Designing housing to meet the needs of the dairy cow: What characteristics do cows value in a lying area?

By

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“There’s nothing like sitting back and talking to your cows”

- Russell Crowe

To the cows.

DECLARATION

I declare that the work presented in this thesis is my own original work, written by myself, which has not been accepted in any previous application for a degree. I have acknowledged all sources of information and assistance which have been used in this thesis.

Laura Shewbridge Carter

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ABSTRACT

Dairy cows are motivated to access pasture, potentially driven by the need for a comfortable lying area. However, most cows experience indoor cubicle housing annually which may not meet the behavioural or welfare needs of cows. The studies reported here set out to improve our knowledge on the lying conditions provided by pasture that makes it attractive for cows to lie down, with the hope that the findings will help influence the design of future dairy cow housing.

The space available and surface type are two qualities that have been identified previously as affecting cow lying behaviour. The first study used preference testing and a trade-off test to investigate which quality cows valued more. Cows traded lying on their preferred surface with a cubicle for lying on their second preferred surface as an open-lying area (P=0.02), demonstrating the importance of space when choosing where to lie down.

To quantify this preference, a second study measured cow motivation for an open-lying area, using walking distance as an indicator, when cows had ‘free’ access to mattress cubicles. Two different surface types were used, mattress and straw, to identify if surface type affected motivation. Although cows reduced lying times on the open-lying areas at the longest distance tested (P<0.001), they did continue to walk this distance to lie down on the open lying areas for >60% of their total lying time, indicating a high motivation. Surface type had a limited effect on motivation, with lying space the main motivation for accessing these open-lying areas.

The final study investigated cow preference and motivation for lying outdoors, when the lying space and surface type indoors and outdoors was equal. An interaction was found between preference and motivation for lying indoors and outdoors (P<0.001), with cows showing no preference between locations, and low motivation to lie down outdoors.

The original findings of this thesis have identified features of the lying area that are important for cows. The results have implications for lying area provisions by farmers and show that housed cow welfare can be improved with innovative housing design.

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CHAPTER 1: LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

Cattle have evolved as grazing animals, spending most of their domestic history being grazed on pasture (Epstein and Mason, 1984). Generally, the public believe that dairy cows should be at pasture, as it is their ‘natural’ environment and perceived as best for welfare (Cardoso *et al*., 2016). In the last 50 years, dairy farming has intensified, with the development of ever improving milking machines (Ekesbo, 2011). The modern dairy cow has high nutritional needs, due to increased milk yields, which is difficult to fulfil on a pasture-based diet (Kolver and Muller, 1998). All-year-round housing, whereby cows are housed indoors all year round to control feed intake, is increasing in practice in the UK, where traditionally cows were given seasonal pasture access (Haskell *et al*., 2006), and across Europe (van den Pol-van Dessellaar et al., 2008).

When housed, cubicle housing, known as free-stall housing in North America, is most common (Margerison, 2011), with cows having free access to cubicles for lying down, designed to discourage cows from urinating and defecating on the lying area, promoting cow hygiene. Studies have investigated various aspects of cubicles (***section 1.5.1***) and have compared them against pasture access (***section 1.5.2***), the majority finding that cows have a preference for and are motivated to access pasture (***section 1.5.2.1***). Open pack areas, discussed in ***section 1.5.3***, are thought to be the future of cow housing, offering housed cows some of the benefits of pasture, although pasture has been found to still be preferred.

For reasons discussed in ***section 1.5.2.2*** and ***section 1.5.2.3***, it is thought that the driving force behind this pasture preference and motivation is the lying behaviour of cows, with pasture better meeting the behavioural and welfare needs of dairy cows than indoor housing. However, due to confounding factors in previous studies, it is unclear what qualities of pasture cows’ value for lying down. If these qualities could be identified, they could be applied to the design of indoor housing, addressing some of the welfare concerns around housing dairy cows.

1.2 WELFARE ASSESSMENT

1.2.1 *Defining Animal Welfare*

The definition of "animal welfare" has evolved with time, being modified as humans begin to understand welfare and what it means to the animal (Broom, 2014).

The standard of living for animals kept under our care became of particular public interest in the mid 1960's, sparked by the publication of Ruth Harrison's "Animal Machines" (Harrison, 1964). Harrison's book highlighted the then recent and relatively rapid development of industrialised agricultural practices she termed 'factory farms'. This was the livestock industry's response to the population increase brought about after World War II, with "Animal Machines" centring on a main theme of animal suffering in such unnatural environments (Fraser, 2008c).

The UK government responded to public concern following the publication of Harrison's book by setting up the Brambell Committee (1965). The committee was to investigate and set out recommendations for the welfare of intensively farmed animals (Webster, 2005), concepts that are now referred to as the 'Five Freedoms' (1. Freedom from hunger and thirst; 2. Freedom from discomfort; 3. Freedom from pain, injury or disease; 4. Freedom to express normal behaviour; 5. Freedom from fear and distress). These were published in the "Brambell Report" (1965) and influenced both UK and European animal welfare legislation, as well as the focus of scientific research, with the 'Five Freedoms' continuing to have an influence on how we measure, research and manage animal welfare (Rushen, 2008; Veissier *et al*., 2008). Different ways in which we are able to assess welfare will be briefly discussed in terms of how we define welfare, before focusing on the use of animal behaviour in the assessment of welfare.

In an attempt to define "animal welfare", Broom (1986) described it as 'an animal's ability to cope with its environment' on a sliding scale from very good to very bad. Primarily, when an animal is in adverse conditions, they use various methods to counteract these conditions in order to cope. This definition allows for the scientific measurement of how well an animal is coping, but it is a moral decision of the observer to deem the amount of effort exerted to cope, and for how long, that is acceptable before welfare is compromised and considered poor (Broom, 1988; Dawkins, 1988). Additionally, it does not take into account the positive aspects of animal life, beyond just coping and not suffering. The term "quality of life" considers whether "coping" with one's environment is enough to constitute good welfare, asking what the animal likes or wants, as well as needs and focusing on the quality and the how a higher level of welfare is achieved (Wemelsfelder, 2007). The Five Domains, originally formulated in 1994 (Mellor and Reid, 1994) are regularly updated to include recent developments in animal welfare science, with the ability to take into account positive aspects of animal life (Mellor et al., 2020; Current Five Domains: 1. Nutrition; 2. Environment; 3. Health; 4. Behaviour; 5. Mental State).

Although there is yet to exist one universally accepted definition for ‘animal welfare’, Fraser (2003, 2008c) describes three overlapping ethical concerns which have evolved, attempting to define animal welfare in a measurable sense (Figure 1). The following section describes techniques used to assess animal welfare under each of these three headings.

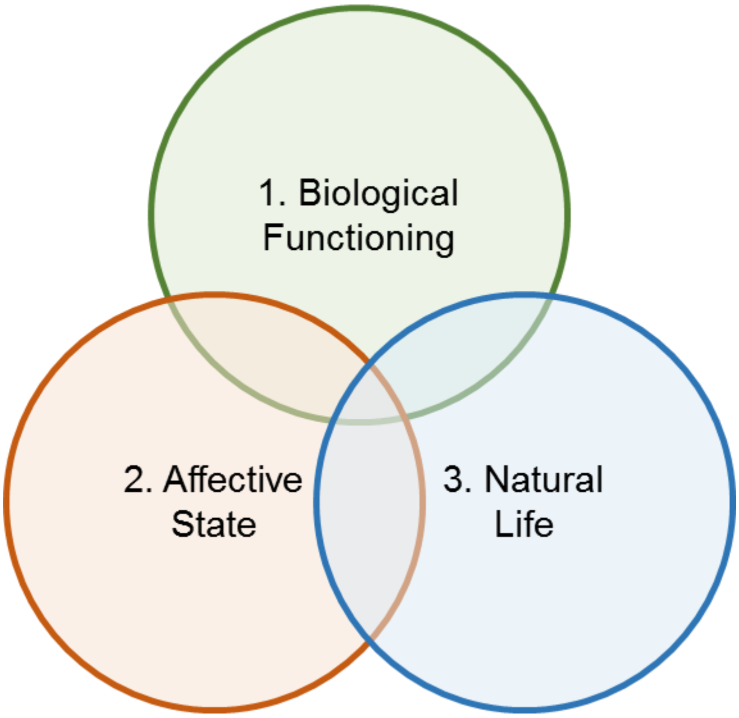

Figure 1. Graphical depiction of three overlapping ethical concerns attempting to define animal welfare in a measurable sense (Fraser, 2008c).

1.2.2 *Assessment of Animal Welfare*

1.2.2.1 Biological Functioning

The first of these ethical concerns considers an animal’s welfare high in terms of biological functioning, concerned with good health, reproductive success and performance in terms of yield (von Keyserlingk and Weary, 2017). In some cases, poor welfare can adversely affect productivity. Resource allocation within the body can be affected by stress, which can be observed as reduced production in livestock, such as poorer growth rates, body condition and yield, indicating poor welfare (Blache *et al*., 2011; Broom, 2009). Milk yield has been shown to decrease for lame dairy cows, with more severe lameness cases having a larger decrease in yield (Warnick *et al*., 2001). However, production rates on their own are not always a reliable welfare indicator, with high production rates for livestock often not related to good welfare at all but rather genetic selection for high yield. In selecting for high yielding animals, with the neglect for other traits, welfare can in fact be impaired (Rauw *et al*., 1998). This can also be the case for captive wild animals; for example, the lions at Dublin Zoological Gardens had very high reproductive success rates in the 1800's (Ball, 1880). Despite the animal's good physical health and biological functioning, those lions today would not be considered to have a high, or even a good, standard of welfare, being kept in barren, overstocked, cages in unsuitable social groups, common to zoos during the Victorian Era (Hosey *et al*., 2013b). This highlights the importance of the overlapping aspect of these three ethical considerations when assessing an animal's welfare state.

Although biological functioning and productivity require careful consideration before being used as welfare indicators, health indicators are the least controversial when studying animal welfare, with disease and injury being widely accepted as evidence for poor welfare (Dawkins, 1988).

1.2.2.2 Affective State

The second of these ethical concerns is interested in the affective states of the animal, referring to "emotions and other feelings that are experienced as pleasant or unpleasant" (Fraser, 2008c). In 1980, Dawkins published her book "Animal Suffering", highlighting the importance of animals' subjective, emotive states, or 'feelings', and that evidence of a long term negative affective state is poor welfare, regardless of an animals physical health (Dawkins, 1980). For Dawkins, "to be concerned about animal welfare is to be concerned with the subjective feelings of animals, particularly the unpleasant subjective feelings of suffering and pain" (Dawkins, 1988). Mendl (2001) also acknowledged that animals in good physical and biological health may experience "subjective suffering", and therefore poor welfare.

Positivism in the 20th century, a view that science only dealt with the material world, had a large effect on how subjective animal emotions and feelings were measured objectively (Fraser, 2009). Scientists were encouraged to establish rules to gather quantitative data using highly controlled experimental environments, irrelevant to real life scenarios, intended to make "the affective states of animals irrelevant to scientific explanation"

(Fraser, 2009). Scientists characterising animal behaviour qualitatively and using subjective language to describe animal feelings, such as the work of Jane Goodall (Goodall, 1971), were often dismissed during this time. The development of physiological indicators became a popular branch of animal welfare science to ascertain states of negative welfare (Duncan and Petherick, 1991). Principally, during stressful situations, the body responds in various physiological ways that can be measured (Blache et al, 2011). For instance, a study on the effect of administering and not administering local anaesthetic to calves during dehorning, using cortisol as an indicator of stress, found a large increase of plasma cortisol after disbudding in calves without pain relief (Petrie et al, 1996). But such physiological measures are not confined to stressful events, occurring during activities which could not be considered aversive, such as courtship, and are often quite difficult to interpret, with studies getting contradictory results and questioning the validity of these measures (Broom, 1988; Rushen, 1991). Two hours after disbudding, the study above found that the calves which received local anaesthetic, as it began to wear off, started to show an increase in cortisol levels. These levels surpassed that of the calves without any pain relief, whose cortisol levels had decreased. It's suggested that without pain relief, the calves' initial increase in cortisol may have in turn helped to suppress inflammation, resulting in experiencing less pain in total than calves administered short term pain relief. A study looking at intensively housed pigs found no physiological differences between indoor tethered pigs in a concrete floor stall and those kept in a group, outdoor paddock when analysing corticosteroid concentrations, the maximum corticosteroid binding capacity (MCBC), and free corticosteroid concentrations (Barnett *et al*., 1984).

Although highly objective and quantifiable, it became apparent that, physiological measures could not completely define an animal's state of welfare. Aspects such as animal behaviour began to be considered to measure affective states. For instance, a study published in the same year as Barnett *et al* (1984) by Blackshaw and McVeigh (1984) showed that group housed sows did not exhibit the same stereotypic behaviour as tethered sows, suggesting decreased welfare in tethered sows. The study of animal welfare began to move towards finding and verifying behavioural measures for welfare to gain an insight into subjective, internal animal states (Dawkins, 2004).

One such measure is that of abnormal behaviour, referring to activity levels or specific behaviours redirected inappropriately, which can then be used as an indicator of poor welfare (Fraser and Broom, 1990). Tail biting in pigs, whereby one pig manipulates the tail of another in their mouth, is referred to as an abnormal behaviour with multifactorial origins (Sonoda et al., 2013). Over time, continuation of the behaviour damages the tail and is a widely accepted welfare concern, an indicator of a negative affective state in pigs and a measure for poor welfare on commercial pig farms (van Putten, 1969; Schrøder-Petersen and Simonsen, 2001). Feather plucking, despite being a regular behaviour, can become a deleterious abnormal behaviour, in the form of self-mutilation, and is an accepted indicator of poor welfare for animals displaying a negative affective state. This behaviour is believed to be performed in response to the negative affective state of boredom and is common in birds housed individually with little or no enrichment, such as domestic parrots (Owen and Lane, 2006). Feather plucking in laying hens is well researched, with the provision of substrate as a foraging material being cited as an effective solution to reducing this behaviour, and therefore boredom, improving the animals affective state and, therefore, welfare (Blokhuis and Wiepkema 1998; Aerni et al, 2000; El-Lethey et al, 2000).

Stereotypic behaviours are a type of abnormal behaviour and can be defined as behaviours from the normal repertoire of an animal which are repeated with little variation and seemingly no function (Mason, 1991b). The cause is often linked with poor or inadequate environmental conditions, manifesting in the outward expression of stress in the form of repetitive pacing, swaying and oral stereotypies, among others (Mason, 1991a). Terlouw and Lawrence (1993) reported that food deprivation, ingestion and housing interact together to affect the development of stereotypies in farmed sows, with only feed deprived sows developing stereotypies, compared to those not deprived of food. Keiper (1969) reported that pacing observed in caged birds is related to the physical restraints of the cage, both size and lack of swinging perch in the cage, and that spot-picking, where a bird repeatedly taps the side of their beak against a particular spot either on themselves or their environment, is associated with laboratory conditions. It has been suggested that stereotypies serve as coping mechanism in stressful environments (Wurbel *et al*., 2006; Olsson, *et al*., 2001). In a review investigating the relationship between suffering and stereotypies, they have been described as ‘do it yourself enrichment’ and ‘repetitive mantras’ (Mason and Latham, 2004). Despite this, the authors emphasize that until more research is conducted to increase the understanding of the role of stereotypies for animals, their appearance should be taken seriously as being related to poor welfare.

Preference testing, a behavioural assessment approach whereby animals are given a choice of variations on an environmental factor and tell us which their favourite is, is a reliable method to understand how an animal perceives their environment. Motivation tests are able to assess the strength of preferences, whereby the animal gains access to the resource by exerting effort, increasing in effort until the animal gives up. Preference and motivation testing are the main experimental methods used in this thesis and therefore will be described in greater detail in ***section 1.3***.

1.2.2.3 Natural Life

The third and final ethical concern is that of living a natural life. One of the main concerns expressed in the Brambell report was the unnatural conditions of intensified animal farming which may lead to the restriction of natural behaviours to such an extent that welfare would be compromised (Keeling et al., 2011). The assumption is that an animal's welfare in an unnatural environment may be compromised if not living a natural life, as they would do in the wild, focusing on the behavioural repertoire of the animal (Mason and Burn, 2018). Welfare can be assessed by studying animal behaviour in the natural environment and if behaviours are absent in captivity, welfare is assumed to be impaired. One of the best known examples, domestic pigs were let loose in a wooded area on the hills near Edinburgh. Their behaviour was monitored over a substantial period of time, and was found to be highly similar to the behavioural repertoire of wild boars despite many generations of housing indoors (Stolba and Wood-Gush, 1984; Stolba and Wood-Gush, 1989). This helped to validate the study of wild conspecifics in order to build a data base of natural behaviours, which could then be used to assess welfare. Based on their observations, the 'Edinburgh family pens' were created to house pigs commercially, allowing them to behave similarly to the semi-wild pigs living on the Edinburgh hills and thus improving welfare (Kerr et al, 1988). Although proving useful, captive animals not performing a full behavioural repertoire as their wild counterparts may not necessarily be experiencing poor welfare (Dawkins, 1980) nor may this be due to environmental insufficiencies. Some wild behaviours, if expressed in captivity, may indicate poor welfare, such as fear from predators, and therefore being absent improves welfare (Keeling and Jensen, 2017).

Animal welfare science today recognises the need for a holistic view to encapsulate and define animal welfare, with the use of various assessment tools. With this in mind, recent welfare assessments for various animals under our care include health, resource, physiological and behavioural parameters (Welfare Quality®, 2009; Barnard et al., 2016; Czycholl et al., 2017; Righi etal., 2019).

1.2.3 *Positive Welfare Measures*

The term quality of life, previously reserved for humans, has found its way into animal welfare science and refers to welfare in the long term, focusing on the presence of positive welfare indicators rather than just the absence of poor welfare indicators and the quality rather than the quantity of these (Wemelsfelder, 2007). It means to consider that the absence of suffering in itself is not indicative of good welfare, but to be concerned with the presence of pleasurable states, with these positive states outweighing negative states over a long period of time (Broom, 2007). There has been a shift in welfare science towards an emphasis on such positive, pleasurable states.

In a review of positive welfare assessment, Yeates and Main (2008) discuss the use of physiological, cognitive processes and behavioural outputs to quantify positive welfare. The authors describe physiological measures as being underdeveloped but that cognitive processes, with our increasing understanding, can become a promising welfare assessment tool. Cognitive bias, referring to how emotional states can alter informational processes, such as attention, memory and judgement (Olsson et al., 2001), have been shown to occur in negative affective states in animals (Mendl, 1999). This has also been shown for positive affective states in humans (Ashby et al., 1999; Isen et al., 1987), with Yeates and Main (2008) indicating that a cognitive bias can also exist in animals in a positive affective state. A review by Boissy et al. (2007) suggests that there is potential for the use of cognitive processes to be used as an assessment of positive emotions in animals. Harding et al. (2004) were able to demonstrate that rats in unpredictable housing, which induced a depression like state in the rats, were more pessimistic, showing reduced anticipation for a positive event. Rats in predictable housing were found to be more optimistic, which is comparable to humans, and could be used to assess positive states in animals. van der Harst et al. (2003) demonstrated that rats kept in standard laboratory housing conditions exhibited an increased sensitivity to reward than animals housed in more enriching conditions, implying that affective state again alters anticipation. In contrast, cognitive bias can also be used to assess emotions on a sliding scale in animals, from positive to negative. Dairy cows have been shown to increase the amount of vigilance behaviour they exhibit when more fearful of a particular person or unfamiliar environment (Welp et al., 2004). The authors suggest a vigilance scale could be applied to quantify the degree of fearlessness to fearfulness for animals. Although this study investigated a negative affective state, that of fear, it could lead to the formation of a positive welfare indicator for assessing fearlessness.

A partially understood behavioural marker for positive welfare assessment has been that of play behaviour. Play behaviour can be defined as (1) not completely functional; (2) spontaneous and pleasurable; (3) appearing to mimic regular behaviours but differing in structure and/or timing; (4) repeated behaviours, but not in a stereotypical fashion; and (5) only occurring when the animal is in a relaxed state, i.e. when survival needs have been met, such as hunger, thirst, rest and health (Burghardt, 2005, cited in Oliveira et al., 2010). Although we can define play behaviour above, there is disagreement over statement (1), with Špinka et al. (2001) proposing various theories supporting the functionality of play, including self-assessment, socialising, training for the unexpected or a multitude of these functions (Špinka et al., 2001). As per point (5) of Burghardt's (2005) definition of play, as play only occurs when an animal is in a relaxed state and with its immediate survival needs met, this would suggest play behaviour as having a direct link with animal welfare, and could be used as a positive welfare indicator. But studies have shown that play can occur or increase with stress. It has been shown that during the decline in maternal care that occurs during the weaning period of rats, considered as a stressful period for the pups, an increase in play behaviour is shown amongst the pups (Smith, 1991). A study examining play behaviour in captive bonobos has shown that play increases before feeding to reduce social tensions (Palagi, 2006). A rebound effect, whereby a behaviour increases when conditions are improved, has been seen for play behaviour in both calves and heifers (Jensen, 1999). Those which were confined with restricted ability to perform locomotive activities for 4, 3 and 1 week were reported to perform more locomotive play behaviour than unconfined controls when given access to an open field. In a review by Held and Špinka (2011), other difficulties with using play as a welfare indicator are highlighted, such as play varying between and within species, differing with age and sex, and variation occurring due to individual personalities. Specifically reviewing dog play behaviour and its welfare implications, Sommerville et al. (2017) imply that the association between play and welfare is multifaceted, concluding that "play might be a useful positive welfare indicator only for some play types and contexts and more research is required to identify these for different species and life stages"

Finally, Qualitative Behavioural Analysis (QBA) is a technique developed for welfare assessment, both positive and negative. This technique asks observers to subjectively assess overall animal behaviour, the animal's body language, how they do things rather than focusing on what they do. Essentially it looks at the quality of animal behaviour and was originally developed by Joan Stevenson-Hinde using rhesus monkeys (Stevenson-Hinde and Zunz, 1978). From her experience of behavioural observations, she noted that observers "end up not only with behavioural data, but also with clear impressions of individuals" and began generating subjective adjectives for the animals, considering herself, and extending to all observers, "another kind of recording instrument" (Stevenson-Hinde et al., 1980). Wemelsfelder (2007) reviewed work her and that of colleagues at SRUC carried out over 10 years using a Free Choice Profiling (FCP) methodology of QBA. FCP allows observers to generate their own descriptive terms for behaviour when observing clips of animals, then, in a follow up session, watch the same clips and use their adjectives to score the intensity of the behaviours. For an instrument to be used to record data, for instance observers used for QBA, they must be shown to be reliable, being able to repeat measures while showing agreement among observers, and valid, recording meaningful results which, in this case, correlate to quantitative assessments of behaviour (Wemelsfelder, 1997). When assessing dairy cow group social behaviour using FCP, Rousing and Wemelsfelder (2006) found agreement among observer results with observers accurately repeating the assessment and reported a significant correlation between the qualitative observer assessments and a quantitative assessment using an ethogram to record behaviours. It was argued that the background in such video clips used for QBA could influence observer judgements. When using FCP to assess pigs, Wemelsfelder et al. (2009) did find an effect of background (indoor vs. outdoor) on the second consensus dimension (confident/content – cautions/nervous), but when both dimensions were considered, the effect was relatively small, and unlikely to cause an overall effect on the assessment. A study investigating the validity of free choice profiling QBA for pig behaviour using observers of different backgrounds, pig farmers, large animal veterinarians and animal activists, found that although the observers differed in background and outlook, established via a questionnaire during the study, there was a high inter- and intra-observer reliability (Wemelsfelder et al., 2012). Similarly, QBA has shown to be a suitable tool for assessing the welfare for a range of animals; horses (Fleming et al., 2013; Napolitano et al., 2008), dogs (Walker et al., 2010), goats (Grosso et al., 2016), dairy buffaloes (Napolitano et al., 2012), donkeys (Minero et al., 2016) and sheep (Phythian et al., 2016).

Although these studies are promising for using QBA, when a QBA and Welfare Quality® protocol, shown to have relatively high validity as an on-farm welfare assessment (Keeling, 2009), were carried out on 43 commercial Danish dairy cow farms by trained assessors, only weak correlations were found (Andreasen et al., 2013). The conclusion from the study was that the quicker QBA was not able to predict the same outcomes as the longer Welfare Quality® protocol, but the authors suggest that there are still validity and reliability concerns with the latter, with not all aspects being validated on farm (Forkman and Keeling, 2009), and that the farm sample size, along with the similarity of farm standards, may have been too small, making it unclear whether it was the QBA or Welfare Quality® protocol which was underperforming (Andreasen et al., 2013).

1.3 PREFERENCE AND MOTIVATION TESTING

Preference testing is a commonly used method to assess animal welfare. Dawkins (2004) stated that behaviour is an important indicator for ‘what animals want’, giving appeal to preference and motivation testing.

As mentioned previously in ***section 1.2.2.2***, preference and motivation tests are the main experimental procedures used in this thesis and therefore will be described in greater detail below as a measure for animal behaviour and welfare indicator, as well as the limitations of such tests.

1.3.1 Preference Testing

Preference testing aims to ask the animals what they prefer when presented with a number of possibilities for a particular aspect of their environment, making the assumption that the choice the animal makes is in their best interest and therefore providing high welfare (Fraser and Nicol, 2011). The possibility of 'asking' animals to tell us what they prefer as a factor of animal welfare research was first proposed by William Thorp in an essay to the Brambell Committee (Fraser, 2008b). According to Fraser and Nicol (2018), the first preference test to attempt to resolve a farm animal welfare issue arose from a criticism by the committee about the type of flooring used in cages for lying hens, which they suggested be replaced by a heavier mesh. It was Hughes and Black (1973) that conducted the preference test using four different floor types: fine-gauge 'chicken wire', which the committee were critical of; a rectangular mesh wire, 2.0 mm thick; a similar rectangular mesh wire, 3.25 mm thick; galvanised steel sheet with circular holes. The hens were presented with a choice of two surfaces at a time and how much time they spent on each was used as the measure of preference. Although hens exhibited no strong preference for one material above all others, they had a tendency to spend more time on the 'chicken wire', previously criticised by the committee. It was suggested that the shape of the mesh, hexagonal opposed to rectangular, offered the hens feet more points of contact and therefore more support and comfort. Additionally, Hughes and Black (1973) expressed excitement for "a new approach to animal welfare; objective assessment of animals' preferences should ultimately make subjective value judgements superfluous".

Since this first example of a preference test resolving a farm welfare issue, preference research has been used for a remarkable number of measures across a range of animals, a few of which are shown in the table below, from Fraser and Nicol (2018).

Table 1. Adapted from Fraser and Nicol (2018) showing examples of the use of preference research to study a range of animals' preferences across a multitude of environmental variates.

Variable	Species	Reference
Preferences for:		
Ambient temperature	Piglets	Morrison *et al*. (1987); Vasdal *et al*. (2010)
	Lobsters	Nielsen and McGaw (2016)
Illumination level	Pigs	Baldwin and Start (1985)
	Gerbils	van den Broek *et al*. (1995)
	Cattle	Phillips and Morris (2001)
	Fish	Gaffney *et al*. (2016)
Social contact	Pigs	Matthews and Ladewig (1994)
	Sows	Kirkden and Pajor (2006)
	Rats	Patterson-Kane *et al*. (2004)
	Horses	Sondergaard *et al*. (2011)
Bedding	Pigs	Fraser (1985)
	Horses	Hunter and Houpt (1989)
	Rodents	Blom *et al*. (1993)
	Cattle	Tucker *et al*. (2003)
Flooring	Pigs	Farmer and Christison (1982)
	Sows	Phillips *et al*. (1996)
	Cattle	Telezhenko *et al*. (2007)
Nesting materials	Mice	van de Weerd *et al*. (1998)
Dust-bathing materials	Hens	van Liere *et al*. (1990)
Shade	Cows	Schütz *et al*. (2011)
Pasture	Cows	Charlton *et al*. (2013)
Roughage structure	Calves	Webb *et al*. (2014)
Multiple resources	Blue foxes	Koistinen *et al*. (2016)
Analgesic drugs	Hens	Nasr *et al*. (2013)
Real and artificial plants	Fish	Sullivan *et al*. (2016)
Preferred design features of:		
Loading ramps	Pigs	Phillips *et al*. (1988, 1989)
Roosts	Hens	Muiruri *et al*. (1990); Schrader and Mueller (2009)

For example, Phillips et al (1996) gave sows a choice between three different lying surfaces in farrowing crates, metal, concrete and plastic. About 5 days before and 2 weeks after farrowing, sows were moved from their gestation room and housed in the testing area, which contained three farrowing crates, each with a different floor. The sows had free choice between the three crates and were found to lie down the most, and therefore have a preference for, the concrete floor crate, although this preference did decrease with time. In a follow up study, Phillips *et al*. (2000) presented sows with three identical farrowing crates at three different temperatures about 7 days before and 2 weeks after farrowing. Sows showed no preference between the surface temperatures before farrowing but preferred a warm surface (35°c) for the first 3 days after. As time progressed, they found that sows preferred a cooler surface (22°c). The authors suggest that this preference for a warm lying surface after farrowing could have influenced sow preference for a concrete floor in the previous study, as concrete is an insulating material.

Although preference tests do come with limitations, which have been outlined in reviews (Fraser and Nicol, 2018; Duncan, 1992), these limitations are relatively easy to address within experimental design and will be discussed in greater detail in ***section 1.3.4***.

1.3.2 Motivation Testing

Preference tests can be expanded upon to gain information on how motivated an animal is to gain access to their preferred resource. As preference does not necessarily infer improved welfare, testing the strength of this preference helps to identify any benefits to an animal's welfare (Fraser, 2008b). Motivation for a preferred resource must be further investigated in order to establish whether it actually does provide for an ethological need, leading to improved welfare (Jensen and Pedersen, 2008), or whether its absence leads to animal suffering (Dawkins, 1988). Simply, motivation tests require animals to work to gain access to a perceived valued resource, increasing this work until the animal gives up or reaches a ceiling effect, whereby they cannot physically work any longer. Schütz et al. (2006) demonstrated that lactating cows deprived of food for 6 or 9 hrs walked more than double the distance to gain access to feed than cows not deprived of food. In this study, a maximum distance walked was reached for each cow, when the cows gave up (average maximum distance walked after: 0 h deprivation = 30.7 m; 6 h deprivation = 64.7 m; 9 h deprivation = 76.9 m). Alternatively, von Keyserlingk et al. (2017) measured lactating cow motivation for access to fresh feed after 1.5 hrs of feed deprivation using a weighted gate. The weight on the gate was increased by 7kg every 24 hrs until no cow within the group successfully used the gate for two consecutive days. The maximum weight pushed for access to feed in this study was 70 kg, at which point it is unclear whether cows gave up or a ceiling effect was met, whereby they could no longer physically push a gate any heavier than this. This method was repeated for pasture access, after both morning and evening milking, allowing motivation for feed and pasture access to be directly compared. This study showed that there was no difference in cow motivation for fresh feed and pasture access, with cows pushing through the weighted gate as hard to access pasture as they did to access fresh feed. Additionally, cows pushed harder in the evening than in the morning for pasture access, demonstrating an increased motivation to access pasture at night.

Asking the animal to pay an ever increasing “price” for access is based on consumer demand theory, whereby a resource is said to have an inelastic demand if motivation for access does not decline, or declines very little, when the price increases (Dawkins, 1983b). Whereas if access declines as the price to access a resource increases, the resource is thought to have an elastic demand. For example, when farmed mink had to push a weighted door to gain access to different resources, with the weight increasing each week throughout the study, Mason *et al*. (2001) reported that mink preferred and were most motivated to gain access to the water pool, by visiting it the most and willing to work harder, more often, to continue to gain access. The authors then denied access to the water pool for 24 hours and monitored urinary cortisol levels, which are associated with stress, reporting an increase and suggesting a high level of stress was being experienced by mink when denied access to a water pool, with the provision of one potentially improving farmed mink welfare (Mason *et al*., 2001).

Operant conditioning can also be used to measure an animal’s motivation for access to a particular resource, whereby an animal learns to perform a behaviour in order to gain a reward (Mendl and Nicol, 2017). For example, Holm *et al*. (2002) conditioned calves to press a panel of fixed ratio (FR; a fixed number of times) with their heads to gain a reward of partial or full social contact, varying the FR throughout the study. The study found that the calves’ value and are more motivated to access an opportunity of full social contact, rather than partial social contact.

