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Factors that influence dairy cow preference to be indoors or at pasture

By

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“Who was the guy who first looked at a cow and said I think I’ll drink whatever comes out of these when I squeeze ’em?”

-Bill Watterson

To everyone past and present that helped me along the way.

DECLARATION

I declare that this thesis has been composed entirely by me and that it has not been accepted in any previous application for a degree. The work, of which is a record, has been done by me. Sources of information and assistance have been specifically acknowledged.

Priya Motupalli

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Many factors influence dairy cow preference to be at pasture. The studies reported here investigated whether herbage mass and previous experience affected preference. The first study offered a high (3000 ± 200 kg DM) vs. low (1800 ± 200 kg DM) mass at a near (38 m) vs. far (254 m) distance to 16 Holstein-Friesian dairy cows. Masses were offered at two distances to determine motivation. A continuously housed control group (n =16) was also compared to cows with free access to pasture. Video recordings and scan-sampling with five-minute intervals revealed that mass did not affect preference ($P > 0.05$), but the proportion of time cows spent at pasture during the day was more at the near distance (73.7% vs. 28.8%, $P < 0.05$). Night-time pasture use was not affected by distance. Continuously housed cows produced 6.7 kg less milk/day than free-access cows ($P < 0.05$).

To determine the effect of previous experience, two groups of 12 Holstein-Friesian dairy heifers were reared with or without exposure to pasture and tested for their preference for pasture at 16 months in 2012. In 2013, when lactating, a similar study was conducted with the same treatment groups in addition to a group that was reared without exposure in their first grazing season, but with exposure in their second grazing season. Indoor-reared heifers spent more time indoors (82.6 vs. 55.6%, $P < 0.05$), and investigating grass (5.07% vs. 2.39%, $P < 0.05$) than heifers with experience of pasture. As the measurement period progressed, indoor-reared heifers spent more time at pasture ($P < 0.05$). Similar results were reported for lactating cows, but no effect of time was recorded for cows without exposure to pasture ($P > 0.05$).

The original findings of this thesis show that herbage mass does not affect high yielding dairy cow preference for pasture, but pasture access can have a beneficial effect on production. Dairy cattle without experience of pasture show a decreased preference for it, but depending on age of exposure this changes over time.

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Rutter, S.M., **Motupalli, P.R**. and Charlton, G.L. 2014. Many factors affect the preference of high-yielding dairy cows for pasture vs cubicle housing. *Proceedings of the First DairyCare (COST Action FA1308) Conference, Copenhagen*, Denmark, 2014.

Motupalli, PR., Rutter, S.M., Sinclair, L.A., Bleach, E.C. The effect of herbage allowance and previous experience on dairy cattle preference to be at pasture. *Proceedings of the International Society for Applied Ethology Regional Meeting*, Edinburgh, UK, 2014.

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LIST OF ABBREVIATIONS

ANOVA: analysis of variance
BCS: body condition score
CP: crude protein
d: day
DIM: days in milk
DM: dry matter
DMI: dry matter intake
EFSA: The European Food Safety Authority
FAWC: Farm Animal Welfare Council
h: hour
ha: hectare
HAU: Harper Adams University
hd: head
kg: kilogram
LS: locomotion score
NDF: neutral detergent fibre
OIE: The World Organization for Animal Health
OM: organic matter
SD: standard deviation
SEM: standard error of the mean
t: tonne
THI: temperature-humidity index
TMR: total mixed ration
WSC: water soluble carbohydrate

1.1 MODERN DAIRY INDUSTRY

Taurine cattle (*Bos taurus*) were domesticated in two events: one in Africa and one in Eurasia about 8000 to 10,000 years ago (Clutton-Brock, 1999). Through selective breeding, specialised breeds for milk production have been created including the Holstein-Friesian. The use of Holstein Friesian genetics of Northern American ancestry (for high milk production and durability) has been pervasive making the Holstein-Friesian the most commonly used dairy cow in temperate countries including the United States, New Zealand, and the United Kingdom (McCarthy et al., 2007).

The modern dairy industry uses several types of housing systems including tie-stalls, free-stalls, loose-housing, dry-lots (in warm climates) and pasture (Cook and Nordlund, 2009). With tie-stalls, cows are typically kept indoors and tied in a specific location—cows will be milked, fed, and allowed to rest in their individual stall or in some instances cows will be brought to a parlor to be milked (Tucker, 2009). Both free-stall and loose-housing systems allow for free movement to and from feeding and resting areas. Cows will typically be brought to and from a central milking parlor, but in systems that utilize automatic milking cows can access the parlor without human intervention (Rushen et al., 2008). In free-stall systems cows have lying areas divided by partitions and different management practices dictate the bedding on which they lie down: rubber or geotextile mattresses, deeply bedded sand, and sawdust are all used (Tucker, 2009). Loose-housing systems allow areas for cows to lie down without any partitions and also vary in the substrate provided: straw, sand, and compost can all be used. Finally, dairy cows may also be kept exclusively at pasture or for certain times during the year (Tucker, 2009). Holstein-Friesians are typically fed a nutritionally balanced total mixed ration (**TMR**) indoors (Coppock et al., 1981) as they have difficulty meeting their nutritional requirements on grass alone (Fike et al., 2003) since the intake rate of grazed herbage is lower than can be achieved from a TMR (Kolver and Muller, 1998). If high-yielding dairy cows (producing > 25 kg/d in early lactation and about 20 kg/d in late lactation) are kept exclusively at pasture, it is recommended that they are provided with supplemental feed in order to increase dry matter intake (**DMI**) to support body condition and maintain milk yields (Bargo et al., 2003).

1.2 CONTINOUSLY HOUSED VS PASTURE-BASED SYSTEMS

Dairy systems in the United Kingdom vary, but traditionally dairy cattle are housed in free-stalls or loose-housing in the winter and allowed to graze in the summer at pasture (Haskell et al., 2006) as climate and soil conditions tend to be poor in the winter, and grass growth is seasonal (O' Driscoll et al., 2009). In recent years the use of continuously housed systems, where dairy cows have no access to pasture has increased in the UK (Haskell et al., 2006). Pol-van Dasselaar et al. (2008) reported that the majority of European countries (including the UK) are moving towards restricted or zero-grazing systems for a variety of reasons including: to have greater control over feed intake for high yielding dairy cows, to cope with increased herd sizes (and lack of accessible grazing land), to accommodate the increased use of automatic milking systems, and to mitigate the effects of an uncertain grass supply.

As continuously housed systems become more prevalent, there are concerns regarding dairy cow health and welfare (Rushen et al., 2008). The European Food Safety Authority (**EFSA**) (2009) identified zero access to pasture as a hazard (factors that have a negative impact on welfare), as animals kept at pasture were less likely to have metabolic and reproductive disorders as well as leg and locomotion problems and they recommend that "when possible dairy cows and heifers should be given access to well managed pasture or other suitable outdoor conditions at least during summer time or dry weather." Haskell et al. (2006) reported that incidences of lameness were reduced at pasture while Hernandez-Mendo et al. (2007) reported that even limited access to pasture in dry conditions (4 weeks) improved foot health. Additionally, Olmos et al. (2009) found that hoof disorders were less prevalent at pasture when compared to cubicle housing, and that cows at pasture were less likely to develop clinical lameness. Washburn et al. (2002) reported lower incidences of mastitis at pasture and Goldberg et al. (1992) reported that udder health was negatively influenced by zero-access to pasture. These results are of importance as both lameness (Shearer et al., 2013) and mastitis (Medrano-Galarza et al., 2012) are considered to pose significant welfare and production risks in dairy systems. Finally, it has been reported that intensively managed indoor systems cause increased incidences of aggression as reduced space allowance can cause more competition for resources (DeVries and von Keyserlingk, 2005) and agonistic encounters are more easily avoided at pasture (Philips, 2002).

There is evidence however, that cows on pasture-based systems are susceptible to increased parasite loads which affect health and can cause decreases in productivity (Bloemhoff et al., 2014) and painful foot infections that lead to severe lameness can be a product of wet summer pastures (Dhawi et al., 2005). It has also been reported that access to pasture does not mitigate the negative consequences of indoor flooring (asphalt, slatted concrete, and rubber) including sole haemorrhages, ulcers and heel horn erosions (Haufe et al., 2012) but the restorative effects of pasture reported by Hernandez-Mendo et al. (2007) occurred over a longer period of pasture access. Additionally, pasture-based systems that do not provide shelter from inclement weather can cause both heat (Kendall et al., 2007, Tucker et al., 2008) and cold stress (Van laer et al., 2014). Holstein-Friesian cattle are particularly susceptible to heat stress which causes both a reduction in feed intake, milk production and welfare via reduced immune function (Lacetera et al., 2006), increased lameness due to behavioral adaptations (reduced lying times) to increased heat load (Cook et al., 2007), and potentially increased prevalence of infection (Dohoo and Meek, 1982)—in some cases heat stress may result in death (Stull et al., 2008). Additionally, dairy cows are highly motivated to access shade to reduce these negative consequences of heat load (Schütz et al., 2008) and if given a choice will move indoors as thermal comfort decreases (Legrand et al., 2009). Finally, indoor housing typically provides continuous access to a high quality diet (Legrand et al., 2009) which is necessary to maintain body condition and meet production demands (Kristensen et al., 2007). Ketelaar-de Lauwere et al. (1999) reported that dairy cows will move indoors to consume a mixed ration as availability of fresh food at pasture declines. However, a mixed ration may compromise animal welfare as dairy cows prefer to select their feed (Rutter, 2010) which is prevented by mixed rations as they are formulated to ensure every mouthful is uniform (Coppock et al., 1981), and individual animals may be able to combat nutritional deficiencies that occur with diets formulated for the average animal when they are given the opportunity for diet selection (Atwood, 2001).

1.3 PUBLIC OPINON OF CONTINUOUSLY HOUSED SYSTEMS

Despite the conflicting evidence, continuously housed systems are viewed negatively by consumers due to welfare concerns and some EU member countries (Norway, Sweden, and Finland) have enacted welfare legislation mandating access to pasture for dairy cows (Pol-van Dasselaar et al., 2008). Additionally, the Farm Animal Welfare Council (1997) expressed concerns that zero-grazing systems would restrict the ability to engage in natural behaviour. When asked if keeping cows continuously housed was acceptable, 95 % (of the 363 members of the UK public that responded) of consumers felt zero-grazing for dairy cattle was “unacceptable” (Ellis et al., 2009). Ellis et al. (2009) also reported that among other factors respondents associated “access to outside,” “freedom to roam/free-range,” “fresh air,” and “freedom to express normal behaviour” with good animal welfare. In Canada, when members of the public (including farmers, veterinarians, teachers, students, industry professionals, and participants with no connection to dairy farming) were asked if dairy cows should be given access to pasture, out of 178 participants, 73 % answered "yes" (across all groups) (Weary et al., 2012). Spooner et al. (2014) reported similar results with participants almost exclusively relating good animal welfare with access to natural environments and the ability to engage in natural behaviour: phrases including "just being outside," "grazing out," and "choice" were frequently used in conjunction with good animal welfare. Consumers in the United States also ranked the ability to engage in normal behaviour and exercise outdoors as the most important allowances for farm animals after receiving adequate food, water, and necessary medical treatments (all factors associated with basic survival) (Prickett et al., 2010) which is an important societal development as concern for animal welfare in the US has typically lagged behind Europe (Mench, 2008). Given these opinions, the negative public view of continuously housed systems, and welfare legislation regarding pasture access is likely in large part due to the perceived "unnaturalness" of modern housing conditions (von Keyserlingk et al., 2009). However, Rushen et al. (2008) argued that determining what is natural for modern dairy cows is difficult given the genetic changes dairy cows have undergone via artificial selection for increased milk yield.

1.3.1. Natural behaviour of dairy cattle

Unlike other domesticated farm species, the ancestor of domestic cattle (aurochs) have been extinct for centuries (Clutton-Brock, 1999) and modern cattle breeds have been shown to have diverged from wild Bovine species (bison and buffalo) hundreds of thousands of years ago (Ritz et al., 2000) reducing our ability to understand the impact of domestication on dairy cattle behaviour, and whether modern breeds even have the same behavioural repertoire as that of their wild counterparts or of their ancestors (Rushen et al., 2008).

Some comparisons of behaviour can be made however, with domestic cattle living with limited human contact (Kilgour, 2012) or feral cattle populations living today and this may be useful in our understanding of welfare problems that occur in modern farming systems (Špinka, 2006). Kilgour et al. (2012) reviewed 22 studies assessing the behaviour of beef and dairy cattle at pasture (with limited human intervention) as well as Chillingham cattle (feral cattle with no human intervention) and reported that cattle have a complex behavioural repertoire and up to 40 behavioural categories have been defined. The three main behaviours (that take up about 90-95% of a 24 h day) cattle engage in include grazing, ruminating, and resting, and on average grazing was the most prevalent of the three. Over a 24 h period, cattle spent between 6.8 – 13.0 h engaged in grazing behaviour and exhibited a diurnal eating pattern where the amount of time spent grazing during the daylight hours was greater than when it was dark. In particular when examining dairy breeds (Holsteins and Holstein crosses) approximately 40 % of their time was occupied by grazing behaviour. Rumination was shown to occur from 4.7 to 10.2 h/d, while resting (both lying and standing) ranged from 3.6 to 10.3 h/d. There was a tendency for rumination to occur more often at night, but no clear diurnal pattern was seen with resting behaviour. This is likely to be the case because Kilgour et al. (2012) did not differentiate between lying and standing when comparing resting behaviour during the day vs. night. Tucker et al. (2009) reported that at pasture, cows spend more time lying down at night, and that feeding and lying behaviour (feeding during the day and lying at night) is highly synchronized at pasture. When strictly examining Chillingham cattle, 58 % and 75 % of their time was spent engaged in ingestion behaviour (grazing and ruminating) during the summer and winter respectively (Hall, 1983).

In contrast, Gomez and Cook (2010) examined the time budgets of 205 lactating dairy cows in free-stall barns (rubber mattresses or sand bedding) and reported that dairy cows spent between 1.1 to 8.1 h/ d feeding and 3.9 -17.6 h/day lying. Tucker et al. (2009) also reported lower feeding times when cows were housed (between 4-6 h/d) principally because cows must spend a longer period of time grazing to achieve sufficient intake rates to meet nutrient needs than when provided with a total mixed ration, which can be consumed more rapidly. The increased time spent resting indoors is possibly due to the decreased feeding time seen indoors when compared to pasture. Arguably, the main "natural" behavior that is restricted therefore, is the ability to graze and it has been argued that denying dairy cows access to pasture may reduce their welfare (Hemsworth et al., 1995).

