article 5502), which sets out minimum space requirements for dairy cattle, does not provide a clear overall number. The standards state that cows should have ‘a minimum of one cubicle per cow with 3.0m² per cow loafing area’. Since loafing area is not clearly defined, the statement is opaque, but on the assumption that a cubicle provides approximately 3.0m²/cow, the recommendations equate to approximately 6.0m² total area per cow (British Standards (BS:5502), 1990). These standards suggest that any space not considered as bedded area could be classed as loafing space although does not state this specifically.

Red Tractor, the most subscribed assurance scheme in the UK, does not state specific sizes for areas in cubicle housing. The Red Tractor baseline recommendations are for 5% more cubicles than cows based on a cubicle size of 2.5m length by 1.2m width which produces a slightly greater area than the British Standards minimum at approximately 6.15m² per cow (Red Tractor, 2020). They also state “passages must be wide enough to facilitate cow movement and the loafing area must be at least 120% of the cubicle lying area in size” (Red Tractor, 2020) but do not define exactly what comprises loafing area. Therefore, loafing space allowance is dependent on the size of lying area which seems counterintuitive since farms with very small cubicles would be allowed to have very small loafing spaces. For illustration, in a cubicle building with a cubicle size of 3.0m² and a stocking density of 1.05 cubicles per cow, the Red Tractor calculation would require 3.15m² of loafing space per cow.

The code of recommendations issued by the RSPCA (Royal Society for the Prevention of Cruelty to Animals, a charity organisation) sets a minimum total area of 9.15m² per cow with non-bedding area making up 6m² of this space (RSPCA, 2018). The RSCPA definition of loafing space includes all other space in the housing excluding the cubicle area.

The UK source recommending the largest amount of space for dairy cows when housed, at 10.4m² per cow, is AHDB (Agriculture and Horticulture Development Board), the dairy levy-board. Cubicle area guidance remains similar to the others at 5% more cubicles than animals in the housing. AHDB have split floor space recommendations into feed-passageways and other areas (including cubicle passages and loafing areas), which combine to give a total non-bedded allowance of 7.25m² per cow (AHDB Dairy, 2018). AHDB's current definition of a loafing area is "an area which cows can use for behaviours other than feeding or lying" and "passageways other than those not immediately adjacent to feeding areas" and state the Red Tractor value of 120% of cubicle lying area as the target for loafing space (AHDB Dairy, 2018). Therefore, from these definitions it is apparent that the UK levy board (AHDB) believe housed cows require more space than stipulated by main UK assurance scheme (Red Tractor). Using AHDB's definition and housing recommendations, around 3.8m² of the total recommended non-bedded space of 7.25m² would be classed as loafing space.

The 'Dairy Herd Health' book provides a different interpretation of loafing space in comparison to the other sources presented above (Green et al., 2012). Loafing area here is defined as "an area to which cows have access during housing, which is outside of the cubicle passageways and feed area". This book also discussed the use of outdoor loafing areas. The figure for loafing space outlined in this book states that a minimum of 2-3m² per cow is required but with no clear scientific rationale (Green et al., 2012).

In terms of research, the Animals (Scientific Procedures) Act 1986 (ASPA) code of practice sets out housing space requirements for animals being used for scientific procedures. These regulations state a value for total area per cow, stipulating a minimum overall space of 8.75m² per cow, of which non-bedded areas should make up at least 5.45m² of the total space (Home Office, 2014). Current UK recommendations for cow space allowances are summarised in Table 1.1.

Table 1.1. Summary of recommendations for space allowances in cubicle housing from sources in Great Britain.

Source	Bedded area (m ² per cow)	Other areas (m ² per cow)	Total area (m ² per cow)
British Standards (5502)	One cubicle** per cow (3.0m ²)	3.0	6.0
Red tractor	One cubicle per cow plus 5% more cubicles (2.5x1.2= 3.0m ²)	120% cubicle lying area (3.15m ²)	6.15
ASPA	One cubicle** per cow plus 10% more cubicles (3.3m ²)	5.45	8.75
RSPCA	One cubicle per cow plus 5-20% (3.15-3.6m ²)	6.0	9.15-9.6
AHDB	One cubicle** per cow plus 5% (3.15m ²)	120% of cubicle lying area plus feed passageways*** (7.25m ²)	10.4

*Based on 750kg dairy cow

**Cubicle size based on AHDB Cubicle Design Recommendations for 750kg dairy cow

***AHDB feed-passage guideline is 4.6m wide with 0.75m width for simultaneous cow feeding
(4.6x0.75= 3.45m²)

1.4.3.2 Worldwide definitions and recommendations

The use of the term “loafing” area for dairy cows is also widely used globally. However, as in the UK, definitions and recommendations are inconsistent, vague and with few scientific references.

Sources from North America, such as the Dairyland Initiative at the University of Wisconsin describes building layouts which would aid the free movement of cattle and states specific recommendations for passageway widths and well as providing information around

use of outdoor access for housed cows. However this source does not provide minimum overall space allowance figures (University of Wisconsin, 2021).

A certification body in the USA, the American Humane Certified™ programme, produce a dairy cow welfare-standards audit sheet which sets out specific measurement methods for housing space allowance but does not give a minimum or recommended figure to compare this against. This organisation places emphasis on housing design for maximising animal comfort. It states that given the range in size and bodyweights of dairy cows it can't prescribe specific dimensions on how to achieve appropriate cow comfort. Instead, it refers to stocking densities as a minimum standard of one cow per available cubicle and that a "loafing" area must be provided but does not provide any definition or description of what they consider a loafing area is. For loose housing they specify a minimum loafing space to be 40-50ft² per cow (American Humane, 2020).

The Society for the Prevention of Cruelty to Animals (SPCA), the Canadian certified standards body, recommends 11m² of resting area per cow in loose housing but does not state a figure within freestall housing instead advising a one stall per cow with no information relating to the rest of the housed area. In terms of "loafing" areas, there are statements around the use of exercise yards and outdoor areas for housed cows. Their description of this area is: "Outdoor areas must allow all cattle to easily stand up, lie down and adopt normal resting postures in addition to allowing them to perform natural social behaviours" (SPCA, 2018). However, even when recommendations are made, they may not be met as one study showed that 80% of a sample cohort did not meet the requirement of feed passageways being of >4.3m set in the Canadian DFC code of practice (Vasseur et al., 2015).

The Australian Animal Welfare Standards and Guidelines give no specific mention of space allowance recommendations, using the advisory line only of: "sufficient space to stand, lie and stretch their limbs and perform normal patterns of behaviour". For freestall housing they say these systems may "possibly have a loafing area for cattle to stand" but again do not

give a definition of loafing area (*Australian Animal Welfare Standards and Guidelines for Cattle Edition One Version One Endorsed*, 2016).

The National Code of Practices for management of dairy animals in India are centred around the minimum standard 4.3, which states “the floor, feeding, watering space and air space available for each animal must be adequate for standing, resting, loafing, exercising, feeding, watering and ventilation”. Although no specific space requirements for freestall housing are specified, this source does provide advice regarding loafing space areas “where feed and water troughs are provided in the loafing area, the access areas should be sufficiently wide to permit free movement of animals and prevent routes becoming wet and slippery” and “about one half of the open (loafing area) should preferably be katcha (earthen) or sand bedded and the remaining half should be brick paved” (*National Code of Practices for Management of Dairy Animals in India*, 2014).

1.4.3.3 Summary of industry recommendations for dairy cow space allowances

Current definitions of loafing space for adult dairy cattle are ambiguous and variable between countries and sources. Existing definitions for loafing space may include or exclude the following: outdoor areas of space (including pasture, concrete or bedded packs), specific indoor areas, feed passageways, cubicle passageways and cross-over passageways. The lack of evidence informing these policies undermines recommendations for best practice for the management of dairy cattle. Fundamental questions remain, such as whether passageways are classed as loafing space, the importance of outdoor versus indoor loafing areas and the optimal and cost-effective space requirements for housed dairy cattle. Such questions are difficult to answer with limited research evidence and variation of opinion between key opinion leaders and organisations.

The following sections present relevant research literature on how the housed environment impacts the health, fertility and welfare of adult dairy cows.

1.5 Impact of the housed environment on the health, fertility, productivity and welfare of dairy cows

This section will focus on research relating to the impact of the housed infrastructure of dairy cows on health, fertility, production and welfare. Housed infrastructure in this instance will be deemed as any fixed apparatus within dairy cow sheds associated with any of the housing systems described above. This infrastructure includes space allowance, stocking density, building materials, shed layout design and environmental enrichment as well as feed and water access. This section will not discuss cubicle design or management practices associated with housing of dairy cows as these are not within the scope of this thesis. Notably, there are lack of randomised controlled trials relating to infrastructure and even other types of research study are relatively sparse.

1.5.1 Impact of housed infrastructure on cow health

There a small number of studies showing direct impacts of housed infrastructure on the health of dairy cows with most studies combining multiple types of disease under the umbrella term of general health. Studies have reported that indoor housing, in certain unspecified environments, appear to have deleterious effects on health (Oltenacu and Broom, 2010). The European Food Safety Authority (EFSA) state that cows are limited by housing, including exercise, foraging activities and novel stimuli to promote investigation of the environment and thus believe that “when possible cows and heifers should be given access to well-maintained pasture” (EFSA, 2009). Housed cows will also be in closer contact and ventilation may be restricted and of poorer quality leading to increased risk of respiratory disease due to irritants such as dust and ammonia (Nalon and Stevenson, 2019).

Due to the restriction of space compared to pasture, housing cows for extended periods is considered to reduce cow “fitness” and longevity (Horn et al., 2012). When a group of cows were purposefully exercised and compared to a control group, they were less

likely to have a treatment or veterinary intervention during that lactation (Gustafson, 1993). Cow locomotor activity changes with housing type, with the addition of “exercise areas” or “loafing areas” important for increasing activity (Shepley et al., 2020). This increase in activity is linked to improved cow fitness shown by lower resting heart rates (Davidson and Beede, 2003) and respiratory rates (Blake et al., 1982). Furthermore, a scientific report conducted by EFSA reported that if cows had no access to pasture during each annual cycle, they would be at increased risk of a number of important diseases, including lameness, mastitis and peri-parturient disease such as ketosis and metritis (EFSA, 2009), although this was supported by a small, weak study comparing health in pasture based systems to housing (Arnott et al., 2016).

Mastitis is important to the dairy industry as it causes pain and economic loss. Inadequate housing is considered likely to be an important factor for increasing the risk of environmental mastitis (Blowey and Edmondson, 2010) with recent literature placing a focus on the environment for prevention (De Vliegher et al., 2018). Prevention is the key for control of mastitis in dairy herds (van Soest et al., 2016), thus ensuring appropriate housing facilities will aid this process. High stocking densities or narrow passageways may lead to dirtier mammary glands and limbs due reduction in available space per cow leading to increased faecal build up in the environment. Dirty udders have been shown to increase rates of clinical *E.coli* mastitis (Breen et al., 2009). However, no research has investigated the impact of general non-bedded space within freestall housing on cases of mastitis.

Poor housing designs such as floorplan layout (e.g. sharp corners between narrow passageways) are considered to increase the risk of lameness because excessive forces on the solar corium and white-line from tight turning movements can lead to claw horn lesions (Flower et al., 2007). Infrastructure related to cow comfort and building design may increase time spent standing on concrete in passageways, prolonging pressure on the solar corium, a significant risk factor for claw horn lesions (Chapinal et al., 2013; Galindo and Broom, 2000). Narrow passageways, independent of management regimes, may promote slurry build up in the environment, a key risk factor for digital dermatitis and slurry heel due to prolonged

exposure to pathogens (Endres, 2017). Slippery feed-passageway surfaces have been associated with increased herd lameness prevalence (Bran et al., 2019). When lame cows were moved to a 'hospital pen' which had softer floor surface, increased space and easier access to resources such as feed and water, cure rates following a lameness treatment were improved compared to a control group, showing links with lameness outcomes and the housed infrastructure (Thomsen et al., 2019). Ultimately, a building design which predisposes cows to lameness is likely to lead to decreased profitability of the farm via increased culling rates, reproductive failures and reduction in milk yields (Archer et al., 2010; Booth et al., 2004; Juarez et al., 2003).

1.5.2 Impact of the housed infrastructure on dairy cow reproduction

Reproductive performance is critical for dairy businesses to be sustainable. On USA dairy farms, reproduction failure was the second highest reason for cows leaving the herd, with the first being death on farm (Shahid et al., 2015). Reproductive performance in dairy cows is often based on the interval between two calvings, with the aim of having a median calving interval of 365 days (Esslemont et al., 2001; Olori et al., 2002), meaning a cow will be in peak lactation once per year. To achieve this a cow must conceive within the first three months of lactation, when metabolic demands are high. Negative energy balance in the early post-partum period has been shown to reduce the odds of pregnancy to the first insemination (Macmillan et al., 2020). Longer calving intervals have been linked with increased odds of being removed from a herd (Shahid et al., 2015). Conception is the critical event that occurs but is affected by many factors of which the housed environment is likely to impact as described below.

A crucial time that affects dairy cow fertility is the transition period (LeBlanc, 2010; Seifi et al., 2011; Walsh et al., 2007). Periparturient disease and negative energy balance can impact on hormonal concentrations of luteinizing hormone and oestradiol, reduce corpora lutea activity and force cows into a state of anoestrous (Bollwein and Lüttgenau, 2013). The

environment a cow is placed within may impact this for example through limiting feed access (Maltz et al., 2013; Moore and DeVries, 2020) or through increased competition for and access to resources (J. M. Huzzey et al., 2012; McGuffey, 2017). Competition for space will sequester energy from desired behaviours such as feeding and lying, as shown by Crossley et al. (2017), where the time taken by cows to carry out these behaviours was reduced when competition was increased. Thus, this competition for resources may mean a cow spends less time eating and consequently spends more time and energy trying to get a place at the feed-face (Crossley et al., 2017). The transition period is a time when cows are under great metabolic stress and is also a time for key physiological reproductive events, such as oocyte recruitment (Lucy, 2000). A cow's environment around this period is therefore of extreme importance for determining reproductive success.

After the transition period, a cow becomes eligible to be served in early lactation. Housing can play an important role at this time, for example, slippery flooring has been shown to discourage oestrous behaviours such as mounting (Orihuela, 2000; Stevenson, 2001). Housing layout is important for expression of oestrous and can be limited by space availability (Diskin et al., 2000). This is important as more farmers rely on technological aids such as pedometers and thus cows need conditions to move around and express such behaviours to reach the sensitivity of these devices (Roelofs et al., 2017). Thus, investigation into how cows utilise space within the housed environment is crucial for developing more appropriate building designs.

Although little research on space allowances has been undertaken for housed dairy cows, a study assessing buffalos housed using two different space allowances reported that conception rate and hormone profiles were not significantly different between the two groups (Salzano et al., 2017). A study which investigated the effects of building design on timing of first serve used data from a national recording system and on-farm measurements of 232 herds showed that time to first serve interval appeared to be shortened when dairy herds were given greater than 8.8m² of space allowance (Martin et al., 2015). Other findings in this study included the hazard of first serve being reduced as stocking density increased

and that slatted floors reduced the hazard and rubber matting increased the hazard compared to solid concrete (Martin et al., 2015). This study provides observational evidence to support the hypothesis that the different conditions produced by environmental infrastructure can alter fertility success in dairy cows.

1.5.3 Impact of housed infrastructure on dairy cow production

Milk yield (volume) is the principle productive outcome for dairy units because many dairy enterprises are paid on a per litre basis, although some contracts apply additional incentives for milk quality. Rumination is a physiological mechanism that involves cows chewing on regurgitated fibre (Beauchemin, 2018) and rumination times have been associated with the level of milk production (Kaufman et al., 2018). Cows ruminate for up to 10.5h/d given certain diets and conditions (De Boever et al., 1990) and rumination mainly occurs when cows are at rest, peaking around 4 hours post feeding (Schirrmann et al., 2012). The environment a cow inhabits as well as genetic factors and diet have been shown to influence rumination time (Beauchemin, 2018).

There is a limited amount of research, however, that directly links milk production or rumination times with dairy cow housing, and these all are over short time periods. For example, two trials which assessed different stocking densities over 14 day periods did not find differences in milk production (Krawczel et al., 2012; Wang et al., 2016). A four week trial comparing straw yards and freestall sheds with different space allowances found no significant effect on milk yield, body condition score, lying or feeding times (Fregonesi and Leaver, 2002a). A 12 week trial assessing unmatched groups of cows given access to a pasture paddock versus a feeding paddock of the same size whilst housed in the same environment reported that providing outdoor access space which was not intended for pasture grazing resulted higher milk yields compared to a pasture paddock (Kismul et al., 2019) although this was most likely due to differences in feeding regimes rather than environment.

A retrospective observational analysis comparing the impact of space allowance on cow production from greater than 200 Norwegian dairy farms reported that space allowance was associated with a greater first lactation milk volume production, the model estimates suggest that for every increase in square metre allocation a primiparous cow would produce 28.84L (Naess et al., 2011). This trial also used mixed effects modelling to show that shed design layout is associated with milk production of 204 relatively small herds, over a 10-year period. Milking system, dead-end passageways, water trough location were all associated with milk production but variables such as feed-face space and number of freestall rows did not influence milk volume production (Naess et al., 2011). A trial involving buffalo cows given a greater indoor space allowance plus outdoor access (10m² total compared to 254m² total, of which 10.5m² was indoor area) observed that there was an increase in milk yield compared with lower allowances without changes to fat and protein percentages (De Rosa et al., 2009).

1.5.4 Impact of housing on dairy cow behaviour

There is a limited research base that directly relates to housing design and infrastructure including space allowances with regards to dairy cow behaviour. Although there is more literature than for health, reproduction or production, there is a notable absence of controlled trials or intervention studies that are known to provide strong evidence of causality. Additionally, many observational research studies that include different farm environments fail to give adequate descriptions of the housing conditions, such as overall space availability, stocking density, feed-face length, environmental enrichment and ventilation, even though these may have a substantial influence on the outcomes being considered.

Animal welfare has long been linked to the physical health of animals but it is argued that assessment of behaviour and asking whether the animal ‘has what it wants’ or ‘a life worth living’ should be taken into account (Dawkins, 2003). Assessment of dairy cow

welfare is difficult, judging what an animal ‘wants’ or ‘needs’ is a complex question and will differ by individual animal, as well as farm environment and resource availability. For example, studying motivation for one specific resource may be dependent on whether another resource (not being investigated) is present, since this may affect the animals response towards the resource of interest (Franks, 2019). Welfare concerns are often raised about housing because the environment is more confined compared to pasture (Robbins et al., 2016).

Pasture during times of minimal rainfall is often considered a “gold standard” for dairy cows, based on evolutionary beliefs, that physiologically and behaviourally, cows are designed for pasture (Knaus, 2016). Many countries worldwide have indicated that dairy cows must or should have access to pasture (Boogaard et al., 2008; Schuppli et al., 2014). The two main drivers of this belief appear to be consumer and animal welfare (European Commission, 2007; Haskell et al., 2006; Vanhonacker et al., 2008). Some organisations and countries have either made legislation to enforce grazing or pay a premium for milk derived from pasture, based on thinking that exercise paddocks improve cow health and welfare whilst appeasing public animal welfare concerns (Bewley et al., 2017; Kismul et al., 2019). However, with a trend of dairy herds moving towards housing-based systems especially within the UK, EU and North America, it is important to understand the impacts housing has on the behaviours and well-being of dairy cows.

Research comparing housing to pasture, report locomotor activity being influenced by factors such as presence of obstacles, floor surface and space allowance per cow (Shepley et al., 2020). Reduction in socialisation, play and comfort behaviours have been linked with the housed environment (Boyle et al., 2008). Cows will utilise more space when they are given access to it through changes to locomotive activity. Activity measured by step count changes according to housing type, with tie-stall identified as lowest compared to freestall and pasture environments (Shepley et al., 2020). Mean step counts on pasture were reported to be higher (2,353 steps/d) than in freestall housing (3,390 steps/d), although the ranges in step count were comparable (range 1,120 – 4,918 for freestall cows and 2,715 – 4,064 for

pasture cows) (Black and Krawczel, 2016; Brzozowska et al., 2014; Dohme-Meier et al., 2014; Platz et al., 2008; Shepley et al., 2020).

Choice is considered important in determining welfare of cows since this allows individual expression of need. When given the choice, dairy cows have been shown to utilise pasture during the night but reduce this behaviour when temperature-humidity index (THI) or rainfall occurs (Falk et al., 2012; Haskell et al., 2013). However, cows are less likely to access outdoor areas during the day, even if high stocking densities were present (Falk et al., 2012). Seasonal effects have also been observed to cow behaviour, in summer months cows chose to spend over 3 times the amount of time outdoors compared to winter (Krohn et al., 1992).

A key difference between dairy cows at pasture and when housed is the total space available. Suggestions have been made that this reduction in space could impact on cow behaviour (Haskell et al., 2013). Typically, outdoor areas given to cows in the UK when housed would be classed as loafing areas, whether that be a pasture paddock or concrete yards, that allow extra space for cows which may also allow routes to extra feed areas. The provision of outdoor areas to enhance housing has come into focus following a number research trials that have shown behaviours change between cows with and without access to these areas. Firstly, there are a numbers of papers that have shown cows appearing to have a preference for outdoor areas during the night (Charlton et al., 2013; Legrand et al., 2009; Smid et al., 2020, 2019; Von Keyserlingk et al., 2017), which coincides with cows avoiding solar radiation if given the choice (Schütz et al., 2009). Suggestions as to why cows access these areas with no apparent function includes avoidance of other cows by subordinate animals to reduce agonistic interactions (Schütz et al., 2015; Smid et al., 2020; Talebi et al., 2014; Val-Laillet et al., 2008) or for thermoregulation (Haskell et al., 2013). Interestingly, space allowance of these outdoor areas also effects the time spent utilising them; one study reported that as outdoor area space allowance increased, usage also increased (Smid et al., 2020).

Since they are herd animals, grouping of cows is important and isolating a cow from a group has been shown to increase cortisol levels, an indicator of stress (Albright and Arave, 1990). However, if given greater space cows will utilise the area and spread out more, leading to fewer agonistic interactions (Kondo et al., 1989; Telezhenko et al., 2012). This has also been shown to occur in buffalos who use additional space to express investigative behaviour (De Rosa et al., 2009).

Providing more space and increasing cow comfort are aspects of housing believed to be beneficial for cow welfare (Blanco-Penedo et al., 2020), yet there is a void of research aiming to optimise these areas even though there is research to suggest that housing standards are not currently appropriate. A trial investigating lying times when cow housing included rubber matting with different space allowances showed positive correlation between increased space and overall lying times (Schütz et al., 2015). This suggests that stocking rates and space allowances in dairy cow housing will affect cow choice and timing of certain behaviours. If space is restricted for high competition resources subordinate cows will tend to move away into places such as loafing areas to avoid agonistic interactions (Haskell et al., 2013), which reduces their ability to utilise important resources such as feeding and lying. These negative cow-cow interactions use up energy which could be partitioned into other productive behaviours such as producing milk and oestrous activity. These interactions also use up time, of which is already restricted in terms of daily time budgets. The reason why loafing areas are hypothesised to aid with reduction to agonistic interactions is by providing cows with a large enough area to avoid dominant cows and allow access to more high traffic areas of the building without the requirement for direct contact with other inhabitants of the building. This facilitates easier access to key areas such as lying and feeding areas. This is particularly important for dairy cows, as they tend to synchronise behaviours if given the opportunity and the environment has been shown to influence this since cows are more likely to do this at pasture than when housed (Krohn et al., 1992). Thus by providing a greater amount of space cows when housed, they will be able to synchronise their behaviours more efficiently, like they would naturally do at pasture, without being

forced to perform the behaviours in confined areas which creates an environment where agonistic interactions are increased, thus reducing their ability to synchronise their oestrous, feeding and lying behaviours.

Increased stocking densities have been shown to increase the number of agonistic interactions between cows leading to increased feed bunk displacements and reduction in lying times (Talebi et al., 2014). Stocking density, in terms of cubicle to cow ratio, can increase competition for this area leading to reduction in lying times and forcing cows to spend time elsewhere in the shed (Fregonesi et al., 2007). Increased stocking densities have been shown to be detrimental to cows, with research showing that the reduction in space leads to more feed-face displacements and reduced lying times when cows are added or removed from a group (Talebi et al., 2014). Furthermore, greater space allowance around the feed-face has shown cows will utilise this space and consequently overall feeding activity also increases (DeVries et al., 2004). Cows have been shown to change behaviour when competition for resources is changed, for example consuming feed in shorter periods and decreasing lying times in order to gain access to feed when this resource is restricted (Crossley et al., 2017).

Behaviour of dairy cows is quite consistent and is linked to their environment. This can be assessed through observing their time budgets which are dominated by eating or lying behaviours. One of the largest differences that the environment can have on time budgets is through eating time. At pasture cows spend up to 9h/day eating compared to a 3-5h/day when fed a total mixed ration when housed (Beggs et al., 2018; Cook, 2020; DeVries, 2019; Gomez and Cook, 2010; Phillips and Rind, 2001). This provides time for alternative activities such as lying, exercising, oestrous activity and socialisation (Cook, 2020). Increased feed-face length has been shown to be correlated with increased lying bout-lengths, likely due to reduced resource competition (Deming et al., 2013). From this, lying times have become an indicator of cow comfort however this could equally be a boredom behaviour. Reports have shown individual cows can range between 4-18h/day dependent of

cow and environment factors but a definition of optimal time is still disputed (Bewley et al., 2010; Cook, 2020; Ito et al., 2009).

Another aspect of the environment which cows utilise are scratching areas and the environment should support this need. Research has indicated that if a mechanical brush is provided all cows will use it (DeVries et al., 2007). Cows are particularly incentivised to use loafing areas when it is not raining and when grooming brushes are present (Haskell et al., 2010). However, even based on this evidence not all dairy farms or assurance schemes require access to a brush and the importance of environmental enrichment for dairy cows remains unclear.

To summarise, when cows are in competition for resources and space within their housed environment, their daily time budgets will be change and time which could otherwise be spent on typical behaviours such as feeding, lying or showing oestrous behaviour may be replaced by agonistic interactions with other cows.

1.6 Importance of data in the dairy industry

Advances in computer processing power and storage has seen the term “big data” increasingly used (VanderWaal et al., 2017). Alongside this, the cost of sensor equipment has reduced and increased uptake of robotic milking has improved access to large quantities of data in the livestock industry (Lokhorst et al., 2019). This gives farmers and researchers non-invasive tools to monitor cows without impacting on their day-to-day routines, and in particular, a possibility of monitoring health and wellbeing.

In a research context, this provides the opportunity to collect larger quantities of potentially unbiased data to study aspects of cow health, production and welfare. Sensors are currently widely used for optimisation of fertility and to some extent health (Harty and Healy, 2016). The increase in data from continuous monitoring has led to the term “smart farming” which uses on-farm technologies to enhance management decisions based on a specific farm’s data (Kamilaris et al., 2017; Newton et al., 2020). Precision dairy farming

and big data are being increasingly utilised in the industry, shown by the increased use of these terms in scientific papers in recent times (Lokhorst et al., 2019). The aim of “Precision Dairy Farming” is to move from a group-orientated response back towards individual animals but with an intensive focus on each animal’s normal (Schulze et al., 2007). It has been hypothesised that this individualisation of farming using data may help to diagnose disease earlier and minimise antibiotic usage requirements (Spilke et al., 2003). However, farmers who have access to this abundance of data require education, support and suitable tools to enable them to use data appropriately and effectively for on-farm decisions (Evans et al., 2017). Furthermore, care should be taken when considering the generalisability of data for event prediction or management since there are limited numbers of research trials to underpin such predictions. For example, individual concentrate feeding of dairy cows based on milk yield is common in automated feed systems, but these commercial products appear to have no scientific basis which evaluates the computational models (da Rosa Righi et al., 2020).

Therefore, ever increasing quantities of data within the industry will provide the potential for development of sophisticated tools to inform decisions on cow health and behaviour; such technology and data will be important for improvements in management of livestock. However, to date such improvements are somewhat constrained by a limited collation and sharing of well-recorded data and a lack of high-quality published research.

1.7 Summary of knowledge gaps, objectives of the PhD and thesis structure

To conclude, current literature is sparse in terms of how housing affects dairy cow production, reproduction, health and wellbeing. This lack of information is notable and even the simplest environment characteristics, such as space allowances, lack robust scientific evidence for their impacts on dairy cattle. Although recommendations and regulations exist, these are varied and the extent to which they meet the needs of the modern dairy cow, dairy

farmer or consumer remains unclear. The literature review highlights that there has been published research regarding aspects of dairy cow housing such as cubicle design, bedding materials, feed-face space and stocking densities which confirms their importance when discussing the housed environment. However, it is even more clear that the most fundamental of all aspects of the housed environment, space allowances (including total area, loafing and living spaces) are noticeably lacking published evidence of the potential effects to inhabitants. Consequently, space allowances was selected on the basis that it is a fundamental area of dairy cow housing which required novel investigation. This was also likely to be of great importance considering that the provision of different space allowances could also contribute to confounding interactions with these other measurements.

In Great Britain, there is an appetite to improve the understanding of how the housed environment affects the health and welfare of dairy cows and a stakeholder group convened in 2017 determined that space allowances and loafing areas for adult cows was the highest priority for research. Therefore, in light of scientific literature and requirements of the GB dairy sector, there were two general areas in which questions were to be addressed in this research:

1. How much and what types of space are currently provided for dairy cattle in British dairy herds and how much variation exists between herds?
2. To what extent does an increase in living space influence productivity, fertility, behaviour and economic returns of dairy cows.

The purpose of this PhD was to answer these questions using the following aims.

1.7.1 Aims and objectives

- i. To quantify and define key housing areas utilised on commercial GB dairy farms (Chapter 2)

- ii. To evaluate farmer understanding of space allowance recommendations and loafing space area importance (Chapter 2)
- iii. Undertake a long-term randomised controlled trial to investigate the impact of living space on (Chapter 3):
 - a. The production of housed dairy cows (Chapter 4)
 - b. The reproductive performance and physiology of housed dairy cows (Chapter 5)
 - c. The behaviour of housed dairy cows (Chapter 6)
- iv. Evaluate the farm economics associated with providing additional living space for housed dairy cows (Chapter 7)

Chapter 2. Field survey to evaluate space allowances and obtain farmer perceptions of dairy cow housing in Great Britain

**This Chapter is based on a published paper in the Journal of Dairy Science: Field survey to evaluate space allowances for dairy cows in Great Britain. JDS, Volume 103, Issue 4, April 2020, Pages 3745-3759. <https://doi.org/10.3168/jds.2019-17004>*

2.1 Introduction

Given the lack of literature describing housed infrastructure globally, this Chapter focusses on investigating the housed infrastructure that is currently available for dairy cows in Great Britain, as well as farmer perceptions of adult cow housing.

Currently, literature on associations between livestock and their housed environment is sparse. Research on human links with the built environment show that it is important for the physical and mental health of the inhabitants (Gan et al., 2017; Hancock, 2002). Further studies have shown increased incidences of disease due to a high chance of exposure to biological, chemical and physical hazards which worsens with poorer quality (Bonney, 2007; Hood, 2005; Jacobs, 2011; Krieger and Higgins, 2002). Interestingly, although poorly explored in livestock species, it has been reported that inadequate space allowances in human housing is considered as the most important factor when assessing housing poverty (Blake et al., 2007; WHO, 2016).

The importance of the housed environment for dairy cows is highlighted by reports that in the USA 80% of the dairy cow population are housed during a lactation (USDA, 2016) and in the UK it has been shown that 99% of the dairy cow population will be housed during winter months (March et al., 2014). Furthermore, the trend is continuing to towards use of indoor housing systems for more farms and longer durations, this is despite recognition that grazing is perceived to be important by consumers (Barkema et al., 2015;

Ventura et al., 2016). The requirement for the housed environment to guarantee a sustainable, welfare friendly industry has been discussed (Place and Mitloehner, 2014) but recommendations are still mainly based on minimum standards and sparse (Bewley et al., 2017; Smith and Cook, 2019).

Loafing space (in this case defined as non-feed, non-lying and non-high traffic areas of the housed environment) has been considered an important component of housing for dairy cows however the scientific literature associated with this subject is minimal (Haskell et al., 2013). Similarly, there are virtually no data available on the amount of, and variability in, space allowances in commercial dairy herds globally.

The aim of this Chapter was to explore the current housing infrastructure used for dairy cows across a random sample of farms in Great Britain, with a specific focus to provide underpinning evidence on the quantity and variability of space provision for dairy cattle, including areas for eating, lying and passageways. A secondary aim was to explore farmer opinion and knowledge on the value of living space for dairy cows.

2.2 Materials and methods

2.2.1 Study design

This was an observational, cross-sectional study to evaluate the variability in living space allowances for dairy cows in Great Britain and undertaken in alignment with the STROBE reporting guidelines (STROBE, 2019; von Elm et al., 2008). The University of Nottingham's Ethical Review Panel granted permission for this research prior to study commencement. Informed consent was obtained from all participants following the provision of research details about the study purpose and data use.

2.2.2 Farm selection

The target population for this study was comprised of dairy farms which supplied a single large British food retailer (n= 739; ~12% of all GB dairy farms). The retailer's initial

pool of farms was originally selected based on milk tanker routes in 2007 (no selection criteria based on farm characteristics were used) thus providing a population which was considered representative of dairy farms in Great Britain.

Model based sample size calculations indicated a sample of 50 farms would provide an estimate of the population mean total space allowance per cow to be estimated with a 95% confidence interval of $\pm 0.5\text{m}^2$ and the population standard deviation to be estimated with a 95% confidence interval of $\pm 0.5\text{m}^2$, using a power set at 0.8 and a significance probability at 0.05.

Initially, 100 farms were randomly selected, using random number generation in Microsoft Excel (Microsoft, 2016); farms were randomly ranked from first to last. During this process the farm identification and details were blinded. Farms were contacted in order of ranking and invited to participate in the study. Selected farmers were invited to participate in the study by letter and email and those agreeing to participate were telephoned to organise a date for visitation. Initially, the top 50 ranked farms were contacted for recruitment. A subsequent 20 farms were selected for contact 2 weeks later based on the response rate of the initial group to ensure a sample size of at least 50 was obtained. Overall, 53 farms agreed to participate out of the 70 approached (75.7% response rate).

2.2.3 Data collection

A single researcher (JT) visited all 53 farms once between 9th November 2017 and 19th January 2018 for approximately 3 hours in duration; the data collection window occurred during the winter housing period for all farms. Data collection consisted of three elements; collation of basic farm details, precise measurement of all adult dairy cow accommodation and a questionnaire to capture farmer opinions on space allowances for cows.

2.2.3.1 Farm characteristics

Farmers provided detail about the management of the farm, including breed of cows, calving pattern, and number of adult dairy stock. Details on the management of cows during the housed period were obtained, including how cows were grouped and the duration and timing of groupings. Milk sold per cow per year and the most recently recorded bulk milk somatic cell count was also obtained.

2.2.3.2 Housing area dimensions

Measurements were taken using a Leica Disto D510 laser measuring device (Range: 200m, Tolerance $\pm 0.10\text{mm}/10\text{m}$ between 10-30m) of all accommodation used for adult dairy cows in lactation. Measurements were recorded on a standardised data capture form for each building or group of animals. Dimensions recorded comprised all areas available to cows: feed passageways, cubicle passageways, cross-over passageways with and without-water areas, collecting yards (if accessible to cows in addition to the time waiting for parlour entry), bedded areas, loafing areas and any other living areas available to the lactating herd.

2.2.3.3 Loafing space opinions

An eleven question, face to face interview was conducted with the main herdsman or farm owner. Closed questions were styled to gain understanding of farmer views about loafing space and housing conditions for dairy cows. Farmers were asked to rank the overall importance of loafing space, as well as importance in terms of production, health and welfare, on a scale from 0-10. Farmers were asked to indicate their preferred option for floor surface in loafing space areas. Farmers also ranked the importance of outdoor access for housed dairy cows. Finally, farmers were asked how much total and loafing area space they believed housed dairy cows required as a minimum and optimum. The questionnaire is available in Appendix – Chapter 2.

2.2.4 Study definitions

Total area:

- This incorporated all floor and bedded areas which a group of dairy cows had access to whilst housed (including cow access areas only such as cubicle bedding area, all passageways and indoor/outdoor loafing spaces but excluding areas in a building which did not permit cow entry such as feed alleys for tractor use only).

Passageway:

- Defined as areas used by cows to move through the accommodation; comprised of cubicle passageways, feed passageways and cross-over passageways.

Loafing space:

- A non-bedded, non-passageway area where cows could roam freely which included indoor or outdoor areas. Examples include:
 - i. Parlour collecting yards which are open and available to cows for a minimum of 4 hours / day, excluding the time prior to milking.
 - ii. Indoor or outdoor concrete/ sand/ straw/ woodchip pad/ pasture areas, not used for bedding and which didn't act as an access area between the cubicles and feed-face.

Living space:

- A novel bespoke definition of the space within the dairy cow accommodation that was greater than that considered a baseline requirement for movement around and feeding within the overall accommodation area, excluding lying

areas. The calculation of this definition was based on bespoke parameters for this study and was estimated as follows:

$$\begin{aligned}
 &\text{Total feed-passageway area over and above a baseline allocation of } 2.4\text{m}^2* \text{ per cow} \\
 &\quad + \\
 &\text{Total cubicle-passageway area over and above a baseline allocation of } 1.32\text{m}^{2**} \text{ per cubicle} \\
 &\quad + \\
 &\text{Total loafing space area (defined above)}
 \end{aligned}$$

** 2.4m² feed-passageway: deemed in this study to be a minimal baseline space required for a cow to move into and stand at the feed-face*

***1.32m² cubicle -passageway: deemed in this study to be a minimal baseline passageway requirement for a cow to move in and out of a cubicle (calculated using an estimated cubicle width of 1.10m multiplied half a minimum width deemed necessary for a cubicle passageway of 1.20m)*

These calculations mean that a minimum cubicle passageway width was taken to be 2.4m (to allow simultaneous exit from opposing cubicles) and the minimum feed-passageway which included access to cubicles to be 3.6m wide (to allow for simultaneous entry/exit to a cubicle whilst another cow was feeding).

Maximum pen stocking density:

- A farmer stated figure for the maximum number of cows that would ever be accommodated in a defined area of the farm.

2.2.5 Statistical analysis

Data analysis was carried out using the R statistical package, version R-3.5.2 (R Core Team, 2020). Data were restructured to consist of farm averages for milking cows in cubicles across all cow groups housed in cubicles on farm. Three farms were omitted from analysis because all milking cows were housed exclusively in straw yards. Descriptive analysis was undertaken to visualise data and linear models were used to explore the relationships between farm building features and farmer opinions on living space measurements.

2.2.5.1 Descriptive analysis

Variability between herds was evaluated using summary statistics to define distributional characteristics. Histograms, scatterplots and box and whisker plots were used to visualise the data. Conventional bootstrapping (Kuhn and Johnson, 2016) of the building measurement data was carried out to estimate the 95% confidence intervals of the mean, median and IQR's.

2.2.5.2 Model 1: Estimation of living space from housing features

The aim of Model 1 was to evaluate the associations between the area defined as “living space” and other measurements made of the housed environment. The purpose was to evaluate whether total area per cow and total cubicle area per cow (total base area: bedded area + lunge area) would provide a good estimate of total living space.

The linear regression model took the form:

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \varepsilon_i$$

Where subscript i denoted the i th farm, Y_i was the living space area per cow for farm i , β_0 was the intercept, x_{1i} was the total building space allowance area per cow for the i th farm, β_1 the coefficient for x_{1i} , x_{2i} the cubicle area per cow (total base area: bedded area + lunge area) for the i th farm, β_2 the coefficient for x_{2i} and ε was the residual error assumed to have a mean of zero and variance σ^2 .

To explore the potential for generalisability of this model to new data, a leave-one-out-cross-validation (LOOCV) (Kuhn and Johnson, 2016) was performed. Model predictions were assessed using r^2 and mean absolute error (MAE). Fit was also assessed using conventional residual analysis and Cook's distance (Petrie and Watson, 2006).

2.2.5.3 Latent class analysis of farmer opinions on space allowances

A latent class analysis of farmer opinions on loafing space was conducted using the Mclust package (Scrucca et al., 2016) in R, to explore whether farmers tended to be grouped within the data in terms of their views. Data from all 53 farmers surveyed were used for analysis. The following five themes were used to evaluate farmer opinions: Importance of loafing space in general for housed dairy cows, importance of loafing space for productivity, importance of loafing space for health, importance of loafing space for welfare and importance of outdoor areas for housed dairy cows. All five questions were presented to participants on an 11-point scale (0 = completely unimportant to 10 = critically important). The unsupervised latent class analysis was conducted using model-based clustering (Fraley and Raftery, 2002). The method comprised three elements; initialisation using model-based hierarchical agglomerative, use of an expectation-maximization algorithm for likelihood and cluster selection using Bayesian Information Criterion approximation (Fraley and Raftery, 2002). Using the technique, first the number of clusters within the data was identified and second the posterior probability of each farmer being in each latent class was estimated. Farmers were allocated to a cluster based on the maximum posterior probability; the Bayesian Information Criterion was used to identify the best model and hence optimum number of latent classes (Fraley and Raftery, 2002).

2.2.5.4 Model 2: Factors associated with availability of living space

A second generalised linear model (Marschner, 2018) was built using living space at maximum stocking density for cows in milk as the outcome variable. The aim was to explore which farm and farmer related variables were associated with availability of living space. Predictor variables tested were milk sold per cow per year, age of building, maximum number of milking cows on farm, duration of housing period, cow breed, calving pattern, farm region and latent class of farmer opinion. A forward stepwise procedure was undertaken with each variable tested and retained in model when $P < 0.05$. One farm was omitted from this model due to missing data for one of the covariates. The model took the form:

$$Y_i = \beta_0 + \beta_n x_n + \varepsilon_i$$

Where subscript i denoted the i^{th} farm, Y_i was the living space area per cow for farm I , β_0 was the intercept value, x_n were model covariates for i^{th} farm, β_n were the coefficients for x_n , and ε was the residual error assumed to have a mean of zero and variance σ^2 .

Model predictions were assessed using R^2 and MAE. Model fit was evaluated based on R-squared interpretation, with outliers assessed using conventional residual analysis and Cook's distance (Dennis Cook, 2000). LOOCV was performed to assess potential for generalisability of the model.

2.3 Results

2.3.1 Farm characteristics

Fifty-three farms participated in the study giving a response rate of 75.7%. Three farms were removed from the data for assessment of space allowances because they did not house cows in cubicles during lactation. Consequently, the minimum target sample size which was prospectively estimated by power analysis prior to commencing the study, was

fulfilled. The 50 farms were spread across Great Britain as shown in Figure 2.1, with the majority of farms within the South-West (18) or Midlands (19) areas. The main breed identified on each farm were Holsteins (18) or Holstein-Friesian types (27) with the remaining 5 consisting of crossbreeds or a mix of breeds without a majority. Twenty-eight farms contained between 201-400 adult dairy stock, there were 4, 10 and 8 farms with <100, 101-200 and >400 respectively (minimum = 60, maximum = 901, mean = 288, median = 265). Most farms stocked all of their cows in lactation in cubicle buildings (26), with 2 farms housing <75% of their milking cows in cubicles (in these herds cows were divided between cubicle and straw yard accommodation). For herds with a proportion of cows in non-cubicle accommodation, calculations were based solely on measurements of, and cow numbers in, the cubicle accommodation only. Calving patterns were split into 3 groups, block calving, double block calving and year-round with 9, 2 & 39 farms respectively. Nineteen farms housed the milking herd year-round, with 23 farms housing cows for 6 months or less on an average year. The highest yielding herd sold 12,650 litres per cow per year, lowest 5,850L with a mean of 8,979L and median of 9,150L (the national UK mean annual milk yield in 2017 was 7893L (AHDB, 2019b)). Ten of the herds produced over 10,000L per cow per year.



Figure 2.1. Representation of the geographical locations of the 53 farms which participated in the survey to investigate space allowance allocations for housed dairy cows in Great Britain (Google Maps, 2019)

2.3.2 Housing infrastructure

Total area per cow showed large variability between farms, with a minimum of 5.4m^2 and maximum of 12.7m^2 , mean = 8.3m^2 (95% CI = 7.90-8.80), median = 8.2m^2 (95% CI = 7.8-8.8), SD = 1.64m^2 (95% CI = 1.26-1.96) and IQR: 1.85m^2 (95% CI = 1.25-2.70).

Specific loafing space per cow across the farms also varied, with a minimum observed of 0m^2 , and a maximum of 5.4m^2 . The mean loafing space was 1.1m^2 (95% CI = $0.76 - 1.54$), median 0.5m^2 (95% CI = $0.0-1.3$), SD = 1.44m^2 (95% CI = $1.06-1.77$) and IQR 1.9m^2 (95% CI = $1.2-2.65$). Approximately one third farms were above a specific UK recommended target of 3m^2 for loafing space (Red Tractor, 2020).

The mean living space across the 50 farms was 2.5m^2 per cow at maximum stocking density (95% CI = $2.15-2.92$), with a minimum of 0.5m^2 and maximum of 6.4m^2 . The median was 2.4m^2 (95% CI = $1.95-2.75$), SD = 1.40m^2 (95% CI = $1.07-1.68$) and IQR 1.6m^2 (95% CI = $1.08-2.30$). The association between total space available per cow and living space per cow at maximum stocking is illustrated in Figure 2.2.

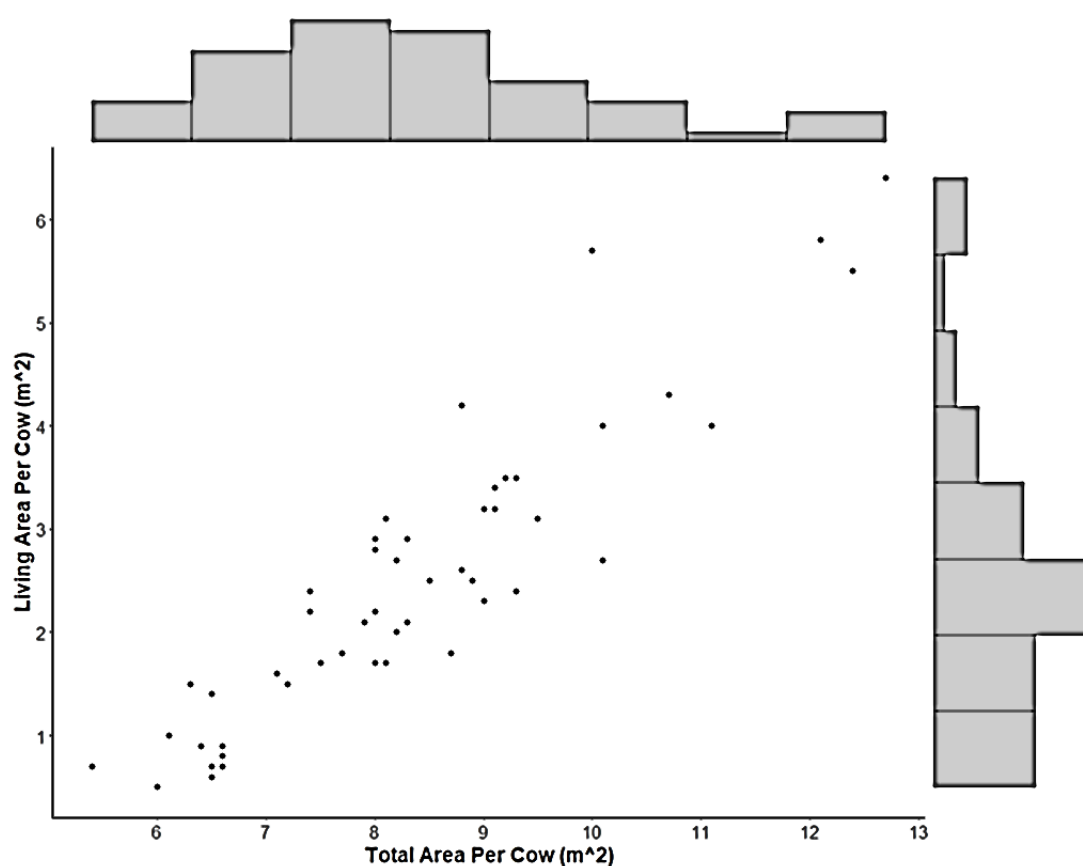


Figure 2.2. The histograms present the distributions of the measurements, in 1m^2 blocks, for total housing area per cow (m^2) and the living space per cow (m^2) for the 50 randomly selected farms recruited onto this study. The associated scatterplot shows how the two measures are associated.

The variability in living space between geographical regions is illustrated in Figure 2.3; 7/10 farms with the most living space area per cow were in the South-West of England. All farms in the north of England and Scotland were in the bottom 50% of study farms for amount of living space area per cow.