Lastly, basic preference tests can be further refined in the form of trade-off motivational studies, where animals must choose between different valued resources, gaining access to one while being excluded from another (Garland, 2014). This is often observed in the wild, for example when Lima *et al*. (1985) showed that grey squirrels were willing to trade foraging time for decreased predation risk. The authors found that grey squirrels will carry food to cover before consuming it, giving them less time to forage but a decreased chance of predation. However, when further from cover, the squirrels display more risky behaviour and forage for longer, likely due to considering the energetic costs. This concept can be used in studies whereby an animal is asked to choose between two or more variations of a commodity, varying in one quality, of which their preferred is known and made less attractive, often by the absence of a second valued quality, determining if the animal is willing to trade one valued quality for another and therefore telling us which quality the animal values more. For example, van de Weerd *et al*. (1998) investigated the preference of laboratory mice for two cages with two different nesting environments. A trade-off was created between their preferred nesting material (tissue paper) with an aversive floor type (grid floor) vs. a less preferred bedding material (sawdust) but in a nest box, found to increase mouse preference for a cage. The authors reported that even with the aversive floor type, mice chose the cage with the tissue over the cage with a nest box and sawdust, concluding that nesting material, rather than just providing a nest box and bedding, may influence laboratory mouse welfare (van de Weerd *et al*., 1998). Similarly, van Rooijen (1980); cited in Broom and Fraser, 2015b) used the knowledge that gilts preferred to lie beside other gilts to valuate preference for floor type against social preference.

Although preference tests are a useful tool, measuring the strength of a preference through motivational studies gives us more information about the preference to allow us to apply it correctly to improve animal welfare.

1.3.3 Choice Improves Welfare

Effectiveness is a framework used in human psychology which describes control, aided by the facilitation of choice over one's environment. It has been theorised effectiveness could be a component of well-being and welfare which can be applied to animals (Franks and Higgins, 2012). Addessi *et al*. (2010) reported that capuchin monkeys (*Cebus apella*) would rather have free choice of different foods, many of which were not well liked by the monkeys, instead of being limited to their favourite food treat. In a study by Makoto Endo *et al*. (2002) Mango fish (*Nile tilapia*) were given the opportunity to 'self-feed', by being trained to pull/push a switch to release food into the tank. They were identified as having a less stressed physiological state than fish with a scheduled feeding regime, which were being fed the same amount as the self-fed fish. This included having lower blood plasma cortisol, paler skin colour, and an increased immune response, suggesting an increase in welfare when in control over their feeding schedule. Taylor *et al*. (2001) conducted a study whereby they gave hen's operant control over the timing of their feeding and light regime, choosing when to gain access to extra food and light, both of which are highly controlled in a commercial environment by the farmer. These hens were yoked with non-control hens, which received the same feed and light outcomes as the hens with control, but didn't have the control over their occurrence. Hens with control exhibited significantly lower levels of preening with decreased resting behaviour compared to hens without control. This suggests that hens with more control over their environment are less stressed and therefore have better welfare than hens with no control. The authors also found that the hens without control had an increase in production, lying more eggs than hens with control. In this instance, if production is to be linked to improved welfare, this result contradicts the behavioural assessment and warrants further research into the effect control over ones environment has on the welfare of a lying hen. It has been suggested that allowing dairy cows the ability to have choice and control over their environment, in itself, may improve welfare, such as a choice to be indoors or outdoors (Motupalli *et al*., 2014; Webster, 2016; Charlton and Rutter, 2017). The full extent of offering animals a choice, and therefore an amount of control, over their environment is yet unclear, but is something to consider in future welfare discussions.

1.3.4 Limitations of Preference and Motivation Testing

Preference and motivation tests have to be carefully planned and designed in order to give an accurate insight into animal welfare, allowing for the outcomes to be interpreted correctly and not be mistaken (Fraser and Nicol, 2011). Something to consider is whether animals are able to weigh up short-term versus long-term benefits and consequences of their decision (Fraser and Nicol, 2011). Abeyesinghe *et al*. (2005) investigated 'self-control' in hens, testing whether hens were willing to wait for a larger food reward or be impulsive and accept a smaller food reward, reporting that when the reward was sufficient enough, hens could rationally differentiate future events and exhibit self-control, waiting for a larger reward. When the difference between the food rewards was less significant, the hens acted with more impulse, suggesting a limit to a hen's ability to rationally differentiate future consequences to their choices, although the same cannot be said for all species (Špinka *et al*., 1998).

An animal's previous experience, or lack thereof, regarding a choice in a preference test can influence the results and needs to be included as a feature of study design in order to account for this (Fraser and Nicol, 2011; Kirkden and Pajor, 2006). For example, guppies have been shown to have a negative frequency-dependent mate choice, in that females are more likely to mate with males of a novel colouration than those they are previously familiar with, contributing to the high variation in male colouration (Hughes *et al*., 1999). In ***section 1.3.1***, the first part of a study by Phillips et al (1996) was discussed, in which they found that sows preferred a farrowing crate with a concrete floor. The sows had previously been housed in a gestation area with a concrete floor and the authors proposed that this experience of a concrete floor, rather than a metal or plastic floor, influenced their preference. A similar preference test was repeated for the second part of the study, but one week beforehand, sows were housed on one of the three different floor types. Sows still exhibited an avoidance of the metal flooring with a preference for concrete after farrowing, regardless of pre-exposure. However, preference increased for the metal floor type before farrowing when pre-exposed to the metal or plastic flooring, with sows pre-exposed to concrete expressing as similar preference as per the first part of the study. This gave the authors valuable insight into sow preference for flooring type during different times of farrowing, demonstrating the importance of previous experience on preference and that, for sows, one week exposure to an unfamiliar floor type effectively increases acceptance of that floor type.

Following on from the first preference test, discussed in ***section 1.3***, a lot of early preference research, before the turn of the century, focused on laying hens. These studies found that a hen's preference is confounded by her previous experience for many different environmental factors. For example, chickens reared on litter, in comparison to those reared on wire flooring, are more likely to choose a peat floor than wire (Petherick *et al*., 1990). Previous experience was also found to confound preference for a run, which chickens eventually prefer to a cage after experience (Dawkins, 1977). Previous experience of conspecifics has shown to influence hen preference, with hens choosing a cage of familiar conspecifics over a cage of unfamiliar conspecifics, with single versus group rearing effecting preference for an empty versus occupied cages (Hughes *et al*., 1999). But Dawkins (1983a) reported that not all hen preference is effected by previous experience. Dawkins presented litter-reared and cage-reared hens with large and small cages with and without deep litter, measuring the time it took for them to leave the starting box and enter the test cage. A preference was found for a larger cage with litter, with no difference found in preference between litter-reared and cage-reared hens, despite cage-reared hens never having experienced litter before.

Preference, influenced by previous experience, can also change with increased familiarity. Tucker *et al*. (2003) reported that cows with previous experience of deep bedded sawdust cubicles, preferred them to sand cubicles when given a choice, but those familiar with sand cubicles showed a partial preference for both sand and sawdust cubicles, even after a restricted phase on all surfaces before the choice phase. The authors suggest that cows need time to adjust when switching lying surfaces to deep bed sand, but after a period of exposure, cows will accept it as an appropriate lying surface. Manninen *et al*. (2002) reported similar findings, with cows less accepting of sand in the beginning when compared to straw and mattress, but reported an increase in the use of sand as cows became more familiar with the lying surface.

When investigating an animal's motivation using consumer demand theory, the amount of reward can influence the elasticity of demand and give an inaccurate interpretation of the animal's motivation. Jensen *et al*. (2005) aimed to measure how long heifers were motivated to lie down in a 24 hr period using consumer demand theory, but first needed to identify an appropriate length of reward period, whereby the elasticity of the demand for lying down was constant. In order to do this, heifers were restricted from lying 9 hrs a day, had 9 hrs of free access to lying a day and were then asked to work for the remaining 6 hrs of potential lying time. The animals were trained to press a panel a number of times (10, 20, 30 ,40 or 50 times) to obtain more rest periods, of either 20, 30, 50 or 80 minute long. When given a reward of 30, 50 or 80 minutes of lying time, demand was inelastic, with heifers working for a similar total lying time regardless of how many times they needed to press the panel. However, demand became more elastic when the reward was just 20 minutes of uninterrupted rest, with heifers reducing the amount of times they were willing to work for a 20 minute lying reward when they had to press the panel more times. This would suggest that for heifers, 20 minutes is an inadequate reward in terms of amount of lying time to measure true motivation for lying. In part two of this study, the author's use 50 minutes of lying time as a fixed reward to measure inelastic demand for total lying time, found to be 12-13 hrs per day. Had they only used 20 minute rewards, demand for total lying time would have been found to be lower, demonstrating the effect reward magnitude can have on the measure of an animal's motivation. The level of reward in any animal preference or motivational study is important and the interpretation of the results should bear this in mind.

Studies where animals need to be trained and conditioned to perform a specific behaviour in order to receive a reward can have implications for interpretation of the results when studying motivation and preference if the animal is unable to learn to perform the behaviour correctly or associate the behaviour with the reward (Fraser, 2008b). Knowing that hens have a preference for a cage floor with litter as opposed to a bare floor, regardless of their previous experience (Dawkins, 1983a), Dawkins and Beardsley (1986) investigated hen motivation for a cage with litter versus a wire floor cage by training the hens to peck two different coloured keys to open a door to the different cages. The birds appeared not to learn or associate pecking the keys to open the doors, with the authors suggesting that this behaviour is performed in relation to food and can be used to access a food reward, but is an inappropriate behaviour to use to associate with a bedding reward. They performed the experiment again, replacing the pecking behaviour with the breaking of a photo-beam when the hens walked towards the cages, finding that, with time, the hens became significantly more likely to break the photo-beam for the cage with litter than the wire floor cage This demonstrated that the apparent lack of motivation to access a resource or perform a behaviour through an operant conditioning experimental design may be caused by the inappropriateness or inability for the animal to be conditioned to perform the necessary behaviour.

Although there are various limitations to preference and motivation tests, a well-designed and informed experimental design can overcome a lot of these limitations. Preference tests still do have value within applied animal behaviour research and have shown that when animals are provided with their preferred option, welfare benefits can occur (von Keyserlingk et al., 2009).

1.4 COW BEHAVIOUR AND WELARE

Having discussed different approaches to measuring and assessing animal welfare in the previous sections, specifically behavioural measures, we must now consider how to use these methods to assess cow welfare. In order to do this, we must first understand a cow's behaviour, discussed in ***section 1.4.1***, before we discuss the use of a behavioural welfare indicator for cows. The methodology used in this thesis focuses on the lying behaviour of the cow as a welfare indicator and therefore is discussed in greater detail in ***section 1.4.2***.

1.4.1 Natural Behaviour of Dairy Cows

It was once thought that modern cattle breeds diverged from wild Bovine species in a single domestication event, but phylogenetic studies using mitochondrial DNA of European, Indian and African breeds show that at least two separate domestication events took place (Loftus *et al*., 1994), if not more (Götherström *et al*., 2005; Zeder *et al*., 2006). All domestic cattle are derived from a single ancestral species in Asia, the wild aurochs, which has been extinct for more than 300 years (Mason, 1984).Today there is only a single species, *Bos primigenius*, with two distinct taxa existing; the humpless European taxa, *Bos taurus*, found primarily in the Northern Hemisphere, South America, Australia and New Zealand, and a humped, or zebu, taxa, *Bos indicus*, with Asiatic origin, found predominantly in Southern and Southeast Asia, the Middle East, and Africa (Phillips, 1961).

Cattle were originally domesticated and bred as draught animals, with countries having local breeds, which, when replaced with horses, allowed them to be farmed and exploited for production (Mason, 1984). Humpless European cattle have been bred primarily for milk and meat production, along with selected behavioural characteristics in the early stages of breeding, giving them a placid disposition (Albright and Arave, 1997b). Today's British Friesian, primarily a dairy cow, was bred at the end of the 19th century from Dutch breeds and British Shorthorn cattle (Mason, 1984).

With the domestication and selective breeding of cattle for thousands of years, and the extinction of their wild ancestors, it is unclear to what extent domestication has affected cow behaviour and whether the modern domestic cow retains any of its ancestor's behavioural repertoire, making it difficult to assess 'natural' behaviour in domestic cattle (Rushen *et al*., 2008b). Cunningham *et al*. (2001) analysed mitochondrial DNA of excavated skeletal remains of British aurochs and a range of modern domestic cattle breeds from across Europe, Africa and the Near East and found that the aurochs falls outside of the genetic range of present day domestic cattle. This suggests that even if the aurochs were still extant, the domestication process may have changed modern cow behaviour beyond comparison with their wild ancestors (Rushen *et al*., 2008b).

Populations of feral cattle and those which have had minimal human interference for hundreds of years have been used to draw comparisons from in order to better recognise the 'natural' behaviour of modern domestic cattle (Kilgour, 2012), which may be a useful technique to understand the welfare problems that exist on the modern farm (Špinka, 2006). In a review compiled of 22 studies assessing the behaviour of cattle at pasture with little human interference, Kilgour (2012) reported that cattle have an extensive behavioural repertoire comprising of 40 behavioural categories. Three of these behavioural categories take up between 90-95% of an individual's day; grazing, ruminating and resting, with grazing being the most common of these, which ranged between 6.8 – 13 h over a 24 h period and displaying a diurnal pattern, with less grazing occurring during the dark than during the daylight hours. Linnane *et al*. (2001) found seasonal changes to the diurnal grazing pattern for Kerry cattle in the south west of Ireland that lived in semi-feral conditions, with cows grazing mostly during the day in summer months, with night grazing increasing in frequency during winter months. Kilgour (2012) reported that rumination was shown to range from 4.7 – 10.2 h over 24 hours, with the majority performed while lying down and at night, as opposed to standing or during daylight. No diurnal pattern was observed for resting behaviour, which ranged from 3.6 – 10.3 h per day, possibly because resting while standing and resting while lying down was not differentiated for night vs. day in this review, but there was a tendency for the majority of resting to be performed while lying down. Tucker (2017) reports that, although there is a lying period during the day, cows spend more time lying down at night when at pasture and the diurnal patterns of grazing during the day and lying down at night are highly synchronized at pasture.

When looking at the feeding behaviour of cows, a study by Roca Fernández *et al*. (2013) investigating the behavioural activities of dairy cows in a pasture-based system compared to dairy cows in a continuously housed, total mixed ration (TMR) fed, cubicle system. This found that cows in the pasture system spend more time grazing at pasture (68% of daily budget) and in a synchronized fashion than housed cows feeding on TMR (22% of daily budget). In this pasture based system, cows grazed for more than 8 hours, resembling that of ‘normal’ grazing behaviour presented by Kilgour (2012) and Arnott *et al*. (2017). In comparison, a study of 205 lactating dairy cows in continuously housed cubicle systems, on both sand bedding and rubber mattress, in the United States found that time spent feeding had a mean of just 4.3 h/day (Gomez and Cook, 2010). The nutritional requirements of the modern dairy cow has increased with increasing milk yields through selective breeding (Phillips, 2002a), with TMR better meeting the nutritional needs of this modern cow. It is possible that cows spend longer grazing at pasture compared to feeding on TMR because they must eat relatively more at pasture to meet their nutritional needs than feeding on TMR.

Roca Fernández *et al*. (2013) found that the amount of time cows spent lying down at pasture was significantly less than housed cows, possibly due to increased grazing times at pasture. In contrast, Olmos *et al*. (2009) found that cows at pasture spent more time lying down (10.25 h/24h) than those continuously housed (9.05 h/24h), as did O'Connell *et al*. (1989), also noting the behavioural synchrony of pasture-based cows compared to housed cows. The inconsistency of time allocation to lying down at pasture could be due to different environmental conditions and feed qualities, as pasture quality can change feeding behaviour (Gibb *et al*., 1997) and has a tendency to influence grazing time (Hendricksen and Minson, 1980; O'Driscoll *et al*., 2008).

Both Roca Fernández *et al*. (2013) and O'Connell *et al*. (1989) found that the amount of time spent standing idle by cows was lower for cows at pasture when compared to housed cows. This could be due to grazing being more time consuming than eating TMR (Charlton and Rutter, 2017), with natural grazing behaviour requiring time to walk, investigate and make decisions before eating (Broom and Fraser, 2015a).

1.4.2 Use of Cow Lying Behaviour as a Welfare Indicator

Rest is important to animals, in order to conserve energy and allow for metabolic recoveries (Fraser, 1983), with cows mostly resting while lying down, as opposed to standing (Arnold, 1984; Kilgour, 2012). Studies show that cows at pasture or loose housing lie down for about 7-13 hrs/day (Arave and Walters, 1980; Arnold, 1984; Kilgour, 2012; Maselyne *et al*., 2017). When lying down, cows ruminate and sleep, which was thought could be differentiated by lying position; cows in rapid-eye movement sleep (REM) lie down with their head against their flank, or in a position where the neck muscles are supported (Girard *et al*., 1993; Albright and Arave, 1997a). However, recent research combining behavioural and physiological measures found that lying position cannot accurately predict sleep stage in dairy cows (Hunter et al., 2021). Rumination cannot occur during REM sleep, but can take place while the cow is in a state of drowsiness, when resting on the sternum (Fraser, 1983; Phillips, 2002b). Additionally, studies have shown that when cows are lying down, there is an increase in blood flow to the mammary glands compared to when cows are standing (Metcalf *et al*., 1992; Rulquin and Caudal, 1992), with studies reporting that increased blood flow is positively correlated to milk yield (Fullerton *et al*., 1989; Metcalf *et al*., 1992; Berger *et al*., 2016). This is supported in part by Munksgaard and Løvendahl (1993), reporting that over time, lying deprivation is likely to negatively affect milk yield as it reduces the plasma concentration of growth hormone, therefore suggesting that not only does increased lying behaviour improve dairy cow welfare, but can potentially be linked to increased milk production.

With lying behaviour demonstrated to be important for different biological functions for cows, a number of studies have used various methods to assess cow motivation for lying down. In a study in which cows had to press a panel a number of times in order to gain lying permission, Jensen *et al*. (2005) demonstrated that dairy heifer's had a high motivation for lying down and showed that they worked to lie down for an inelastic period totalling 12-13 hr/day. A study by Metz (1985) reported that cows that were prevented from lying down for 3 hours in the morning, greatly increased the time they spent lying down in the subsequent hours compared to when the same cows were not deprived of being able to lie down. When investigating the effect of food deprivation on cow lying behaviour, Metz (1985) found that time spent lying down decreased in favour of feeding in the first hour after receiving feed permission. However, when both feeding and the ability to lie down were prevented, lying behaviour of the cows was similar to when only lying was deprived, compared to when only feed was deprived. The author suggests that these results show that cows aim to lie down for a fixed amount of time per day, are highly motivated to do so and preventing them to lie down negatively affects their welfare. When both early and late lactation cows were deprived of lying, eating and social contact for 1hr, 9hrs and 12 hrs, Munksgaard *et al*. (2005) reported that with less time to access the resources, the proportion of time cows spent lying down increased (1hr = 56%; 9hrs= 64%; 12hrs = 71%), suggesting that lying down has a higher priority for cows over feeding and social contact, with no effect of lactation stage. There was no difference found between the amount of time cows spent lying down when deprived for 9 hours and 12 hours, again suggesting cows work towards a set amount of time lying down per day and that any less would be compromising welfare (Munksgaard *et al*. (2005).

With cows actively working to lie down and for a set amount of time each day, total lying times are a useful indicator for cow welfare, although it is often accompanied with lying bout frequency and lying bout duration to get a comprehensive understanding of the lying behaviour (Tucker et al., 2021). Generally, longer but fewer lying bouts are associated with harder lying surfaces, with cows reluctant to get up once lying down due to the discomfort experienced in the process of lying down and getting up (Herlin, 1997; Haley et al., 2000; Tucker et al. 2003; Rushen et al., 2007). Haley et al. (2001) found that cows had shorter, more frequent lying bouts on a mattress compared to a concrete lying surface, overall accounting for longer lying times on the mattress. In a study investigating the effect of bedding material and quantity on lying behaviour, Tucker et al. (2009) reported that lying bout durations on a rubber mattress increased as straw was added, improving cow comfort as surface compressibility increased with bedding quantity. Additionally, lying space can affect lying behaviour, with cows exhibiting longer lying bouts in larger cubicles (Tucker et al., 2004) and when stocking density is lower on an open rubber mattress (Schütz et al., 2015).

It is important to include lying bout measures when using lying behaviour to assess welfare, as higher lying times do not always indicate higher welfare. Krohn and Munksgaard (1993) found that cows housed in tie-stall systems have higher total lying times than cows housed in cubicles, accompanied by an increase in lying bout frequency. If using total lying time alone, this would indicate that tie stall housing provides cows with a higher level of welfare. However, it is suggested that because cows in tie stall systems do not spend time performing a more full repertoire of natural behaviours (such as walking, searching for food and grazing), they perform more lying events, leading to higher total lying times (Krohn and Munksgaard, 1993). Therefore, in the context of tie stall housing vs. cubicle housing, higher lying times indicates poor welfare. Likewise, cows that have higher locomotion scores, indicating high levels of lameness and therefore compromised welfare, lie down for longer than cows with low locomotion scores (Blackie et al., 2011a; Blackie et al., 2011b; Ito et al., 2010). However, high lying times caused by lameness can be characterised with longer and fewer lying bouts (Solano et al, 2016; Westin et al, 2016). When Ito et al. (2010) evaluated the relationship between lameness and lying behaviour of cows on 11 Canadian farms using deep-bedded cubicles, they found that not only did lame cows lie down for longer and have longer lying bouts, but they also had a larger variation between lying bout durations. Similarly, Solano et al. (2016) also reported lame cows having more varied lying bout durations than non-lame cows, accompanied with higher lying times, longer lying bout durations and fewer lying bouts. These studies highlight the importance of using other lying behaviour measures along with total lying time in order to fully understand the welfare state of cows based on their lying times.

Although total lying times, lying bout duration and lying bout frequency are most commonly measured when using cow lying behaviour as a welfare indicator, other behavioural and physiological indicators can also be used. A quick latency to lie down after a period deprivation has shown to demonstrate a high motivation for cows to lie down. After 4 h of forced standing, Krebs et al. (2011) found that after obtaining access to a lying area, cows lay down within 5 minutes. Norring and Valros (2016) found that after 4 h of lying deprivation before milking, cow latency to lie down was shorter compared to cows that were not deprived, concluding that a 4 h standing period was sufficient to increase cow motivation for lying down. Similarly, Tucker at al. (2018) reported that cows deprived of lying for 4 h before milking lay down over an hour sooner after milking than cows that had continuous access to lie down. A study investigating the effects of lying deprivation on cow behaviour reported that in the first hour of deprivation, restless behaviour, such as leg stomping, weight shifting and head swinging, increased compared to control cows, which were not deprived, and that these behaviours tended to increase over the deprivation period (Cooper *et al*., 2007). Munksgaard and Simonsen (1996) reported both a behavioural and physiological change in cows deprived of lying for 14 h/d for 3 weeks, changing the cow's reaction to a novel environment and affecting hypothalamic-pituitary activity, increasing ATCH concentrations (adrenocorticotropic hormone), further supporting that lying time can be used as a welfare indicator.

Finally, cows display a clear diurnal lying pattern, primarily lying down during the night time (Tucker at al., 2017; Tucker at al., 2021). This pattern of behaviour has been observed both for cows at pasture and in cubicle housing (Legrand et al., 2009; Winckler et al., 2015). Therefore, it is important when using cow lying behaviour as an indicator of welfare to record behaviour parameters over at least a 24 h period.

As mentioned previously in ***section 1.2***, welfare indicators must be carefully interpreted, using more than one when possible. For the most part, high lying times are associated with cow comfort and welfare, however other aspects of lying behaviour should be assessed in conjunction with total lying times, to ensure a comprehensive interpretation of the behaviour, which should be assessed for at least a 24 h period.

1.5 DAIRY COW HOUSING

The development of different housing systems has more recently been driven by cow requirements, farmer demands, societal pressures and environmental impact, producing technical innovations to find solutions (Galama et al., 2020). These driving forces differ greatly between countries, resulting in several different management and housing systems used in modern dairy practices, including tie stalls, cubicles and pasture (Rushen *et al*., 2008a). Rushen et al. (2008a) reported that the most common housing practice in North America is the tie stall, whereby cows are restrained around the neck in their own cubicle stall, consisting of metal partitions, where they lie down, eat, drink and on some farms are milked from (Rushen et al., 2008a). The Canadian Government reported that in 2020 over 70% of Canada's milking herd were housed in tie stalls (AAFC, 2021). In 2001, the United States Department of Agriculture reported that more than half of lactating dairy cows in the USA were housed in tie-stall systems (52.5%), with only 30.8% in cubicles (USDA, 2002). However, the number of cows housed in tie-stall systems in the USA has decreased in recent years (38.9%), with cubicle housing systems becoming more common (39.7%; USDA 2016). Technical innovations in milking and feeding practices (Galama et al., 2020) allowed for the transition from tie stalls housing to cubicle housing, which have been popular since the 1970's (Bewley et al., 2017). When housed, cubicle housing is the most common housing practice globally (Margerison, 2011), with cows having free access to a number of traditionally metal cubicles in a barn for which to lie down on, similarly designed to tie stalls in order to discourage cows from urinating and defecating on the lying area and promoting cow hygiene. In these systems, cow comfort should be a priority, using soft bedding materials, such as rubber cow mattress or deep-bedded sand, with cubicle measurements meeting recommendations based on cow size (Margerison, 2011). Cows in New Zealand and Australia are typically kept permanently on pasture due to the favourable climate and availability of land, while in more temperate climates, such as in Europe and North America, pasture access is seasonal, with cows being brought in during the wet and cold winters (Rushen *et al*., 2008a). In some management systems, cows are housed permanently indoors, a practice which is increasing in the UK, whereas traditionally cows were given seasonal pasture access (Haskell *et al*., 2006). A farmer questionnaire distributed in 2012 across Britain reported that traditional seasonal pasture access with winter-only indoor feeding was practiced by less than one third of respondents (March *et al*., 2014).

Below are described the most relevant housing systems in the UK, cubicle housing and pasture, as well as indoor open pack areas, which have been adopted by a minority of UK dairy farms (Fujiwara, 2018), but are suggested to be part of the future of dairy cow housing (Bewley et al., 2017; Galama et al., 2020). Excluded from in depth discussion is tie-stall management systems, which, despite popularity in North America, are rare in the UK..

1.5.1 Cubicle Housing

Cubicles are used today in dairy cow housing as an alternative to tie stalls to offer cows more space and freedom while discouraging cows defecating in stalls and on bedding, improving cow cleanliness (Rushen *et al*., 2008a; Ruud *et al*., 2011). Cleanliness has been linked to improved cow health (Barkema *et al*., 1998; Barkema *et al*., 1999), as well as reducing the maintenance time (Fregonesi *et al*., 2009) and cost of bedding for management (Schmisseur *et al*., 1966). Due to the popularity of the cubicle, and to an extent tie stall cubicles, numerous studies have investigated cow lying preference for various cubicle modifications with the aim to increase cow lying times via increased cubicle comfort. A feature of the cubicle to position the cow to reduce soiling on the lying surface is a brisket board. However, the presence of brisket boards has been shown to reduce cow lying times in cubicles, with cows having a preference for lying in cubicles without brisket boards (Tucker et al., 2006). With regards to the size dimensions of cubicles, Tucker et al. (2004) investigated the effect of cubicle width and length on the behaviour of dairy cows. In a first experiment where two cubicle lengths (229 and 274 cm from curb to wall) and widths (112 and 132 cm) were compared in a 2 x 2 factorial design, cows showed no preference for cubicle size in terms of lying time. The authors suggest that because the ancestors of cattle were plain-dwelling animals who did not have to consider special restraints, the modern cow lacks the ability, or priority, to distinguish the difference between the cubicle sizes presented. However, cows lay down for longer on wider cubicles during a no-choice phase. In a follow up experiment, Tucker et al. (2004) investigated cow preference for stalls of three different widths (106, 116 and 126 cm), finding that cows lay down longer in the widest cubicles. However, these larger cubicles were more likely to become soiled with faeces, increasing exposure of teats to bacteria and the probability of cows developing mastitis (Tucker et al., 2004). However, cubicles that are most commonly used are more likely to become soiled (Gaworski et al., 2003), and due to the nature of Tucker et al. (2004) preference test, this may be the cause of increased soiling rather than cubicle size. Cubicles commonly have a ‘neck rail’ which controls the position of cows both standing and lying in the cubicle so that it minimises soiling of the lying surface. Tucker et al. (2005) performed two experiments to explore cow preference for cubicles with a variety of neck rail placements. The first experiment explored the effect neck rail placement in terms of height (102, 114, 127 cm and no neck rail) by distance to the curb (160 or 180 cm, respectively), with the second experiment investigating the distance of the neck rail from the curb (140, 175 and 233 cm) when the height of the neck rail was constant (131 cm). In both experiments, there was no effect of neck rail placement on the lying behaviour of cows. However, cows did spend less time standing with four hooves on the cubicle when the neck rail was lower and closer to the curb, resulting in cleaner cubicles (Tucker et al., 2005). Although cleaner cubicles are favourable, cows that spend more time with all four hooves on the cubicle reduces the time spent standing on concrete, which is associated with an increase in lameness (Vokey et al., 2001; Somers et al., 2003). Additionally, Fulwider et al. (2007) found a correlation between lameness and a restrictive neck rail hight. Tucker et al. (2005) suggests avoiding the use of restrictive neck rails to allow cows a comfortable place to stand and maintain stall cleanliness with frequent cleaning, or provide cows with a more comfortable area to stand outside of cubicles and thus use restrictive neck rails to keep cubicles clean. Similarly, Tucker et al. (2004) found that wider stalls accommodated for an increase in standing with all four hooves in the cubicle while decreases time spent standing with just the front two hooves in the cubicle, a behaviour known as perching. Increased perching behaviour has been shown to predispose cows to claw lesions, again due to increased standing on concrete flooring (Colam-Ainsworth et al., 1989; Galindo and Broom, 2000). Additionally, due to the function of cubicle design to promote a clean cubicle surface, the alleyway directly behind the cubicle where a cows' two hind hooves are situated when perching increases hind hoof exposure to faecal material and a moist environment. Increased hoof exposure to such environments has been linked to an increased prevalence of lameness (Bergsten and Pettersson, 1992; Fitzgerald et al., 2000).

In an attempt to cater for cow comfort while maintaining more restrictive stall measurements to promote cubicle cleanliness alternative cubicle designs have been investigated. One solution was the development of flexible, plastic cubicle partitions, with companies patenting designs from 2009 (Bewley et al., 2017). Ruud and Bøe (2011) were the first to investigate cow lying behaviour in cubicles with plastic hardware compared to conventional metal. They investigated cow lying times and preference for both cubicle types, finding that when cows did not have a choice between the two, they lay down for a similar amount of time in both cubicle types. However, when cows had a choice, they displayed a preference for lying down in cubicles with plastic hardware (65.2% lying time) over metal hardware (34.8%), demonstrating that the flexibility that plastic cubicle hardware provides is important to cows. Abade et al. (2015) investigated cow preference for cubicles compared to an alternative cubicle design, in which neck rails were removed and the metal cubicle partitions replaced with wooden boards protruding from the deep-sand bedding, finding that cows choose to lie in the cubicle over this alternative design. Unlike the flexible cubicle design, this protruding wooden board design still restricted lying area, which may explain the lack of preference cows showed towards the design.