As previously mentioned, the presence of total mixed rations decrease feeding times when compared to cows that consume the majority of their feed at pasture, and it has been reported by Redbo (1990) that time spent eating is negatively correlated with stereotypic tongue rolling behaviour. Additionally, in captive ungulates the more artificial the foraging regime, the greater the likelihood of the appearance of stereotypic behaviours (Mason and Rushen, 2006). Stereotypies are abnormal, reptetitive behaviours that serve no obvious purpose and occur in the presence of frustrated environmental conditions, so in conjunction with other measures they can be used to assess the extent to which an environment is fulfilling the behavioural needs of an animal (Mason, 1991, Mason et al., 2007). Interestingly, dairy heifers that engage in stereotypic tongue rolling behaviour indoors (11 % of their time), do not engage in this behaviour at all after being put out to pasture. When these heifers were confined to housing again, they showed a significant increase in tongue rolling behaviour (25 % of their total time) when compared to their behavior prior to being at pasture (Redbo, 1990). It is not immediately clear however, what aspect of pasture (i.e. grazing or simply being at pasture) caused the elimination of these abnormal behaviours as heifers in the study of Redbo (1990) were confined to restrictive tie-stalls when indoors.

1.4 METHODOLOGY FOR WELFARE ASSESSEMENT

As the evidence regarding animal welfare in continuously housed vs. systems providing access to pasture is conflicting, and the importance of pasture access is unclear (von Keyserlingk et al., 2009), it is essential to try and evaluate how access to pasture is perceived by the dairy cows themselves, and exactly what aspects of pasture are important to dairy cows. As argued by Duncan (2005) decisions regarding animal welfare should be based on how the animals feel, and as we cannot do this directly there are a number of methods that have been developed to indirectly determine how they feel about their environment. Animal welfare and the scientific methodology used to assess animal welfare have been subject to a number of extensive reviews and articles (Smidt et al., 1983, Broom, 1988, Mason and Mendl, 1993, Duncan, 2005, Dawkins, 2006, Carenzi and Verga, 2009) including reviews specific to dairy cattle (Rushen et al., 2008, von Keyserlingk et al., 2009) and a brief summary will be provided in the next section.

1.4.1 Animal welfare

In general, three schools of thought have developed regarding the definition of animal welfare: biological functioning (normal physiological and behavioural responses, satisfactory health and growth, and in the case of farm animals, good productivity); natural living (living lives as close to their natural state as possible and being given the ability to develop and perform their full behavioural repertoire); and affective state (the emotional state of the animal and associated subjective experiences—animals should experience positive feelings including pleasure and be able to avoid unpleasant feelings associated with pain, fear, or hunger). Individually, these definitions have been criticized. For example, allowing animals to perform their full behavioural repertoire would likely include behaviours that are exhibited during times of great distress (e.g. piglets panting to cope with heat stress) and environments that encourage these behaviours are likely to increase suffering rather than mitigate it. Additionally, a definition of welfare that only includes subjective feelings would separate welfare from health in a negative manner, and an animal that exhibits good biological function i.e. is producing well does not necessarily indicate an absence of welfare problems.

Fraser (2003) argued that even when taking all three factors into account, animal welfare scientists and other individuals can still reach different conclusions when addressing issues in farm animal welfare based on the importance they place on each factor. Von Keyserlingk et al. (2009) reported that these factors often overlap: a lactating dairy cow without shade on a hot day (natural living), will feel uncomfortable (affective state), and this inability to cope with the heat load can result in hyperthermia and ultimately decreased milk production (biological functioning). In this case, if there is an improvement in one factor, it is likely that there will be an improvement in all three: if there is a reduction in the physiological signs of hyperthermia, the negative feelings that arose as a result of discomfort will disappear. Von Keyserlingk et al. (2009) cautioned however, that these factors may actually conflict and produces the example of poorly managed group housing for calves: although calves may be able to perform natural social behaviours, increased incidences of disease and aggression may occur. Thus, solutions to animal welfare that focus on simply one factor of animal welfare can be misleading (Dawkins, 1999), and the use of multiple factors to address welfare problems in the environment is essential (Duncan, 1992).

When these three approaches are taken together, a holistic view of animal welfare is achieved where emphasis is placed on freedom from suffering i.e. negative feelings, high biological functioning (via absence of injuries, disease, hunger etc.) and positive experiences (via the ability to express natural behavior) (Veissier et al., 2008). Official definitions tend to incorporate this holistic view: The World Organization for Animal Health (**OIE**) (2013) asserts that “an animal is in a good state of welfare (as indicated by scientific evidence) if it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress." Additionally, the Farm Animal Welfare Council (**FAWC**) (2011) recently recommended that when assessing welfare, a greater emphasis should be placed on a "life worth living" for animals on farm, suggesting that good biological function and the absence of negative feelings are not enough to provide for good welfare.

1.4.2 Methods of assessing each component of animal welfare.

1.4.2.1 Biological functioning. Duncan and Fraser (1997) outlined a number of measures of biological function used to assess welfare. Reproduction and growth rates, specific health threats such as injury and disease based on housing, disturbances to endocrine and immune function, longevity and reproductive success, physiological indicators of stress (heart rate/cortisol levels), and behavioral disturbances have all been employed.

For example, in order to assess whether automatic milking systems were potentially more stressful than conventional ones, Hopster et al. (2002) measured the heart rate of cows during milking in both systems to contribute to the conclusion that either system was acceptable and normal heart rate responses were present in both systems. Measures of biological functioning can also be used as a useful indicator of affective states encouraging the overlap of the different factors associated with animal welfare. For example, Laister et al. (2011) used heart rate to investigate the calming effects of social licking in dairy cows: a decline in heart rate for individuals being groomed indicated an effect of relaxation and a positive affective state.

1.4.2.1.1 Limitations of individual measures of biological functioning. As suggested previously, measures of good biological functioning can be useful in assessing welfare but individual measures have been criticized. Production losses can signify poor welfare if they are a result of injury, disease, malnutrition etc. but a decrease in production may not necessarily indicate poor welfare (Duncan and Fraser, 1997) and conversely, increases in production or maintenance of production, may not indicate good welfare as genetic selection for increased production has made livestock more susceptible to health problems (Rauw et al., 1998). Specifically, dairy cattle are more susceptible to metabolic stress, lameness, and reproductive difficulties as a result of selection for increased production (Oltenacu and Algers, 2005). The use of injury or disease as an indicator of poor welfare cannot be refuted, but welfare may be compromised before there are clinical signs. For example, Bruijnis et al. (2012) reported that the total welfare impact of subclinical foot disorders was equivalent to the impact of clinical foot disorders. Sub-clinical disorders can be determined by changes in physiology, but the use of certain physiological measures including cortisol levels as sole indicators of distress have been questioned. Although the secretion of cortisol can indicate levels of stress that contribute to poor welfare under highly controlled conditions it is often secreted in response to innocuous or positive stimuli (Moberg and Mench, 2001). Colborn et al. (1991) reported that stallions produced similar concentrations of cortisol when being restrained, exercising, and when given an opportunity to mate, making it difficult to differentiate between stress leading to poor welfare and stress that does not necessarily influence welfare (Moberg and Mench, 2001). Finally, changes in normal behavioral patterns can help contribute to the assessment of welfare, but it is poorly understood if abnormal behavior actually decreases welfare unless physical harm occurs as a result of a change in behaviour (Mason, 1991).

1.4.2.2 Affective state and natural living. Direct measurements of the subjective experience of animals are impossible, but techniques have been developed to indirectly assess their experience (Duncan, 1992). There have been some attempts to simply observe animals in as “natural” an environment as possible and then attempt to recreate an environment that satisfies the key elements of their natural behaviour (Stolba and Wood-Gush, 1989). As previously discussed, this approach tends to be problematic because the whole behavioural repertoire may not be appropriate in captive management systems

(Špinka, 2006) and because management practices allowing these may be perceived as uneconomical or impractical (von Keyserlingk et al., 2009). More recently, cognitive bias has been used to attempt to directly assess valence (positive or negative emotional state), where animals are trained to associate a cue with either a negative or positive event. The general concept is that animals that have been exposed to a cue associated with a negative event will then judge an ambiguous cue in a "pessimistic" manner and an animal that has been exposed to a cue associated with a positive event will judge an ambiguous cue in a "optimistic" manner (Mendl et al., 2009). It has been used to show that dairy calves experience a negative emotional state after disbudding (Neave et al., 2013) and that early cow-calf separation leads to a negative emotional state in calves (Daros et al., 2014) amongst other studies.

Another method of assessing feelings, aversion testing, developed from the idea that negative feelings have evolved to help animals avoid harm. For example, if an animal is put in a situation where it experiences fear, distress, or pain, it will try and remove itself from the negative stimulus (Widowski, 2009). To test aversion, either choice or operant tests can be employed (Widowski, 2009). For example, cattle have been shown to avoid going down a runway where rough handling has previously been experienced (Pajor et al., 2000). Duncan (1992) stressed that tests where animals are making a positive choice, or working to gain access to a preferred resource tend to be easier to interpret than tests where animals are actively avoiding painful or stressful stimuli.

Preference testing is perhaps the most widely used method to help us understand how animals feel about their environment, and is the main method used in the studies presented in this thesis. Animals are given the opportunity to choose between aspects of their environment and the assumption is that the choice they make will be in the interest of their welfare. For example, Kristensen et al. (2000) investigated the preference of laying hens for different concentrations of ammonia in housing systems to identify if environments with high concentrations of ammonia was aversive to laying hens while Borderas et al. (2009) determined that dairy calves removed from their mother preferred areas in the barn with heat lamps over areas without a source of heat. Reviews of preference testing have identified key limitations which will be briefly discussed in ***section 1.4.2.2.1*** (Dawkins, 1983a, Duncan, 1992, Fraser and Matthews, 1997) but preference testing can be the most straight-forward way of asking animals how they feel and often welfare benefits can arise from providing them access to their preferred option (von Keyserlingk et al., 2009). Furthermore, limitations to preference testing can be addressed relatively easily (Dawkins, 1983a, Duncan, 1992, Kirkden and Pajor, 2006). Additionally, it can be used to understand what aspects of a natural life are of most importance to livestock, which has been shown in ***section 1.3*** to be of great concern to the public.

1.4.2.2.1 Main limitations of preference testing. Preference testing gives insight into how animals perceive their environment, and what resources they prefer. However, it doesn't indicate how important a resource is, it simply indicates that of the options presented one is preferred over the other. The preferred option may therefore not necessarily improve welfare, while the least preferred option may not necessarily result in poor welfare. In order to elucidate the importance of the preferred choice motivation testing is used as a follow-up to the preference test (Jensen and Pedersen, 2008). Motivation or demand testing is used to determine the strength of a preference by asking the animal to "pay a price" to gain access to a preferred resource, and increasing the price incrementally until the animal refuses to "pay" anymore. Both operant techniques and obstruction techniques are employed in motivation testing: animals can be taught to perform a particular task including pressing a panel to gain access to a social companion (Holm et al., 2002), pushing through a weighted door to gain access to a perch (Olsson and Keeling, 2002), walking a particular distance to gain access to pasture (Charlton et al., 2013), or they may be asked to give up something that is known to be important (Munksgaard et al., 2005).

The difficulty of these tasks is increased (Kirkden and Pajor, 2006) and the response to these increases in difficulty can be used to understand the importance of a particular resource. If the animal is willing to work hard to gain access to the preferred resource, then it can be reasonably assumed that welfare will be improved in the presence of the preferred resource (Broom, 1988).

Adapted from the field of economics, preferred resources can either be classified as having elastic or inelastic demand. Elastic demand indicates that as the "price" increases the demand will decrease while inelastic demand indicates that as the "price" increases, the demand will stay the same. (Dawkins, 1983c). If the resource is said to have inelastic demand, then it can be inferred that depriving the animal of this resource will cause it to suffer—a conclusion that would not be possible via preference testing alone (Dawkins, 1983c). As with preference testing, limitations exist but can be overcome with careful experimental design. For example, rewards must remain constant regardless of the price, and demand curves are dependent on open vs. closed economies—i.e. the motivational priorities of an animal may change depending on whether it lives in the experimental set-up or whether there is a separate home space (Dawkins, 1988).

Duncan (1992) discussed that animals do not always make choices that are in their best interest for the long-term. Care must be taken therefore when providing a choice between resources that may benefit the animal in the short-term, but may negatively impact the animal in the long-term. For example, animals selected for fast-growth (e.g. broiler breeders) and as a consequence large appetites, will over-consume food if given *ad libitum* access leading to poor welfare as a result of obesity, decreased reproductive ability and eventual morbidity. Although they may prefer *ad libitum* feed, and short-term needs would be met, it would compromise their welfare in the long-term. Dawkins (1983a) argued that this does not invalidate the use of preference testing, but highlights the importance of using multiple methods of measuring welfare.

Previous experience (effects of rearing and short-term exposure to resources) can also influence the results of preference testing, but it is not always clear which factors will be influenced. Lentfer et al. (2011) reported that nest site preferences in laying hens are affected by early life experience and rearing in laying hens while Dawkins (1983b) reported that hen preference for litter flooring was not influenced by rearing. Dawkins (1983a) argued that if it is suspected that preference for a particular resource is sensitive to rearing conditions, this can easily be addressed by conducting an experiment to determine if this is the case.

Preferences may change depending on a number of factors including age, circadian rhythm, or physiological state and this must be taken into account when making conclusions. Telezhenko et al. (2007) reported that when given a choice, lame cows did not express a preference for soft flooring when compared to non-lame cows. However, because they were lame, they were in competition for the resource with healthy herd-mates that were of a higher rank within the social hierarchy and presumably were not able to spend as much time on soft flooring as they would if they were not lame. Davis et al. (1999) reported that the light intensity preferred by chickens differed with age and with behavioural activity: younger birds preferred brighter environments, while older birds preferred dim light during periods of inactivity vs. bright light during periods of activity.

1.4.3 Thesis methodology

As discussed throughout ***section 1.4***, a number of methods have been used to help understand how animals perceive their environment. Preference testing remains a valid method to help gain insight on which aspects of natural living dairy cows deem most important especially when used in conjunction with motivation testing and biological methods of assessing welfare. The main method used in this thesis therefore is preference testing coupled with motivation testing and the quantification of behaviour (location, posture and jaw activity) and production measures (feed intake, milk yield, and milk composition). Additionally, specific factors are tested: herbage allowance, distance, and previous experience rather than testing each environment as a whole, which is useful as it helps us to pinpoint exactly which features of the environment influence preference.

1.5 PREFERENCE FOR PASTURE AND FACTORS INFLUENCING PREFERENCE

Preference testing and demand testing have been used in a limited number of studies directly assessing cattle preference between indoor housing and pasture. These studies have yielded complex results: in general, dairy cows prefer to be at pasture when given free-choice, but a number of factors appear to influence this preference.