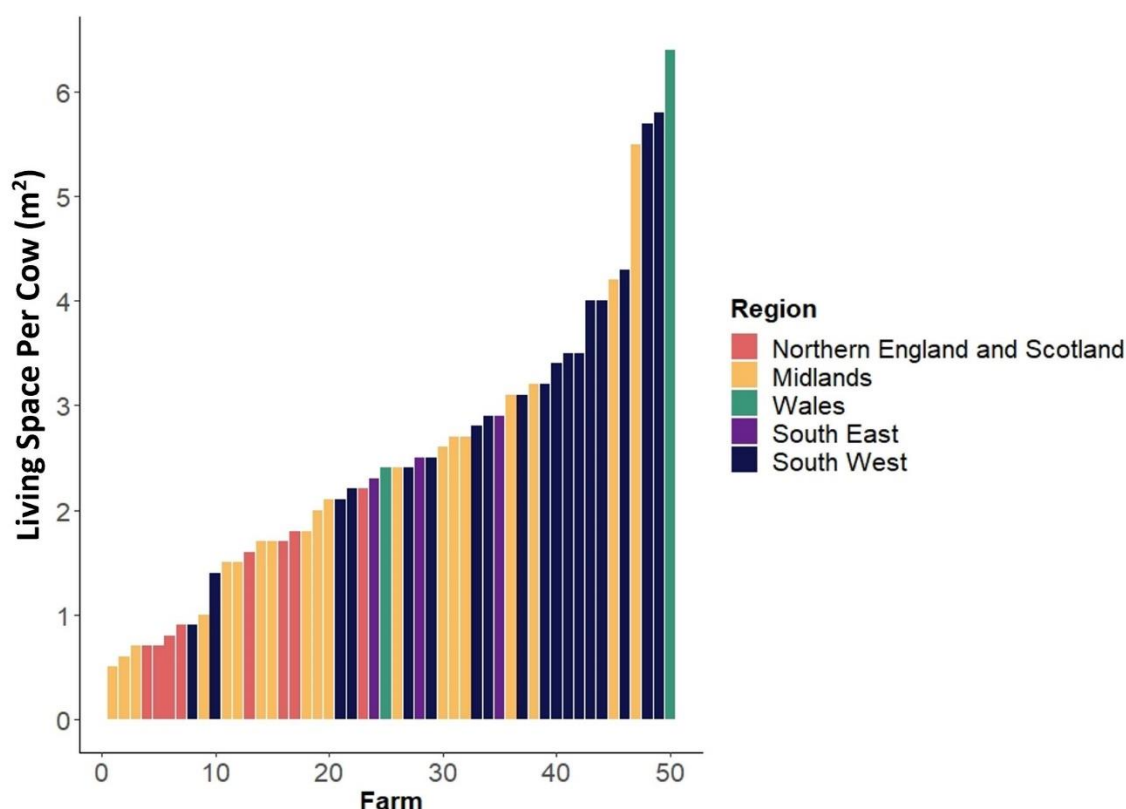


Figure 2.3. This presents the living space measurements (m^2 per cow per farm) for the 50 randomly selected dairy farm in Great Britain, arranged in ascending order. The colours represent how the geographical region in which the farm is located is associated with observed living space.

2.3.3 Farmer opinion questionnaire; descriptive results.

Results of the farmer opinion questionnaire are summarised in Figure 2.4. In general, answers to the five 11-point questions were relatively high indicating farmers valued loafing space and outdoor access as relatively important for housed dairy cows. The importance of loafing space for cow welfare received the highest score, with a median of 8 and interquartile

range of 8-10. The importance of outdoor access for housed dairy cows had most variation in scores (minimum = 0, maximum =10, IQR = 5-8).

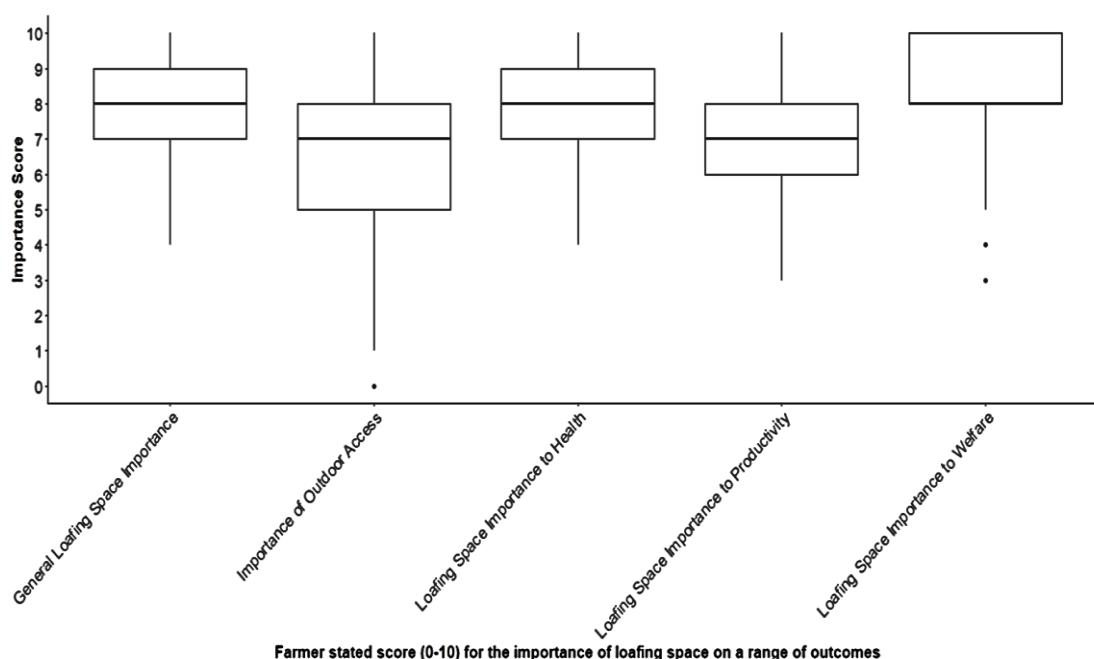


Figure 2.4. The five boxplots show the distributions of answers for the 11-point questions based on the perception of loafing space and outdoor access of the 53 participant dairy farmers in Great Britain. The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

When answering questions about the definitions of space there was substantial disagreement between respondents, twenty-two of 53 farmers (42%) stated that they thought all passageways (cubicle, feed and cross-over passages) were defined as loafing space whereas 16 farmers stated that none of these comprised loafing space.

Twenty-one farmers (40%) identified grooved concrete as the best surface for a loafing space, sixteen (30%) farmers stated this should be rubber matting and the remaining farmers selected either pasture, sand, slatted rubber matting, straw or woodchip pads.

Seventeen (50%) of the farmers responded that housed dairy cows should have greater than 2m² as a minimum loafing space (Min: 0.00m²; Median: 2.00m²; Mean: 2.65m²; Max: 8.00m²; IQR: 1.40-4.00m²; NA: 20) but thought that ideally cows would be given more

loafing space with a median of 5.00m² stated as the ideal situation (Figure 2.5; Min: 0.57m²; Mean: 5.04m²; Max: 12.00m²; IQR: 2.95-6.13m²; NA: 21).

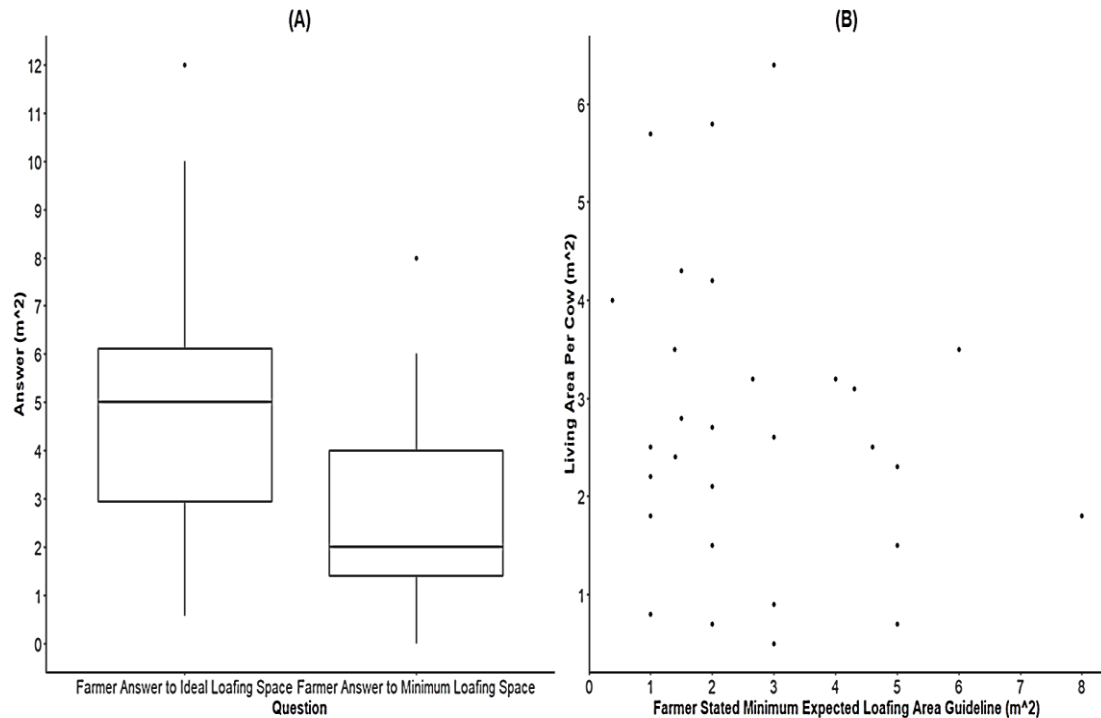


Figure 2.5. A) The two boxplots show the distributions of answers from the 53 participant dairy farmers from Great Britain to the questions about how much loafing space should be given to housed dairy cows: i) in an ideal scenario (left boxplot; NA (farmer declined to state answer for this question) = 20/53), ii) as a minimum (right boxplot; NA = 21/53). The boxplots show the median value (midline) and 25th and 75th percentiles (boxes). The whiskers contain data within 1.5× interquartile range, with outlying data shown as individual points. B) The scatterplot compares the observed living space per cow on the farm against the farmers-stated minimum amount of loafing space that housed dairy cows should have access to from the loafing space questionnaire.

2.3.4 Model 1: estimation of living space from housing features

The final model to predict living space from total area per cow and cubicle bedded area per cow, both at maximum stocking was:

$$\text{Living space at maximum stocking} = 0.83 T - 1.28 C - 0.79$$

Where T was the total area per cow at maximum stocking and C was the cubicle bedded area per cow at maximum stocking.

This model has a MAE of 0.39m and an r^2 of 0.87. Results of the LOOCV are illustrated in Figure 2.6. The LOOCV model produced a MAE of 0.42m and an r^2 of 0.85.

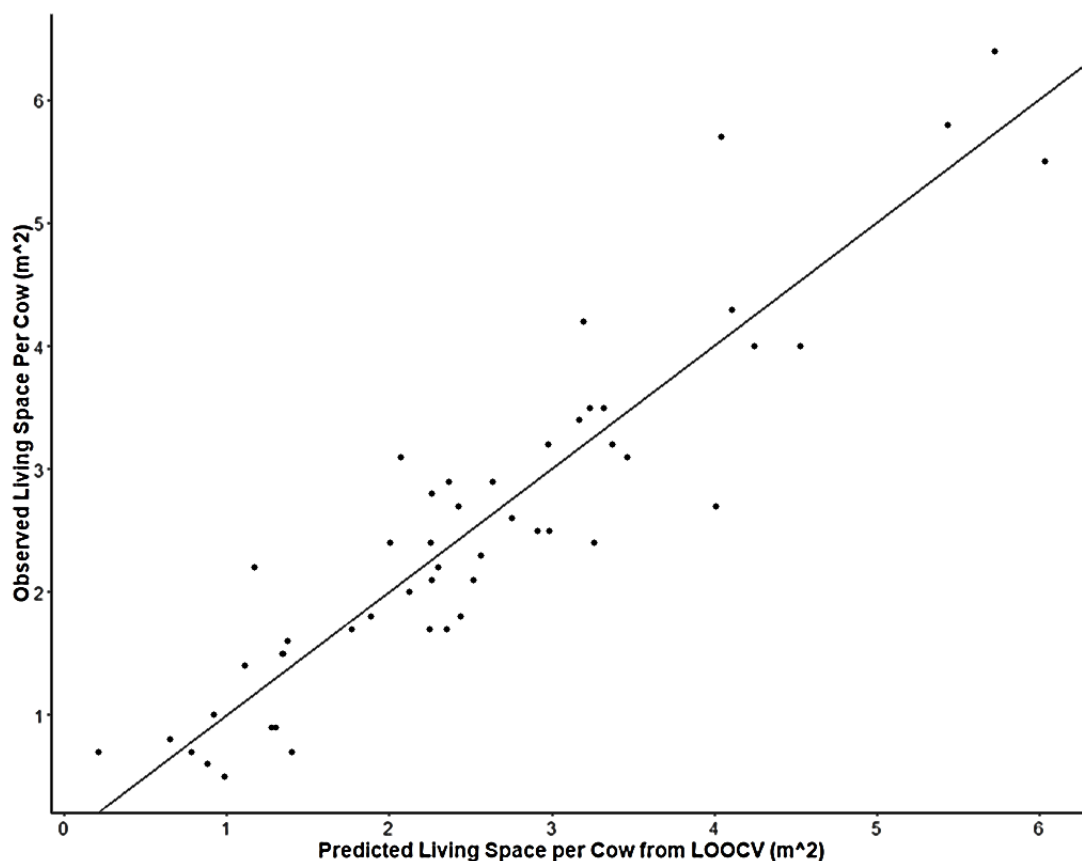


Figure 2.6. This scatterplot compares the observed living space calculated per cow per farm (y-axis) against the predicted living space per cow per farms from the leave-one-out cross-validation (LOOCV) from model 1 ($R^2 = 0.85$) of the 50 farms recruited to the study investigating space allowances in dairy cow housing within Great Britain.

2.3.5 Latent class analysis of farmer opinions on space allowances

The 52 farms were categorised into 4 latent classes as illustrated in Figure 2.7. The classes contained 22, 6, 13 and 11 farms respectively. Latent class 2 contained farmers which gave the lowest scores for the importance of loafing space and outdoor access but these were generally around a neutral score of 5. Conversely latent class 4 farmers gave the highest score for all 5 questions. Farmers in latent class 1 gave intermediate scores for loafing space

importance but scored more highly than latent class 3 farmers, who scored outdoor access more highly than latent class 1 farmers. Thus, latent lass 1 farmers appeared to believe that space was important for housed dairy cows but believed this space did not have to provide outdoor access to be beneficial.

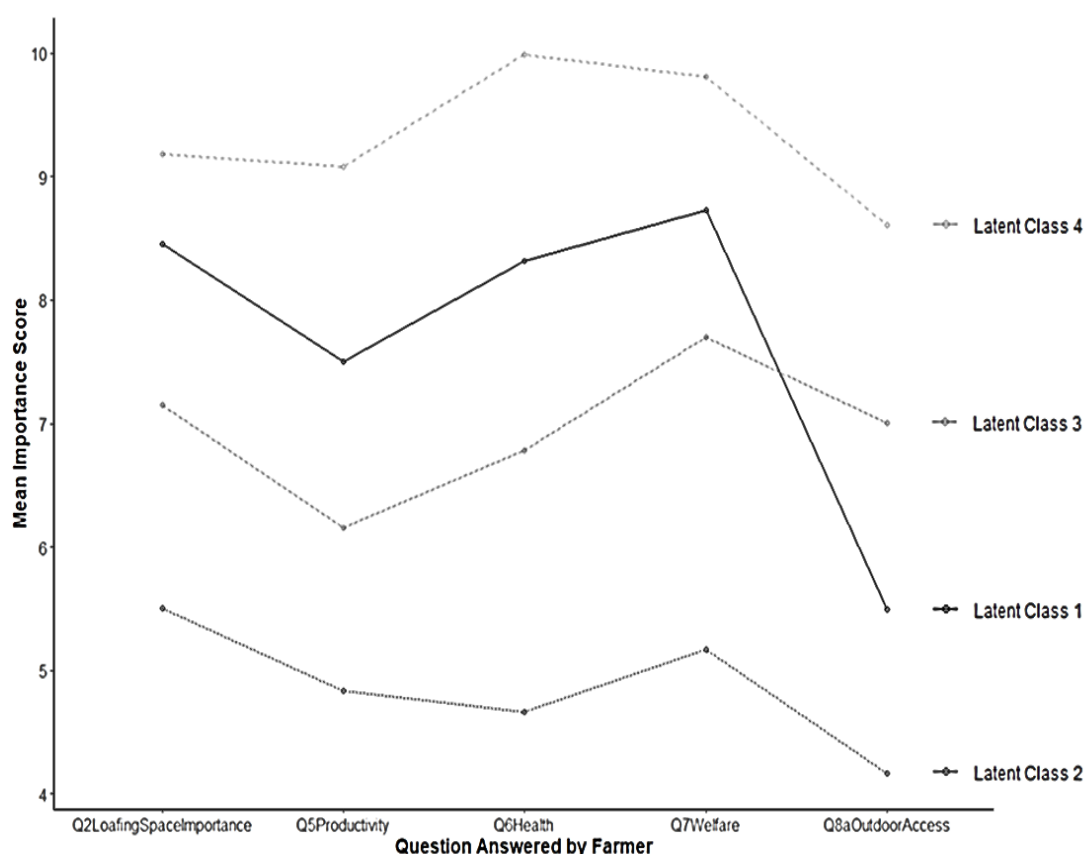


Figure 2.7. Shows the mean farmer opinion score of the five 11-point questions from the loafing space farmer questionnaire completed by 53 GB dairy farmers based on which of the 4 latent class groups they were assigned to. Latent class 1: $n = 22$, all farmers gave intermediate scores for the four loafing space importance questions but scored the importance of outdoor access for housed dairy cows low in comparison to latent class 3. Latent class 2: $n = 6$, contained farmers which ranked the 4 loafing space importance questions and importance of outdoor access lowest of all four latent classes. Latent class 3: $n = 13$, linked farmers who gave intermediate scores to all the questions but a higher score for importance of outdoor access for housed dairy cows in comparison to latent class 1. Latent class 4: $n = 11$, were a group of farmers who scored all 5 questions highly in comparison to the other latent classes.

2.3.6 Model 2: Factors associated with availability of living space

Results of Model 2 are presented in

Table 2.1. Region and farmer latent class were significant variables, with farms in the South West, Wales and the South East being associated with greater amounts of living space per cow than farms in Northern England and Scotland. Farmer opinion latent class 3 (higher outdoor access importance answers but lower loafing space importance answers) had farms with more living space per cow in comparison to farmer latent class 1 (higher loafing space importance answers with lower outdoor access importance answers) ($P < 0.05$). Farmers in latent class 1 stated that they thought space was important generally but the model estimated them as giving the least amount of space than all other groups, with even farmers in latent class 2, which answered with the lowest scores to each of the questions, appearing to provide more space for their housed dairy cows. The mean absolute error for the model was 0.78m and $r^2 = 0.39$. Post fit analyses indicated that model fit was good.

Table 2.1. The results from statistical model 2 which assessed factors associated with the amount of living space available for lactating cows on farm, in a study investigating space allowance in dairy cow housing in Great Britain.

Model outcome: Observed living space allowance (m² per cow)			
	Estimate	Std. error	P
Intercept	0.84	0.51	0.1119
Farm Region			
Northern England and Scotland	(ref)		
Midlands	0.83	0.55	0.1356
South East	1.51	0.84	0.0796
South West	2.01	0.52	0.0004
Wales	3.28	0.97	0.0017
Farmer Opinion Latent Classes			
Latent Class 1	(ref)		
Latent Class 2	0.34	0.60	0.5743
Latent Class 3	1.03	0.44	0.0238
Latent Class 4	0.59	0.46	0.2098

2.4 Discussion

This study is the first worldwide to report the quantity and variation in living space availability for a sampled national population of dairy herds. The variation in space allowance spanned all current GB recommendations; 10% farms exceeded the largest GB guideline (AHDB: 10.5m²), and one farm was below the minimum guideline set in British standards (6.5m²). Farms with higher space allowance and living space area per cow typically had outdoor loafing or feeding areas as well as wider passageways. The farms with accommodation with the least space had narrow passageways and fewer cross-over passages. Given the extent of variation observed, (the standard deviation was 20% of the mean for total space allowance per cow), the biological importance of the distribution of data is potentially of great significance although its impact is currently uncertain.

Although measuring health outcomes were not an objective of this study, previous research suggests that living conditions are likely to impact upon the health of inhabitants (Bonnefoy, 2007; Jacobs, 2011). It has been hypothesised that variation in the housed environment on farms has a relationship with health, welfare and productivity of the cows; a study looking into alternative housing systems for dairy cows has shown that the environment could influence somatic cell counts, mastitis infection rates and reproductive performance (Barberg et al., 2007). Housing has been reported to influence lameness incidence through inappropriate design as a risk factor that could predispose cows to white line lesions, sole haemorrhage (Flower et al., 2007), or digital dermatitis (Endres, 2017). Milk production and quality has been reported to be influenced by space allowance via competition for resources, for example at the feed-face or stocking density in automatic milking systems (Crossley et al., 2017; Deming et al., 2013), however the short term nature of such trials make these difficult to interpret. Space availability is likely to influence cow behaviour; spatial distribution analysis has shown dominant cows to have lower cow-to-cow distances and that lower ranking cows may want to space themselves further from other animals within a group (Syme et al., 1975). More recently, stocking density (defined as the

number of cows to cubicle spaces) has been shown to decrease lying times, cause cows to spend more time in passageways and alter their social structure (Hill et al., 2009; Krawczel et al., 2012). Therefore, current literature suggests that stocking density, feed-face and shed design can influence cow health, welfare and production. Surprisingly, no studies to date have evaluated the direct impact of living space allowance on cow health and welfare.

This study proposes “living space” as a novel and valuable measure of space availability to cows on farm. Many farm regulatory bodies and assurance schemes have recommendations for loafing space availability in cubicle systems, however, imprecise definitions of loafing space make it difficult for assessors to measure and for farmers to modify their buildings appropriately and to some extent, the sparsity of research into loafing areas may be caused by the ambiguity around its definition. The novel definition of living space, an area above that considered essential to gain access to lying and feeding areas, provides a more intuitive framework to define space availability for cows. This definition of living space encompasses all fundamental areas of a cubicle building, including accounting for wider passageways and additional outdoor/indoor areas, thus providing a useful estimate of the true available space using a single parameter. Importantly, this study identified that definition of living space could be accurately estimated from two simple measurements; total housed area and total cubicle bedding area (Model 1) which means it can be readily estimated under commercial conditions using a standard laser measurer. Since indoor housed cubicle systems are used across many large dairying nations, this definition of living space is likely to be useful for assessing the housed environment for cubicle buildings on a widespread basis, including the standardisation of research studies.

One limitation of the study and indeed the living space calculation is that it does not directly account for other infrastructure in the building that would be important for housed dairy cows. This includes aspects such as cubicle size, bedding type, stocking density, feeding space, water access and flooring type. These could all be classed as important contextual variables that could influence interpretation of the quality of the housed environment for dairy cows but these parameters have not been described in this study as

they are out of the scope of this work. However, it is important to note that these are additional parameters that should be noted when assessing the housed infrastructure.

Farmers who participated in this survey considered loafing space to be relatively important to the health, welfare and productivity of their herd, as observed in the questionnaire results. However, answers to questions based around their knowledge of loafing space were very variable, for example 38% (20/53) were unable to give an answer for the minimum amount of loafing space requirements (range: 0.3m² to 8m² per cow), suggesting poor knowledge of this topic. Some of this variation could arise from different perceptions of what constitutes loafing space, especially given the different views identified in the survey on whether all passageways should be counted as part of loafing space or not. Currently, farmer attitudes towards loafing space haven't been investigated widely; it would be of interest to understand whether farmer views about the importance of loafing space is markedly different in different cultures and countries.

The observed living space measured on each farm was noticeably different to the farmer's stated minimum acceptable loafing space, with around half of farmers providing their cows less than their cited minimum value. This is an example of cognitive dissonance, where a belief does not match with behaviour (Bandura, 2000); there is evidence to show that behaviour does not always match with cognitive components of belief (LaPiere, 1934). A previous example of this occurring has been shown with footrot management in sheep (O'Kane et al., 2017) when farmer behaviour has been identified to be effected by many factors, such as habit, emotions, social norms and social capital (O'Kane et al., 2017; Shortall et al., 2018). This raises questions as to what the social norm for provision of loafing space to dairy cows is within the industry or, in terms of perceived behavioural control, how difficult farmers believe the provision of extra space to be in relation to its benefits. Our results indicate that many farmers believe loafing space is important for their cows but barriers must exist which prevent implementation, further exploration of these barriers would be beneficial but are likely to include cost and practical difficulties associated with making significant changes to existing building and farm layouts. Plausible theories preventing

farmers moving between the stage of wanting to implement change to actually undertaking it may include motivation, competition with other initiatives on farm, or anticipation of potential difficulties (Green et al., 2012).

Region and latent classes of farmer beliefs were both factors associated with the amount of living space per cow observed on farm. Farms in the south of England provided more living space than those in the north. A possible explanation for this may be that farms with greater amounts of space tended to have additional outdoor loafing areas. From further meteorological investigation it was found that the farms in this study from the more northern areas of GB generally had higher rainfall on average than those located in the southern parts. Farms in Northern England and Scotland region grouping may be less likely to opt for outdoor loafing areas due to the higher average rainfall recorded in these areas, as this could increase the volume of slurry and therefore time and expense to manage it. Farmers in latent class 3 (intermediate loafing space importance scores, higher outdoor access score) provided more living space for cows than those in latent class 1 farmers (intermediate loafing space scores, lower outdoor access score). The main difference between these two classes was the stated importance of outdoor access for housed dairy cows with latent class 3 farmers scoring this feature higher. Therefore, it is more likely that farmers in this group would commit to providing outdoor areas for their livestock and consequently specific loafing spaces, which tend to inflate the total living space availability.

A limitation associated with the questionnaire is the potential for social desirability bias; it was known to the respondent that the researcher was from a university and loafing space was a key theme of the study. Therefore, farmers may have believed that loafing space should be important for housed dairy cows and may have inflated their scores in response to this. Although a high response rate (76.7%) was achieved from study farms, we cannot be sure that non-responders would be the same as those responding. Reasons given for non-participation included time availability (n=2), exiting the dairy business (n=2), illness (n=2), biosecurity (n=1) and non-interest (n=5). Three of the selected farmers were uncontactable despite at least 3 attempts of contact via email and telephone and the remaining farmers did

not provide a reason for non-participation. It is likely that sample group was representative of the target sample because of the robust randomisation method of selection and very high response rate, however, data protection issues meant it was not possible to compare demographics of the non-responder group and study participant group meaning a degree of selection bias cannot be ruled out.

The target population in this study was all dairy producers supplying a large British retailer and it is unclear how this group relates to other British farmers. How this sample relates to other dairying nations is also unknown due to differences in management practices and legislation/guidelines. It will also be important to greatly improve the understanding of the quality of space provided to cows. For example, this could include investigations of the types of spaces required for displaying natural behaviours, improving access to feed and lying areas, design of cubicle layout and the quality of floor surfaces for walkability. By combining assessment into quantity, quality and types of space provided to indoor housed dairy cows, the impact of building design should be evaluated in the context of ammonia emissions, cow health, welfare and productivity. Such research would provide a scientific basis to advise international industry standards on the quantity and quality of living space for dairy cows.

2.5 Conclusion

This research has demonstrated that there is important variation in the housing conditions of dairy cows across Great Britain. It is unknown to what extent this variation impacts upon health, welfare and productivity; this is the subject of the following chapters.

Chapter 3. Randomised controlled trial: Materials and methods

The results observed in Chapter 2 became the basis of a randomised controlled trial to explore the effect that living space has on housed dairy cows. This section provides an overview of the materials and methods for the randomised controlled trial, for the data collection and analyses undertaken in Chapters 4, 5 and 6. This chapter will provide an overview of the study design employed, with more specific details described in respective chapters where needed.

3.1 Ethical review and study guidelines

This study was designed using the CONSORT 10 guidelines (Moher et al., 2010). Ethical permission was granted by the Home Office and the University of Nottingham; the study's license number was MG_P07992717 and was conducted under the Animal [Scientific Procedures] Act 1986.

3.2 Study design

Data collection took place between 16th July 2018 and 15th July 2019 at the Sutton Bonington Dairy Centre, Leicestershire, UK. The research comprised a randomised, controlled [1:1], long-term (1 year), longitudinal, parallel-group (pairs of adult dairy cows matched by parity and days in milk), cross-over (group location within facility) study to evaluate superiority/inferiority of a space allowance intervention for housed dairy cattle.

This study used a single, novel, state-of-the-art dairy cow cubicle shed which allowed internal layout modification of the housed infrastructure. The shed was set up to incorporate 2 groups of cows during the trial phase. One half contained the intervention group ('high space' group) of animals with the 'control space' group acting as the control. The experimental unit of the analysis was at cow level, with cows matched as pairs to control for differences between the trial groups. The two pens in the research building were a precise

mirror image except for the experimental intervention to modify the floor space availability in each pen.

Dairy cows from The University of Nottingham's Sutton Bonington dairy farm were the sole pool for the selection group. Characteristics included Holstein-type dairy cows in lactation and of good health after a veterinary clinical examination on the day of study commencement, or enrolment. Details of cow selection are provided below in Section 3.7.

3.3 Details of the intervention

The flexible housing unit allowed changes to the overall floor space available to dairy cows when in housed conditions; a change in floor area per cow was the intervention for the experiment. Trial cows were moved into the research housing 3 weeks prior to the beginning of the study for habituation but space was only restricted on the day of trial commencement.

The control space group had a total space allowance, including bedded area, of 9m² per cow of which 3m² was classed as living space. This space allowance was based on the approximate median space provision observed on commercial dairy farms in Chapter 2. The high space (intervention) group had a larger overall space allowance at 14m² per cow with 6.5m² of this space classed as living space. This was perceived to be the high end of what cows would have available, based on the farm measurements surveyed in Chapter 2. The two space allowances selected were marginally greater than the median and maximum range of the farms in Chapter 2 because it was observed that the farms with milking robots (as with cows in this RCT) had more space than conventional parlour milked herds due to the need of an in-situ 'collecting yard' area. The extra space in the high space group was created by using wider passageways (feedface passageway width: 4.5m vs. 3m, cubicle passageway width 3.5m vs 3m) and an indoor loafing space area (Figure 3.1). No other differences existed between the two pens.

The remainder of the facility and all management procedures were identical for both groups. Feed-face length and location, bedded area and location, water trough volume and location, and environmental enrichment (cow brush and mineral licks) were the same for both groups. Daily and weekly management routines were also matched for both groups of cows.

Every 91 days the cow group and housing set-up were changed to the opposite side of the flexible housing shed to account for possible environmental variability from seasonal change. Individual cows allocated to either the high space or control space groups remained within these groups and were exposed to the same housed conditions throughout their time within the study Figure 3.2.

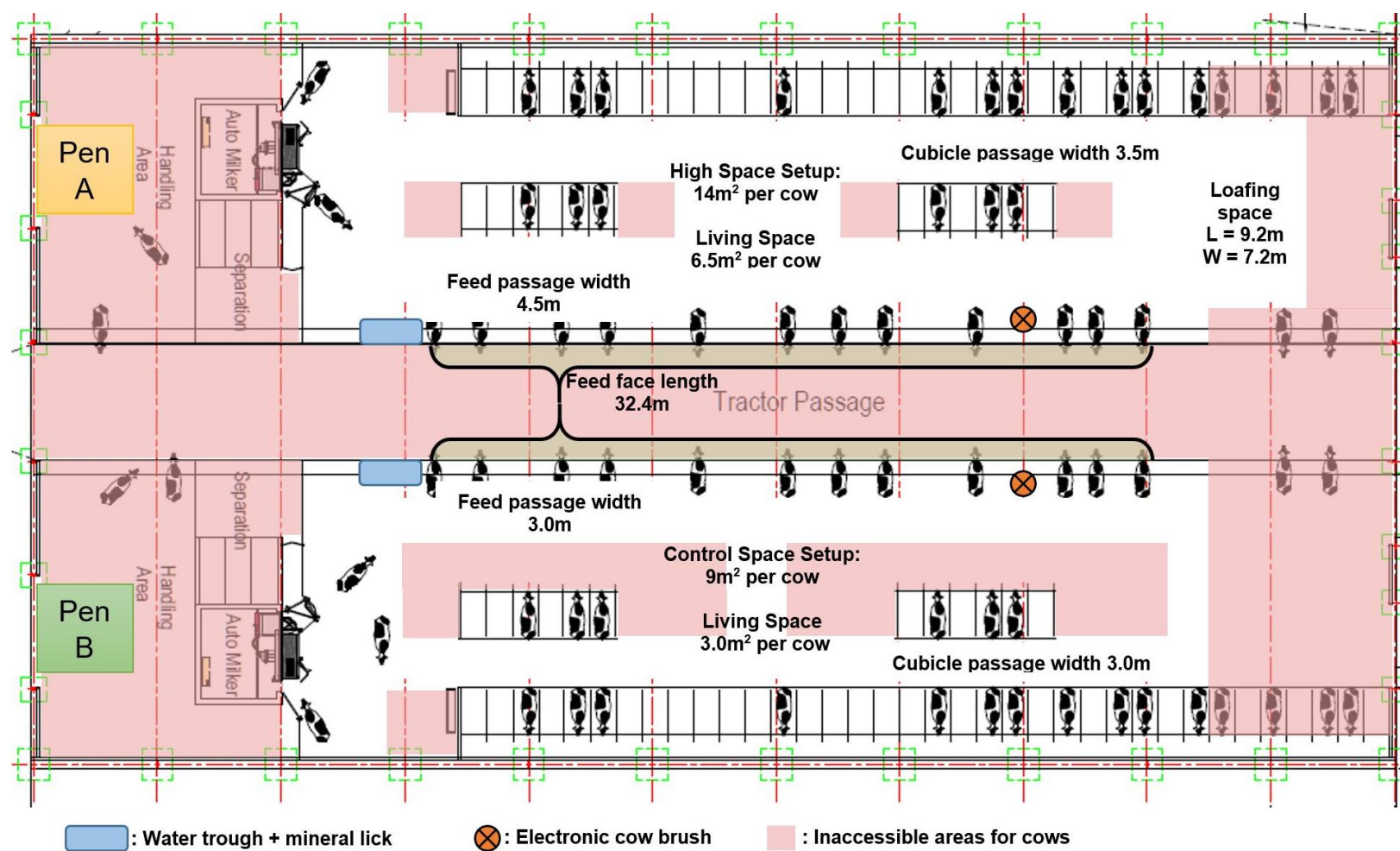


Figure 3.1. Schematic of randomised controlled trial cubicle shed floorplan with associated space comparisons. Top: High-space group (intervention) with 14m² per cow total space (6.5m² living space). Bottom: Control space group with 9m² per cow total space (3m² living space).

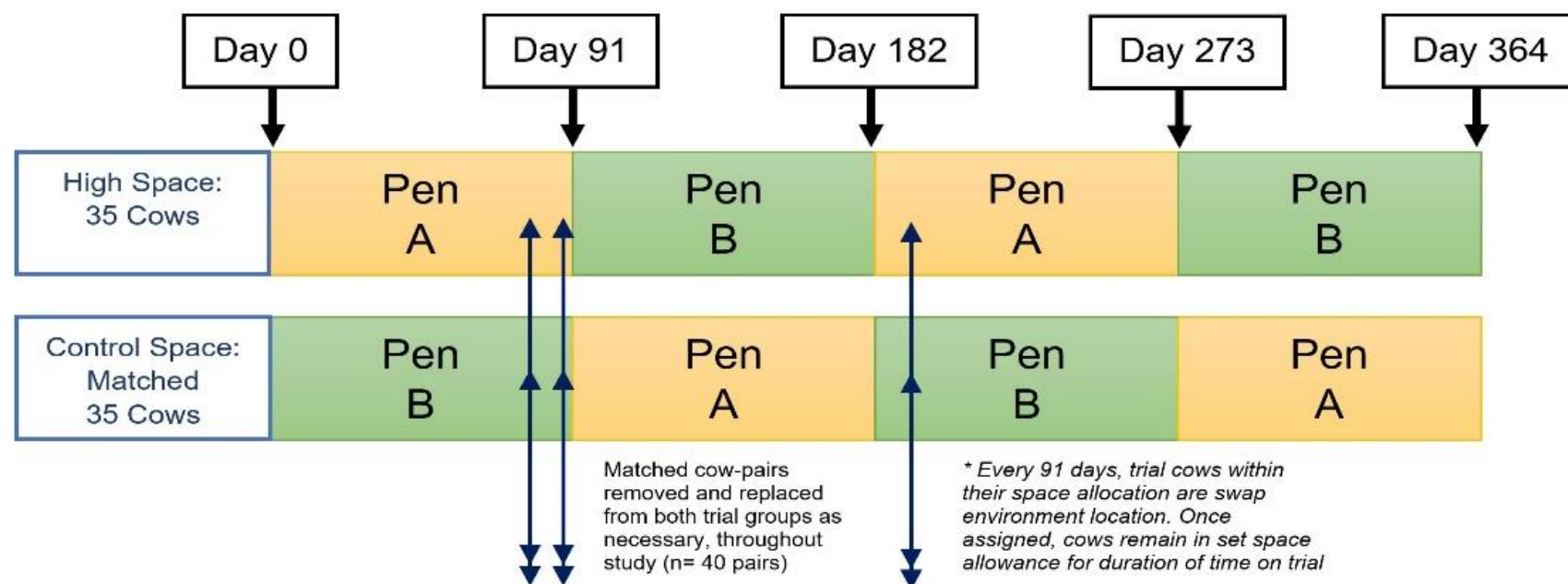


Figure 3.2. Schematic which shows the cross-over and trial cow entry-exit timelines for the 364-day period of the randomised controlled trial to investigate space allowances of housed dairy cows.

3.4 Housing and husbandry

The housing was set as a mirror image and included the following infrastructure. Each pen contained one Lely Milking Robot (Lely Astronaut A4 milking robots). The trial group of cows had access to 39 deep sand cubicles; 27 fixed cubicles running along the barns outside edge with 2 sets of 6 space cubicle bays centred in the middle of each pen (Figure 3.1). The feedface (32.4m) was located in the centre of the shed for both pens with access through head yokes. Cows were fed a fresh partial mixed ration each day between 7.30-8.30am. Two water troughs were accessible in both pens (183x61cm). Each pen had one electric cow brush located at the feed face barrier. Safesite® 2000 crash barriers and gates (Figure 3.3) were used to restrict space at the end of each pen (Safesite, n.d.). Motorway crash barriers were also used to create the specified cubicle, feed and cross-over passageway dimensions around the moveable cubicle pods.



Figure 3.3. Picture example of the water-filled safesite® 2000 crash barrier used to restrict space for the randomised controlled trial to investigate the impact of space allowances on housed dairy cows (Safesite, n.d.).

Environmental conditions included artificial lighting from the Lely Light 4 Cows system (Lely, n.d.). Temperature and humidity were monitored via four sensor locations (ALTA 900MHz Industrial Humidity Sensors with Probes; +/- 3% under normal conditions: 10% - 90% RH, optimal battery operating temperature: +10°C to +50°C). To ensure environmental conditions remained even throughout the research facility, a variable ventilation modular side curtain system (Galebreaker Agri, VVS®) was used. THI was monitored throughout the entirety of the trial and was shown to be similar across the trial locations in each time period, as shown in Figure 3.4. Therefore, it was not considered to be a confounder and thus was not tested further in any of the analyses undertaken.

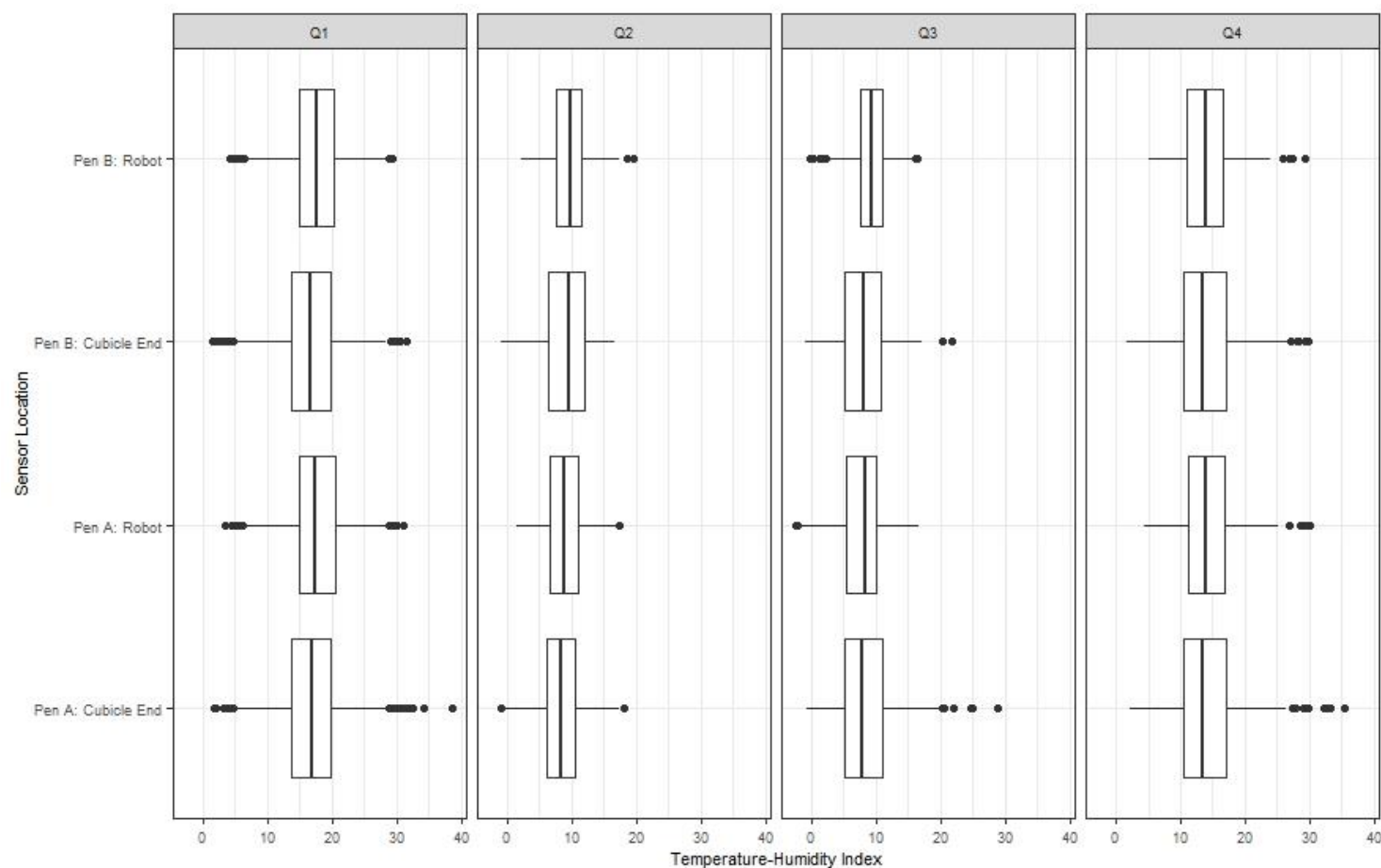


Figure 3.4. Boxplots to illustrate the distributions of the temperature-humidity indices (x-axis) at each 2-hour timepoint of the data recordings during the randomised controlled trial to investigate space allowances for housed dairy cows. The boxplots are specific for the sensor location (Pen A: Robot; Pen A: Cubicle End; Pen B: Robot; Pen B: Cubicle End) and quarter-year time periods (Q1-4). The median values are represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

Animals were observed twice daily for health and welfare checks by trained farm staff. If an animal was identified as having a problem a general clinical examination was undertaken by a qualified veterinarian and appropriate treatment prescribed or the cow was removed from the trial if deemed necessary for welfare purposes. For any cow removed from the study, the matched cow in the other trial group was removed at the same time.

Daily and weekly management routines were identical for both groups of cows and in brief were as follows. The daily feeding routine consisted of a single fresh ad libitum partial mixed ration fed in the morning, with feed ‘pushed up’ (moved back towards the cows using a tractor) every 2 hours during the day. The free stall bedding comprised deep sand which was raked twice per day to remove any soiling.

3.5 Experimental procedures

The following section describes the experimental procedures which required contact with the cows or the environment to obtain data. Details on the eligible populations and analyses are described in the relevant chapter containing topic results.

3.5.1 Animal Scientific Procedure Act (ASPA) procedures

A trained researcher, JT or MG, performed all ASPA procedures for the duration of the trial. All ASPA procedures were undertaken weekly on a specified day to limit differences to daily management since cows were also being monitored for behaviour. Procedures were undertaken in weekly blocks (7-14DIM or 15-21DIM), the first timepoint a cow was sampled was within the block on the specified procedure day rather than on a set DIM. To be eligible for the procedures, cows had to enter the trial at <14 days in milk.

3.5.1.1 Blood sampling

Blood samples were taken within 3 time-blocks for each cow; 7-14, 15-21, 22-28 days in milk. Blood was taken from the coccygeal vein using a 20 gauge 1” needle and vacutainer blood tube. A single tube (10ml) was taken at the first two time points and 2 tubes (20ml) at the final time point. Cows were restrained in head yokes at the feedface. The method involved washing the sample site with Hibiscrub detergent soaked cotton wool. Venepuncture was then performed. After sampling, pressure was applied at the sample site and released when bleeding ceased. The blood samples were taken in the morning between 8.30am and 10am.

Laboratory analysis of blood samples was undertaken for beta-hydroxybutyrates (BHB) and non-esterified fatty acids (NEFA) at all three time points (see Chapter 5). At the final time point the extra sample was used for analysis of Anti-Mullerian Hormone (AMH, see Chapter 5).

3.5.1.2 Transrectal ultrasound examinations

Transrectal ultrasound examinations (U/S Scan) were undertaken at 4 time points: U/S Scan 1: 14-21 DIM, U/S Scan 2: 22-28 DIM, U/S Scan 3: 29-35 DIM and U/S Scan 4: 36-42 DIM. The researcher (JT) performed all transrectal ultrasound scans using a BCF Easi-Scan Linear (“Easi-Scan Linear | BCF Technology | Cattle Scanning Machines,” n.d.). Measurements taken included the cross-sectional widths of the left and right uterine horns (1-4cms caudal to the bifurcation), the length and width of both ovaries, a count of the number of antral follicles (between 2-10mm) and a count of follicles >10mm (these were recorded with approximate sizes). The presence of corpora lutea and cystic structures were also noted. Scans were undertaken with cows restrained at the feed-face in head yokes between 8.30am and 11am.

3.5.1.3 Vaginal examinations

Vaginal examinations were performed at 3 sampling points between day 21-28, 29-35 and 36-42 in milk. This was undertaken between 8.30-11am in head yokes at the feedface. To minimise contamination of the vagina, the vulva and perineum were wiped using cotton wool soaked in Hibiscrub whilst the tail was held aloft. A metricheck device (a 4-cm hemisphere of silicon cup on the end of a 50cm long stainless-steel pipe) was cleaned with Hibiscrub and lubricated prior to insertion. Steps were carried out as described by Pleticha et al (2009) with insertion of the device through the vulval lips, prior to advancement to the cranial fornix of the vagina. To obtain a sample the metricheck was retracted caudally to allow for visual assessment of any discharge held within the silicon cup. The scoring system previously reported (Pleticha et al., 2009) was used as follows:

- Score 0: Clear or translucent mucus, for healthy cows.
- Score 1: Mucus containing flecks of white or off-white pus.
- Score 2: Discharge containing less than 50% white or off-white mucopurulent material.
- Score 3: Discharge composed of more than 50% white or yellow pus or was sanguineous

Results obtained from these data are described in Chapter 5.

3.5.2 Non-ASPA sample collection

3.5.2.1 Milk samples

Milk samples were collected 3 times each week during Monday daytime, Wednesday daytime and Thursday evening/Friday morning. To achieve this a Lely shuttle milk sampling machine was attached to the robotic milker at the start of each sampling

period and set to collect 25ml of milk per cow. Automated sampling had specific built-in restrictions (Lely, 2020), the most notable of which was that a sample could only be collected >4 hours after a previous successful milking. Samples were taken on all three days for progesterone testing (further details in Chapter 5).