Cows show stronger preferences when it comes to the cubicle surface type, with cows consistently spending less time lying on hard concrete cubicles with a small amount of bedding compared to rubber mats and mattresses (Chaplin *et al*., 2000; Haley et al., 2001; Rushen et al., 2007). During preference tests, cows also exhibit a preference for softer rubber mat and mattress lying surfaces compared to concrete (Natzke *et al*., 1982; Herlin, 1997; Norring et al., 2010). Herlin (1997) studied cow preference for different surfaces of varying softness; concrete, conventional hard rubber mats with a softness of 65-85 (on a soft-hard scale where 0 equates to water and 100 equates to steel) and soft rubber mats with a softness of 35-45. The study reported cows preferring to lie down on the soft rubber mat compared to both the concrete and hard rubber mats, with cows displacing one another significantly more from the soft rubber mats than other surfaces. However, bedding quantity can influence cow preference for a lying surface. Gebremedhin *et al*. (1985) reported that the dislike cows have for concrete can be minimised by the amount of bedding used, finding that increasing the use of sawdust on concrete stalls increased stall use and with 15cm of sawdust, cows preferred concrete stalls over rubber mats. Similarly, Manninen et al. (2002) found that cows lay down for similar total lying times on concrete cubicles bedded with 6.5 kg of straw per cubicle (deep-bedded straw) and soft rubber mats, bedded with a small amount of straw, and exhibited a preference for deep-bedded straw cubicles in the winter. Manninen et al. (2002) also reported that cows preferred both the deep-bedded straw and the lightly bedded rubber mats over deep-bedded sand (20 cm). This is in agreement with Norring et al. (2008), finding that not only did cows lie down longer in deep-bedded straw cubicles (6.5 kg) compared to deep-bedded sand cubicles (20 cm), but cows that had previous experience of deep-bedded sand showed no preference between the two bedding types when given the choice, whereas cows with previous experience of deep-bedded straw preferred lying in the straw cubicles. Additionally, in deep-bedded sand cubicles, Drissler et al. (2005) found that the bedding depth changes over a 10 d period, with daily lying times decreasing by 10-11 min per 1 cm decrease in bedding over a 24 h period. Likewise, Jensen *et al*. (1998) reported cows having a partial preference for concrete with 4-5 kg of fresh straw (53%) but found that as time progressed and stalls had less straw, cows showed a preference for a soft triple layer synthetic mat. Lying times on mattresses have also been shown to be affected by the amount of bedding, with Tucker and Weary (2004) reporting cows lying down for 1.5 h longer on mattresses bedded with 7.5 kg of sawdust compared to mattresses without bedding. These cows also showed a preference for lying in the mattresses with more bedding, with all cows participating in the study (n = 11) spending the majority of their lying time on the 7.5 kg sawdust bedded option. Similarly, Tucker et al. (2009) found that cows increased their lying times on mattresses by 3 and 12 min for every additional kilogram of shavings (range: 3-24 kg/cubicle) and straw (1-7 kg/cubicle), respectively. However, Fregonesi et al. (2007b) demonstrated that the quality of the bedding material is important, as cows spent 5 h less lying down on mattresses cubicles bedded with wet sawdust (26.5% dry matter) compared to dry sawdust (86.4% dry matter), with all cows expressing a large preference to lie down on the dry bedded cubicles when given the choice (Dry: 12.5 h/d; Wet: 0.9 h/d). When investigating a larger range of 5 different moisture levels in sawdust bedding (89.9, 74.2, 62.2, 43.9, and 34.7% dry matter), Reich et al. (2010) also found that cows lay down longer on the dryer bedded cubicles, further demonstrating the importance of the quality of bedding, as well as the amount, for cow comfort.

Not only does a softer cubicle lying surface increase lying times, indicating increased cow comfort resulting in improved welfare, but harder lying surfaces can have negative health implications on cows (de Vries et al., 2015). Rushen et al. (2007) reported that cows housed on concrete cubicles had a higher incidence of swollen knees compared to cows housed on soft rubber mats. Likewise, Kielland et al. (2009) found that cows housed on compact rubber mats or mattresses had fewer lesions on the knee, and that those housed on mattresses had fewer hock lesions when compared to those on compact rubber mats. These findings are consistent with the compressibility of these lying surfaces, with cow mattress found to be more compressible than rubber mats (Fulwider and Palmer, 2004). When comparing the prevalence and severity of hock lesions on different bedding surfaces, Weary and Taszkun (2000) found that lesions were less prevalent, numerous and severe on cows from sand-bedded farms compared to cows from farms using mattresses. The authors suggest that the friction between the leg and the mattress, leading to a build-up of heat, reduces the strength of the skin, causing an increase in lesions. Furthermore, cows housed on deep-bedded sand cubicles have been shown to have a lower prevalence of lameness than cows housed on mat or mattress cubicles (Cook, 2003; Cook et al., 2004). A study of 94 farms found that cows housed on sand were dirtier than cows housed on mattresses, which has been associated with poorer health (Ward et al., 2002; Schreiner and Ruegg, 2003; Reneau et al., 2005). However, there was no difference in somatic cell count (SCC) between the two housing systems, suggesting the health and welfare of the sand housed cows was not compromised (Fulwider et al., 2007). This may be due to the farms in the study housing cows on mattresses bedded up the cubicles with fresh bedding more often than the farms using sand.

Another management practice that can effect lying times in cubicles is stocking density. Fregonesi et al., (2007a) manipulated stocking density for a group of 12 cows, creating stocking levels of 100, 109, 120, 133 and 150%, finding that at higher stocking densities, there was an increase in competition for cubicles resulting with reduced lying times for the herd. Similarly, Winckler et al. (2015) found that cows lay down 1 h less at 150% stocking density compared to 100%, although cows only lay down for 0.2 h longer when understocked at 75%, compared to 100% stocking. Winckler *et al*. (2015) also reported an increase in lying synchrony at lower stocking densities, which has been shown to occur at pasture (Stoye *et al*., 2012) and is considered an indicator of good welfare (Napolitano *et al*., 2009; O'Driscoll *et al*., 2008).

Many factors can affect lying times in cubicles, but generally less restrictive, softer cubicles that are not overstocked are more comfortable for cows, increasing cow lying times, which can have health benefits and overall improve cubicle housed cow welfare.

1.5.2 Pasture

In a review by Arnott *et al*. (2017), the authors highlight that all-year-round housing and pasture access systems differ in two main ways, nutrition and housing, affecting cow health, behaviour and physiology. In terms of nutrition on pasture-based systems, the nutritional requirements of the modern dairy cow has increased with increasing milk yields through selective breeding (Phillips, 2002a) and meeting a high yielding (>30 kg/d of milk) dairy cow's nutritional needs has become more problematic for pasture based systems. Cows grazed on high quality pasture have found to be producing less milk, receiving a poorer body condition score and weighing less than those housed and fed TMR (Kolver and Muller, 1998). When fed a concentrate with pasture, high yielding cows continue to perform worse than those fed TMR, loosing body condition, gaining less weight and producing less milk (Bargo *et al*., 2002), compromising the health and welfare of the cows. A total mixed ration (TMR), which generally includes forages, protein and cereals with calcium carbonate for lactating cows along with straw to contribute functional fibre intake, optimising rumen function, can be used to fulfil this nutritional demand (Margerison, 2011).

With regards to housing, cow health, physiology and behaviour can all be affected with pasture access. A major health concern across the dairy industry is the prevalence of lameness, a symptom of a variety of diseases, which has a significant impact on milk yield as well as welfare (Huxley, 2013). A study conducted by Hernandez-Mendo *et al*. (2007) showed that, when given access to pasture, clinical lameness in cows was reduced and locomotion scores improved. Haskell *et al*. (2006) reported that farms where cows were given pasture access during the summer months and housed in the winter had a lower prevalence of lameness than all-year-round housed systems. de Vries *et al*. (2015) reported similar findings, both of which were conducted in the winter and therefore demonstrating a potential long-term benefit to pasture access on the prevalence of lameness. In contrast, studies have found poorer claw health and increases in white line disease, both contributing to lameness, in cows out on pasture compared to those continuously housed (Baird *et al*., 2009; Barker *et al*., 2009). Burow *et al*. (2014) suggests that increased lameness at pasture may not be caused by the pasture itself but rather the condition of the cover on the path to pasture, reporting that tracks with no prepared cover, such as grass, sand and soil, can increase lameness in a herd, compared to tracks with prepared cover, such as asphalt, gravel, slag, concrete and/or rubber. A study of pasture-based systems in Australia found a significant relationship between the prevalence of lameness and stock handling; lameness increased with crowding in the holding yard before milking and inappropriate handling of cows, such as constant pushing of cows on the way to the dairy (Ranjbar *et al*., 2016), demonstrating that the causes of lameness are multi-factorial. However, there is a general consensus that pasture access reduces lameness in dairy cows (Olmos *et al*., 2009; Arnott *et al*., 2017; Charlton and Rutter, 2017).

A cow's physiology while at pasture is mostly affected by the climate, either by heat stress in warm climates or effected by cold and wet conditions in more temperate climates, such as in New Zealand and Ireland (Arnott *et al*., 2017). Indoors, cows are often protected from such environmental extremes, but in a UK preference study, as the temperature humidity index (THI) increased, both indoors and outdoors, cows spent more time outside at pasture (Charlton *et al*., 2011a). Charlton et al. (2011a) reported THI values of less than 72 throughout the study, which is usually considered the upper critical climate for cows, based on a decline in milk yield above this level (Igono et al., 1992; Ravagnolo et al., 2000; Spiers et al., 2004). In a North American preference study where the THI reached a level of 78, Legrand *et al*. (2009) reported that as the THI increased outdoors, time spent on pasture decreased, with cows opting to go indoors for shade. It's been found that the amount of shade available effects both the physiological and behavioural response of dairy cattle at pasture; cows with access to more shaded space (9.6 m^2 shade/cow) spent more time in it, were less aggressive, spent less time around the water trough and had a reduced respiration rate compared to cows with little (2.4 m^2 shade/cow) or no available shaded space (Schütz *et al*., 2010). Schütz *et al*. (2011) also showed that cows prefer a shaded area than sprinklers, despite sprinklers being a more effective method of cooling. A trade-off study between shade and the opportunity to lie down after 12 hours of deprivation showed that cows highly value shade, choosing it over lying down at a high air temperature (>25° c; Schütz *et al*., 2008). Considering the importance of lying down to cows after just 4 hours of deprivation, as previously discussed in ***section 1.4.2***, this further highlights the effects of warm weather and the importance of providing cows shade at pasture. Cows are also affected by cold, wet and windy weather when at pasture, having evolved behavioural adaptations in order to mitigate the effects of exposure to such weather conditions (Olson and Wallander, 2002). This includes cows lying in positions which expose a reduced surface area coupled with lying down less (Tucker *et al*., 2007) and choosing to go inside when rainfall is above average (>1.8mm; Charlton *et al*., 2013). Studies done with beef cattle to study the effects of sudden and cold intermittent weather, which cows can be exposed to in some countries while at pasture, demonstrate that physiological adaptation to such sporadic, adverse weather may not be possible for cows (Bergen *et al*., 2001; Kennedy *et al*., 2005). Adverse cold weather not only effects a cow's physiology negatively, by increasing cortisol and glucocorticoid levels, but changes their lying behaviour (Tucker *et al*., 2007). Cows adopted a position to reduce surface area ratio in cold and wet weather, rather than a position where neck muscles are supported, which was more commonly observed indoors and effects the amount of rapid eye movement (REM) sleep obtained, ultimately decreasing welfare (Tucker *et al*., 2007).

When comparing the lying behaviour of cows at pasture and those that are housed, O'Connell *et al*. (1989) found that cows at pasture had increased lying times compared to those housed in cubicles with concrete surfaces. Considering that cows have been found to lie down longer (Haley et al., 2001; Rushen et al., 2007) and have a preference for softer cubicle lying surface compared to concrete (Herlin, 1997; Norring et al., 2010), as discussed in ***section 1.5.1***, the results of O'Connell *et al*. (1989) are not all that surprising. However, Olmos et al. (2009) similarly found that when comparing the lying times of cows housed at pasture and in cubicles with soft rubber mats, cows lay down for longer at pasture, despite the soft lying surface provided indoors. O'Connell *et al*. (1989) also reported increased lying synchrony at pasture, with 90% of cows lying down at any one time at pasture, compared to just 45% indoors. Synchrony of lying down has been proposed as a positive welfare indicator for cows as it conveys reduced competition for resources (Færevik *et al*., 2008) and increased lying comfort (Fregonesi et al., 2001). Additionally, synchrony of behaviour has shown to be a characteristic of semi-natural cow herds and herds at pasture (Flury and Gygax, 2016; Kilgour, 2012) and therefore has been suggested as a positive welfare indicator (Phillips et al., 2013; Napoliano et al., 2009).

When examining pasture access and evaluating the implications it has for dairy cow welfare, there is supporting evidence both for and against access based on health, physiological and behavioural indicators (Arnott et al., 2017) and ultimately management of the system can be as important as the system itself (Mee and Boyle, 2020). However, when cows are asked to choose between indoor cubicle housing and pasture, cows almost always show a preference and motivation for pasture access (Arnott et al., 2017; Charlton and Rutter, 2017), providing further evidence that pasture access improves dairy cow welfare. The following sections will discuss cow preference and motivation for pasture access, the influencing factors involved and what is thought to be the two main driving forces, grazing and lying behaviour.

1.5.2.1 Preference and Motivation for Pasture

As a tool to assess welfare, studies have asked cows what they prefer; going out to pasture or indoor housing, with the majority reporting a preference and motivation for pasture access (Legrand *et al*., 2009; Charlton *et al*., 2011a; Falk et al., 2012; Charlton *et al*., 2013; Motupalli *et al*., 2014; Shepley *et al*., 2016; von Keyserlingk *et al*., 2017). Additionally, Smid et al. (2018) found that cows had a preference for pasture access over access to an outdoor sand pack area. Cows are highly motivated to access food when freshly delivered and after milking (de Vries *et al*., 2003) and von Keyserlingk *et al*. (2017)

reported that, against expectations, cows were just as motivated, if not more, to access pasture as they were to eat fresh feed after milking, demonstrating the value of pasture to cows. Despite this strong motivation for pasture access, cow preference for pasture is multi-factorial, with many different influential factors effecting individual cow and herd preference (Arnott *et al*., 2017; Charlton and Rutter, 2017). It was thought that decreasing stocking density might affect cow pasture preference, but Falk *et al*. (2012) reported that availability of stalls did not influence time spent indoors, suggesting that cows would rather lie down at pasture than indoors in cubicles, even when there is ample lying space indoors.

Krohn *et al*. (1992) studied the behaviour and preference of 12 cows in a 'dairy cow park' in Denmark over 2.5 years, where cows had free access to pasture (excluding the month of April), indoor deep bedding and a yard. This study showed that cows have a seasonal preference for pasture, spending the majority of their time at pasture from May – September with more time being spent indoors in the winter months. Charlton *et al*. (2011a) also found a seasonal effect for cow pasture preference in the UK, with cows spending less time on pasture as the season progressed (August – November). This may be due to weather conditions or, as Charlton *et al*. (2011a) considers, due to a decrease in grass quality, and therefore pasture use due to decreased time spent grazing.

As previously discussed in ***section 1.5.2.***, adverse weather conditions can change cow lying behaviour at pasture and when given a choice, they will retreat indoors from adverse weather conditions. Legrand *et al*. (2009) reported that as THI increased outdoors, cows chose to spend more time indoors. Additionally, Tucker *et al*. (2008) reported that as solar radiation increased, so did the time that cows on pasture spent in the shade, particularity under structures that provide a higher degree of shade. In contrast, Motupalli *et al*. (2014) reported no effect of THI on pasture preference, possibly because THI ranged from 56-66, which is considered low when the upper critical climate for cows is 72, whereas Legrand *et al*. (2009) recorded a more varied THI (52-78). Charlton *et al*. (2013) reported a similar THI range as Motupalli *et al*. (2014), averaging 59-62, and again reported having no effect on pasture preference. Wet weather conditions have been found to also negatively affect cow preference for pasture (Legrand *et al*., 2009; Charlton *et al*., 2011b; Falk *et al*., 2012), with Charlton *et al*. (2013) finding that with above an average rainfall of >1.8mm, more cows spent time indoors. Ketelaar-de Lauwere *et al*. (1999) also reported that cows moved indoors from pasture during times of heavy rain and when the black globe humidity index, which takes into account THI, increased. Finally, Krohn *et al*. (1992) reported that cows always spent some time at pasture during the winter months, except on frosty days, when cows stayed solely indoors.

Although the majority of such pasture preference studies have found that cows have a preference for pasture, a study by Charlton et al. (2011b) is an exception to this. Charlton *et al*. (2011b) suggests that their results, cows having a preference to be indoors, is due to the previous experience of the cows, having all been reared indoors with just two weeks pasture access before the study began. As discussed in ***section 1.3.4.***, an animal's preference can be influenced by their previous experience and has been shown to effect dairy cow preference for different lying surfaces (Tucker *et al*., 2003; Norring *et al*., 2008; Norring *et al*., 2010). Additionally, Motupalli (2014) reported that cows that had previous experience of pasture choose to spend more time at pasture than indoor housing, exhibiting a partial preference for pasture, compared to cows without previous pasture experience. From this study, it was also noted that cows over two years of age (in their third grazing season) with no pasture experience, did not increase their time at pasture as the study progressed, spending their time nearly exclusively indoors (97%), whereas cows exposed to pasture in their second grazing season were found to change their preference from indoor housing (80%) in year one to pasture (68%) the following year. These results clearly shows the effect previous experience and the effect of age when exposed has on cow preference for pasture access.

Cow's diurnal activity patterns have been shown to influence their preference for pasture access, with multiple studies showing a preference for pasture over indoor housing primarily at night (Legrand *et al*., 2009; Charlton *et al*., 2011a; Falk *et al*., 2012). When using increasing walking distances as an indicator of motivation, Charlton et al. (2013) found that the time cows spent at pasture during the day was influenced by distance, spending less time at pasture as the distance to access it increased. However, at night, no effect of distance was found, suggesting that cows had a higher motivation to access pasture at night than during the day. Additionally, von Keyserlingk *et al*. (2017) investigated cow motivation for pasture access using a weighted gate of increasing weight to gauge cow motivation for pasture access. Similarly, von Keyserlingk *et al*. (2017) found that cows pushed harder in the evening than in the morning to gain access to pasture, further supporting the idea that cows value and are more motivated to access pasture in the evening and at night than during the day.

Due the high nutritional needs of modern high yielding dairy cows (Phillips, 2002a), feeding high yielding dairy cows solely at pasture can impact their health and yield (Kolver and Muller, 1998). Therefore, when given a choice, it is not unexpected that high yielding cows (>26.9 kg/day) change their behaviour, spending more time indoors than at pasture compared to lower yielding cows, presumably to supplement their nutritional intake. Studies have shown that cows with a lower body condition score (BCS) produce more milk after calving (Garnsworthy and Topps, 1982; Treacher *et al*., 1986; Garnsworthy and

Jones, 1987), eating more than cows with higher BCS. Charlton *et al*. (2011b) reported that cows with lower BCS tended to spent less time at pasture, potentially due to their increased nutritional requirements, which can be met with TMR provided indoors (Charlton and Rutter, 2017).

1.5.2.2 Pasture for Grazing

Popular public opinion is that pasture access improves dairy cow welfare because they have the ability to express natural behaviours, such as a natural grazing behaviour (Schuppli *et al*., 2014; Hötzel *et al*., 2017). However, the effect of time of day on dairy cow preference for access to pasture, with cows having a stronger preference and motivation at night than during the day (previously discussed in ***section 1.5.2.1.***). This would appear to contradict this public opinion, given that cows mostly perform grazing behaviours during the day (Kilgour, 2012). Alternatively, Charlton *et al*. (2011a) hypothesised that a previous finding for cow preference for indoor housing, in particular high yielding cows (Charlton *et al*., 2011b), may have been due to the provision of TMR indoors and not being able to meet their nutritional needs from grazing. Charlton *et al*. (2011a) further investigated the pasture preference of cows when TMR was offered both indoors and at pasture. The authors reported that there was no influence of TMR found on a partial preference for pasture, suggesting that the provision of feed indoors did not affect pasture preference. Furthermore, pasture quality can influence feeding behaviour (Gibb *et al*., 1997), with Hendricksen and Minson (1980) finding that starting out at a high sward height (2230 kg/ha), time spent grazing was low (465 min on day 1) and increased with time (685 min on day 6), before declining again (490 min on day 12). Motupalli *et al*. (2014) studied the effect of two different herbage masses on dairy cow motivation for pasture access, using walking distance as an indicator of motivation. They found that there was no effect of herbage mass on cow motivation for pasture, with cows continuing to exhibit a higher level of motivation at night than during the day. These findings, coupled with the knowledge that cows have a stronger motivation to access pasture at night compared to during the day, would suggest that cow preference for pasture is not primarily linked to being able to express a natural grazing behaviour or meeting nutritional demands from pasture.

1.5.2.3 Pasture for Lying Down

Tucker (2017) reported that cows at pasture exhibit distinctive diurnal rhythms, feeding during the day and, although having a bout of lying during the day, the majority of their time spent lying down being observed at night. It has been suggested then that cows value pasture access for the properties it offers for lying down, rather than for grazing.

This is supported in part by the pasture preference studies previously discussed in ***section 1.5.2.1.***, reporting that the majority of cows have a stronger preference and higher level of motivation for pasture access at night compared to during the day. Cows having a high motivation to access pasture at night (Charlton *et al*., 2013; Motupalli et al., 2014; von Keyserlingk *et al*., 2017) would suggest that the properties it offers for lying down are important to cows and are potentially a factor in promoting better welfare.

Two main differing factors between indoor cubicle housing and pasture have been identified as possible driving factors for cow pasture preference and motivation in terms of lying behaviour: surface type and space available for lying down. Social encounters may influence cow preference for the space pasture offers for lying down, as studies have shown that more antagonistic behaviours occur between cows indoors than at pasture, as well as an increased level of avoidance of cows that block the movement of others when indoors (O'Connell *et al*., 1989; Miller and Wood-Gush, 1991). Rioja-Lang *et al*. (2009) reported that when cows were paired, and one identified as dominant over the other at the feed face, cows not only showed a preference for feeding alone than with the dominant cow, but were willing to trade-off food of a higher feed quality for feeding alone. This study highlights the importance of avoidance behaviours for cows, which may be facilitated by the increased space offered at pasture, giving cows a greater choice of who they lie beside. Additionally, Abade *et al*. (2015) reported that cows were able to get up quicker from lying down in an alternative cubicle design (removing all cubicle hardware, replaced with wooden boards protruding from the lying surface) compared to conventional cubicles. This might help in facilitating cows to avoid conflict, removing themselves quicker from conflict when lying down in a less restrictive lying area with more space.

Knowing that cows prefer softer lying surfaces (as discussed previously in ***section 1.5.1***; Natzke *et al*., 1982; Herlin, 1997; Norring et al., 2010), it is possible that pasture offers cows a softer lying surface than indoor housing alternatives. Hernandez-Mendo *et al*. (2007) reported cows having a higher frequency of lying bouts at pasture when compared to deep-sand bedded cubicles, a higher measure of which often indicates increased cow comfort (as previously discussed in ***section 1.4.2***; Herlin, 1997; Haley et al., 2000; Haley et al., 2001; Tucker et al. 2003; Rushen et al., 2007). In contrast to this, during a no-choice phase, Legrand *et al*. (2009) reported no difference in lying bout frequency between pasture and cubicle housing when the cubicle surface was a rubber mattress bedded with sand. This would suggest that the cubicles were as comfortable as pasture to lie down on when the lying surface is a mattress as opposed to sand. However, when given the choice, cows showed a preference for lying at pasture (Legrand et al., 2009). When given the opportunity to go outside to pasture or outside to a sand pack, cows showed a preference for pasture (Smid et al., 2018), again suggesting that surface type might be driving pasture preference. However, there is a confounding factor of space, with the area of pasture access larger than the sand pack (Smid et al., 2018). The confounding factor of surface type and space is a common theme among pasture preference and motivation studies, comparing mattress cubicle housing against a larger area of pasture (Legrand et al., 2009; Charlton et al., 2011a; Falk et al., 2012; Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al., 2017).

1.5.3 Open Pack Areas

Much like cubicles providing a less restrictive alternative to tie stalls, open pack areas, providing cows with a large open lying area, are viewed as a less restrictive alternative to cubicles (Fregonesi et al., 2009), providing cows overall with more space (Galama et al., 2020) and improved health (Barberg et al., 2007). Traditionally, these open pack areas were bedded using straw (referred to as straw yards; Bewley et al., 2017) and have behavioural benefits, such as increased lying time, lying synchrony and number of lying bouts compared to cubicles (Phillips and Schofield, 1994; Fregonesi and Leaver, 2001; Shepley et al., 2020).

More recently, open pack areas have been bedded using sand (Smid et al., 2018), wood chips (Smid et al., 2019; Smid et al., 2020), comprised of mattresses (Fregonesi et al., 2009) and, most notably, as compost bedded pack areas (CBP; Barberg et al., 2007). CBP systems differ to other open pack surfaces as the organic bedding material is tilled a few times throughout the day, promoting microbial activity, heating and drying the substrate, providing a dry and comfortable lying area (Bewley et al., 2017; Leso et al., 2020). Despite excrement being mixed in with lying substrate, in a well-managed system, cow hygiene and health are good (Black et al., 2013), with Eckelkamp et al. (2016) reporting low levels of clinical mastitis in 8 CBP farming systems in Kentucky. Traditional straw yards may also have benefits for cow health, with no difference in observed lameness between cows housed in straw yards and cubicles (Phillips and Schofield, 1994; Livesey et al., 1998; Fregonesi and Leaver, 2001), and cows housed in straw yards having a lower level of claw disorders compared to a range of other housing systems (Somers et al., 2003). Additionally, cows in straw yards have been shown to have lower hock lesion scores than cows house in cubicles with either a mat or mattress lying surface (Livesey et al., 2002). Fregonesi and Leaver (2001) also reported an increase in lying times for cows in a straw yard when compared to conventional cubicles, but only for a 4-week period, with a second experiment carried out over 17 weeks showing no difference between the two housing systems. The authors suggest that the results from the second experiment were potentially due to a change in the layout of the straw yard, which is supported in part by similar studies reporting cows having a stronger preference for lying in straw yards than cubicles (Phillips and Schofield, 1994; Leaver and Fregonesi, 2002). Fregonesi and Leaver (2001) also noted that when cows were moved from the cubicles to the straw yards after the first experimental period, they exhibited play behaviour which was not observed when switching cows back from straw yards to cubicles, and although the type of play behaviour is not specified, play behaviour is considered a positive welfare indicator (Špinka *et al*., 2001). However, Fregonesi and Leaver (2001) reported that cows housed on straw yards were observed to be dirtier, have higher cell counts and a greater incidence of clinical mastitis. The authors suggest that the decrease in cleanliness, and in turn the associated health disadvantages, were related to the poor design of the straw yard. A study investigating cow behaviour on cubicles, open pack and CBP systems found that cows lay down longer and had fewer and less severe visible bodily lesions/swellings on both the open pack and CBP systems compared to cows housed in cubicles (Fernández et al., 2020).

Not only have cows been shown to have higher lying times on open pack areas, but they also display a preference for them. Fregonesi et al. (2009) found that cows had a preference to lie down on an open pack area, comprised of a cow mattress, over lying in cubicles of the same lying surface. When investigating an outdoor pack area, comprised of a base of sand covered in woodchips, Smid et al. (2020) found that time of day affected the use of the open pack. Cows mostly accessed the pack at night, when cows primarily lie down, and the authors found no effect of the size of the open pack area on lying time. In a similar study, Smid et al. (2019) reported a seasonal effect on the use of an outdoor pack area, with cows lying down less during the winter on the open pack. However, Smid et al. (2018), still found that cows had a preference for pasture when given the choice between pasture and an outdoor pack area.

These studies demonstrate that an open pack area may be a good alternative to cubicle housing, offering cows a good indoor alternative for lying down when pasture is unavailable, catering to their behavioural needs without compromising health when properly managed, and are possibly the future of dairy cow housing (Bewley et al., 2017; Galama et al., 2020; Leso et al., 2020).

1.5.4 Public Opinion

Despite a decreasing trend in pasture-based systems and all-year-round housing growing in use (Van den Pol-van Dasselaar et al., 2008; March et al., 2014), public opinion tends to emphasise the importance of naturalness, often manifesting in terms of pasture access been seen as desirable (Schuppli et al., 2014; Ventura et al., 2015; Cardoso et al., 2016;

Beaver et al., 2020). When asked directly, 95% of UK respondents thought it was unacceptable for cows to never access pasture (Ellis et al., 2009). Boogaard et al. (2008) conducted farm visits with Dutch citizens, asking for their on-farm observations and what they thought would be valuable for the future of dairy farms. Although the cubicle was perceived positively, there was still concerns about decreasing pasture access. Jackson et al. (2020) found similar results, with UK citizens mostly concerned with pasture access. However both Jackson et al. (2020) and Boogaard et al. (2008) found that public concern for pasture access is linked to naturalness and the grazing behaviour, which, as discussed previously in ***section 1.5.2***, may not be the driving factor behind cow pasture preference.

Studies have shown that farmers are likely to disregard public opinion on current farming practices due to perceived public ignorance of modern farms and their anthropomorphising of animals (Hubbard *et al*., 2007; Vanhonacker et al., 2008; Spooner *et al*., 2014). When surveying Dutch farmers and citizens on the welfare of pigs, Benard and de Cock Buning, (2013) reported similar opinions, with farmers regarding citizen knowledge and opinion as irrelevant, but the authors argue that disregarding these opinions would fail in any attempt to regain citizen acceptance of modern farming practices. Specifically in relation to dairy farming, when asked to fill out a welfare questionnaire before and after being given a tour of a dairy farm, some citizen concerns were mitigated, such as access to adequate food and human care (Ventura et al., 2016). However, other concerns were reinforced, in particular the lack of access to pasture, with regards to natural living, freedom of movement and ability to express natural behaviours (Ventura *et al*., 2016). The authors conclude that because, even when educated on farm practices, citizens still have particular concerns about cow welfare, that engagement should be a two-way system, not a one-way educational approach supported by stakeholders, to address public concerns for farm animal welfare. This is supported in part by a survey distributed to urban citizens in Brazil, which reported that, although participants were naive of zero-grazing and cow-calf separation practices, they rejected the use of these practices both before and after being given supporting information justifying both. This indicates that a lack of public awareness and knowledge cannot be used to dismiss such farming welfare concerns (Hötzel *et al*., 2017).

A study looking at participant views specifically about pasture access found that those in support were for reasons of 'naturalness' for the animal, which some expressed went beyond natural grazing behaviours, such as "to feel sunshine on her back, to feel earth beneath her feet, to breathe fresh air", emphasizing the importance of the outdoor environment and experience (Schuppli *et al*., 2014). The authors reported more neutral responses on the concern of pasture access, 16.7%, than were found in a previous study on the issue of cow-calf separation, which reported 9% responding neutrally (Ventura *et al*., 2013), concluding that the issue of pasture access is a less clear-cut welfare issue.

1.6 CONCLUSIONS

After reviewing animal welfare and its assessment, preference and motivation testing, when well designed to overcome limitations, is a valid and useful assessment of animal welfare, with these being the main experimental procedures used in this thesis.

With lying behaviour being an important behaviour for cows to be able to perform, it has been shown to be a useful indicator for welfare. However, total lying times alone are not a reliable welfare indicator and need to be accompanied by a more detailed description of the lying behaviour. As cow lying behaviour is often directed by the housing management system, great consideration needs to be given to how cows' are housed and whether they have access to pasture.

There are disparities between public opinion of dairy cow management, which emphasises access to pasture in order to display natural grazing behaviour, and scientific studies of dairy cow preference and motivation for pasture, suggesting lying behaviour, as opposed to grazing, as a driving factor. However, current pasture preference and motivation studies, as well as open pack area studies, have a number of gaps in the knowledge caused by a few confounding factors, including: space, surface type and location (indoors vs. outdoors). Considering the importance cows place on lying down, and the growing interest to provide housed dairy cows with lying areas that better meet their behavioural needs, an enhanced understanding of cow lying preferences and motivations could help to shape the future design of dairy cow housing and welfare. The remainder of this thesis will attempt to address some of these gaps.

1.7 THESIS OBJECTIVES

1. To determine which lying quality cows value more for lying down: surface type or lying space, via a trade-off preference study.
2. To investigate how motivated cows are to access an open lying area when they have free access to cubicles, using increasing walking distances to measure motivation, for two different surface types: one to be the same as the cubicles and one to be different.
3. To measure cow preference and motivation for lying outdoors, when an indoor and outdoor open lying area are of the same size and surface type, using increasing walking distances as a measure of motivation.

CHAPTER 2: DAIRY COW TRADE-OFF PREFERENCE FOR 2 DIFFERENT LYING QUALITIES: LYING SURFACE AND LYING SPACE

2.1 INTRODUCTION

Rest is important to animals, in order to conserve energy and allow for metabolic recoveries (Fraser, 1983), with cows mostly resting while lying down, as opposed to standing (Kilgour, 2012). Dairy cows deprived of lying down have been shown to prioritise the behaviour over other deprived behaviours, such as feeding and socialising, and appear to work towards a set amount of time to lie down per day (Metz, 1985; Munksgaard et al., 2005). Cows deprived of lying are more likely to shift their weight and foot stomp, indicating discomfort (Cooper et al., 2007) and when deprived for 4hrs, are quicker to push a weighted pneumatic gate to gain access to a deep-bedded lying area, indicating a motivation for a comfortable lying area (Tucker et al., 2018).