1.5.1 Overall preference to be indoors or at pasture

Krohn et al. (1992) reported that when given free access between deeply bedded (long straw) free-stalls and a yard or pasture, cows spent the majority of their time at pasture (17.2 h/day) during the summer. This is in agreement with both Charlton et al. (2011b) and Charlton et al. (2013) who reported that when given free choice between free-stall housing with rubber mattresses or pasture, cows expressed a partial preference to be at pasture (71 % and 58 % respectively). Additionally, when given a choice between an open feedlot environment (gravel base with 250 mm thick feedlot compost) and pasture, beef cattle also preferred to be at pasture, spending 75 % of their time there (Lee et al., 2013). Some studies have not reported an overall preference for pasture, but rather a preference for pasture during the night-time (20:00 – 06:00 h) when given the choice between free-stalls with geotextile mattresses deeply bedded with washed river sand and pasture (0.1 m depth) (Legrand et al., 2009, Falk et al., 2012). It appears that only one study to date that directly assessed preference between pasture and free stall housing (with rubber mattresses) reported that dairy cows showed an almost exclusive preference to be indoors, spending about 92 % of their time there (Charlton et al., 2011a). Charlton et al. (2011a) speculated that this result may have been due to the cows' limited prior experience of pasture.

Additionally, a few studies have quantified time spent in cubicle housing vs. pasture when given 24 h access to both locations while not directly assessing preference. Ketelaar-de Lauwere et al. (1999), Ketelaar-de Lauwere et al. (2000), and Spörndly and Wredle (2004) reported that dairy cows spend approximately 76 %, 85 %, and 68 % of their time respectively at pasture over indoor cubicle housing.

Finally, it appears only a single study has attempted to quantify dairy cow motivation for access to pasture using motivation testing. Charlton et al. (2013) reported that when cows were asked to walk 60, 140, or 260 m to gain access to pasture, the cows reduced pasture use as the "price" to gain access to pasture increased. Cows were willing to walk up to 260 m to gain access to pasture during the night however, and overall pasture use (24 h) was not influenced by distance.

Although the majority of these studies reported that dairy cows express a partial preference to be at pasture and there is emerging evidence that cows are motivated to gain access to pasture at night, this preference is conditional on a number of factors. As this preference is not straightforward, it is important to try and understand which factors influence preference for pasture rather than purely quantifying where they spend their time. To date, the studies assessing preference have directly shown that the following factors influence dairy cow preference for pasture.

1.5.2 Season

Two of the previously conducted preference experiments assessed seasonal differences in preference for pasture. Krohn et al. (1992) reported that in Denmark during May-

September cows expressed a preference for pasture, while during November-March cows spent only about 5 h /day outdoors. This is in agreement with Charlton et al (2011b) who also reported that as the season progressed in the UK, pasture use decreased with cows spending 86.7 % of their time at pasture during August-September, 68.3 % during September-October, and 58.3 % from October-November. It can be argued that this change in pasture may be due to weather conditions (including ground conditions) as discussed in the following section, but it may also be due to the change in grass quality and quantity as discussed in ***section 1.6.1***.

1.5.3 Weather

Previous research indicates that cattle behaviour is affected by weather conditions and availability of shelter (Redbo et al., 2001, Tucker et al., 2008). In hot weather, cattle actively seek shade (Blackshaw and Blackshaw, 1994) and *Bos taurus* breeds tend to be more sensitive to heat rather than cold (Hemsworth et al., 1995). Several studies mentioned in ***section 1.5.1*** indicated that dairy cows changed their location in response to both humidity and rainfall. Legrand et al. (2009) and Falk et al. (2012) both observed a decrease in pasture use as the temperature humidity index (**THI**) increased at pasture (range: 49.9-74.6 and 54-68 respectively) during the day-time and as rainfall increased during the night-time (range: 0-65.4 and 0-25 mm/d respectively). Charlton et al. (2011a) and Charlton et al. (2013) did not find an effect of humidity on preference for pasture, but reported that on days with greater than average rainfall cows spent less time at pasture. Charlton et al. (2011b) found that when the THI increased indoors, cows spent more time at pasture. The apparent inconsistencies in the literature with respect to THI are likely to be due to the comparatively low mean THI values reported in both Charlton et al. (2011a) and Charlton et al. (2013) of 59.9 and 59.6 respectively at pasture as the upper critical THI limit for dairy cows (the point at which milk production levels are compromised) is reported to be 72 (Tucker et al., 2008). The decline in pasture use during the day due to heat (using the black globe humidity index as a measure of heat stress) as well as heavy rain overall was also reported by Ketelaar-de Lauwere et al. (1999) and Ketelaar-de Lauwere et al. (2000). Finally, Krohn et al. (1992) observed that when the ground was frozen at pasture, cows stayed exclusively indoors.

1.5.4 Time of day

Cows used in preference studies spent more time at pasture during the night (after evening milking). Legrand et al. (2009) reported that between morning and evening milking (08:00 to16:00 h) cows were outside for less than 3 h. In contrast, after 16:00 h cows spent the majority of their time at pasture, and most cows were exclusively on pasture between 00:00 to 04:00 h. This is in agreement with Krohn et al. (1992) who reported that during the summer cows spent all their time at pasture from dusk until dawn. Falk et al. (2012) also reported similar results with cows spending 78 % of their time at pasture between 20:00 to 06:00 h and only 41 % of their time at pasture between 08:35 to 15:00 h. Finally, Charlton et al. (2013) reported that dairy cows spent about 80 % of their time at pasture during the night.

1.5.5 Performance

Three of the preference studies examined the effect of performance factors (body condition score, milk yield, lactation number, lameness, and weight) on preference to be at pasture with differing results. Charlton et al. (2011a) and Charlton et al. (2011b) reported that cows with a body condition score (**BCS**) greater than 2.7 and 3 respectively had a tendency to spend less time at pasture while Charlton et al. (2013) found no effect of BCS. Charlton et al. (2011a) also reported that cows producing greater than 26.9 kg of milk per day spent less time at pasture while (Charlton et al., 2011b) and (Charlton et al., 2013) reported no effect of milk yield on preference to be at pasture. Mean lactation number, reported to be 1.8 (Charlton et al., 2011a), 2.3 (Charlton et al., 2011b), and 2.6 (Charlton et al., 2013) had no impact on time spent at pasture. Lameness score (1.5) and live-weight (644 kg) did not influence preference according to Charlton et al. (2011a), but cows with a lameness score greater than 1.5 spent less time at pasture (Charlton et al., 2011b).

1.6 GAPS EMERGING FROM PREVIOUS RESEARCH INVESTIGATING PREFERENCE FOR PASURE

1.6.1 Grazing behaviour and herbage mass

The impact of season on dairy cow preference to be at pasture suggests that herbage mass and quality may influence where dairy cows spend their time. Herbage mass is defined as the amount of herbage per unit of area (Hodgson, 1979) and is usually expressed in kg DM/ha. It has been reported that grazing animals spend more time grazing in the beginning of the season (May – July) than late in the season (August onwards) possibly due to herbage availability and quality (Spörndly and Burstedt, 1996). Since cows in the preference studies had a choice to consume TMR indoors they were able to spend more time consuming a mixed ration as the season progressed when herbage quality was likely to have decreased (Krohn et al., 1992, Charlton et al., 2011b).

Charlton et al. (2011a) found that dairy cows only spent about 8 % of their overall time at pasture, but when they did spend time at pasture about 50 % of their time was spent grazing. This indicates that although cows did not prefer to be at pasture, they were actively choosing to access pasture to graze. Other preference studies that recorded grazing time have reported that cows spent 32 % (Charlton et al., 2011b), 35 % (Charlton et al., 2013), and 24%, (Krohn et al., 1992) of their time at pasture grazing. Although this is less than 50 %, grazing was still in the top four activities performed at pasture and if combined with rumination (ingestion behaviour) it became the most prevalent behaviour performed at pasture.

Krohn et al. (1992) reported that during the summer, dairy cows preferred to graze rather than consume a mixed ration (although they always consumed some of both feed sources) and Ketelaar-de Lauwere et al. (2000) reported that when given an opportunity to eat forage indoors vs. outdoors, cows preferred to graze. Grazing time was not influenced by distance to pasture which was up to 296 m. Since cows were willing to walk increasing distances to graze, it suggests that the ability to graze was particularly important to them. In contrast, Charlton et al. (2013) reported that as distance to pasture increased (up to 260 m) time spent at pasture decreased. In the study conducted by Ketelaar-de Lauwere (2000), sward height varied between 7.3-14.5 cm while herbage mass provided by Charlton et al. (2013) varied between 2000-3500 kg DM/ha. Although it is difficult to make direct comparisons, the sward height of 14.5 cm. would likely equate to under 3000 kg DM/ha.

Given this information, it is unclear whether or not the ability to graze influences preference for pasture and further work is necessary to elucidate this. Additionally, there may have been some interaction between herbage mass (or sward height) and distance that affected cows in the studies conducted by Charlton et al. (2013) and Ketelaar-de Lauwere et al. (2000) as it might be expected that cows would be willing to walk a longer distance (or to “pay a price” to gain access to lush pasture (a high herbage mass)) if grazing is important.

In order to determine if preference for pasture is influenced by the ability to graze, it is important to understand what might affect dry matter intake (**DMI**) at pasture. DMI is constrained by three main behavioural factors: bite mass (DM/bite), biting rate (bites/minute) and grazing time (Kolver and Muller, 1998). Of these factors, bite mass is generally considered to be the most important in determining DMI from temperate pastures (which is the pasture type generally used for dairy cows in the UK) and bite mass is primarily affected by pasture height (McGilloway et al., 1999) and density (Rook, 2000). Dairy cows tend to remove about one-third of the pasture height, but bite mass will decrease as pasture height decreases (Bargo et al., 2003). Gibb (1997) reported that when cows continuously grazed ryegrass, bite mass decreased from 0.31 g of organic matter (**OM**)/bite when pasture height (or sward surface height) was at 7 or 9 cm to 0.23 g OM/bite when pasture height was 5 cm. Pasture height did not influence biting rate or grazing time. In agreement with Gibb (1997), two experiments reported by McGilloway et al. (1999) reported that bite mass decreased from 1.28 to 0.85 g DM/bite when sward height decreased from 21 to 7 cm, and from 1.0 to 0.66 g DM/bite when sward height decreased from 11 to 6 cm respectively. A sward height x pasture density interaction was also reported with bite mass: bite mass at low sward heights were reduced more when there was a low pasture density (1.02 to 0.47 g DM/bite) when compared to a high pasture density (0.97 to 0.63 g DM/bite).

Biting rate and grazing time are more influenced by animal-related factors rather than factors associated with the sward including genetic merit and milk production, although all three behavioural factors (bite mass, bite rate, and grazing time) are negatively influenced at very short pastures (Bargo et al., 2003). When bite mass decreases, both biting rate and grazing time increase in an attempt to maintain necessary DMI. For example, Wims et al. (2014) reported that at a low herbage masses (1150 kg DM/ha), lactating cows (without supplementation at pasture) spent more time grazing to ensure sufficient intake to support milk yield. However, this compensatory increase will decline when the time for other high priority activities begins to suffer as a result (i.e. ruminating time) (Rook, 2000). High genetic merit cows have been reported to graze ryegrass pastures longer than low genetic merit cows to (218 vs. 204 min/7 h period respectively) and have a higher biting rate (64 vs. 61 bites/min) (Bao et al., 1992). In general, it has been reported that cows producing >

25 kg milk/d had greater grazing times, number of bites per day, and intake rates than low producing cows (< 25 kg milk /d) (Pulido and Leaver, 2001, Bargo et al., 2002a), possibly due to their increased metabolic demands.

However, simply increasing the sward height as a means to ensure maximum bite mass is not sufficient as research has reported that herbage mass is likely to have a greater effect on bite mass than sward height for ruminants, particularly on continuously grazed swards (Penning et al., 1994), and herbage mass, structure and allowance are limiting factors for dairy cow bite mass (Chilibroste et al., 2012). Wales et al. (1999) reported that early-lactation Holstein Friesian dairy cows on ryegrass and white clover pastures increased DMI by 0.12 – 0.35 kg DM/cow/day for every increase in kg DM of herbage allowance. Herbage allowance is usually defined as the weight of herbage per unit of animal live weight at a point in time, usually expressed as kg DM per cow per day (Hodgson, 1979).

Additionally, for every t DM/ha increase in herbage mass, DMI increased by 1.3 kg DM per cow per day. These results are in agreement with Stockdale (1985) who reported that although herbage mass was a less consistent indicator of DMI than herbage allowance (i.e. increases in DMI were seen at a wider range), for every t DM/ha increase in herbage mass, lactating dairy cows consumed 3.2 to 5.1 kg DM/cow/day more. It has been suggested that higher herbage masses positively influence DMI as there is more harvestable material available than at lower masses where higher proportions of stem and dead material exist (Peyraud et al., 1996). There are some contrasting reports in the literature with Holstein-Friesians grazing perennial ryegrass (or ryegrass x white-clover mixed swards) where DMI declines at a high herbage mass (Hodgson and Wilkinson, 1968, Wims et al., 2010) and Curran et al. (2010) reported no difference in DMI at a low vs. high herbage mass. However, it is generally agreed that manipulating herbage mass will manipulate DMI. Additionally, there is no clear definition or consistency in the literature of a “low” vs. “high” mass. Conflicting results are likely to be a function of this: Wales et al. (1999)

conducted experiments using a low herbage mass of 3100 kg DM/ha and a medium herbage mass of 4900 kg DM/ha; Wims et al. (2010) reported high herbage masses at 1993 kg DM/ha and low masses at 1075 kg DM/ha; and Curran et al. (2010) reported a mean high mass of 2278 kg DM/ha vs. a low mass at 1551 kg DM/ha amongst other studies.

Pérez-Prieto and Delagarde (2013) reviewed 56 papers discussing the relationship between pasture allowance (a function of herbage mass) and DMI and reported that variations in intake associated with pasture allowance were highly associated with pasture intake rate from low to medium pasture allowance. From medium to high pasture allowances, intake was more associated with grazing time. However, estimated mass and subsequently allowance vary based on estimation height—in Australia and New Zealand, estimation height is from ground level (Stockdale, 1985, Wales et al., 1999), while in Europe height can be variable (ground level, above 2.5 to 3.5 cm, above 4 to 5 cm) (Ribeiro Filho et al., 2005, McEvoy et al., 2009, Pérez-Prieto et al., 2011). Although Pérez-Prieto and Delegarde (2013) reported that the relationship between pasture allowance and intake as well as grazing behaviour was strong and was independent of estimation height, it may explain the differences found when results are reported. For example, when estimation height is from ground level, a daily pasture allowance of 20 to 25 kg DM/d is considered a low allowance (Wales et al., 1999) while it is considered high when measured from 5 cm (Delagarde et al., 2011). Consequently, differences in DMI as mass or allowance increase will be affected by the defined “low” “medium” and “high” thresholds.

In addition to these factors, supplementation of pasture-based diets has been reported to affect grazing behaviour and has been reviewed by Bargo et al. (2003). In general, it was reported that concentrate supplementation reduced grazing time, but did not influence biting rate or mass. There was also some evidence that bites per day and grazing time increased when given access to a fibre-based concentrate (beet pulp, barley etc.) rather than a starch-based concentrate (corn).