3.5.2.2 Omnisense location data

Selected pairs of cows were fitted with a neck collar containing a location tracking device. These devices provided coordinates for each cow every 7 seconds as well as other parameters such as area zones and orientation (Omnisense, 2018) and allowed for continuous location monitoring of the cows. Further details will be described in Chapter 6. During the trial battery changes occurred during the weekly sampling procedure whilst cows were restrained in feed-face head yokes.

3.6 Sample size

Sample size calculations were based on the use of repeated measures models (i.e. multiple measurements conducted on each cow over time) with a power set at 0.8 and a significance probability at 0.05. Example power calculations for one experiment are provided below; separate power analyses were conducted for each experimental outcome to ensure the number of animals used was both minimal and sufficient. The most limiting outcome variables in terms of sample size were those relating to milk production. Sample size estimates are illustrated here using the outcomes of milk yield and somatic cell count (a proxy for immune status of the mammary gland), this took into account the repeated measurement of these over a minimum of 14 occasions. For milk yield (average daily cow yield of approximately 33kg /d), based on a detectable difference between treatment groups of 0.5kg/d, a standard deviation between days within cow (corrected for days in milk and parity, based on current herd data) of 0.5kg/d, a standard deviation between cows (corrected for days in milk and parity, based on current data) of 2kg/d, a minimum group size of 30

cows was required. Sample size calculations for the analysis using a Cox proportional-hazard model for the time-to-conception data using the package “powerSurvEpi” and function “ssizeT.default” in R-statistical software (Qiu et al., 2021). This calculation indicated that 38 cows in each group would be required to obtain a statistically significant difference for a hazard ratio of 0.5, using a power of 0.8 and type I error rate of 0.05.

To allow for the unplanned loss of cows (for example to disease events) during the study period and to maintain a minimum group size of 30 cows, a starting sample size of 35 cows per group was used. Sample size calculations based on blood and cytological parameters indicated that smaller sample sizes were needed compared to milk yield because between and within cow variability was expected to be relatively small and the detectable biologically important differences between treatment groups, relatively large. Calculations indicated that 15 cows in each group was sufficient to detect significant differences for these parameters. Multiple measurements on each animal (e.g. blood sampling at the same time as reproductive tract scanning) added little extra discomfort to individuals but reduced the total numbers of animals required. To minimise the burden on individuals, sampling occasions per animal were restricted to those essential for testing hypotheses. Control animals were required to make a statistically valid comparison, i.e. so that the effect of a specified housing condition was clearly evaluated against a known comparator. Control groups were needed for each experiment since there will be potentially confounding variables (such as climatic conditions) that could vary from one experiment to the next which meant a single group to represent a ‘control’ for all subsequent studies would not have been scientifically robust.

Therefore, for the experiment a starting population of 70 animals was used, 35 in each of two groups. To retain target stocking rates and space allowances, the number of animals in each group had to remain the same whilst at the same time animals had to be dried off at the end of their lactations. Consequently, some animals entered and left the study during the trial period but replacement of cows meant the sample sizes of each remained consistent.

3.7 Experimental animals

All cows were selected from the University of Nottingham dairy herd. Seventy Holstein-Friesian female dairy cows were on trial at all times. A total of 134 individual cows were used during the 364-day period (16 cows were used during two different lactations). A total of 33 cows were a “re-use” having been included in a previous home office study prior to enrolment. All animals were deemed healthy by a veterinarian before entering onto study.

To recruit the starting population of cows. All adult cows in the University of Nottingham dairy herd ($n=300$) were paired, matched on parity then by days in milk in the current lactation; parity $< 3 \pm 3$ DIM, parity $\geq 3 \pm 5$ DIM. If a cow could be matched with more than one other, then the closer match in terms of DIM was used. In this instance, if DIM were identical, then reproductive status was taken accounted for matching (pregnant/ inseminated/ non-pregnant).

Initially all eligible cows in the herd were matched based on the parameters above. More than 35 pairs were identified. Cows were excluded from selection if they were greater than 270DIM, clinically unwell (received an antibiotic or anti-inflammatory treatment for clinical disease within 4 weeks of the trial start date or commencement onto trial) or had undergone a surgical procedure in their current lactation. Using the current herd parity structure as a template to select the parity structure for study cows, pairs of cows were selected to fit this structure. This meant all pairs of cows in their 2nd or higher parity were selected ($n=15$ pairs). There were more than the required number of primiparous cows available and a stratified procedure for sampling was used for these. Pairs of primiparous cows were split into sub-population groups depending on DIM (1-40DIM, 41-80DIM, 81-120DIM and 121+DIM). From this a maximum 4 cow pairs were chosen from each block. If there were more than 4 potential cow-pairs in a block 4 pairs were chosen at random using random number generation in Microsoft Excel (Microsoft, 2016). Through this process, 35 pairs of cows were initially selected to start the study.

During the study period pairs of cows moved in and out of the study. The two reasons for moving out of the study were for drying off (± 2 weeks from 60 days prior to calving, $n = 36$) or for health issues, which meant a cow could not continue on trial for welfare reasons ($n = 4$; 1- severe lameness, 3-severe fatty liver/non-responsive to treatment). All pairs of cows that moved out at drying off were replaced immediately with a matched pair of recently calved cows ($<14\text{DIM}$). If a pair exited the study because of the poor health of one cow, they were replaced within 3 days, again with a matched pair of recently calved cows ($<14\text{DIM}$). To ensure cows were in good health all received a veterinary clinical examination on the day of enrolment.

A total of 38x 1st parity heifers, 22x 2nd parity cows, 8x 3rd parity cows, 4x 4th parity cows and 3x 5th parity cow pairs were used during the one-year study.

3.8 Blinding

The researchers could not be blinded to the group allocation of study cows during the project but most of the outcome measurements were made by sensor or laboratory equipment which were free from operator bias.

Blinding of all raw data was undertaken before data analysis; trial group and cow identification numbers were all changed such that the data analyst could not identify cows or experimental groupings. Once analysis was completed the identification process was reversed to obtain the trial group results.

3.9 Experimental outcomes

The purpose of the experimental study was to quantify the impact of adult dairy cow living space on:

- a. Milk production (Chapter 4)
- b. Reproductive performance and physiology (Chapter 5),

c. Behaviour (Chapter 6).

Key outcome measures used:

a. Production (Chapter 4)

- i. Daily and lactation milk yield
- ii. Daily and lactation weight of milk solids butterfat
- iii. Daily and lactation rumination times
- iv. Bodyweights
- v. Concentrate feed intake

b. Reproductive (Chapter 5)

- i. The time taken to conception after calving
- ii. The time to return to normal oestrus cyclicity post calving
- iii. The time to return to a normal uterine environment post calving
- iv. The proportion of oestrus cycles that occur with normal hormonal dynamics and normal inter-oestrus intervals
- v. Effect on and of transition cow indicators such as NEFAs and BHBs

c. Behavioural (Chapter 6)

- i. Daily time budgets
 - i. Lying time duration
 - ii. Time spent at feed-face
 - iii. Time spent in specified living space areas

3.10 Chapter summary

This chapter has described the overarching methodology for the experimental trial undertaken to assess the impact of living space on dairy cow production, reproduction and behaviour. Additional details of data collection methods, statistical analyses and results are provided in Chapters 4, 5 and 6.

Chapter 4. Randomised controlled trial results: Production data

4.1 Introduction

Milk yield is the principal production outcome of dairy enterprises as many are paid on a per litre basis, although some contracts apply additional incentives dependent on milk quality. Estimating the volume of milk production can be useful for estimating future income (Ehrlich, 2013; Liseune et al., 2021) and optimising cow management. Essential to this is the ability of a cow to convert energy from nutrients into milk whilst limiting the metabolic impact which could cause weight loss and disease. Milk solids in terms of protein and fat content can be an indicator of milk quality and indicative of nutrition.

An important physiological determinant of milk volume is rumination which has been shown to be positively correlated with increased production, especially in early lactation (Kaufman et al., 2018). Rumination is a physiological mechanism, that involves cows chewing on regurgitated fibre (Beauchemin, 2018). Cows can ruminate for up to 10.5h/d given certain diets and conditions (De Boever et al., 1990) and rumination mainly occurs when cows are at rest, peaking around 4 hours post feeding (Schirrmann et al., 2012). Environmental, genetic and dietary factors can all influence rumination time (Beauchemin, 2018).

Importantly, information on milk production is now much more accessible due to the implementation of sensor technology onto farms. For example, robotic milk machines calculate milk volume and bodyweight at every milking. Rumination times can also be monitored non-invasively by external cow collar microphones (Ambriz-Vilchis et al., 2015; Schirrmann et al., 2009).

As noted in the literature review in Chapter 1, there is limited research that has been undertaken to assess the impact that the housed infrastructure has on dairy cow production. However, this research does suggest that housing can influence production and that infrastructure currently found on many farms is likely to be a limiting factor. For example, a

retrospective analysis showed an association between space allowances above and below a set threshold of 7.94m² of free space allocation and subsequent milk production in first lactation animals (Naess et al., 2011). However, short terms studies (<12 weeks) assessing stocking density and space allowance have failed to replicate these findings (Fregonesi and Leaver, 2002b; Krawczel et al., 2012; Wang et al., 2016).

The primary aim of this chapter was to conduct a randomised controlled trial to evaluate whether a specified increase in living space in adult dairy cows would lead to increased milk production. The expected biological hypothesis was cows housed in the high space group would increase 305day milk volume production, with as associated increase in rumination time without losing bodyweight to compensate, compared to cows in the control space group. This was hypothesised on the basis that the historical literature suggests dairy cows improve production when under less competition, thus the high space cows should be able to utilise key areas such as cubicles, feed-face and the milking robot more efficiently and thus the energy saved from competition would be partitioned into milk volume production. Living space allowances were defined from results obtained in Chapter 2; a comparison was made between the median (9m²) and the higher end (14m²) space allowances identified in British dairy herds. Secondary aims were to evaluate the impact of living space on physiological measurements related to productivity, including rumination times and bodyweight changes.

4.2 Materials and methods

4.2.1 Trial design

Data were collected using the study design, materials and methods described in Chapter 3. Data from all cows on trial were used for production data analyses. Data collected for these purposes included individual cow daily milk volume, daily rumination time, monthly milk solids, daily bodyweights and daily quantity of concentrate fed.

Statistical Analysis Software: All analyses were conducted using R statistical software. The following packages were used: tidyverse(Wickham, 2019), gridExtra(Auguie, 2017), lme4 (Bates et al., 2015), minpack.lm (Elzhov et al., 2016), influence.ME (Nieuwenhuis et al., 2012).

4.2.2 Production Data Analysis: Outcome variables

Primary outcome:

- Volume of milk production, daily and per 305-day lactation

Secondary outcomes:

- a. Production of milk solids (weight of fat and protein), monthly and per 305-day lactation
- b. Daily rumination times
- c. Bodyweight changes
- d. Feed concentrates used per cow

4.2.3 Data collection and statistical analysis

Data were collected during the randomised controlled trial as described in Chapter 3. The trial was undertaken at the University of Nottingham Centre of Innovation and Excellence Dairy Farm for a 364-day period between 16th July 2018 and 15th July 2019. This Chapter evaluates 150 Holstein cows during full or part lactations on trial, to analyse the effect of living space on study outcomes. The following section provides further details on data collection and statistical methods used.

4.2.3.1 Milk yield analysis

Data were sourced from the Lely Time 4 Cows (T4C) software which collated and stored daily milk yield recordings from the robotic milking system. The daily yield data for every cow on every day of the trial was used in the analysis. In total 150 different cow-lactations were used (75 pairs). Data were omitted from analysis for the three housing cross-over days when cows were disrupted due to readjustment of house layout and movement of cows within the housed environment.

Each time a cow entered the study in a different parity they were classed as a “new” individual in the trial; the term ‘cow-lactation’ is used as a unique cow identifier for each lactation during the trial. For example, if cow X started the initial study as a parity 2 cow at 250 DIM then was dried off and became eligible to re-enter the study as a fresh cow in the subsequent lactation, the cow was renamed X-3 rather than X-2. Only 16 cows contributed more than one part-lactation to the study (cow-lactations were accounted for statistically as random-effects but this subgroup was too small to add as a further random effect).

Graphical analysis was undertaken initially to assess raw data for completeness and to visualise patterns. The raw data were compared for individual cows and between trial groups. Following this assessment of possible confounders such as DIM and parity were investigated through statistical modelling methods. Milk volume records between 1-305DIM only were considered in these analyses since most cows completed lactations within or close to this period. The total number of litres of milk produced during the study was calculated for each trial group and a further comparison was made by parity (primiparous cows (heifers) versus multiparous cows).

4.2.3.1.1 Milk Production Mixed Effects Models

Initially, mixed effects models were built using R statistical lme4 package, using milk volume per day (L) as the outcome variable and ‘cow-lactation’ as a random effect to

account for repeated measures within cow. Models were created using a forward stepwise method; predictor variables were retained in the model based on significance ($P < 0.05$), model complexity and model fit. Fixed effects tested were trial group (2 level factor: High space group, Control space Group), DIM (continuous variable) and parity category (2 level factor: primiparous, multiparous). Parity category was tested in the model to confirm whether this was a statistically significant covariate. Polynomials for DIM up to a power 4 were tested, to account for the non-linearity of milk yield over lactation. The model was assessed with and without interactions between fixed effects. The model with interactions took the form:

$$\begin{aligned}
 V_{ij} = & a + \beta_1 G_j DIM_{ij} + \beta_2 G_j DIM_{ij}^2 + \beta_3 G_j DIM_{ij}^3 + \beta_4 G_j DIM_{ij}^4 + \beta_5 P_j DIM_{ij} \\
 & + u_j + e_{ij} \\
 u_j \sim & N(0, \sigma_v^2)
 \end{aligned}$$

where subscripts i and j denoted the i^{th} daily milk yield of the j^{th} cow respectively. V_{ij} = daily milk volume for the i^{th} observation of the j^{th} cow, a = model intercept, DIM_{ij} = the days in milk at the i^{th} milk yield of the j^{th} cow, DIM was included as polynomial functions to power 2-4, G_j = trial group for the j^{th} cow (categorical: high vs control space), β_{1-4} = vector of coefficients for $G_j DIM_{ij}^{1-4}$, P_j = categorical variable to represent cow parity (primiparous vs. multiparous), β_5 = coefficient for $P_j DIM_{ij}$, u_j = a random effect to account for residual variation between individual cows (assumed to be normally distributed with mean = 0 and variance = σ_v^2), e_{ij} = the residual model error.

Model fit was assessed using R-squared outputs and outliers evaluated using the ‘influence.ME’ package in R, in which the influence and DFbetas of datapoints are checked (Nieuwenhuis et al., 2012). QQplots were used to visually assess model residuals.

Unfortunately, the fit of these initial random effects models was poor. Peak lactation yields were predicted to be later than observed in the actual data (~100DIM rather than 60DIM). Therefore, alternative methods of modelling were investigated to achieve better fit to the data and safer model inference.

4.2.3.1.2 Non-Linear Milk Yield Models

A non-linear approach was used to model milk yields and make predictions for cow lactation curves using a published formula (Ehrlich, 2013). Data were analysed using non-linear models (Elzhov et al., 2016) and took the form:

$$V_{ij} = a \left(1 - \left(\frac{e^{\left(\frac{c-M_{ij}}{b} \right)}}{2} \right) \right) (e^{-d*M_{ij}})$$

where subscripts i and j denote the i^{th} observation for the j^{th} cow respectively. V was the 7-day rolling milk volume in litres, M the corresponding days in milk for each cow at each daily recording, model parameters were depicted as; a = scale; b = ramp; c = offset; d = decay.

Model parameters a , b , c and d were initially set at $a=49$, $b=27$, $c=4$, $d=0.00206$ as suggested by Ehrlich (2013). Parameter estimates were derived for a , b , c and d using the Levenberg-Marquardt non-linear least-squares algorithm (Elzhov et al., 2016). The parameters a , b , c and d provide a predicted daily cow milk volume curve. A prediction for each trial-parity group was made based on the expected lactation curve for each study population (high space multiparous cows, control space multiparous cows, high space primiparous cows, control space primiparous cows). From this, the 305-day milk yield was predicted for each group.

The fit of the non-linear milk yield model was superior to the random effects models with predicted lactation curves fitting more closely to the lactation shape of observed raw milk yield data. However, unlike the mixed effect models, in the non-linear model it was not possible to account for repeated measures of milk yield. Therefore, individual cows could heavily influence group predictions and from investigating model fit, it was found that there were cows that had a high influence on group predicted mean milk yields.

To account for repeated measures within the non-linear model, a further analytic step was taken incorporating residuals from the non-linear model analysis. This is described in the section below.

4.2.3.1.3 *Final Model: Non-Linear Model Residuals within a Mixed Effects Model*

A non-linear milk yield model was built, as described in section 4.2.3.1.2, using all available milk volume data from trial cows. A residual for each data point was calculated as the difference between the observed daily milk yield and the corresponding predicted yield from the non-linear model. Therefore, each new data point (residual value) represented the difference in milk yield, on a specified day of lactation, between one specific cow and the average cow in the study. For example, a residual value of +3 at 20 days in milk meant that this cow had a predicted milk yield 3 litres above the predicted mean value of all cows at 20 days in milk. The residual values were used as the outcome variables in a mixed effects models in which a random effect for cow-lactation was incorporated to account for repeated measures of milk yield over time.

As for the models above, covariate selection was conducted using a forward stepwise method, and predictor variables retained when $P < 0.05$. Predictor variables included were DIM (continuous) and trial-parity groups (categorical: high space primiparous cow; control space primiparous cow; high space multiparous cow; control space multiparous cow). Interactions between trial-parity group and DIM were tested. Cow-lactation was set as random effect for all models to account for repeated measures.

The model took the form:

$$O_{ij} = a + \beta_1 F_j DIM_{ij} + \beta_2 G_j DIM_{ij} + \beta_3 H_j DIM_{ij} + u_j + e_{ij}$$

$$u_j \sim N(0, \sigma_v^2)$$

where subscripts i and j denoted the i^{th} residual of the j^{th} cow respectively. O_{ij} = non-linear model residual (milk deviation) for the i^{th} observation of the j^{th} cow, a = model intercept, DIM_{ij} = the days in milk at the i^{th} milk yield of the j^{th} cow, F_j = categorical variable to represent primiparous cows in high space trial group, G_j = categorical variable to represent multiparous cows in control space trial group, H_j = categorical variable to represent multiparous cows in high space trial group, β_{1-3} = fixed effect coefficients, u_j = a random effect to account for residual variation between cow-parity (assumed to be normally distributed with mean = 0 and variance = σ_v^2), e_{ij} = the residual model error.

Model fit was assessed using R-squared outputs and outliers by evaluating covariate influence and delta betas (Nieuwenhuis et al., 2012). Q-Qplots were used to visualise and assess the residuals of both the non-linear and mixed effect models.

Model predicted lactation curves were created for each parity-trial group, which now accounted for repeated measures of milk yields from individual cows. The residual based outputs were converted back to absolute litres per day by adding the model output to the non-linear model residual line output for the corresponding DIM. Model predictions were tabulated to facilitate comparisons in predicted 305-day yields between groups.

4.2.3.2 Analysis of milk solids

A monthly milk sample was collected from each cow on trial and processed in an accredited external laboratory (QMMS Ltd, Somerset, UK) for quantification of protein and fat content. Results were combined with milk yield records and used to estimate the corresponding total kilograms of solids a cow would have produced for each sample. The formula used was:

$$\text{Total kilograms milk solids} = \text{total solid \%} \times 1.03 \text{ (milk density)} \times \text{daily milk volume} \\ \text{(ICAR, 2017).}$$

Samples recorded as having 0% of milk solids were omitted from the dataset as missing (40 out of 828 samples). A mixed effects model was used to evaluate the effect of treatment group on total milk constituents (kg). Predictor variables tested were trial group (high space and control space), DIM (continuous variable), and polynomials of DIM to

power 4 (continuous variables). Interactions between group and DIM polynomials were tested and retained when $P < 0.05$. A random effect was included for cow-lactation and model fit was assessed as described in section 4.2.3.1.3. The model took the form:

$$S_{ij} = a + \beta_1 G_j DIM_{ij} + \beta_2 G_j DIM_{ij}^2 + \beta_3 G_j DIM_{ij}^3 + \beta_4 G_j DIM_{ij}^4 + u_j + e_{ij}$$

$$u_j \sim N(0, \sigma_v^2)$$

where subscripts i and j denoted the i^{th} monthly record of the j^{th} cow respectively. S_{ij} = monthly weight of milk solids for the i^{th} observation of the j^{th} cow, a = model intercept; DIM = the days in milk at the i^{th} observation for the j^{th} cow, DIM was tested as polynomial functions to power 4, G_j = trial group for the j^{th} cow (categorical: high vs control space), β_{1-4} = vector of coefficients for $G_j DIM_{ij}^{1-4}$, u_j = random effect to account for residual variation between cow-lactation (assumed to be normally distributed with mean = 0 and variance = σ_v^2), e_{ij} = residual model error.

Total milk constituents produced per cow-lactation were estimated from model predictions for 1-305 DIM. Patterns of predicted constituents were assessed graphically to allow a visual comparison between trial groups.

4.2.3.3 Rumination

Daily rumination times were collected from all cows throughout the study using sound monitor microphones in Lely neck-collars (Schirmann et al. (2009); Ambriz-Vilchis et al. (2015)). A mixed effects model was used to evaluate the daily rumination times between trial group. Trial-parity group and DIM were tested in the model individually and as interaction terms, and the final model was specified as:

$$R_{ij} = a + \beta_1 F_j DIM_{ij} + \beta_2 G_j DIM_{ij} + \beta_3 H_j DIM_{ij} + u_j + e_{ij}$$

$$u_j \sim N(0, \sigma_v^2)$$

where subscripts i and j denoted the i^{th} record of the j^{th} cow respectively. R_{ij} = daily rumination time in minutes for the i^{th} observation of the j^{th} cow, a = model intercept, DIM_{ij} = the days in milk of the i^{th} observation of the j^{th} cow, F_j = categorical variable to represent p cows in high space trial group, G_j = categorical variable to represent multiparous cows in control space trial group, H_j = categorical variable to represent multiparous cows in high space trial group, β_{1-3} = fixed effect coefficients, u_j = random effect to account for residual variation between cow-parity (assumed to be normally distributed with mean = 0 and variance = σ^2_v), e_{ij} = residual model error.

From the final model parameters, predictions of mean daily rumination times were made for the period of 1-305 days in milk for each parity-trial group.

4.2.3.4 Bodyweights

Cows were weighed at each milking on weigh-scales within the Lely Astronaut T4C robot and data used to calculate the mean weight per cow. A comparison of daily weights and the final weight of each cow at trial exit was made using Kruskal-Wallis rank sum tests. Exit bodyweights were chosen to be analysed to assess whether the two populations differed in terms of mass given the primary assessment of milk yield production and the effects that could have on body condition.

4.2.3.5 Concentrate feeding

Concentrate feed intake was monitored daily for each cow on trial. Cows were fed concentrate to according to milk yield automatically via the Lely Astronaut T4C system which dispensed concentrate at each milking. The daily weight of concentrate eaten by each cow was collated and boxplots used to visualise the data to allow comparisons between trial groups.

4.3 Results

4.3.1 Milk yield

4.3.1.1 Descriptive statistics

Figure 4.1 illustrates the raw observed daily milk yields of the 150 cow-parities in the randomised controlled trial (n= 25614 observations). Cows in the high space group produced 17,673 more litres than cows in the control space group over the duration of the trial period (an estimated difference of 1.40L per cow per day), a total of 574,692L compared to 557,289L. When only milk volume data between 1-305 days in milk were considered (24,449 observations); 556042L (12,224 observations) was produced by the high space group and 539,840L (12,225 observations) by the control space group. Primiparous cows accounted for 268,442L (multiparous = 287,600L) and 251,179L (multiparous = 288,661L), for the high space and control groups respectively.

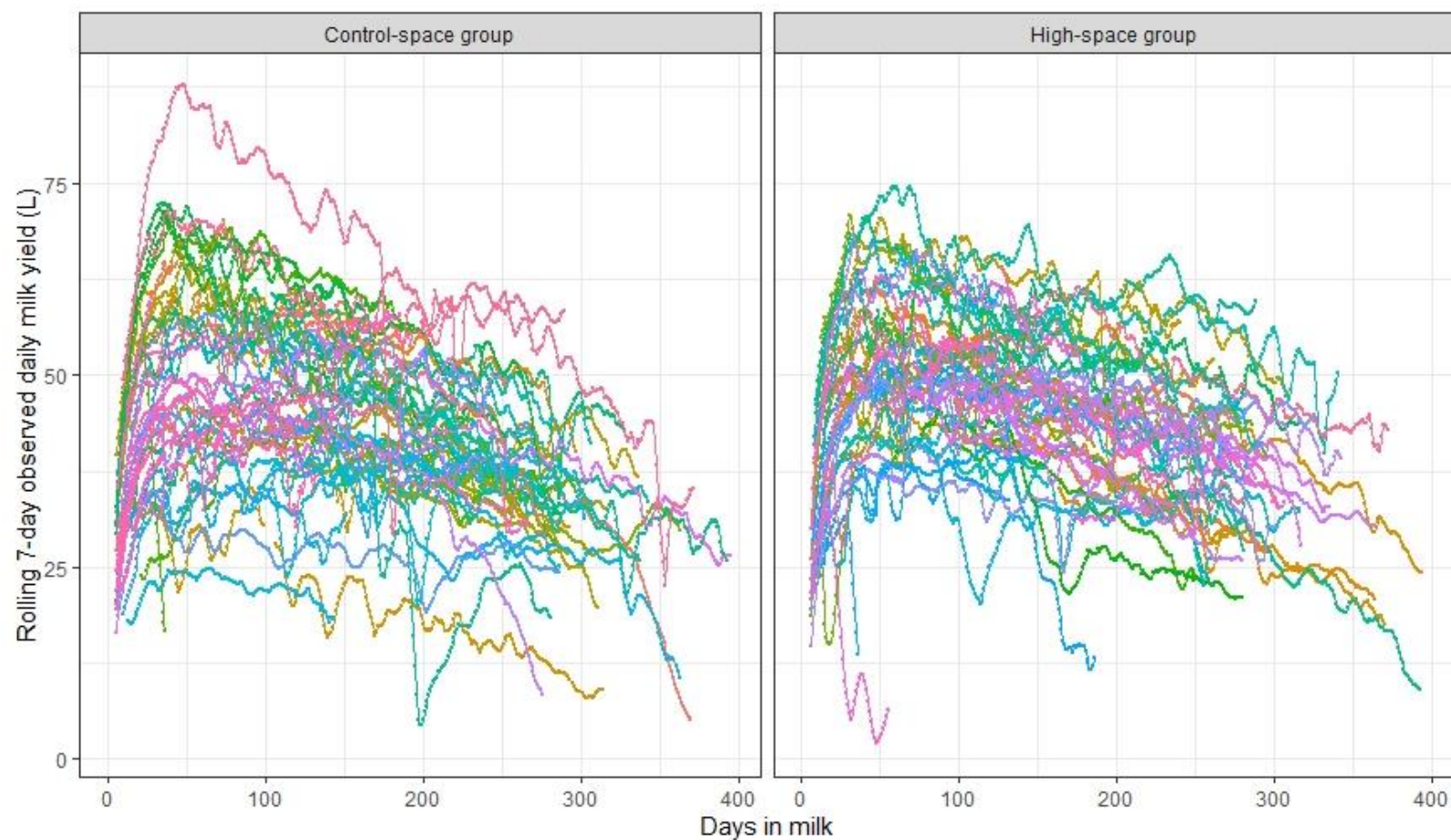


Figure 4.1. Line-plots to show of the 7-day rolling mean milk yields (y-axis) of all cows during the trial period by days in milk (x-axis). The different colours represent each individual cow on trial and their associated lactation curve during the trial period during the randomised controlled trial to investigate the impact of space allowances for housed dairy cows. Left: includes the cows in the control-space group. Right: includes cows in the high-space group.

4.3.1.2 Mixed effects models

The initial mixed effects model created using daily milk volume (L) as the outcome variable was of poor fit and failed to produce realistic predicted lactation curves. Therefore, alternative methods of modelling the data were investigated.

4.3.1.3 Non-linear models

Model parameter estimates from the final non-linear model incorporating all available milk yield data from trial cows are provided in Table 4.1. The model predicted lactation curve (and raw data) are illustrated in Figure 4.2; the predicted lactation curve was an appropriate shape for the data with lactation yield peaking at approximately 60 DIM. Some outlying data points were evident, with relatively few unusually high values (>75 litres) that related to one specific cow and a set of low values (<10 litres) that reflected cows that became unwell. From this model, all data were used to calculate residuals for each data point – the residual for a data point was the observed milk yield recording for that cow minus the predicted mean model value for the specified day in milk (Figure 4.2). These residual values were subsequently used in a mixed-effects model to account for repeated milk yield recordings within cows (Section 4.2.3.1.3).

Table 4.1. Parameter estimates for the non-linear model (4.2.3.1.2) to predict the average lactation curve for all cows on trial for the randomised controlled trial. Model parameters are depicted as; a = scale; b = ramp; c = offset; d = decay.

Parameter	Estimate	Standard Error	t value
a	60.19	0.4326	139.124****
b	30.20	1.685	17.928***
c	-4.328	1.000	-4.327***
d	0.001782	0.0003419	52.128***
Residual standard error: 10.19 on 25610 degrees of freedom			

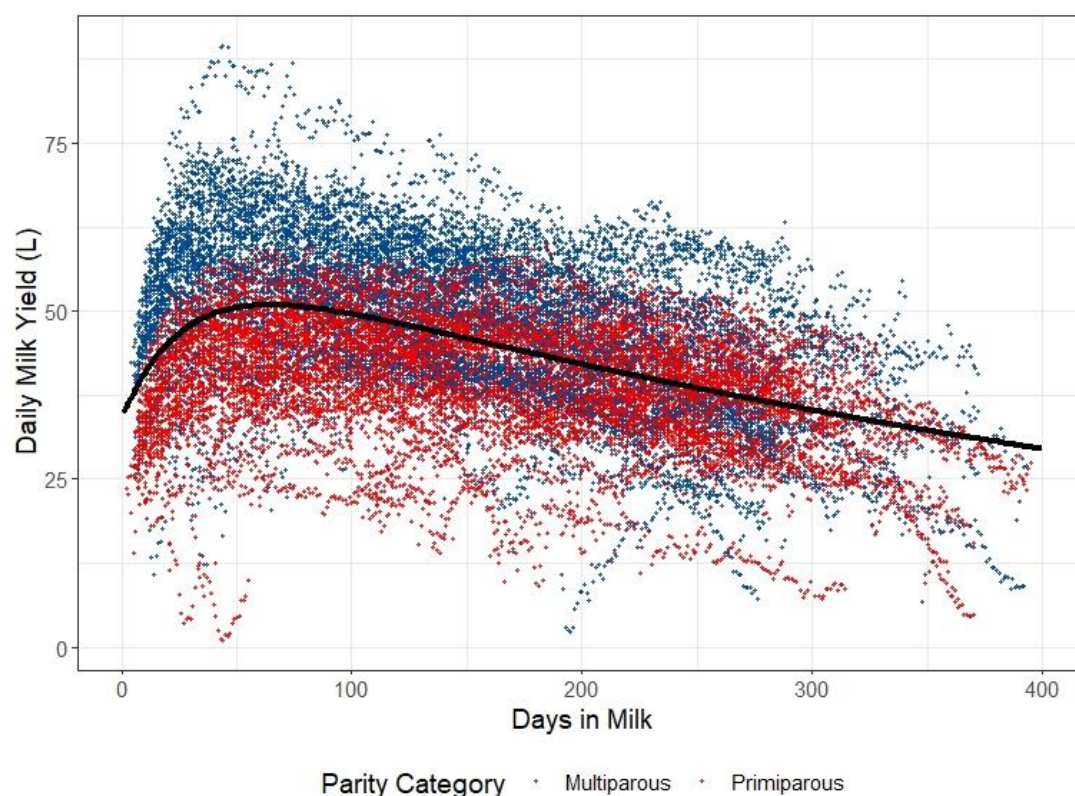


Figure 4.2. Scatterplot of all daily milk yields in litres (y-axis) by days in milk (x-axis) throughout the randomised controlled trial period. The blue points represent multiparous cows and the red points are primiparous cows. All data were used in the non-linear model to predict the lactation curve for the average cow on trial for both trial groups, indicated by the green line which was based on the parameter estimates in Table 4.1.

4.3.1.3.1 Mixed effects models of residuals

The final mixed effect model incorporating residual milk yield values from the non-linear model is provided in Table 4.2. The model included interactions between trial group and DIM, and this reflected the fact that different shaped lactation curves occurred between cows in the different groups. To describe and illustrate the final model, predictions of 305-day milk yields were made for each trial parity group (Figure 4.3). Parity category was confirmed to be significant ($P < 0.05$) as a covariate within the model, thus was included in the final model for milk volume predictions. This gave final estimates of 305-day milk yields

as 12,235L for primiparous cows in the high space group and 11,592L for primiparous cows in the control space yielding (a difference of 643L). Multiparous cows in the high space produced 14,746L and those in the control space produced 14,644L (a difference of -102L).

After exploration of the impact of outlying data points it was found that their impact on model coefficients was negligible and model fit was deemed to be good.

Table 4.2. Table of mixed effects model (4.2.3.1.3) estimates for prediction of the daily milk volume residuals calculated from the non-linear model, using days in milk, parity and trial group (with interactions) as covariates.

Model outcome: Daily milk yield residual (L)			
Fixed effects	Estimates	CI	P
(Intercept)	-10.31	-12.88 – -7.75	
DIM	0.03	0.03 – 0.03	<0.001
Primiparous- Control space	(ref)		
Primiparous- High space	2.64	-0.98 – 6.27	0.153
Primiparous- High space * DIM	0	-0.01 – -0.00	0.002
Multiparous- High space	16.01	12.36 – 19.66	<0.001
Multiparous- High space * DIM	-0.04	-0.04 – -0.03	<0.001
Multiparous- Control space	17.96	14.32 – 21.61	<0.001
Multiparous- Control space * DIM	-0.05	-0.05 – -0.05	<0.001
Random effects			
Residual variance	16.37		
Random effect variance	64.23 _{cow_parity}		
Intraclass correlation	0.8		
Number of random effect groups	150 _{cow_parity}		
Number of observations	25594		
Marginal R² / Conditional R²	0.233 / 0.844		

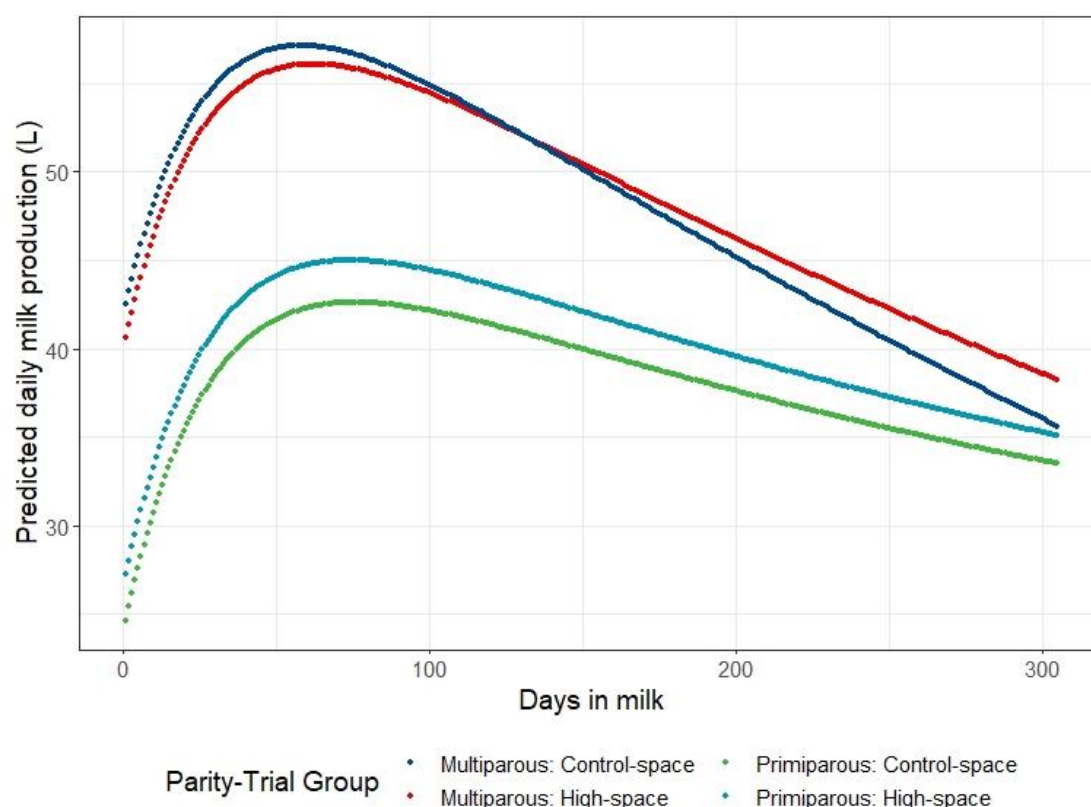


Figure 4.3. Dotplot depicting the predicted 305-day lactation curves for the four groups of cows investigated: Primiparous- High-space (light blue); Primiparous- Control-space (green); Multiparous- High-space (red); Multiparous- Control-space (dark blue). Predictions were created from significant parameters in the mixed effects model (4.2.3.1.3) containing parity-trial group * DIM interactions with cow-lactation as a random effect.

4.3.1.4 Comparison of all milk yield models

A summary of the trial group comparisons in milk yield from raw data and from each of the modelling methods employed, are shown in Table 4.3. In all cases, primiparous cows in the high space group were estimated to produce substantially more milk than their control space counterparts. For multiparous cows, there was notable variation between method and this arose from the influence of multiple recordings from one specific cow with unusually high milk yields. Therefore, when repeated measures were accounted for, yield differences between group were more similar across methods. The final model selected

accounted for repeated measures of individual cows and was deemed the most biologically plausible; it also provided a conservative estimate of milk volume differences between both trial groups. This is highlighted by the MAE of each method; the mixed effects model using the non-linear model residuals had a MAE of 6.72 compared to the initial model without using the non-linear model of 7.00.

Table 4.3. This table shows the comparative predicted 305-day milk yields outcomes for each parity-trial group dependent on analysis: raw data (4.2.3.1), initial mixed effects model (4.2.3.1.1) and the final mixed effects model (4.2.3.1.3). The $\pm$ symbols represent the relative difference of the high space group result compared to the control space group.

			Raw data	Mixed effects model (using observed data)		Mixed effects model (using non-linear model residuals)	
305 Day Yield	Multiparous	High-space Group	15159		14971	14746	
		Control Group	15189	-31	14633	14644	+102
	Primiparous	High-space Group	12719		12186	12235	
		Control Group	11926	+793	11847	11592	+643
	Multiparous	High-space Group	49.7		49.1	48.3	
		Control Group	49.8	0	48	48	+0.3
Avg Production Per Day	Primiparous	High-space Group	41.7		40	40.1	
		Control Group	39.1	+3	38.8	38	+2.1

4.3.2 Milk solids

The patterns observed from modelling total milk solids were similar to that for milk volume. The final mixed effects model to predicted the expected total milk solid production per sample dependent on trial group and DIM is shown in Table 4.5. As shown in Figure 4.4 milk solid production peaked at a similar time to milk volume production.

Table 4.4. Results from the mixed effects model (4.2.3.2) to predict daily milk solid production from monthly milk samples based on trial group and days in milk.

Model outcome: Daily total milk solid production (kg)			
Fixed effects	Estimates	CI	P
(Intercept)	3.26	2.93 – 3.60	
High space	(ref)		
Control space	0.31	-0.16 – 0.78	0.198
DIM	0.012	0.0010 – 0.023	0.032
DIM²	-0.00015	-0.00027 – -0.000026	0.017
DIM³	0.00000059	0.000000077 – 0.0000011	0.024
DIM⁴	-0.0000000008	-0.0000000015 – -0.0000000001	0.027
Control space * DIM	-0.017	-0.032 – -0.0010	0.037
Control space * DIM²	0.00017	-0.0000021 – 0.00035	0.053
Control space * DIM³	-0.00000065	-0.0000014 – 0.000000084	0.083
Control space * DIM⁴	0.0000000008	-0.0000000002 – 0.0000000018	0.112
Random effects			
Residual variance	0.26		
Random effect variance	0.33		
Intraclass correlation	0.56		
Number of random effect groups	145		
Number of observations	788		
Marginal R² / Conditional R²	0.076 / 0.593		

The predictions obtained from this model indicated that cows in the high space group produced 968.2kg of milk solids compared to 951.9kg in the control group over a 305-day lactation. Model fit was judged to be good.

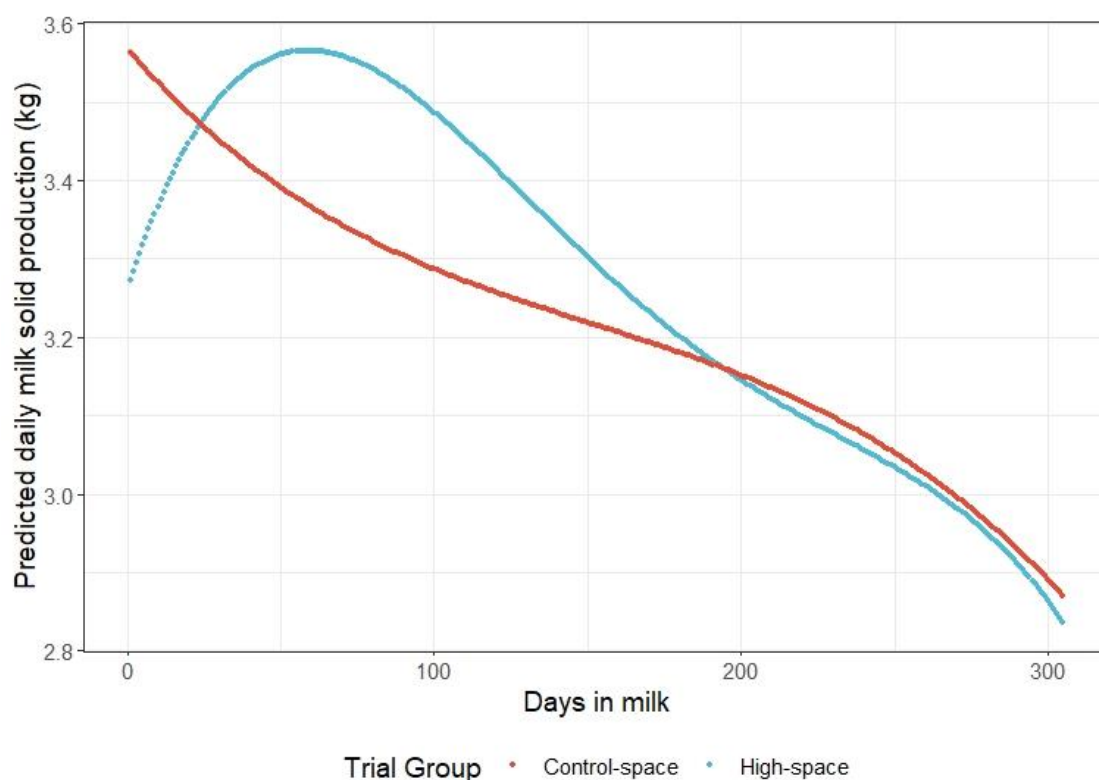


Figure 4.4. Plot to illustrate the predicted total solid production curves for the high (blue) and control (red) space groups based on the mixed effects model parameter estimates using monthly milk solid samples. This shows the total milk solids in kilograms (y-axis) against days in milk from 0-305 days (x-axis).

4.3.3 Rumination

Raw rumination data is illustrated in Figure 4.5. Cows in the high space group ruminated for a total of 5,928,891 minutes during the trial (12804 observations; 465.4 mins/cow/day) compared to 5,831,561 minutes (12790 observations; 457.7mins/cow/day) for cows in the control group.

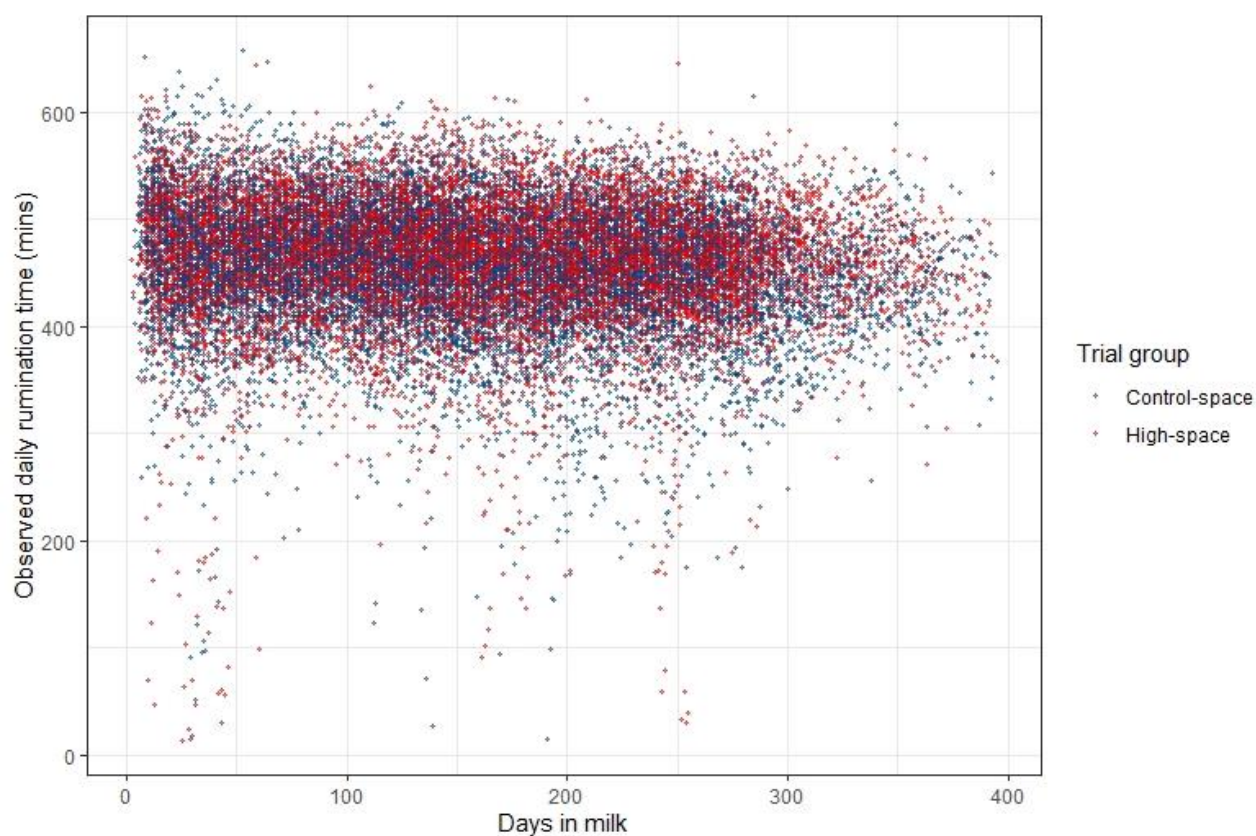


Figure 4.5. Scatterplot of the raw data for daily rumination times in minutes per day (y-axis) against days in milk (x-axis) for the whole trial period. The data for the high space group are shown in red with control space data in blue.

Results of mixed effects model with daily rumination time as the outcome variable is shown in Table 4.5. Each trial-parity group ruminated for different amounts of time but the relationship between daily rumination times and trial group was complex because of a significant interaction between trial group and days in milk; rumination time changed over the course of a lactation.

Table 4.5. Results from the mixed effects model (4.3.3) to predict daily rumination time (minutes) based on parity-trial group and days in milk (DIM).

Model outcome: Daily rumination time (minutes)			
Fixed effects	Estimates	CI	P
(Intercept)	462.936	449.159 – 476.713	
DIM	-0.039	-0.056 – -0.022	<0.001
Primiparous, control space	(ref)		
Primiparous, high space	13.048	-6.435 – 32.531	0.189
Multiparous, control space	16.078	-3.531 – 35.686	0.108
Multiparous, high space	32.256	12.646 – 51.866	0.001
DIM * Primiparous, high space	-0.101	-0.125 – -0.077	<0.001
DIM * Multiparous, control space	-0.179	-0.204 – -0.153	<0.001
DIM * Multiparous, high space	-0.166	-0.192 – -0.141	<0.001

To illustrate the final model, a graph of model predictions is presented in Figure 4.6. The mean daily rumination time for the multiparous cows in the high space group, were consistently higher than the multiparous cows in the control group. The trend was different for primiparous cows since the mean rumination time for each group differed dependent on stage of lactation. The primiparous cows in the high space group had higher rumination times until approximately 130 days into lactation at which time the effect was reversed (Figure 4.6). Notably, where cows moved from commencement of lactation to peak yield during the first 100 days of lactation, the predicted mean rumination times per group were; high space primiparous cows 469 minutes/day, control primiparous cows 462 minutes/day, high space multiparous cows 485 minutes/day and control space multiparous: 469 minutes/day.