With the majority of dairy cows experiencing indoor housing at some point throughout their lives, (99% of British cows and >99% of cows in the United States are housed for some period within each year (March et al., 2014; USDA, 2016)) and year-round housing growing in popularity (Haskell et al., 2006; Van den Pol-van Dasselaar et al., 2008), it is more relevant than ever to ensure housed environments are meeting the behavioural and welfare needs of cows. When cows are housed, cubicle housing is most common, with the cubicle design developing from original tie stall designs (Margerison, 2011). Knowing the importance of lying down for cows, much research has been done on cow lying preference for various cubicle modifications, such as stall size, (width and length: Tucker et al., 2004; neck rail placement: Tucker et al., 2005), surface type (Manninen et al., 2002; Tucker et al., 2003), bedding type (Norring et al., 2010), and alternative stall design (Abade et al., 2015). Cow preference for the cubicle has also been tested against open lying spaces, such as pasture (Legrand et al., 2009; Charlton et al., 2011; Charlton et al., 2013; Motupalli et al., 2014), indoor open pack areas (Fregonesi et al., 2009) and outdoor open pack areas (Smid et al., 2019). However these studies have confounded the factors of surface type, size of total lying area and indoor vs. outdoor conditions. In general, cows prefer to lie on a soft surface, a stall with larger dimensions or an open lying surface. However it is unclear which of these lying qualities is most important to cows and where the focus should be when improving cow lying comfort when housed.

In the current study, two different lying qualities that appear to be important to cows were selected as the focus: surface type and open space. The aim was to investigate the importance a cow puts on these two different aspects of a lying area. This was done by establishing their preference between three different lying surfaces, deep-bed sand (SA), rubber mattress (M) and deep-bed straw (ST), both with and without a cubicle on them. Once surface preference was established, the cows were given a choice to lie down on their most preferred surface with a cubicle or the lesser preferred surfaces with no cubicle. This presented the cow with a trade-off between surface type and open space when lying down to establish whether lying space or surface was more important to them. Based on these previous studies, we predicted that during the trade-off, cows would trade lying on their preferred surface with a cubicle and lie down for longer periods on either of the two less preferred surfaces without cubicles, indicating a preference for an open lying area over a preferred lying surface.

2.2 MATERIALS AND METHODS

Ethical approval for this study was given by Scotland's Rural College (SRUC) Animal Ethics Committee (ED AE 12-2018) and the work was conducted under the authority of the UK Animal (Scientific Procedures) Act 1986 (Home Office, 1986).

2.2.1 Animals and Management

The study was carried out at Crichton Farm, SRUC Dumfries, Scotland, United Kingdom, in an open-sided barn. Twenty-four pregnant Holstein dairy cows (3 primiparous and 21 multiparous) with an average lactation number of 2.75 (± 0.3, ± SEM) in mid to late lactation (271 ± 14.8 DIM; range 142 to 412 DIM), with a milk yield between 12.2 and 29.4 kg/d (mean 20.8 ± 0.79 kg/d) and weighed on average 728 kg (± 11.7 kg; range 643 – 847 kg) were selected for the study. Cows were selected based on milk yield <35 kg, with a body condition score (BCS) between 2.75 and 3.5 (mean 3 ± 0.03), as described by the Penn State method (Ferguson et al., 1994), and a lameness score (LS) no greater than 2, (mean 2 ± 0.1; 2 = imperfect locomotion but ability to move freely not diminished; Flower and Weary, 2006). BCS and LS were assessed by the same person (LSC) while cows walked across a concrete floor after their ~1500hr milking one week before cows came on trial.

The cows were allocated to 1 of 6 experimental periods according to their stage of lactation (n = 4 x 6), which were carried out from July 10th to November 12th, 2018 (study period 1: July 10th to July 30th; study period 2: July 31st to August 20th; study period 3: August 21st to September 10th; study period 4: September 11th to October 1st; study period 5: October 2nd to October 22nd; study period 6: October 23rd to November 12th).

Each experimental period lasted for a total of 21-d: 1-d set up, 6-d training and 2-d choice periods before a day to move and reset equipment, followed by another 6-d training and 2-d choice, finishing with a 3-d trade-off choice period (Figure 2).

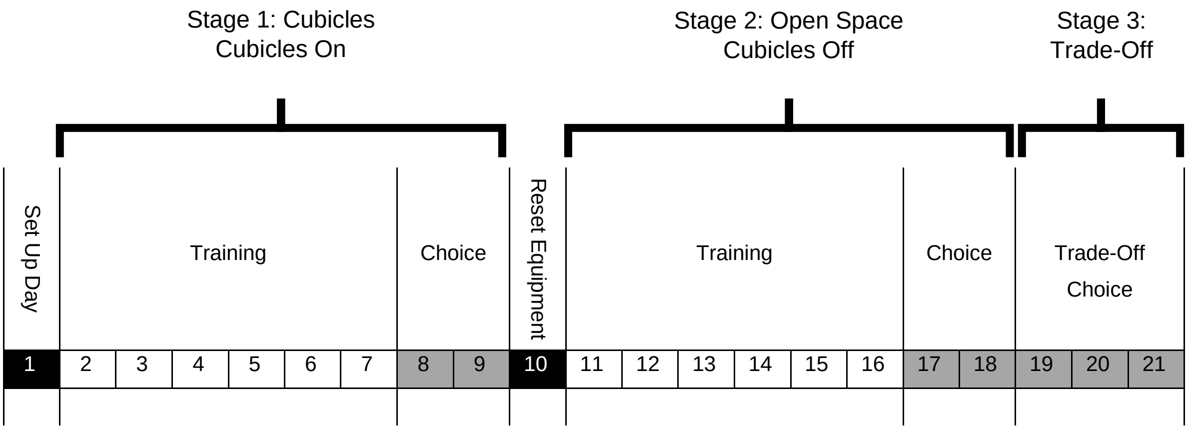

Figure 2. Representation of the 21-d experimental period, which was repeated 6 times using 4 cows. Days shaded in black represent equipment set up days when cows were not present in the pens. Days shaded in grey represent days when cows had a choice of all lying surfaces.

Cows were individually penned while participating in the study, to ensure that during the free-choice periods, their choice was not influenced by the presence of other animals that might potentially be competing for the same resource or otherwise influence their choice of where to lie down. All cows were assigned to one pen for the duration of the study and had visual and tactile contact with a test cow in the adjacent pen.

Before the study, the cows in this herd had been housed indoors in a cubicle barn on mattresses, milked 3 times a day (0700, 1500 and 2200 h), with experience of straw pens during the pre-calving period, and had pasture access during the day from July to October or November, depending on weather conditions and harvesting schedules. This ensured that they had experience of lying on mattress-bedded cubicles and in straw. One week before the start of a new experimental period, cows due to go on trial were housed together in a pen with a deep-bed sand area for lying, to allow them to experience sand as a lying surface. During this week, the cows were also brought down from three to two milkings a day, to better emulate common practice on British farms.

2.2.2 Performance and Lameness

Throughout the study, milk yield was recorded automatically at each milking for individual cows and was used to calculate average yield per cow for the duration of their time on the study.

BCS and LS were recorded when cows were on trial on day 1, 10 and 20 while cows were returning to trial pens after the morning milk, at approximately 0700 h. The average BCS and LS of each cow was calculated as the mean of these 3 scores.

After each milking, cows were automatically weighed while leaving the parlour. Data recorded after both morning and evening milkings were used to calculate the average weight for each cow for their duration on the study.

2.2.3 Experimental Design and Housing

Twice a day, at around 0600 h and 1830 h, the cows were collected and taken to the milking parlour (DeLaval 14:14 herringbone parlour) and milked after the main herd in the morning and before the main herd in the evening, to allow for a near 12hr:12hr split. No concentrates were fed during milking. Following the morning (approximately 0700 h) and evening (approximately 1900 h) milkings, the cows were returned to their pens and manually separated into their own pen. Cows experienced the lying treatments within the pens for approximately 11 h between morning and evening milking and 11.5 h between evening and morning milking. Eight security cameras (Viewlog, GeoVision Inc., Taiwan), two per pen, were set up to continuously record cow behaviour within the pens and each cow wore an accelerometer (IceTag; Ice Robotics, Edinburgh, Scotland, UK) on their hind leg to automatically record lying behaviour. Cows first entered the pens after a morning milking and, from there on, each experimental “day” began when the cows were put into their pens after the morning milking and ended with the start of morning milking the following day.

2.2.3.1 Pen Housing Layout

A 365m² area of an open-sided barn, separate from the barn where the main herd was housed, was divided into 4 pens to house each cow individually (6.0m x 15.2m) (Figure 3). Each pen had three different lying surfaces (20cm deep-bed sand (SA); rubber mattress bedded with sawdust (M) (Pasture Mat; Wilson Agri, Coleraine, Northern Ireland, UK); and 20cm deep-bed wheat straw (ST)) contained in wooden boxes, 2.4m x 2.4m x 0.2m in size, with each being able to have a cubicle and rounded plastic brisket board fitted or removed, depending on the experimental stage (Figure 4). Each surface had a 2.0m distance between one another and a 1.8m distance from the edge of the pen, to allow cow access from any side when cubicles were removed, as well as discouraging a cow using multiple surfaces at the one time (Figure 3).

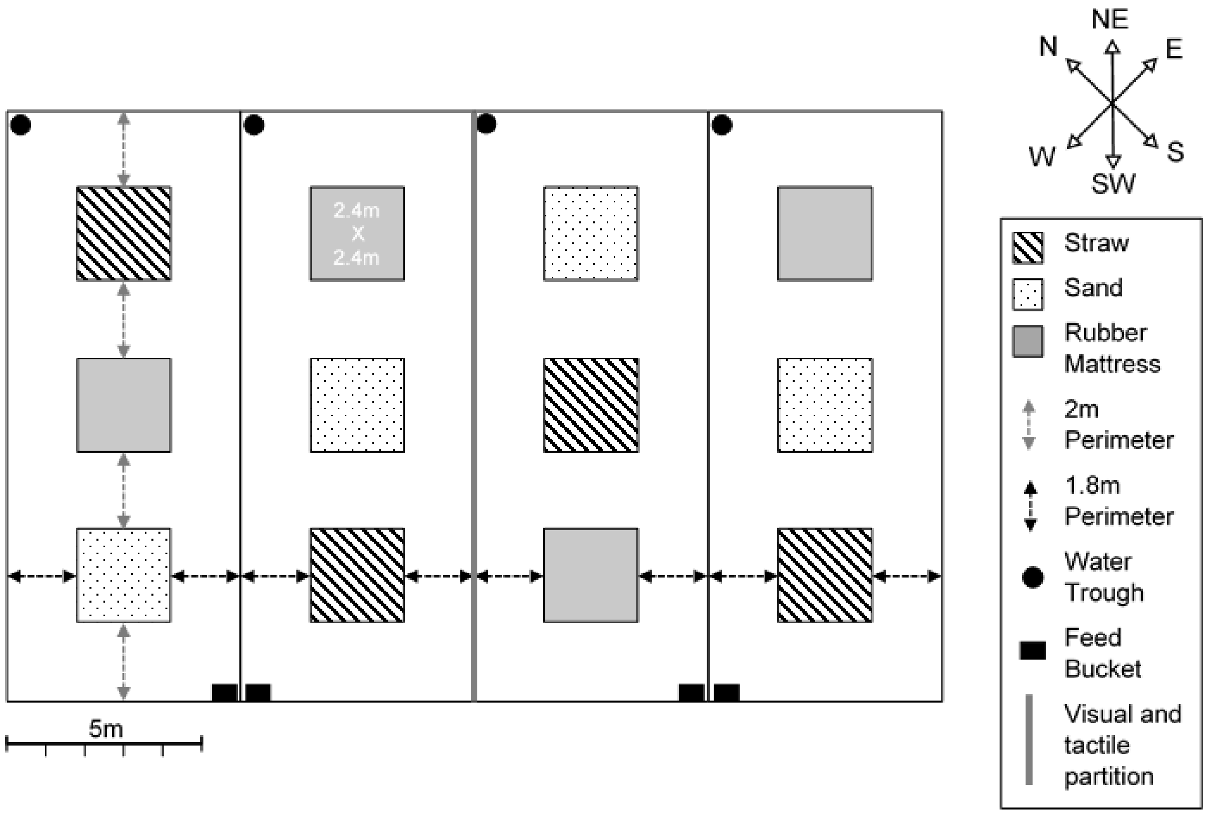

Figure 3. Plan of barn, divided into 4 pens with three lying surfaces in each, used for the experiment.

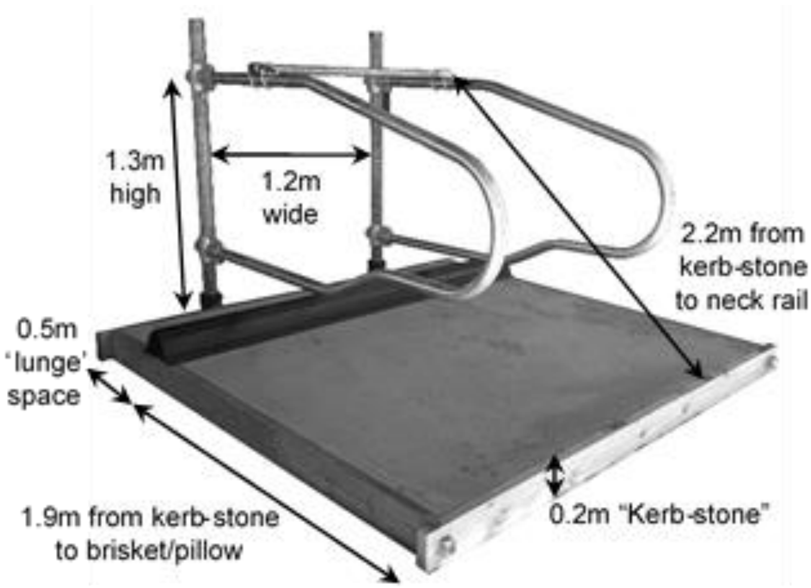

Figure 4. Photograph of cubicle design over deep-bedded sand surface with cubicle dimensions.

A Latin square design was used to allocate the surfaces to the three locations in each pen, such that each surface occurred in each location at least once and that no cow had the same layout of surfaces as her neighbour (Figure 3). This was to take into account order effect of the training stages, whereby cows had access to one lying surface at a time and were encouraged to lie down on each so that they had experience of all the lying surfaces.

After the second and fourth experimental periods, the location of surfaces within pens were moved to account for location effect within the barn, as per another Latin square design (Figure 5).

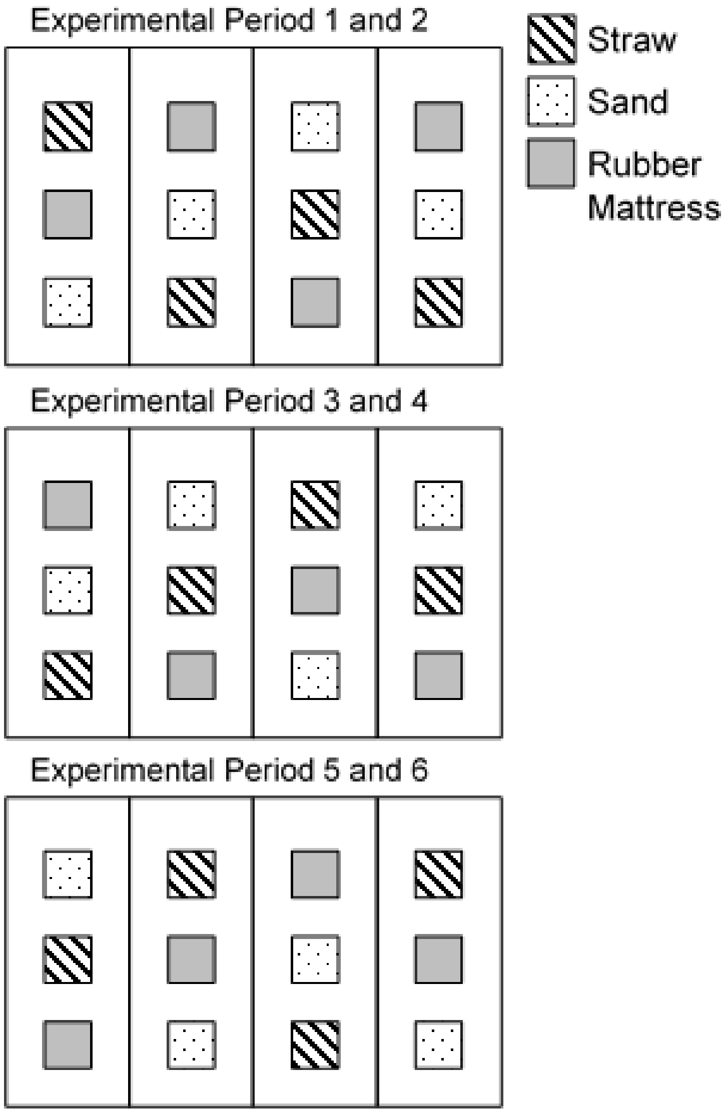

Figure 5. Plan of barn surface layout demonstrating the latin square design for changing surface location after every two experimental periods.

Pens provided individual ad libitum access to feed and water. An ad libitum total mixed ration was provided daily at approximately 1000 h, with feed refusals being removed every day before the fresh feed was provided. Water buckets were emptied and refilled every other day. Pens were cleaned out once a day at approximately 0900 h, with lying surfaces being tended to at this time i.e. sand flattened, rubber mattress re-covered with sawdust and fresh straw topped up. Following the evening milking, lying surfaces were cleaned where necessary.

2.2.3.2 Experimental Design

Preference studies used as a measure of welfare run the risk of either measuring a preference for the 'lesser of two evils' or indeed choosing the better of two good options. An animal forced to take the less preferred option does not necessarily experience good animal welfare (Duncan, 1992). By giving a choice of more than two options, the range of choice is widened to help overcome these risks when interpreting the results. A preference study with just two choices requires preference to be defined as >50% 'use' of one of the options, with random choice being 50:50. Throughout the current study, for one lying option to be preferred out of the three possible options, the percentage of total lying time for the most preferred must at least be > 33⅓ %. However, we considered that cows showed a strong preference when the percentage of lying time on any one surface was >60%, with the maximum combined total for the remaining two surfaces being 40% of lying time.

Each experimental period was comprised of three stages (Stage 1: Cubicles, Stage 2: Open Space and Stage 3: Trade-off), lasting a total of 20 days, not including the initial set up day (Refer to Figure 1.). Below is a description of the three stages for one cow:

Stage 1: Cubicles

Cubicles were fitted to each lying surface in an orientation that allowed companion cows to face one another when lying down. The introduction of the cubicle on the lying surfaces was to control lying posture and orientation, as a regular cubicle would.

A training period of 6-d consisted of the cow having access to one surface at a time for two consecutive days, with the other two surfaces blocked off using sheep pen hurdles. Training began with cows having access to the surface in their pen on the North East side of the barn, followed by the middle surface and lastly having access to the surfaces on the South West side of the barn. This allowed the cow to experience each of the three lying surfaces with a cubicle. A training protocol was in place for cows that did not understand that they could lie down in the cubicle. This consisted of training the cow to follow a bucket of concentrated pellets into the cubicle so that all four hooves were on the lying surface. Cows were rewarded here with pellets left in the lunge area, the bucket removed and the following behaviours observed. For cows that did not lie down within the first 10 minutes, or left the lying surface, this was repeated multiple times. All cows did lie down on each lying surface during this training period.

After the 6-day training period, all hurdles were removed and the cow was given free choice between all three lying surfaces with cubicles on for two days.

Stage 2: Open Space

A day was taken in between Stage 1 and Stage 2 to remove all cubicles and brisket boards from the pens, with the cows kept on cubicles in the main herd barn overnight.

When the cow returned to the pen the following day, with all cubicle dividers and brisket boards removed, a training period equivalent to that in Stage 1 began, with the cow having access to one surface at a time, in the same order as previously, for two consecutive days, with the other two surfaces blocked off with sheep pen hurdles.

This allowed the cow to experience each of the lying surfaces without a cubicle and allowing the cow an opportunity to express a range of different lying postures and orientations that might be expressed in an open lying space.

After the 6-day training period, all sheep pen hurdles were removed and the cow was given free choice between all three lying surfaces for two days.

The video footage of the middle 24 hours of this choice period was analysed to determine the cow's most preferred lying surface (largest percentage of lying time, with a minimum threshold of 60%) to determine where to re-fit the cubicle in Stage 3. This was due to the time constraint between the end of Stage 2 and the need to re-fit a cubicle before the beginning of Stage 3. The full 48 hours of video footage was used for in the final analysis of Stage 2. It also showed that for most cows (but see below) the preference exhibited in the middles 24hr represented the choice over the whole period.

After viewing the middle 24 hours of video footage, three cows did not have a preferred lying surface, with no lying surface meeting the minimum threshold of 60% total lying time, and so the full 48 hour period was analysed for these cows. From this, a preferred lying surface was determined for two of the cows (>60%) and one cow did not meet the minimum threshold of 60% for one lying surface. The cow for which a preferred surface could not be determined was excluded from the statistical analysis for Stage 3.

Stage 3: Trade-Off

The cow's most preferred lying surface, as determined in the previous stage, had a cubicle and brisket board refitted (P1 + Cubicle), with the two lesser preferred surfaces left without (P2 + Open and P3 + Open, the second and third preferred surfaces, respectively). The cow then had free choice between these lying options for 3-d, giving her the choice between whether lying surface or space for lying down was more important to her.

2.2.4 Measurements

2.2.4.1 Behavioural Measurements

Time in and out of the pen each day (i.e. at milking) was recorded from the video data to get a total time in pen per day for each cow. For each cow during the training periods, when there was no choice for lying location, the IceTag data was analysed to obtain lying bout start and end time. For choice periods, video data was used to obtain start time, end time and location of each lying bout for each cow. From this, the proportion of each day spent lying on each of the surfaces was calculated along with the frequency and duration of each lying bout for training and choice periods.

2.2.4.2 Weather Conditions

Weather conditions were recorded daily at 1000 h automatically throughout the study period using a Met Office weather station ~220m from the barn. Outdoor dry temperature (°C), rainfall (mm), wind speed (Beaufort Scale) and wind direction (on a 32 point scale with N = 1/32, E = 8, S = 16 and W = 24) was recorded.

2.2.5 Statistical Analysis

2.2.5.1 Training Stage 1 Cubicle On and Stage 2 Cubicle Off

For the training period, the time spent lying down on each surface was analysed as a percentage of total time in the pen for the second day of training on each surface for Stage 1 and Stage 2, as all cows had been successfully trained after the first 24 hours. A general linear model was used to analyse lying behaviours during training (percentage of time spent lying, lying bout duration, and lying bout frequency). This model was created to test the effect of surface type, stage, order of training, and for a surface type x stage interaction, blocked by cow ID.

2.2.5.2 Choice Periods Stages 1, 2, and 3

During the choice period of Stage 1 and Stage 2, cow preference for surface type was determined by analysing the percentage of total time lying on each surface during the 2 days of choice for each stage, applying a mixed model using the REML algorithm. The fixed-effects were cow ID, repetition, pen location, surface type, location of surface, and for a surface type x location interaction. Surface type x repetition, surface type x pen location, and surface type x cow ID interactions were used as the random effects. When analysing Stage 2, the location of the surface and surface type x location of surface interaction was dropped from the model when found to be non-significant.

The total percentage of time spent lying down was calculated for the training and choice periods of Stage 1 and Stage 2. The data were normally distributed and a two-way analysis of variance was used to test for a difference in time spent lying down between stages 1 vs. 2 and training periods vs. choice periods, blocked by cow ID, and determined whether there was an interaction between time spent lying down during stage 1 and 2 x training and choice periods. Paired t-tests were used to determine whether there was a difference between a) lying bout frequencies and b) lying bout durations between the choice periods of Stage 1 and Stage 2.

For Stage 3, lying time in minutes was analysed using a series of Wilcoxon matched pair signed ranks test, paired for each cow, for a) P1 + Cubicle against P2 + Open and b) P1 + Cubicle against P3 + Open, as the data were not normally distributed, even following a transformation. Six cows had no preference between P2 and P3 and either one of the two surfaces could have been preferred in principal. All possible combinations were considered and the Wilcoxon test was applied 64 times ($2^6 = 64$) to each possible combination to test for a) and b), with the mean values of these two series compiled.

Five cows in total were not included in the analysis for Stage 3; of these five cows, data from four cows were lost due to the failure of video recording equipment. The fifth cow had not made a clear choice, >60%, for one surface during Stage 2 (percentage of lying time on each surface during 48hrs of Stage 2 for cow 5 as per Figure 5 – SA: 0%; ST: 54.2%; M: 45.8%). These 5 cows are included in the analysis for Stage 1 and Stage 2, but not Stage 3.

To investigate whether cows that had a very high percentage of total lying time for one surface during Stage 2 (>80%; n=11) showed a similarly high percentage for one lying option in Stage 3, a Spearman's rank was performed on the lying option that had the largest percentage of total lying time for these eleven cows for Stage 2 and Stage 3.

2.2.5.3 Weather and Performance Factors

Weather factors and surface choice during choice periods for Stage 1, 2 and 3 were averaged per repetition and linear regressions, corrected using Bonferroni corrections, used to assess the effect of weather on choice. This weather data is summarised in Table

2.

Table 2. Summary of weather data, consisting of temperature (°C), rainfall (mm), wind speed (Beaufort Scale), and wind direction (32 point scale), averaged (mean ± SEM) and full range (Range) for the three day choice periods for Stage 1 (Cubicles), Stage 2 (Open Space) and Stage 3 (Trade-off).

	Stage 1		Stage 2		Stage 3	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Temperature (°C)	12.9 ± 1.78	4.6 - 16.6	13.6 ± 1.64	8 - 18.9	12.9 ± 1.3	9.1 - 16.2
Rainfall (mm)	5.2 ± 1.44	1.1 – 11.3	2.5 ± 1.56	0 – 9.5	6.8 ± 3.46	0.3 – 18.2
Wind Speed (Beaufort Scale)	1.6 ± 1.5	1 – 5.5	3.2 ± 0.6	1.5 – 5.5	3.1 ± 0.3	2.3 – 4
Wind Direction (32 point scale)	24.3 ± 1.9	22.5 - 32	20.8 ± 2	14 - 27	24.3 ± 1.8	18.3 – 27.3

Multiple regressions, corrected using Bonferroni corrections, were used to test for an effect of cow performance factors on surface choice during choice periods for Stage 1, 2 and 3. These factors were BCS, LS, DIM, days in calf, lactation number, weight and yield.

All data were analysed using GenStat (18th Edition, Lawes Agricultural Trust, Rothamsted, UK).

2.3 RESULTS

2.3.1 Training Stage 1 Cubicle On and Stage 2 Cubicle Off

The average time spent lying (hr), percentage of total time spent lying (%), average lying bout duration (min) and average lying bout frequency for the second day of training for Stage 1 and Stage 2 is presented in Table 3.

Table 3. Summary, averaged for all cows, of lying time (h), percentage of total time spent lying (%), lying bout duration (min) and lying bout frequency (number) for the second day of training of Stage 1(Cubicles; n=24) and Stage 2 (Open Space; n=24), when cow only had access to one lying surface at a time, for each lying surface and average (Average) for Stage 1 and Stage 2 (± SEM).

	Sand	Straw	Mattress	Average
Stage 1				
Lying Time (hr)	13.9 ± 0.47	13.14± 0.78	14.55 ± 0.42	13.86 ± 0.34
% of total time spent lying	62.5 ± 2.1	60.3 ± 3.6	65.7 ± 1.8	62.8 ± 1.7
Lying bout duration (min)	101.38 ± 6.56	75.42 ± 4.11	89.41 ± 3.61	88.74 ± 3.08
Lying bout frequency (number)	8.9 ± 0.5	10.6 ± 0.7	10 ± 0.3	9.8 ± 0.3
Stage 2				
Lying Time (hr)	14.93 ± 0.44	15.48 ± 0.21	14.46 ± 0.4	14.96 ± 0.21
% of total time spent lying	66.9 ± 1.9	69.2 ± 0.9	64.8 ± 1.7	66.9 ± 1.1
Lying bout duration (min)	98.64 ± 4.33	89.85 ± 3.94	92.65 ± 5.64	93.71 ± 2.7
Lying bout frequency (number)	9.4 ± 0.4	10.8 ± 0.4	10 ± 0.6	10.1 ± 0.3

There was no interaction between surface type x stage and no effect of order on percentage of time spent lying down, lying bout duration or lying bout frequency during the training periods, and these were therefore dropped from the models.

Overall, during the training periods, cows spent 1.1hrs longer lying down during Stage 2, without cubicles, (W = 5.343; P = 0.022; d.f. = 1; 67.1 ± 0.9%) than during Stage 1, with cubicles, (63.6 ± 1.2%). Surface type had no effect on percentage of time spent lying (W = 0.067; P = 0.967; d.f. = 2). Surface type had an effect on lying bout duration and lying bout frequency, with cows lying for longer but in fewer lying bouts on SA (lying bout duration: W = 12.975; P = 0.002; d.f. = 2; ST: 83 ± 3min; M: 91 ± 3.3min; SA: 100 ± 3.9min; lying bout frequency: W = 9.573; P = 0.01; d.f. = 2; ST: 10.7 ± 0.4; M: 10 ± 0.33; SA: 9.1 ± 0.32). There was no difference between Stage 1 and Stage 2 on either lying bout duration (W = 1.593; P = 0.209; d.f. = 1) or frequency (W = 0.335, P = 0.564; d.f. = 1).

2.3.2 Choice Periods Stages 1, 2, and 3

The average time spent lying (hr) per day, percentage of total time spent lying (%) and percentage of total lying time (%) for Stage 1, Stage 2 and Stage 3, averaged per cow, is presented in Table 4. The percentage of total time spent lying on each surface during the choice periods of Stage 1, Stage 2 and Stage 3 are presented in Figure 6 for each individual cow.

Table 4. Summary, averaged for all cows, of average lying time per day (h), average percentage of total time spent lying per day (%) and average percentage of total lying time per day on each lying option and an average daily total for all lying options combined (Daily Average) for the choice periods of Stage 1 (Cubicles; n = 24), Stage 2 (Open Space; n = 24) and Stage 3 (Trade-off; n = 19) (±SEM).

		Sand	Straw	Mattress	Daily Average
Stage 1	Lying time (hr)	1.29 ± 0.32	6.62 ± 1.09	6.65 ± 1.15	14.55 ± 0.27
	% of total time spent lying	5.8 ± 1.5	29.9 ± 4.9	30.2 ± 5.3	65.9 ± 1.3
	% of total lying time	9.1 ± 2.4	46.6 ± 7.8	44.3 ± 12.4	–
Stage 2	Lying time (hr)	1.9 ± 0.79	10.01 ± 1.14	3.64 ± 1.06	15.55 ± 0.28
	% of total time spent lying	9.0 ± 3.8	45.8 ± 5.1	16.5 ± 4.7	71.3 ± 3.7
	% of total lying time	12.4 ± 5.2	64.4 ± 7.2	23.2 ± 6.7	–
		P1 + Cubicle	P2 + Open	P3 + Open	Average
Stage 3	Lying time (hr)	3.02 ± 0.85	9.78 ± 1.02	2.08 ± 0.55	14.87 ± 0.22
	% of total time spent lying	13.9 ± 3.9	44.8 ± 4.7	9.5 ± 2.5	68.2 ± 1.0
	% of total lying time	20.5 ± 5.9	65.7 ± 6.9	13.8 ± 3.7	–

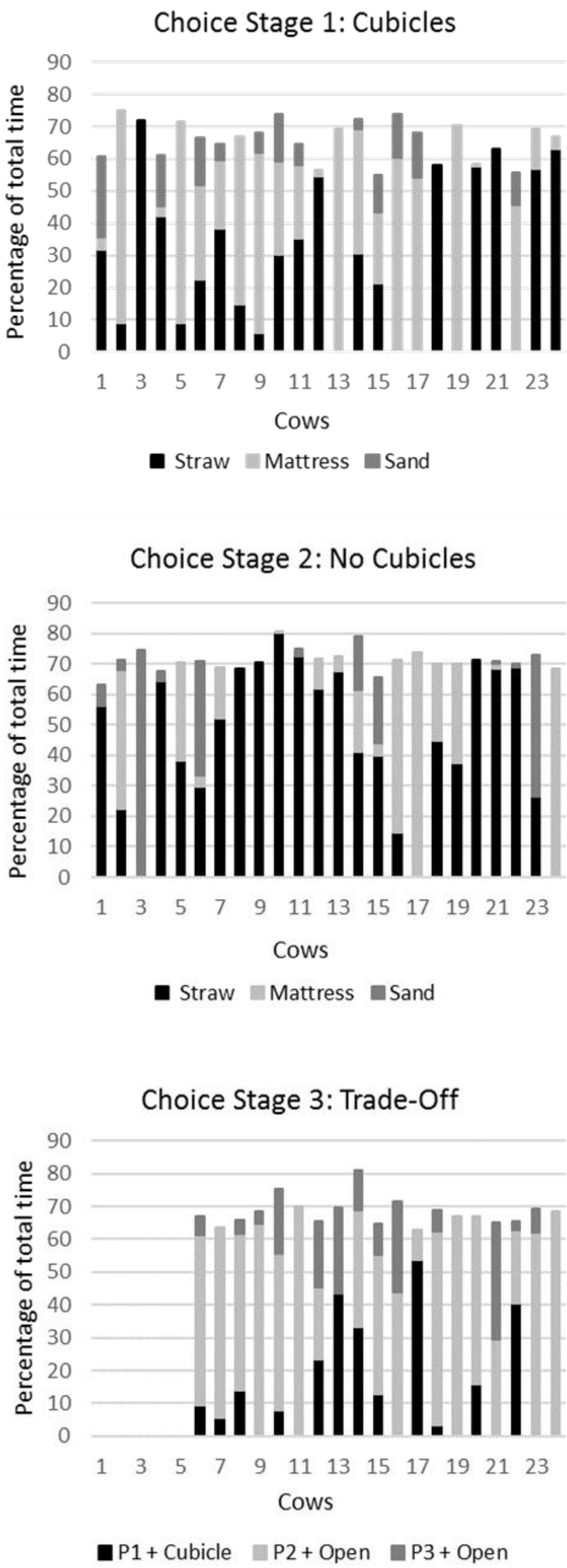

Figure 6. Percentage of total time spent lying on each surface during the choice periods of Stage 1 (Cubicles; n=24), Stage 2 (Open Space; n=24) and Stage 3 (Trade-off; n=19) for each individual cow.