Given that herbage mass affects grazing behaviour (bite mass and time) and subsequently DMI, preference for pasture may be influenced by the provision of a high herbage mass that provides for an optimal allowance/cow and is maintained to promote a greater proportion of harvestable material.

1.6.2 Lactation stage

Dairy cow lactation is typically divided into three stages: early, mid, and late. During early lactation, peak milk production levels are achieved, but DMI cannot keep up with production so a nutrient deficit occurs (Weber et al., 2013). In order to mitigate the deficit between the nutrients that are required for milk production and the nutrients available from DMI body tissue is mobilised (Drackley et al., 2001). During mid-lactation peak DMI is achieved and the balance between available nutrients and required nutrients is restored (Fuller, 2004). After peak yield is achieved, milk production declines by about 2.5 %/week (Philips, 2010). During late lactation production levels continue to decline and body condition lost during early lactation should recover (Philips, 2010).

For primiparous cows, the lactation curve is different as they do not have the same capacity for milk production as multiparous cows and nutrients are utilized for weight gain as well as milk production (Philips, 2010). Therefore, their lactation curve is flatter and the increase to peak lactation is much less pronounced.

For high yielding dairy cows, the consumption of grass alone does not support their nutritional requirements. Leaver (1985) suggested that high producing dairy cows (> 25 kg/d of milk) were not able to achieve their maximum genetic potential for DMI under grazing conditions. Particularly, early lactation cows, even when grazing high quality pasture have difficulty achieving a sufficient DMI to support production levels and to mitigate health problems associated with excess mobilisation of body tissue to compensate for high milk yields (Herdt, 2000). Kolver and Muller (1998) reported that cows in early lactation, grazing a high quality pasture had a pasture DMI of 19.0 vs. 23.4 kg DM/d when cows were fed a TMR, corresponding to 29.6 vs. 44.1 kg/d of milk respectively. This difference is due to the intake rate of grazed herbage being lower than can be achieved from a TMR. On average, the intake rate of conserved forage is approximately twice the rate of grass intake at pasture (40 g DM/min vs. 20 g DM/min) (Philips, 2010). Therefore, it is generally agreed that high producing dairy cows on pasture need supplemental energy to achieve maximum intake and production levels (Bargo et al., 2003) and to help the body restore nutrient balance at a faster rate (Fuller, 2004).

Studies have reported that by manipulating daily herbage allowance (***see section 1.6.1)*** and providing supplementary concentrate at pasture, genetic potential for DMI (i.e. optimum performance based on genetic merit) can be achieved (Peyraud et al., 1996, Wales et al., 1998, Bargo et al., 2002a). However, these studies were conducted on cows in mid-lactation where metabolic demands are not as high and there is limited evidence that early-lactation cows, even when supplemented at pasture, can successfully achieve sufficient intake rates (McEvoy et al., 2008). Additionally, early lactation cows that are in their first lactation may have increased difficulty if they have not had previous experience of pasture (***see section 1.6.3***). Chilibroste et al. (2012) reported that early lactation, primiparous cows were not able to meet their energy requirements on grass even when supplemented due to low grazing times and bite rates associated with behavioral adaptation to pasture. Lactation number has not previously been shown to affect preference for pasture (***see section 1.5.5***), but it appears only three studies have attempted to determine an association between lactation number and preference, and the instances where dairy cows preferred to be at pasture cow were on average beyond their first lactation (mean lactation number of 2.6 and 2.3) (Charlton et al., 2011b, Charlton et al., 2013) while when cows preferred to be indoors the mean lactation number was 1.8 (Charlton et al., 2011a).

All preference studies that reported lactation stage have generally used dairy cows in late lactation: Legrand et al. (2009), Charlton et al. (2011a), Charlton et al. (2011b), Falk et al. (2012) and Charlton et al. (2013) reported mean days in milk (**DIM**) as 266 ± 81.8, 294 ± 19.6, 240 ± 8.46, 276 ± 72.6, and 270 ± 7.80 respectively in lactation typically expected to last for 305 d. As milk production begins to decline in late lactation and feed intake is greater than can be achieved in early lactation (Philips, 2010), dairy cows are more likely to achieve an intake level at pasture that will allow them to reach their genetic potential for yield. Therefore, putting dairy cows out at pasture under optimal conditions in late lactation would logically appear to be an acceptable management practice. As the preference studies offered access to ad libitum TMR as well as pasture it may be expected that cows would achieve higher DMI than they would if given only a TMR or a pasture-based diet at a similar lactation stage, but as none of the preference studies reported herbage intake this assertion is unclear, and warrants further investigation.

As late lactation cows can achieve a higher DMI than early lactation cows, and late lactation cows have decreased metabolic demands (Weber et al., 2013), it might be expected that they would spend more time at pasture than early lactation cows that may prefer to spend more time indoors consuming a TMR rather than use energy to graze. Further research on preference for pasture in early-lactation cows should be conducted to elucidate this.

Furthermore, if early-lactation cows can achieve an increased DMI by consuming a full TMR ration in addition to having access to pasture, and they prefer being at pasture, then pasture access for these cows may be a feasible management option. Bargo et al. (2002b) reported that Holsteins in early-mid lactation (109 ± 39 DIM) were able to achieve an overall DMI of 25.2 kg/d and produce 32.0 kg/d, when confined to pasture during the day, and housed with TMR during the night. Although this was less than milk production (38.1 kg/d) and intake (26.7 kg/d) reported with a TMR only diet, cows were not given 24 h access to pasture and TMR indoors as they would during a preference study which may have altered the results. Given this information, it is possible that both lactation number and lactation stage will affect preference for pasture and should be investigated further in a preference study.

1.6.3. Previous experience and preference

Previous experience and rearing has previously been shown to affect preference: hens tested for preference for unfamiliar or familiar conspecifics initially chose to spend more time with familiar conspecifics, but as they gained more experience with unfamiliar birds their preference changed, indicating that early preference was modified as a result of specific experience (Bradshaw, 2001). Similarly, hens given a choice between cages and open runs preferred the environment they had the most experience with. Hens reared in a garden, chose the open run, while hens reared in cages chose the cage. However, the preference of cage-reared hens changed with experience, and they eventually preferred to be in the run (Dawkins, 1980). Dairy cows reared in free-stalls bedded with sawdust preferred sawdust bedding over sand bedding, but cows reared on sand bedding did not show a preference for either substrate (Tucker et al., 2003). Dawkins (1983a) also reported that previous experience does not always affect preference: hens reared on wire flooring preferred litter flooring when given the choice between the two even when they had no experience of it.

The lone preference study to report that dairy cows preferred to be almost exclusively indoors over 24 h was conducted with dairy cows reared indoors and only given experience with pasture for 14 d prior to the study (Charlton et al., 2011a). Other preference studies reported about 5 months of exposure to pasture (Spring-Autumn) either during rearing (Krohn et al., 1992) or prior to the experiment (Charlton et al., 2011b, Charlton et al., 2013). Legrand et al. (2009) reported that study cows were kept on pasture as heifers and during previous dry periods while Falk et al. (2012) stated that cows were experienced with pasture. Therefore, although these studies suggest that previous exposure to pasture during rearing influences preference for pasture later in life, this should be investigated by directly comparing the preference of cows reared with and without previous experience of pasture.

1.6.3.1 The influence of previous experience on grazing behaviour The ability to engage in grazing behaviour may affect preference for pasture (***see section 1.6.1***), and a body of research has indicated that early life experience causes morphological, neurological, and physiological changes in animals which influence behaviour as adults (Provenza and Balph, 1990, Piersma and Lindstrom, 1997, Provenza et al., 1998). Experiences early in life for ruminants affect forage harvesting techniques, food preference, and adaptation to specific environments that they are reared in (Provenza et al., 2003).

Imprinting occurs during a specific period of time in an animals' life and once the necessary information has been acquired, it persists. This information cannot be obtained once the sensitive period is over (Hess, 1973). Initially, when calves are born and left with their mother, the innate "food finding behaviour" that occurs is teat-finding and suckling (Broom and Fraser, 2007). If given access to pasture immediately after being born, calves will begin to manipulate grass within the first few days of life and grazing time gradually increases during their first four months corresponding with the development of the rumen and weaning (Tucker, 2009).

Orphaned lambs exposed to forage shrub during 1 to 5 weeks of age (pre-ruminants, and would normally be dependent on their mother) or during 8-12 weeks of age (ruminants) spent less time browsing, had a slower bite rate, and consumed less of the shrub than lambs exposed to the shrub during 4-8 weeks of age (during the transition period to ruminants associated with weaning). In general, pre-ruminant lambs had more difficulty learning to consume the shrub (Squibb et al., 1990). It has been suggested that a sensitive period during weaning is adaptive as milk yield from the mother wanes and the animal is in transition between a monogastric animal to a ruminant: this is because learning may be enhanced by the urgency surrounding a lack of milk supply and positive feedback from the developing rumen may occur as a result of ingesting novel forages (Provenza and Balph, 1987).

Additionally, the rate at which goats developed foraging skills was affected by age: when goats were exposed to shrub blackbrush, bite rate increased with experience when exposed at both 6 months and 18 months. However, goats exposed at 6 months showed a much greater increase in bite rate than goats exposed at 18 months. After 30 d, the bite rate of goats exposed at 6 months and 18 months was 29 bites/min vs. 22 bites/min respectively (Ortega-Reyes and Provenza, 1993). Lobato et al. (1980) reported that sheep more readily accepted a novel food when they were young vs. mature and the willingness to accept a novel food decreased during the first year of life while Arnold and Maller (1977) reported that sheep reared on hay, spent 20% longer grazing when moved to rangeland than sheep reared on pasture, but intake was 40 % less. This suggests that skills manipulating particular foods were obtained during the rearing period, and grazing efficiency is affected by early life experience. Flores et al. (1989) reported that inexperienced lambs (given 15 times less exposure to a shrub than experienced lambs) were able to ingest pelleted food and cut shrub with a similar efficiency to experienced lambs, but lacked the physical skills associated with foraging (prehension ability) to be able to harvest forage at the same level as experienced sheep. Similarly, sheep reared for three years with no experience of grazing grazed much less efficiently and had lower intakes per hour of grazing than experienced sheep. Additionally, previous experience also affected sheep preference for specific pasture plants (Broom and Fraser, 2007). However, after 10 weeks of experience on pasture intake rates improved (Broom and Fraser, 2007) suggesting that initially, the lack of experience during rearing was detrimental to grazing ability, but over time grazing ability could still be acquired after rearing.

Provenza and Balph (1987) indicated that a sensitive period may exist during weaning, but it is less clear whether or not food imprinting actually occurs. What is clear however, is that young animals are able to learn more readily and more efficiently about which foods to eat than older animals and this is modulated in particular by social models. Although evidence exists that young animals can learn from any experienced mature animal, and will learn more efficiently with any experienced animal than when alone (Chapple et al., 1987b, Thorhallsdottir et al., 1990), the mother is generally considered the best model by which to learn about food due to the bond developed between mother and young that would not normally develop with another adult (Nowak et al., 2008). Young animals must both observe their mother and engage in exploratory behaviour (investigation of the food source and imitation of the mother's behaviour) in the presence of their mother to learn which foods to avoid and which foods to consume (Thorhallsdottir et al., 1990). Interestingly, while exploratory behavior is essential to learning, actual ingestion does not necessarily need to take place (Lynch et al., 1983). Additionally, there may be an interaction between previous grazing experience during rearing and the presence of an experienced animal that promotes increased grazing time, bite rate and rumination in lambs turned out at three months of age after weaning (Phillips and Youssef, 2003).

Goats reared with their mother from 1-4 months of age on blackbrush consumed 2.5 times more than goats without experience of blackbrush when tested as adults (Distel and Provenza, 1991), and lambs given exposure to wheat at six weeks of age for one hour/day for five days consumed more wheat as adults than both adult sheep exposed to wheat early in life without their mother and sheep that were never exposed to wheat (Green et al., 1984). Additionally, sheep that had no prior experience with wheat had to acquire skills associated with wheat consumption including how to prehend, chew and swallow it

(Chapple et al., 1987a). Ramos and Tennessen (1992) reported that exposure to grazing before or after weaning had no effect on subsequent grazing success, but lambs given exposure to grazing with their dams grazed for twice as long as lambs without exposure to grazing and preference for white clover or ryegrass pasture was affected by experience.

Individual learning is also a recognized process by which ruminants develop their foraging ability and dietary preference. Young animals develop preferences for particular feed types as a result of their experience with sensory, nutritional, and physiological consequences associated with each feed (Arnold and Maller, 1977). Booth (1985) and Burritt and Provenza (1989) reported that young livestock will learn which foods to eat and which to avoid by ingesting small amounts of a novel food and incrementally increasing the consumption if there are positive nutritional consequences and no adverse effects due to either nutrient deficiency or toxicity of the plant. Distel and Provenza (1991) reported that goats were able to remember positive or negative consequences associated with particular foods for up to three years, and Provenza and Balph (1987) asserted that individuals could acquire the ability to seek out specific feed to supplement diets that were nutritionally deficient. This would potentially explain why in the same environment, in addition to needing to acquire motor skills associated with grazing (Provenza and Balph, 1987), inexperienced sheep and goats (Arnold and Maller, 1977, Gluesing and Balph, 1980, Provenza and Malechek, 1986) and cattle (Pfister et al., 1997) may graze less than experienced animals as they are gaining experience with the consequences of ingesting particular feeds.

1.6.3.2 Previous experience and cattle. As discussed in ***section 1.6.3***, a large body of knowledge exists in sheep and goats regarding the effect of early experience with food on subsequent behaviour later in life. Additionally, the majority of this research has been conducted on rangeland pastures with variable sward types and quality. In general, early experience as a young animal with grazing both in the presence of their mother and in the absence of their mother promotes grazing efficiency and encourages specific dietary preferences later in life. Limited research has been conducted with cattle, and even less on dairy cattle grazing high quality pasture.

Beef heifers allowed to graze from breeding to weaning during the winter retained grazing skills into the next grazing season and had greater average daily gains than heifers reared according to normal practice (moved to a dry-lot after weaning with no access to grazing) (Olson et al., 1992). Similarly, Wiedmeier et al. (2002) reported that beef calves exposed to straw as calves, consumed more straw as adults and also maintained better body condition, produced more milk, and were able to be bred back sooner than inexperienced cows. Orr et al. (2013) reported that beef calves reared extensively with their dams performed better as adults on unimproved grassland than calves reared intensively, potentially due to the ability of the experienced animals to forage more efficiently. Finally, Boland et al. (2011) revealed that the preference of beef steers for alfalfa was affected by prior experience with this forage.