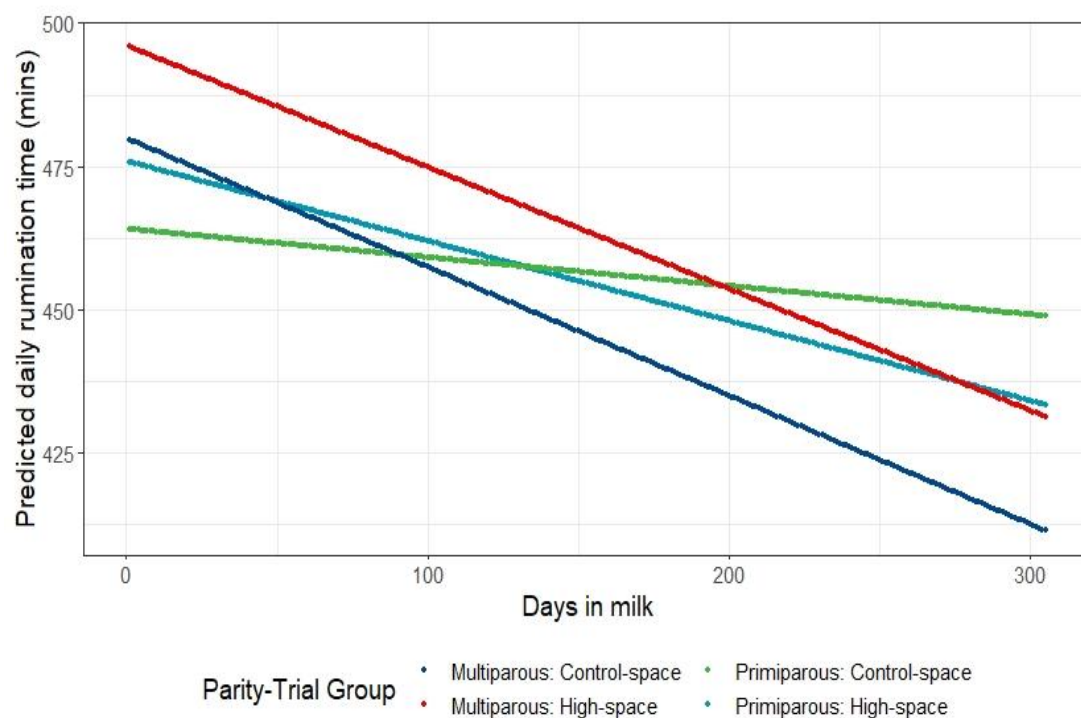


Figure 4.6. Plot to illustrate the predicted rumination time in minutes per day (y-axis) by days in milk (x-axis) for the four parity-trial groups investigated: Primiparous high-space (light blue); Primiparous control-space (green); Multiparous high-space (red); Multiparous control-space (dark blue). These are based on the estimates calculated from the mixed effects model (4.2.3.3) for rumination times.

4.3.4 Bodyweight analysis

The distribution of cow weights at the point of trial entry were almost identical between both trial groups, as can be seen in Figure 4.7. Cows in the high space group had a median weight of 774kg (IQR: 718-839kg) on the day of exit from the trial, compared to cows in the control space that had a median weight of 770kg (IQR: 716-839kg). A Kruskal-Wallis rank sum-test revealed that cow bodyweights were not significantly different between trial group at study exit ($P = 0.71$).

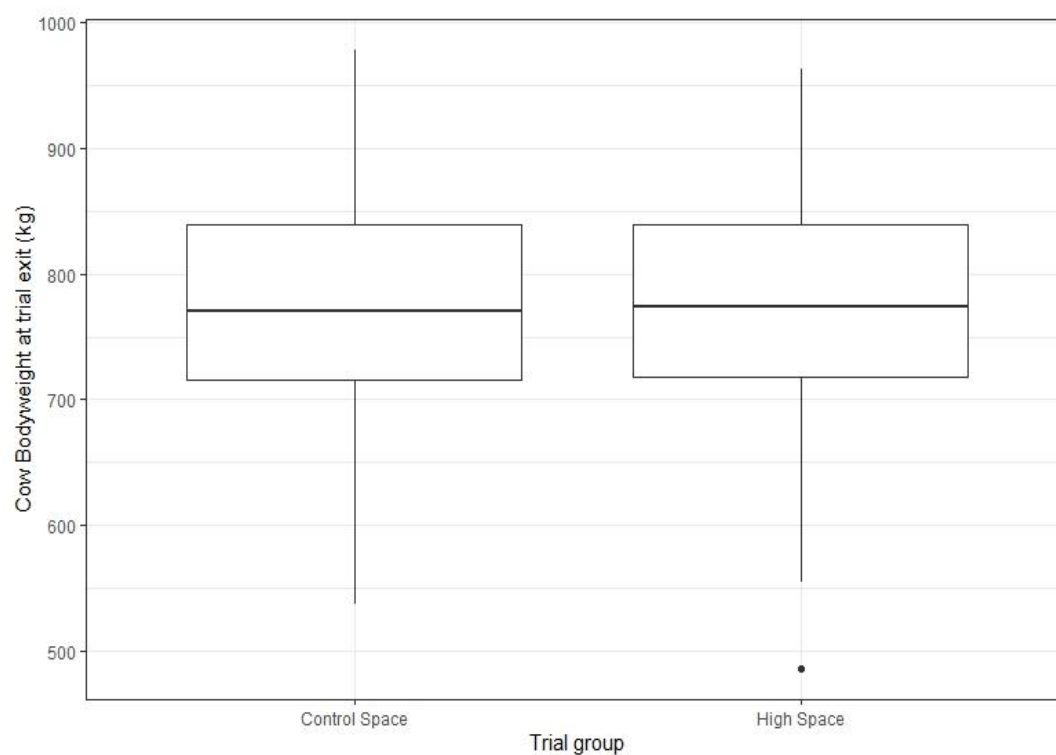


Figure 4.7. Boxplots to show the distribution of cow bodyweights at the point of trial exit. The left boxplot includes the control space group cows ($n=75$) and the right boxplot contains the high space group cows ($n=75$). The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

This distribution of daily bodyweight measurements recorded for all cows during the trial is presented in Figure 4.8. There were no significant differences in bodyweight identified between trial groups for either primiparous or multiparous cows.

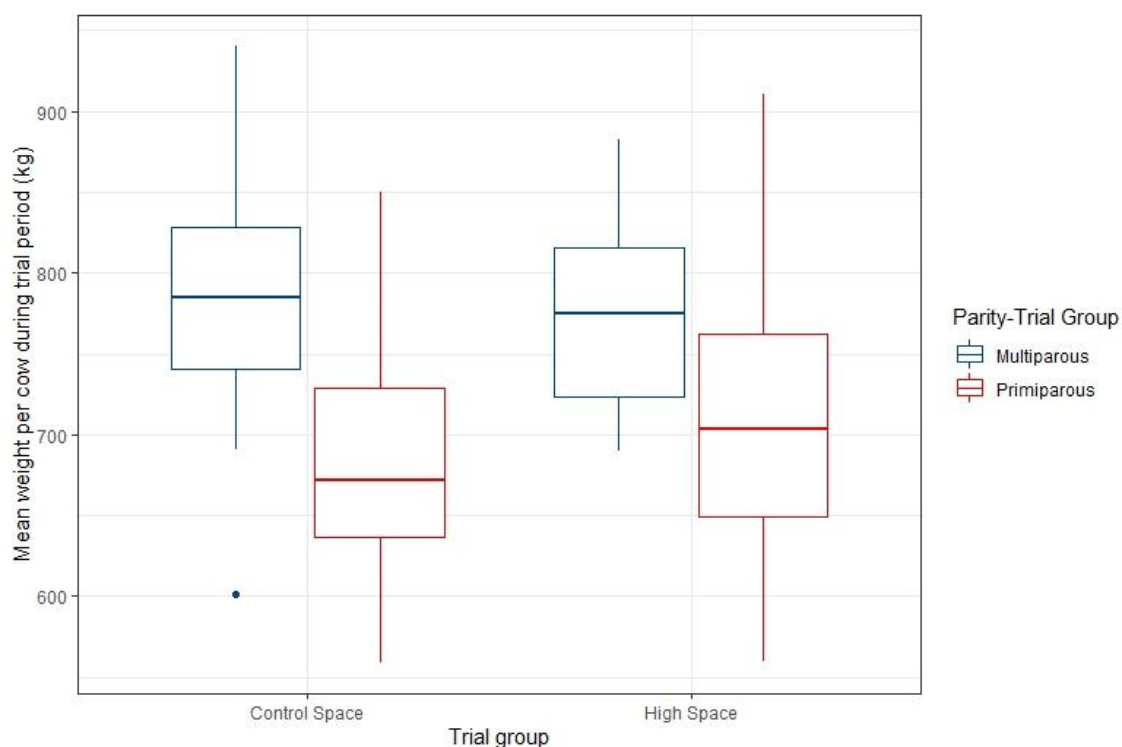


Figure 4.8. Boxplots to show the distributions of each cow's mean daily bodyweight records throughout the trial period. The primiparous cows are shown in blue and multiparous cows shown in red. The two boxplots on the left are from control space cows, whilst the right sides show only high space cows. The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

4.3.5 Concentrate feeding

It is important to note that all cows were fed concentrate in the robot according to milk yield and this was the same for both trial groups. This was at a rate of 0.45kg/L above an M+ level of 30L for multiparous cows and 26L for primiparous cows. Therefore, it is intuitive that cows in the high space group ingested slightly more concentrate (median: 8.55kg per day; mean: 8.2kg per day) compared to the control space cows with a median of 8.17kg (mean: 7.8kg) since their milk production was higher, these results are shown in more detail in Figure 4.9.

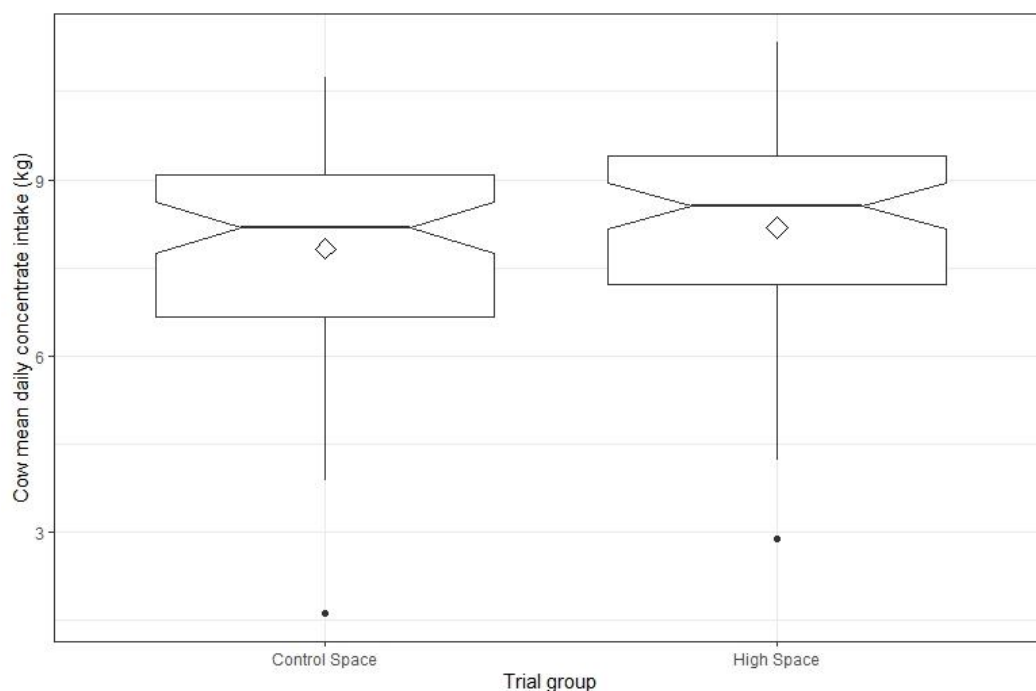


Figure 4.9. Indent boxplots to show the distributions of each cow's mean daily concentrate intake throughout their trial period. The left boxplot includes the control space group cows ($n=75$) and the right boxplot contains the high space group cows ($n=75$). The median value is represented by the midline, with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points. The notches in the boxes represent a confidence interval around the median ($\pm 1.57 \cdot \sqrt{n}$). The diamond shape represents the mean daily concentrate intake of the trial group.

4.4 Discussion

This research represents the first medium to long-term and first randomised controlled study of the impact of housing on dairy cow milk production. The findings indicate that by giving cows more living space in the housed environment, milk yield will increase without there being a detrimental impact on milk solids or the body condition of the cows. Given that space allowance was the only difference between groups in the controlled trial, the difference in milk yield is striking from such a simple change to the environment. These findings do not concur with other studies that have suggested that space allowances or

stocking densities have little effect on dairy cow production (Fregonesi and Leaver, 2002a; Krawczel et al., 2012; Wang et al., 2016) but it is important to note, these previous studies have all been of short duration. The current research suggests that over a longer time period milk production is affected by differences in living space; it could mean that the short-term nature of previous trials was unable to detect this significant difference. An additional important difference between the current study and previous research is study design. The current study was a randomised controlled trial and such a design is known to limit the effects of confounders and produce a greater strength of evidence than observational field trials (Oxman, 2004).

A possible reason for why cows in the high space group produced more milk than those with less space could be partly explained by the increase in daily rumination times for cows in this group. Rumination times and milk yield are known to be positively associated (Kaufman et al., 2018; Stone et al., 2017) and an increase in rumination time is likely to be directly associated with the higher milk production, especially in early lactation.

Rumination times have been linked with the environment a cow inhabits, most notably through increased cow comfort and longer lying times (Schirrmann et al., 2012). Research also suggests that with increasing temperature-humidity indexes in housing, rumination times decrease (Ji et al., 2020; Moretti et al., 2017; Müschner-Siemens et al., 2020) and reductions in rumination time have been linked with disease (Calamari et al., 2014; Dittrich et al., 2019). Therefore, increased rumination times could be indicative of dairy cows being provided with a more appropriate environment to ruminate.

It is noteworthy that the bodyweights of cows in the two trial group populations did not differ, which suggests that the increased milk yields had not been to the detriment of metabolic status; the increased production did not appear to put the cows under excess nutritional stress. Some of the additional nutritional requirements would have been met by additional concentrate intakes and/or through increased consumption of the partial mixed forage ration (intakes of this could not be measured).

It is important to appreciate that the largest difference in milk production identified between trial groups in this research occurred in the primiparous cow populations; it appears that these less mature cows benefit most from the increase living space. These results support conclusions from a recent observational study that reported that when primiparous cows are housed with greater than 7.94m² (compared to less space than 7.94m²), they are more likely to have greater milk production in that lactation (Naess et al., 2011). The hypothesis for this improvement in primiparous cows is that it leads to a reduction in competition for resources with older cows and thus dry matter intakes and subsequently milk yields are increased (Grant and Albright, 2001). Overall, these studies support the view that maintaining primiparous cow-only groups (and prioritising them with additional space) is likely to improve productivity. However, it should be noted that this hypothesis was not specifically tested in the current research.

This chapter did not specifically assess the external environmental impacts of changing living space allowances but in general, intensification of farming through increased efficiency of feed conversion into milk is likely to improve environmental parameters due to measurements being centred on emissions per unit of milk produced (Lillford and Hermansson, 2020; Thornton and Herrero, 2010). Thus, the increased milk volume output observed in this trial could be indicative of possible improvements with regards to emission efficiency and add to the sustainability of milk production.

Results presented in Chapter 2 showed substantial variability in the housing space provided for cows in dairy farms in Great Britain. Similar research has also shown this variability in housing exists in other countries, specifically Norway (Naess, 2010). This huge variation in dairy cow housing indicates a need for more appropriate, evidence-based recommendations and legislature on the subject. Although the results of the current trial are based on the comparison of only two very specific environmental conditions, it highlights the critical need for further investigations into housing infrastructure for dairy cattle, particularly to ensure dairy farm sustainability is not being limited by the housed environment (Blanc et al., 2006; VandeHaar et al., 2016).

4.5 Conclusion

To conclude, the milk yield data observed in this long-term randomised controlled trial suggested that an increase in space allowance was associated with an increase in milk volume production per 305-day lactation, most notably within the primiparous cow sub-population with 643 more litres produced by the high-space primiparous cows than the control group primiparous cows within their first lactation. There appeared to be a positive association with rumination time and milk solids which increased in line with milk yield and that the increase in milk yield did not impact on bodyweight.

Chapter 5. Randomised controlled trial results: Reproductive performance

5.1 Introduction

A key part of dairy business sustainability is reproductive performance and efficiency. Financial losses associated with poor fertility can be mainly attributed to a loss in milk production due to a longer time in lactation resulting in fewer returns to peak yield, expenses associated with costs of insemination (Craig et al., 2018) and culling cows due to infertility (Philipsson and Lindhé, 2003). To attain good reproductive performance in a herd, there are many factors that must be combined to enable a cow to be served early in lactation with a high chance of conceiving to the insemination, these include excellent transition and calving management (LeBlanc, 2010), accurate heat detection (LeRoy et al., 2018; Roelofs et al., 2017; Stevenson, 2001) and a suitable environment (Diskin et al., 2000; Orihuela, 2000). Farmers invest large amounts of time and money into achieving better fertility and improving genetics within their herds, including investments in technologies such as pedometers and automatic heat detection. The combination of genomic selection and advancements in technology has accelerated progress of reproductive performance (Bouquet and Juga, 2013; Lucy, 2019; Thomassen et al., 2016) but it is important that these improved outcomes are not limited by barriers such as the housed environment.

As noted in the literature review in Chapter 1, the housed infrastructure has been shown to impact on different aspects of the reproductive timelines (Crossley et al., 2017; Martin et al., 2015), from the transition period (LeBlanc, 2010; Seifi et al., 2011; Walsh et al., 2007), to oestrous detection (Diskin et al., 2000; Orihuela, 2000; Stevenson, 2001) to ovarian activity and uterine environments (Bollwein and Lüttgenau, 2013; Pleticha et al., 2009). The transition period is the most likely time for events to compromise a cow's reproductive capacity due to an increased likelihood of peri-parturient diseases, changes to metabolic and

immunological profiles leading to predisposition to infectious disease such as endometritis which can all lead to a reduction in conception rates (LeBlanc, 2010; Macmillan et al., 2020; Ribeiro et al., 2016, 2013).

Thus, the housed infrastructure relating to space allowances and more specifically living space is likely to influence reproductive performance of dairy cows. A genotype-fertility environment interaction ($G \times E$) has been described that influences reproductive variation between cows; it is suggested that environmental factors may affect genotypes to varying degrees or that genotypes could be reranked in different environmental conditions (Craig et al., 2018). Given the variability observed in space allowances of housed dairy cows in Great Britain (Chapter 2), it is important to understand if this could be a limiting factor for reproductive success.

The primary aim of this chapter was to conduct a randomised controlled trial to evaluate the effect of living space on the reproductive performance of housed dairy cows. The primary biological hypothesis of this Chapter centred around cows in the high space group having a shorter time to conception compared to the control space group. This hypothesis was based on research suggesting that greater space allows for better detection and expression of oestrous activity, which would result in better timing of artificial insemination and thus an increased chance of conception. Living space allowances were defined from results obtained in Chapter 2; a comparison was made between the median space allowance identified in British dairy herds (9m^2) and the upper 95th percentile allowance (14m^2). A secondary aim of the research was to measure key physiological markers that underpin reproductive performance to assess differences between trial groups.

5.2 Materials and methods

5.2.1 Trial overview

The study design and key elements of the materials and methods are described in Chapter 3. For the reproductive outcomes of interest, analyses were conducted on data available over the entire 364-day study period. Cows that were pregnant at the start of the study were not eligible for inclusion. For different reproductive outcomes, different subsets of cows were eligible for analysis, these populations are described in the relevant sections below.

To investigate physiological changes underpinning reproductive performance, the following were measured; progesterone profiles (14-84 days after calving), Anti-Mullerian Hormone (22-28 days after calving), ultrasonic measurements of uterus and ovaries (14-42 days after calving), the number and timing of artificial inseminations.

Statistical Analysis Software: All analyses were conducted using R statistical software. The following packages were used: Tidyverse (Wickham, 2019), gridExtra (Auguie, 2017), lme4 (Bates et al., 2015), minpack.lm (Elzhov et al., 2016), influence.ME (Nieuwenhuis et al., 2012), survival (Therneau, 2020), survminer (Kassambara, n.d.).

5.2.2 Reproduction Data Analysis Outcomes

The following outcomes were used to evaluate reproductive performance between trial groups:

Primary outcome:

- Time to conception after calving

Secondary outcomes:

- a. Time to return to normal oestrus cyclicity after calving

- b. The proportion of oestrus cycles that occurred with normal hormonal dynamics and normal inter-oestrus intervals
- c. Occurrence of endometritis after calving
- d. Ovarian dynamics measured by follicular development
- e. Energy balance indicators

The secondary outcomes were used to assess potential underlying physiological changes that explained differences in the primary outcome.

5.2.3 Data collection and statistical analysis

5.2.3.1 Time to conception and artificial insemination data; collection and processing

Timing of insemination was identified exclusively by trained farm staff using the Lely T4C heat recognition software (Lely, 2019) and inseminations were carried out by a trained external AI technician. Pregnancy diagnosis was undertaken weekly during a veterinary routine fertility visit with cows checked between 32-39 days post insemination. The veterinarian made decisions at the visit entirely based on set of a pre-defined standard operating procedures (Appendix- Chapter 5) to ensure no differences in reproductive management occurred between trial groups. Veterinary routine fertility visit findings were entered into the on-farms recording system and collated for subsequent analysis.

At the end of the study period, the following data were collected from the on-farm recording system; cow ID, reproduction events (calving, insemination, pregnancy diagnosis, heat detection), event remark (e.g. for pregnancy diagnosis: pregnant/non-pregnant) and event date. A conception date was identified by finding the positive pregnancy diagnosis post calving and identifying the previous insemination date prior to the identification of pregnancy. The difference between the conception date and corresponding calving date was calculated to provide the figure for days to conception for each individual cow on trial. If a

cow conceived during the trial period but her matched pair was already pregnant prior to study enrolment both cows were ineligible for reproductive analysis.

5.2.3.2 Conception time-to-event analysis

Time-to-event (survival) analysis was undertaken to evaluate times to conception (days) of eligible cows. Cows that did not conceive on trial were right censored, with time to censoring set from calving to cow exit or study end (days).

A Kaplan-Meier graph (Altman, 1992) was used to visualise survival curves between intervention groups. A Cox proportional hazards model (Cox, 1972) was built to compare the high space with the control group for time to conception. The conventional Cox proportional hazards model took the form:

$$h(t) = h_0(t) \times \exp(\beta_1 x_1 \dots \beta_p x_p)$$

where t was the time to a conception event, $h(t)$ the hazard function which was dependent on a baseline hazard $h_0(t)$, and p covariates that are depicted $(x_1 \dots x_p)$ with related coefficients $\beta_1 \dots \beta_p$.

Covariates were retained in the model when $P < 0.05$. Visual assessment of Schoenfeld residuals, the log-log curves and delta betas were used to evaluate model fit and assumptions (Grambsch and Therneau, 1994; Schoenfeld, 1982). The median time to conception and survival percentages to key time points of 100, 150 and 200 days after calving were calculated from the Cox proportional hazards model.

To enhance the understanding of the time to conception analysis, two additional analyses were conducted. The first analysis was conducted on a subset of data that included only pairs of cows that entered the trial almost immediately after calving ($<14\text{DIM}$). Time to event analysis was repeated as described above on these cow pairs ($n=39$) to evaluate whether the effect seen in all trial cows was predominantly a result of cow pairs that had spent the majority of lactation in the trial groups.

A further survival analysis was conducted to assess the effect of the proportion of time spent in each side of the building, to rule out a possible confounding effect. To do this

the proportion of time a cow spent in a section of the building prior to conception was calculated as:

$$\text{Proportion of days spent in Pen-A} = \frac{\text{Number days in Pen-A}}{(\text{number of days in Pen-A} + \text{number of days in Pen-B})} \times 100$$

If a cow did not conceive on trial (censored data) this was calculated in the same way but the cut off was the day of exit from the trial. Based on these proportions, cows were placed into four categories: 0-25% Time in *Pen-A*, 25-50% Time in *Pen-A*, 50-75% Time in *Pen-A* and 75-100% Time in *Pen-A*. The survival analysis was repeated using ‘Time in *Pen-A*’ as a categorical explanatory variable to test for any confounding influence (change in the hazard ratio of the effect of trial group).

5.2.3.3 Artificial insemination outcome analysis

Insemination outcomes were analysed by assessing the percentage of successful artificial inseminations given to cows whilst they were on trial. Data for cow pairs where at least one cow was pregnant prior to study enrolment were removed from analysis (leaving 48 cow pairs eligible). The total number of serves and number of conceptions were calculated for each trial group and converted to percentage as follows:

$$\text{Insemination success percentage} = \frac{(\text{Number of Conceptions} / \text{Total Serves})}{1} \times 100$$

This procedure was repeated solely for cows that entered the study immediately after calving (<14DIM).

5.2.3.4 Time to first serve data analysis

Time to first service data were collated for all cows during the study period. Cow pairs entering the study where one or both cows were pregnant or received a first serve prior

to study enrolment were excluded from this analysis (n=43 pairs included). Time to event (survival) analysis was used to assess the difference between trial groups in time to first service. Cows that had not received a first service before being removed from the trial were censored on the day in milk at removal. Time to first service data were visualised and assessed using Kaplan-Meier plots and Cox proportional hazards models as described in Section 5.2.3.2.

5.2.3.5 Inter-service interval (ISI) analysis

Inter-service intervals were calculated between consecutive services occurring in cows during the trial period, where both cows were non-pregnant at entry onto study (n=48 pairs). Intervals were categorised into the following ISI blocks for analysis: 1-17days, 18-26days, 27-35days, 36-52days and 52+days for both trial groups. A bar chart was produced to visualise the proportion of serves occurring in each category per trial group to assess the patterns against commercial targets and evaluate the accuracy and rate of oestrous detection.

5.2.3.6 Progesterone profiles

Milk samples (25ml) were obtained 3 times each week (Monday, Wednesday and Friday) from cows between 14-84 days in milk using the Lely Milk Sampling Shuttles. A total of 80 cows (40 pairs) were eligible for analysis. Milk samples were stored at -20°C prior to laboratory analysis.

The Ridgeway Milk Progesterone Enzyme Immunoassay Procedure (a competitive indirect solid phase assay) was used to analyse the samples for progesterone, carried out according to manufacturer instructions and quality control standards (Appendix- Chapter 5). Results for progesterone concentration were obtained in mg/ml to a maximum value of 20mg/ml.

5.2.3.7 Progesterone profile analysis

A graph was plotted to visualise each cow's profile individually (n=80). From these graphs the profiles were categorised by type and the day of commencement of luteal activity (CLA) was designated. The selection of the day for CLA was taken as the day when progesterone concentration increased above 5ng/ml for at least 2 consecutive sampling timepoints. If commencement of luteal activity did not occur during the trial-period, the cow was censored at the timepoint of the last collected sample (max: 84DIM). Survival analysis was conducted (as described for the time to conception data above) using time to CLA as the outcome variable.

Progesterone profiles were categorised into 3 groups as previously described (Mohammed et al., 2019): Normal, Abnormal or Delayed Ovulation (DOV). Definitions for each of the categories are summarised below:

Normal:

- First rise in progesterone occurred ≤ 45 days post-partum and was followed by normal cyclicity (a short luteal phase < 10 days after the first ovulation was considered typical).

Delayed ovulation (DOV):

- The first rise in progesterone (> 3 ng/ml) occurred more than 45 days after calving.

Abnormal (this category was made up of three different profile definitions):

- Cessation of cyclicity: The first progesterone rise occurred within the normal period (≤ 45 days) but was followed by a period of progesterone concentrations < 5 ng/ml for > 12 days.

- Prolonged luteal activity: Progesterone concentration >5ng/ml for >21 days in the absence of AI
- Short luteal phase: Progesterone concentration >5ng/ml for less than 10 days (excluding the first oestrous cycle).

The total numbers of each profile were calculated by trial group and tabulated. Chi-squared analysis was undertaken to assess differences between groups. As a proxy for total time spent in luteal phase, the mean progesterone was calculated for individual cows across the 14-84DIM sampling window. Cow pairs were removed at this stage if at least one of the cows in the pair had fewer than 25 out of the expected 30 samples. A boxplot was produced to compare the median, mean and range of cow mean progesterone per trial group. A Kruskal-Wallis rank sum test was used to analyse the difference between trial groups.

5.2.3.8 AMH blood samples

Blood samples were collected at a single timepoint when cows were between 28-35 days in milk on the weekly procedure day as described in Chapter 3.5.1.1. The blood samples were processed for bovine specific Anti-Mullerian Hormone analysis by the following method. Blood was collected from the coccygeal vein into 2 vacutainers. The samples were left to clot in a fridge overnight then serum was collected and frozen in Eppendorf tubes at -20°C. All samples from cows in the trial were processed in a single run. The serum samples were analysed using a bovine specific AMH sandwich ELISA (Ansh Lab, USA, Cat no AL-114) according to manufacturer's instructions. This ELISA was a quantitative 3-step sandwich type immunoassay. Antibody-antigen-biotin conjugate-SHRP complex bound to the well was detected by enzyme-substrate reaction. Dual wavelength absorbance at 450 nm (primary test filter) measured the degree of enzymatic turnover of the substrate. The quantified absorbance was directly proportional to AMH concentration of the samples.

Data were collated in Microsoft Excel to calculate concentrations from standards and sample absorbance values. The standards ranged from 16.5 to 2050pg/ml with samples analysed in duplicate. Summary statistics were calculated, and a Kruskal-Wallis rank sum test used to compare values between groups. Additional analyses were conducted to evaluate AMH concentration by cow parity and trial group.

5.2.3.9 Reproductive procedure data

Cows entering the study <14DIM were eligible for the reproductive procedure protocols. This was formed of two parts, a standardised transrectal ultrasound examination of the reproductive tract and a vaginal examination using a 'metricheck' device to assess for endometritis as described in Chapter 3.5.1.2 and 3.5.1.3.

5.2.3.9.1 *Transrectal ultrasound examination*

Transrectal ultrasound examinations were undertaken at 4 time points as described in Chapter 3.

For the uterine measurements, the area of each horn was calculated using the formula for the area of an oval (length x width x π). The area measurements for both horns were then added to give a single measurement per timepoint per cow. Antral follicle count was the total number follicles identified of less than 10mm diameter on both ovaries combined. The mean, median, IQR, minimum and maximum number per timepoint per group were calculated. Corpora lutea data were identified on one or both ovaries and a cumulative assessment used to measure the proportion of cows that had a CL at any time point prior to or at each sampling period.

Summary statistics were calculated for all reproductive parameters. Kruskal wall tests were used to compare the distributions of antral follicle counts and uterine measurements between groups. Chi-squared tests were undertaken to assess whether there was difference between trial group in the presence of a CL by 42DIM.

5.2.3.9.2 Vaginal examination:

Vaginal examinations were performed at 3 sample points between day 21-28, 29-35 and 36-42 in milk using a 'metrheck' device and visual examination of the discharge to assign an endometritis score between 0-3 (Pleticha et al., 2009)

Analysis of endometritis scores was conducted at each sampling timepoint to evaluate differences between trial groups; summary statistics and a chi-squared tests were used assess differences.

5.2.3.10 Energy balance status

Blood sampling was undertaken at 3 time points for all cows; 7-14, 15-21, 22-28 days after parturition using venepuncture as described in Chapter 3. Samples were stored and tested within a University laboratory facility (NUVETNA laboratory, Sutton Bonington, UK). Non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (BHB) levels were determined according to manufacturer's methods using Randox standardised colorimetric kits for BHBs and NEFAs (Randox Laboratories, Crumlin, UK) with the RX IMOLA machine (Randox Laboratories, Crumlin, UK)(RANDOX, 2019).

Summary statistics were calculated for BHB and NEFA by trial group. The number of 'high' BHB or NEFA results in each group were assessed; BHBs were classed as high when $\geq 1.2\text{mmol/l}$ and NEFAs when $\geq 0.8\text{mmol/l}$ (Ospina et al., 2013). The effect of potential energy balance on reproductive performance was assessed by removing cow-pairs from the time-to-conception model when at least 1 individual had a minimum of 1 high NEFA or BHB results. The time-to-conception was re-run using this smaller sub-sample of eligible cow pairs (n= 24 pairs). A Kaplan-Meier graph and Cox proportional hazards model were repeated as described above for the time-to-conception analysis using only animals that were not diagnosed with a high NEFA or BHB result.

5.2.4 Milk yield and time to conception; investigation of associations

To evaluate possible relationships between reproductive performance and milk yield, data were re-structured and additional statistical models constructed. The methods used for this are detailed below.

5.2.4.1 Evaluation of the impact of reproduction on milk yield

The daily milk yield data (Chapter 5) were combined with reproductive conception data. A new variable was created for identification of pregnancy status for each cow by day of lactation; when a cow was not pregnant a zero was assigned and from the day of conception to trial exit, a cow was assigned a continuous integer corresponding to the number of days pregnant (DP). This DP variable was recoded to a categorical covariate as follows: 0 (non-pregnant), 1 (1-50 DP), 2 (51-100 DP), 3 (101-150 DP), 4 (151-200 DP) and 5 (201-250 DP). A 7-day rolling mean milk yield was calculated for each cow and used as the outcome variable.

The methods implemented for non-linear model residual analysis and mixed effects modelling was as described in Chapter 4.2.3.1.3. A mixed effects model was used with the addition of the DP covariate to assess the impact of stage of pregnancy on milk production. The model took the form:

$$O_{ij} = a + \beta_1 F_{ij}DIM_{ij} + \beta_2 G_{ij}DIM_{ij} + \beta_3 H_{ij}DIM_{ij} + \beta_4 DP_{ij} + u_j + e_{ij}$$

$$u_j \sim N(0, \sigma_v^2)$$

where subscripts i and j denoted the i^{th} residual of the j^{th} cow respectively. O_{ij} = non-linear model residual (milk deviation in litres) for the i^{th} observation of the j^{th} cow, a = model intercept, DIM_{ij} = the days in milk at the i^{th} milk yield recording of the j^{th} cow, F_{ij} = categorical variable to represent primiparous cows in high space trial group, G_{ij} = categorical variable to represent multiparous cows in control space trial group, H_{ij} = categorical variable to represent multiparous cows in high space trial group, DP_{ij} = the days pregnant categorical variable for the i^{th} milk yield of the j^{th} cow, β_{1-4} = fixed effect coefficients, u_j = a random effect to account for residual variation between cow-parity (assumed to be normally distributed with mean = 0 and variance = σ_v^2), e_{ij} = the residual model error.

Model fit was assessed as described in Chapter 4.2.3.1.3. Predictions of 305-day milk yields from the final model were made to allow a comparison of trial and parity groups.

5.2.4.2 Evaluation of the impact of milk yield on time to conception

This analysis included pregnancy status as a binary variable (0= non-pregnant, 1= pregnant) alongside the rolling mean 7-day milk yield for each cow. Pregnancy status was assigned a zero for cows up to the time of conception and at conception was assigned a one. Records for each cow were included until the day of conception or, for cows that did not conceive, until removal from the study. Data for 48 cow pairs, where both were non-pregnant at trial entry, were selected for analysis.

A conventional random effects binomial logistic regression model was used evaluate whether time to conception was associated with the previous 7-day rolling milk yield. The outcome was conception as a binary factor (0/1) with a random effect included for ‘cow’ to account for repeated measures. Explanatory variables incorporated were 7-day rolling milk yield (continuous), $\ln(DIM)$ (continuous) and trial group (binary factor: high space or control space group). DIM was also tested in model with polynomials up to a power of 4.

Model fit was tested by comparing predicted accumulated probabilities to actual observed events for parity 1-4 and by trial group (Gelman et al., 1996).

5.3 Results

5.3.1 Time to conception analysis

5.3.1.1 All eligible cows

Survival analysis for time to conception for eligible cows (n=48 cows per group) identified that cows in the high space group took longer to conceive than control space cows, as shown by the Kaplan-Meier survival graph in Figure 5.1. The median days to conception for cows in the control group was 101 (95% CI: 82-143, pregnancies = 37/48), and in the high space group was 135 days (95% CI: 105-202, pregnancies= 29/48). Compared to cows in the control group, those in the high space group had a reduced hazard of conception (hazard ratio = 0.58, 95%CI: 0.35-0.94, P = 0.029), as shown in Table 5.1.

Model fit was good. The assumption of proportionality of hazards was not violated (Schoenfeld t-test P = 0.65) and deviance and dfbeta residuals indicated no evidence of an unduly large influence of any observations on model coefficients.

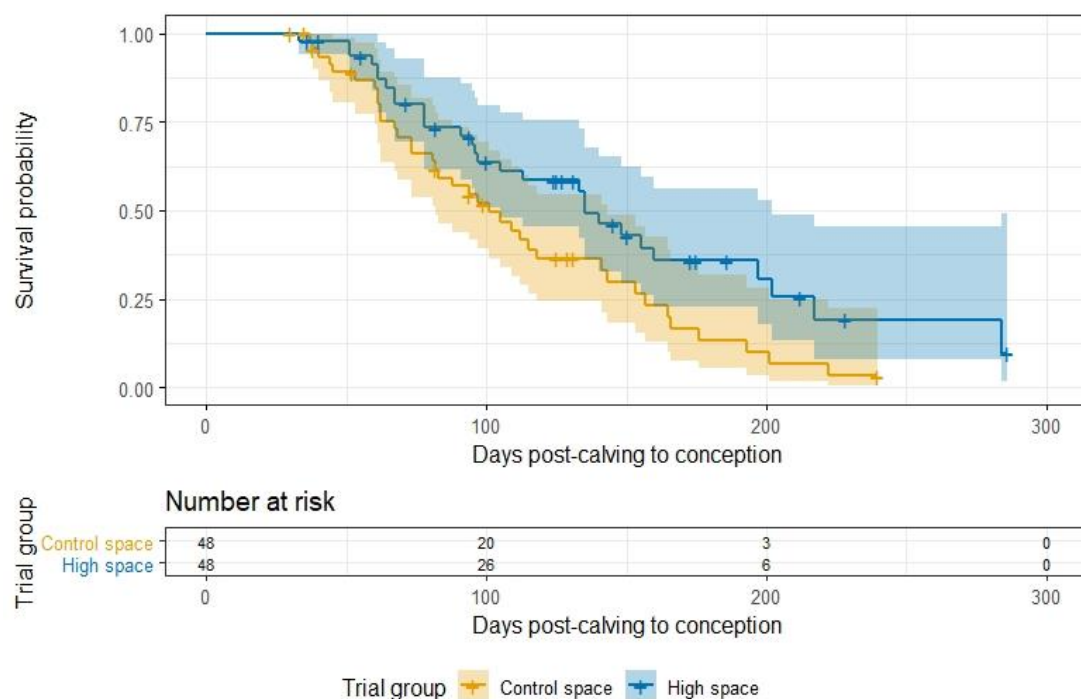


Figure 5.1. Results of time to conception analysis for matched cows that entered the trial non-pregnant (n=48pairs). Top: Kaplan-Meier plot of survival to conception for the high space (blue) and control space (yellow) trial groups. Respective colour shading represents the 95% confidence interval. Bottom: Risk table showing the associated number of cows non-pregnant at 0-, 100-, 200- and 300-days post-calving for each trial group.

Table 5.1. Parameter estimates from the Cox proportional hazards model (5.3.1.1) results by trial group, using data from cow-pairs that were non-pregnant at trial entry.

Term	Estimate	Std. error	Statistic	P value	Lower CI	Higher CI
Control space group	(ref)					
High space group	0.58	0.25	-2.19	0.03	0.35	0.94

Further analysis of the rate of conception by days in milk revealed that 48% of cows in the control space group had conceived by 100DIM compared to only 36.4% of the high space group. Additional details are provided in Table 5.2.

Table 5.2. Results of the Cox proportional hazards model (5.3.1.1) detailing the numbers and percentages of cows that were non-pregnant (survival %) at 100, 150 and 200 days in milk by trial group.

Days in Milk	Trial Group	At Risk (n)	Event (n)	Survival (%)	Standard Error	95% C.I (%)
100	Control space	20	21	52.0	0.076	39.1, 69.5
	High space	26	16	63.6	0.073	50.8, 79.7
150	Control space	9	29	29.8	0.075	18.2, 48.7
	High space	13	23	42.9	0.082	29.6, 62.3
200	Control space	3	35	9.9	0.053	3.5, 28.3
	High space	6	26	30.7	0.085	17.8, 52.7

With parity incorporated as a covariate in the Cox proportional hazards model (Figure 5.2, Table 5.3), primiparous cows were found to be the main influence on the difference in time to conception between cows in the high and control space groups. In the

high space group, primiparous cows had a significantly lower hazard of conception than those in the control space group (Hazard ratio = 0.48, 95% CI: 0.24 - 0.95, $P=0.04$). This resulted in a median time to conception of 155 days for primiparous cows in the high space group compared to 83 days for those in the control group. Multiparous cows had less difference in times to conception, with cows in the high space having a median time to conception of 133 days compared to 109 days for cows in the control group. Model fit was deemed to be good.

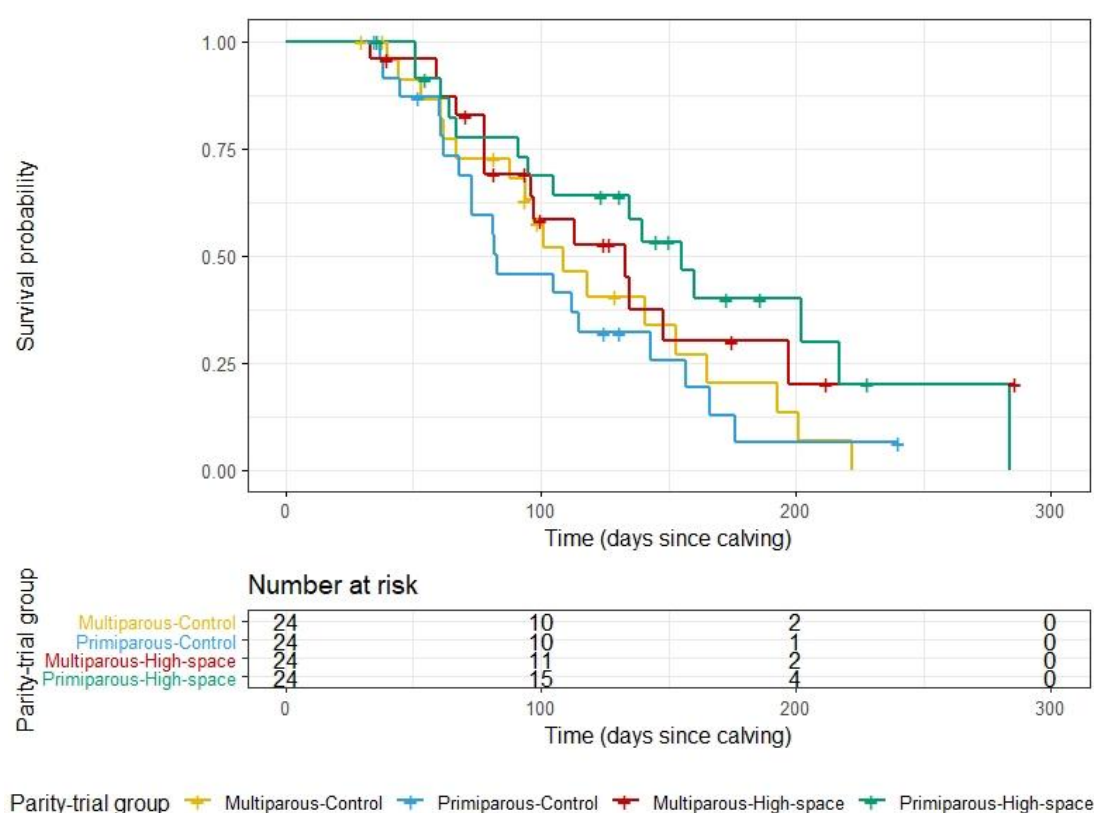


Figure 5.2. Results of time to conception analysis for matched cows that entered the trial non-pregnant ($n=48$ pairs). Top: Kaplan-Meier plot of survival to conception for the four trial-parity sub-groups (Primiparous-high space (green); Primiparous-control space (blue); Multiparous-high space (red); Multiparous-control space (yellow)). Bottom: Risk table showing the associated number of cows non-pregnant at 0-, 100-, 200- and 300-days post-calving for each parity-trial group.

Table 5.3. Parameter estimates from the Cox proportional hazards model 5.3.1.1 using data from cow-pairs that were non-pregnant at trial entry by trial-parity group.

Term	Estimate	Std error	Statistic	P value	Lower CI	Higher CI
Primiparous control space	(ref)					
Primiparous high space	0.48	0.35	-2.09	0.036	0.24	0.95
Multiparous high space	0.58	0.36	-1.52	0.13	0.29	1.17
Multiparous control space	0.84	0.33	-0.54	0.59	0.44	1.60

The effect of the proportion of time spent in each of the two experimental pens prior to conception had no confounding effect on the survival analyses; there was only a very small alteration (maximum of 0.17%) in the hazard ratios which made no meaningful difference in biological interpretation.

5.3.1.2 Time to conception for cows entering the study <14 days in milk

Results of the survival analysis for pairs of cows that entered the study at a maximum of 14DIM (n=39 cows per group) revealed similar patterns to the analysis with all eligible cows (Figure 5.3). Cows in the control group had a median time to conception of 97 days (95% CI: 81 – 118, pregnancies = 28/39), compared to the high space group median of 140 days (95% CI: 105 – NA, pregnancies = 20/39). Compared to cows in the control group, those in the high space group had a reduced hazard of conception (Table 5.4: hazard ratio = 0.51 (95%CI: 0.29-0.91, P=0.027).

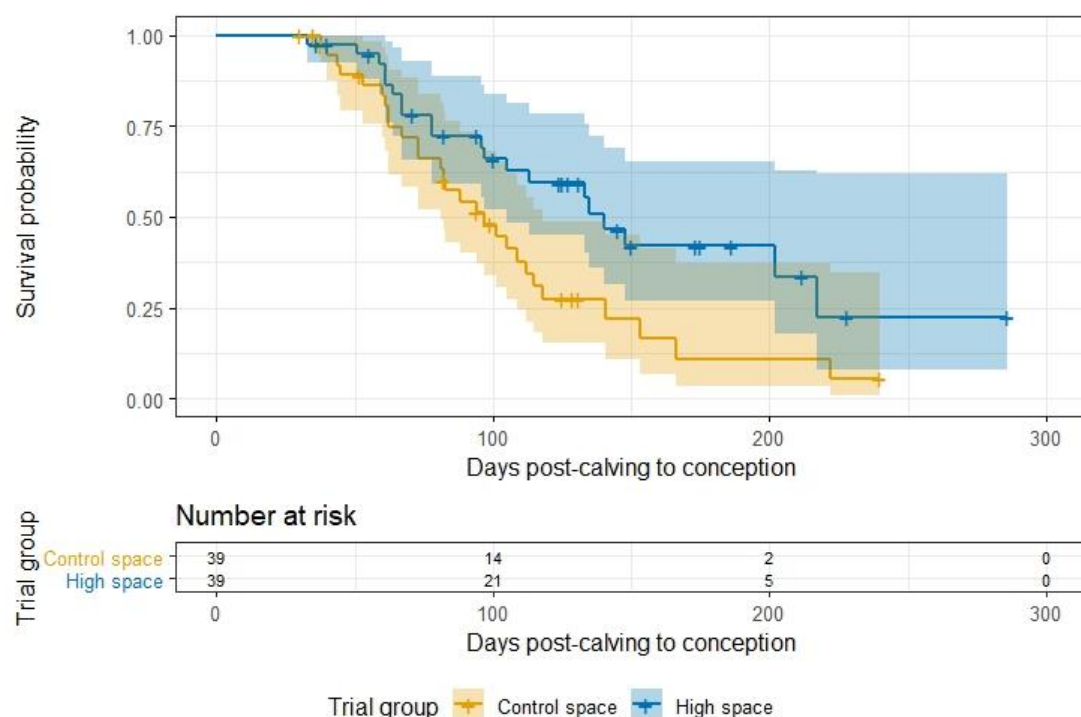


Figure 5.3. Results of time to conception analysis for matched pairs that entered trial at less than 14 days in milk ($n = 39$ pairs). Top: Kaplan-Meier plot of survival to conception for the high space (blue) and control space (yellow) trial groups. Respective colour shading represents the 95% confidence interval. Bottom: Risk table showing the associated number of cows non-pregnant at 0-, 100-, 200- and 300-days post-calving for each trial group.