During the 48-hr choice period for Stage 1, an interaction was found between surface type and surface location for ST and M (W = 11.93; P = 0.03; d.d.f. = 42). Cows lay down longest on M when in the middle of the pen (North East: 18.6 ± 9.5%; middle: 46.1 ± 6.8%; South West: 25.8 ± 8.9%) but longest on ST when in the South West of the pen (North East: 17.6 ± 7.2; middle: 31 ± 8%; South West: 41 ± 9.1%).

Overall, cows lay down for >5 hrs longer on ST and M than on SA (W = 11.45; P = 0.02; d.d.f. = 11; SA: 5.8 ± 1.5%; ST: 29.9 ± 4.9%; M: 30.2 ± 5.3%). This is due to some cows having a strong preference for ST (9 cows >60% of lying time) and some for M (9 cows > 60% of lying time) as opposed to cows splitting their time between ST and M (Figure 5).

During the Stage 2 48-hr choice period, no interaction was found between surface type x surface location and the interaction was dropped from the model. A difference was found between the percentage of time spent lying on the different surface types (W = 66.82; P = 0.027; d.d.f. = 2.1) with cows spending more time lying down on ST than on M or SA (SA: 9 ± 3.8%; ST: 45.8 ± 5.1%; M: 16.5 ± 4.7%). Cows were found to spend a greater percentage of their time lying down during Stage 2 than Stage 1 (F = 22.16; P < 0.001; d.f. = 1; 71.3 ± 0.8% vs. 65.9 ± 1.3%). Percentage of time spent lying in the training periods were found to be lower than during the choice periods for Stage 1 and 2 (F = 13.43; P < 0.001; d.f. = 1; 64.9 ± 1.1% vs. 68.6 ± 0.8%), a difference of <1hr. There was no interaction found between stage 1 and 2 x training and choice periods (F = 0.37; P = 0.543; d.f. = 1). There was no difference in lying bout frequency between the choice periods of Stage 1 and Stage 2 (t = -1.1; P = 0.284; d.f. = 23; 20 ± 0.7 and 20.6 ± 0.6, respectively) and no difference in lying bout duration between the choice periods of Stage 1 and Stage 2 (t = -1.42; P = 0.168; d.f. = 23; 90mins ± 3.1 and 94mins ± 2.5).

For the trade-off choice period of Stage 3, the 64 combinations of Wilcoxon tests were averaged to get a mean p-value. The results showed that cows lay down on average for >6hr longer on P2 + Open than P1 + Cubicle (W = 35.25; P = 0.023; P1: 13.9 ± 3.9%; P2: 44.8 ± 4.7%), expressing a strong preference to lie down on P2 + Open (65.7% of total lying time), compared to lying on P1 + Cubicle (20.5%). There was no difference in lying times between P1 + Cubicle and P3 + Open (W = 64.44; P = 0.730; P3: 9.5 ± 2.5%).

For cows that expressed a high percentage of lying time on one surface (>80%) during Stage 2 (n = 11), no correlation was found for the largest percentage of total lying time for one lying option between Stage 2 and Stage 3 (ρ = 0.471; p = 0.144). These cows had an average lying time on one surface of 97.3% during Stage 2, which dropped to an average of 73.3% during Stage 3.

2.3.3 Weather and Performance Factors

There was no effect of any weather factors on cow surface choice for Stage 1, 2 and 3. No cow performance effects on cow surface choice were found Stage 1 and Stage 2.

An interaction between number of lactations and cow choice during Stage 3 was the only cow performance effect at this stage (P = 0.015; 3 ± 0.3). Cows with fewer lactations (≤ 2.9) spent a larger percentage of time lying on P2 + Open than higher lactation cows (57.8 ± 3.2% vs. 33.2 ± 6.6%, for below-average and above-average lactation number, respectively) but spent a shorter percentage of time on P1 + Cubicle (4.9 ± 2.2% vs. 19.8 ± 6.2%) and P3 + Open (5 ± 3% vs. 13.6 ± 3.6%).

2.4 DISCUSSION

The objective was to establish what aspect of a lying area was more important to a cow, the surface type or an open space, using a preference trade-of. The majority of cows in this study had a strong preference to lie down in an open space on a surface they had not shown a strong preference for previously, suggesting that they were prepared to give up their preferred surface in order to have an open space to lie in. There was no difference in lying time found between P1 + Cubicle and P3 + Open, suggesting that lying in a cubicle on their preferred surface is as favourable as lying on their least preferred surface. However, the difference in lying time between the two least preferred options in Stage 3 (P1 + Cubicle and P3 + Open) was ~1hr. Differences of this magnitude were significant in the analysis of Stage 1 and Stage 2 (n=24), but may not have been detectable in Stage 3 due to a smaller sample size (n=19). There was no preference found during Stage 1 when cubicles were on the surfaces, with cows on average lying for a similar amount of time on both ST and M. However, Figure 7 illustrates that it is due to some cows choosing to spend the majority of their lying time on ST and some on M, as opposed to most cows splitting their time between both surfaces. During Stage 2, the majority of cows had a strong preference for ST.

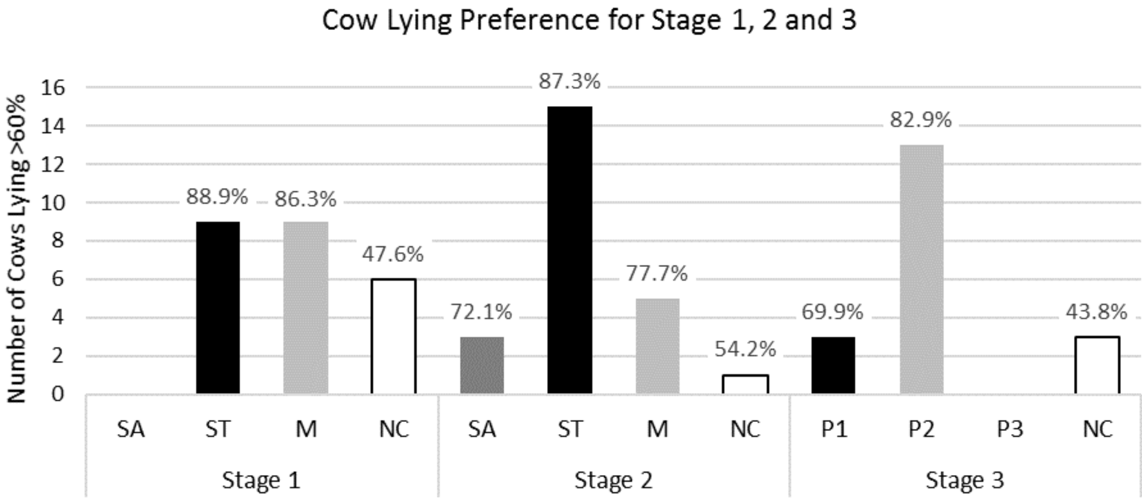

Figure 7. Number of cows that had a strong preference (>60% total lying time) on each lying option (SA = sand; ST = straw; M = mattress; P1 = P1 + Cubicle; P2 = P2 + Open; P3 = P3 + Open) with the average percentage of total lying time for that lying option and number of cows with no clear choice for just one surface (NC) with the average percentage of total lying time for the surface they lay the most on during Stage 1 (Cubicles; n = 24), Stage 2 (Open Space; n = 24) and Stage 3 (Trade-off, n = 19).

As predicted for this current study, these cows did choose to trade their preferred surface type for an open lying space. However, it has been suggested in other studies that cows are less focused on the spatial constraints of a cubicle when deciding where to lie down and more so on the cubicle surface. Studies investigating the effect of aspects of the cubicle structure, such as stall length, width and neck rail placement, on lying time in cows (Tucker et al., 2004; Tucker et al., 2005) tend to yield less definitive results compared to studies investigating the effect of stall surface on cow lying preference (Tucker et al., 2003, Manninen et al., 2002, Norring et al., 2010). This was further demonstrated in a study whereby cows chose to lie in cubicles over alternative cubicles, with neck rails removed and stall dividers replaced with a wooden board protruding from the deep-sand bedding, eluding to an open space (Abade et al., 2015). However, the changes made to the total lying space in these studies assessing use of 'adjusted' cubicles are relatively small compared to offering cows a true open lying area, as was done in the present study. Fregonesi et al. (2009) found that cows had a preference for an open lying area, of the same total lying area and same lying surface, over cubicles, showing the value of a true open lying space to cows, which is supported by the results in the current study.

Overall, in the current study, when cows had a choice, they generally preferred lying on surfaces other than sand, which could be due to the overall lack of previous experience on sand bedding compared to M and ST. When Manninen et al. (2002) gave four groups of cows, with no previous experience of sand, a free choice of sand, straw and soft rubber mats in cubicles, the cows avoided the sand cubicles, sometimes even choosing to lie in the passageways to avoid them. In the Manninen et al. (2002) study, after the first two groups of cows had refused to use the sand cubicles at all, the second two groups were given a 3 day forced period on each of the different beds before the choice period. These cows were found to use the sand cubicles more often, demonstrating the importance of resource exposure before preference tests. Similarly, Tucker et al. (2003) found that cows previously housed on deep-bedded sawdust cubicles had a preference for sawdust over cubicles with deep-bedded sand or with rubber mattresses bedded with sawdust, but after a 2 day forced period on each surface, two out of the twelve cows switched their preference to sand. In Tucker et al., (2003) the rubber mattress cubicles were the least preferred and were the surface the cows had the least experience with prior to the study. It is worth noting that in the current study, when cows had no choice in lying surface (in the training period), there was no difference in total lying times between sand and the other surfaces, suggesting that although these cows did not have a preference for sand, it was not sufficiently aversive when they had no other lying option to cause animal welfare challenges associated with reduced lying times.

Cows lay down for ~1hr longer during Stage 2, when cubicles were removed, compared to Stage 1, when cubicles were present, both when cows had no choice of surface (training periods) and a choice of surface (choice periods). Using time spent lying down as an indicator of comfort (Haley et al., 2000), this would suggest that cows find lying down in a cubicle less comfortable than lying down in a more open space, even when given a choice of lying surface types. Although these results are significant, we must consider whether a difference of ~1hr is biologically significant. Studies have shown that cows have a daily lying time ranging between 11.1hr – 12.5hr, depending on stage in lactation (Maselyne et al., 2017), and heifers have an inelastic demand to lie down for between 12-13hrs (Jensen et al., 2005). Lying times reported for all stages of this study are greater than 13hrs, suggesting that lying comfort is not compromised in a cubicle during this study.

In the current study, during the training periods, when there was no choice of lying surface, surface type had an effect on lying behaviour, with cows on SA having the longest but least frequent lying bouts. However, the special constraints of a cubicle did not have an effect on lying behaviour, with no difference of lying bout duration or frequency found between Stage 1 (cubicles on) and Stage 2 (no cubicle). Longer but fewer lying bouts are generally reported on harder lying surfaces, with cows more reluctant to stand up and lie down on harder surfaces due to the discomfort experienced during the process (Herlin, 1997; Haley et al., 2000; Haley et al., 2001; Tucker et al., 2003). This would indicate that the cows in this study found sand an uncomfortable surface on which to lie down on and from which to get back up from, which is supported by cow avoidance of SA during the choice periods. Additionally, lying space did not have an effect on this lying behaviour, suggesting that the cubicle does not impede on the process of lying down and getting up for these cows. Contradictory to these results, studies have reported that compared to an open lying area (such as pasture or an open indoor lying area), when in a cubicle cows do exhibit longer but fewer lying bouts (Haley et al., 2000; Hernandez-Mendo et al., 2007) and that the cubicle is impeding the lying down and getting up motion. However, unlike the current study, those studies had a confounding factor of surface type, further suggesting that surface type can effect lying behaviour, with lying bout duration and frequency an indicator of lying comfort, but only in terms of surface and not necessarily space.

An interaction was found between cow choice for lying surface and surface location within the pen during the Stage 1 choice period. As this interaction was only seen for Stage 1, it could be linked to the training protocol, whereby cows were always trained on the NE surface when first introduced into the pen. Taking into account their general avoidance of SA, and possible avoidance of the first surface they were trained on when they entered the pens, when these options are removed (see Figure 4) the locations of M and ST are for the majority in the middle and SW, respectively. Had the cows been given a couple of days to adjust to the pens before data collection began, this interaction may have been minimised.

Improved welfare aided simply by having control over one's environment, described as 'agency' (Wemelsfelder, 1997; Špinka, 2019), has been proposed as a reference point for welfare enhancement (Mellor, 2015; Mellor and Beausoleil, 2015; Mellor, 2016). It has been suggested that giving cows the ability to have choice within their environment, with even a perceived sense of control, may improve welfare (Motupalli et al., 2014; Webster, 2016; Charlton and Rutter, 2017). In the current study, cows lay down longer when given a choice of surfaces compared to during the training stages, when they only had access to one lying surface at a time. Similarly, Legrand et al. (2009) found that when given the choice of indoor cubicles and pasture, cows spent longer lying down compared to when they were confined to pasture alone. However, the difference in both studies was relatively small and highlights that the full extent of the effects of offering animals choice over their environment is unknown and requires further study.

2.5 CONCLUSIONS

On average, when a cubicle was refitted onto the cow's preferred surface and these cows were presented with a trade-off between lying on their preferred surface or an open lying space of less preferred surface, the majority of these cows chose the open lying space. These cows made no choice between ST and M when a cubicle were present, however this was most likely due to some cows having a strong preference for ST and some for M, as opposed to most cows splitting their time evenly between the two surfaces. When the cubicle was removed, the majority of cows had a strong preference for ST. These results suggest that when choosing where to lie down, these cows valued an open lying space, without a cubicle, over their preferred surface type. More work is needed to investigate cow motivation for open lying space and the relationship between this and surface type.

CHAPTER 3: DAIRY COWS VALUE AN OPEN LYING AREA FOR LYING DOWN

3.1 INTRODUCTION

The majority of dairy cows are housed at some point throughout their lives (99% of British cows are housed at some point throughout the year, March et al., 2014) and many farmers are moving to year-round housing (Haskell et al., 2006; van den Pol-van Dasselaar et al., 2008). When housed, dairy cows are most commonly housed in cubicles, known as free-stalls in North America, a housing system which originated form tie-stall designs (Margerison, 2011). Various studies have shown that lying down is an important behaviour to cows (Rebound response: Munksgaard and Simonsen, 1996; Norring and Valros, 2016. Trade-off: Metz, 1985; Munksgaard et al., 2005. Operant conditioning: Jensen et al., 2004; Jensen et al., 2005. Consumer demand: Tucker at al., 2018). Therefore, it has become increasingly important to establish whether cubicles are the best system to meet the behavioural and welfare needs of cows.

Motivation tests require animals to work to gain access to a valued resource in order to quantify the value the animal places on that resource. Tests of motivation are commonly preceded by a preference test (Fraser and Nicol, 2018). Preference tests allow an animal to choose between differing variations of one resource or between different resources, with the assumption that the animal chooses in a way that best provides for their own welfare (Fraser and Nicol, 2018). A great deal of research has been carried out on cow lying preference investigating a range of cubicle modifications (stall width and length: Tucker et al., 2004; neck rail placement: Tucker et al., 2005; surface type: Manninen et al., 2002; Tucker et al., 2003). Although cows appear more focused on the lying surface of a cubicle, preferring softer lying surfaces, a trade-off preference study found that when choosing where to lie down, an open lying space is more important to cows than their preferred lying surface (Shewbridge Carter et al., 2021). Cow preference for cubicles has been compared against open lying areas, such as pasture (Legrand et al., 2009; Charlton et al., 2011) and pack areas, both indoors (Fregonesi et al., 2009) and outdoors (Smid et al., 2019), with the results supporting the proposition that cows have a preference for an open lying area.

However, preference tests do not differentiate between the potential preference for one resource (suggesting preference is based on perceived benefits) versus potential avoidance of another (suggesting preference is based on choosing the lesser of two evils) (Fraser and Nicol, 2018; Fraser 2008). Therefore, the motivation for a preference choice must be further investigated in order to establish whether it actually does provide for an ethological need, leading to improved welfare (Jensen and Pedersen, 2008), or whether its absence leads to animal suffering (Dawkins, 1988). For dairy cows, weighted gates (von Keyserlingk et al., 2017; Tucker et al., 2018) and distance walked (Schütz et al 2006; Charlton et al 2013; Motupalli et al 2014) to gain access to a resource has been used to measure motivation, with increasing weight or distance, respectively, being the cost to cows for access. Studies have shown that cows are motivated to gain access to pasture, walking long distances (Charlton et al., 2013; Motupalli et al., 2014) and pushing weighted gates for access (von Keyserlingk et al., 2017), and in particular at night, which may be driven by the motivation to lie down on pasture. However, cow motivation for pasture access has always been tested against indoor cubicles, with the results confounded by surface type and/or location of the lying areas provided (i.e. pasture outdoors vs mattress cubicles indoors). Therefore, it is unclear whether cows are motivated to access pasture as an open lying area because it is outdoors or because of the surface it provides for lying down.

The aim of the current study was to measure cow motivation for lying down on an indoor open mattress lying area [MAT] when cows had free access to indoor mattress-bedded cubicles, thus removing the confounding factors of surface type and location. This was repeated for a deep-bedded straw yard (ST) of an identical size to the open mattress, to further investigate the value cows place on lying surface type and the relationship between this and the value cows place on lying space. Walking distance was used as a measure of cow motivation to access these open lying areas via an indoor raceway, set at three different increasing distances (Short, 34.5 m; Medium, 80.5 m; Long 126.5 m). We predicted that cows would spend more time lying in the open lying areas compared to the cubicles and that surface type would play in underlying role in cow choice, with cows being more motivated to access the deep-bedded straw than the mattress.

3.2 MATERIALS AND METHODS

Ethical approval for this study was given by the Harper Adams University Research Ethics Committee (0488-201905-PGMPHD).

3.2.1 Animals and Management

The study was carried out at the Agri-EPI Midlands Dairy Research Centre at Harper Adams University, Shropshire, United Kingdom, in an open-sided barn. Thirty pregnant

Holstein-Friesian dairy cows (see Table 5 for cow details) were selected for the study. Cows were selected based on a confirmed pregnancy status by the farm veterinarian, had to have body condition score (BCS) between 2.75 and 3.5, as described by the Penn State method (Ferguson et al., 1994), and a lameness score (LS) no greater than 2 (2 = imperfect locomotion but ability to move freely not diminished; Flower and Weary, 2006). BCS and LS were assessed by the same person (LSC) while cows walked across a concrete floor before cows came on trial. The cows were allocated to 1 of 6 experimental periods according to their stage of lactation (n = 5 x 6), which were carried out from August 31st 2019 to July 21st 2020 (study period 1: August 31st to October 1st, 2019; study period 2: October 5th to November 5th, 2019; study period 3: November 9th to December 10th, 2019; study period 4: January 11th to February 11th, 2020; study period 5: February 15th to March 17th, 2020; study period 6: June 20th to July 21st, 2020). Before the study, the cows in this herd had been housed indoors in a cubicle barn on bedded rubber mats with plastic, flexible cubicle dividers and with experience of a straw yard during the pre-calving period and metal cubicles as heifers. They had free access to one milking robot (VMS V200, De Laval).

Table 5. Mean (± SEM) and range of lactation number, days in milk, milk yield (kg/d), body condition score and lameness score for all cows in the study. * This study included 6 primiparous and 24 multiparous cows.

	Mean (± SEM)	**Min**	**Max**
Lactation Number *	2.8 ± 0.2	1	6
Days In Milk	260 ± 15.5	130	466
Milk Yield (kg/d)	24.13 ± 1.84	1.78	42.26
Body Condition Score	3 ± 0.03	2.75	3.5
Lameness Score	1.62 ± 0.04	1.5	2

3.2.2 Trial Area Housing

The trial area was located in an area of 453-m^2 at the south-east end of an open sided barn. Trial cows were separated from the main herd, of approximately 50 cows, into the trial area using cow hurdles. The trial area was split into two main areas (Figure 8). The 'Cubicle Area' included six sawdust-bedded cubicles with a mattress (2.7 x 1.2 m; Super Comfort Cubicles, Intershape Ltd., Daventry, England, UK; Pasture Mat; Wilson Agri, Coleraine, Northern Ireland, UK), access to the milking robot, and a feed-face and water-trough providing ad libitum Partial Mixed Ration (PMR) and clean water, respectively.

PMR was provided daily at approximately 0730 h, with feed refusals being removed every day before fresh feed was provided. Cows were fed concentrates, based on milk yield, during milking in the milking robot.

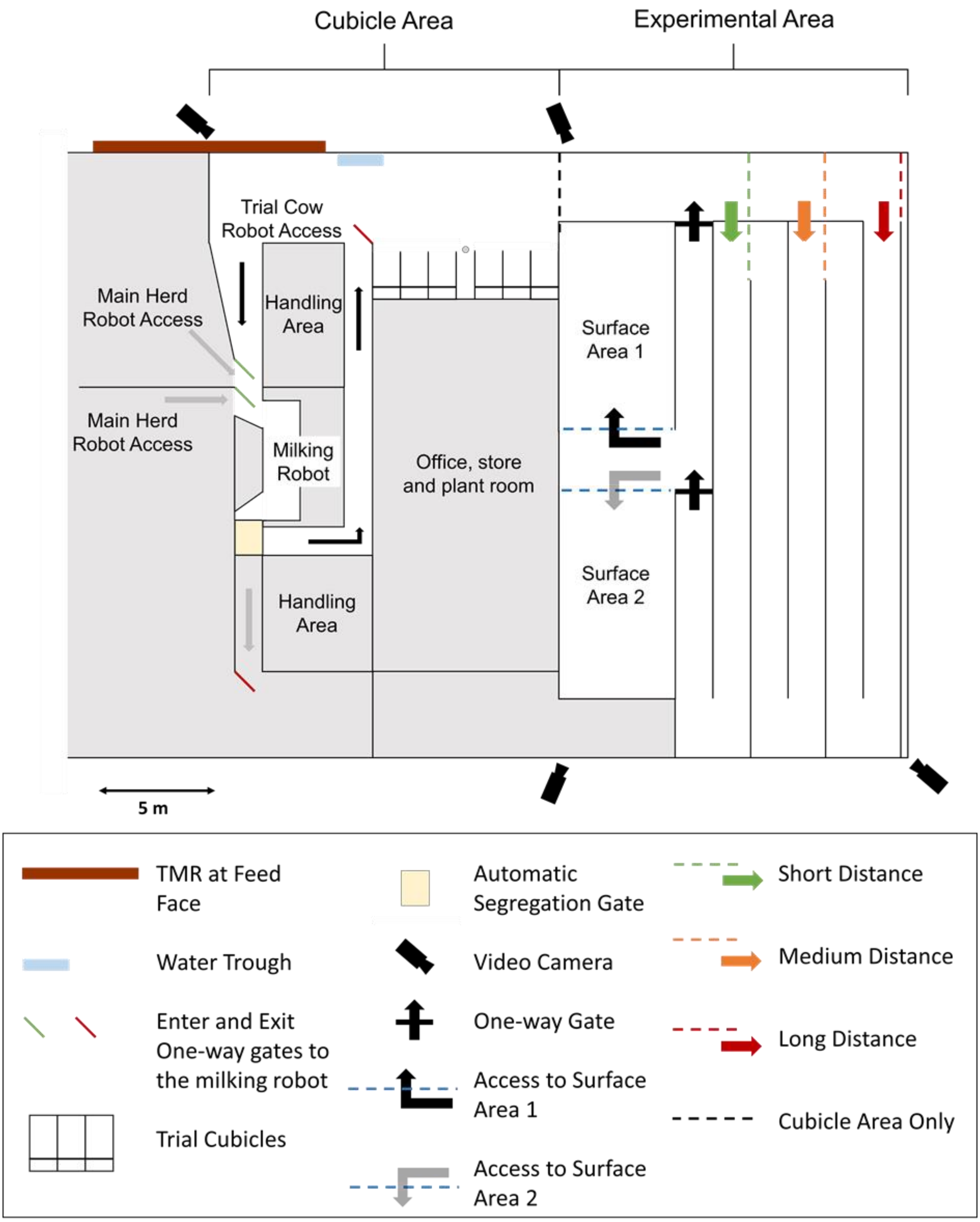

Figure 8. Plan of the trial area. The area is split into two main components; the 'Cubicle Area' and 'Experimental Area'. Within the 'Cubicle Area' cows had access to six cubicles, feed face, a water trough and the milking robot. The 'Experimental Area' contained an indoor raceway which allowed access to one of two open lying areas at a time, 'Surface Area 1' and 'Surface Area 2'.The raceway could be adjusted to three different distances, via the three difference entrances indicated, to a Short (34.5 m), Medium (80.5 m), and Long (126.5 m) distance.

The ‘Experimental Area’ (Figure 8) comprised of an indoor raceway (1.5 m wide) on a concrete floor, which could be adjusted to three different distances (Short; 34.5 m, Medium; 80.5 m, Long; 126.5 m), and incorporated a number of 180 degree turns (Short: 1 turn; Medium: 3 turns; Long: 5 turns). Each turning area was 2.5 m x 3m and had a rubber mat floor (EASYFIX MG Max 4, Agri & Industrial Rubber Ltd, Galway, Ireland). The raceway led to two open lying areas (9.0 x 5.0m) of different surface types (deep-bedded straw [ST] and sawdust bedded mattress [MAT] (Pasture Mat; Wilson Agri, Coleraine, Northern Ireland, UK)), separated by a central access point. Cows only ever had access to one of the open lying areas at a time. The raceway had two one-way gates to ensure that once a cow walked the length of the raceway and entered the surface area, via a first one-way gate, she could not walk back the length of the raceway to return to the ‘Cubicle Area’, but returned to the ‘Cubicle Area’ via a short race (9 m) through a second one-way gate, thus setting up a one-way system in and out of the surface areas.

3.2.3 Experimental Design

Throughout the study cows had continuous access to cubicles, with each experimental period lasting a total of 31-d (Figure 9): 3-d of familiarization (cubicle access only), followed by 14-d of access to the first open lying area and 14-d of access to the second open lying area.

Cubicles Only			Surface A														Surface B													
Familiarization			Short						Medium				Long				Short						Medium				Long			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

Figure 9. Representation for the 31-d study period, which was repeated 6 times using 5 cows per experimental period. Unshaded days represent training days and days shaded in grey represent choice days. Days shaded in black represent the familiarization period, when cows didn’t have access to either open lying area. Short, Medium and Long describe the raceway distance in order to access the open lying areas, with Surface A being the first surface and Surface B being the second surface.

While on trial, cows continued to have free access to the milking robot, were fed concentrates during milking, and were segregated into the trial area after milking via an automatic segregation gate (Figure 8). At approximately 0800 h each day, the trial area was cleaned out. When cows had access to the open lying areas, they were moved and kept in the cubicle area during cleaning.

Four video cameras (Swann, Milton Keynes, UK), were set up to continuously record cow behaviour within the trial area (see Figure 8). Each experimental "day" began when the cows had access to the open lying areas after cleaning and ended when cleaning began the following day (approximately 1030 – 0800 h). Behaviour was only recorded during choice periods, as per Figure 9.

Each experimental period began with a 3-d familiarization period. During this time, cows only had access to the 'Cubicle Area' so that they could familiarize themselves with their new grouping, robot access and the cubicles. For the duration of the study, cows had 'free' access to the cubicles at all times and did not have to work to gain access to them.

During training periods, each cow was encouraged to walk the raceway distance, with a researcher walking quietly behind them, to gain access to the open lying space they had access to at the time. Following the familiarization period, the first training period, which always occurred at the Short distance, was 3-d long, to allow for one-way gate training (see APPENDIX 1 for a detailed training protocol) as well as to allow cows to become familiar with the raceway length which had to be walked in order to access the first open lying area, Surface A. This training period was followed by a 3-d choice period, whereby cows had the choice to use the raceway to gain access to the open lying area available, paying the price of walking the Short distance (34.5 m), or could access the cubicles for 'free'. The raceway length was then changed to the Medium distance (80.5 m), as per Figure 8, and cows had 1-d training, as per Figure 9. This was followed by another 3-d choice period. Finally, the raceway length was changed to the Long distance (126.5 m), as per Figure 8, and cows had a 1-d training period followed by a 3-d choice period. Then the lying surface access was changed from Surface A to Surface B and the above series of training and choice periods repeated.

For experimental periods 1-3, the open lying area marked 'Surface Area 1' as per Figure 8 was a mattress bedded with sawdust (MAT) and the open lying area marked 'Surface Area 2' was deep-bedded straw (ST). These surface locations were reversed for experimental periods 4-6; 'Surface Area 1' was ST and 'Surface Area 2' was MAT. For each experimental period, the order in which cows had access to these open lying areas alternated; Group 1 had ST followed by MAT, Group 2 had MAT followed by ST, and so on.

3.2.4 Performance and Lameness

Throughout the study, milk yield was recorded automatically at each milking for individual cows via the robotic milking system and was used to calculate average yield per cow for the duration of their time on the study. Milking permission, in terms of milking frequency and latency between milkings, was set by the herd manager depending on a cow's stage of lactation. The milking status of trial cows was checked regularly by the herd manager and LSC, with any trial cows that had not successfully taken themselves to be milked for >12 h being encouraged to the robot for milking.

LS was recorded after the trial area was cleaned each morning, at approximately 1000 h, on the first day of training for Surface A and Surface B (day 4 and 18 as per Figure 9) and on the first and last day of each choice period (the first and last day in the grey shaded blocks as per Figure 9).

3.2.5 Measurements

3.2.5.1 Behavioural Measurements

Total experimental time varied slightly each day depending on the duration of cleaning. In order to measure different behaviours during the days of choice periods as a proportion of total time, the beginning and ending of each experimental day (i.e. when cleaning finished and then started the following day) was determined using the video footage to obtain the total time for each experimental day. For the 'Cubicle Area' during the choice periods, video footage was recorded from a camera located above the 'Cubicle Area' (see Figure 8) and was used to obtain cubicle lying bout start and end time for each cow. From this, total time spent lying and the number of lying bouts in the cubicles was calculated. For the 'Experimental Area' during the choice periods, video footage was analysed from cameras located above each surface area and above the raceway to obtain the enter and exit time to the open lying areas and the lying bout start and end time on the open lying areas. From this, time spent lying down and non-lying time (time spent standing and performing other behaviours while standing) in the open lying areas was calculated for each choice period for each cow. Additionally, frequency of raceway completions per choice period for each cow was recorded from this video data.

To account for changing daylight photoperiod during the trial, the time of sunrise and sunset was obtained from Time and Date (Thorsen, 2021), using geographical coordinates for the site of the trial area (52°46’52.8” N, 2°25’52.3” W). Time spent lying down during the day was defined as being between sunrise and sunset and time spent lying down during the night was defined as being between sunset and sunrise.

3.2.6 Statistical Analysis

Dependent variables were time spent lying, and not lying down on the open lying areas, and the time spent lying down in the cubicles versus open areas. The number of lying bouts was measured as the total lying bout frequency for each choice period. Lying bout duration was calculated as the average lying bout duration for each choice period. Lying bout frequency and average lying bout duration were calculated separately for open lying areas and the cubicles. Frequency of raceway completion on the open lying areas was measured as total raceway completion frequency for each choice period. Explanatory variables included chosen lying location (open lying, or cubicle), raceway distance (Short, Medium, or Long), and surface type for open areas (mat, or straw).