Specific to dairy cows, (Hodgson, 1971) reported that male Friesian dairy calves given access to pelleted herbage or untreated dried herbage prior to weaning subsequently had an increased intake of the untreated dried herbage post-weaning compared to those without prior experience, but no differences were reported with the pelleted herbage. Phillips (2004) reported that the provision of cut perennial ryegrass to female calves (Friesians crossed with various beef breeds) increased grazing time at turnout and reduced biting rate when compared to calves without the provision of grass—suggesting that experience of grass (even when it was not grazed) helped promote grazing after weaning. In contrast, Miller-Cushon and DeVries (2011) reported that early exposure to specific feed types at birth for Holstein bull calves affected diet selection just after weaning, but did not have a lasting influence as all calves developed a similar diet selection after four weeks.

1.6.3.3. Heifer rearing and subsequent effect on grazing ability and preference. Although it is evident from ***section 1.6.3*** that young ruminants acquire skills from their mother, modern dairy calf management typically dictates that the calf is removed from their mother soon after birth in order to prevent disease transmission and milk loss (Webster, 1996). Although management varies, in temperate regions like the UK, calves born outside the grazing season (Autumn-born) are generally fed indoors for about 6 months: initially hay and straw is provided, and post-weaning either silage or a TMR is provided (Philips, 2010). As they age, the rumen microflora becomes optimized for the silage or TMR diet (Philips, 2010). In the summer they are typically turned out to pasture either with or without buffer feed (Garnsworthy, 2005). Spring-born calves may be given access to pasture from the first few days of their life (Tucker, 2009). Growing heifers at pasture require high quality pasture and an appropriate herbage allowance to maintain body condition and ensure optimum growth but often they do not receive the same accurately rationed pasture allowance that lactating cows are provided with (Philips, 2010). As discussed in ***section 1.6.1*** cows must work harder at lower allowances to achieve sufficient intake—this might prove particularly difficult if heifers are also learning to graze.

If heifers are reared indoors initially without prior experience of grass and then subsequently turned out without buffer feed, it is likely that an adaptation period is necessary where they develop the motor skills associated with grazing which are quite different from the skills associated with consuming conserved forage. Grazing is a complicated process by which cattle collect herbage into their mouth using high mobility of their tongue (Broom and Fraser, 2007). It is compressed against the dental pad with both their tongue and lower incisors and subsequently severed from the plant with an upward jerk motion of the head (Ekesbo, 2011). This comprises one bite, and after a series of these bites are completed, cattle will chew and swallow the plant material (Broom and Fraser, 2007). As lactation number increases, and udders become larger and teats fatter, dairy calves have difficulty in finding the teats—a problem that is rare for beef cattle or any other farm animal (Broom and Fraser, 2007). If calves have difficulty with the initial hard-wired “food-finding” behaviour, lack of experience of grazing compounded with potentially poor pasture allowances, no buffer feed, and in the absence of their mother might lead to both negative welfare and production consequences. Recently, Chilibroste et al. (2012) reported that inexperienced primiparous cows had difficulty maintaining milk yield compared to multiparous cows under grazing conditions, potentially due to a slower adaptation period to grass: grazing time (< 35%) and bite rate (< 25 bites/min) were low even at a high herbage allowance. Additionally, abrupt changes in diet (indoor ration to pasture) also require a period of adaptation where rumen microflora adapts to the new diet which may take from three days to three weeks (Grubb and Dehority, 1975).

To date, it appears only one study has assessed the effect of prior experience of pasture on subsequent adaptation to pasture in dairy cows. Lopes et al. (2013) conducted a study investigating the effects of being reared on high quality pasture on subsequent adaptation to grazing as dairy heifers and lactating cows and reported early experience did affect both grazing behaviour and performance but that heifers without experience of pasture showed similar grazing behaviour to experienced heifers after one day, and lactating cows needed a longer adaptation period, but they expressed similar behaviour and production levels after three days. Even though inexperienced heifers and lactating cows expressed similar performance and behaviour in less than one week, Lopes et al. (2013) recommended an adaptation period of 10-14 d for both inexperienced and experienced heifers to fully adapt to pasture and for performance to stabilise. It is interesting therefore, that in the study of Charlton et al. (2011a) when cows were given an adaptation period of 14 d where cows were housed on pasture for 20 h/d prior to being tested, they still expressed a preference to be indoors. Additionally, Lopes et al. (2013) conducted their study using both Holstein and Holstein x Jersey crosses but did not assess a breed affect. As Jersey cows are better adapted to utilize grazing to achieve maximum intakes and milk yields than Holstein-Friesians, this may have affected adaptation to grazing (Prendiville et al., 2010). Research also suggests that Holstein-Jerseys in particular are well suited to intensive grazing environments when compared to Holstein-Friesians as evidenced by increased intake capacity at pasture (Prendiville et al., 2009). Further research is necessary therefore, to understand how previous experience relates to both grazing behaviour and preference for pasture for high-yielding Holstein-Friesian dairy cows.

1.6.4 Lying behaviour

As reported in ***section 1.3.1***, lying behaviour is one of the three most common behaviours dairy cows engage in. Reduced lying times are associated with injuries (Rushen et al., 2007) and Munksgaard et al. (2005) reported that when deprived of lying, dairy cows will give up the opportunity to feed in order to lie down, providing insight into the importance of this behaviour for dairy cows. Lying deprivation is also associated with behavioural and physiological indicators of stress which can have negative long term impacts on both welfare and production (Munksgaard and Simonsen, 1996, Fisher et al., 2002). Therefore, total lying time in dairy cows can be a useful indicator of welfare (Fregonesi and Leaver, 2001) and it has been suggested that high yielding dairy cows must optimize resting times to ensure adequate welfare and production levels (Rushen et al., 2007). Dairy cows show distinct preferences with regard to lying environment: they prefer dry bedding to wet bedding (Fregonesi et al., 2007), and spend more time lying down on soft substrates (Tucker et al., 2003, Drissler et al., 2005).

Some evidence exists that lying behaviour is connected to milk yield. Rulquin and Caudal (1992) and Metcalf et al. (1992) reported that mammary blood flow to the udder increased by 24 and 28 % respectively when dairy cows were lying down vs. when they were standing. Since the necessary substrates for milk synthesis are provided by blood flow to the udder (Davis and Collier, 1985, Prosser et al., 1996), blood flow is deemed particularly important for milk yield. Despite being widely cited, both studies assessing blood flow and lying behaviour were conducted with very low sample sizes: Rulquin and Caudal (1992) used only one lactating cow and Metcalf et al. (1992) used five. Additionally, studies directly assessing the correlation between blood flow and milk yield have also used very small sample sizes. Peeters et al. (1979) conducted their study on three cows and Delamaire and Guinard-Flament (2006) used four cows, while studies with larger sample sizes (40 cows) only found a moderate correlation between blood flow and milk yield and reported a large variation in mammary blood flow among cows (Götze et al., 2010). Given this information it is unclear whether there is a direct association between lying behaviour and milk yield, although reductions in lying time still have indirect effects on production (Rushen et al., 2007).

Preference studies that reported a preference for pasture at night, or increased pasture-usage at night have generally suggested that this may be the case due to pasture being a more comfortable lying area for dairy cows and they choose to access pasture at night vs. the day because that is when the majority of their resting behaviour occurs (Tucker, 2009). Falk et al. (2012) suggested that having an unconstrained lying surface might be a factor that encourages lying on pasture as cows are less restricted by cubicle design and cannot lie down in stretched positions. Cows have been observed to lie in as many as 410 different lying positions when given an unconstrained lying area such as loose housing (Endres and Barberg, 2007). In contrast, Haley et al. (2000) reported that reduced lying time in restrictive tie-stalls vs. loose-housing in box stalls (which would allow for stretched lying positions) was not a function of the ability to lie down in specific lying postures.

Of the preference studies reported in ***section 1.5***, only Krohn et al. (1992), Legrand et al. (2009), and Falk et al. (2012) reported that cows preferred to lie down at pasture. In contrast, Krohn and Munksgaard (1993) reported that cows spent less time lying down at pasture vs. deep bedding and Charlton al. (2011a) reported that cows spent more time lying indoors. Although Falk et al. (2012) and Legrand et al. (2009) used cubicle housing indoors, the lying substrate was deeply bedded sand which has been shown to be a comparably comfortable substrate for dairy cows as the deeply bedded straw used in the experiment conducted by Krohn and Munksgaard (1993) if they have prior experience with sand (Norring et al., 2008). It is interesting therefore, that cows would prefer to lie down at pasture vs. sand, but would find straw more comfortable than pasture. It should be noted however, that while sand bedding promoted normal lying times in dairy cows, and prior experience with sand promoted acceptance of sand as a bedding material, straw bedding was still preferred over sand (Norring et al., 2008), which may help explain some of the conflicting results. Additionally, the cows used by (Charlton et al., 2011a) preferred to be indoors over a 24 h period which would explain their preference to lie down indoors. Charlton et al. (2011b) reported no difference in lying time at pasture vs. indoors in cubicle housing, while Charlton et al. (2013) reported that there was a difference in time spent lying on pasture (16.6 ± 2.32 %), on the track (3.4 ± 1.08), and indoors (25.0 ± 2.72) but it was not reported what the difference actually was. Based on the reported standard error of the mean (**SEM**) values, it appears that all three means were significantly different. Given this information, further investigation of preference for lying on pasture or indoors in cubicles is necessary, and to further elucidate why a particular environment might encourage lying behaviour, lying posture can be quantified. It has been suggested that particular lying positions, especially with the head curled back onto their flank, or resting on the ground, and lying completely flat on their sides is important for comfortable sleep and is associated with REM sleep (Merrick and Scharp, 1971, Albright and Arave, 1997).

Limited studies have quantified lying postures in dairy cows: (Krohn and Munksgaard, 1993, Haley et al., 2000, Haley et al., 2001, Endres and Barberg, 2007). In general, the most common lying position was with their heads upright, front legs tucked underneath the body, and one hind leg tucked under and one not fully tucked under or fully extended. Although observed, lying completely flat out on their sides was uncommon in all studies. Only Krohn and Munksgaard (1993) recorded lying postures at pasture however, and only one study recorded postures in free –stalls (Haley et al., 2000). Endres and Barberg (2007) assessed lying posture in a loose-housing system which may be comparable to lying postures assumed at pasture, but none of the studies have directly compared lying posture in free-stalls vs. pasture. It is unclear therefore whether or not there are differences in lying posture between free-stall housing and pasture, and if this is potentially another factor that influences where dairy cows prefer to spend their time. Further research is therefore necessary to elucidate this.

1.6.5 Production and economic implications of confinement, grazing, and combined systems

A number of studies have assessed milk production per cow (Holstein-Friesians) in confinement systems vs. grazing systems and the majority have reported that production is lower in grazing systems. (Kolver and Muller, 1998) reported that cows on pasture (without supplementation) produced 15 kg/d less than cows confined with a TMR. Tucker et al. (2001) reported that as cows transitioned from mid-late lactation, cows confined and fed a TMR had consistent milk yield (22.5-27.2 kg/d) while cows at pasture decreased from 26.6 to 15.9 kg/d. Bargo et al. (2002b) reported that cows on pasture (with concentrate supplementation) consumed 21.6 kg DM/d and produced 28.5 kg/d when compared with 26.7 kg DM/d and 38.1 kg/day in a confinement system with TMR. White (2002) reported that pasture-based cows (supplemented with grain and haylage depending on herbage availability) produced 11.1 % less milk than confinement cows with a TMR. However, this does not necessarily mean, that overall, grazing systems are less profitable. It has also been reported that although production levels were consistently higher in confinement systems, they also incurred the most operating expenses and particularly when net farm income per cow and the rate of return to assets on pasture-based vs. confinement systems was compared, pasture-based systems were an economically viable option (Winsten et al., 2000). Tucker et al. (2001) concluded that although milk production was reduced in pasture based cows, the grazing system was still economically competitive when compared to the confinement system due to the decreased feed costs, and White (2002) concluded that the loss of milk yield was offset by other economic factors including culling rates, feed costs, and labour which all favoured the pasture-based system. Given this information, both systems appear to be economically viable, although they are more likely to be viable in areas where there is ample available grassland (e.g. New Zealand, Australia, Ireland) (Philips, 2010) or areas with legislation mandating cows must be at pasture (Sweden, Finland, Netherlands) (Pol-van Dasselaar et al., 2008). With herd sizes generally increasing and grazing land being scarce it is difficult to incorporate pasture management into areas without availability.

Some research has investigated production and profitability of combined systems (both TMR and pasture). Soriano et al. (2001) compared the performance and profitability of feeding lactating Holsteins in three systems: a TMR only diet, pasture in the morning (8 h/d) and TMR in the afternoon, and TMR in the morning vs. pasture in the afternoon (8 h/d). The TMR only treatment consumed the greatest amount of feed (26.6 kg DM/d) and had a greater BCS change than either of the other two treatments (0.14). However, it was concluded that both pasture treatments were more economical when compared to the confinement only system, with the TMR in the morning and pasture in the afternoon treatment have an 18.6 % higher income-over-feed cost.

Tozer et al. (2003) argued that Soriano et al. (2001) underestimated the total cost of pasture as they did not allow for less than 100 % pasture utilization or take into account costs for pasture establishment and maintenance, thereby overestimating the net returns. In order to overcome these limitations, Tozer et al. (2003) conducted a similar study with three treatments: pasture plus concentrate, TMR only, and pasture between am and pm milking and TMR during the night. (Tozer et al., 2003) reported that although the expenses per day on the TMR only treatment were the highest ($ 4.12), the TMR only treatment had the highest net income per cow per day due to having the highest milk yield (38.1 kg/d) and optimum levels of the different milk components (1.24 kg /d fat and 1.13 kg/d of protein). The pasture plus concentrate system was found to be generally less profitable than the pasture plus TMR system.

It is important to consider that the methods used in these studies differed considerably (sward type, average herd production, year-round vs. limited grazing seasons) and as such are difficult to compare directly. Additionally, Tozer et al. (2003) noted that the profitability of each treatment system is affected by milk and milk component prices. Although less than in TMR only treatments, both Soriano et al. (2001) and Tozer et al. (2003) still reported high intakes and milk yields when cows had both TMR and pasture. It should be noted that cows in these studies were restricted as to when they could consume either TMR or be at pasture. Additionally, both studies also only provided TMR during the night, when generally cows spend the majority of their time resting (Tucker, 2009). Giving cows unrestricted free access between pasture and indoor housing with ad lib TMR may therefore positively affect intake, milk production level, and subsequently profitability. To date, there are no studies comparing the production differences in dairy cows with free access to pasture and ad lib TMR vs. confined dairy cows with TMR. Further research is necessary to determine the viability of a system where unrestricted access to high quality grass (via the management of herbage mass) as well as TMR is provided.

As discussed in this literature review, the current preference studies to date have a number of gaps in the knowledge surrounding dairy cow preference for pasture, and the remainder of this thesis will attempt to systematically address some of these gaps.

1.7 THESIS OBJECTIVES

1. To determine whether a high vs. low herbage mass influences dairy cow preference to be indoors or at pasture, and if there is an interaction between herbage mass and distance.