Table 5.4. Parameter estimates from the Cox proportional hazards model (5.3.1.2) using data from cow-pairs which entered the trial at less than 14 days in milk.

Term	Estimate	Std. error	Statistic	P value	Lower CI	Higher CI
Control space group	<i>(ref)</i>					
High space group	0.51	0.30	-2.28	0.022	0.29	0.91

When analysed to investigate the effect of parity, control space primiparous cows were set as the reference group. The median time to conception for high space multiparous cows was 113 days versus 101 days for control space multiparous cows. Primiparous in the high space group took 202 days to conceive compared to 83 days for control space

primiparous cows. Results of the Cox proportional hazards model including parity are provided in Table 5.5.

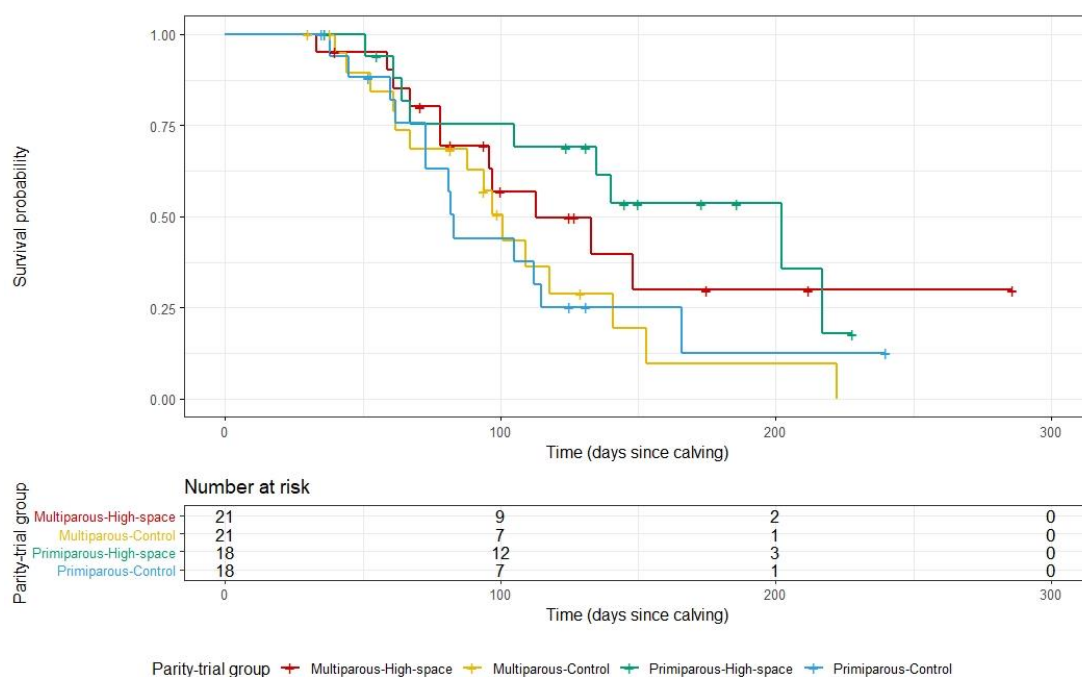


Figure 5.4. Results of time to conception analysis for matched cows that entered trial at less than 14 days in milk ($n = 39$ pairs). Top: Kaplan-Meier plot of survival to conception for the four trial-parity sub-groups (Primiparous-high space (green); Primiparous-control space (blue); Multiparous-high space (red); Multiparous-control space (yellow)). Bottom: Risk table showing the associated number of cows non-pregnant at 0-, 100-, 200- and 300-days post-calving for each parity-trial group.

Table 5.5. Parameter estimates from the Cox proportional hazards model (5.3.1.2) using data from cow-pairs which entered the trial at less than 14 days in milk, by parity-trial group

Term	Estimate	Std error	Statistic	P value	Lower CI	Higher CI
Primiparous control space	<i>(ref)</i>					
Primiparous high space	0.43	0.44	-1.94	0.052	0.18	1.01
Multiparous high space	0.60	0.41	-1.25	0.21	0.27	1.34
Multiparous control space	1.00	0.38	-0.007	0.99	0.47	2.10

5.3.2 Artificial insemination analysis

There were a total 219 inseminations given to cows on trial where both were non-pregnant at time of entry into the trial ($n = 48$ pairs). Of the 219 serves, 120 were given to cows in the high space group, of which 24.2% ($n=29$) resulted in confirmed pregnancies. In comparison, 37.5% of the 99 serves administered to the control group resulted in confirmed pregnancies, which was significantly greater than for the cows in the control group (Chi-squared test, $P = 0.049$).

When only cows that entered the trial <14 DIM were considered ($n = 39$ pairs), these accounted for 173 of the 219 serves. A similar pattern emerged with the high space group having significantly more serves, 97, but fewer confirmed pregnancies, 20 (20.6%), compared to the control group with 76 serves resulting in 28 (36.8%) confirmed pregnancies (Chi-squared test, $P = 0.028$).

5.3.3 Time to first service analysis

The survival analysis of time to first service identified that cows in the high space group cows took a median time of 56 days after calving to receive a first service (95% CI: 51 – 64, services = 40/43) which was sooner than cows in the control group that took a median of 63 days (95% CI: 59 – 73, services= 42/43). Figure 5.5 illustrates the full Kaplan-Meier survival plot for both trial groups. The Cox proportional hazards model indicated that compared to cows in the control group, those in the high space group had an increased hazard of receiving a first service (hazard ratio of 1.27) but this was a non-significant difference ($P = 0.29$).

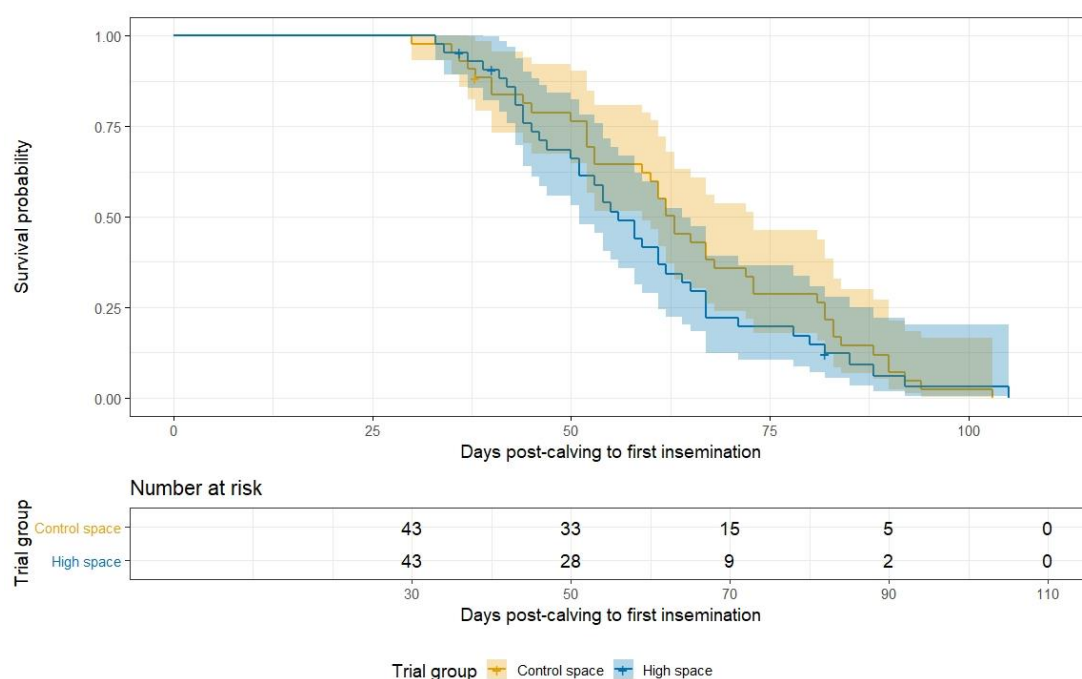


Figure 5.5. Results of time to first insemination analysis for matched cows that entered the trial without a first-serve record ($n=43$ pairs). Top: Kaplan-Meier plot of survival to first-service for the high space (blue) and control space (yellow) trial groups. Respective colour shading represents the 95% confidence interval. Bottom: Risk table showing the associated number of cows without a recorded first-service at 30-110 days (in 20-day periods) post-calving for each trial group.

5.3.4 Inter-service intervals

Figure 5.6 shows the distribution of the inter-service intervals for both trial groups. Many intervals aligned with the accepted 18-26day oestrous period in both groups, with cows in the control group attaining 36% and the high space group 50% of returns to oestrus in this period. The remaining interval groups were deemed consistent with distributions of inter-service intervals commonly seen on commercial farms.

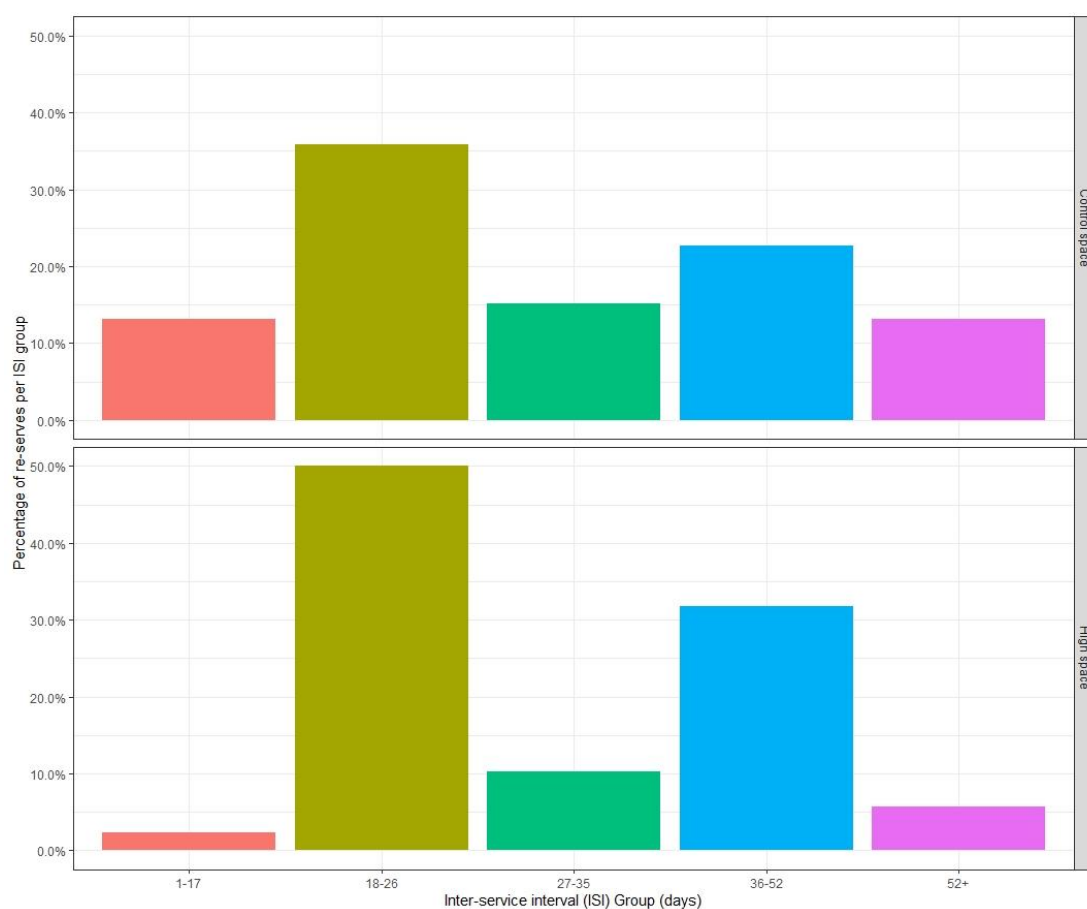


Figure 5.6. Bar charts of inter-service intervals (ISI) by trial group (Top: control space; Bottom: high space). The height of each bar represents the percentage of all return serves (y-axis) in each time period (x-axis). A higher percentage in the 18-26day period (yellow bars) indicates normal cow cyclicity and appropriate sensitivity of oestrous detection.

5.3.5 Progesterone profiles

A total of 80 cows had milk samples taken for progesterone analysis during the trial. The survival analysis for commencement of luteal activity (Figure 5.7) shows that there was minimal difference between the two groups, with a median of 39 days for cows in the control group (95% CI: 23 – 61, commencements = 34/40) and 38 days for cows in the high space group (95% CI: 24 – 46, commencements = 35/40). A Cox proportional hazards model indicated no significant difference between trial groups in time to commencement of luteal activity (hazard ratio for the high space group = 1.38, $P = 0.18$).

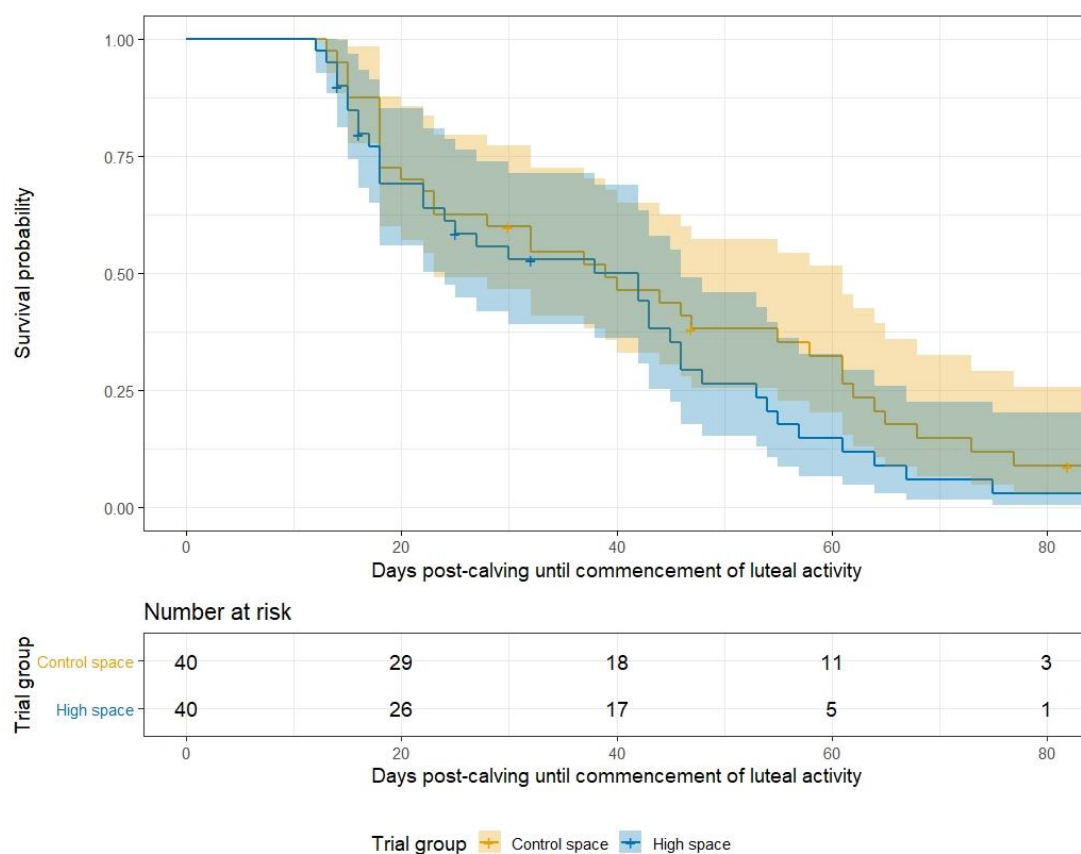


Figure 5.7. Results of time to commencement of luteal activity (CLA) analysis ($n=40$ pairs). Top: Kaplan-Meier plot of survival to CLA for the high space (blue) and control space (yellow) trial groups. Respective colour shading represents the 95% confidence interval. Bottom: Risk table showing the associated number of cows yet to commence luteal activity at 0-80 days (in 20-day periods) post-calving for each trial group.

When each individual cow's progesterone measurements were assigned a profile (Normal, Abnormal or Delayed Ovulation), 78 of the 80 profiles were available (n = 2 missing profiles due to lack of sufficient data due to time on trial). Results of categorisations are presented in Table 5.6. A similar distribution of profiles was identified in cows from both trial groups and a Chi-squared test indicated there was no significant difference between groups ($P = 0.73$). Examples of plotted profiles and evidence of group classification are provided in (Appendix – Chapter 5).

Table 5.6. Table to show the number of observed progesterone profile category types by trial group.

Profile category	Control space group	High space group
Normal	11	8
Abnormal	11	12
Delayed ovulation	17	19

5.3.5.1 Cow progesterone concentrations

Mean cow progesterone levels, between day 14-84 in milk, for cow pairs with ≥ 25 progesterone samples each (n=32 pairs) is illustrated in a box and whisker plot in Figure 5.8. The median progesterone concentration was 7.04mg/ml for the cows in the control group (mean = 7.25, IQR = 3.98-9.87) and 7.12mg/ml for the cows in the high space group (mean = 7.29mg/ml, IQR = 4.83-9.88). This difference was non-significant (Kruskal-Wallis rank sum test, $P = 0.89$).

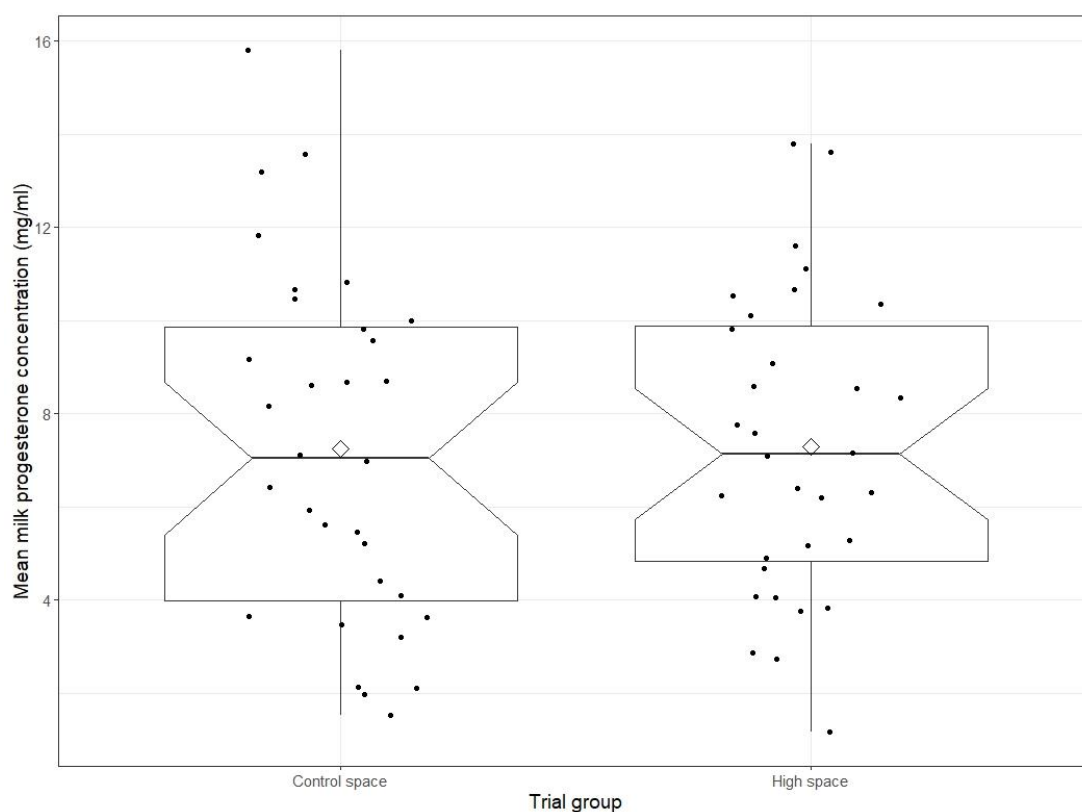


Figure 5.8. Indent boxplots to illustrate distributions of cow mean progesterone concentrations between 14-84 days in milk (LEFT: Control space group (n=32), RIGHT: High space group (n=32)). The median value is represented by the midline and 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range and outliers are shown as distinct points. The notches in the boxes represent a confidence interval around the median ($\pm 1.57 \cdot \sqrt{n}$). The diamond shape represents the mean progesterone concentration of the trial group.

5.3.6 Cow Anti-Mullerian Hormone (AMH) concentrations

Figure 5.9 illustrates the AMH concentrations for cows in both trial groups. Cows in the control group had a median AMH concentration of 150pg/ml and a mean of 197.9pg/ml (IQR: 98-254.5). Cows in the high space group had a median AMH concentration of 122pg/ml and a mean of 180.2pg/ml (IQR: 73.5-271). This difference was non-significant (Kruskal-Wallis rank sum test, $p=0.40$).

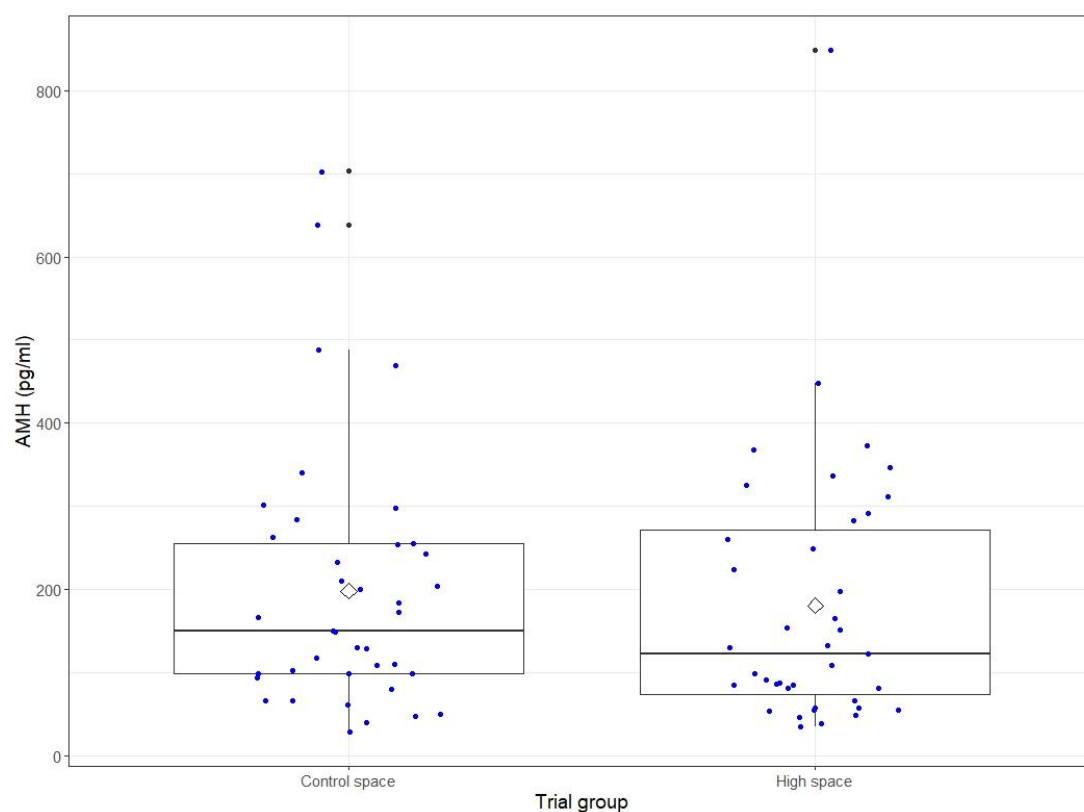


Figure 5.9. Boxplots to show the distribution of Anti-Müllerian Hormone (AMH) concentrations by trial group (LEFT: Control space group; RIGHT: High space group). The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points (black). The jitter points (blue) represent each cow's observed AMH recording.

The distribution of AMH concentration by parity and trial group is illustrated in Figure 5.10. In general, primiparous cows had lower AMH concentrations compared to multiparous cows, and the variation within parity between trial groups was similar.

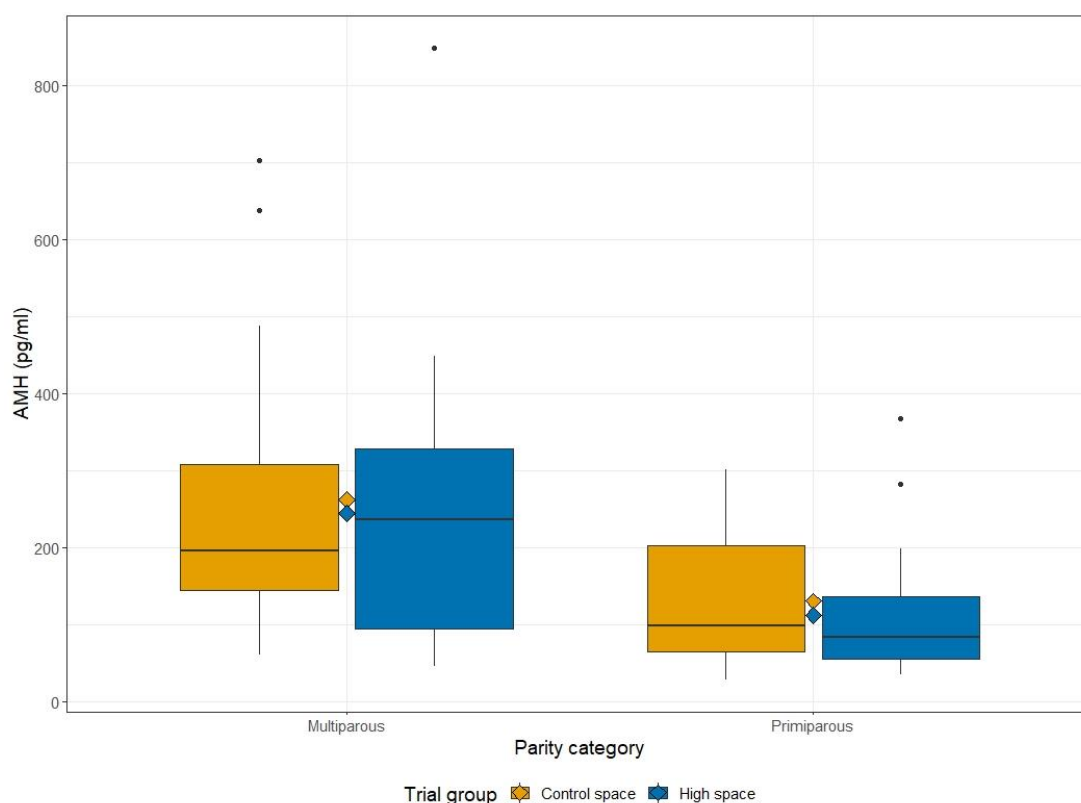


Figure 5.10. Boxplots to show distributions of the Anti-Mullerian Hormone (AMH) results by parity-trial group (Yellow: Control space group; BLUE: High space group). The two boxplots on the left are from multiparous cows, whilst the right sides show only primiparous cows. The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points. The respective coloured diamonds indicate the mean AMH in each parity-trial group.

5.3.7 Uterine and ovarian observational data

A summary of the uterine and ovarian observational data is provided in Table 5.7. No significant differences were identified between trial groups in the presence of endometritis ($P = 0.583$), uterine measurements ($P = 0.509$), maximum antral follicle count ($P = 0.853$) or presence of a corpora lutea by 42 DIM ($P = 1.00$); these were all found to be similar between groups throughout the trial.

Table 5.7. Results of the observational ovarian and uterine procedure data for both study trial groups.

Measurement	Timepoint	Trial group	Number of cows	Median	Mean	IQR	Minimum	Maximum
Presence of endometritis detected by 42DIM*	22-42DIM	Control space	10/39					
		High space	7/39					
Uterine Measurements (Area, mm²)*	14-21DIM	Control space		663	770	343	507	850
		High space		644	719	324	549	873
	22-28DIM	Control space		660	714	370	446	816
		High space		628	688	346	478	824
	29-35DIM	Control space		600	630	279	481	760
		High space		600	664	258	509	767
	36-42DIM	Control space		600	623	301	378	679
		High space		694	738	247	571	818
Antral Follicle Count*	14-21DIM	Control space		9	9.1	3	4	13
		High space		7	9.6	10.5	1	23
	22-28DIM	Control space		9	10.5	6.5	1	21
		High space		10	9.7	6	1	18
	29-35DIM	Control space		11	10.8	7	2	23
		High space		9	11.1	7	2	25
	36-42DIM	Control space		10	11.3	7.5	4	24
		High space		12	12.1	6.5	3	24
Corpus Luteum detected on cow ovaries by 42DIM	22-42DIM	Control space	22/35					
		High space	22/35					

*Definitions provided in section 5.2.3.9

5.3.8 Energy balance

Negative energy balance was assessed through BHB and NEFA in blood samples taken in weeks 2, 3 and 4 post-partum. The laboratory normal range for BHB was up to 1.2mm/l and for NEFA was up to 0.8mm/l. The majority of results were normal; however, some cows did present with above normal threshold values as illustrated in Figure 5.11 and Figure 5.12.

Both trial groups presented with a similar number of high results for each time-point. In terms of BHBs, over the three sampling timepoints 15 high space cows and 17 control space groups were found to have a high reading ($n = 38$ total animals per group), which was found to be a non-significant difference on a chi-squared test ($P = 0.787$). Results for the NEFAs showed that 10 high space cows and 8 control space cows tested positive at least once in the three sampling timepoints ($n = 38$ total animals per group), which was also found to be non-significant on a chi-squared test ($P = 0.816$).

When concentrations of BHB and NEFA were tested as a variable in the survival analyses described above, no biologically meaningful impact on model parameters was identified. When cow pairs where 1 cow had a high NEFA or BHB result were removed from the analysis ($n = 24$ pairs remaining), time to conception remained similar: high space cow median = 148 days, control-space cow = 118 days, high space primiparous cows = 202 days and control space primiparous cows 105 days.

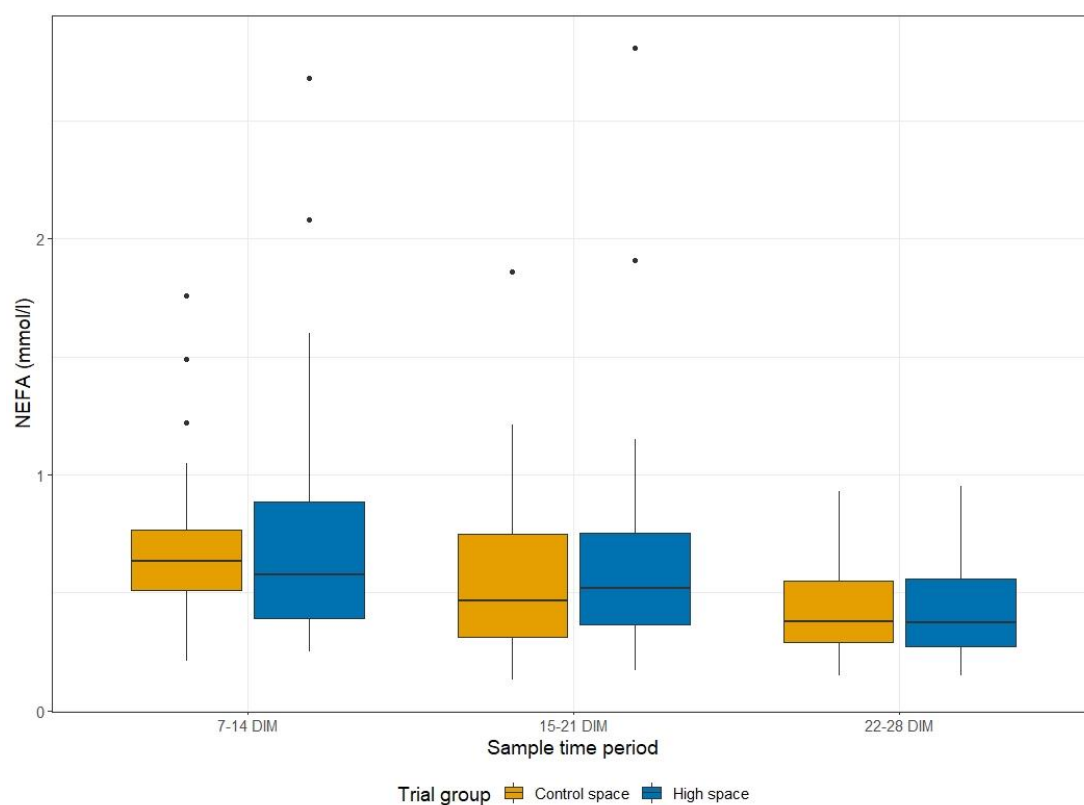


Figure 5.11. Boxplots to show distributions of the non-esterified fatty acid (NEFA) results by sampling time period and trial group (Yellow: Control space group; Blue: High space group). The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

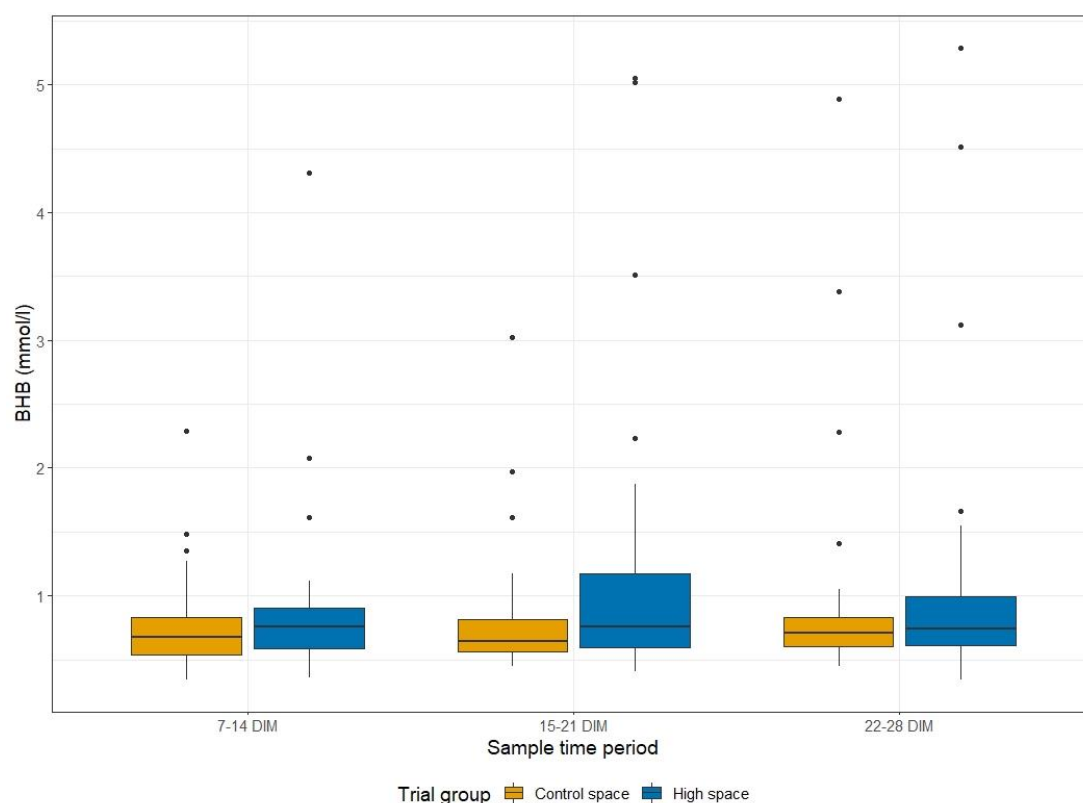


Figure 5.12. Boxplots to show distributions of the beta-hydroxybutyrate (BHB) results by sampling time period and trial group (Yellow: Control space group; Blue: High space group). The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

5.3.9 Associations between milk yield and conception

5.3.9.1 The effect of pregnancy status on milk yield: Does the difference in trial group reproductive performance contribute to a difference in milk production?

Results of the final random effects model that evaluated the association between milk yield and pregnancy status are provided in Table 5.8. In early pregnancy (up to 100 days after conception) there was a relatively small difference in milk volume between pregnant and non-pregnant cows, but as pregnancy progressed milk volume decreased with

increasing days pregnant, such that by 201-250 days pregnant, this accounted for a reduction of 6.7L per cow per day ($P < 0.001$).

Table 5.8. Results of the mixed effects model (5.2.4.1) to investigate the impact of pregnancy status and trial-parity group on daily milk production.

Model outcome: Daily milk yield residual (L)			
Fixed effects	Estimates	CI	P
(Intercept)	-12.03	-14.56 – -9.49	
DIM	0.04	0.04 – 0.05	<0.001
Primiparous- control space	(ref)		
Primiparous- high space	3.08	-0.51 – 6.66	0.092
Multiparous- control space	18.63	15.02 – 22.23	<0.001
Multiparous- high space	16.8	13.20 – 20.41	<0.001
DIM * Primiparous- high space	-0.01	-0.01 – -0.00	<0.001
DIM * Multiparous- control space	-0.06	-0.06 – -0.05	<0.001
DIM * Multiparous- high space	-0.04	-0.05 – -0.04	<0.001
Non-pregnant	(ref)		
Days pregnant (1-50)	0.45	0.25 – 0.65	<0.001
Days pregnant (51-100)	-0.27	-0.53 – -0.00	0.047
Days pregnant (101-150)	-1.06	-1.41 – -0.71	<0.001
Days pregnant (151-200)	-3.25	-3.69 – -2.80	<0.001
Days pregnant (201-250)	-6.72	-7.35 – -6.09	<0.001
Random Effects			
Residual variance	15.71		
Random effect variance	62.76		
Intraclass correlation	0.8		
Number of random effect groups	150		
Number of observations	25594		
Marginal R² / Conditional R²	0.251 / 0.850		

When this model was used to predict milk volume per cow over a 305 day lactation using reproductive inputs (Table 5.9 and Figure 5.13), it was identified that the first parity animals in the high space group continued to outperform the control space group by 615L even once the difference in median days to conception of 72 days was taken into account.

This would mean a milk difference associated with the reproductive performance would equate to only 28L.

Similarly, when the 24-day delay in median days to conception for multiparous cows in the high space group compared to the control space group was taken into account, a 69L increase in milk volume per animal was still observed. This shows that the differences in reproductive performance accounted for 35L of the total observed difference between the two group for multiparous cows.

Table 5.9. Predicted 305-day milk yields according to days in milk (DIM) at conception and trial-parity group. DIM at conception indicated when a cow conceived and incorporates the results from the days to conception survival analyses.

		DIM at conception									
		80	83	101	109	120	130	133	135	155	160
Trial-parity group	Primiparous: Control space	11364	11385	11505	11549	11584	11617	11627	11633	11698	11706
	Primiparous: High space	11979	12000	12120	12164	12200	12232	12242	12248	12313	12321
	Multiparous: Control space	14427	14447	14568	14611	14647	14679	14689	14695	14760	14768
	Multiparous: High space	14495	14516	14636	14680	14715	14748	14758	14764	14829	14837

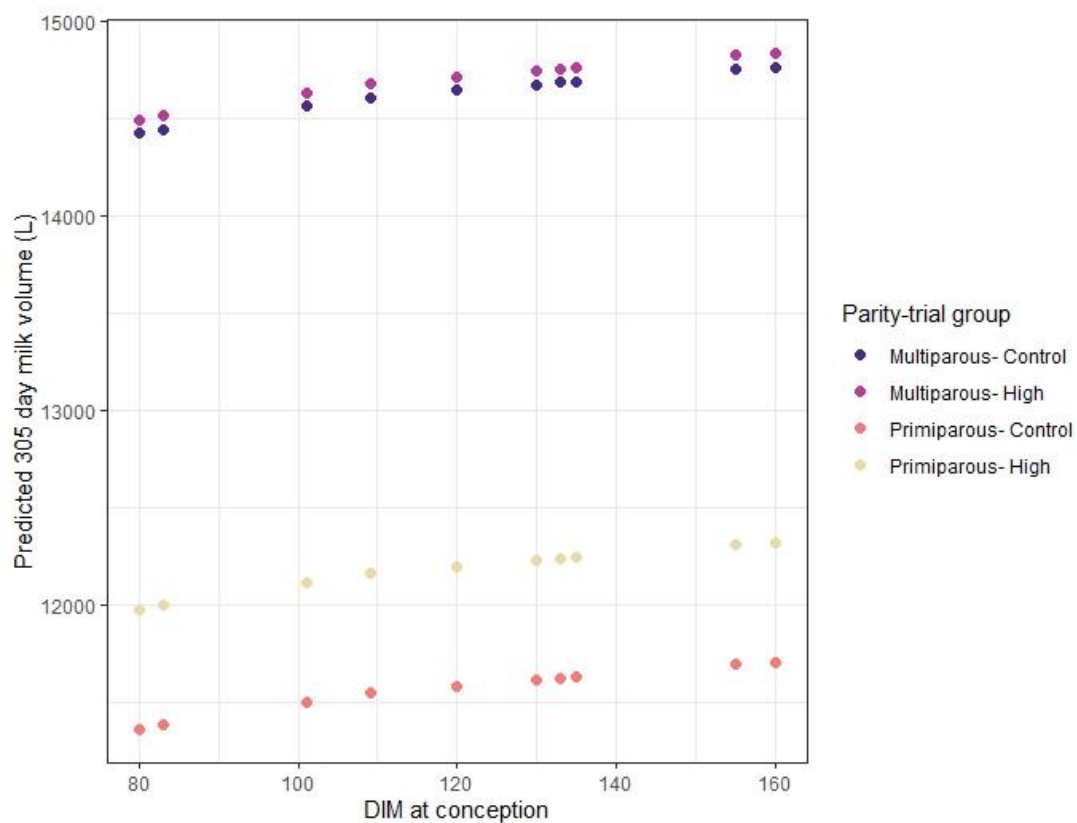


Figure 5.13. Dot-plot to illustrate the predicted 305-day milk yields (y-axis) by DIM at which a cow conceived (x-axis). The predictions are grouped by parity-trial group.

5.3.9.2 The effect of milk yield time to conception: Are differences in times to conception between trial group associated with differences in milk yield prior to conception?

The final random effects logistic regression model is presented in Table 5.10. No effect of milk volume was identified on the odds of conception (odds ratio = 1.00, $P = 0.87$), which indicated that increased milk volume did not influence the probability of conception. The effect of trial group remained significant with the odds of conception at a service being reduced in the high space compared to the control group (odds ratio = 2.68, $P = 0.026$). Model fit was deemed to be good.

Table 5.10. Results of the final binomial regression model (5.2.4.2) to estimate the effect of the previous 7-day milk yield on conception.

Model outcome: Conception			
Predictors	Odds ratios	CI	P
(Intercept)	0	0.00 – 0.00	
High space	(ref)		
Control space	2.68	1.13 – 6.31	0.025
Mean rolling 7-day milk volume	1.00	0.96 – 1.03	0.869
ln(DIM)	7.30	2.30 – 23.13	0.001
Random Effects			
Residual variance	3.29		
Random effect variance	1.67		
Intraclass correlation	0.34		

5.4 Discussion

This chapter aimed to investigate the effect of increasing living space on reproductive performance of housed dairy cows. The key result was that the time to conception was substantially longer for cows that were housed in a greater amount of living space. Since most literature suggests that improvements to environmental conditions should reduce stress and improve fertility (Dobson and Smith, 2000; Ewing et al., 1999; von Borell et al., 2007), the findings of the current study were unexpected.

There is, however, literature to support the view that reproductive performance in livestock is complex and that the impact of the environment (or other stressors) is not always clear cut. It is accepted that stress can elicit diverse (sometimes unpredictable) responses that can either increase or decrease the probability of an animal to reproduce (von Borell et al., 2007). For example, it has been reported (currently only in pigs) that some types of stressors do not result in any deterioration in reproductive performance (Turner et al., 2005), thus ensuring production of progeny and perpetuation of genetic material. For dairy cows, it has been suggested that maintaining an adaptive ability in times of stress is important for survival (Blanc et al., 2006). These authors conclude that the normal response to prioritise individual survival rather than invest in pregnancy and the associated energy requirements is less important for dairy cows because prioritising reproductive performance ensures that they are not selectively culled from a herd. It may be, therefore, that we have selected cows that are capable of breeding under some conditions of stress.

In general, however, reproductive performance is worsening in the worldwide dairy cow population, this is a multifactorial problem but management and environment are considered to be key contributing factors (Lucy, 2001). It is known that fertility is impacted on by nutrition (Buckley et al., 2003; Butler, 2014) and that key metabolic hormones are associated with reproductive physiological mechanisms (Garnsworthy et al., 2008). In the current study, nutritional constituents and feeding processes were identical between trial groups and hormonal concentrations appeared to differ very little. Although there have also

been reports of increased metabolism of progesterone and oestradiol associated with higher feed intakes and milk yields with the potential to impact on fertility (Sangsritavong et al., 2002), this is an unlikely explanation in this research because the progesterone profiles observed between the groups were similar. Thus, the pathway behind this mechanism causing the increased time to conception in the high space cows remains unclear.

Potential physiological pathways that underpin reproduction were investigated in this study; progesterone profiles, AMH, antral follicle counts and oestrous interval analysis. No differences were found between cows in the two trial groups which meant no clear evidence was provided that living space had an impact on any of these physiological processes. Although, these processes have been reported to affect reproductive outcomes (Bekara and Bareille, 2019; Rojas Canadas et al., 2020) it appears in this study, that they were not responsible for the differences identified in reproductive outcomes. Indeed, all of the data collected to investigate reproductive pathways showed no differences between the trial groups suggesting underlying physiology was not greatly impacted by the difference in living space allowances.

The similarity in progesterone profiles between trial groups is particularly interesting. Progesterone profiles have been shown to help with reproduction monitoring of dairy cows by taking the individual and unique cycles of each cow to diagnose abnormal cyclicity or pregnancy status and is considered the gold standard for evaluating reproductive status in research (Adriaens et al., 2017). Firstly, shorter calving to conception intervals and conception rates have been associated with earlier resumption of ovarian cyclicity (Bittar et al., 2014; Galvão et al., 2010). Secondly, cows identified as having atypical profiles have been shown to have around an 18-day increase in time between calving to conception in comparison to cow with normal profiles (Petersson et al., 2006a). The proportion of atypical profiles has been linked to the housed environment, with one study reporting that atypical profiles were more likely to occur in cow housing in tie-stalls than loose housing (Petersson et al., 2006a). Further environmental effects associated with commencement of luteal activity were housing type, winter calvings, and treatment for disease such as mastitis or

lameness (Petersson et al., 2006b). Higher milk yields have also been associated with increased abnormal profiles (Lucy, 2001). Considering there were differences in reproduction, milk yield and the housed environment between trial groups in this study, it is noteworthy that progesterone profiles appeared to remain very similar between trial groups.

Another parameter measured in this study, AMH, has been reported to be a useful indicator of reproductive performance (Akbarinejad et al., 2020) and pregnancy rates have been positively associated with AMH concentrations (Ribeiro et al., 2014). However, no differences in AMH were observed between the two trial groups in this study despite the median times to conception being different and these results align with two previous studies in which fertility in dairy cows was not associated with AMH concentrations (Baruselli et al., 2018; Gobikrushanth et al., 2019).

The genetic potential of the cows in the study population was considered as a possible confounding factor for the difference in days to conception between two trial groups. However, the recruited population were from the same closed herd with similar sire selection which alongside a randomisation process for allocation into the control or high space group should have meant that the distributions of genetic potential were small and equally balanced between the two groups. Thus, the genotypes of this sample population were assumed to have had no impact on the observed results.

The difference in milk volume produced between the two trial groups was investigated to assess whether this was impacting on reproduction performance, but it was shown to have little influence. This aligns with previous research where milk production was reported to be positively associated with likelihood of artificial insemination and pregnancy (Buckley et al., 2003). Other studies have found that the level of milk production in early lactation did not affect subsequent reproductive performance of dairy cows (Gröhn and Rajala-Schultz, 2000). Conversely, there have been reports that suggest that improvement of milk production traits can have negative impacts on fertility (Albarrán-Portillo and Pollott, 2013; Pryce et al., 2004), and that these animals are more heavily impacted when subjected to inadequate environments (Blanc et al., 2006; Mendes et al., 2021).

The design of this study, a randomised controlled trial following the CONSORT guidelines (Moher et al., 2010), means that differences between the trial groups were unlikely to be caused by confounding or bias and that evidence for causality can be considered to be strong (Oxman, 2004). Nonetheless, there are important limitations of the study that should be considered. Due to its nature, full blinding of researchers was not possible; some of the measurements were subjective and the data collection of these could not be blinded, such as endometritis scores and reproductive organ scanning. To mitigate this issue, it was decided that the majority of key outcome data were based on laboratory analysis, sensor data or other objective data (e.g. date of conception) to help remove unconscious bias. In addition, to remove bias at the analysis stage the researchers were blinded to cow identification number and trial group and analyses conducted without this knowledge.