Linear mixed effects models were used to analyse the (fixed) effects of chosen lying location, raceway distance, and surface type. Assumptions of Gaussian residual distribution and homoscedasticity were examined and met for our analyses. To meet the assumption of independence of observations for individual cows (n individual cows = 30) and experimental groups (n groups = 6), group and cow ID nested in group were treated as random effects. All data were analysed using R version 4.1.0 [32] using the “lme4” package [33]. Main effects were evaluated using the Wald chi-squared test statistic for mixed effects models [34] using standard methods [35]. Comparisons of pairwise *post hoc* differences were made using the Sidak correction [36].

3.3 RESULTS

The main descriptive results are summarised in Table 6, averaged for a day across all cows.

Table 6. Summary, averaged for all cows, of time spent lying (h/d), lying bout frequency per day, lying bout duration (hr), time spent lying during the day (h/d), and time spent lying during the night (h/d) on MAT, ST, Open Lying Surfaces and Cubicles at the Short, Medium and Long distances and time spent not lying (h/d) and raceway completion frequency per day for ST and MAT at the Short, Medium and Long distance (± SEM).

	Short	Medium	Long
MAT			
Lying (h/d)	10.3 ± 0.9	9.0 ± 1.0	6.7 ± 1.0
Lying Bout Frequency (per day)	7.2 ± 0.7	6.7 ± 0.7	4.6 ± 0.8
Lying Bout Duration (h)	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
Lying Day (h/d)	3.4 ± 0.4	3.0 ± 0.4	2.3 ± 0.4
Lying Night (h/d)	6.7 ± 0.6	6.0 ± 0.7	4.5 ± 0.7
Not Lying (h/d)	1.5 ± 0.3	1.1 ± 0.2	0.7 ± 0.1
Raceway Completion Frequency (per day)	3.6 ± 0.4	2.7 ± 0.3	1.8 ± 0.3
ST			
Lying	12.4 ± 0.6	11.3 ± 0.9	8.2 ± 1.0
Lying Bout Frequency	8.5 ± 0.5	7.4 ± 0.6	5.2 ± 0.7
Lying Bout Duration	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Lying Day	4.3 ± 0.4	3.6 ± 0.4	2.8 ± 0.4
Lying Night	8.2 ± 0.4	7.6 ± 0.6	5.5 ± 0.7
Not Lying	1.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
Raceway Completion Frequency (per day)	4.3 ± 0.2	3.3 ± 0.3	2.1 ± 0.3
Open Lying Surfaces			
Lying	11.4 ± 0.6	10.1 ± 0.7	7.5 ± 0.7
Lying Bout Frequency	7.9 ± 0.6	7.0 ± 0.6	4.9 ± 0.7
Lying Bout Duration	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
Lying Day	3.8 ± 0.3	3.3 ± 0.3	2.5 ± 0.5
Lying Night	7.5 ± 0.4	6.8 ± 0.5	5.0 ± 0.3
Cubicles			
Lying	2.3 ± 0.5	3.1 ± 0.5	4.8 ± 0.6
Lying Bout Frequency	2.1 ± 0.4	2.9 ± 0.5	4.7 ± 0.6
Lying Bout Duration	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
Lying Day	1.1 ± 0.2	1.2 ± 0.2	1.9 ± 0.3
Lying Night	1.3 ± 0.3	1.9 ± 0.2	2.9 ± 0.3

3.3.1 Open Lying Areas versus Cubicles

Overall lying time, lying bout frequency and lying bout duration results are shown in Fig 3 and were analysed in order to evaluate the effect of distance with respect to choices made while cows had access to the open lying areas. A strong overall effect of location on lying time per 24 h was found, being higher in open lying areas than in cubicles (W^2_1 = 247.1, P < 0.0001; Fig 3A). Cows also spent more time lying at night than during the day (W^2_1 = 93.5, P < 0.0001; Fig 3B). No significant average effect of distance on total lying time was found (W^2_2 = 1.9, P = 0.39), but a significant interaction was detected between distance and location (W^2_2 = 43.7, P < 0.0001). As distance to access the open lying areas increased, lying time on the open lying areas decreased and increased on the cubicles. A strong overall effect of location on the frequency of lying bouts per 24 h was found, with more lying bouts on open lying areas than in cubicles (W^2_1 = 71.1, P < 0.0001; Fig 3C). No significant average effect of distance was found on the frequency of total lying bouts (W^2_2 = 0.2, P = 0.92), but there was a significant interaction between distance and location (W^2_2 = 34.4, P < 0.0001). A strong effect of location was also found on lying bout duration, with cows exhibiting longer lying bouts on the open lying areas than in the cubicles (W^2_1 = 132.0, P < 0.0001; Fig 3D). Here also, there was no significant average effect of distance found on the duration of lying bouts (W^2_2 = 0.9, P = 0.65). There was however a weak significant interaction between distance and location (W^2_2 = 34.4, P = 0.01).

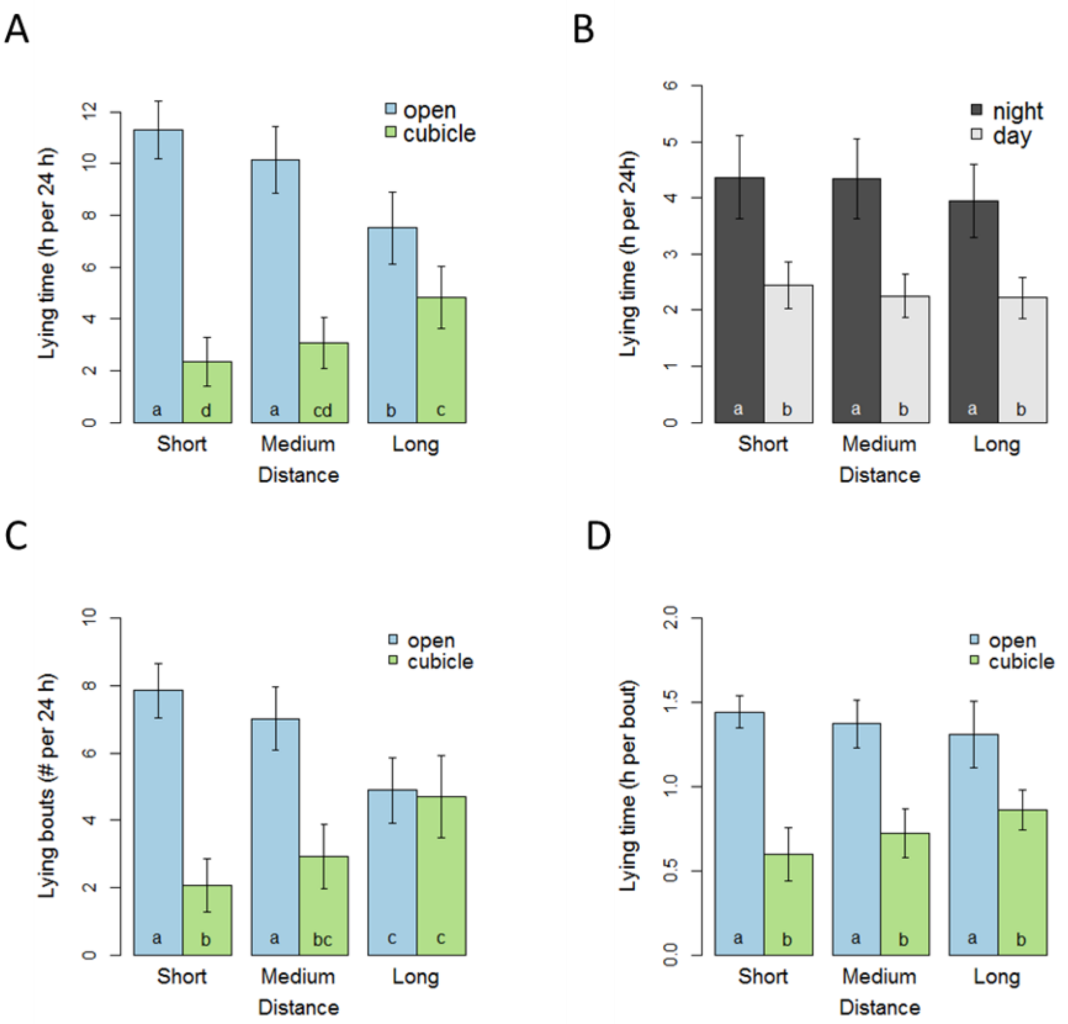

Figure 10. Lying as a function of distance choice treatment. Bar height represents the category mean and error bars represent the category 95% confidence interval. Letters represent mean differences based on post hoc pairwise tests (Sidak corrected alpha = 0.05). A. Lying time per 24 h in open lying areas versus cubicles. B. Lying time per 24 h at night versus daytime. C. Frequency of lying bouts per 24 h in open lying areas versus cubicles. D. Average duration of lying bout per 24 h in open lying areas versus cubicles.

3.3.2 MAT versus ST

Lying time and other behaviors were also analysed with respect to choices made while in the open lying areas in order to evaluate the effect of surface type and distance (Fig 4). Distance was found to have a strong overall effect on lying time per 24 h, with lying time decreasing with increasing distance ($W^2_2 = 50.2$, $P < 0.0001$; Fig 4A). Cows spent more time lying at night than during the day ($W^2_1 = 209.0$, $P < 0.0001$; Fig 4B). Surface also had a strong effect on lying time, with cows choosing to lie longer on ST compared to MAT ($W^2_1 = 21.3$, $P < 0.001$), and no significant interaction was found between distance and surface ($W^2_2 = 0.6$, $P = 0.73$). Distance also strongly influenced the frequency of lying bouts in the open lying areas, with the number of lying bouts decreasing with distance ($W^2_2 = 49.8$, $P < 0.0001$; Fig 4C). There was a significant, small increase in the frequency of lying bouts on ST compared to MAT ($W^2_1 = 6.6$, $P = 0.01$), and no significant interaction between distance and surface ($W^2_2 = 0.9$, $P = 0.64$). The mean duration of a lying bout was also higher for ST versus MAT ($W^2_2 = 9.9$, $P = 0.002$; Fig 4D), but there was no significant influence of distance ($W^2_1 = 3.1$, $P = 0.21$) nor an interaction between distance and surface ($W^2_2 = 1.5$, $P = 0.48$). We found that the time spent not lying was also influenced by distance, with less time spent not lying as distance increased ($W^2_2 = 24.7$, $P < 0.0001$; Fig 4E). However, we did not detect a significant overall association between time spent not lying and surface type ($W^2_1 = 0.6$, $P = 0.44$) nor an interaction between distance and surface ($W^2_2 = 0.7$, $P = 0.69$). Finally, the frequency of raceway completions was analysed and a strong decrease in the number of completions as distance increased was found ($W^2_2 = 90.3$, $P < 0.0001$; Fig 4F), and less completions for MAT compared to ST ($W^2_1 = 11.1$, $P = 0.001$). No interaction effect was found between distance and surface for the frequency of raceway completions ($W^2_2 = 1.0$, $P = 0.60$). Pairwise post hoc comparison of means is indicated in each figure panel, with letters indicating significantly different means (Sidak corrected alpha = 0.05).

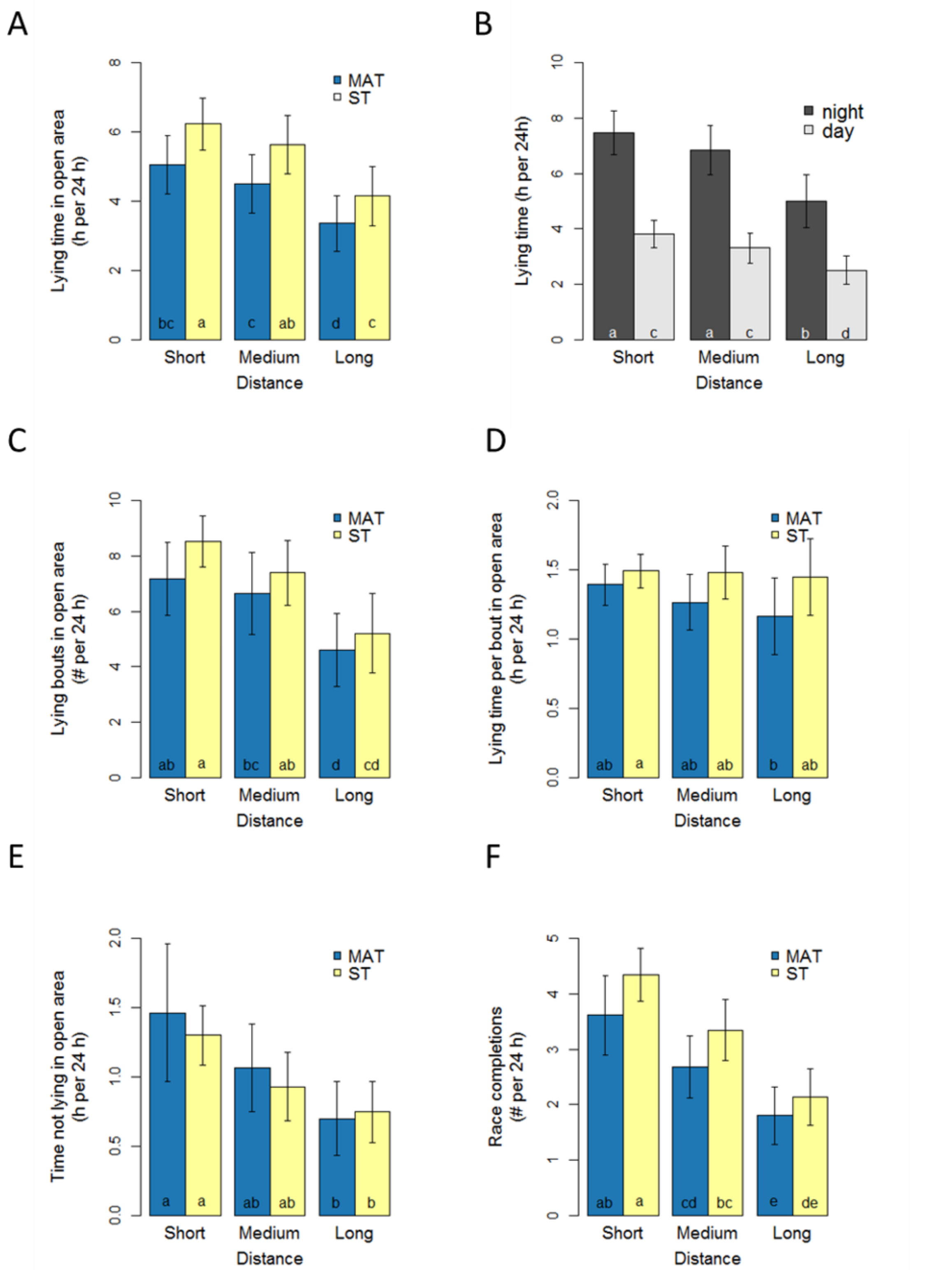

Figure 11. Lying, not lying and race completions as a function of distance choice treatment in open lying areas. Bar height represents the category mean and error bars represent the category 95% confidence interval. Letters represent mean differences based on post hoc pairwise tests (Sidak corrected alpha = 0.05). Lying time is shown separately for when MAT or ST was available. A. Lying time per 24 h in open lying areas with MAT versus ST. B. Lying time per 24 h in open lying areas as night versus daytime. C. Frequency of lying bouts per 24 h with MAT versus ST. D. Average duration of lying bouts per 24 h in open lying areas with MAT versus ST. E. Time not lying per 24 h in open lying areas with MAT versus ST. F. Raceway completions per 24 h with MAT versus ST.

3.4 DISCUSSION

The main objective of the study was to establish the extent of dairy cow motivation for lying on an open lying area when the cows had free access to cubicles, both of which were indoors and of the same surface type, (MAT; mattress bedded with sawdust), so as to remove these as confounding factors. This was repeated for a different surface type (ST; deep-bedded straw) to investigate the influence surface type has on motivation for an open lying area. Overall, time spent lying on the open lying areas declined as distance to access the open lying areas increased to the Long distance. However, cows still chose to lie down for longer on the open lying areas at this distance (>60% of lying time), compared to the free access cubicles, showing they were motivated to access the open lying areas rather than lying in cubicles. With a longer raceway we have observed shorter lying times for the open lying surfaces, but a ceiling effect (when the cost is too much and never paid) was not reached in the current study. Surface type did influence motivation, with cows expressing a higher motivation to lie down on the open lying area when it was a deep-bedded straw yard (ST) compared to MAT. On average, cows had more frequent and longer lying bouts on ST compared to MAT and completed the raceway more often to access the ST than MAT, which would explain the increase in lying time for ST over MAT. However, there was no interaction between surface type and distance to access the open lying areas, suggesting that surface type has a limited effect on motivation and that access to an open lying area was the main driving factor for motivation in the current study. This is in agreement with a previous study whereby cows were found to trade lying down on their preferred lying surface with a cubicle for lying on an open lying space with a less preferred lying surface, demonstrating the increased value cows place on an open lying area than the lying surface (Shewbridge Carter et al., 2021).

Unlike the relatively high motivation for an open lying area found in the current study, Fregonesi et al. (2009) found that cows showed a relatively small and varied preference to lie down on a mattress open lying area (created by removing all cubicle hardware except for the brisket board) compared to cubicles of the same surface. The difference in results between that study and the relatively high motivation found in the current study may be due to the physical differences between the open lying areas. The current study offered cows a flat open lying area without obstruction, whereas Fregonesi et al. (2009) offered cows a sloped lying area, due to the area’s previous function as cubicles, and the lying area was obstructed with the brisket board, limiting where the cows could lie down. Additionally, there was a disparity in stocking densities between the two studies, on both the open lying areas and the cubicles. The current study allocated 9 m^2 per cow on the open lying surfaces and had an 83% cubicle stocking density, compared to 3.1 m^2 per cow on the open mattress area and 67% cubicle stocking density in the other study

(Fregonesi et al. (2009). Cows have been shown to lie down for longer at lower cubicle stocking densities (Telezhenko et al., 2012; Krawczel and Lee, 2019) and value space for lying down (Shewbridge Carter et al., 2021). The lower stocking density of the open lying area in the current study would make the open lying area more attractive for lying down, and subsequently the cubicles less attractive, compared to Fregonesi et al. (2009). This demonstrates the importance of the stocking density of an open lying area when promoting the use of an open lying area by cows and must be considered when practiced on farm.

The total time cows spent in the open lying areas and lying on the open lying surfaces in the current study are similar to motivational studies for pasture access that also use walking distance as an indicator for motivation (Charlton et al., 2013; Motupalli et al., 2014). Motupalli et al. (2014) reported that, at a distance similar to the Short distance in the current study, cows spent longer at pasture compared to the open lying areas in the current study. This difference may be due to the dual function of pasture as a lying and grazing area, resulting with cows spending more time at pasture. Charlton et al. (2013) measured cow motivation for pasture access and used three different distances, with the two shorter distances being similar to the Medium and Long distance in the current study. That study found that cows spent less time lying on pasture compared to the open lying areas in the current study. As that study only recorded lying times during the day, it is not surprising that, at similar walking distances, the lying times were lower than those recorded in the current study for the open lying areas. Additionally, Charlton et al. (2013) found an effect of rain on cow motivation to go out to pasture, which may explain why cows spent less time at pasture at a similar distance compared to the current study, which took place indoors. It is possible that cow motivation to lie down at pasture or on an open lying area indoors is similar, however without knowing whether a ceiling effect exists for either lying option, we cannot be certain which cows find the most attractive.

Cows in the current study lay down more often and had longer lying bouts on the open lying areas compared to the cubicles, which can account for longer overall lying times on the open lying surfaces compared to the cubicles (Tucker et al., 2021). However, longer lying bouts are generally reported on harder lying surfaces (Haley et al., 2001), as cows experience discomfort in the process of lying down and getting up and therefore are reluctant to get up once lying down (Haley et al., 2000; Rushen et al., 2007). In the current study, the lying surfaces of the open lying areas offered were at least as soft as the cubicles. When straw is added to a rubber mattresses, lying bout durations are increased with each kg added and improves cow comfort as the surface is more compressible (Tucker et al., 2009). In a study investigating the lying behaviour of dairy cows in different cubicle sizes, cows had longer lying bouts in larger cubicles (Tucker et al., 2004).

Additionally, a study showed that cows have longer lying bouts when offered more space per cow on open rubber matting (Schütz et al., 2015). Therefore, the increase in lying bout duration in the current study on the open lying surfaces compared to the cubicles could be interpreted as a response to access to increased lying space, rather than in indicator of a lack of surface comfort on the open lying areas.

Cows spent longer lying down on the open lying surfaces at night compared to in the day-time in the current study. Cows exhibit a clear diurnal lying pattern, with cows lying down primarily at night (Tucker at al., 2017; Tucker at al., 2021), which has been observed at pasture (Legrand et al., 2009) and in cubicles (Winckler et al., 2015). Motivation for the open lying areas in the current study was higher at night compared to the day, which is similar to diurnal patterns shown in cow motivation for pasture access (Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al., 2017). However, time spent lying down at night on the open lying areas did decrease with increasing distance, which was not found to be the case in the previous pasture motivation studies mentioned, which found lying at pasture at night did not change with the increased cost of access. This might partly be accounted for by how day-time and night-time were calculated in the current study, using the time of sunrise and sunset, to adjust for the seasons. Previous pasture motivation studies have used a fixed time point throughout the study to differentiate between night-time and day-time (Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al 2017).

Activities such as eating, drinking and sleeping are said to be 'resilient' activities which animals tend to show inelastic demand to perform, meaning that an animal will continue to work to perform such activities despite an increasing cost (Dawkins, 1990; Fraser and Nicol, 2018). Lying behaviour in cows has been shown to have an inelastic demand, with heifers and cows working to lie down for between 12 - 13 h/d (Jensen et al., 2005; Tucker at al., 2018), although this can fluctuate within lactation (Maselyne et al., 2017). Studies measuring cow motivation for lying down found that as the workload to access an area to lie down increased, time spent on other behaviours decreased (Tucker at al., 2018; Norring and Valros, 2016). Although the current study is not an example of a true demand type study (cows had unlimited access to the open lying area after paying the price for access), a similar result was found. Cows in the current study spent a smaller proportion of their time in the open lying areas performing activities other than lying down at the Long distance, compared to the Short and Medium distances, where time spent on these activities was higher. This highlights that although lying time decreased on the open lying areas at the Long distance, lying down became a more important behaviour to perform after paying the higher price for access.

An additional indicator of motivation is the number of times the cows in the current study completed the raceway to gain access to the open lying areas. Tucker at al (2018) used a pneumatic push gate to measure cow motivation to lie down in a deep-bedded area when cows were deprived and not deprived of lying down. They found that as the force required by the cows to push the gate open increased, the cows deprived of lying used the gate more frequently compared to non-deprived cows, demonstrating their motivation for lying. Furthermore, successful passes through the pneumatic gate decreased as it became more difficult to open. Similarly, in the current study, cows made more raceway completions to access ST than MAT and as the distance to access the open lying areas increased, the number of successful raceway completions to access them decreased. This further supports the idea that the cows in the current study were more motivated to access and lie down on ST than MAT as they were not prepared to exert as much effort to access MAT as ST.

3.5 CONCLUSIONS

Cows in the current study were motivated to access and lie down in the open lying areas compared to the cubicles, with cows having a slightly higher motivation for an open deep-bedded straw area than an open cow mattress. Surface type had a limited effect on motivation, demonstrating the value cows place on an open lying area regardless of the surface type in the current study. Given cows value access to open lying areas, the provision of such areas has the potential to improve cow welfare in commercial housing systems in the future.

CHAPTER 4: THE MOTIVATION OF DAIRY COWS TO ACCESS AN OUTDOOR LYING AREA

4.1 INTRODUCTION

According to public opinion, the opportunity for dairy cows to live a natural life is an important contributor to their welfare (Vanhonacker et al., 2008; Beaver et al., 2020). Access to pasture and open space is thought to be particularly important (Ventura et al., 2016; Ellis et al., 2009, Cardoso et al, 2016), with the public placing emphasis on a cow's ability to perform grazing behaviour (Boogaard et al., 2008; Jackson et al., 2020). However, the importance the public place on pasture access and naturalness often refers to more than just cows being able to graze, with perceived benefits to cows of fresh air, sunshine and the ability to roam, a sentiment which is not new ("they were individuals, allowed their birthright of green fields, sunlight and fresh air" – Harrison, 1964) and is recurring in recent research ("every being deserves to feel sunshine on her back, to feel earth beneath her feet, to breathe fresh air" – Schuppli et al., 2014; "being able to get outside and breathe fresh air and feel daylight" – Spooner et al., 2014; "to let the cows free range on pasture" – Cardoso et al., 2016). Despite this, the practice of continuous housing systems, whereby cows are housed indoors all-year-round without access to pasture, is a prominent practice in North America (>80% in the US (USDA, 2016); >75% in Canada (Denis-Robichaud et al., 2016)) and is becoming more popular in the UK (March et al., 2014) and Europe (van den Pol-van Dessellaar et al., 2008).

A number of research projects have investigated whether cows value access to pasture. When given the choice to access pasture outdoors or cubicle housing (known as free-stall housing in North America; Margerison, 2011), cows show a preference to go outside to pasture, particularly at night (Legrand et al., 2009; Charlton et al., 2011a). Other studies have quantified this preference, showing that cows are motivated to access pasture by walking long distances (Charlton et al., 2013) and pushing on a weighted gate for access (von Keyserlingk et al., 2017), and, again, showing a stronger motivation at night. It has been suggested from these studies that cows are driven to go out to pasture primarily to lie down, rather than primarily to graze, as cows primarily graze during the day and lie down at night (Tucker, 2017). This is supported by a study which tested cow motivation for pasture at a high and low herbage mass, finding that herbage mass did not affect pasture use and motivation for pasture access was higher at night (Motupalli et al., 2014).

To better understand why cows value pasture access, further studies have been carried out to explore the relative importance of different features of pasture to cows. Shewbridge Carter et al., (2021) found that cows valued an open lying space more than lying on a preferred surface type. When given the opportunity to go outside to pasture or outside to a sand pack, cows spent more time at pasture than on the sand pack and when they had a choice of both, they showed a preference for pasture (Smid et al., 2018). This would suggest that surface type or grazing behaviour might be driving pasture preference. However, there was a confounding factor of space, with the area of pasture being larger than the sand pack (Smid et al., 2018). There is a similar confounding factor in the previous pasture studies mentioned, as they compared a large area of pasture against a smaller area of indoor housing and cubicles (Legrand et al, 2009; Charlton et al, 2011a; Charlton et al, 2013; von Keyserlingk et al, 2017).

In the current study, the confounding factors of both space and surface type were removed, and cows were presented with an open mattress of the same type and size located indoors and outdoors. The aim was to measure cow preference and motivation for access to an open mattress outdoors [OUT] or indoors [IN]. To measure preference, cows were presented with both the indoor and outdoor open mattresses from an intermediate choice point. Cow motivation for lying down on the outdoor open mattress was then measured, using walking distance as an indicator for motivation at a Short (74.5 m) followed by a Long (120.5 m) distance, while cows had free access to the indoor open mattress. Based on previous pasture studies, we predicted that cows would have a preference for lying on the outdoor mattress and would be motivated to lie down on the outdoor mattress, particularly at night, when weather conditions were favourable.

4.2 MATERIALS AND METHODS

Ethical approval for this study was given by the Harper Adams University (HAU) Research Ethics Committee (0488-201905-PGMPHD-CO2).

4.2.1 Animals and Management

The study was carried out at the Agri-EPI Midlands Dairy Research Centre at Harper Adams University, Shropshire, United Kingdom, in an open-sided barn. Twenty Holstein-Friesian dairy cows (see Table 7 for cow details) were selected for the study. Cows were selected based on time in lactation (mid-late lactation), with a body condition score (BCS) between 2.75 and 3.5 (mean 3 ± 0.06), as described by the Penn State method (Ferguson et al., 1994), and a lameness score (LS) no greater than 2, (mean 1.68 ± 0.05; 2 = imperfect locomotion but ability to move freely not diminished; Flower and Weary, 2006). BCS and LS were assessed by the same person (LSC) while cows walked across a concrete floor before they came on trial. The cows were allocated to one of four experimental periods according to their stage of lactation (n = 5 x 4), which were carried out from September 21st 2020 to November 15th 2020 (study period 1: September 21st – October 4th; study period 2: October 5th – October 18th; study period 3: October 19th – November 1st; study period 4: November 2nd – November 15th). Before the study, the cows in this herd had been housed indoors in a cubicle barn on bedded rubber mats with plastic, flexible cubicle partitions, with experience of a straw yard during the pre-calving period and metal cubicles as heifers. Due to the relatively recent recruitment of cows to this herd, these cows had various levels of previous experience at pasture. However, all cows at least had experienced access to pasture as heifers. They had free access to one milking robot (VMS V200, De Laval).

Table 7. Animal Details. Mean (± SEM) and range of lactation number, days in milk, milk yield (kg/d), body condition score and lameness score for all cows in the study, which included 19 pregnant cows and 1 non-pregnant cow (hormone controlled to prevent the occurrence of oestrus). * This study included 9 primiparous and 11 multiparous cows.

	Mean (± SEM)	Min	Max
Lactation Number *	2.3 ± 0.3	1	5
Days in Milk	266 ± 29.6	128	732
Milk Yield (kg/d)	23.58 ± 2.35	6.80	37.63
Body Condition Score	3 ± 0.06	2.75	3.5
Lameness Score	1.68 ± 0.05	1.5	2

4.2.2 Trial Area Housing

The trial area was located in an area of 503.5 m^2 at the south-east end of an open sided barn. Trial cows were separated from the main herd, of approximately 50 cows, into the trial area using cow hurdles. The trial area included access to two open mattresses (9.0 x 5.0m; Pasture Mat; Wilson Agri, Coleraine, Northern Ireland, UK), one located indoors [IN] and one located outdoors [OUT] on the south west side of the barn, the milking robot, a feed face and water trough providing ad libitum Partial Mixed Ration (PMR) and clean water, respectively (Figure 15). PMR was provided daily at approximately 0730 h, with feed refusals being removed every day before fresh feed was provided. Cows were fed concentrates, based on milk yield, during milking in the milking robot.

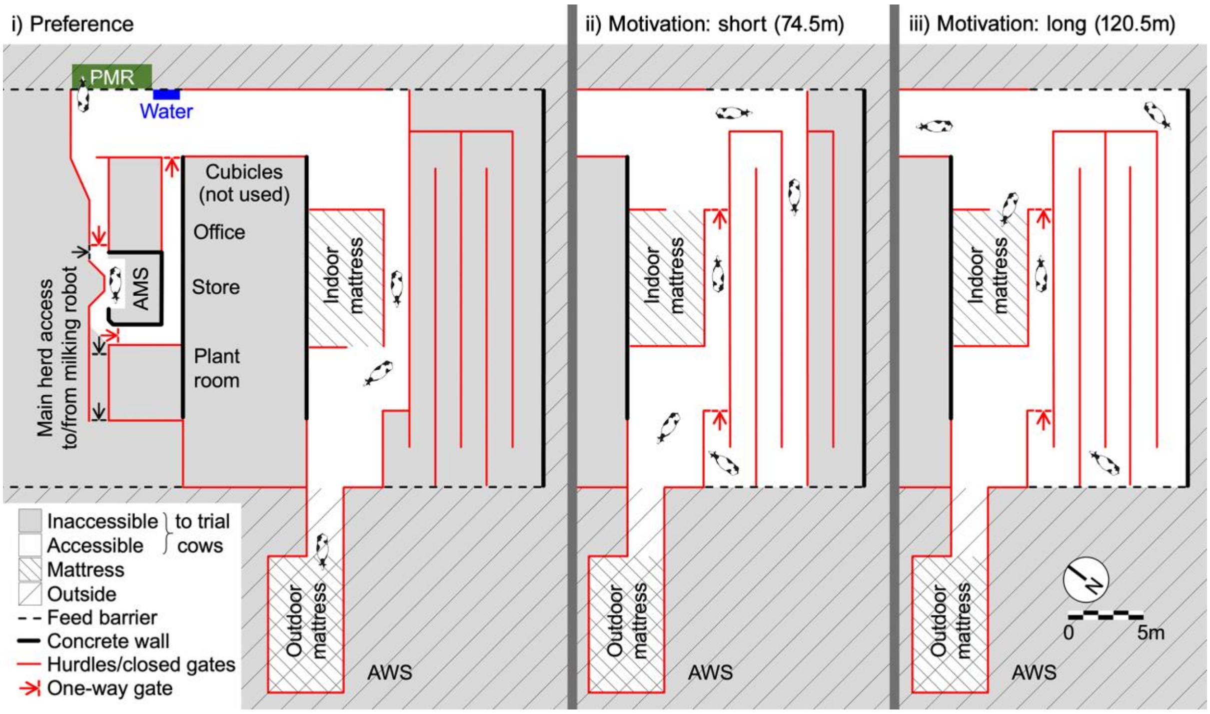

Figure 12. Plan of trial area set up for i) the preference stage, ii) the short and iii) the long motivation stages. During both the Preference (i) and Motivation stages (ii and iii), cows had free access to a partial mixed ration (PMR) at the feed face, water trough and the milking robot (AMS). Cows had free access to the indoor and outdoor mattresses from an intermediate choice point during the Preference Stage [i]. The trial area layout was changed during the Motivation Stages [ii and iii] so that cows had 'free' access to the indoor mattress and access to the outdoor mattress via an indoor raceway, the length of which could be adjusted to a Short (74.5 m) or a Long (120.5 m) distance. AWS = automatic weather station.