2. To determine whether behavioural and/or production differences exist between cows that have free access to pasture (with *ad lib* access to TMR indoors) and cows that are continuously housed.

3. To determine whether previous exposure to pasture during rearing influences dairy heifer and dairy cow preference for pasture (and grazing behaviour) and to elucidate if a sensitive period exists where dairy cows are encouraged to use pasture.

4. To characterize lying postures on pasture vs. in cubicle housing

CHAPTER 2: Effect of herbage mass and distance on preference for pasture and behavioural and production differences between lactating Holstein Friesian cows with free access to pasture vs. housed cows

2.1 INTRODUCTION

Dairy cows have evolved as grazers, so providing access to pasture allows the expression of natural behavior (Rutter, 2010). Modern cattle however, have gone through genetic selection which has increased milk yields, and resulted in higher nutrient requirements than their ancestors (Webster, 1996). As a consequence, cows may not be able to meet their nutritional demands from grass alone (Fike et al., 2003). Additionally, grass growth is seasonal and climate and soil conditions often require cows to be housed for at least part of the year in some parts of the world (O' Driscoll et al., 2009). Therefore, it has been general practice to house animals over the winter, but some dairy management practices include housing cows all year round (Haskell et al., 2006). Indoor housing provides greater control over feed intake which helps to maintain high production levels. Total mixed rations are thought to facilitate a more balanced and constant nutrient intake for lactating cows (Coppock et al., 1981) as keeping cows on pasture can result in a loss of body weight and also cause a reduction in milk yield compared to continuously housed cattle (Fontaneli et al., 2005), due to the DMI rate of grazed herbage being lower than can be achieved from TMR (Kolver and Muller, 1998). Although cows can compensate for a lower intake rate of grass to some extent by increasing the time spent grazing (Wims et al., 2014), this compensatory increase will fall when the time for high priority activities begins to suffer as a result (i.e. ruminating time) (Rook, 2000).

Preference testing, or giving the animal an opportunity to choose between resources, provides insight into how animals perceive their environment and is a common method of assessing animal welfare (Webster, 1996). There is emerging evidence that dairy cows prefer to be at pasture and a number of factors influence this preference including weather, BCS, season, time of day, and distance (Legrand et al., 2009, Charlton et al., 2011a, Charlton et al., 2013, Falk et al., 2012). Herbage mass in these studies was maintained at a wide range, sometimes differing by more than 1000 kg DM/ha throughout the study. It has been reported that manipulating herbage mass (McEvoy et al., 2009) and herbage allowance (a function of herbage mass) affects pasture DMI (Moate et al., 1999) and grazing time in dairy cows with restricted access to pasture (Perez-Ramirez et al., 2009), so it may be an important driving factor in dairy cow preference to be indoors or at pasture. The objectives of the current study were to determine to what extent herbage mass influenced dairy cow preference and motivation for access to pasture.

It was hypothesized that herbage mass would influence preference for pasture, with cows spending more time on pasture at the high vs. low mass.

2.2 MATERIALS AND METHODS

Ethical approval for this study was given by the Harper Adams University (**HAU**) Research Ethics Committee.

2.2.1 Animals and management

Thirty two (12 primiparous and 20 multiparous) in-calf, mid-late lactation, Holstein-Friesian dairy cows that were 229 ± 82.9 (mean ± SD) DIM, producing 34.1 ± 6.98 kg/d, with a locomotion score (**LS**) (Flower and Weary, 2007) of 2.19 ± 0.64, BCS of 2.5 ± 0.35 (Edmonson et al., 1989), and a weight of 670.8 ± 69.4 were chosen from the HAU dairy herd for this study. Sixteen animals were tested during study period A: July 16 to August 21, 2011. The second set of 16 animals was tested during study period B: August 24 to September 29, 2011. Animals were randomly allocated to 3 treatment groups during each study period: Group 1 (n=4) and Group 2 (n=4) were given free choice to move indoors and outdoors (free-choice cows) and Group 3 (n=8) was continuously housed (housed cows). The groups were balanced for milk yield, DIM, BCS, and LS at the start of each period. These variables were all still balanced at the beginning of each measurement period.

Study cows were milked with the main herd, and after both AM and PM milkings (approximately 04:30h and 15:30h), study cows were automatically separated into a holding area via an automatic segregation gate (GEA Farm Technologies Bönen, Germany). At approximately 06:45 and 16:30 h, study cows in group 3 were led into their housing area which they shared with limited members of the main herd that were not used as part of the experiment (611m^2 free-stall housing; 1.5 cubicles (2.7 x 1.2 m)/cow with 3 cm thick rubber mattresses, bedded with lime as 3x/week, Fig. 1) with *ad libitum* access to TMR composed of 6.60 kg DM/head (**hd**) maize silage, 2.40 kg DM/hd grass silage, 1.68 kg DM/hd Lucerne silage 1st cut, 0.43 kg DM/hd chopped wheat straw, 1.49 kg DM/hd Rouxminate, 0.17 kg DM/hd Rouxminate Premix, 4.00 kg/hd water, 2.95 kg DM/hd rape blend, 2.43 kg DM/hd Alkagrain, 2.03 kg DM/hd soya hulls, 1.52 kg DM/hd hipro soya, 0.30 kg DM/hd ruminer fat, 0.15 kg DM/hd dairy minerals, 0.10 kg DM/hd limestone flour, 0.10 kg DM/hd acid buffer, 0.04 kg DM/hd Vistacell 8 % yeast, 0.05 kg DM/hd feed grade urea (407 g/kg DM, 175 g/kg DM crude protein (**CP**), and 356 g/kg DM neutral detergent fibre (**NDF)** from 12 electronic RIC feed bins (1.0 x 0.9 x 0.8 m; Insentec, Marknesse, the Netherlands). Water was provided *ad libitum* at either end of the allotted area from water troughs.

Study cows in groups 1 and 2 were led to their indoor area where they were physically separated from the main herd (they were still able to engage in auditory, visual, and limited physical contact) which was at the end of the indoor housing facility (210 m^2 divided into two equal areas by a swing gate, free-stall housing: 1.5 cubicles (2.7 x 1.2 m)/cow with 3 cm thick rubber mattresses, bedded with lime ash 3x/week). Here they had access to *ad libitum* TMR via 4 feed bins (0.7 x 0.6 x 0.4 m) placed inside 4 (1.2 x 1.4 m) Calan gate feeders (American Calan, Inc., Northwood, NH) on each side (8 calan gates in total, 4 per group). Water was available *ad libitum* from two water troughs on each side of the swing gate. After being separated into their respective areas, gates were opened to allow cows access outside onto a track that lead to the pasture (predominantly a mix of perennial ryegrass and white clover, Figure 1) at approximately 07:30 h.

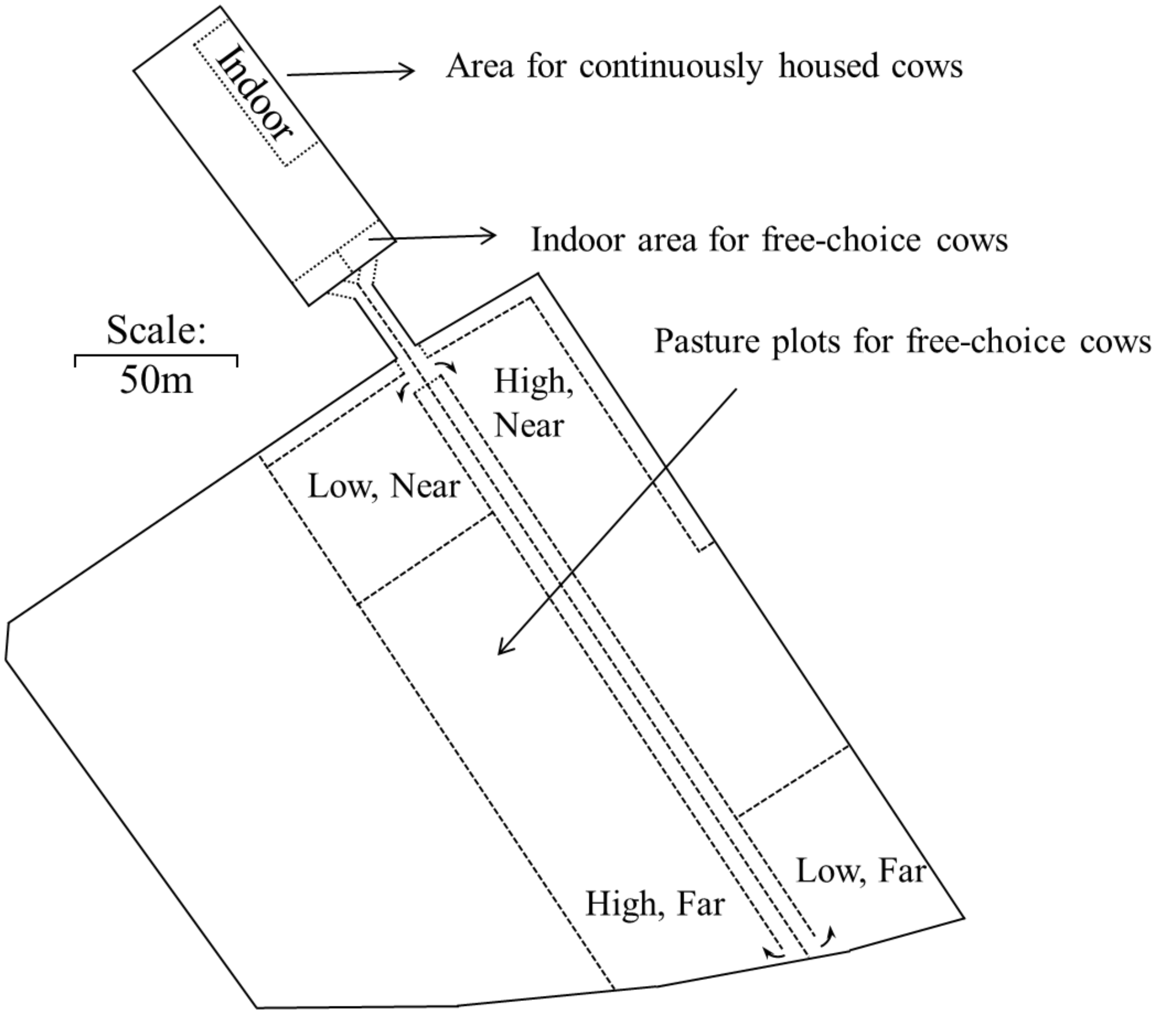


Figure 1. Plan of the experimental area showing the position of the indoor housing for the continuously housed cows and the indoor housing for cows with free access to pasture in relation to the near pasture at 38 m from the housing (with a high—3000 ± 200 kg DM/ha vs. low—1800 ± 200 kg DM/ha herbage mass) and the far pasture at 254 m from the housing (with low vs. high herbage).

The 1.8 ha field used for the study was cut for silage on May 19, 2011 and 20 kg/ha nitrogen (N) (Lithan 34.5%N) applied. Application of N was continued at this rate on a monthly basis throughout the trial. On average over each study period, herbage offered consisted of 243 g/kg DM, 122 g/kg DM CP and 534 g/kg DM NDF. Electric fencing was erected to divide the study area into four plots (Figure 1): high herbage mass (maintained at 3000 ± 200 kg DM/ha) at 38m from the cubicle housing (high/near), high herbage mass at 254 m from the cubicle housing (high/far), low herbage mass at 38m (low/near) and low herbage mass (maintained at 1800 ± 200 kg DM/ha) at 254m (low/far). Note that herbage mass here is defined as the total mass of herbage per unit area of ground (Allen et al., 2011). The high herbage mass was chosen to provide lush pasture for cows to consume all of their feed from grass alone if they chose and the low herbage mass was chosen to allow for grazing and for use as a loafing paddock. Additionally, herbage masses were chosen to try and account for the extremes offered in previous preference studies discussed in ***section 2.1***.

Each herbage mass was offered at two distances in order to determine dairy cow motivation for access to pasture as well as to investigate whether an interaction existed between herbage mass and distance. Distances were chosen to offer the nearest and furthest possible access to pasture from the cubicle housing area. The high herbage mass plots were approximately 0.66 ha and the low herbage mass plots approximately 0.22 ha. All four plots had self-filling water troughs, and the troughs in the 0.22 ha plots straddled the fence-line of the 0.66 ha plots, so cows in the high herbage plots would have access to water at both the top and bottom of the grazing area. Additionally, a back-fence was installed in the high herbage mass plots to ensure consistent herbage mass throughout the study. An electronic rising plate meter with a built in regression equation (Herbage mass = [sward height (cm) x 125] +640, Farmworks, F200, New Zealand, plate meter reading) was used for 2 months prior to the start of the study and during the study to monitor herbage mass daily. The plate meter was placed randomly around the field 30 times in a W pattern, with 5 paces between each measurement. Three weeks prior to the start of the study, 11 heifers were used to graze down the low herbage areas, and 60 low-yielding cows from the HAU milking herd were used to graze down the high herbage areas, in order to have the required herbage mass for each plot at the start of the study. Additionally, the field was topped throughout the study to maintain appropriate herbage mass. In practice, the high herbage mass plots ranged from 2740 to 3209 kg DM/ha while the low herbage plots ranged from 1634 to 2208 kg DM/ha.

2.2.2 Experimental routine

2.2.2.1 Training. Each group of cows was given a ten day training period in order to ensure that each animal could access their individual feed bin via a Calan gate without assistance. Free-choice cows were also trained to use the indoor and pasture experimental area (described in ***Animals and Management)***. Free-choice cows were locked into the housing area after milking during the first 5 d during which time they were trained to access their individual Calan bin. During the last 5 d of training cows were herded up the track into the area of pasture they would have access to during the measurement period. They were left for one hour intervals, approximately 3x/d and periodically observed and herded in and out until all cows successfully entered and exited on their own. Cows were given free access to pasture at night. Cows were also given a seven day re- training period to the feed bins on the opposite side of the building (to allow them access to the remaining 2 plots—***section 2.2.1***) in the same manner.

2.2.2.2 Measurement period. Following each training period, all groups had a five day measurement period where groups 1 and 2 (or 4 and 5) had access to one of the four plots, while group 3 (or 6) remained indoors (Table 1). During each measurement period, groups 1 and 2 were provided with different herbage masses, but were always at the same distance from the housing in order to remove weather effects. A Latin square was used to allocate each group to the order of low/high herbage and near/far distances (Table 1).

Table 1. Group allocations to high (3000 ± 200 kg DM/ha) or low (1800 ± 200 kg DM/ha) herbage masses at near (38 m) or far (254 m) distances and to the continuously housed group during study period A (July 16 to August 21) and B (August 23 to September 29)

Group	Study Period	Training period	Measurement period		Training period	Measurement period	
		d 1-10	d 11-15	d 16-20	d 21-27	d 28-32	d 33-37
1	A		high/near	low/far		low/near	high/far
2	A		low/near	high/far		high/near	low/far
3	A		CONTINOUSLY HOUSED				
4	B		high/near	low/far		low/near	high/far
5	B		low/near	high/far		high/near	low/far
6	B		CONTINUOUSLY HOUSED				

2.2.3 Measurements

2.2.3.1 Total time spent at pasture or indoors. A Voltek night vision video camera (KT&C Co Ltd, Seoul, South Korea) connected to a digital video recorder was set up to continuously record cow movement from indoor housing to the track.