Standard operating procedures were put into place for general farm management and reproductive interventions to reduce bias. However not all tasks were undertaken under direction of the researcher and it was assumed that daily management tasks undertaken by farm staff (such as cleaning cubicles and moving cows to the robot) were, as requested, performed to the same standard for both trial groups.

A limitation of some of the statistical analyses undertaken could have been the sample size. The sample size calculations for the randomised controlled trial were set to investigate all cows on trial irrespective of characteristics (e.g. parity). This may explain a lack of power of the sample sizes using small sub-groups of data when populations were split into smaller groups such as primiparous and multiparous cows. Furthermore, when some of the physiological parameters were tested, for example progesterone profiles (3 categories) the power would have been reduced. Given that this was a potential limitation of this trial, further work to explain the underlying physiological changes which occurred are of critical importance.

5.5 Conclusion

In conclusion, the findings in this chapter suggest that increased living space may have a deleterious impact on dairy cow reproduction although the reasons for this are unclear. Given the study design and that such a substantial difference in median time to conception was observed, the evidence for a true effect is strong and makes an important case for additional research in this area. The fundamental reproductive pathways analysed in this study failed to present a plausible explanation for a causal pathway and, alongside current literature, the reasons for the large deterioration in reproductive performance associated with increased living space remains unclear.

Chapter 6. Randomised controlled trial results: Behavioural data

6.1 Introduction

Following the important results observed in Chapters 4 and 5, for dairy cow production and reproduction respectively, this Chapter continues to investigate how living space impacts housed dairy cows but with a focus on how cows interact with different living space allowances in terms of behaviour and time budgets.

It is well recorded that dairy cow time budgets are restricted, mainly due to the time required for eating, lying and being milked (Gomez and Cook, 2010; Krohn et al., 1992; Norring et al., 2012). This limits time available for other behaviours such as play, use of environmental enrichments (DeVries et al., 2007), drinking, oestrous activity (Zebari et al., 2018) and generally moving between areas. Space availability for housed dairy cows has been reported to be associated with changes in behaviour such as, decreased lying times, decreased activity and increased agonistic interactions when space is limited (Hill et al., 2009; Kondo et al., 1989; Shepley et al., 2020; Wierenga et al., 1991), indicating housed infrastructure is of fundamental importance

Due to these reported observations, the behaviour of dairy cows, especially in housed conditions, have been widely used in welfare assessments (Robbins et al., 2016). When assessing welfare, a commonly used gold standard question is now: “does an animal have a good life” (Yeates, 2017), which has superseded “does the animal have a life worth living?” (Dawkins, 2003). This is a hugely emotive subject within the livestock industry (Jackson et al., 2020) and fundamental to answering this question is whether the animal inhabits an appropriate environment. Other welfare assessments have centred around the ‘five freedoms’ (Webster, 2001) and whether cows have access to basic necessities, although consideration is increasingly moving towards ‘what animals want’ (Franks, 2019),

commonly referred to as ‘positive welfare’. Examples of positive welfare include use of environmental enrichment, novel stimuli and choice (DeVries et al., 2007). Such beliefs have developed from the idea of cows being bored within the housed environment because of a lack of stimuli in comparison to pasture grazing (Crump et al., 2019; DeVries et al., 2007).

Many measurements currently used to make a quantitative assessment of welfare involve the use of sensors, for example, for disease detection (Gusterer et al., 2020; Hut et al., 2021) or to classify behaviours such as lying times (Barker et al., 2018; Vázquez Diosdado et al., 2015). Unfortunately, even though such measurements can be quantified, interpretation remains difficult in terms of what constitutes optimal cow welfare.

There have been no long-term randomised controlled trials to explore how cow behaviours or time budgets change when given extra living space. Thus, even the effect of a most basic difference in housing is currently unknown. Concerningly, behaviour-based measurements are becoming more prominent in regulations and recommendations even though there are many confounders that commonly affect them. In the example of lying times, higher lying times are generally seen as positive although cow level lying times are hugely variable on a day to day basis (Bewley et al., 2010; Cook, 2020; Ito et al., 2009). Thus, the use of average lying time is an oversimplified measurement of an extremely complex behaviour. There is also research to suggest that some increases in lying times are not positive for cows, for example lameness increases lying time (Ito et al., 2010; Walker et al., 2008). Furthermore, farm practices and conditions such as frequency of milking, stocking density, cubicle design and climate are all known to influence lying time and lying area preference (Bach et al., 2016; Charlton et al., 2014; Haskell et al., 2013; Hill et al., 2009; Robles et al., 2021). Therefore, it is important that a more complete understanding is gained of how cows interact with their environment to enhance welfare and that we make improvements to the housed infrastructure that will positively affect cow wellbeing.

The aim of this chapter was to evaluate whether and how the daily time budgets of cows are influenced by living space availability. Specifically, the purpose was to quantify where cows spend time in the housed environment and whether this alters with living space

allowance. It was hypothesised that cows within the high space group would utilise the cubicles and feed-face for longer periods per day than the control space cows, as cows in the high space group would face less competition and fewer agonistic interactions when travelling around the pen and thus benefit by being able to spend more time in high resource reward areas.

6.2 Materials and methods

A general overview of study design, materials and methods has been described in Chapter 3. Additional materials and methods used to address the specific research question in this chapter are described below.

6.2.1 Data software

A wireless sensor-based system (Omnisense Cluster Geolocation System, UK) was used to collect location co-ordinates of dairy cows during the trial period (Omnisense, 2018). This system was validated previously in a study to classify dairy cow behaviour in a housed environment (Vázquez Diosdado et al., 2015).

The Omnisense system used a wireless mesh network which measured the time of arrival from periodic signal messaging between sensors to determine the relative location of each sensor from known fixed sensor positions. Each point in the building was given an x and y co-ordinate that described its position relative to a specified fixed-point set as $x=0$, $y=0$. The sensors remotely transmitted ultra-wideband radiowaves from 3.5-6.5GHz, IEEE802.15.4, to connect to the location server. The fixed and on-cow sensors formed a mesh network which allowed for the relative spatial location of the on-cow sensors to be determined by assessing the time duration between emitting and receiving signals. This provided an accuracy of $\pm 50\text{cm}$ at 95% confidence (which improves to $\pm 20\text{cm}$ under good conditions). Location data were obtained every 9s on average (0.11Hz) over the sampling time period, to give appropriate battery function of ~ 7 days before replacement was

necessary. The wireless system instantaneously stored location data onto disc which allowed real-time observation of cow location. However, all data were stored and analysed at the end of the trial.

Sensors were placed onto study participant cows using a neck collar. The cow sensor was fixed in position on the dorsal aspect of the cow's neck (to enhance positioning relative to fixed sensors) using a counterweight (0.5kg).

6.2.2 Data collection

The system was used to track cow movement and location of a subset of cows for 5 months at the end of the study period (14/02/2019-11/07/2019). A subset of paired cows ($n=16$) was selected and comprised those earliest in lactation when the sampling period commenced; selected cows were between 9 and 77 days in milk. During this 5-month period, x and y co-ordinates were collected every 9 seconds except for brief periods when sensor transmission failed (details below).

6.2.3 Data handling

A total of 29.7 million data points were collected. Initial visualisation of all datapoints was undertaken using heatmaps in R (Wickham, 2019). To improve data reliability and handling capacity, a mean coordinate position was calculated for each 1-minute time interval ($n = 3738739$ data points). Each individual cow's data was partitioned into the 1,440 minutes over each 24-hour period, if there were multiple measurements in each minute time period the mean of each x and y co-ordinate was calculated and this was then assigned as the cow's location during that minute of the trial. This was based on previous behavioural research that studied frequency of cow movements and reported that positional data measured each minute captured sufficient granularity to provide an accurate representation of cow movements. Since the hypotheses being testing in this chapter was based around time budgets in key zones (which did not require exact positioning every 9

seconds), it was considered that the averaging of co-ordinates per minute would provide sufficient detail for analysis whilst limiting computer processing power required.

6.2.3.1 Data coverage per cow

Coverage of each cow sensor was assessed over time based on the 1-minute time periods and proportions of coverage (proportion of eligible time periods with records) were assigned to each animal for the entire data collection period. Missing data were either due to loss of battery charge or failure of the sensor to be detected by the system.

The data for pairs of cows were classified as partial or full-matched by calculating the percentage of 1-minute intervals the cow had sensor data for over the sampling period (147days x 24hours x 60minutes), which equalled: no. observations / 211,680. Examples of the data are shown in Figure 6.1.a (partial coverage; example = 43.2%) and Figure 6.1.b (full coverage; example = 89.8%).

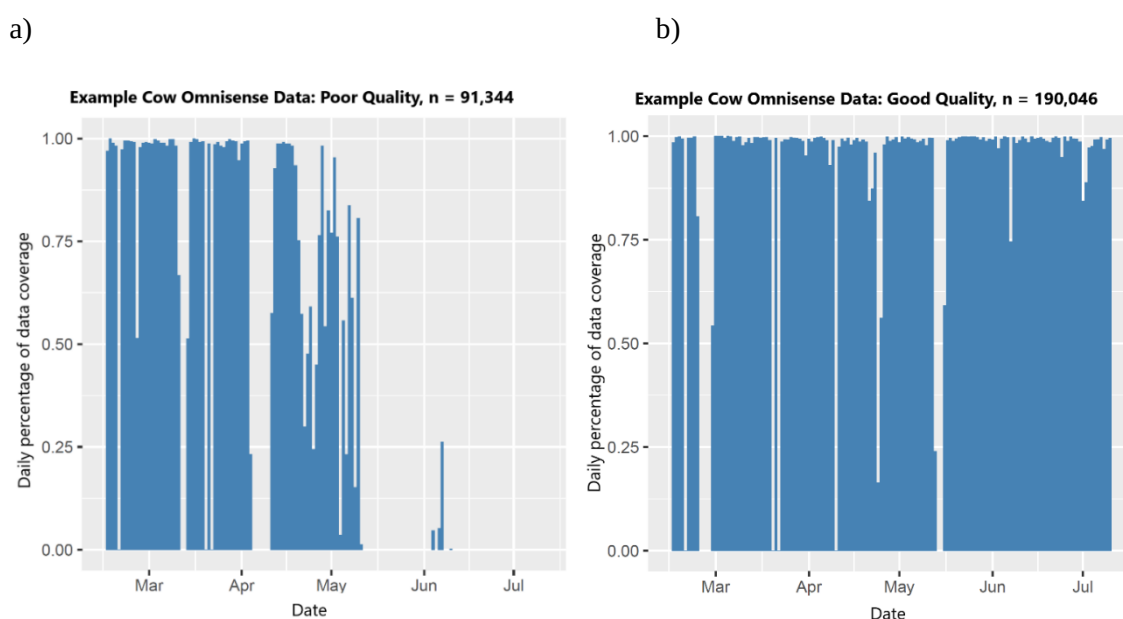


Figure 6.1. Plot to illustrate the percentage of data coverage for a cow per sampling day. Bars (blue) closer to 1.00 indicate 100% coverage for every 15-minute time block during a 24hour period. Missing data was caused by loss of battery or poor connection of cow sensor to the system (grey area). a) Example of a cow dataset classed as poor quality due to multiple extended periods of battery loss as well as total loss of sensor connectivity during latter stages of trial. b) Example of a cow dataset classed as good quality with almost complete coverage of the 5-month data collection period.

Overall, there were 5 pairs of trial cows judged to have full-matched coverage based on the percentage of 1-minute time period with data. These pairs had greater than 64% of expected 1-minute time interval data for both cows individually (min = 64.3%, max= 90.8%, mean = 83.8%, median= 85.9%). The remaining 11 pairs had at least one cow with less than 55% data coverage.

6.2.4 Data analysis

The research buildings were divided into key zonal areas which were defined by the underpinning grid of x and y coordinates; cubicles, robot, water trough, brush, feedface, and other (movement area- i.e. non-feeding area of feed passageway) as well as the loafing area for high space group. These were physically measured by the researcher during the trial period to ensure co-ordinates were correctly positioned; the measurements were compared to the geolocation set base co-ordinate (0,0) and the corresponding co-ordinate of the zone was calculated. Co-ordinates were then assigned to each zone. Using the R “fuzzyjoin” package (Robinson et al., 2020), cow location co-ordinates were allocated to the corresponding key zonal area for each observed datapoint, which can be visualised in Figure 6.2.

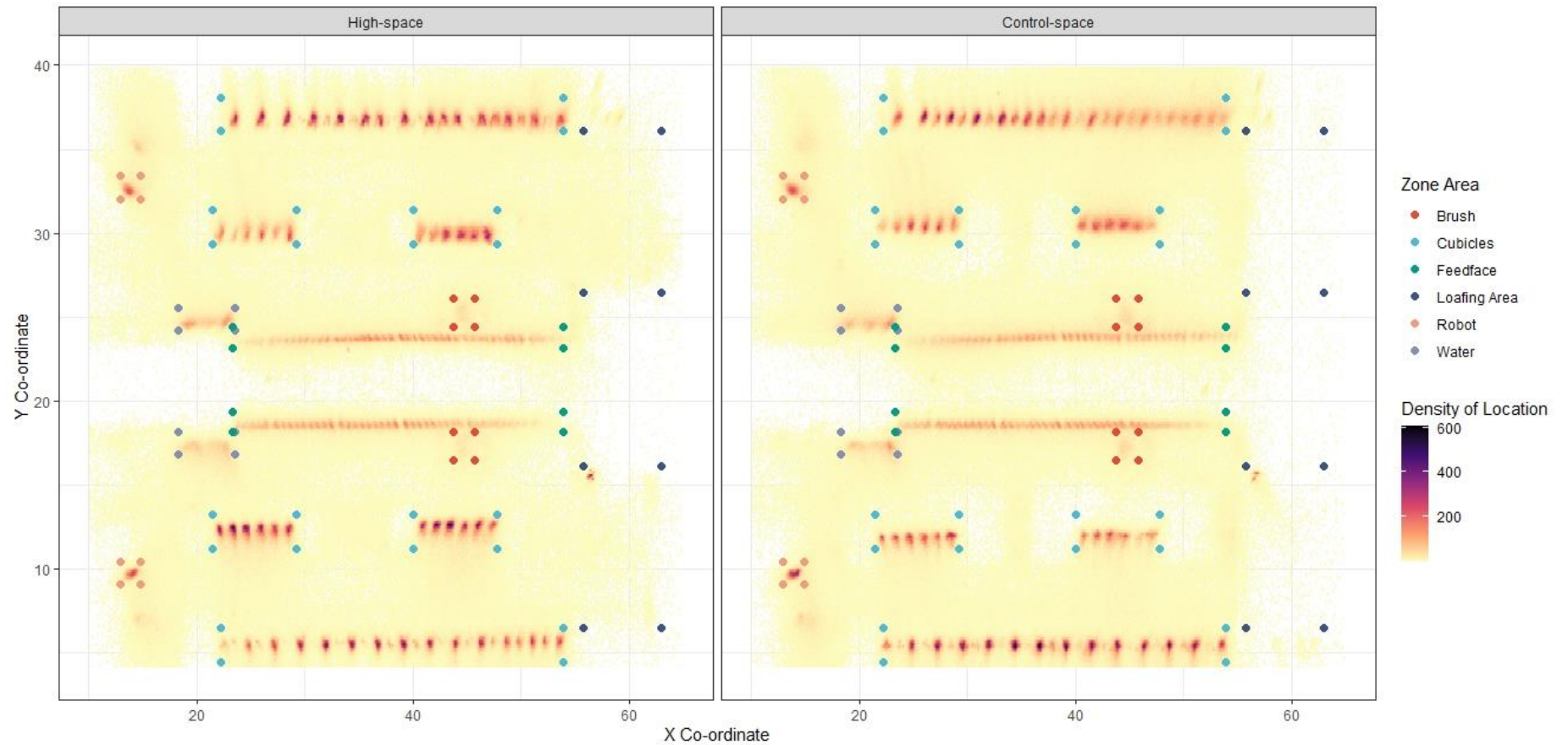


Figure 6.2. Heatmap depicting the location of the key area zones for the respective trial groups. The areas, shown by the dark purple and pink, represent areas more frequently utilised by the inhabitants such as the cubicles (light blue dots), feedface (green dots) and milking robot (pink dots). The loafing area (dark blue dots) was not available for the control-space side which is shown by the lack of activity here (white) compared to the high space group (yellow).

Direct comparisons of daily time budgets were made between trial groups based on proportions of time per day spent within each zonal area. The percentage of time in each zone out of the total datapoints for each trial group was calculated. This percentage was rescaled into minutes out of a 24-hour period.

Analyses were undertaken on different subsets of data to check consistency of outcomes. The subsets used were; i) all data (n= 3738739; cow pairs = 16), ii) data match exactly by minute for both cows in a pair (n= 2410756; cow pairs = 16), and iii) data including only matched pairs of cows with full-matched coverage (n= 1768094; cow pairs = 5). Statistical comparisons were made between times cows spent in designated areas such as living space areas, cubicles (as a proxy for lying time) and environmental enrichment use.

Bootstrapping of the data was undertaken using two methods, to assess the uncertainty associated with times cows spent in different zonal areas. Re-sampling was undertaken 500 times per trial group for both methods. From the bootstrap resampling, the mean and 95% quantiles were calculated for each trial group in each area zone. The first bootstrap method used was sampling with replacement within each cow's data to ensure all cows remained equally represented within the bootstrapped data. The second method was to sample with replacement from all data rows so that bootstrapping methods could be compared to assess consistency in outputs, dependent on method used. From the 500 bootstrap samples, confidence intervals were produced for the mean time spent in each zone by cows in each trial group.

6.3 Results

6.3.1 Descriptive results

6.3.1.1 Raw data

Heatmaps to illustrate the difference between data collected every 9 seconds and data averaged over 1-minute periods are shown in Figure 6.3. The heatmap in Figure 6.3.a contains 29,766,608 datapoints which comprises all raw (~9 second interval) datapoints for 16 cows. The heatmap in Figure 6.3.b comprises the same data averaged over 1 minute-intervals for each cow and contains 3,738,787 datapoints. Density patterns are similar in both heatmaps and illustrate the increased use of lying and feeding areas (lighter blue/green regions) within the housing facility.

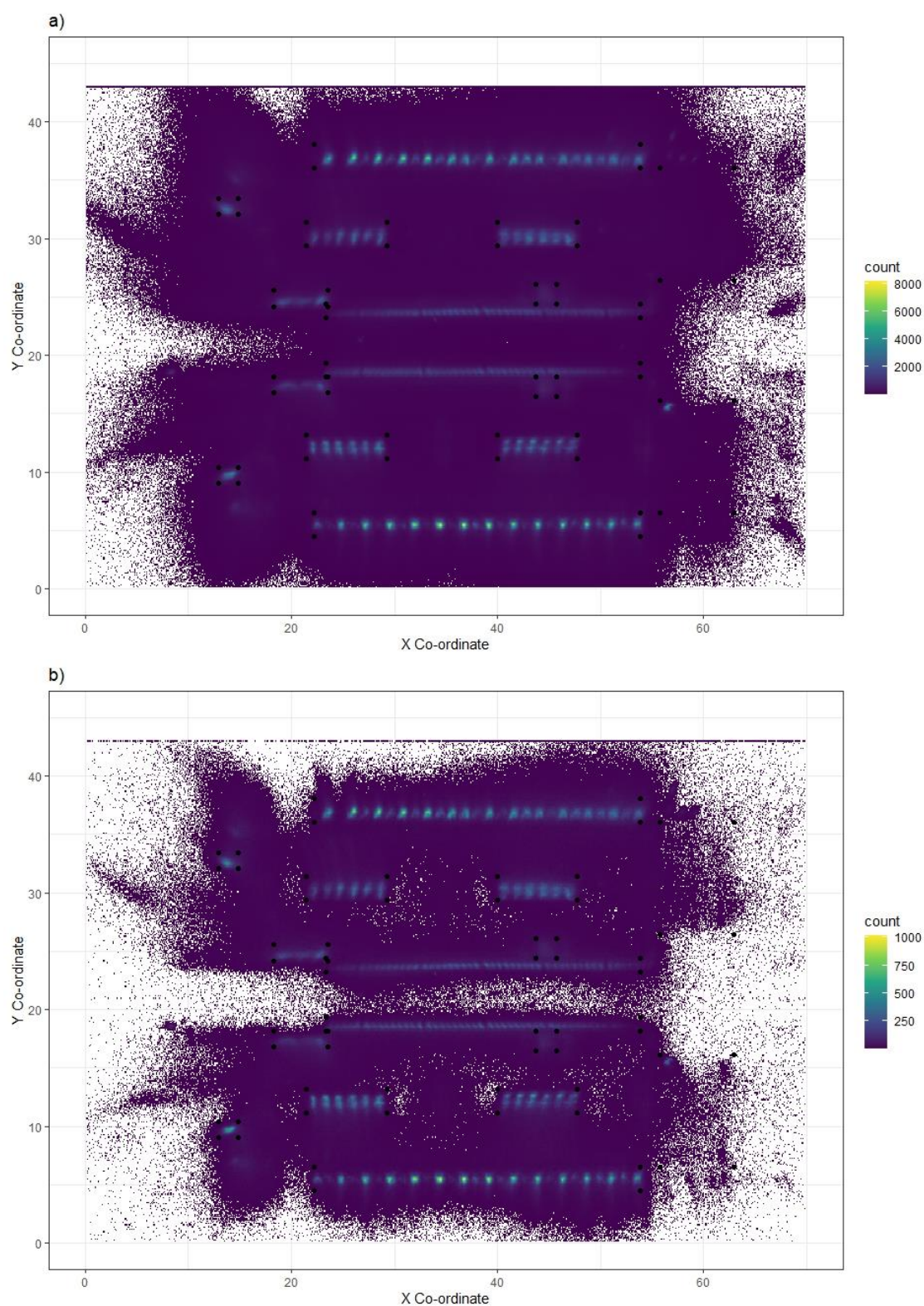


Figure 6.3. Heatmaps of x,y co-ordinates for every cow's location data during the sample period. This image allows for direct comparison of a) every datapoint recorded ($n=29,766,608$) and b) mean x,y coordinate per minute block ($n=3,738,787$). The areas with the most data density are shown by the lighter yellow, green then blue colours, which are consistent between heatmaps.

6.3.2 Analysis of daily time budgets

The final results of the daily time budgets are illustrated in Figure 6.4, these are based on the full dataset using bootstrapping by individual cows. Significant differences were identified in two behaviours. Cows in the high space group spent 63 minutes per day longer in the lying areas (12.43 hours/day, 95% CI = 11.70-13.29), than cows in the control group (11.35 hours/day, CI = 10.73-12.12). Cows in the control group spent 87 minutes per day longer in the passageway areas (8.17 hours/day, 95% CI: 7.48-8.88) than the high space group (7.10 hours/day, 95% CI: 6.48-7.53). Times spent on other activities, feeding, drinking, use of a mechanical brush and milking were similar between groups.

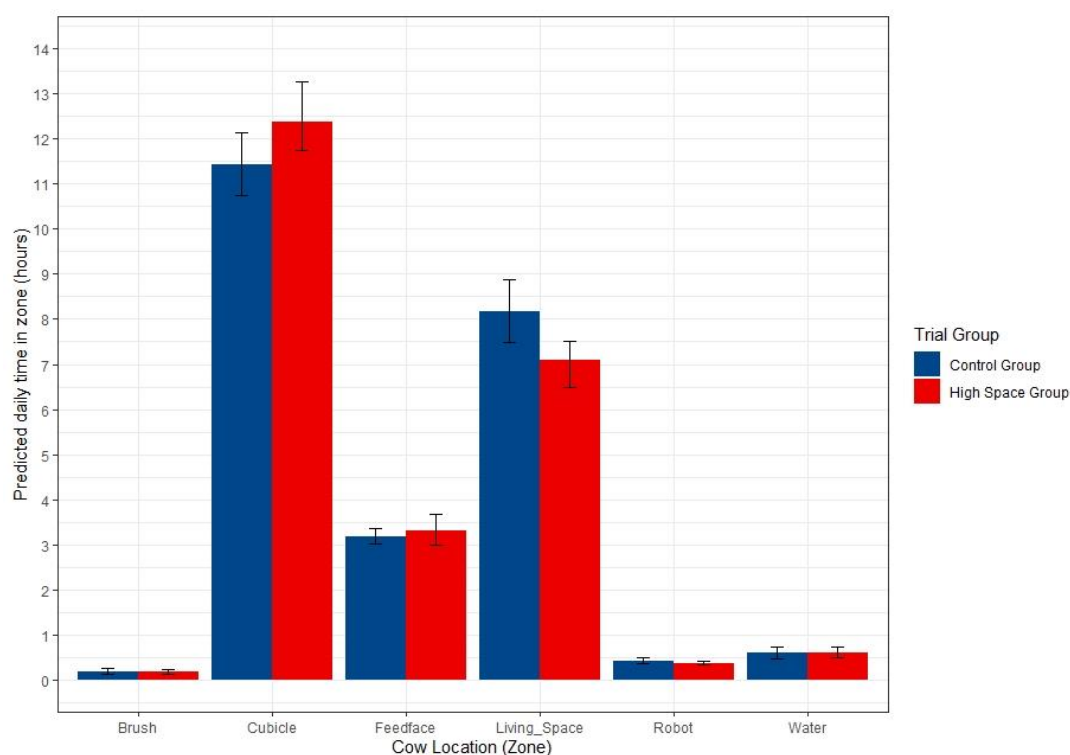


Figure 6.4. Boxplot of the final results of the estimated time budgets for cows in each trial group during the sampling period. Cows in the high space group (red) spent significantly more time in cubicles and less time in living space areas in comparison to cows in the control space group (blue). The error bars (black lines) represent the 95% confidence interval around the observed key zone time budget.

6.3.3 Comparisons between subsets of dataset and bootstrap methods

Figure 6.5 shows the graphical representations of the time-budget results when the full dataset and the two data subsets were bootstrapped by the two methods described (by cow or by row of data). Results indicate the findings were not heavily influenced by either missing data or bootstrap method. Compared to the results described in the section above (6.3.2) for lying times (High space: 12.43 hours/day, 95% CI = 11.70-13.29; Control space: 11.35 hours/day, CI = 10.73-12.12), shown in Figure 6.5.a) and Figure 6.5.d). When the subset was used where both cows had a recording for every 1-minute time period, lying times were 12.48 hours/day for the high space and 11.14 hours/day for the control space group (Figure 6.5.b & e). When the subset of 5 pairs of cows classed as having full-data for the whole sampling period lying times was used, lying times were 12.78 hours/day for the high space group and 11.24 hours/day for the control space group (Figure 6.5.c & f). Bootstrap confidence intervals for these different subsets of data yielded consistent significance or non-significance for zonal use between trial groups, independent of method as illustrated in Figure 6.5.a-c for bootstrap by line and Figure 6.5.d-f for bootstrap by cow.

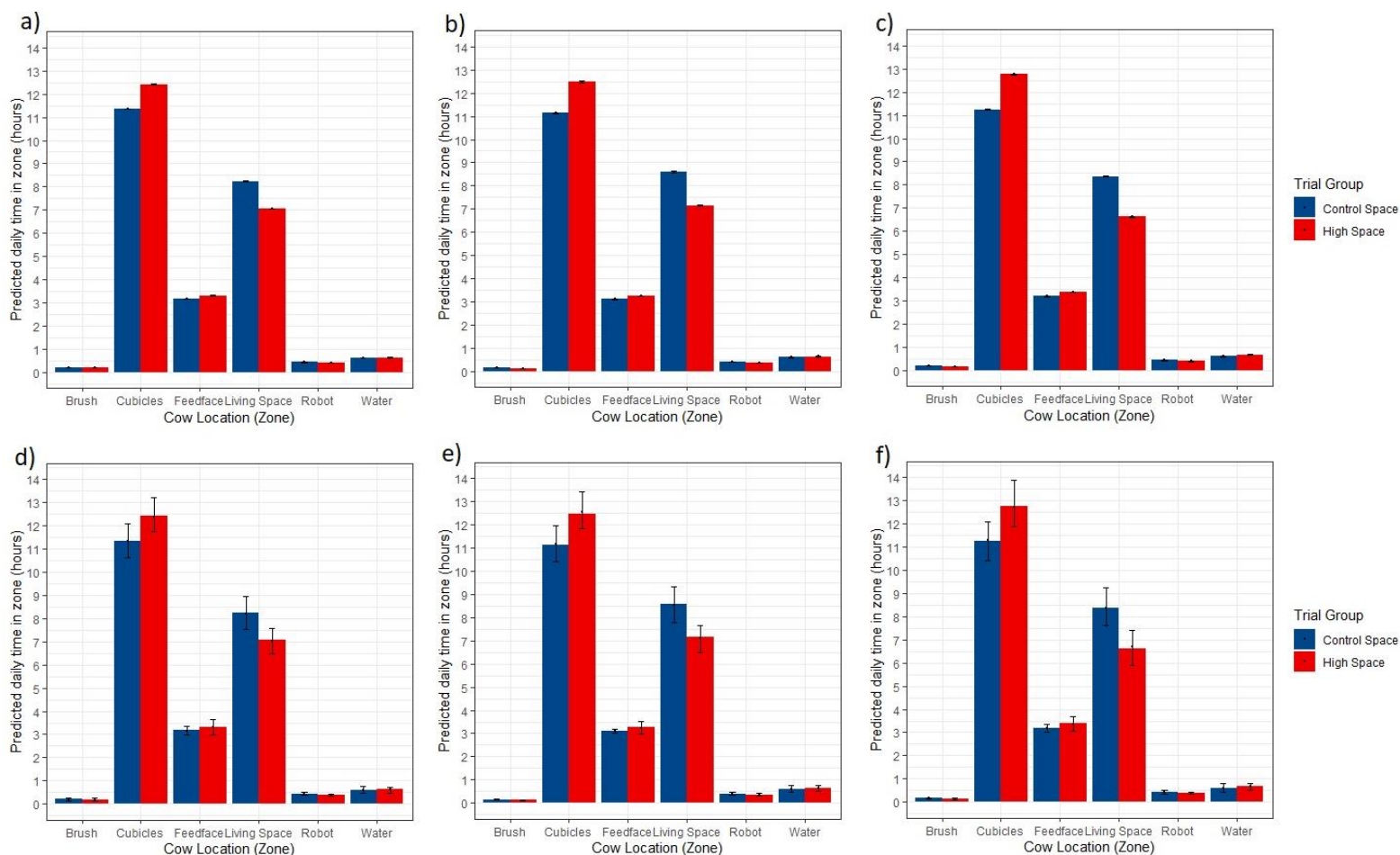


Figure 6.5. Boxplots to illustrate the estimated time budgets for cows in each trial group during the sampling period to allow comparisons between the subsets of data (Red: High space group; Blue: Control space group). The error bars (black lines) represent the 95% confidence interval around the observed key zone time budget, based on bootstrap method. The dataset and bootstrap method for each graph is as follows: a) Full dataset with row level bootstrap; b) Matched cow pair time period data timepoint, bootstrap by row; c) Full coverage cow pair data only ($n = 5$), bootstrap by row; d) Full dataset with cow level bootstrap (final outcome result); e) Matched cow pair time period data, bootstrap by cow; f) Full coverage cow pair data only ($n = 5$), bootstrap by cow.

6.4 Discussion

This chapter has identified key changes in dairy cow behaviour, measured by daily time budgets, that occurred with an alteration of living space availability. Whilst these changes to a cow's daily behaviour were not unexpected, the magnitude of the differences was surprising. Given there were no differences in cubicle design, location or stocking density, an observed increase in daily lying time of more than one hour per day shows the large effect additional living space had on cow behaviour. Cows in the high space group also spent nearly one and a half hours less in passageway areas even though they had greater access to these areas. The results demonstrate that cows use the facilities available to them differently dependent on living space availability and that restrictions in one area (for example narrower passageways) may cause changes in use of other areas (for example cubicles) even when these other areas themselves do not change.

A key point of difference in welfare often cited between cows housed or at pasture is the space to exhibit normal behaviours (Arnott et al., 2016; Crump et al., 2019; EFSA, 2009; Wierenga et al., 1991). Increased lying times are generally considered an indicator of enhanced welfare (J M Huzzey et al., 2012; Plesch et al., 2010; Vasseur et al., 2012) and time in passageway areas could represent difficulty of free movement when less space is available (DeVries et al., 2004; Kondo et al., 1989; Telezhenko et al., 2012; Templeton et al., 2014). Therefore the behaviour changes associated with additional living space in this study may represent enhanced welfare for the cattle and could be viewed positively by consumers and citizens who deem the welfare of housed cows an important issue (Jackson et al., 2020; Lillford and Hermansson, 2020). Indeed, beyond improvements to productivity and financial returns, the expectations of the general public regarding the ethical state of dairy cow farming must be met in order to maintain a societal licence to continue (Ventura et al., 2016) and increased living space may be one route to address such ethical concerns.

Furthermore, these changes in lying times are likely to be linked to the increased rumination and milk volume production in the same group of animals as previously

discussed in Chapter 4. Associations between lying times and rumination have been extensively reported as rumination is most likely to occur whilst a cow is lying down (Schirrmann et al., 2012). Additionally, although not specifically studied in this trial, there are likely to be further important effects on dairy cow health as a consequence of the increased lying times and reduction in resource competition. For example, increased lying times and the reduction of time spent in passageways are linked to a decreased risk of lameness in dairy cows (Solano et al., 2016). It is known that increased pressure on the solar corium of cows' feet predisposes to claw horn disease (Randall et al., 2018). Thus, reducing the time spent standing on concrete by providing more living space is likely to reduce lameness incidence in a herd. Additionally, in terms of health, providing greater living space through wider passageways will help to reduce the accumulation of slurry in the environment. This should result in a more hygienic environment and thus less infection pressure for example for digital dermatitis within slurry pooling (Endres, 2017; Somers et al., 2003) and cleaner cows which has been linked to reduce mastitis incidence (Breen et al., 2009).

It should be noted that results of the behavioural analyses in this study were only on a subpopulation of 16 cows out of a group of 35 per trial group, for 5 months of the 12-month study. This is of importance because there have been reports to suggest that individual cow behaviours can be highly varied in terms of activity, and to a lesser extent also influenced by time since calving, pregnancy status, parity and health (Müller and Schrader, 2005). Therefore, depending on the social hierarchy of each trial group, it could be that some behaviours were missed or over represented, for example it is known that less dominant cows are more likely to spend time in loafing areas (Haskell et al., 2013). A baseline assumption of the study was that the behaviours of the subpopulation of 16 cows was representative of the whole group. Furthermore, there were some missing data due to sensor connectivity and battery life, although analyses of different subsets of data did not yield different results suggesting that the missing data did not cause serious bias.

A final limitation of the study was that behaviours within the specified zones were implied rather than directly observed (e.g. the feed face zone indicated feeding and the

cubicle zone indicated lying). For example, lying times were based on sensors detecting an X,Y co-ordinate for the front region of the cubicle zones, rather than detection of a lying behaviour specifically. Therefore, there was potential for some of this data to be of alternative behaviours such as perching or standing in cubicles. This is negated by the fact that the cubicles were designed based on gold standard specifications for dimensions and deep-sand bedded to enhance comfort characteristics. Consequently, the time budgets observed do not have the detail of specific behaviours or, for example, how cows interacted with their pen mates (i.e. social hierarchy) and lying versus other behaviours in cubicles. Despite these limitations and lack of granularity of data, the differences observed in zonal time budgets provides strong evidence that the housed environment greatly affects cow behaviour and suggests additional research on this topic would be beneficial to improve the understanding of how this might be used to enhance cow wellbeing.

6.5 Conclusion

In conclusion, the results observed for the daily time budgets between trial groups suggest that an increase in living space allows cows easier access to the resources that they prioritise (lying areas) and consequently reduces the amount of time they need to spend navigating resulting in a reduction of time spent in passageways.

Chapter 7. An economic evaluation of increased living space for adult dairy cows

7.1 Introduction

Results from Chapters 4 and 5 suggest that financial returns associated with increased milk production and increased time to conception when cows were given greater living space are worthy of consideration. The results observed in these previous Chapters were further explored using simulation modelling to investigate plausible economic differences between the two living space scenarios. Reproductive performance, in terms of delayed time to conception, has been associated with negative impacts on financial returns due to cows being delayed in return to peak lactation (Britt, 1985). However, the increased milk yield, which has been shown not be associated with the reproductive differences in this study, could increase profitability. Therefore, to explore the economic interplay between the increased milk production and poorer reproductive performance observed in Chapters 4 and 5, theoretical simulation modelling was undertaken.

Finances are important for sustainability and, in particular, the costs involved in building or adjusting dairy cow housing can be substantial. The large capital investment required to fund new buildings is important, as if this investment does not create an optimal environment for its inhabitants, this could be a limiting factor for outputs that can be achieved. Thus, further investment into options such as improving genetics and nutrition, could be wasted if the housed environment remains a limiting factor. The financial implications of providing increased living space are important because the investment is substantial. Therefore, an assessment of the required building investment in relation to any expected additional income from the provision of additional living space was also explored in this chapter.

The aim of this chapter was to investigate the theoretical financial differences between the two living space scenarios defined in Chapter 3, based on results from Chapters 4 and 5. A secondary aim was to place this in the context of the investment required to build or modify cow housing to provide the additional space.

7.2 Materials and methods

7.2.1 Model overview

The aim of the modelling process was to simulate lifetime milk yields and financial returns for cows given one of two space allowances, 14m² or 9m² (Living space: 6.5m² versus 3m²), based on the production and reproduction models in Chapters 4 and 5. Using predicted lifetime milk yields and specific associated production costs, a financial benefit associated with each space allowance was derived for all cows. Since lifetime yield depends on the length of time a cow spends in the herd, differences in times of removal were incorporated in the model. The two scenarios considered for a cow to be removed from a herd were i) to be culled at the end of a lactation or ii) to die on farm. A “random death” was defined as a cow dying on farm or being euthanised with no value due to a problem such as disease or injury and could occur at any point in life. When the term “culled at end of lactation” was used, this was based on a farm management decision (such as reproduction failure, age, or chronic disease) to remove the cow once her current lactation had finished (which would account for the majority of dairy cow removals in the UK). The basis of the model structure is illustrated in Figure 7.1.

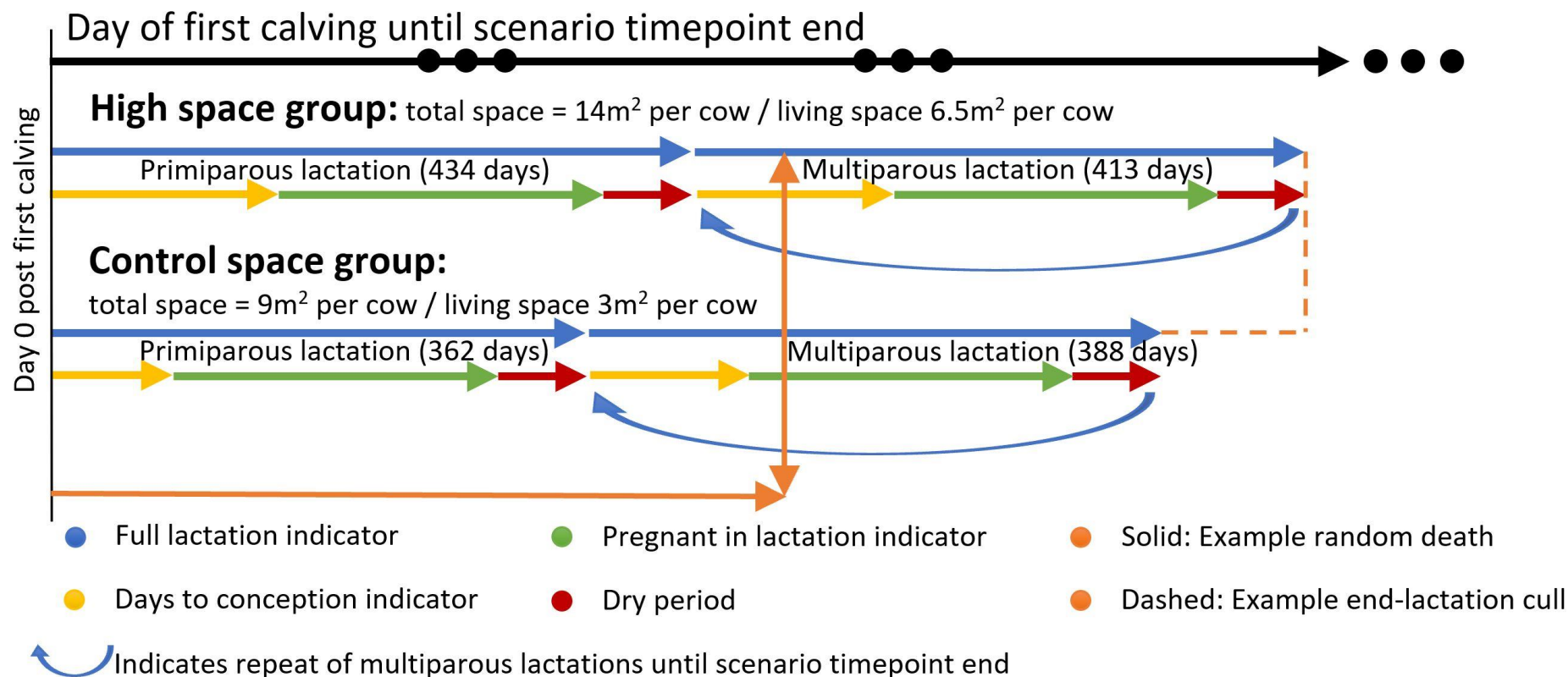


Figure 7.1. Schematic to illustrate the underlying principles of the simulation models. The black line represents a continuous time scale over which events occur post first calving. The respective blue lines show the time frame of full calving intervals (dependent on time to conception results in Chapter 5), which are made up from yellow (time non-pregnant in lactation), green (pregnant in lactation) and red (dry period) lines. Cow lactations are continually repeated until the end of the simulated scenario is reached. The solid orange line represents the time of a random death and indicates that the respective cow in the high space and control space groups would be at different stages of a lactation. The end of lactation cull scenario is represented by the time difference between the end of a control space group lactation and a high space group lactation (dashed orange line), the cow being immediately replaced with a newly calved primiparous cow.

7.2.1.1 Model set up and input variables

Model building was undertaken in R statistical software, version 3.6.1. R packages tidyverse (Wickham, 2019), lme4 (Bates et al., 2015), minpack.lm (Elzhov et al., 2016) and gridExtra (Auguie, 2017) were used.

The models incorporated outputs from the reproduction survival analysis (Chapter 5), which evaluated time to conception according to space allowance, and the milk volume prediction curves, which described the milk yield by DIM and days pregnant (DP). Multiple theoretical cow lifetimes were simulated such that herds of 100 cows were repeatedly generated. The main basis of the model was prediction of daily milk volume for all cows, which was achieved using predictions from the final mixed effects model described in Chapter 4. Additional assumptions were necessary, and these are described at relevant points below.

Initial assumptions included that all animals would have an exact gestation period of 280 days of which the final 60 days would be a dry period. Based on the median times to conception estimated in Chapter 5, the days to conception for different groups of cows were as follows. Control space (9m²) primiparous = 82 days, high space (14m²) primiparous = 155 days, control space multiparous = 108 days and high space multiparous = 133 days.

Economic values were set for factors that would affect the outcome of interest which was termed the “financial return”. For the purposes of this chapter, the term “financial return” relates to the margin of income per cow per year over purchased feed. Economic values (e.g. milk price) were set to be the same for both trial groups and in all model scenarios. Only economic values that were necessary to calculate the difference in financial return between the two living space scenarios were used in the modelling. Values that were not deemed to be directly dependent on living space provision (e.g. income from a cull cow sale) were assumed to be identical between scenarios. Thus, the financial return estimates are the predicted differences of margin over purchased feed between the two living space scenarios and are not absolute values.

Milk margin was based on the average margin over purchased feed in the UK using data published by Promar milkminder costings between July 2017 to January 2021 and set as 20.49ppL income margin per litre of milk over purchased feed (Promar, 2021).

Since a key difference between the living space scenarios being considered was the calving interval and hence the number of calvings in a lifetime, the cost of a transition and calving period was incorporated in the models. The cost of a transition period was calculated using figures provided by Liang (2017) for common transition diseases (incl. metritis, mastitis, ketosis, lameness, dystocia etc.) based on stochastic modelling (Liang et al., 2017), values are provided in Table 7.1. These costs per disease incident originating from the transition period were combined with estimated disease incidences appropriate for UK dairy farms (Dobson and Esslemont, 2002). Calculations of costs are provided in Table 7.1. For primiparous cows the mean cost of a transition period was estimated at £64.86 per cow per transition and for multiparous cows at £99.08 per cow per transition. These figures were inclusive of the death risk associated with transition cow disease and thus incorporated the financial aspect of cows being at higher risk of disease and death at this time.

Table 7.1. Table to describe the costs expected from transition periods which were used to calculate the expected cost of transition period per cow used in the simulation model.

Parity group	Disease	Total costs (\$) (<i>Liang 2020</i>)	Total cost (£)	Assumed disease incidence	100 cow farm cost
Primiparous	Mastitis	\$325.76	£254.09	15	£3,811.39
	Lameness	\$185.10	£144.38	10	£1,443.78
	Metritis	\$171.69	£133.92	4	£535.67
	Retained placenta	\$150.41	£117.32	1	£117.32
	Left-displaced abomasum	\$432.48	£337.33	1	£337.33
	Ketosis	\$77.00	£60.06	4	£240.24
	100x primiparous cost				
Cost risk per primiparous transition					£64.86
Multiparous	Mastitis	\$426.50	£332.67	15	£4,990.05
	Lameness	\$333.17	£259.87	10	£2,598.73
	Metritis	\$262.65	£204.87	4	£819.47
	Retained placenta	\$313.49	£244.52	1	£244.52
	Left-displaced abomasum	\$639.51	£498.82	1	£498.82
	Ketosis	\$180.91	£141.11	4	£564.44
	Hypocalcaemia	\$246.23	£192.06	1	£192.06
100x multiparous cost:					£9,908.08
Cost risk per multiparous transition:					£99.08

7.2.2 Model building

7.2.2.1 Initial single time frame model

To explain the underlying predictive mechanism of the model this section describes how outputs were calculated for cows that left a herd at a single, defined time point. For this example, the cow lifetime after first calving was set at 1460 days (4 years). The model started with a cow at first calving in each living space group and subsequent milk production and fertility were simulated over the 1460-day period independently. The model

incorporated variables required for the milk volume mixed effects and time to conception survival models described in Chapters 4 and 5 to predict daily milk yield, time to conception, time to drying off and time to subsequent calving. This provided an estimate for milk production on each day of a cow's life for 4 years. The daily predicted milk yield was cumulatively added to produce an estimate of the lifetime production of a cow within the 4-year timeframe. The milk volume was calculated and multiplied by milk price margin (20.49ppl) to find total income of for each cow in the two living space scenarios. The number of dry periods that occurred in the time period were calculated and multiplied by the assumed transition period cost (£64.86 for primiparous cows and £99.08 for multiparous cows). This transition cost was subtracted from the milk income value to estimate a mean financial return for each living space group. This single time frame simulation is illustrated in Figure 7.2.

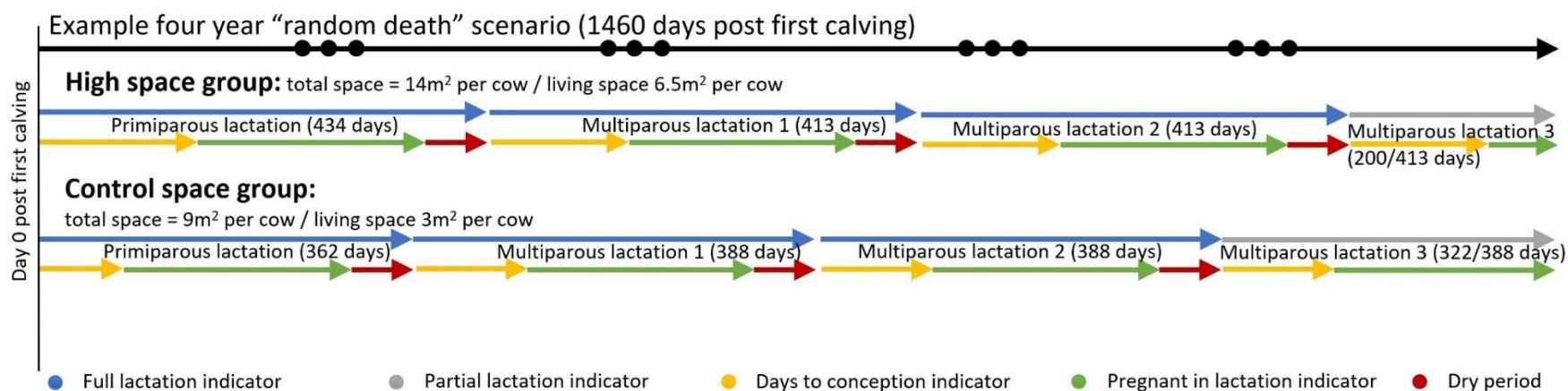


Figure 7.2. Schematic to illustrate reproductive differences between the high space and control space trial groups in the example of a random death at 1460 days post first calving. The cow in each trial group would have three full lactations (blue line: 1x primiparous cow, 2x multiparous cow) and would reach a different point in their fourth lactation (grey line).