Bedding was not used on either open mattress throughout the study. Both mattresses had a 5% south east facing slope to encourage liquid to run off the mattress surface. The mattresses were surrounded by cow hurdles with a 2.5m wide access point. The bottom of the hurdles surrounding the highest half of the mattresses were covered in rubber sheeting reinforced with plywood, with these hurdles being fixed to the frame of the mattress to prevent injury which may have occurred if there had been a space between the edge of the mattress and the hurdles (see Figure 16).

A. Indoor Mattress

B. Outdoor Mattress

Figure 13. Photographs depicting the slope and hurdle arrangement to the A. Indoor mattress and B. Outdoor mattress.

During the Preference Stage, cows had free access to both the indoor and outdoor open mattresses from an intermediate choice point (Figure 15). During the Motivation Stage, access to the mattress changed, allowing the cows to have free access to the indoor mattress but access to the outdoor mattress was via an indoor raceway. The length of the raceway was adjustable to two different distances (Short: 74.5 m; Long: 120.5 m) and incorporated a number of 180 degree turns (Short: 3 turns; Long: 5 turns). Each turning area was 2.5 m x 3m and had a rubber mat floor (EASYFIX MG Max 4, Agri & Industrial Rubber Ltd, Galway, Ireland). The raceway had two one-way gates to ensure that cows needed to walk the full length of the raceway to access the outdoor mattress, but then only had a short (9m) walk to return to the feed, water and milking robot area.

4.2.3 Experimental Design

Each experimental period lasted for a total of 14-d (Figure 17), consisting of a 5-d Preference Stage and a 9-d Motivation Stage, within which were training periods and three 3-d choice periods when cow behaviour was recorded ("Preference" during the Preference Stage, "Short" and "Long" during the Motivation Stage, as per Figure 17).

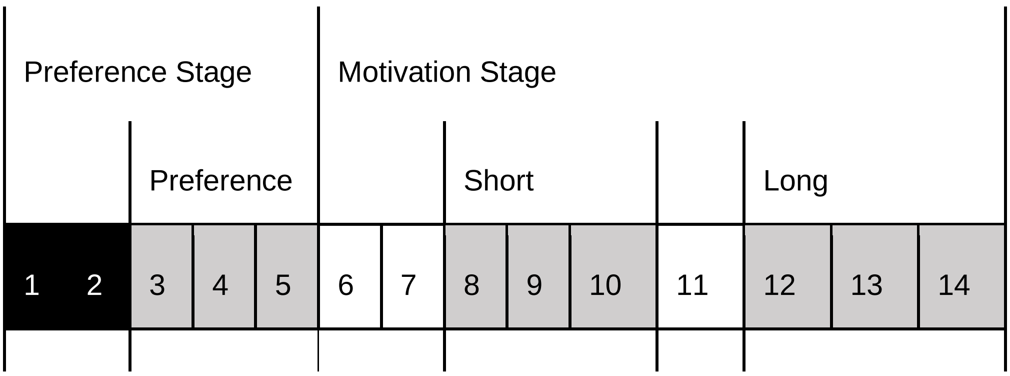

Figure 14. Representation for the 14-d study period, which was repeated 4 times using 5 cows. Unshaded days represent training days and days shaded in grey represent choice days. Days shaded in black represent an initial familiarization period to allow cows to become accustomed with their new surroundings and grouping. Throughout the 'Preference Stage' cows had free access to both the indoor and outdoor mattress via a neutral choice point. Throughout the 'Motivation Stage', cows had free access to the indoor mattress and access to the outdoor mattress via a raceway at a Short distance (74.5 m) followed by a Long distance (120.5). 'Preference', 'Short', and 'Long' characterize the choice periods as: outdoor access from a neutral choice point, via the raceway at a relatively short distance, and via the raceway at a relatively long distance, respectively.

While on trial, cows continued to have free access to the milking robot, were fed concentrates during milking, and were segregated into the trial area after milking via an automatic segregation gate (Figure 15). At approximately 0650 h each day, any cows on the outside mattress were brought inside and the outdoor mattress closed off from the cows to allow a feed wagon around the shed. The whole trial area was cleaned out from approximately 0700 – 0830 h each day, after which, access to the outdoor mattress was reopened. At approximately 1700 h each day, any cows on the mattresses were encouraged off and the indoor and outdoor mattresses were cleaned again.

Three video cameras (Swann, Milton Keynes, UK) were set up to continuously record cow behaviour within the trial area. Each experimental "day" began at the time when the cows first had access to the outdoor mattress, after the trial area was cleaned in the morning, and ended when access to the outdoor area was closed off for cleaning the following morning (approximately 0830 – 0700 h). Behaviour was only recorded (details below) during choice periods, as per Figure 17.

4.2.3.1 Preference Stage

During the Preference Stage, cows had free access to both the indoor and outdoor open mattress from an intermediate choice point for a total of 5-d. For the first 2-d, cow behaviour was not recorded, whilst the cows became familiar with their new surroundings and cow grouping. Cow behaviour was recorded for the final 3-d of the Preference Stage, the 'Preference' choice period (see Figure 17).

4.2.3.2 Motivation Stage

During the Motivation Stage, cows had free access to the indoor mattress and access to the outdoor mattress via the indoor raceway for a total of 9-d, divided into 5-d at the Short distance and 4-d at the Long distance (see Figure 17). The first two days at the Short distance consisted of training days, whereby each cow was encouraged to walk the raceway, with a researcher walking quietly behind them, on a number of occasions throughout the days to gain access to the outdoor open mattress (see APPENDIX 2 for a detailed training protocol). This allowed the cows to become familiar with the one-way gate system and the raceway length. These training days were followed by a 3-d choice period at the Short Distance ('Short' as per Figure 15), whereby cows had the choice to use the raceway to gain access to the outdoor mattress, paying the price of walking the Short distance (74.5 m), or could access the indoor mattress for 'free'. The raceway length was then changed to the Long Distance (120.5 m), and cows had 1-d training, to become familiar with the new raceway access length to the outdoor mattress, followed by a final 3-

d choice period (‘Long’ as per Figure 15).

4.2.4 Performance and Lameness

Throughout the study, milk yield was recorded automatically at each milking for individual cows via the robotic milking system and was used to calculate average yield per cow for the duration of their time on the study. Milking permission, in terms of milking frequency and latency between milkings, was set by the herd manager depending on a cow’s stage of lactation. The milking status of trial cows was checked regularly by the herd manager and LSC, with any trial cows that had not successfully taken themselves to be milked for >12 h being encouraged to the robot to get milked.

LS was recorded after the trial area was cleaned each morning, at approximately 0830 h, on the first and last day of each of the choice periods (the first and last day in the grey shaded blocks as per Figure 15).

4.2.5 Measurements

4.2.5.1 Behavioural Measures

Total experimental time varied slightly each day depending on the duration of cleaning. In order to measure different behaviours during the days of choice periods as a proportion of total time, the beginning and ending of each experimental day (i.e. when cleaning finished and then started the following day) was determined using the video footage to obtain the total time during which cows had access to the mattresses for each experimental day.

Video footage was analysed during the choice periods to obtain the time that each cow got on and off each mattress and the start and end times of all lying bouts on both the indoor and outdoor mattresses. From this, total time spent on, and time spent lying on the indoor and outdoor mattresses was calculated for each cow for each day of the choice periods.

For each day within each choice period, the time of sunrise and sunset was obtained online from Time and Date (Thorsen, 2021), using the coordinates for the location of the trial (52°46’52.8” N, 2°25’52.3” W). Time spent lying down during the day was defined as being between sunrise and sunset and time spent lying down during the night was defined as being between sunset and sunrise. Time spent lying down during the day and night was analysed as a percentage of total time during the choice periods for the open lying areas and the cubicles.

4.2.5.2 Weather Conditions

A weather station (Davis Vantage Pro2, Davis Instruments, CA, USA) was set up beside the outdoor mattress (see Figure 15) to record wind speed, wind direction, outdoor temperature and solar radiation every 15 minutes. This weather station was linked to an indoor recorder (Davis Vantage Vue Console, Davis Instruments, CA, USA), which recorded the temperature within the barn. Rainfall was recorded every hour throughout the study at a Met Office weather station on the campus of Harper Adams University, ~460 m from the barn. From these devices, indoor temperature, outdoor temperature, solar radiation, wind speed, wind direction, rainfall could be calculated for each day of the choice periods.

For each day during the choice periods, the surface temperature of the indoor and outdoor mattress was recorded using a handheld laser thermometer after morning cleaning (approximately 0830 h), at approximately solar noon and after evening cleaning (approximately 1730 h). The temperature was taken roughly from 5 similar points on each mattress; in ~1 m from each corner of the mattresses and the mattress centre. Surface temperature was then averaged for each day of each choice period.

4.2.6 Statistical Analysis

All data were analysed using RStudio (RStudio Inc., Boston, MA, USA) using the “nlme” package (Pinheiro et al., 2019).

4.2.6.1 Behaviour

Total time spent on the indoor and outdoor mattress each day of the choice periods was analysed as a percentage of total time per experimental day. A linear mixed effects model was created to test the effect of choice period (Preference, Short and Long), mattress location (IN and OUT), and a distance x choice period interaction on percentage of total time per day spent on the mattresses, with cow ID nested in experimental period as the random effect. Three Mann-Whitney U-tests, with Bonferroni post-hoc corrections, were performed to further investigate whether there was a difference in total time spent indoors and outdoors at the Preference, Short and Long choice periods, respectively.

Time spent lying down on the indoor and outdoor mattresses were analysed as a percentage of total time for each choice period. A linear mixed effects model was created to test the effect of choice period (Preference, Short and Long), mattress location (IN and OUT), time of day (Day and Night), and a choice period x location x time of day interaction on percentage of total time spent lying, with cow ID nested in experimental period as a random effect. Three Mann-Whitney U-tests, with Bonferroni post-hoc corrections, were performed to further investigate whether there was a difference in total time spent lying down indoors and outdoors at the Preference, Short and Long choice periods, respectively.

Lying bout frequency was analysed as total lying bout frequency for each choice period and lying bout duration was analysed as average lying bout duration for each choice period for the indoor and outdoor mattresses. Two linear mixed effects models were created to test lying about frequency and duration, respectively, for an effect of choice period (Preference, Short and Long), mattress location (IN and OUT), and a choice period x location interaction, with cow ID nested in experimental period as the random effect.

4.2.6.2 Weather Conditions

A linear regression model was created to test the relationship between the weather variables (indoor temperature, outdoor temperature, solar radiation, wind speed, wind direction, rainfall, and surface temperature of outdoor mattress) and the percentage of time spent lying on the outdoor mattress each day. A Spearman correlation was used to investigate the correlation between the weather variables, and those that were found to be highly correlated ($r > 0.7$; e.g. indoor and outdoor temperature), and did not significantly change the model when dropped, were removed from the model. This resulted in dropping indoor and outdoor temperature from the final model.

4.3 RESULTS

The main descriptive results are summarised in Table 8, averaged for a day across all cows.

Table 8. Results Summary. Summary, averaged for all cows, of total time spent (h/d), time spent lying (h/d), time spent lying during the day (h/d), time spent lying during the night (h/d), lying bout frequency per day, and lying bout duration (hr), on the Indoor and Outdoor mattresses at the Preference, Short and Long choice period (± SEM).

	Preference	Short	Long
INDOORS			
Total Time (h/d)	7.1 ± 1.1	11.6 ± 0.7	12.6 ± 0.7
Lying (h/d)	6.0 ± 1.0	9.1 ± 0.6	10.3 ± 0.6
Lying Day (h/d)	1.6 ± 0.3	3.0 ± 0.2	4.1 ± 0.4
Lying Night (h/d)	4.4 ± 0.7	6.1 ± 0.5	6.2 ± 0.5
Lying Bout Frequency (per day)	12.1 ± 1.9	19.8 ± 1.7	19.7 ± 1.7
Lying Bout Duration (h)	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
OUTDOORS			
Total Time (h/d)	9.3 ± 1.1	2.6 ± 0.6	2.6 ± 0.6
Lying (h/d)	6.7 ± 0.9	2.1 ± 0.5	2.1 ± 0.5
Lying Day (h/d)	2.8 ± 0.4	0.8 ± 0.1	1.0 ± 0.3
Lying Night (h/d)	3.9 ± 0.9	1.3 ± 0.5	1.2 ± 0.5
Lying Bout Frequency (per day)	13.4 ± 1.6	4.1 ± 0.8	4.3 ± 1.2
Lying Bout Duration (h)	1.4 ± 0.1	1.2 ± 0.2	1.4 ± 0.2

4.3.1 Behaviour

An interaction was found between the choice periods and mattress location for percentage of time per day cows spent on the mattresses, showing that as distance to access the outdoor mattress increased, time spent on it by the cows decreased ($F_{2,335}$ = 66.1; P <0.001). Cows spent on average 2.2 h/d longer on the outdoor mattress compared to the indoor mattress during the Preference choice period (W = 1395; P = 0.03; IN: 31.2 ± 4.6%; OUT: 40.7 ± 4.9%), but time spent on the indoor mattress increased and decreased on the outdoor mattress during the Short (W = 3350; P < 0.001; IN: 50.8 ± 3.0%; OUT: 11.5 ± 2.7%) and Long (W =3375; P < 0.001; IN: 54.4 ± 3.0%; OUT: 11.2 ± 2.7%) choice periods (Figure 18).

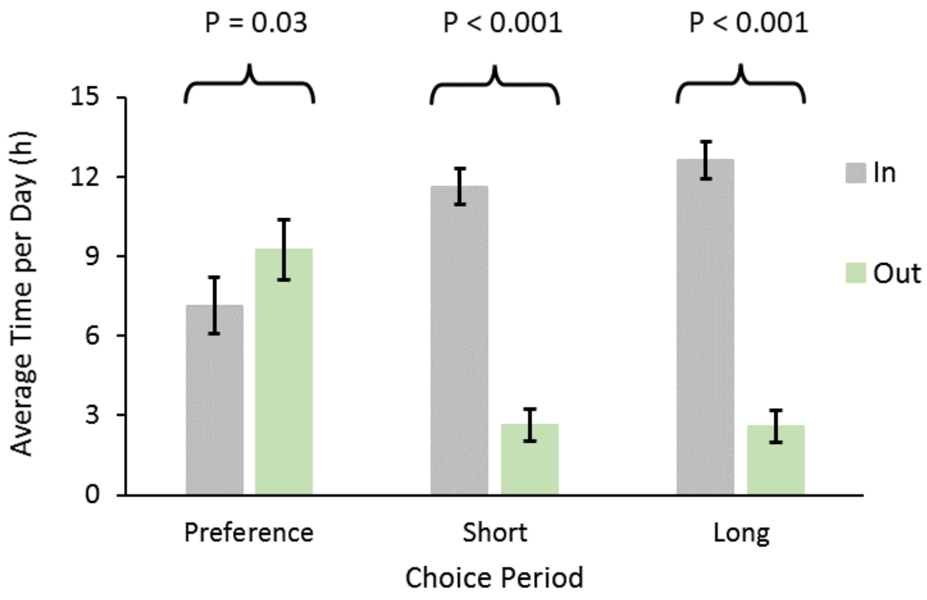

Figure 1512. Average percentage of time (h/d; mean ± SEM) spent on the indoor and outdoor mattresses during the choice periods (Preference, Short and Long).

When the total percentage of time spent lying on the indoor and outdoor mattresses was compared for each choice period, an interaction was found between the choice periods and mattress location ($F_{2,689}$ = 56.9; P < 0.001; Figure 19). Lying time indoors and outdoors was similar during the Preference choice period (W = 6448; P = 0.16; IN: 13.1 ± 1.5%; OUT: 14.7 ± 1.5%), but time spent on the indoor mattress increased and decreased on the outdoor mattress during the Short (W = 3275; P < 0.001; IN: 19.8 ± 1.3%; OUT: 4.6 ± 1.1%) and Long choice periods (W = 3346; P < 0.001; IN: 22.1 ± 1.5%; OUT: 4.6 ± 1.1%).

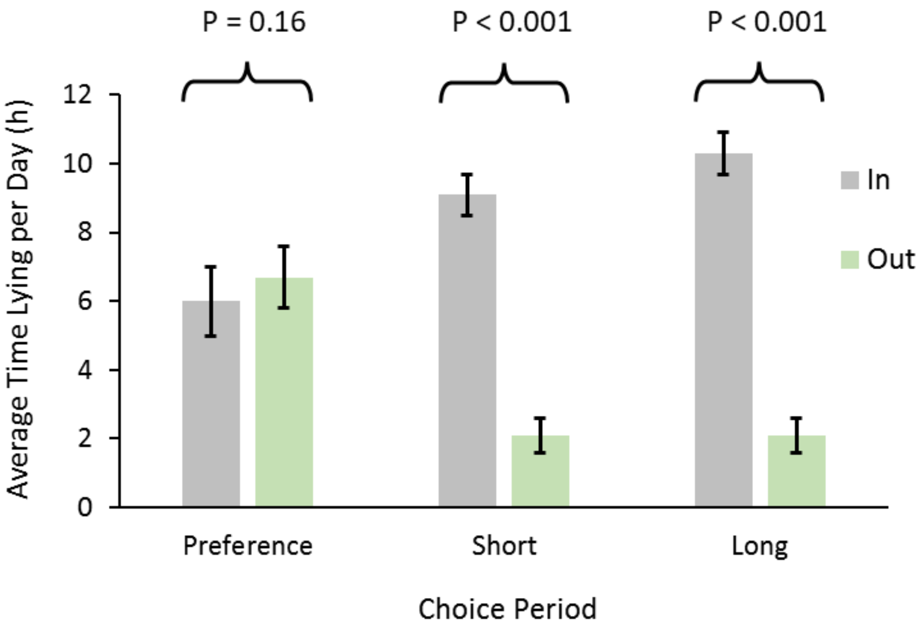

Figure 16. Difference in time spent lying (h/d; mean ± SEM) between the indoor and outdoor mattresses, averaged per cow, during the Preference, Short and Long choice periods (Padj = Bonferroni adjusted P value).

Although the percentage of time spent lying during the night (16.7 ± 1.6%) was higher than during the day (9.6 ± 1.0%; $F_{1,689} = 78.9$; $P < 0.001$), an interaction was found between location and time of day ($F_{1,689} = 31.2$; $P < 0.001$; Figure 20). The difference between the percentage of time lying during the day and during the night was greater for the indoor mattress (Day: 12.6 ± 1.1%; Night: 24.1 ± 1.8 %) than for the outdoor mattress (Day: 6.7 ± 0.9%; Night: 9.3 ± 1.5%).

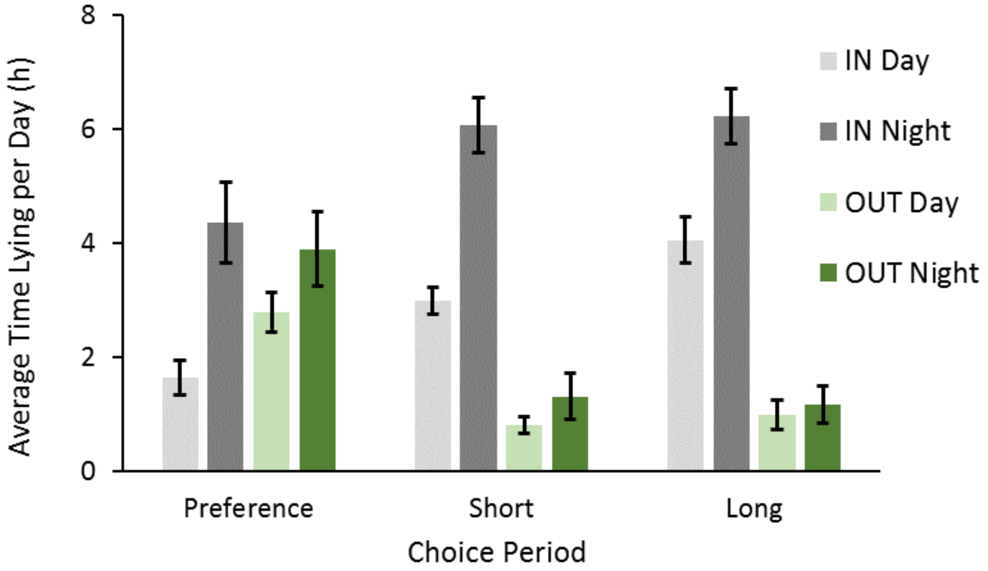

Figure 17. Difference in time spent lying (h/d; mean ± SEM) between the indoor and outdoor mattresses during the Day and Night, averaged per cow for the Preference, Short and Long choice periods.

No interaction was found between the choice periods and time of day for percentage of time spent lying on the mattresses ($F_{2,689}$ = 1.7 P = 0.18), with cows always lying down for a similar amount for time during the day and during the night throughout each choice period.

An interaction was found between mattress location and choice period for lying bout frequency ($F_{2,95}$ = 21.7; P < 0.001), with cows exhibiting a similar amount of lying bouts on the indoor and outdoor mattress during the Preference choice period, but with the number of lying bouts decreasing for the outdoor mattress and increasing for the indoor mattress during both the Short and Long choice periods (see Table 8). No interaction was found between mattress location and choice period for lying bout duration ($F_{2,95}$ = 0.4; P = 0.65), however, on average, cows had longer lying bouts indoors compared to outdoors (IN: 1.6 ± 0.1 h; OUT: 1.3 ± 0.2 h; P = 0.014).

4.3.2 Weather Conditions

Overall, weather conditions accounted for just 13% of the variation for percentage of time spent lying down on the outdoor mattress (R^2 = 0.13; $F_{5,174}$ = 6.28; P < 0.001). The average solar radiation was 51.6 W/m² (± 2.1 W/m²; range = 15.8 to 138.8) during choice periods, which influence where cows lay down. On days during the choice periods with above-average solar radiation, cows would spend 2.1 h longer lying outdoors ($t = 2.6$; $P = 0.009$). Wind speed (1.0 ± 0.0 km/h; range = 0.1 to 2.5; $t = -3.2$; $P = 0.001$) and wind direction ($t = 2.1$; $P = 0.03$) both had an effect on outdoor lying times. Cows would spend 1.2 h less lying down on the outdoor mattress when the wind speed was above average and coming from a south westerly direction, compared to a north easterly direction. The surface temperature of the outdoor mattress had the smallest effect on outdoor lying times (13.8 ± 0.3°c; range from 6.2 to 27.4°c; $t = -2.6$; $P = 0.01$), with cows spending just 0.1 h longer lying outdoors when the surface temperature was above average. Rainfall (2.6 ± 0.4mm; range = 0 to 29 mm) had no effect on the percentage of time cows spent lying on the outdoor mattress ($t = -1.0$; $P = 0.33$).

4.4 DISCUSSION

In this study, we investigated cow preference for lying down on an open mattress located indoors [IN] or outdoors [OUT]. This was followed by measuring cow motivation to access the outdoor mattress, using walking distance as an indicator for motivation at a Short (74.5 m) and Long (120.5 m) distance, while cows had free access to the indoor open mattress. We predicted that cows would have a preference for and be motivated to lie down outside. Although cows spent >2h longer outdoors than indoors during the Preference stage, on average, cows in the current study did not have a clear preference for lying down inside or outside and there was a big reduction in both the time spent outside and the time lying outside when they had to work to gain access to it. This large reduction in lying time outside was seen at both the Short and Long distance, with no 'dose' response experienced. This result was consistent, with 95% of the cows lying down for longer indoors than outdoors during this motivation stage; one cow at the Short distance (cow number '13' in Figure 21. B.) and one cow at the Long distance (cow number '7' in Figure 21. C) lay down longer outdoors. In the previous motivational study (Chapter 3) investigating cow motivation for an indoor open lying area when given free access to cubicles, the same raceway and walking distances were used. A 'dose' response was found, with cows reducing their lying time on the open lying areas at the Long distance. However, the reduction in lying time was relatively small compared to the current study, with cows lying down for >60% of their total lying time on the open lying areas, compared to <20% outdoors in the current study. This comparison further highlights the low level of motivation cows in the current study had to lie down outdoors.

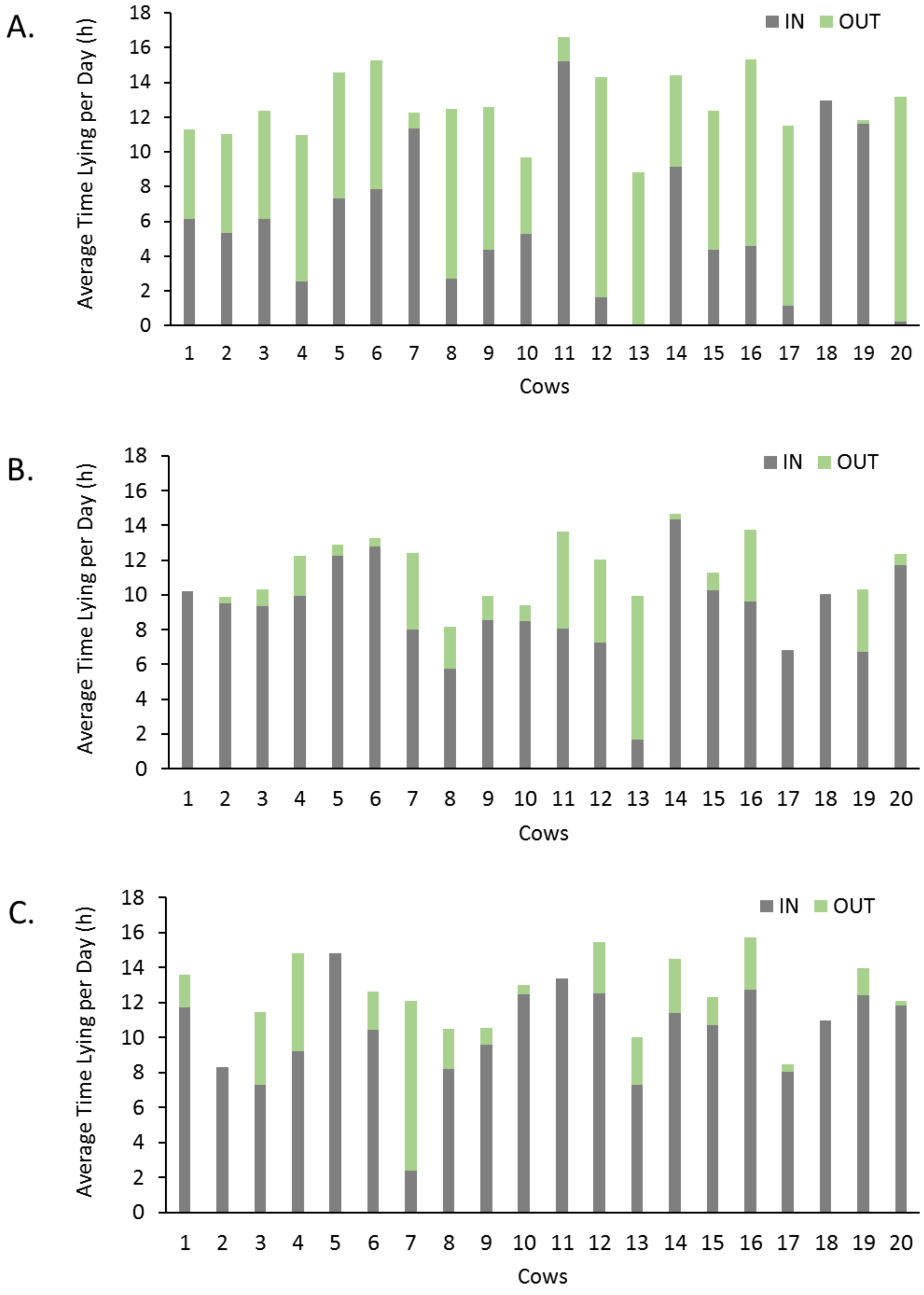

Figure 18. Average time (h per day) each cow spent lying down on the indoor (IN) and outdoor (OUT) open mattress during the Preference Stage (A.) and the Motivation Stage, at the Short (B.) and Long (C.) distances.

Overall, cows spent a similar amount of time lying down on the indoor and outdoor mattresses during the Preference choice period, not displaying a clear preference for lying in one location over the other. Similarly, Charlton et al. (2011a) found no difference in cow lying times when cows were given the choice between indoor cubicle housing and outdoor pasture access. However, lying times were only recorded by Charlton et al. (2011a) during daylight and the cows were found to spend most of their time during the night at pasture. As cows lie down longer at night than during the day (Tucker at al., 2021), total lying time may have been higher on pasture than indoors. Cows in the current study chose to lie down outdoors less during the Motivation Stage compared to the Preference Stage, indicating a low motivation to lie down on the outdoor mattress. Using similar walking distances to access pasture as the Motivation Stage during current study, Charlton et al. (2013) found that percentage of total lying time spent lying at pasture was higher than in the current study. However, the lying times reported by Charlton et al. (2013) were also only recorded during the day and the inclusion of night lying times may have increased overall percentage of time spent lying at pasture. This is supported in a study by Legrand et al. (2009), which recorded cow lying times during both the day and night time for indoor cubicle housing and outdoor pasture access, finding that cows chose to lie down more than twice as long at pasture.

In previous pasture preference and motivation studies reporting cows having a preference for pasture access and a relatively high motivation for pasture, the size of the outdoor pasture access provided was much greater than the area of the outdoor lying area provided in the current study, having a space allowance of 9 m^2 per cow (Legrand et al., 2009; Charlton et al., 2011a; Charlton et al., 2013; Motupalli et al., 2014). This discrepancy in size may explain the lack of preference and lower motivation for accessing the outdoor lying area in the current study. This is partly supported by a study showing that cows lie down for longer on an outdoor pack area when the space allowance per cow is increased from 4 to 16 m^2 per cow (Smid et al., 2020). However, a study providing cows a choice between a large area of pasture outdoors (1750 m^2/cow) and a smaller outdoor pack area (12 m^2/cow) found no difference in lying times, suggesting the difference in area per cow was not a factor when choosing where to lie down (Smid et al, 2018).

The current study provided cows with the same lying option indoors and outdoors. This differs from previous pasture preference studies, in which pasture access has been compared to indoor cubicles, reporting that cows have a preference for pasture (Legrand et al, 2009; Charlton et al, 2011a; Smid et al, 2018). In addition to pasture studies, cows have shown to prefer an open lying area rather than cubicles for lying down (Fregonesi et al, 2009; Smid et al., 2019; Shewbridge Carter et al, 2021). Furthermore, pasture motivation studies comparing pasture against cubicles have shown that cows are highly motivated to access pasture, in comparison to the current study (Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al., 2017). Cubicles are designed to control cow lying postures in such a way as to promote cow cleanliness (Margerison, 2011), restricting the lying freedom of a cow more so than an open lying area (van Erp-van der Kooil et al., 2019). Unlike the results of these previous pasture motivation studies, cows exhibited low motivation to access the outdoor mattress during the Motivation Stage of the current study. Cows went outdoors less and spent less time lying down outdoors compared to the Preference Stage, indicating a low motivation to access the outdoor mattress when a cost was imposed. The difference in cow motivation between the current study and previous pasture motivation studies may be attributed to the indoor lying option in the current study being more attractive to cows i.e. an open lying area rather than cubicles. A future study investigating cow preference between and cow motivation for an indoor open mattress and outdoor pasture access of the same size is needed to better understand this relationship, investigating whether the surface type of pasture is a driving factor for its preference.