2.2.3.2 Manual behavioral observations. Observations occurred on days two and four of each measurement period. Observations took place between 10:00 to 14:00 h, on day 2 and from 18:00 to 22:00 h on day four. Five minute scan sampling was used. Location (indoors, track, or pasture), posture (lying, standing, walking), and jaw activity (grazing, ruminating, eating TMR, drinking and idling) were recorded for each cow via manual observation (Charlton et al., 2011a). Four observers were responsible for recording behavioural activity over the course of the experiment and inter-observer reliability was measured at 100 % prior to the first measurement period. One observer recorded behavioural activity for the free-choice cows while another observer simultaneously recorded activity for the housed cows during the measurement periods.

2.2.3.3 TMR intake. Access to TMR for Group 1 and Group 2 (or 3 and 4) was controlled using Calan gates. Each cow wore a Calan collar with a transponder which only allowed access to a specific feed bin. Refusals were disposed of at 08:30 h every morning and were weighed during measurement periods. A weighed amount of fresh feed was provided at 09:30 h daily at 105% of *ad libitum* intake. Feed intake (TMR) for continuously housed cows (Groups 3 or 6) was measured using RIC bins (Sinclair et al., 2005). Fresh feed was provided at approximately 10:00 h daily at 105% of *ad libitum* intake, and refusals were removed 3x/week at approximately 08:00 h.

2.2.3.4 Herbage intake. Herbage intake was estimated using dosed n-alkanes following the method described by Mayes et al. (1986) and Dove and Mayes (2006) with the following modifications. Cows with access to pasture were dosed with 2 g of n-alkane C_{32} (dotriacontane- Minakem®, France) twice daily from the start of each training period to the end of each experimental period. Cows were given their dosage just after their morning milking at 06:30 h, and just prior to their afternoon milking at 14:30 h. The dose was placed directly on top of the TMR ration inside each individual Calan bin. Each cow was watched carefully to ensure they ingested the full dosage.

At 04:00 h, 14:00 h, and 18:00 h naturally-voided faeces (approximately, 100 ml) were collected from the ground from each individual cow during the last 3 days of each measurement period. In practice, at 04:00 h and at 14:00 h samples were collected almost immediately as cows were usually lying down and generally defecated after standing up when disturbed to go for milking. At the 18:00 sampling time however, the sampler sometimes waited until 20:00 h to collect a sample. Additionally, 3 herbage samples were collected using a circular quadrat (962 cm^2) placed randomly in sections of each of the plots being grazed. Samples were cut down to ground level using scissors. One TMR sample was collected on day 3 of each measurement period for subsequent n-alkane and nutrient analysis. Samples for nutrient analysis were analysed immediately for DM content and then bulked, ground and stored for CP and NDF analysis at a later date (see ***nutrient analysis of TMR and herbage***). Samples used for the estimation of herbage intake were stored in a freezer at -20 °C and subsequently freeze-dried, bulked/cow/day, and ground for analysis at a later date (see Appendix 1).

2.2.3.5 Weather Conditions. A Davis Vantage PRO2 weather recorder (Hayward, California USA) was used to automatically record weather conditions indoors and at pasture. Ambient temperature (°C) and relative humidity (%) were recorded indoors while ambient temperature (°C), relative humidity (%), and rainfall (mm) were recorded outdoors every 15 s for the duration of the study. The temperature-humidity index (**THI**) was calculated as THI = (1.8T + 32) – [(0.55 – 0.0055RH) x (1.8T – 26)] (NOAA, 1976), with T = ambient temperature and RH = relative humidity.

2.2.3.6 Performance. During each experimental period, milk yields for each animal on study were recorded at each milking by an automatic recording system (GEA Farm Technologies, Bönen, Germany). At the beginning and end of each study period, milk samples were taken for fat, protein, and lactose percentages and analysed using near-infrared spectroscopy (Milkoscan Minor, Foss, Hillerød, Denmark).

2.2.3.7 Nutrient analysis of TMR and herbage. For DM determination, samples were weighed directly after collection and immediately oven dried to constant weight at 105 °C. The concentration of CP in the samples was measured by combustion using a LECO FP 528 N analyser (Leco Corporation, St Joseph, MI, USA, AOAC 2000). The concentration of NDF was determined according to the methods of Van Soest et al. (1991).

2.2.4 Statistical design and analysis

The experiment was a 2x2 factorial “within subject” crossover design (groups 1, 2, 4, 5) with a separate control (group 3 and 6). All data that were not normally distributed were transformed using an arc-sin transformation to improve the distribution of the data and subsequently analysed using parametric tests (means reported are of untransformed data).

Overall time spent indoors or at pasture is expressed as a percentage of the total time offered a choice (cows were offered a choice between being indoors or at pasture for approximately 18 h/d—this excludes time being milked). Overall preference for pasture was determined using a one-sample t-test: the percentage of time spent indoors and at pasture was analysed to determine if it was significantly different from 0 (choice to be indoors), 50 (indifference or random choice), and 100 (choice to be outside) % (Charlton et al., 2011a). Analysis of daytime behavioral activity in the cows given access to pasture was conducted using a two-way analysis of variance (**ANOVA**) in GenStat (12^{th} edition; Lawes Agricultural Trust Co. Ltd., Rothamsted, UK) in order to determine effects of herbage mass and distance. The model was created to find a treatment effect, a distance effect, a treatment x distance interaction, and blocked by cow group. Effects of herbage mass and distance on milk yield, milk composition, TMR intake and herbage intake were also analysed in this manner. Comparisons between the behaviour, feed intake, milk yield, and of continuously housed cows and cows given access to pasture at both the near and far pasture were made using a one-way ANOVA followed by a Post-hoc Tukey test. Since no effect of herbage mass was observed, the continuously housed cows were compared separately with those given access to the near pasture and the cows given access to the far pasture. Linear regressions were used to determine effects of weather on preference. Significant differences were determined at $P \leq 0.05$ and descriptive data are presented as mean ± SEM unless otherwise stated.

2.3 RESULTS

2.3.1 Effect of herbage mass and distance on 24 h time free-choice cows spent either indoors or at pasture

Overall, cows showed a partial preference to be at pasture, spending an average of 68.7 % of their time at pasture over a 24 h period. This was different from 0% ($t = 7.88$, $P <$

0.001), 50 % (t = -3.78, P < 0.001), and 100 % (t = -11.1, P < 0.001). Cows spent a greater percentage of their time at pasture when it was provided at the near distance compared with the far distance (F = 7.65, P = 0.022), and this was not influenced by herbage mass (F = 0.02, P = 0.888, Table 2). Distance affected pasture use during the day (P = 0.046), but not at night (P = 0.184), and consequently distance had an overall effect on pasture usage. Herbage mass did not affect day-time (F = 0.01, P = 0.925) or night-time (F = 0.22, P = 0.647) pasture use.

2.3.2 Effect of herbage mass and distance on behavioural activity during the day, feed intake, and performance among free-choice cows and the difference in lying behaviour indoors vs. outdoors during the day

Cows that had access to the high herbage mass grazed more (F = 5.82, P = 0.039) than cows that had access to the low herbage mass, whereas grazing time was not influenced by distance to pasture (F= 4.13, P = 0.073). Distance to pasture affected posture and jaw activity as cows with access to the near pasture spent more time lying (F = 34.22, P < 0.001) and ruminating (F = 22.71, P = 0.001), but less time standing (F = 18.18, P = 0.002), eating TMR (F = 9.61, P = 0.013), and drinking (F = 7.13, P = 0.026).

There were no differences in the average daily TMR consumption when offered either herbage mass (F = 0.21, P = 0.660) or distance to pasture (F = 0.39, P = 0.546). Although no interaction existed between herbage mass and distance for grass intake (F = 0.81, P = 0.390), cows had a greater intake when offered a high mass (F= 21.17 P = 0.001) and a near distance (F = 6.60, P = 0.030). There were no differences in average milk yield per day when offered either herbage mass (P = 0.474) or distance (P = 0.850). No differences (P > 0.05) were found in milk fat, protein, or lactose content when offered either herbage mass or distance, with mean values of 388, 328, and 464 g/kg respectively. Finally, there was no interaction between herbage mass and distance for any of the measured variables (P

> 0.05). Additionally, a paired t-test revealed that overall, cows spent a similar amount of time lying indoors and at pasture during the day (t = 0.32, P = 0.760).

Table 2. Effect of herbage mass (3000 ± 200 kg DM-high or 1800 ± 200 kg DM-low) and distance to pasture (38 m-near or 254 m-far) on overall time spent at pasture, day-time and night-time pasture use, feed intake, milk yield, and behavioral activity during day-light hours. There were no interactions between herbage mass and distance.

Item	Herbage		*P*-value	Distance		*P*-value	SEM
	High	Low		Near	Far		
Time (%)							
Pasture	69.1	68.3	0.888	76.4	60.9	**0.022**	3.96
Daytime pasture use	54.1	53.0	0.925	65.6	41.5	**0.046**	7.38
Night-time pasture use	77.2	75.0	0.647	80.6	71.6	0.184	3.28
DMI and milk yield							
TMR intake, kg DM	21.3	21.5	0.658	21.6	21.2	0.544	0.30
Milk yield, kg	33.1	33.8	0.474	33.6	33.4	0.850	0.64
Grass intake, kg DM	1.61	0.820	**0.001**	1.44	1.00	**0.030**	0.03
Behavioural Activity during manual observations (%)							
Indoors	35.7	39.4	0.619	23.9	51.2	**0.004**	5.11
Track	10.2	12.3	0.562	2.50	20.0	**<.001**	2.43
Pasture	54.1	48.3	0.564	73.7	28.8	**0.001**	6.82
Lying	44.2	44.2	0.985	52.4	36.3	**<.001**	1.90
Standing	51.7	50.8	0.794	44.5	58.0	**0.002**	2.21
Walking	4.1	4.90	0.452	3.40	5.60	0.068	0.83
Eating TMR	14.8	17.8	0.190	13.0	19.6	**0.013**	1.56
Grazing	18.4	11.1	**0.039**	17.8	11.6	0.073	2.24
Drinking	1.2	1.30	0.655	0.80	1.60	**0.026**	0.23
Ruminating	26.5	27.5	0.565	31.0	23.0	**0.001**	1.24
Idling	39.2	42.3	0.395	37.3	44.1	0.086	2.52

2.3.3 Weather

Over the course of the study the mean temperature was 16.7 °C indoors (range: 10.5 – 22.7 °C) and 15.9 °C outdoors (range: 12.2 – 20.2 °C). Indoor THI (61.6 ± 0.59, P = 0.217, range: 57-66) and outdoor THI (60.2 ± 0.59, P = 0.124, range: 56-65) had no effect on preference to be in or out. Rainfall occurred on 18 out of 40 measurement days (0.04 ± 0.01 mm/d) and no effect of rainfall was found (P = 0.451) on time spent at pasture.

2.3.4 Behavioural activity (daylight hours), feed intake, and milk yield between free-choice cows and housed cows

2.3.4.1 Jaw activity. Cows spent a greater percentage of time eating TMR (Figure 2A) when continuously housed than those that had access to the near or far pasture (29.5% vs. 13.0%, F = 18.2, P < 0.001) and (29.5% vs. 19.6%, F = 18.2, P < 0.001) respectively. Additionally, cows spent more time ruminating (Figure 2B) when continuously housed than those that had access to the far pasture (29.3% vs. 23.0%, F = 7.25, P = 0.004) but there was no difference compared to cows that had access to the near pasture. Finally, cows at the near pasture spent less time drinking than continuously housed cows (0.840% vs. 2.11%, F = 4.93, P = 0.018, Figure 2C).

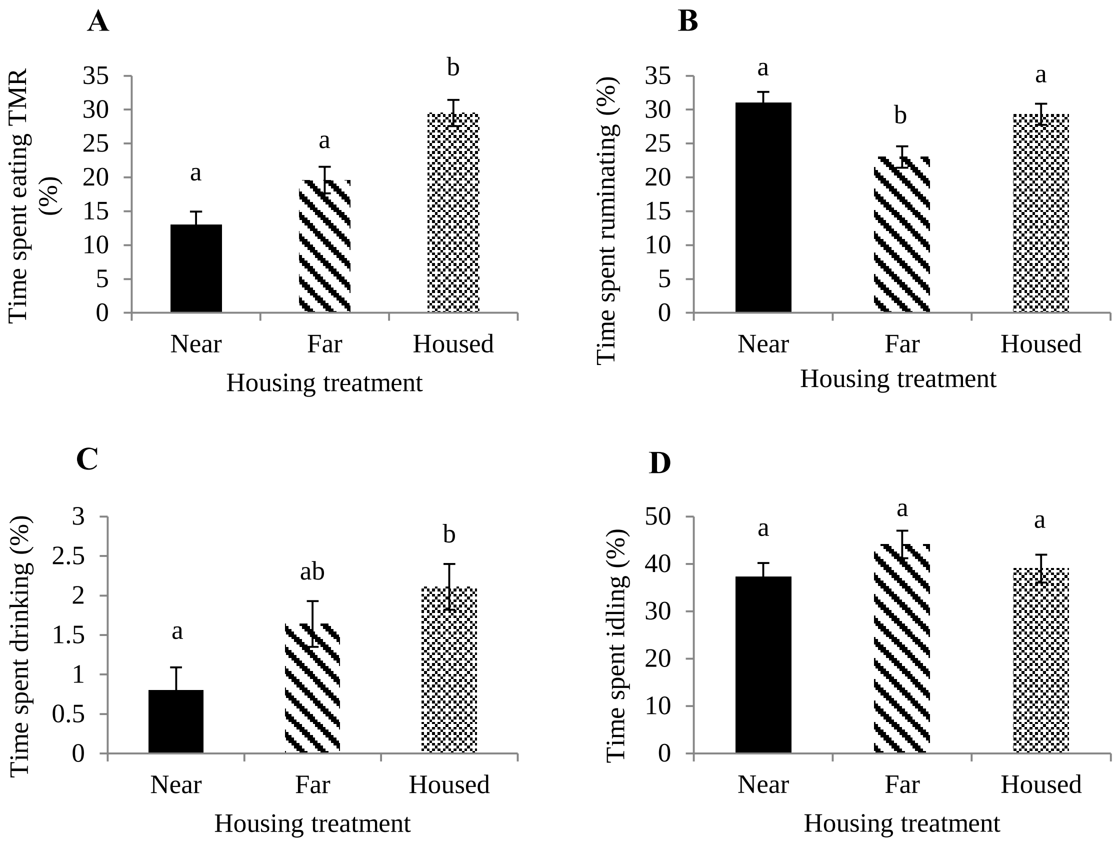


Figure 2. Jaw activity. Differences in percentage of time (mean ± SEM) spent eating TMR (**A**), ruminating (**B**), drinking (**C**), and idling (**D**) between cows that were continuously housed and those given access to pasture (near and far pastures). Cows at the near pasture are the same sample as cows at the far pasture. Means sharing the same letter are not significantly different at the $P \leq 0.05$ level.