7.2.2.2 Random death scenarios

For each farm simulated, a proportion of cows were assumed to have died (or have been euthanised) on-farm with no value. The term “random death” was used to signify this event. To explore the potential differences in financial return depending on the proportion of random death events that occurred, a prediction was made for death on each day from 1-3650 days (10 years) after a first calving.

The random death scenario was assumed to occur in addition to events related to the transition period, which were already represented in the transition cost. Thus, the transition costs were factored into the financial return independently, and the additional random deaths represented events that could occur over and above those associated with transition and calving.

For a cow that randomly died on a specific day, a difference in economic return for that cow was estimated dependent upon which living space conditions were in use. For example, if a cow died on farm 3000 days post first calving, the difference in financial return was estimated for that cow for both high and control living space conditions. Financial outcomes assessed for cows that succumbed to a random death were total financial return per lifetime, total financial return per day of life post first calving and financial return per cow per year.

To provide an estimate of an average value of the financial return from a random death in each living space group, a subsampling procedure was used. The probability of a random death was assumed to be equal throughout a 3650-day period after first calving and the mean cost (and 95% confidence interval) estimated from 100 deaths generated randomly for each of 1 million theoretical farms. To achieve this, repeated subsampling with replacement was undertaken to randomly generate 100 deaths for each farm and the mean financial returns from these deaths per farm was calculated. This procedure was repeated for 1 million herds for each living space scenario and used to calculate a distribution of the mean return attributable to a random death in both scenarios. The difference in the financial return

from a random death between the two living space scenarios was calculated as the median difference between of the mean values estimated for each scenario.

Sensitivity analysis was undertaken to explore whether the cost associated with transition disease impacted on the financial return for the random death scenarios. The transition cost was analysed at double and half the reported cost of £64.86 for primiparous cows and £99.08 for multiparous cows.

7.2.2.3 End-lactation cull scenarios

The majority of adult cows that leave a dairy unit do not die on farm but are culled at the end of lactation based on a management decision such as chronic lameness, mastitis or reproductive disorders. These cows do not fit the above random death scenario and thus alternative scenarios were constructed for end of lactation culls. This part of the model aimed to include likely financial outputs of herds with different culling strategies based on the financial returns of cows being culled at the end of lactation. However, due to reproductive differences between space allowance groups, cows could be culled at different points in time and this difference is accounted for in the model. A baseline assumption used for all scenarios was that every cow culled at the end of a lactation would be replaced immediately with a newly calved primiparous cow (Figure 7.1).

It was considered that a cow could be culled at the end of any lactation (to a maximum of lactation 10) and the financial returns for cows culled at the end of different lactations were estimated for both living space groups. The scenarios investigated were:

- Timepoint 1: One non-pregnant primiparous lactation
- Timepoint 2: One pregnant primiparous lactation + one non-pregnant multiparous cow lactation
- Timepoints 3-10: One pregnant primiparous lactation + n^{2-8} pregnant multiparous cow lactation + one non-pregnant multiparous cow lactation

In order to make comparisons between the living space groups, the following assumptions were made:

- If there was a time difference between living space groups in a cow being culled (that is, the end of the n^{th} lactation did not coincide temporally), the cow culled soonest was immediately replaced by a first lactation primiparous
- Cows did not become pregnant in the lactation in which they were culled and the milk yield for that lactation was predicted for a non-pregnant cow throughout lactation
- Cows were culled at 305 days of lactation

The financial return for each end of lactation cull was calculated as described in section 7.2.2.1, and differences between living space groups estimated.

To evaluate the impact on financial returns of different culling/replacement strategies in 100 cow dairy herds, a simulation procedure was used. Cows were randomly assigned to a time of culling that could occur at the end of any lactation, with equal probability. Initially each herd was assigned to have a maximum number of lactations per cow (to reflect a general difference in culling policy); this was set at a maximum of between 4 and 10 lactations. Therefore, a herd with a maximum of 10 lactations per cow would have a mean number of 5.5 lactations per cow and a herd with a maximum of 4 lactations, would have a mean number of 2.5 lactations per cow. A simulation was run seven times for 20,000 herds for each living space scenario, with the maximum number of lactations set at 4, 5, 6, 7, 8, 9 and 10 respectively per run. Therefore, data for a total of 280,000 herds were generated. From these data, the difference in financial return per cow per year between the two living space groups was calculated in herds for which the maximum number of lactations was between 4 and 10, resulting in 140,000 individual financial return costing differences between living space allowances dependent on cull-scenario explored.

Sensitivity analysis of transition costs were not performed in this dataset as it was assumed that both scenarios would have the same number of dry periods and therefore the same associated costs.

7.2.2.4 Final model

A final model was produced by combining the culling and random death models described above, to evaluate what could occur in herds with different living space allowances, percentage of “random deaths” and herd culling rates. The structure of the model is illustrated in Figure 7.3.

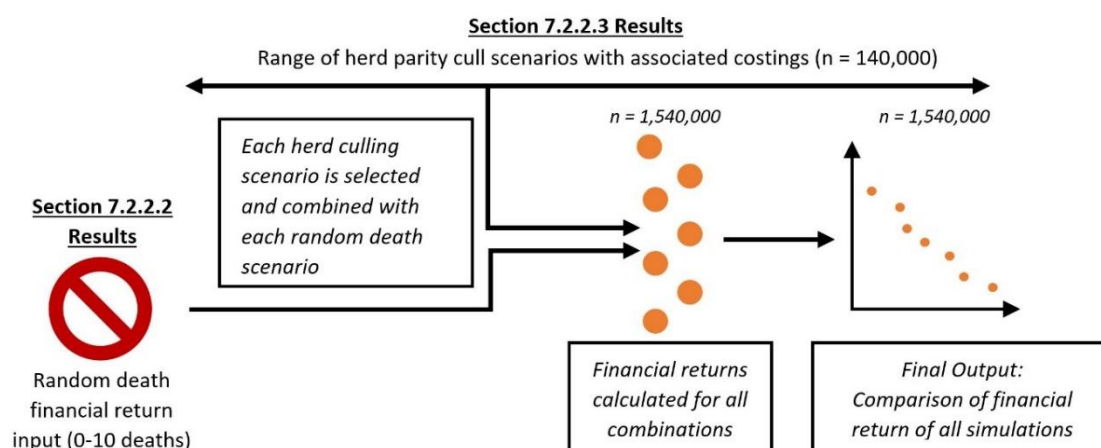


Figure 7.3. Schematic to illustrate how the simulation model linked the random death scenarios with the end-lactation cull scenarios to produce the 1,540,000 simulations. Top: the range of herd culling scenarios and associated financial returns were calculated as stated in section 7.2.2.3. These were duplicated 11 times and combined with the random death associated costs (as calculated in section 7.2.2.2) from 0-10 deaths a year (left). This produced the 1,540,000 scenarios (middle), which were visualised in the final graph (right).

The 14000 end-lactation cull herd scenarios were used as the base for financial calculations and modified with the addition of random deaths on farm. The financial impact of changing the number of random deaths from 0 to 10 cows per year was explored. The financial return for a random death was taken as the median financial return calculated in

section 7.2.2.2 multiplied by the number of “random deaths” occurring in the herd scenario. Random deaths were considered to occur with equal probability across all lactations.

The difference in financial return between living space groups, dependent on herd culling and random death scenarios, was calculated from overall financial margin (described in section 7.2.2.1) divided by the associated time period to obtain a financial return per cow per year for the herd scenario. This figure equated to the difference in financial return per cow per year between herds with higher compared to lower living space provision.

7.2.2.4.1 *Sensitivity analysis: margin over purchased feed per litre of milk*

A sensitivity analysis was conducted to explore how estimated financial returns may be impacted by margin, as milk price margins are known to fluctuate. The simulation was re-run with four new milk margins over purchased feed (16, 18, 22 and 24 pence per litre) to compare with the current margin over purchased feed per litre of milk in Great Britain at 20.49 pence per litre. Graphs were generated as described in 7.2.2.4 to allow direct comparison between model outcomes.

7.2.3 Cost-return scenarios

To supplement the estimated financial returns described above, an estimate was made for the building costs associated with providing extra space for lactating dairy cows (i.e. for the difference in space provision between the higher and lower space scenarios). It was assumed that that additional space could be provided either as a new building or through extension of an existing building. Costs for both of these circumstances are considered below.

7.2.3.1 New build

To estimate the cost of building a new housing facility, with varying space provision, the AHDB housing wizard calculator was used (AHDB, 2021). Total floor space allowances of 9m² and 14m² per cow were used to estimate costs. A scenario was set using a 100-cow herd housed for 365 days per year on bedded on sand cubicles. Costings were undertaken to assess a 2-cubicle row and 3-cubicle row system with feed-face; from the wizard, the cost per m² for a 2-row cubicle shed was set at £140 and for a 3-row cubicle shed £145 per cow. These are base prices for the building shell and flooring. This estimate does not include, cubicle or other internal infrastructural costs but these would be assumed to be the same between trial scenarios as the only difference was total floor area.

The cost of the two scenarios were similar based on the estimates found from the research for this costings calculator, which is to be expected given the internal infrastructure would be the same between two scenarios but the dimensions of the building would change from longer and narrower for a 2-row system compared to shorter and wider for a 3-row system. Therefore, the costing of the 2-row system was used as a comparator as this was the set-up used within the randomised controlled trial. These costs were multiplied by the corresponding space allowances, either 9m² or 14m² per cow. The AHDB calculator separated the cost of housed infrastructure from bedded area and additional features (such as galebreakers, lighting and autoscrapers). The bedded-area and additional features were assumed to have identical costs for both space allowance scenarios. For example, each group would have 1.1 cubicles per cow and therefore the cost of this would be identical between groups.

In reality, farmers often pay for capital investments over prolonged time periods and the cost of these investments was converted to an expected annual repayment with a loan interest rate set at 2%. These repayment costs were compared to the estimated financial returns derived from the final financial returns model to estimate an approximate return on investment.

7.2.3.2 Building extension modification

In this section the cost involved in providing additional living space through modification of a building from a 3m² per cow living space allowance to a 6.5m² is assessed. Two approaches were considered as follows.

The first modification was a building extension to provide an indoor “loafing area” (an open area of concrete) to increase living space allowance. An AHDB housing report (AHDB, n.d.) indicated a general-purpose building steel frame, roofed building with concrete floor would cost £185 per m². This cost appears to be greater on a square metre basis to a new build due to the complexity involved with shed modification in terms of removal of part of existing infrastructure to replace with new materials to allow for joining of the existing building with new extension.

An alternative method of increasing living space from 3m² to 6.5m² was to add 3.5m² of additional outdoor “loafing area” (an open area of concrete). The Agricultural Budgeting and Costing book for UK agricultural buildings (Agro Business Consultants, 2019) provides an indicative cost of a fenced, concrete based, outdoor area, to be in the region of £77.50 per m² per cow.

The cost for increasing the area for both modifications was calculated for a 100-cow herd. The capital investment required was converted to an expected annual repayment, as described for construction of a new building above.

7.2.3.3 Sensitivity analysis: interest rates and amortisation period

For each building scenario investigated, the investment required was tested through sensitivity analysis. Each scenario was testing for interest rates of 0, 1, 2 and 3% APR over a amortisation period of 10 or 20 years. Each of these costings was then compared to the average financial return obtained from the simulation model for 2.5-4 lactations and the UK average cow lactation of 3.7, to estimate the likely net return on investment due to increased milk volume production and reduced time to conception for cows inhabiting the higher living

space scenario, dependent on the cost difference to provide the extra space in comparison to the control space group.

7.3 Results

7.3.1 Single time frame model

Figure 7.4 illustrates model estimates of daily milk yield for cows with 9m² or 14m² total space allowance during 1460 days after first calving. Over this time, in which cows have three dry periods, a cow with 9m² total space is predicted to have produced 57,029L compared to the same cow given 14m², which would have produced 57,806L.

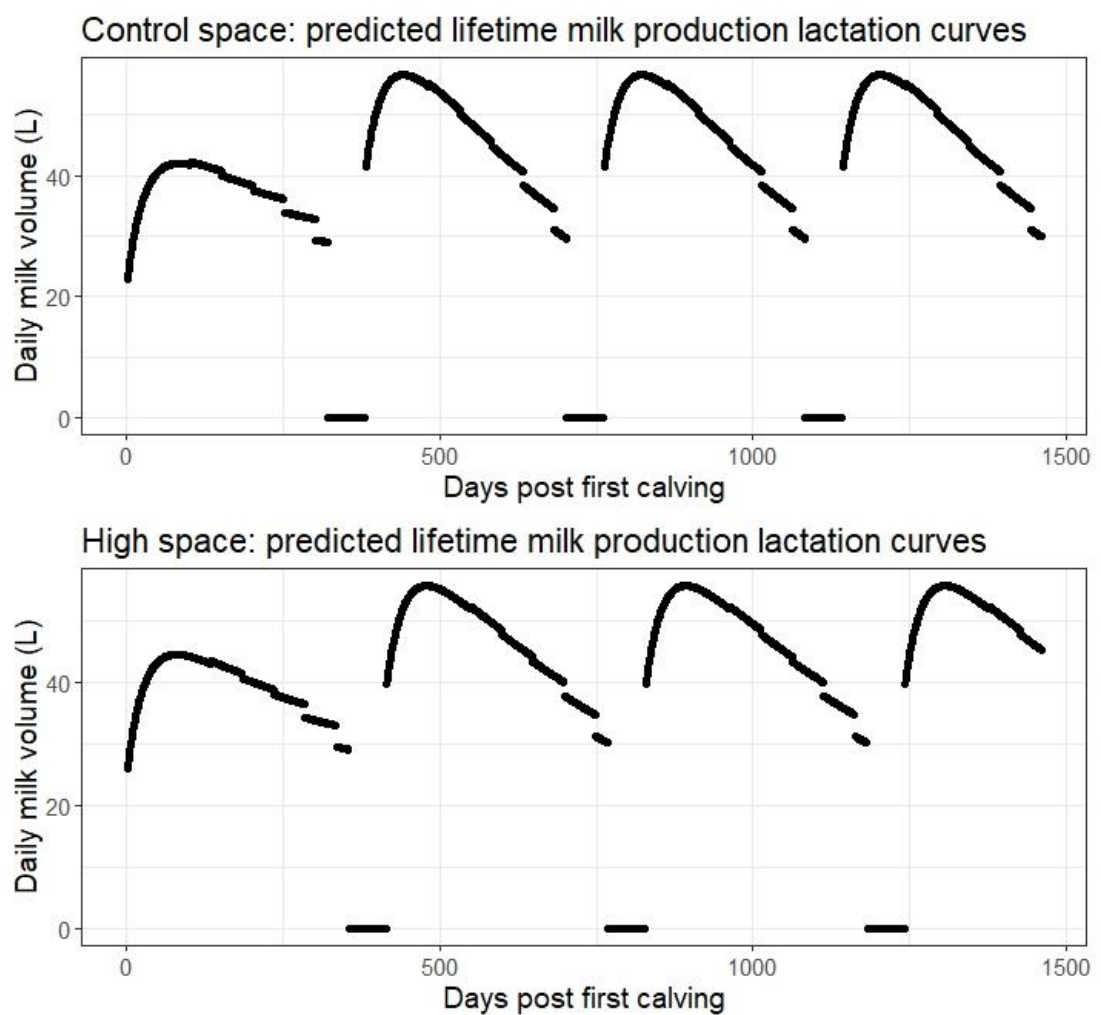


Figure 7.4. Plot to illustrate the respective lifetime milk volume production curves in litres (y-axis) for the 1460 days (x-axis) of the example random death scenario, between the control space group (top) and high space group (bottom). When cows are in the dry period zero litres of milk produced which are represented by the horizontal lines at 0L on the y-axis.

7.3.2 Random death scenarios

Results of the model to estimate financial return associated with cows that randomly died are illustrated in Figure 7.5. The sinusoidal shape of the financial returns and alternating financial benefit between groups reflects different timings of the dry periods for each group of cows due to the differences in reproductive performance. For each day that a random death could occur (between 0 and 3650 days after first calving) the financial return was estimated and for 63.3% of these days, cows in the high space group had a higher return than those with less space.

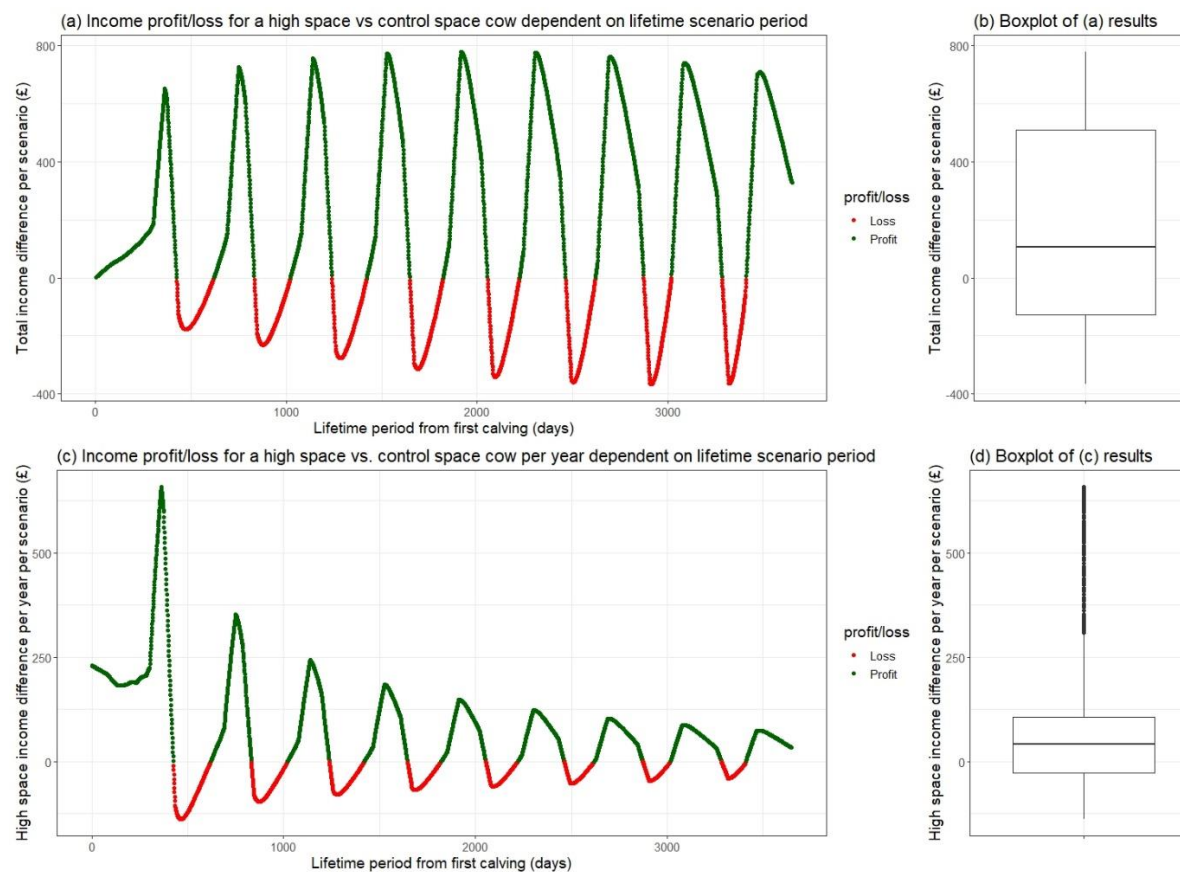


Figure 7.5. Distribution of the estimated financial returns associated with 3650 unique random death scenarios that occurred between 1 and 3650 days after first calving in both trial groups. The outcomes depicted in green are when the time of a random death was more profitable for the high space group (and in red for the control-space group). Top-left (a): the estimated financial outcomes per scenario. Bottom-left (c): the outcome is converted to a financial return per cow per year per scenario. Right: the corresponding boxplots to show the distributions of the financial outcomes shown in (a) and (c) respectively. The median value is represented by the midline with 25th and 75th percentiles as boxes. The whiskers show data within 1.5x interquartile range, with outliers shown as distinct points.

The resampling analysis to calculate a financial return dependent on when random deaths occurred revealed that the median financial return was £112.78 (95% confidence interval: £180.52-£249.02) greater for the high space group compared to the lower space group. When this was calculated as an income per year, the median difference was an increase in the high space group of £57.52 (95% CI: £35.53-£81.93). These figures indicate that for the majority of times when a random death could occur, cows in the high living space group would provide a higher financial return than those in the control space group.

7.3.2.1 Sensitivity analysis: transition disease cost

Further exploration of the impact of transition disease costs in the random death models indicated that if the transition cost per cow was doubled to £129.72 for primiparous cows and £198.16 for cows the financial return difference per year was +£57.52 (CI: 35.51-81.96) per cow for high space individuals, and expected total income difference was +£186.88 (CI: 117.80-257.00) per cow in the high space group. When transition costs were halved to £32.43 for primiparous cows and £59.54 for cows, income difference per year was calculated to be +£57.53 (CI: 35.50-81.91) per cow for high space cows, and expected total income difference was £170.70 (CI: 104.79-237.18) per cow in the high space group.

7.3.3 End of lactation cull scenarios

Results for financial differences between living space groups for culling at each timepoint between 1-10 lactations are shown in Table 7.2. Cows with 14m² were always more profitable than cows with 9m² when culled at the end of a lactation with a range from £23.92-£156.67 per year in milking herd as shown in Table 7.2. In general, the earlier in life a cow is culled the greater the difference between living space allowance groups.

Table 7.2. Results of the financial calculations for the end of lactation culling scenarios, based on the number of lactations a cow spent in the herd. The increased total financial return for high space cows is the calculated as the increase in milk margin over purchased feed (minus transition risk costs) for milk produced by cows in the high space compared to control space group. The increased financial return per annum for high space cows is the increase in total financial return rescaled to represent a return per year of a cow's life.

Parity when culled	Milking herd lifetime duration (days)	Control space income/cow	High space income/cow	Increased total financial return for high space cows	Increased financial return per annum for high space cows	Increased financial return per m² per annum for a high space cow
1	305	£2,375.20	£2,506.95	£131.75	£157.67	£31.53
2	739	£5,823.78	£5,956.82	£133.04	£65.71	£13.14
3	1151	£9,069.09	£9,205.05	£135.96	£43.11	£8.62
4	1563	£12,313.65	£12,453.27	£139.62	£32.61	£6.52
5	1975	£15,552.98	£15,701.50	£148.52	£27.45	£5.49
6	2387	£18,786.55	£18,949.72	£163.17	£24.95	£4.99
7	2799	£22,014.50	£22,197.95	£183.45	£23.92	£4.78
8	3211	£25,236.38	£25,446.17	£209.79	£23.85	£4.77
9	3623	£28,451.19	£28,694.40	£243.21	£24.50	£4.90
10	4035	£31,655.67	£31,942.62	£286.95	£25.96	£5.19

7.3.4 Final simulation model results

Results of the final model to explore multiple scenarios of culling strategy and random deaths are presented in Figure 7.6. For scenarios evaluated, herds with increased living space had greater financial returns. The range for increased gross financial return was £26.82 - £64.77 per cow per year (equating to £5.36-£12.95 per square metre per cow per year, for the 5m² difference of the two scenarios). Variation in return was dependent more on culling strategy than the level of random deaths, although the latter had more of an effect on finance return when cows were older at culling. In many high yielding units, an average cow will exit the herd after 2.5 to 4 lactations. Results from this model suggest that such cows would provide an increased gross financial return of between £33.75-53.34 per cow per year if provided with a total space of 14m² rather than 9m² (equating to £6.75-£10.69 per square

metre per cow per year, for the 5m² difference of the two scenarios). The median lactation number at exit for UK dairy cows is currently 3.7 (Hanks and Kossaibati, 2019) which equates to a difference of £35.97-£39.76 per cow per year, dependent on the level of random deaths (equating to £7.19-£7.95 per square metre per cow per year, for the 5m² difference of the two scenarios).

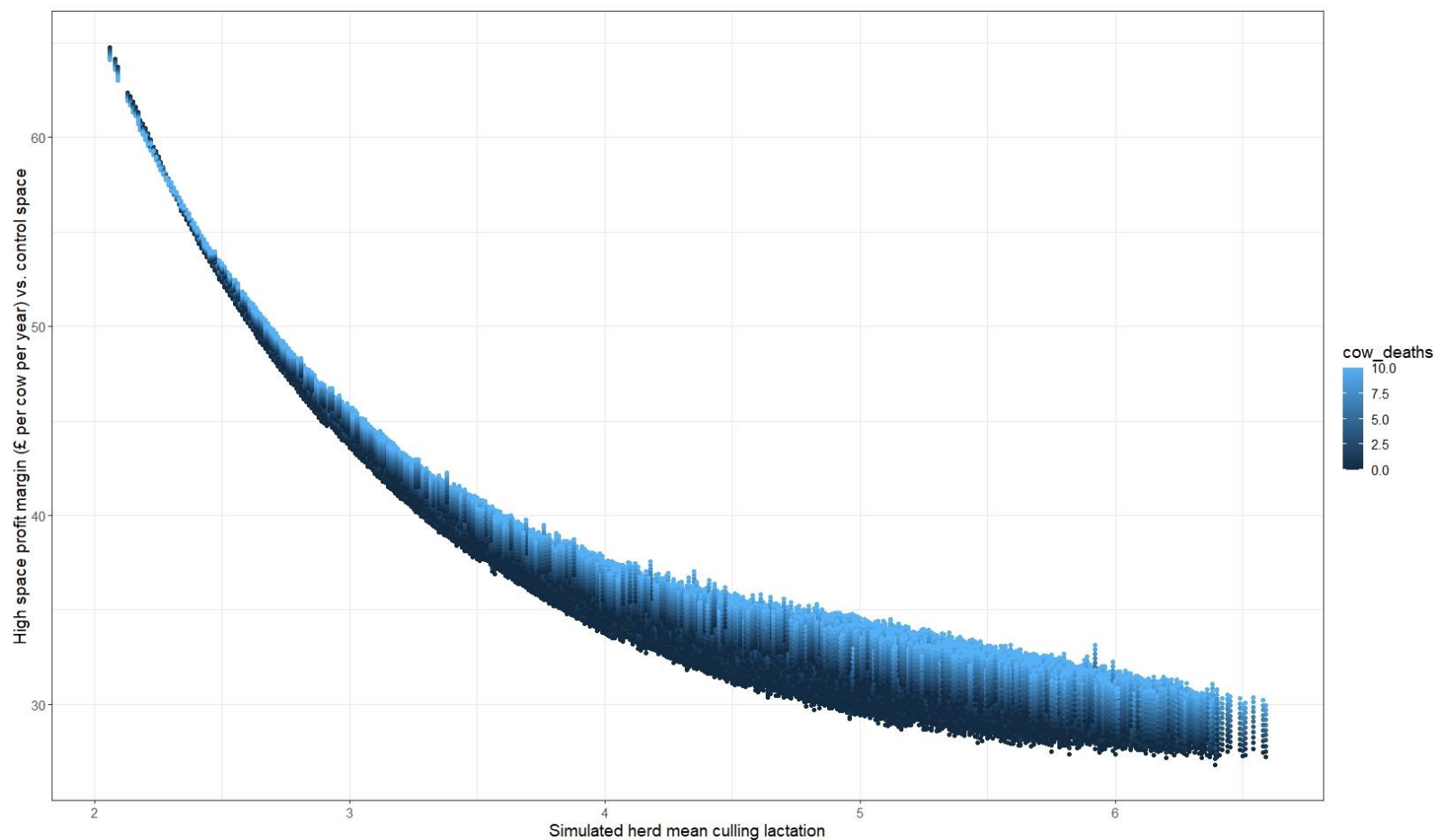
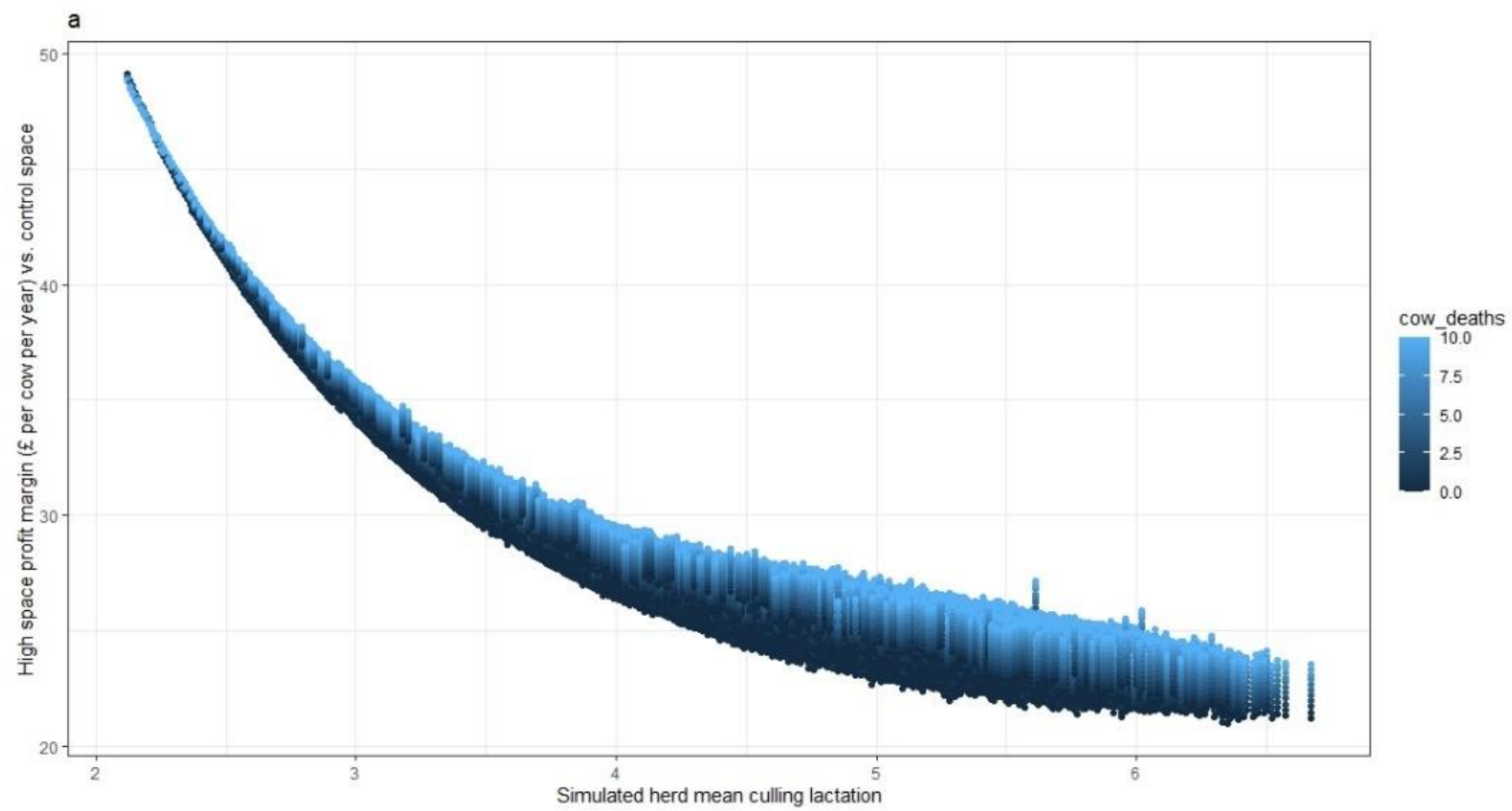


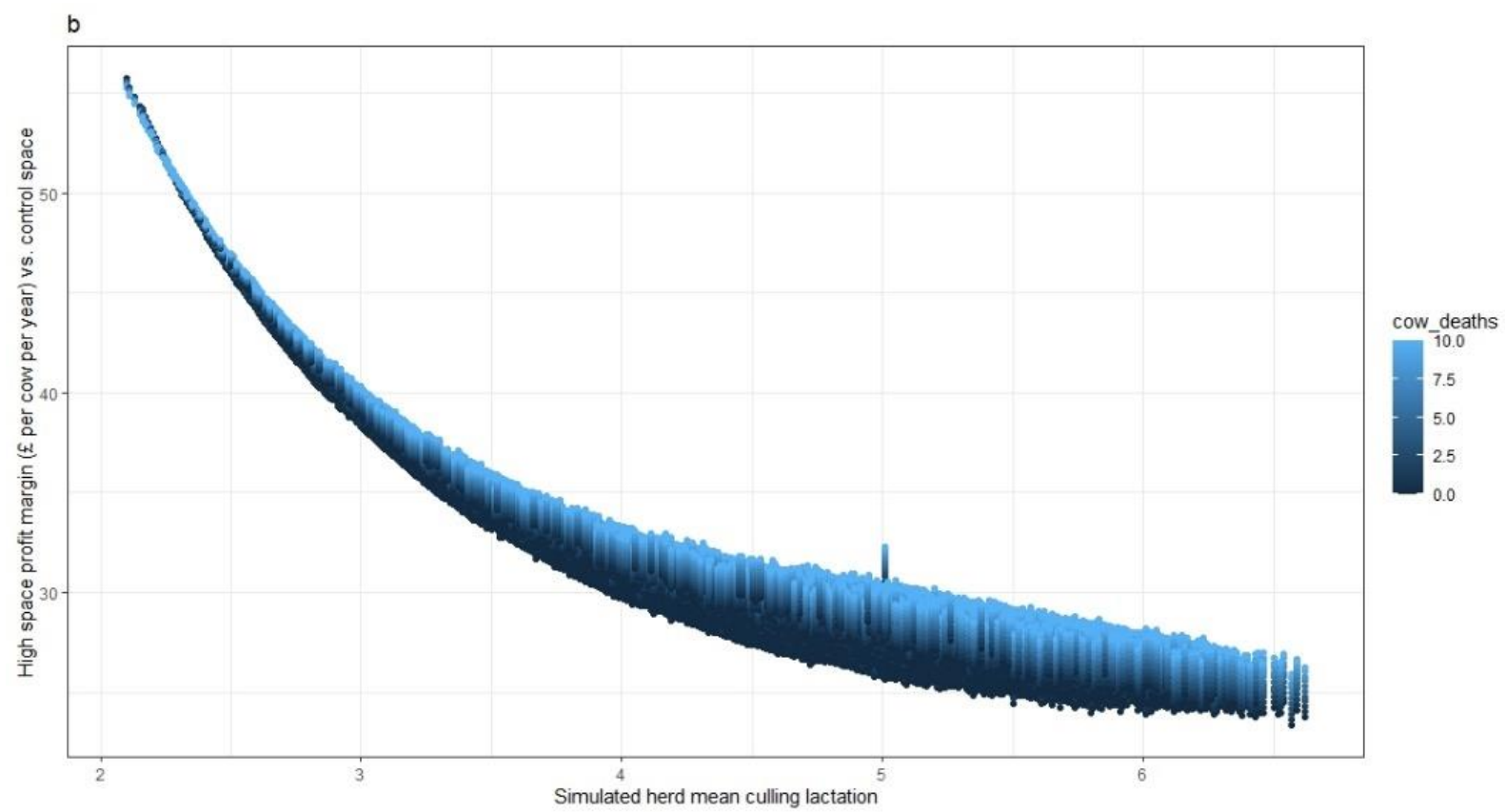
Figure 7.6. Scatterplot to depict the possible financial outcomes across all model scenarios. The y-axis represents the expected financial return per cow per year for cows in the high space group compared to those in the control space group. The x-axis represents the herd's mean cow lactation number at culling. The colour shading (blue) indicates the number of additional random deaths assumed to occur (fewer random deaths are represented by a darker shade).

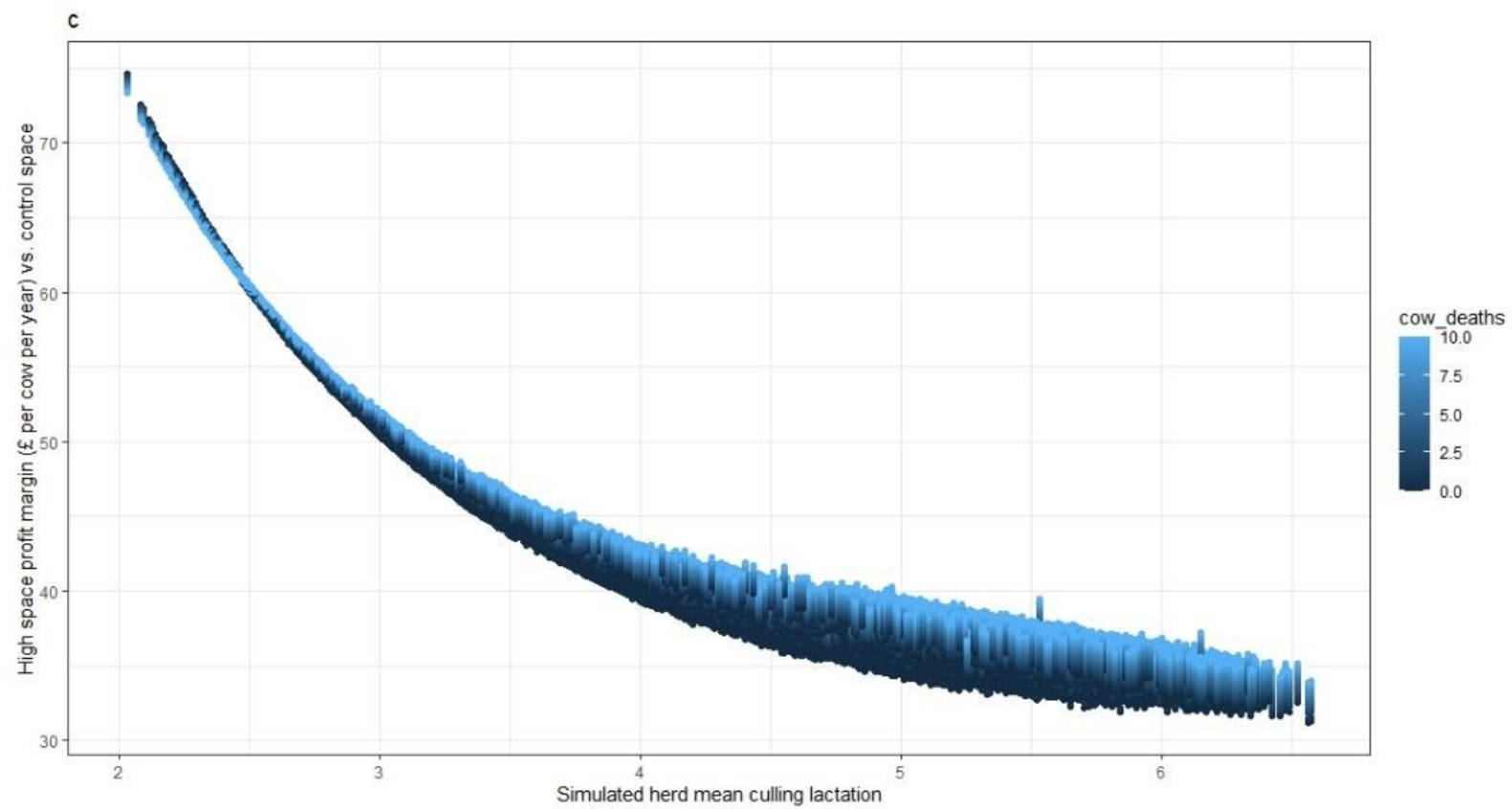
7.3.4.1 Sensitivity analysis: margin over purchased feed per litre of milk results

When each price margin was incorporated into the random death model, the corresponding output costs were as follows; £57.52 per cow per year at 20.49ppl, £46.70 per cow per year at 16ppl; £51.53 per cow per year at 18ppl; £61.15 per cow per year at 22ppl; £65.96 per cow per year at 24ppl. These costs for random deaths were incorporated into the full sensitivity analysis simulation alongside the new margin over purchased feed inputs.

Figure 7.7 illustrates the sensitivity analyses to explore the impact of changing the margin over purchased feed per litre of milk to 16, 18, 22 and 24 pence per litre. All simulations indicate a positive financial return for herds providing the high space allocation compared to herds with control space. For comparison the financial return for the UK average mean culling lactation of 3.7 lactation is £28.09-£31.36 at 16ppl, £31.63-£34.84 at 18ppl, £41.79-£45.30 at 22ppl and £42.14-£46.44 at 24ppl compared to a current margin over purchased feed of 20.49 pence per litre which was calculated at £35.97-£39.76.







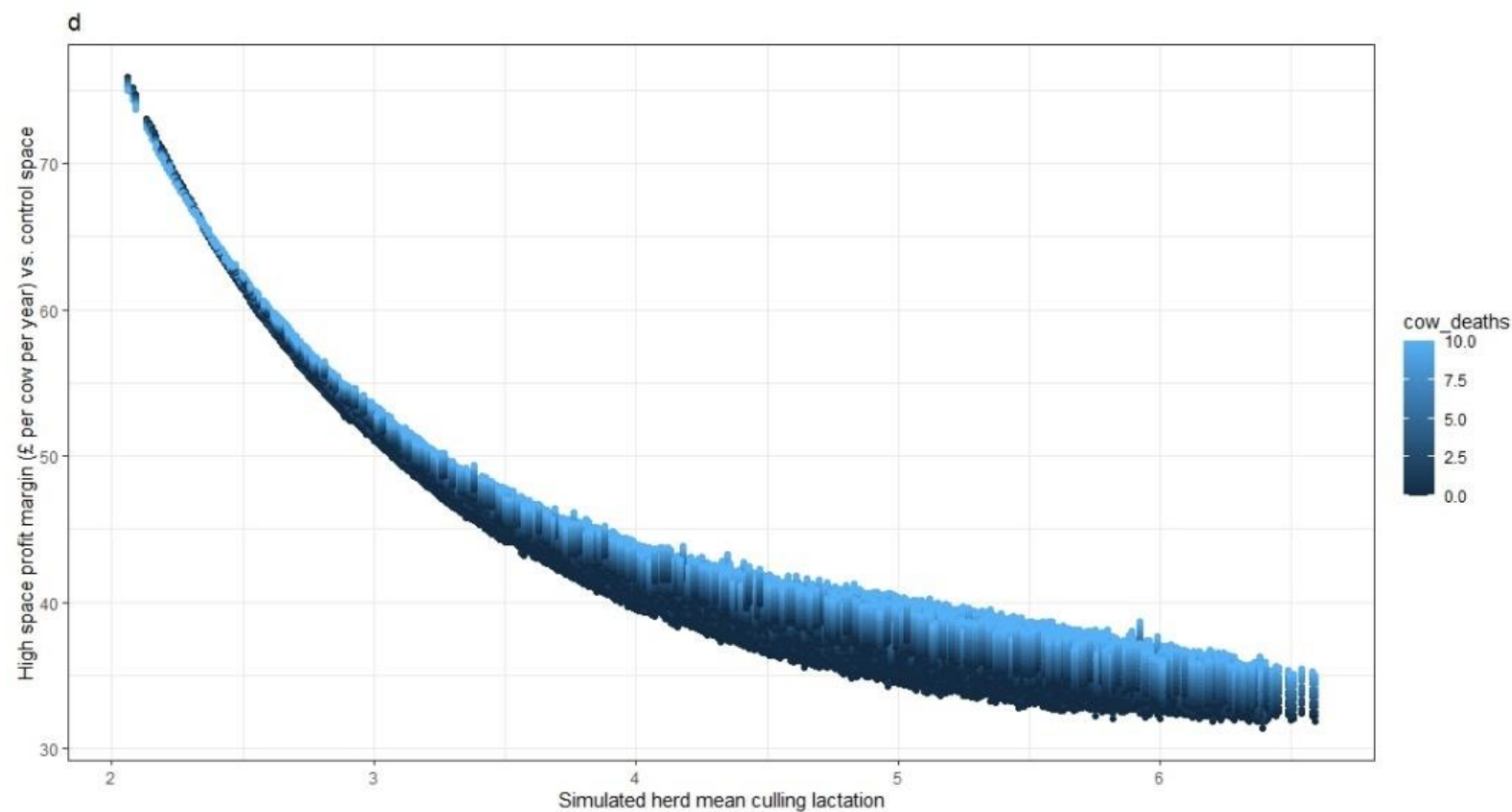


Figure 7.7. Scatterplots to illustrate the possible financial outcomes across all model scenarios for each of the four margins over purchased feed sensitivity analyses undertaken (a) 16ppl; b) 18ppl; c) 22ppl; d) 24ppl). The y-axis represents the expected financial return per cow per year for cows in the high space group compared to those in the control space group. The x-axis represents the herd's mean cow lactation number at culling. The colour shading (blue) indicates the number of additional random deaths assumed to occur (fewer random deaths are represented by a darker shade).

7.3.5 Cost of building comparisons

7.3.5.1 New-build costs

The most expensive scenario for giving cows extra living space would be to build a new building for a herd. Based on the AHDB wizard housing calculator tool, to build a new shed for 100 cows with a total space allowance of 14m² versus 9m² would cost £196,000 vs £126,000 respectively. If this was paid outright the cost per cow per year over a 20-year depreciation period would equate to a cost difference of £35 per cow space per year. If loan repayments were necessary, with a 2% interest rate, it would cost a farm at total of £237,160 (14m²) or £152,460 (9m²) a difference of £84,700. This difference equates to £847 per cow space or £42.35 per cow per year. In terms of net financial return when building a new shed with a 2% interest rate, based on the estimates from the simulation model for an average UK dairy herd with cows averaging 3.7 lactations this would equate to between -£6.38 and -£2.59 per cow per year (between 2.5-4 lactations the range would equate to between -£8.61 to +£10.99 per cow per year). These costs are of a similar magnitude to the estimate financial returns from providing extra living space, for the majority of culling scenarios likely to occur in UK dairy herds.

7.3.5.2 Existing housing modification comparison

A more likely scenario when planning to increase living space availability for housed dairy cows is to modify existing buildings. For the first scenario explored, the cost of providing an indoor loafing area was estimated to be around £185 per m² and would thus gross cost in the region of £647.50 per cow in total or £32.37 per cow per year over 20-year period (£39.17/cow/year with interest payments at 2%). In terms of net financial difference this would equate to a return of between £3.60 - £7.39 per cow per year for the average UK herd (for herds ranging between an average cow lactation of 2.5 - 4 this net financial return would be between £1.37 - £20.97 per cow per year). However, this may be an over-estimate

if some of the infrastructure was in place; the cost quoted would be based on a stand-alone structure.

For the second scenario, provision of an outdoor (unroofed) loafing area, published figures suggest a cost of £77.50 per m². Therefore, for one cow the additional cost to provide 3.5m² extra space would be £271.25 or £13.56 per cow per year over a 20-year period (£16.41/cow/year with interest payments at 2%). In terms of net financial return for this scenario, a return of between £22.41 - £26.20 per cow per year for the average UK herd of 3.7 lactations (for herds ranging between an average cow lactation of 2.5 - 4 this net financial return would be estimated between £20.18 - £39.78 per cow per year).

As expected, modification of existing buildings would appear to present a more cost-effective choice than new buildings for the addition of increased living space. However, when the opportunity for a new building arises, the addition of increased living space should be considered.

7.3.5.3 Sensitivity analysis: Net profit/loss sheet for different amortisation and interest rates on investment

Table 7.3. Table of the net financial returns for the three building scenarios to provide extra living space. This provides details of sensitivity analyses dependent on interest rates from 0-3% and amortisation over a 10- or 20- year period. Results in green show scenarios where investment to increase living space is more profitable for a farm. N.B. Once the amortisation period has finished the investment has been paid off and the estimated financial return post this period would not be required to be offset against the initial investment costs.