In the current study, cows lay down more often and had longer lying bouts on the indoor mattress compared to the outdoor mattress. This can account for cows overall lying down longer indoors than outdoors. Although longer lying bouts can be attributed to harder lying surfaces (Herlin, 1997; Haley et al, 2001; Norring et al., 2010), both the indoor and outdoor lying surface were the same. It is more likely that lying bouts on the outdoor mattress were disrupted due to unfavourable weather conditions, leading to shorter lying bouts overall outdoors. Of the weather conditions that had an effect on outdoor lying time, solar radiation was the most variable and also had the greatest effect on outdoor lying time. In general, cows spent more time lying outdoors as solar radiation increased. This finding is inconsistent with studies that have reported that cows increase their use of a shaded area as solar radiation increases (Schütz et al., 2010) and prefer a shaded area that provides more protection from solar radiation (Tucker at al., 2008; Schütz et al., 2009). However, those studies were conducted during the summer months with an average solar radiation higher than the maximum solar radiation recorded in the current study, which was conducted in the autumn. Cows have been found to spend more time lying down in cubicles with dry bedding compared to wet bedding (Fregonesi et al., 2007; Reich et al., 2010), therefore, the drying effects of an increased solar radiation on the outdoor mattress in the current study might have made the outdoor mattress more favourable for lying down. Additionally, cows spent more time lying down outdoors when the surface temperature of the mattress was higher than average, suggesting that a dry and warm area to lie down was attractive to cows in this study. Increased wind speed and a south westerly wind direction decreased the time cows spent lying outdoor in the current study. A study investigating the behaviour of cows kept outdoors in unfavourable weather conditions (regular wind and rain) compared to those indoors, sheltered from such conditions, found that cows lay down less outdoors and changed their body posture in response (Tucker et al., 2007). The effect of wind direction in the current study may be the result of the positioning of the protective backboard surrounding half of the mattress. This backboard would have protected the cows on the outdoor mattress from northerly winds, but left them exposed when the wind came from a southerly direction. Rainfall has also been found to negatively affect dairy cow preference for outdoor access (Legrand et al., 2009; Charlton et al., 2011b; Charlton and Rutter, 2017; Smid et al., 2018; Smid et al., 2019), however, rainfall did not have an effect on outdoor lying times in the current study. The outdoor surface in the current study was not permeable to water, unlike previous studies, and was sloped to encourage water runoff, which may explain why rainfall did not affect outdoor lying times.

The public perceive pasture access as a positive, being beneficial to the welfare of cows (Schuppli et al., 2014; Ventura et al., 2015; Cardoso et al., 2016; Beaver et al., 2020). A UK survey found the 95% of participants felt it was unacceptable for cows to never access pasture (Ellis et al., 2009). The public's concern surrounding pasture access is focused around a cow's ability to perform 'natural behaviours', often centred on grazing behaviour (Boogaard et al., 2008; Jackson et al., 2020). Additionally, the current study did find that cows spent longer outside during the Preference Stage, which did not affect lying time, and with grazing behaviour not possible, this would suggest that maybe being outdoors provided them with some other form of enrichment. Although, if this was the case, this alternative enrichment from being outdoors was not valued enough by the cows to work to gain access during the Motivation Stages. Furthermore, research would suggest that cow pasture preference and motivation is linked to their lying behaviour, with grazing primarily observed during the day (Kilgour, 2012; Arnott et al., 2017; Charlton and Rutter, 2017) but their motivation to access pasture highest at night (Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al., 2017). With the provision of pasture access in commercial dairy herds declining (March *et al*., 2014) and all-year-round housing becoming more widespread (Haskell *et al*., 2006), for a variety of factors (Van den Pol-van Dasselaar et al., 2008), alternative forms of housing offering cows an open space for lying down is an important consideration in the future of cow housing (Bewley et al., 2017; Galama et al., 2020). This study has shown that outdoor access is not highly valued by cows when the lying area indoors and outdoors is the same, increasing the confidence that a more open housing system could better meet the behavioural needs and improve the welfare of housed cows. The open-sided barn which contained the indoor open lying area in this study was well ventilated and further research is needed to understand how the level of ventilation indoors affects cow preference and motivation to access an outdoor open lying area.

4.5 CONCLUSION

Cows in the current study did not show a preference, nor a strong motivation, for lying down outdoors, when the indoor and outdoor lying areas were of the same size and surface type. Considering that an outdoor mattress is not highly valued by cows for lying down on when the same is provided indoors, the provision of such indoor open mattresses has the potential to improve cow welfare in commercial housing systems in the future.

CHAPTER 5: GENERAL DISCUSSION

5.1 INTRODUCTION

According to public opinion, living a natural life is imperative for dairy cow welfare (Vanhonacker et al., 2008; Schuppli et al., 2014; Spooner et al., 2014; Beaver et al., 2020), with access to pasture particularly important (Ellis et al., 2009; Ventura et al., 2016; Cardoso et al, 2016; Jackson et al., 2020). Additionally, studies have shown that cows have a preference (Legrand *et al*., 2009; Charlton *et al*., 2011a; Falk et al., 2012; Smid et al., 2018) and a relatively strong motivation (Charlton *et al*., 2013; Motupalli *et al*., 2014; von Keyserlingk *et al*., 2017) to access pasture. Furthermore, pasture access has shown to have positive effects on cow health (Haskell et al., 2006; Hernandez-Mendo et al.; 2007; de Vries et al.; 2015) and behaviour (O'Connell et al., 1989; Olmos et al., 2009), indicating improved welfare (Arnott *et al*., 2017; Charlton and Rutter, 2017). Despite this knowledge, all-year-round housing is common practice in North America (USDA, 2016; Denis-Robichaud et al., 2016) and is increasing in use in the UK (March et al., 2014) and Europe (van den Pol-van Dessellaar et al., 2008). All-year-round housing can be beneficial over pasture access as it is difficult to meet the nutritional requirements of the modern high yielding dairy cow on pasture alone (Kolver and Muller, 1998; Bargo *et al*., 2002), potentially compromising cow health and welfare. When housed, cows are often fed a total mixed ration (TMR) to fulfil this high nutritional demand (Margerison, 2011). As there are many drivers to keeping cows indoors, it is more important than ever to ensure that commercial housing systems are meeting the behavioural and welfare needs of dairy cows.

Lying behaviour has been shown to be an important behaviour for cows to be able to perform (Metz, 1985; Jensen *et al*., 2005; Munksgaard *et al*., 2005). Therefore with pressure to improve indoor housing, a lot of research has been conducted on the design of cubicles, both in the context of cubicles and tie stall housing systems (size: Tucker et al., 2004; Tucker et al., 2005; surface type: Haley et al., 2001; Manninen et al., 2002; Rushen et al., 2007; Norring et al., 2010; cubicle hardware: Tucker et al., 2006; Ruud and Bøe, 2011; Abade et al., 2015). However, indoor open pack areas, providing cows with a large open lying area, are viewed as a less restrictive alternative to cubicles and are thought to be the future of cow housing (Bewley et al., 2017; Galama et al., 2020; Leso et al., 2020). These housing systems bring aspects of pasture access, such as space, indoors, and have shown to have behavioural and welfare benefits (Phillips and Schofield, 1994; Fregonesi and Leaver, 2001; Shepley et al., 2020). Additionally, cows have shown a preference for these open pack areas, both indoors (Fregonesi et al., 2009) and outdoors (Smid et al., 2020) compared to cubicles, although not when compared to pasture (Smid et al., 2018).

5.2 ORIGINAL CONTRIBUTION TO KNOWLEDGE

Previous studies have informed us as to what cows may or may not want in a cubicle, but also that cows have a preference for open lying areas, both pasture and open pack areas, over cubicles. However, due to confounding factors in these studies, it is unclear as to what exactly cows value in these open lying areas. The three studies presented in Chapters 2, 3, and 4 investigate cow preference and motivation for different qualities of a lying area, evaluating the value cows place on a lying area through a trade-off preference test design, comparative motivational studies and reducing confounding factors.

In Chapter 2, two main differences identified between pasture and cubicles for lying down were space and surface type, which are often confounding factors in pasture preference studies. For instance, some studies have given cows a choice between pasture with all its varying attributes, such as a soft lying surface, open lying area, that is outdoors, and cubicles, which have a different lying surface, relatively small lying area and are indoors (Legrand *et al*., 2009; Charlton *et al*., 2011a; Falk et al., 2012; Smid et al., 2018). A trade-off study was designed to better understand the value a cow places on these two lying area qualities when choosing where to lie down. Studies investigating cow preference and the spatial dimension of cubicles (Tucker et al., 2004; Tucker et al., 2005) yielded less definitive results compared to studies examining the surface type of cubicles (Tucker et al., 2003, Manninen et al., 2002, Norring et al., 2010), with the studies suggesting that cows are less focused on the spatial constraints of a lying area compared to the surface type. However, the results in Chapter 2 highlight that cows value an open lying area more so than their preferred lying surface. The clear results reported in Chapter 2 may be due to effectively separating space and surface type as lying qualities for cows to choose between. Studies investigating cow preference for different cubicle dimensions had relatively small changes in dimensions, which cows might not have been able to clearly perceive, whereas studies comparing different lying surfaces in cubicles use surface types that are dissimilar in a variety of ways making differences easier to perceive. The trade-off presented to cows in Chapter 2 was easily distinguishable, both in terms of surface type and lying space. The surface types, like previous studies, were dissimilar in different ways, and the amount of space was either denoted by the presence of a cubicle or by completely removing the cubicle hardware to have an open lying space. After establishing this preference, it was important to quantify it with a motivational test, as is best practice to identify whether the preferred quality provides an ethological need and therefore improved welfare (Fraser, 2008b; Jensen and Pedersen, 2008). This was carried out in Chapter 3, first by removing the confounding factor of surface type; motivation for an open cow mattress was compared against cubicles of the same surface type (i.e. mattress), to reveal a relatively strong motivation for access to the open lying area. However, to further investigate the effect of lying surface, motivation was tested for a different open lying surface (i.e. straw) against the cubicles. Although the surface type did affect cow motivation to access the open lying areas, this effect was limited, with access to an open lying area being the main driving factor for cow motivation in that study.

Finally, an important aspect of pasture access is that it is located outdoors, which the public perceive as benefitting the cow ("every being deserves to feel sunshine on her back, to feel earth beneath her feet, to breathe fresh air" – Schuppli et al., 2014; "being able to get outside and breathe fresh air and feel daylight" – Spooner et al., 2014; "to let the cows free range on pasture" – Cardoso et al., 2016). Although cows have a preference (Legrand *et al*., 2009; Charlton *et al*., 2011a) and a relatively strong motivation (Charlton *et al*., 2013; Motupalli *et al*., 2014; von Keyserlingk *et al*., 2017) to access pasture, it is unclear whether being outdoors is the main driving factor for this, with space and surface type of the lying area previously being confounded by indoors vs outdoors in these studies. Chapter 4 describes the first study of cow preference and motivation for two lying areas of the same size and surface type, with the only difference being whether or not it is located outdoors. By removing all other factors, this is the first study to show the value cows place on "the outdoors" as a factor when choosing where to lie down. With cow motivation found to be relatively low for an outdoor lying area, this provides evidence that housing cows indoors with the right design and management may cater well for the needs of the cow. However, whether or not this sufficiently satisfies the public's concern with a lack of outdoor access for cows remains to be seen.

5.3 VALUE OF LYING SURFACE AND SPACE

Previous preference work has been done with dairy cows in relation to cubicle sizes, and although during the no-choice periods in these previous studies, cows lay down for longer in cubicles with larger dimensions compared to those with smaller dimensions, cows showed no preference when given the choice (Tucker et al., 2004; Tucker et al., 2005). However, the adjustments made to cubicle sizes in these studies were relatively small, with cows being offered a choice between cubicles and an open lying space, such as pasture. The results showed that cows preferred to lie at pasture (Legrand et al., 2009). Fregonesi et al. (2009) tested cow preference for cubicles against an open lying area in which the cubicle hardware, except for the brisket board, was removed, creating an equal total lying area of the same surface type. They found that cows did have a preference for the open lying area, showing that cows value an open lying area. The results presented in Chapter 2 further support this, highlighting that cows are willing to trade their preferred lying surface in a cubicle for a less preferred lying surface which is presented as an open lying area.

Chapter 3 further expands upon a cow's preference for additional lying space reported in Chapter 2, showing that cows are motivated to gain access to an open lying area, with the surface type of this open area only having a small influence in the strength of this motivation. When comparing these results to those from pasture motivation studies which also use walking distance as an indicator of motivation, similar lying times are reported for similar distances (Charlton et al., 2013; Motupalli et al., 2014; Figure 22. i.). Motupalli et al. (2014) tested cow motivation for pasture at two distances, the shorter distance being similar to the Short distance reported in Chapter 3 (38 and 34.5 m, respectively). Similarly, Charlton et al. (2013) tested cow motivation for pasture at three distances, with the shortest and the middle distance (60 and 140 m, respectively) being similar to the Medium and Long distance reported in Chapter 3 (80.5 and 126.5 m, respectively). Although the observed percentage of time spent lying per day was higher in Chapter 3 than either of the previous pasture motivation studies, those studies only measured lying time during the day. Considering that cows display a diurnal lying pattern, lying longer at night than during the day (Tucker, 2017), had Charlton et al. (2013) and Motupalli et al. (2014) recorded lying behaviour both during the day and night, those studies may have reported higher lying times at pasture than the lying times reported for the indoor open lying areas in Chapter 3. This is supported in part when comparing these studies for the total time spent at pasture and the open lying areas over a 24 h period, whereby cows spent more time at pasture, in both the Charlton et al., (2013) and Motupalli et al (2014) studies, compared to the open lying areas at similar distances reported in Chapter 3 (Figure 22. ii). Additionally, none of these studies reached a 'giving up point', whereby the distance to access the resource was too great and no cow was willing to pay the price for access. To truly understand which resource cows value more, pasture or an indoor open lying area, a study is needed where the distance to access these resources could be increased until no cow was willing to walk for access. The resource with the average longest distance walked in order to access would indicate that cows had a higher motivation for. Ideally, this would be done using an indoor raceway, as in Chapter 3 and Chapter 4, to eliminate any possible effect of weather on motivation that isn't directly affecting pasture use. However, it likely would not be feasible to create an indoor raceway for an unknown, possibly infinite, distance. One alternative would be to use a remotely controlled one-way gate system which would allow the system to be programmed in such a way that once cows walked the maximum distance to access either resource, the raceway could be lapped. This would double the distance the cows would be required to walk before gaining access to either resource, with infinite additional laps able to be programmed until cows give up and a ceiling effect determined.

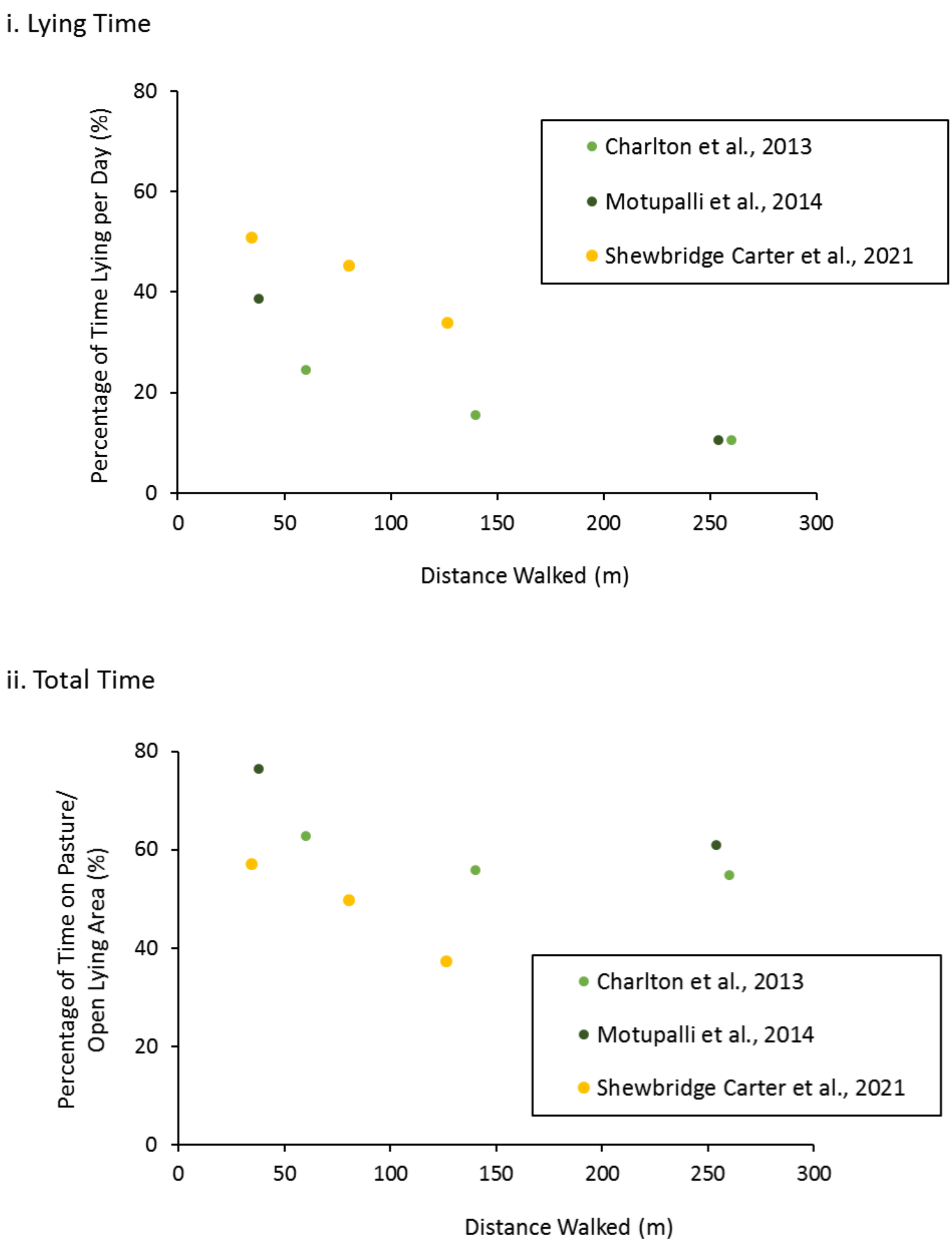

Figure 19. Graphs comparing i) the percentage of time spent lying at pasture during the day as reported by Charlton et al. (2013) and Motupalli et al. (2014) and lying on the open lying areas over a 24 h period as reported by Shewbridge Carter et al. (2021), which appears in Chapter 3, plotted against the distance walked in order to gain access to the pasture/open lying area, and ii) the percentage of time spent at pasture as reported by Charlton et al. (2013) and Motupalli et al. (2014) and on the open lying areas as reported by Shewbridge Carter et al. (2021), all for a 24 h period, plotted against the distance walked in order to gain access to the pasture/open lying area.

5.4 VALUE OF LYING OUTSIDE

The results of the study in Chapter 4 illustrate that, relative to the results discussed in Chapter 3, cows have a low motivation to lie down outside when the lying opportunities are the same indoors and outdoors. This is interesting considering that cows have a relatively strong motivation to access pasture (Charlton et al., 2013; Motupalli et al., 2014; von Keyserlingk et al., 2017) and would suggest that pasture access is either driven primarily by the additional space, unconfined lying areas (i.e. no cubicles) or the surface texture it offers cows for lying. However, in these previous studies, motivation for pasture access has been compared against free access to cubicles. Other studies have already shown that cows value cubicles less than pasture, having a partial preference for pasture in favourable conditions (Legrand et al., 2009; Charlton et al., 2011a; Falk et al., 2012). Therefore, measuring pasture motivation against cubicles probably tells us more about how much cows dislike cubicles, rather than how much cows value pasture. Offering cows something more attractive indoors to compare against would begin to reveal more about what cows' value in a lying area. Having found in Chapter 3 that cows have a high motivation to access an open mattress for lying when they had free access to cubicles, this resulted in a stronger study in Chapter 4, which could reveal more about what cows' value when lying down compared to previous pasture studies. The indoor option offered in Chapter 4, an open mattress, is more attractive to cows than the cubicles offered in the previous pasture motivation studies. The relatively low motivation to access the outdoor lying area in Chapter 4 may be due to this, with outdoor access not being an important driving factor for cows when choosing where to lie down when there is access to an 'attractive' indoor open lying area. It is possible then that the strong motivation cows have shown for pasture in previous studies is not primarily driven by a want to be outside per se, but rather due to other factors.

Interestingly, rainfall had no effect on cow preference or motivation to lie down outside during the study described in Chapter 4, which has shown to have a negative effect on lying times at pasture (Legrand et al., 2009; Charlton et al., 2011b; Charlton and Rutter, 2017) and on an outdoor open pack area (Smid et al., 2019). This was unexpected considering that the average daily rainfall recorded in Chapter 4 was more than that reported by Charlton et al. (2011b). Smid et al. (2019) reported a similar average daily rainfall during the summer as was reported in Chapter 4, but still found that this negatively affected the amount of time cows spent on an outdoor open pack area at night. However, unlike pasture or the outdoor open pack area, the outdoor mattress in this study was not permeable and was sloped to encourage runoff, resulting in a relatively drier lying area after rainfall than pasture, which retains rain water in the soil. This is partly supported by lying times being positively affected by higher solar radiation, which would have a drying effect on the mattress. As cows lie down for longer on dry bedding (Fregonesi et al., 2007b; Reich et al., 2010), the features of the mattress that lend itself to drying off quickly may have made the mattress more attractive than pasture in wet weather. Additionally, cows have been shown to dislike muddy ground conditions, even in the absence of rain (Chen et al., 2017), which was not an issue in the experiment presented in Chapter 4.

5.5 FURTHER RESEARCH

5.5.1 Pasture vs. Indoor Open Mattress

Research is needed to further understand cow preferences and motivations for pasture when given free access to an indoor open lying area, such as the open mattress used in the studies in Chapter 3 and 4. We now know that both this indoor open lying area and pasture are preferred over cubicles. An experiment could be carried out in which both an indoor open lying area and pasture access is provide and dairy cow preference and motivation to access the pasture investigated. Both the indoor open mattress and the area of pasture available should be the same size, to remove this as a factor. The indoor housing should be well ventilated, such as that used throughout this thesis, as poor ventilation may be a factor when cows are choosing where to lie down. Additionally, paddocks of pasture areas would need to be used so that a rotation system could be implemented in order to better control the ground conditions of the pasture being offered. This would tell us whether pasture access is still highly valued when the indoor lying option is an open lying area, or whether the strength of pasture motivation decreases. Furthermore, the effects of the size of the pasture area offered relative to the open indoor option should be investigated in order to increase our understanding of the role space plays in cow lying behaviour. Finally, a long term, parallel study to investigate the effects of an open indoor housing system compared to a cubicle housing system on overall farm productivity and cow health is needed to fully understand the effects of this relatively new housing system.

When considering potential farmer uptake of this new housing system, due to the costs involved, uptake initially may be slow and interim alternatives for improving housed cow welfare will need to be considered. As discussed in ***section 1.3.3***, agency, providing animals with choice over their environment, may in itself improve welfare. This could easily be done using current housing infrastructure in common farming systems by providing cows simultaneous access to pasture and indoor housing options, with the caveat that pasture access is available and easily accessed by cows autonomously. As before, a long term, parallel study to investigate the effects of offering cows agency over their environment against a system, or multiple systems, which restrict cow environmental choice would be proactive. Assessing cow behaviour, health, and productivity during such a study would help us to evaluate whether a housing system which incorporated choice and is relatively easy for farmer implementation to encourage uptake, is beneficial to overall cow welfare.

5.5.2 Barn Quality

The barn used to house cows in Chapter 2 was a converted straw barn, with completely open sides, and the barn used in Chapters 2 and 3 was a new, purpose built, open-sided barn. Both of these housing systems were very well ventilated, which may be unlike older barn designs or other converted buildings. Further research is needed to understand whether this was a factor for cows having a preference for an open lying space, and particularly in Chapter 4, if it was a factor that influenced the cows' lack of motivation for outdoor access. To do this, a study which offered cows the same lying area indoors and outdoors would be tested on a range of different barn types, with different qualities of ventilation. In less well ventilated barns, an outdoor open lying area may become more valuable to cows than was found in Chapter 4.

5.5.3 Stocking Density of Indoor Open Mattress

A relatively unknown factor when considering an open housing system is the space allowance per cow in such a system. Smid et al. (2020) investigated the effect of space allowance on an open pack area, although this was on an outdoor open pack area with a woodchip lying surface. Smid et al. (2020) found that at larger space allowances, cows spent more time on this outdoor open pack area, however time spent lying on the open pack area was not affected by space allowance. It would be useful to conduct a similar experiment with the indoor mattress surface which was used throughout this thesis for comparison.

5.6 CONCLUSIONS

Not only do cows place a higher value on open lying space than on the surface type, but they are willing to work to gain access to open lying areas. Although surface type has an effect on motivation, this effect is limited and secondary to motivation for an open lying space. Additionally, when given access to the same lying option indoors and outdoors, cows do not have a preference for one location over the other. Furthermore, they are generally not willing to work to lie down outside when given free access to the same lying option indoors.

5.6.1 Recommendations for Practical Application

Cows value open lying space when housed, and its provision better meets the behavioural needs of housed dairy cows. Although not investigated in the research reported here, previous studies have shown that lying is an important behaviour to cows (Metz, 1958; Jensen et al., 2005; Munksgaard *et al*., 2005), an increase of which has been associated with higher levels of milk production (Munksgaard and Løvendahl , 1993).Therefore, with cows showing increased lying times when given an open lying space, there are possible positive welfare and production implications of this type of housing system and is a factor to be considered in the design of future cow housing. From the findings in this body of work, it would be recommended that farmers try to house cows in open housing systems. However, further investment from industry is required to design a more viable systems for climates which do not support compost bedded pack areas, the most prevalent type of open housing system (Barberg et al., 2007). This research has shown that an open mattress is favourable to cows and would be a viable surface type in a variety of climates. However, further research is needed to develop an effective automated cleaning system for such a mattress, as this was done manually in the current research and would not be commercially viable (Newman et al., 2018; Blanco-Penedo et al., 2020; Galama et al., 2020).

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APPENDIX 1

Training Protocol.

- 'Day 1' of this training protocol occurred on day 4 as per Fig 2, 'Day 2' as per day 5, and 'Day 3' on every subsequent training day (day 6, 10, 14, 18, 19, 20, 24 and 28).
- At any point during training days, if all cows entered the raceway, lay down in the open space and exited of their own accord, that group of cows were considered trained for that training period and were given free access to the experimental area for the remainder of that training period.

Day 1

- 9am – 2pm: Cows had access to the 'Experimental Area' with the raceway at the Short distance and left to explore for themselves.
    - The first one-way gate to enter the open lying areas was tied open to encourage the cows through, as they had not encountered this type pf one-way gate before and needed to learn to push it.
    - The second one-way to exit to the 'Cubicle Area' was left closed i.e. cows needed to push it to get through it.
    - Cows that did not learn to push the one-way gate to exit to the 'Cubicle Area' during this exploration time were still able to return to the 'Cubicle Area' via the first, tied open one-way gate i.e. they were not confined to the 'Experimental Area'.
- 2pm – 3pm: Any cows in the 'Cubicle Area' were encouraged through the raceway and the first one-way gate (tied open) into the open lying area. The first one-way gate was then closed and the cows left to explore the open lying area for 5 minutes in case some cows had not explored the area themselves before this. The cows were then encouraged to push through the second one-way gate, back into the 'Cubicle Area'.
    - 3pm – 5pm: The first one-way gate was tied back open and cows had free access between the 'Cubicle Area' and the 'Experimental Area'.
- 5pm -6pm: Any cows left in the open lying area were encouraged to push through the second one-way gate, back into the 'Cubicle Area'. All cows were then encouraged through the raceway and the first one-way gate (tied open) into the open lying area.

- Cows had free access over night between the ‘Cubicle Area’ and the ‘Experimental Area’.

Day 2

- The first one-way gate to enter the open lying areas was loosened and tied to half way between closed and open. This was closed enough that it functioned as a one-way gate, but open enough to encourage cows to push through.
- Any cows in the ‘Experimental Area’ were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’ for the duration of cleaning.
- 9am – 10am: All cows were encouraged through the raceway and the first one-way gate (tied half way between closed and open) into the open lying area.
    - 10am – 12pm: Cows had free access between the ‘Cubicle Area’ and the ‘Experimental Area’.
- 12pm – 1pm: Any cows left in the open lying area were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’. All cows were then encouraged through the raceway and the first one-way gate (tied half way between closed and open) into the open lying area.
    - 1pm – 3pm: Cows had free access between the ‘Cubicle Area’ and the ‘Experimental Area’.
- The first one-way gate was untied.
- 3pm -4pm: Any cows left in the open lying area were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’. All cows were then encouraged through the raceway and the first one-way gate (untied) into the open lying area.
    - Cows had free access over night between the ‘Cubicle Area’ and the ‘Experimental Area’.

Day 3

- Any cows in the ‘Experimental Area’ were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’ for the duration of cleaning.
- 9am – 10am: All cows were encouraged through the raceway and the first one-way gate into the open lying area.
    - 10am – 12pm: Cows had free access between the ‘Cubicle Area’ and the ‘Experimental Area’.
- 12pm – 1pm: Any cows left in the open lying area were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’. All cows were then encouraged through the raceway and the first one-way gate into the open lying area.

    - 1pm – 3pm: Cows had free access between the ‘Cubicle Area’ and the ‘Experimental Area’.
- 3pm -4pm: Any cows left in the open lying area were encouraged to push through the second one-way gate, back into the ‘Cubicle Area’. All cows were then encouraged through the raceway and the first one-way gate into the open lying area.
    - Cows had free access over night between the ‘Cubicle Area’ and the ‘Experimental Area’.

APPENDIX 2

Training Protocol.

- The days numbered in this protocol correspond to those outlined in Fig 3.
- At any point during training days, if all cows entered the raceway, lay down in the open space and exited of their own accord, that group of cows were considered trained for that training period and were given free access to the experimental area for the remainder of that training period.

Day 6

- 9am – 2pm: Cows had access to the outdoor open mattress via the raceway at the Short distance and left to explore for themselves.
    - The first one-way gate to enter the open lying areas was tied open to encourage the cows through, as they had not encountered this type of one-way gate before and needed to learn to push through it.
    - The second one-way gate to exit to the indoor trial area was left closed i.e. cows needed to push it to get through it.
    - Cows that did not learn to push the one-way gate to exit to the indoor trial area during this exploration time were still able to return to the indoor trial area via the first, tied open one-way gate i.e. they were not confined to the outdoor open mattress.
- 2pm – 3pm: Any cows in the indoor trial area were encouraged through the raceway and the first one-way gate (tied open) into the open lying area. The first one-way gate was then closed and the cows left to explore the open lying area for 5 minutes in case some cows had not explored the area themselves before this. The cows were then encouraged to push through the second one-way gate, back into the indoor trial area.
    - 3pm – 5pm: The first one-way gate was tied back open and cows had free access between the indoor trial area and the outdoor open mattress.
- 5pm -6pm: Any cows left in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area. All cows were then encouraged through the raceway and the first one-way gate (tied open) into the open lying area.
    - Cows had free access over night between the indoor trial area and the outdoor open mattress.

Day 7

- The first one-way gate to enter the outdoor open mattress was loosened and tied to half way between closed and open. This was closed enough that it functioned as a one-way gate, but open enough to encourage cows to push through.
- Any cows in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area for the duration of cleaning.
- 9am – 10am: All cows were encouraged through the raceway and the first one-way gate (tied half way between closed and open) into the open lying area.
    - 10am – 12pm: Cows had free access between the indoor trial area and the outdoor open mattress.
- 12pm – 1pm: Any cows left in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area. All cows were then encouraged through the raceway and the first one-way gate (tied half way between closed and open) into the outdoor open mattress area.
    - 1pm – 3pm: Cows had free access between the indoor trial area and the outdoor open mattress.
- The first one-way gate was untied and remained untied for the duration of the experimental period.
- 3pm -4pm: Any cows left in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area. All cows were then encouraged through the raceway and the first one-way gate (untied) into the outdoor open mattress area.
    - Cows had free access over night between the indoor trial area and the outdoor open mattress.

Day 11

- 9am – 2pm: Cows had access to the outdoor open mattress via the raceway at the Long distance and left to explore for themselves.
- 12pm – 1pm: Any cows left in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area. All cows were then encouraged through the raceway and the first one-way gate into the outdoor open mattress area.
    - 1pm – 3pm: Cows had free access between the indoor trial area and the outdoor open mattress.
- 3pm -4pm: Any cows left in the outdoor open mattress area were encouraged to push through the second one-way gate, back into the indoor trial area. All cows were then encouraged through the raceway and the first one-way gate (untied) into the outdoor open mattress area.

- Cows had free access over night between the indoor trial area and the outdoor open mattress.