	Interest rate (%)	Actual cost difference per cow		Cost difference per cow per year		Net cost difference per cow per year based on 2.5-4 lactation average				Net cost difference per cow per year based on 3.7 lactation average			
		10years	20years	10years	20years	10years		20years		10years		20years	
						Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
New build shed build cost difference	0	£700.00	£700.00	£70.00	£35.00	-£36.25	-£16.66	-£1.25	£18.34	-£34.03	-£30.24	£0.97	£4.76
	1	£738.50	£773.50	£73.85	£38.68	-£40.10	-£20.51	-£4.93	£14.66	-£37.88	-£34.09	-£2.71	£1.08
	2	£777.00	£847.00	£77.70	£42.35	-£43.95	-£24.36	-£8.60	£10.99	-£41.73	-£37.94	-£6.38	-£2.59
	3	£815.50	£920.50	£81.55	£46.03	-£47.80	-£28.21	-£12.28	£7.31	-£45.58	-£41.79	-£10.06	-£6.27
Indoor living space area extension	0	£647.50	£647.50	£64.75	£32.37	-£31.00	-£11.41	£1.38	£20.97	-£28.78	-£24.99	£3.60	£7.39
	1	£683.11	£715.50	£68.31	£35.77	-£34.56	-£14.97	-£2.02	£17.57	-£32.34	-£28.55	£0.20	£3.99
	2	£718.87	£783.40	£71.87	£39.17	-£38.12	-£18.53	-£5.42	£14.17	-£35.90	-£32.11	-£3.20	£0.59
	3	£754.33	£851.50	£75.43	£42.57	-£41.68	-£22.09	-£8.82	£10.77	-£39.46	-£35.67	-£6.60	-£2.81
Outdoor living space extension	0	£271.25	£271.25	£27.13	£13.56	£6.63	£26.22	£20.19	£39.78	£8.85	£12.64	£22.41	£26.20
	1	£286.17	£299.70	£28.62	£14.99	£5.13	£24.72	£18.76	£38.35	£7.35	£11.14	£20.98	£24.77
	2	£301.09	£328.20	£30.11	£16.41	£3.64	£23.23	£17.34	£36.93	£5.86	£9.65	£19.56	£23.35
	3	£316.01	£356.70	£31.60	£17.83	£2.15	£21.74	£15.92	£35.51	£4.37	£8.16	£18.14	£21.93

7.4 Discussion

The aim of this chapter was to explore possible economic consequences of the two different living space allowances evaluated in Chapters 3-5. Of particular interest, was whether the predicted increase in milk production would financially outweigh the identified deterioration in reproductive performance. Based on the model specifications and assumptions used, the findings suggested that provision of additional space would result in increased financial returns across a wide range of herd scenarios. Different herd culling strategies and death rates were found to influence the extent of the financial return, but returns were generally in favour of additional space.

When herd financial returns were compared to the expense of providing extra living space, it appeared that the increase in returns would generally cover the cost of space provision, although predicted income and costs were of a similar magnitude. Therefore, under the circumstances and contexts studied, the results suggest that provision of additional space for the adult dairy herd should at worst be cost neutral.

It should be noted, however, that these conclusions are based on a variety of important assumptions and may not be generalisable to all dairy herds or systems; with any theoretical models there are limitations from the inputs used and the assumptions that made. In this instance, key assumptions of note were: an expected margin over purchased feed per litre of milk was set at 20.49 and sensitivity was tested between 16-24ppl, that the transition cost estimates were realistic, that there were no extra (unknown) effects of space that may have had a financial impact, and that replacement of culled cows with a newly calved primiparous cow was immediate. For the model outputs to be generalisable, model inputs and assumptions would need to apply to different herds with different systems and yields and whether this is realistic remains unknown; further research would be needed to establish generalisability. Furthermore, there is a potential limitation regarding the use of a fixed margin over milk price, as this assumes that the efficiency of production is linear throughout lactation. Whereas in research the peak production period in the first 100 days of lactation

has been shown to more efficient, in terms of feed inputs to milk volume production conversion. This could mean that there is potential for underestimation of the efficiency of cows in the control space group which have a shorter lactation cycle and thus less time spent in the inefficient late stages of lactation (>200DIM). However, given feed intakes of the partial mixed ration were not measured in this research so an estimate for this would have been difficult to include in this simulation model.

In terms of the sensitivity analyses conducted, the assumptions of the cost-risk for each cow's transition period did not appear to greatly influence the final financial return costings when the cost was doubled and halved. However, this assumption did allow for the increased risk of removal from a herd during this period and thus meant that a distribution input to account for cows being more likely to die around this time period was not required in the random death element of the simulation model. The sensitivity analyses conducted on the margin over purchased feed input suggested that there would be some fluctuation in the associated financial returns. However, even at the low value of 16ppl margin over purchased feed, a positive financial return was identified for herds giving cows the high space compared to the control space group, suggesting that when milk price is not optimal these cows will continue to provide greater on-farm efficiency in terms of production and associated financial returns.

It should also be noted that it is possible that the calculated financial benefits of additional living space could represent an under-estimate. Although not tested in this research, it is biologically plausible that increased living space could result in additional financial benefits not included, such as reduced incidence rates of mastitis, lameness and infectious disease. Such differences could enhance the financial benefits estimated in this chapter, although additional research is required to verify this. Furthermore, the financial results are expressed as 'per cow per year' which is different to the more widely used 'per cubicle place'. However, it was felt that per cubicle place was inappropriate due to the fact that both groups were at a set cubicle stocking density so this could lead to misinterpretation of the reporting of these results by linking these results back to cubicle stocking density

which is a separate parameter to total and living space allowances. This was avoided due to the current unknowns surrounding how an interaction between cubicle stocking density and total space allowance would impact the production and reproduction results, but this is would be important for future research to investigate.

An interesting aspect of the model output was the reduced financial return per cow per year for cows in the high space group, as herd culling rate decreased (Figure 7.6). For primiparous cows culled at the end of first lactation the increase in financial return difference was estimated as £157.67 per cow per year, a much greater difference than if culled at any point as multiparous cows. A similar effect was seen for random deaths that occurred earlier in life compared to later. These reduced financial benefits arise from the difference in predicted milk yield being far greater for primiparous cows than for multiparous cows in the high space group; primiparous cows were predicted to produce 643 litres more milk per lactation and multiparous cows only 102 litres. In reality, however, in most dairy herds, cows will generally be culled (on average) after 3-6 lactations and therefore the cost benefit of additional living space estimated at around £30-£40 per cow per year appears realistic. The results do raise the possibility that if a farm was unable to provide additional space for the whole herd, it may be that the provision of additional living space solely for primiparous cows could provide a cost-effective return on investment. However, this hypothesis was not directly investigated, so caution is needed before extrapolating results to different scenarios.

As well as considering financial aspects of the provision of additional living space, it is important to take into account the potential for wider benefits. In Chapter 6, increased living space was found to change the behaviour of cows resulting in an increase in daily lying times of over one hour, which is deemed to be beneficial (Krawczel and Lee, 2019). Other research has reported that cows will make effective use of extra space (Kondo et al., 1989; Talebi et al., 2014), they are likely to become fitter (Horn et al., 2012; Shepley et al., 2020) and be in less direct competition with each other for resources (Fregonesi et al., 2007; Talebi et al., 2014). Therefore, there may be important welfare benefits associated with increased living space that are as relevant to sustainability as financial returns. Indeed, in

terms of consumer perceptions welfare benefits are likely to outweigh farmer finances (Jackson et al., 2020) and identification of methods that enhance the welfare of housed dairy cows can only be good for the dairy sector. The greater the confidence consumers have in cow welfare, the less likely they are to source substitute products from elsewhere and if improvements to dairy cow housing do not occur, sustainability of the industry could be endangered.

7.5 Conclusion

To conclude, results from this chapter suggest that providing extra space for housed dairy cows (14m^2 as opposed to 9m^2) is likely to be either cost neutral or slightly cost beneficial, although generalisability is dependent on assumptions made. Beyond financial returns, providing extra living space is likely to be beneficial to the sustainability of the dairy industry through improvements to cow welfare which may improve public perception of the industry.

Chapter 8. General Discussion

8.1 Introduction

This chapter provides an overview of the findings of the research in relation to the aims outlined in the opening chapter. This is followed by a wider discussion of elements arising of particular interest, further thoughts around limitations, and suggestions for future research.

8.2 Summary of findings of this thesis

The sparse availability of literature to assess the impact of housed infrastructure on adult dairy cows, as shown in Chapter 1, highlighted the requirement for further research in this area. The field survey in Chapter 2 provided an insight into current housing practices for British dairy farms and perceptions of dairy farmers about housing. Importantly, a large variation in current adult cow housing conditions was observed, especially in total space allowances. Furthermore, the survey highlighted the need for a standardised approach to assess housed conditions for dairy cows in cubicle accommodation, leading to the novel term, living space, being defined. This term enables comparisons to be made of space availability for all types of cubicle housing layout.

A long-term randomised controlled study to investigate the effect of living space on housed dairy cows was described in Chapter 3 and this allowed for quantification of changes to production (Chapter 4), reproduction (Chapter 5) and behaviour (Chapter 6).

Chapter 4 identified substantial changes to milk production over a 305-day lactation; production increased when cows were given more living space, especially the primiparous cow cohort. Furthermore, physiological differences such as rumination time also increased alongside milk volume in this group, mainly within early lactation. Interestingly, these

changes were not associated with loss in bodyweight and were not detrimental to milk solids production over the trial period.

In Chapter 5, the increase in living space was shown to be associated with an increase in time to conception. However, the underlying reproductive parameters investigated did not provide evidence for an underlying mechanism or causative pathway. Furthermore, there was no effect of milk production on conception success. There was found to be minimal effects when the opposite hypothesis was tested indicating that the difference in reproductive performance did not substantively contribute to the increased milk production in the high space group.

Additionally, the randomised controlled trial suggested that increased living space is likely to change the behaviour of housed dairy cows with evidence of increased lying times and decreased time spent in passageways, as described in Chapter 6. This indicates that there is the potential for improved welfare by increasing living space allowances.

The results obtained from the randomised controlled trial showed there are potentially large benefits associated with an increase in living space allowance, namely increased production and improved wellbeing. However, provision of an increase in space allowance would have associated building costs as well as an apparent reduction in fertility performance. In Chapter 7, the economic return of both trial groups was estimated and results indicated that the return on investment was likely to be economically viable in many herd scenarios, based on the results obtained in the production (Chapter 4) and reproduction (Chapter 5) analyses. In conclusion, the provision of more living space for housed dairy cows has been found to be both economically viable and better for cow behaviour and wellbeing, thus providing a more sustainable approach for dairy farming.

8.3 Sustainability of the dairy industry

The dairy industry has an ethical duty of care to ensure housed dairy cows are provided with suitable conditions for their health and welfare. However, as highlighted in

this thesis, there is a minimal body of evidence and therefore no clear understanding of how current commercial housing conditions impacts the inhabitants.

Sustainability of industries and businesses is key to allowing standards to progress. For the dairy industry to be sustainable, changes should be undertaken to ensure increasing alignment with the three pillars of sustainability. These pillars are; i) to be socially acceptable for animal, farmer and society, ii) to have a neutral or positive environmental impact and iii) to provide sufficient economic return for continued development (ten Napel et al., 2011). Interestingly, intensification is a widely accepted method to limit environmental impacts because production can be maintained or increased from fewer animals (Lillford and Hermansson, 2020; Thornton and Herrero, 2010; Willett et al., 2019). However, the intensification of dairy farming is an emotive subject for society, mainly due to links with the concepts of “naturalness” (Jackson et al., 2020). Therefore, understanding the fundamental impacts of housed conditions on animal productivity, wellbeing and underlying physiological responses, as initiated in this thesis, is essential for making progress in the industry, as it is increasingly important that farming practices align with societal, environmental and welfare attitudes (Mandel et al., 2016; Mehrabi et al., 2020; Nannoni et al., 2019).

The current variability in management and housing practices within the industry make the housed infrastructure difficult to assess to a framework and advocate beneficial improvements, but it is clear that housing will influence animal health, welfare, productivity, land use and greenhouse gas (GHG) emissions in their own right (Herrero et al., 2013a). In the case of GHG emissions, there are reports to suggest that the magnitude of greenhouse gas production is directly linked to the environment in which cows are housed in (FAO, 2010; Kristensen et al., 2011). Emissions have been shown to be influenced by different housing infrastructure, systems and configurations, such as floor type and areas causing build-up of excreted material, showing this is an important factor to be accounted for when assessing sustainability of the housing (Baldini et al., 2016). Therefore, the target must be to negate environmental impacts, whilst safeguarding food supplies and animal welfare, using methods

that are socially acceptable (Muller et al., 2017). This research provides results which suggest that our understanding of many management practices is currently inadequate in achieving this aim. Worryingly, more strain will be put on the dairy industry because it is forecasted that outputs from agriculture will have to increase by 50% to sustain the projected 2050 global population of over 9 billion (Alexandratos and Bruinsma, 2012) and trends suggest that demand for livestock products will grow worldwide (Alexandratos and Bruinsma, 2012).

There has been a shift towards developing sustainable intensification which has used advancements in technology to increase production efficiency through livestock feed conversion (Gerber et al., 2013; Valin et al., 2013), genetics (Schultz et al., 2020) and precision livestock technologies (Lovarelli et al., 2020). The assessment of dairy cow longevity has played a role in trying to improve efficiency and improve animal welfare (Schuster et al., 2020). However, housing systems have been shown to have a substantial impact on longevity of dairy cows which has led to the advancements described above (Barberg et al., 2007). There have also been some novel developments into new housing systems, such as freewalk systems with artificial permeable flooring and multi-climate sheds, to combat this trend of reduction in longevity (Galama et al., 2020), but research and development of current systems appear not to have been undertaken to evaluate whether modifications to these systems could improve sustainability. Unfortunately, the randomised controlled trial undertaken within this thesis was not able to assess how the difference in living space allowance would affect the longevity of the cows, but it could be hypothesised that the behavioural changes, specifically the reduction in time cows spend in passageways, could mean that there will be fewer agonistic interactions whilst cows are moving around the environment. Therefore, they would be less likely to suffer from trauma, more likely to be able to access the required resources and less likely to suffer from claw horn lesions leading to chronic lameness. In terms of sustainability, this may be of critical importance as it has often been shown that longevity is beneficial when assessing the GHG emission intensity and profitability of dairy cows (Grandl et al., 2019, 2016)

Sustainable intensification has added that animal welfare and production are inter-linked and therefore higher welfare standards should also be factored into these assessments, alongside the more classical productivity, health and efficiency (Herrero et al., 2013b). More recently, the OIE- World Organisation for Animal Health has declared that production and animal wellbeing are inter-related (OIE, 2019). The concepts of ‘One Health’ and ‘One Welfare’ are becoming widely adopted (Garcia Pinillos, 2018; WHO, 2017) and underline the critical links between animals and humans that consequently protect public health (Nalon and Stevenson, 2019). The housed environment has been considered important for cow health and welfare, with postulations that improvements to housing and husbandry could be of greater benefit to inhabitants than therapeutic measures such as vaccination (Greger, 2006). The conditions in which cows are housed is therefore fundamental to the sustainability of dairy farming and wellbeing of farmed cattle. It is of critical importance that the housed infrastructure for dairy cows, which generally require periods of housing during their productive lifetime (Barkema et al., 2015; March et al., 2014; USDA, 2016), does not conflict or undermine the advancements in genetics and precision livestock technologies. Improvements to housed infrastructure can only be achieved by furthering the understanding of how dairy cows interact with these surroundings.

Despite the perceived importance of housing to the industry, quantitative research to evaluate and optimise housing conditions to achieve sustainable intensification in dairy farming remains virtually non-existent. Consequently, this research is of great relevance and provides evidence of the need for further research in this area. The key areas of research recently suggested as needed to increase efficiency of dairy farming are feeding of specific feedstuffs, regionally appropriate animals, improved health and nutrition (Eisler et al., 2014), and use of technology (Du et al., 2018). However, it is important to note that any changes to these activities are only likely to be successful if the underlying environment of the cows does not act as a limiting factor and this is continually overlooked. The current body of work demonstrates that, given additional living space, dairy cows will increase their milk output, even in comparison to already high yielding animals, which suggests that these animals were

not reaching their genomic potential due to restrictions in their environment, even though they would already have been classed as highly productive animals.

The randomised controlled trial conducted as part of this thesis, was the first of its kind undertaken and has identified that living space, which could be considered the most basic attribute of the housed environment, impacts on a range of key parameters, including productivity, rumination, reproductive performance and behaviour. This offers insight, not only into how the housed environment fundamentally impacts the inhabitants, but also illustrates the importance and urgency of furthering the understanding of animal-environment interactions to improve the sustainability and welfare of dairy cows.

8.4 Implementation of Results

8.4.1 Recommendations and regulations

The highly variable and sparsely detailed recommendations and regulations surrounding dairy cow housing worldwide (Chapter 1) is an area which urgently requires a research base to allow evidence-based decision making. Currently, there is a void of research available for key decision makers and therefore they do not currently have the required support to produce appropriate policies and regulations (Appleby and Mitchell, 2018). This is especially worrying since even a fundamental aspect of the housed environment, the amount total space and living space (Chapter 2), have no compelling data on which to base recommendations. The recommendations currently used for dairy cow space allowances worldwide have little scientific basis and show extreme variability, highlighted by the range which was described in Chapter 1, from 6.0m² up to 11m² (Bewley et al., 2017; Bickert and Light, 1982; McGlone et al., 2010; Red Tractor, 2020; SPCA, 2018). The estimates made for current space provision in British dairy herds in Chapter 2, found a range of 5.4-12.7m² for the 50 randomly selected dairy farms. These farms even provide milk to the same retailer and are thus governed by the same set of regulations. Therefore, the large differences in the

production, reproduction and behavioural results observed in this randomised controlled trial provide the first substantive evidence in showcasing how critical space allowances, specifically living space allowances, can be for housed dairy cows. The control group with 9m² total space per cow used in this research is above the minimum guidelines of most nations for cubicle housing and was shown to limit the behaviour and milk production of inhabitants compared to a high space group (14m²). Additionally, the high space group was at the top end of space provision of farms observed in Great Britain (Chapter 2) and represents a greater amount of space than all recommendations currently being used, which could mean that cows on the vast majority of farms in Great Britain are being limited by their environment. Further reflection should also be made about the farms providing less than the 9m² per cow, more than half of the farms assessed in Chapter 2, where it remains unknown whether these cows would have even more severe limitations than those on farms that provide 9m². Since this research identified substantial differences between space allowances of 9m² and 14m², serious consideration should be given to whether the current minimum baseline of 6m² per cow of total space is appropriate for the dairy industry of today.

In research assessing the effects of housing on humans, space allowance has been classed as the most important characteristic of housing, with correlation between less space and increase to the prevalence of health issues (Blake et al., 2007; Boch et al., 2020; WHO, 2016). Furthermore, within the human literature it has been reported that living space is critical for health and wellbeing of occupants (Bonnefoy, 2007; Jacobs, 2011; Krieger and Higgins, 2002). Given the results described in this thesis it appears that space allowances are of a similar importance for housed dairy cows and that this requires further research to inform appropriate codes of practice and legislation.

8.4.2 On-farm application

For change to occur to the housed environments in commercial settings, evidence of the benefits of providing more space would need to be accepted, regardless of

recommendations and regulations. However, widespread knowledge transfer from research to on-farm solutions is notoriously difficult. It is important to understand what may motivate farmers to embrace change by understanding what they feel is important. The farmer survey in Chapter 2 demonstrated that currently farmers appear to believe that living space is an important element of cattle housing in terms of the health, welfare and productivity of their cows but do not have the available resources to implement positive change. Importantly this research topic (space allowances) was selected by key industry stakeholders who believed that change may be required in this area; this group could help with disseminating the results.

However, it is important to consider possible barriers that could prevent implementation of the findings of this research to avoid pitfalls. The survey and farmer conversations in Chapter 2 highlighted key themes that could present barriers to provision of extra space. These themes were: concerns about providing outdoor space due to climate and slurry restrictions, concerns around the actual impact additional space would have for the cows, and concerns around finding a suitable person to design buildings appropriate for their cows. Understandably, the greatest concern centred around economics and return on investment. The results from Chapter 7, which assessed the associated economic returns from greater living space, found that given the increase to milk yield of primiparous cows, provision of extra space is likely to be profitable. The testing of herd structures through culling patterns also suggested this was likely to be economically viable for the majority of herd management systems in GB. In general, the costs of providing the extra living space for cows, in any of the described methods, should at the very least be cost neutral over a 20-year investment period.

The economic calculations and the apparent benefits to cow behaviour provide highly motivating arguments to persuade farmers and the industry to implement this research on a wide scale by increasing the living space available to cows. Although further work is needed into the optimisation of cubicle housing design (especially in terms of passageway widths and indoor/outdoor loafing areas) there are a number of ways that farmers could

increase access to space on-farm. This includes reducing stocking rates (which may not be as economically viable), providing additional covered indoor areas or outdoor areas, or by designing new-build sheds with wider passageways (cross-over, feed and cubicle passageways). A perceived drawback of using outdoor space is that this space is usually used for farm traffic during the day but if that is the case there is the possibility to open-up space solely over night to alleviate the problem. Another example would be in a twice daily milking system, to give cows access to the collecting yard when not in use between milking times. Having such options may help to encourage farmers to give cows more space.

8.5 Limitations and ideas for future research

This section will focus on the overall limitations of the thesis and use these alongside the results of the thesis to propose ideas for future research.

8.5.1 Generalisability

With any research a key question is how generalisable the outputs are to the target population, in this case housed dairy cows. The randomised controlled trial described in Chapters 3-6 used a single cohort of high yielding Holsteins within all-year round housed conditions.

8.5.1.1 Duration of housing

Although the majority of dairy cows worldwide would be housed at some point in a lactation the practice of all year-round housing is limited to a smaller percentage of farms (March et al., 2014), although the trend for this is increasing. Therefore, the results observed may not be a true reflection for herds that graze their cattle in summer months. It would be difficult to hypothesise how these results could be impacted by this change in management because cows would spend less time in the housing and therefore only part of their lactation

would be affected by these conditions. Although, it is also true that these cows would be used to a much larger space allowance whilst at pasture and therefore the acclimatisation period to a small space allowance in housing may have an even greater effect. Taking this into consideration, the investigation of moving between different space allowances, including following grazing, would be worthy of further research.

8.5.1.2 Breed and genetics

It is important to note that the study population was solely high-yielding Holstein cows. Important breed characteristics to account for are size (Holsteins are generally taller in size than other dairy breeds) and milk production capacity (Holsteins are generally considered to have higher milk volume capacity than other dairy breeds). Therefore, the space allowances used within this trial may cause different interactions with other dairy breeds. Furthermore, the large difference in milk yield observed in the primiparous cows, which would already be considered as very high producing animals in this study, is of significance. Further research would be important to assess whether this difference would be observed in animals of different breeds and predicted milk yield capacity (i.e. a 650L difference to an 8,000L cow is much more substantial than a 12,000L cow).

No genetic or genomic measurements or indices were taken into account in this research. In terms of the current results, this was unlikely to have affected the outcomes of this work for two main reasons. Firstly, since a stratified randomisation procedure was used to select cows for each treatment group, it is unlikely there was a disparity in genetic merit between trial groups; this was the purpose of randomisation. Secondly, since all animals were from a single closed herd with similar sire management over many years, the variation between cows was likely to be limited. An important consideration of genetic potential, however, is that it remains unknown whether the impact of space allowance may vary between cows with a different genetic make-up. Therefore, the extent to which results are generalisable to cows of different genetic potential remains unknown.

8.5.1.3 A single cohort of animals

This work was undertaken on a single cohort of animals, on a single dairy farm, without replication. Replications of the randomised controlled trial would provide further validation to the trial results, especially as the time to conception result in Chapter 5 was unexpected and markedly different in the opposite to hypothesised direction. Unfortunately, the resources needed to replicate such long-term research studies are substantial making this impractical.

8.5.1.4 Parity structure

A further limitation of the randomised controlled trial is that more than 50% of cows in each group were primiparous animals which is a greater proportion that would generally be expected in commercial settings. Given the large differences observed based on parity interactions with both milk yield and days to conception it is important to be aware that group structure could also be associated with the results observed. However, it is unknown how this would alter the biological processes associated with the results described in Chapters 4, 5 6 and 7.

8.5.1.5 Trial space allowances

The randomised controlled trial focussed on only 2 distinct space allowances (9m² and 14m², of which there was 3m² and 6.5m² of living space respectively). Given the vast range of measurements observed for commercial space allowances and living spaces in Chapter 2, it is not possible to know how cows on farms with different space provision may be influenced; results cannot be generalised to other circumstances. Therefore, this work should be seen as a proof of concept that there are important interactions between space allowances and housed dairy cow production, reproduction and behaviour. Firstly, this shows it is important to describe these environmental differences when reporting research trials - there is the possibility for confounding of trial results because of environmental

variation. Furthermore, there is a need for a greater number of scenarios to be investigated, both in terms of living space allowances and also around living space configurations. Subjects to study include passageway widths, bespoke loafing space areas and non-linear layouts (curvature to aspects of the design such as feed face and bedded area). From undertaking new research, a stronger body of evidence would be produced to determine the most appropriate designs and space allowances for housed dairy cows.

8.5.1.6 Trial duration and longevity assessments

The trial duration of one year was long for an RCT on adult dairy cows, especially with such an intense trial that included many protocols and specific management. The research was also conducted in commercial conditions such that it reflected a relatively 'normal' dairy cow life. However, the trial still represented a relatively small proportion of the lifetime of a cow and it is possible the effect of space allowance may change over greater periods of time. The standard lactation lengths of cows meant that there were many cows that contributed part-lactations in the analyses rather than full-lactations, however this did mean that the study was more similar to commercial scenarios where cows would be entering and exiting groups and be at different points in a lactation during the study period.

A longer trial would be able to assess longevity of cows but would be difficult to achieve in such a controlled way for this period of time. There would also be other factors to account for if undertaking a trial for a longer duration, such as, risks of drift (e.g. management, genetics) as well as increased time and costs due to the intensive nature of the trial. A potential study for assessing the longevity of dairy cows given different space allowances would be to assess how long the cows remained in the herd following a lactation in a specific space set-up. This would be less intensive and less scientifically rigorous than an extended RCT (due to many potential confounders) but could provide insight into whether there were longer lasting effects to be observed from providing different space allowances.

8.5.1.7 Economic costings

In Chapter 7, the sensitivity analysis on milk margin and transition disease, as well as the different herd culling scenarios indicated that the economics would consistently show a positive return for cows housed in the high space group. This suggests a return on investment from providing the extra space available is likely. However, it should be acknowledged that these investments, in terms of financial outlays will vary among farms and geographical location. Furthermore, the limitations discussed above around generalisability mean that it is difficult to interpret how cost-effective these changes would be for different systems (i.e. 8000L Friesians housed for 6-month periods). Therefore, any future work undertaken on this topic should also aim to quantify economic returns on investment.

8.5.1.8 Cow management practices

The management of both trial groups was closely controlled and monitored through standard operating procedures, thus minimising impacts of confounding between the two groups. However, there are many different management systems (in addition to the housed infrastructure discussed earlier in this section) such as for reproductive interventions and monitoring, robot versus conventional milking systems, 2 versus 3 times a day milking and numerous bedding systems (e.g. sand, sawdust mattresses, mats), which were all observed in Chapter 2. These could all have an influence on the results observed within the randomised controlled trial. Therefore, it would be important to undertake research to assess how different systems may change the cow-housed infrastructure (space allowance) dynamics.

8.5.2 Primiparous cow-only groups

The results pertaining to the primiparous cows, especially in terms of milk yields offers a specific area of interest. These results bring into focus the use of primiparous cow-only groups with the possibility of providing additional space solely for this group. Given the

costs associated with providing more living space (as detailed in Chapter 7), if financial input was limited then these results suggest that of the population tested in the randomised controlled trial, it would be the primiparous cows that would benefit the most. Even though the high space primiparous cows did not conceive as quickly as the control space group, this did not appear to impact on the financial outputs in Chapter 7. Indeed, if anything it would appear that the majority of the financial return observed was due to the difference in the milk yield and reproductive performance in younger animals. It remains unknown whether the substantial differences, described in Chapters 5 and 6 between the two space allowances would apply to primiparous cow-only groups, but it is possible.

However, it should be noted that this research did not specifically test the hypothesis of increased milk yield for primiparous cow-only groups dependent on space allowances. Thus, care must be taken in extrapolation of these results. There is the potential that primiparous only groups could obviate the difference in yield observed between the two groups as it is currently unknown how the group structure, in terms of primiparous vs. multiparous cows, would modify the observed effect on milk yield. Therefore, investigation of space allowance allocations on primiparous only groups would be worthy of further research.

8.6 Conclusions

Considering the outcomes of each Chapter, the conclusions of this thesis are as follows:

It has been shown, in Chapter 1, that there is sparsity in the literature of how the housed infrastructure impacts upon dairy cows. Alongside this, there are many recommendations and regulations on housing, but they are varied and not based on scientific research. The survey conducted in Chapter 2 demonstrated how the current lack of literature and varied recommendations appear to have led to confusion around housed infrastructure definitions, resulting in a large variety of housing environments being observed, especially

around provision of space. This research led to a novel term, living space, to be defined in order to provide a clear definition of excess space in cubicle accommodation above a baseline minimum.

A randomised controlled trial (described in Chapters 3-6) was conducted to assess how space allowances, and in particular living space, affects housed dairy cows. The data from this trial identified that cows with greater living space allowance produced more milk, ruminated for longer and increased milk solid production without impacting on cow bodyweight (Chapter 4). However, time to conception was found to be extended in cows provided with a greater amount of living space although underlying reproductive physiological parameters remained similar between trial groups meaning a causal pathway was not revealed (Chapter 5). Finally, in Chapter 6 it was identified that cows increased their lying times and reduced the time spent in passageways when given greater space, indicating possible improvements to welfare.

In Chapter 7, the economic viability of providing a 6.5m² of living space versus 3m², given the observed results from the RCT, was shown to be plausible over many herd culling strategies, milk price margins and incidences of transition cows disease.

Overall, this body of work suggests that the housed environment for adult dairy cows has been poorly understood but that it will impact upon productivity, reproductive performance and behaviour. Optimal methods of housing, and especially space provision, are required to prevent the limitation of health, welfare and genomic potential. This thesis provides strong evidence that more research is needed to improve the housed infrastructure for dairy cows and enhance the sustainability of the dairy industry.

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Appendix – Chapter 2

- Opinion questionnaire questions in Chapter 2

Opinion Questionnaire; Loafing Space

Farm ID:

Date:

The term '**loafing space**' is used in the dairy industry to refer to space available for adult cows when animals are housed. In this questionnaire we would like to explore your opinions about loafing space for adult cows.

1. In one sentence, please could you sum up what your understanding of the term 'loafing space' is for your adult dairy cows?

.....

.....

.....

2. Where would you rate the importance of loafing space to adult dairy cows? (*On a scale of 0= completely unimportant to 10= critically important*)

a) 0 1 2 3 4 5 6 7 8 9 10

b) *Why?*

.....

.....

3. When dairy cows are housed, which areas would you consider to be classed as "loafing space"? (*all that apply*)

a)

- | | |
|--|--------------------------------------|
| ☐ Feeding Areas | ☐ Cubicles |
| ☐ Outdoor Yards (non-pasture) | ☐ Pasture |
| ☐ Collecting Yards | ☐ Passageways |
| ☐ Straw Yards | |
| ☐ Other (please specify): | |

.....

- b) With regards to passageways, which of the following would you class as loafing areas, if any?

- | | |
|--|---------------------------------------|
| ☐ Cubicle passage | ☐ Feed passage |
|--|---------------------------------------|

☐ Cross-over passage ☐ None

4. Of the areas you have highlighted in the previous question, which area would you regard as **the best** loafing space for housed dairy cows and why?

a) Which.....

b) Why.....

5. **In terms of herd productivity only**, on a scale of 0 = completely unimportant, 10= critically important, how important do you consider loafing space for your housed dairy cows to be?

0 1 2 3 4 5 6 7 8 9 10

Definition of productivity: volume of milk production and milk solids

6. **In terms of health status only**, on a scale of 0 = completely unimportant, 10= critically important, how important do you consider loafing space for your housed dairy cows to be?

0 1 2 3 4 5 6 7 8 9 10

Definition of health: the state of being free from disease or injury

7. **In terms of welfare only**, on a scale of 0 = completely unimportant, 10= critically important, how important do you consider loafing space for your housed dairy cows to be?

0 1 2 3 4 5 6 7 8 9 10

Definition of welfare: the state or condition of well-being, either mentally or physically. A life worth living.

8. *In an ideal scenario, how important is **outdoor access** for adult dairy cattle when they are housed? Defined as: space open to the elements, both pasture and non-pasture. (Scale: 0 = completely unimportant, 10= critically important)*

a) 0 1 2 3 4 5 6 7 8 9 10

- b) i) Is this something you would consider implementing on a farm in an ideal scenario?

Yes

No

ii) What are the main factors that prevent you from implementing this?

.....

.....

.....

9. Which floor **surfaces** do you consider to be suitable in loafing areas for adult dairy cows (tick all that apply)?

- | | |
|--|---|
| ☐ Concrete (ungrooved) | ☐ Rubber matting |
| ☐ Concrete (grooved) | ☐ Woodchip pad |
| ☐ Hardcore | ☐ Straw |
| ☐ Pasture | ☐ Sand |
| ☐ Recycled manure solids (RMS)/ Green bedding | ☐ Other (please specify) |

.....

10. In an ideal scenario *and with no financial restraints*, which do you consider to be **the best surface** in loafing areas for adult dairy cows?

- | | |
|--|---|
| ☐ Concrete (ungrooved) | ☐ Rubber matting |
| ☐ Concrete (grooved) | ☐ Woodchip pad |
| ☐ Hardcore | ☐ Straw |
| ☐ Pasture | ☐ Sand |
| ☐ Recycled manure solids (RMS)/ Green bedding | ☐ Other (please specify) |

.....

11. Do you take any measures to increase or enhance loafing space areas on your unit? If so, what are they (please specify below)

a)	Yes	No
1. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
2. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
3. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
4. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
5. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
6. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
7. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
8. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
9. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		
10. The company should be required to disclose the names of all directors and officers who have been convicted of a crime involving fraud or dishonesty within the last five years.		

b) Why?

.....

.....

.....

12. When housed, how much "loafing space" do you consider one adult dairy cow requires as a minimum and as an ideal? (please state answer in square metres or square feet, if unsure, please place an "X")

a) Minimum..... **m²/ft²**

i) *Why?*

.....

b) Ideal.....**m²/ft²**

i) *Why?*

.....

c) Are you aware of loafing space being a component of any assurance or health/welfare scheme that you are a part of (circle):

Yes

No

i) If yes, what is the name of the scheme?

.....

13. When housed, how much **space in total** (*all areas available to a cow, including bedded area*) do you consider one adult dairy cow requires as a minimum and as an ideal? (*please state answer in square metres or square feet, if unsure, please place an "X"*)

a) Minimum..... **m²/ft²**

i) *Why?*

.....

b) Ideal.....**m²/ft²**

i) *Why?*

.....

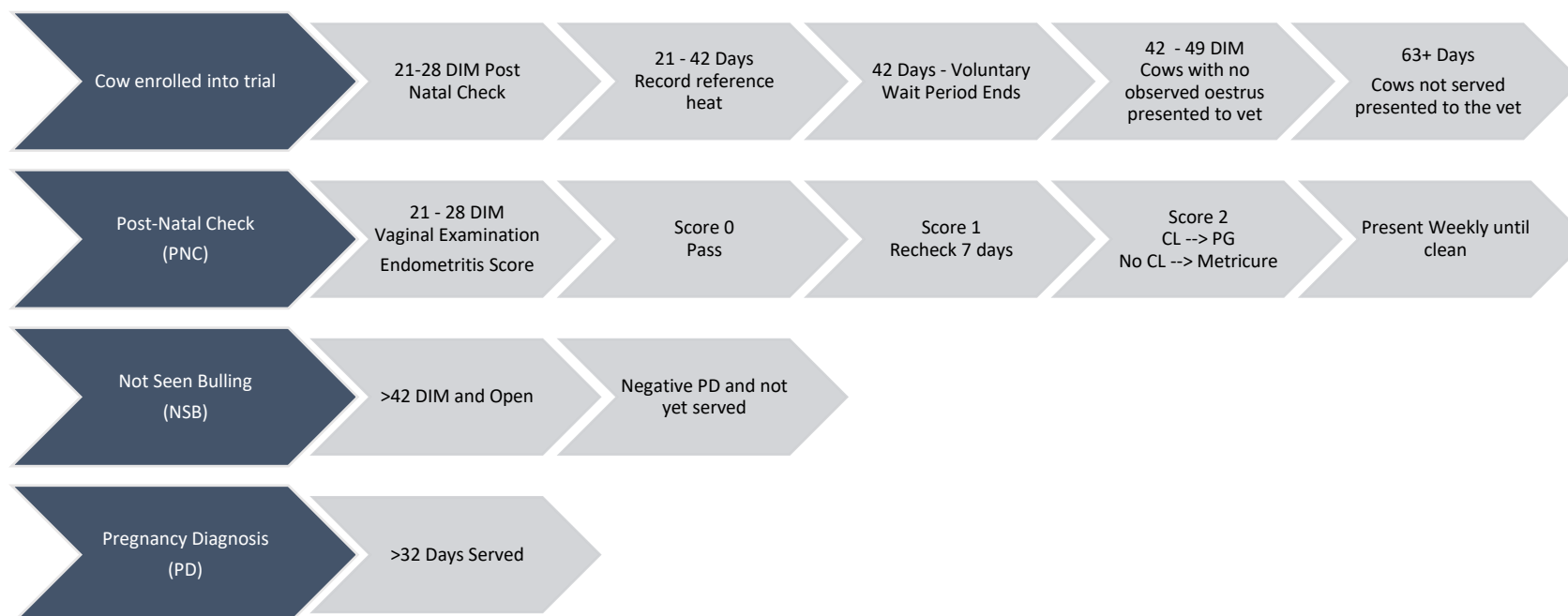
14. Thank you again for agreeing to participate in this research study, your cooperation has been hugely appreciated.

We have had a very good level of engagement for this study, what were your primary motivations for participating?

.....

Appendix – Chapter 5

1) Veterinary fertility visit protocol and standard operating procedure



Routine Fertility Protocol

Post-natal check 21-28 DIM

All cows at 21-28 DIM to be presented to veterinary surgeon at routine fertility visit

Vaginal examination to assess for endometritis.

If endometritis present -> administration of Metricure and recheck in 7 days

No further actions required if endometritis not detected.

Not seen bulling first presentation 42-49 DIM

Dominant structure	Treatment	Action	Represent 7 days
CL	2ml Prostaglandin	Serve to observed oestrus	Not served
Follicle >10mm	None	Serve if seen	Not served
Follicular cyst (25mm)	If no history of cyst → 5ml GnRH If previous treatment for cyst → CIDR 7 Days		For CIDR removal Always recheck
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
None	None	Vaginal to detect recent ovulation	Always recheck
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
Any other reproductive pathology		Treat according to veterinary diagnosis	

Not seen bulling second presentation 63 – 70 DIM

Dominant structure	Treatment	Action	Represent 7 days
CL	2ml Prostaglandin	Serve to observed oestrus	Not served
Follicle >10mm	None	Serve if seen	Not served
Follicular cyst (25mm)	If no history of cyst → 5ml GnRH If previous treatment for cyst → CIDR 7 Days		For CIDR removal Always recheck
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
None	None	Vaginal to detect recent ovulation	Always recheck
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
Any other reproductive pathology		Treat according to veterinary diagnosis	

Not seen bulling third presentation 71 – 78 DIM

Dominant structure	Treatment	Action	Represent 7 days
CL	If previous tx: PG If no previous PG	Commence Ovsynch 2ml PG	For PG if not served If not served
Follicle >10mm	Commence Ovsynch	Serve if seen	For PG
Follicular cyst (25mm)	If no history of cyst → 5ml GnRH If previous treatment for cyst → CIDR 7 Days		For CIDR removal Always recheck
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
None	CIDR 7 days		Always recheck
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
Any other reproductive pathology		Treat according to veterinary diagnosis	

Not seen bulling fourth presentation 79 – 86 DIM

Dominant structure	Treatment	Action	Represent 7 days if
CL	If previous tx: PG If no previous PG	Commence Ovsynch 2ml PG	For PG if not served If not served
Follicle >10mm	Commence Ovsynch	Serve if seen	For PG
Follicular cyst (25mm)	If no history of cyst → CIDR 7 days If CIDR present → Remove		Represent 7 days Always recheck
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
None	CIDR 7 days		Always recheck
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
Any other reproductive pathology		Treat according to veterinary diagnosis	

Negative PD 75 – 100 DIM

Dominant structure	Treatment	Action	Represent 7 days
CL	PG	Serve if seen	If not seen
Follicle >10mm	Commence Ovsynch	Serve if seen	For PG
Follicular cyst (25mm)	If no history of cyst → 5ml GnRH If previous treatment for cyst → CIDR 7 Days		For CIDR removal Always recheck
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
None (Anoestrus)	CIDR 7 days		Always recheck 14 days
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
Any other reproductive pathology		Treat according to veterinary diagnosis	

Negative PD >100 DIM

Dominant structure	Treatment	Action	Represent 7 days
CL	Commence Ovsynch	Serve if seen	For PG, if not served
Follicle >10mm	Commence Ovsynch + CIDR		For PG
Follicular cyst (25mm)	CIDR 7 Days		For CIDR removal
Luteal cyst (>25mm)	2ml PG	Serve if seen	Not served
Endometritis	If CL present-> 2ml PG If no CL present-> Metricure		Recheck in 1 week
None (Anoestrus)	CIDR 7 days		Always recheck in 14 days
Any other reproductive pathology		Treat according to veterinary diagnosis	

Definitions:

Ovsynch: hormonal synchronisation protocol defined as; Day 0: GnRH, Day 7: PGF2a, Day 9: GnRH, Day 10: FTAI.

Summary of veterinary reproductive interventions administered:

Veterinary treatment	High space group	Control space group
5ml GnRH (cyst)	6	4
CIDR only	4	5
CIDR + Ovsynch	2	2
GnRH for Ovsynch commencement	30	23
Estrumate for Ovsynch	24	12
Estrumate only	59	43
Metricure	1	1

*if a cow was served after the first GnRH for Ovsynch the remainder of the Ovsynch was cancelled.

2) Picture of Ridgeway milk progesterone enzyme immunoassay protocol

RIDGEWAY SCIENCE

RIDGEWAY MILK PROGESTERONE ENZYME IMMUNOASSAY PROCEDURE
Store in a cool place (4-8°C)

1. Bring to room temperature and strip foil from required number of wells. Empty wells and tap dry onto tissue. Add 10µl of standard (0-20ng/ml) or sample to each well. Add 10µl PBS to NSB wells
2. Add 200µl progesterone-enzyme label (make sure fully dissolved) to ALL WELLS except NSB
(NSB well is PBS and wash buffer only)

Leave 2-2.5 hours at room temperature (22°C).

3. Wash 3 times in wash buffer (200µl; 2 min each). Tap dry on paper each time.
- *4. Add 200µl alkaline phosphatase substrate
Leave 45-60 minutes.
5. Compare colour development of samples with standards.
Read optical density at 544 nm after zeroing machine on blank well and compute concentration from standard curve. Results are in ng progesterone/ml serum
(Alternative substrate Sigma P4744 at 140 mg/25ml using 405 nm filter).

Milk samples
Store samples frozen. Mix thoroughly after thawing.

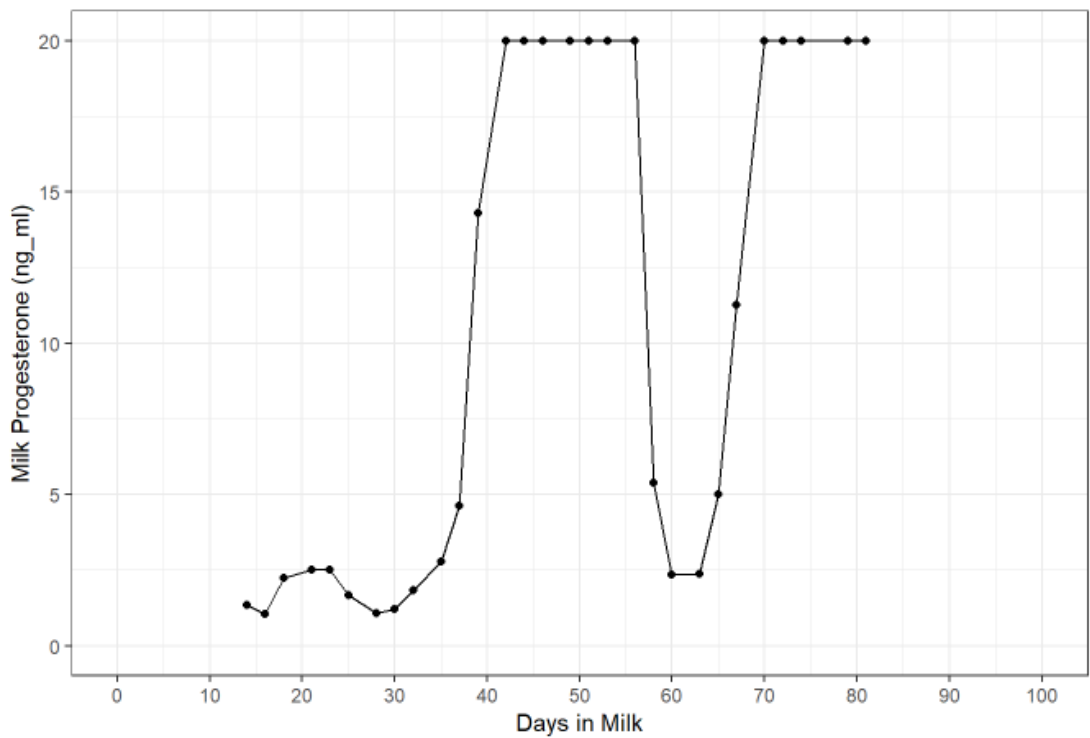
Milk standards
Use 0, 1, 2, 5, 10, 20 and 50ng/ml progesterone standards provided in kit

Substrate
*Dissolve substrate powder in 25ml substrate buffer 2. Sufficient for one plate. Store in the dark. Do not contaminate with progesterone-enzyme label 1. Takes 20-30 minutes to dissolve

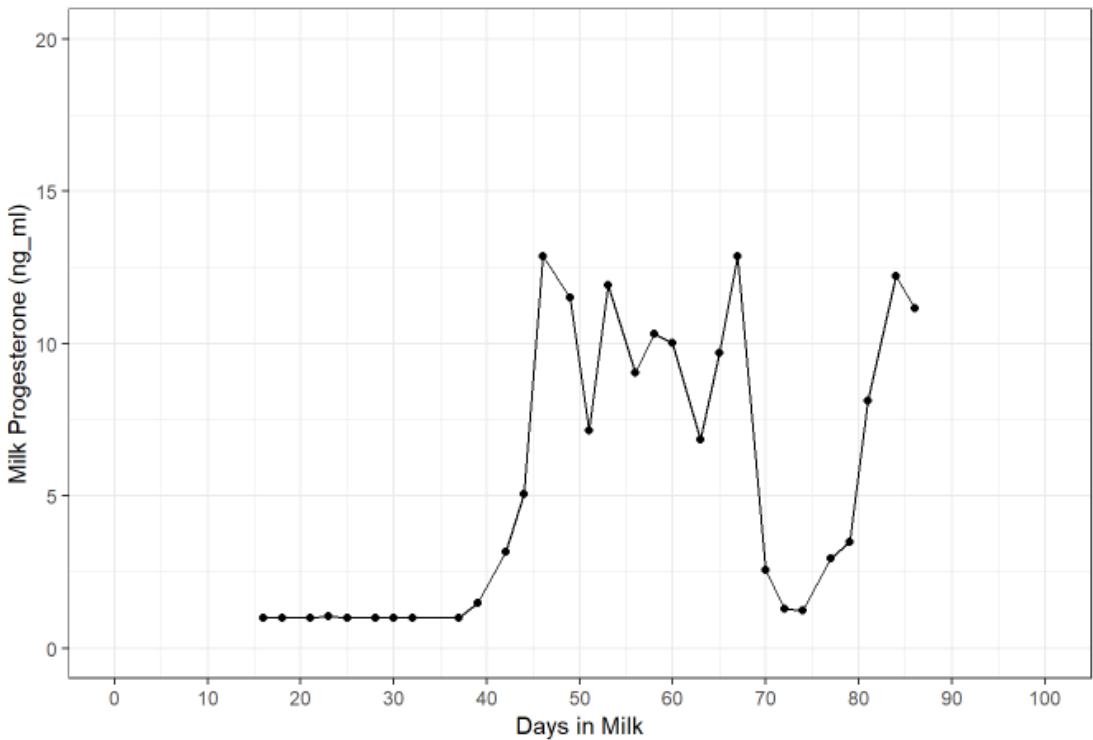
WARNING All solutions contain thimerosal (0.01% w/v). This is toxic. Dispose of carefully and wash down sinks with excess water. Keep away from food and children and wash your hands after use

3) Progesterone profile examples

a) Profile type: Normal



b) Profile type: Abnormal



c) Profile type: Delayed ovulation