2.3.4.2 Posture. Cows spent more time lying when offered access to the near pasture compared to those that were continuously housed (52.1% vs. 34.6%, $F = 18.4$, $P < 0.001$, Figure 3A) but there was no difference between cows that were continuously housed and those that had access to the far pasture (34.6 % vs. 36.3 %, $P > 0.05$). Additionally, cows spent more time standing when continuously housed than when given access to the near pasture (61.8% vs. 44.9%, $F = 15.2$, $P < 0.001$, Figure 3B), but there were no differences between the housed cows or those given access to the far pasture. Finally, there were no differences ($P > 0.05$) in the percentage of time spent walking (Figure 3C) between treatments.

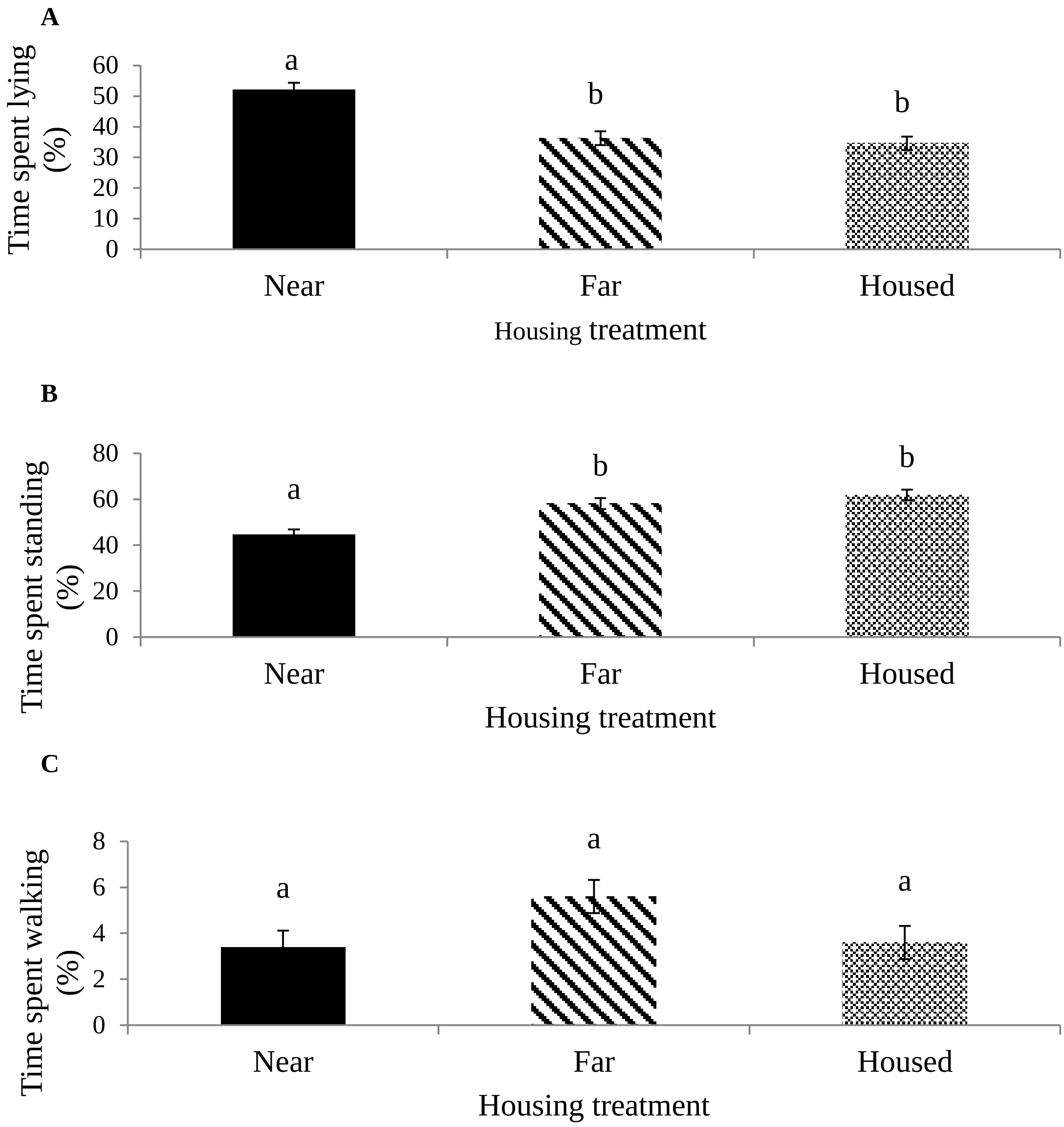


Figure 3. Posture. Differences in percentage of time (mean ± SEM.) spent lying (**A**), standing (**B**), and walking (**C**) between cows that were continuously housed and those given access to pasture (near and far pastures). Cows at the near pasture are the same sample of cows at the far pasture. Means sharing the same letter are not significantly different at the $P \leq 0.05$ level.

2.3.4.3 TMR intake. When comparing TMR consumption for cows that had access to the near or far pasture vs. cows that were continuously housed, no differences were found in the amount consumed (21.6 kg DM/d vs. 21.3 kg DM/d vs. 23.0 kg DM/d respectively, $F = 2.63$, $P = 0.101$).

2.3.4.4 Milk yield. Cows that had access to the near pasture and cows that had access to the far pasture produced more milk on average per day compared to continuously housed cows

(33.6 kg/d. vs. 33.4 kg/d. vs. 26.8 kg/d respectively, F = 28.88, P < 0.001, Figure 4).

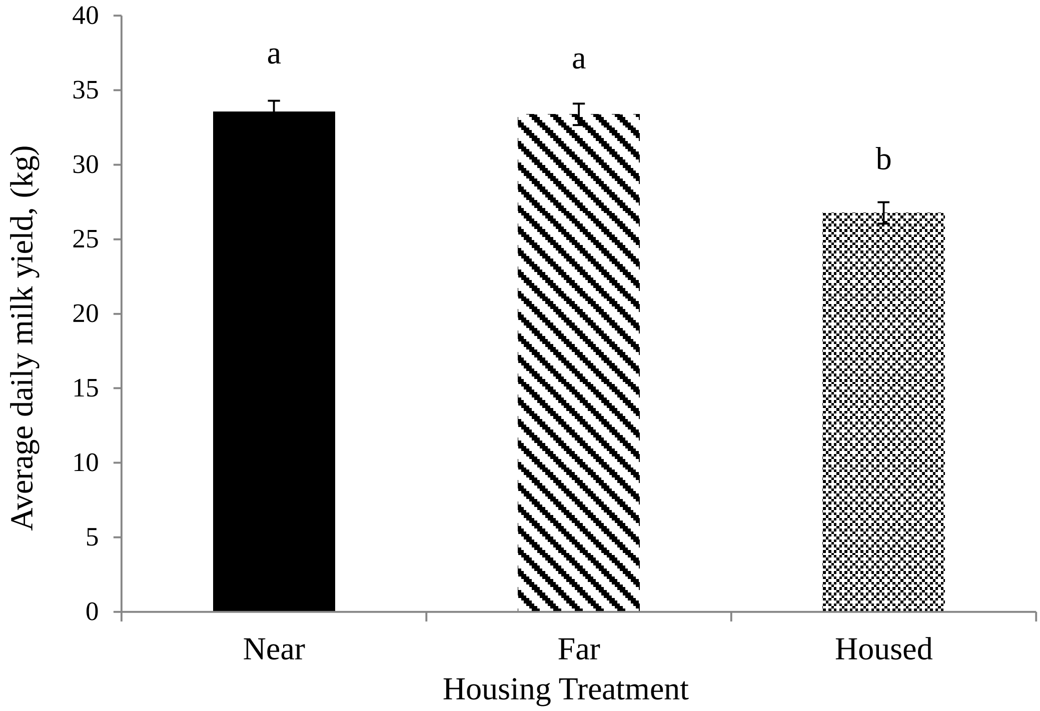


Figure 4. Differences in average daily milk yield (mean ± SEM) between cows that were continuously housed and those given access to near and far pastures. Cows at the near pasture are the same sample of cows at the far pasture. Means sharing the same letter are not significantly different at the $P \leq 0.05$ level.

2.4 DISCUSSION

Overall, cows expressed a partial preference to be at pasture spending almost 70 % of their time outside. Preference for pasture was affected by distance but not herbage mass. The overall partial preference for pasture is similar to findings reported by Krohn et al. (1992) and Charlton et al. (2011b) where dairy cows given a free-choice to be indoors or at pasture during the summer spent 72 % and 71 % of their time outdoors respectively. However, Charlton et al. (2011a) reported that dairy cows preferred to be indoors over 90% of the time. This difference may be due to the cows used by Charlton et al. (2011a) having had limited exposure to pasture during rearing. Falk et al. (2012) and Legrand et al. (2009) reported that cows did not show an overall partial preference to be indoors or at pasture spending 42 and 46 % of their time indoors respectively. The lack of overall preference for pasture in these two studies may be due to the higher maximum THI values reported at pasture than in the current study as heat stress can influence where cattle choose to spend their time (Blackshaw and Blackshaw, 1994).

Both Legrand et al. (2009) and Falk et al. (2012) did report an almost exclusive preference for pasture at night however, which may be due to the cubicle bedding used in those studies which consisted of geotextile mattresses covered with 0.1 m (depth) of soft washed river sand (deeply-bedded) vs. the 3 cm thick rubber mattresses scattered with lime-ash used in the current study. Tucker and Weary (2004) reported that the depth of substrate on mattresses is important to encourage lying behaviour and cows presented with bare mattresses or mattresses with 7.5 kg of sawdust spent more time lying down when given access to the sawdust mattresses. Cows in the studies conducted by Legrand (2009) and (Falk et al., 2012) may have been prepared to spend more time indoors during the day as the cubicles provided enough comfort for the amount of time they typically spend resting during the day, but at night when resting behaviour is more prevalent, they opted to spend the majority of their time at pasture.

Herbage mass did not influence preference under the conditions of this study and there was no interaction between herbage mass and distance to pasture. This suggests that herbage mass is not a key driving factor in dairy cow preference to be indoors or at pasture when a TMR is offered indoors. This is potentially because high yielding dairy cows (> 30 kg milk/d) cannot achieve the same DMI from grazing as cows fed a TMR: they are constrained by DM per bite, bite rate, and grazing time (Kolver and Muller, 1998). Additionally, the relatively low average grass intake of 1.22 kg DM/d suggests that dairy cows were simply supplementing their TMR intake with grass rather than actively using grass as the sole means of nourishment. As it has been shown that dairy cows prefer to select their diet when given a chance, and a TMR provides a balanced nutrient intake for the average cow, rather than the individual (Rutter, 2010), cows may have been using the pasture as a way to fulfil individual nutritional needs.

These results are contradictory to those reported by Ketelaar-de Lauwere et al. (2000) that indicated that at lower sward heights cows spend more time indoors. However, unlike in the current study where the temperature range outside was moderate (12.2-20.2°C), much higher temperatures were reported (18.9 – 32.1°C) in the study of Ketelaar-de Lauwere et al. (2000) and since heat is known to affect time spent at pasture it is possible that some interaction between temperature and sward height affected time spent indoors rather than sward height being the sole reason for increased time indoors. Additionally, the cows used by Ketelaar-de Lauwere et al. (2000) were in early-mid lactation vs. the mid-late lactation cows used in the current study which may have affected their motivation to be at pasture, although yield was higher in the current study.

In the present study, distance to pasture was the main factor that influenced preference, with cows spending more time at pasture when given access to the near vs. far pasture. Similar results were reported by Spörndly and Wredle (2004), when cows given access to a near pasture (50 m) chose to spend 68 % of their time outdoors. Cows were not willing to walk the far distance to gain access to pasture during the day, but they were willing to do so during the night. Charlton et al. (2013) also reported that when cows had to walk a short distance (60 m) to gain access to pasture vs. further distances (140 or 260 m), cows were prepared to walk the distance at night, but not during the day, suggesting that night-time pasture access is particularly important.

The intake of TMR and milk yield were not affected by herbage mass or distance to pasture although cows did spend more time eating TMR when given access to the far pasture, possibly because they spent more time indoors during the daytime which is typically when the majority of feeding behaviour occurs (Tucker, 2009). Both TMR intake and milk yield among free-choice cows were maintained at high levels through-out the study (21.4 kg DM/d and 33.5 kg/d, respectively). The ability to maintain intake and milk yield is supported by previous research that has indicated that giving cows free access to pasture is not detrimental to performance if cows are allowed *ad libitum* access to a TMR ration indoors (Chapinal et al., 2010). In contrast, the present findings are contradictory to results reported by Legrand et al. (2009) where there was a 14 % decrease in TMR intake when cows were given a choice between cubicle housing and pasture. Cows in the current study produced more milk than cows used by Legrand et al. (2009) (34 kg/d vs. 26 kg/d respectively) despite being at approximately the same lactation stage as cows in the current study and because of this it suggests they may have had different metabolic demands which influenced their intake behaviour. Additionally, grass intake was not measured by Legrand et al. (2009), so although TMR intake may have decreased, it is not clear whether overall DMI was reduced as a result of free-access to pasture.

Grass intake in the current study was affected by both herbage mass and distance. Intake was higher on the near pasture with cows consuming 0.44 kg DM/d more than when given access to the far pasture, and cows consumed 0.79 kg DM/d more at the high vs. low herbage allowance. Bargo et al. (2003) reported similar results indicating that pasture DMI increases as herbage mass increases. These results are not surprising, as grazing time was affected by herbage mass with cows spending more time grazing when offered a high allowance and a tendency existed for cows to spend more time grazing at the near distance. Contrary to these findings, Ketelaar-de Lauwere et al. (2000) reported that total time spent grazing was not influenced by sward height. This contrary result may be due to their highest reported sward height of 14.5 cm. being less than the high herbage mass offered in the current experiment, but as they did not report herbage mass or sward type the results are difficult to compare directly.

When offered the high herbage mass, cows spent more time grazing, but did not spend more time at pasture. These results contradict those reported in two studies looking at grazing behaviour in dairy cows at different herbage allowances where herbage allowance did not influence the proportion of time spent grazing (Chilibroste et al., 2012, Pérez-Prieto et al., 2011). The results of the current study however, are supported by Perez-Ramirez et al. (2009) as their study showed a tendency ($p = 0.067$) for the proportion of time spent grazing to increase at a high vs. a low herbage allowance with cows that spent 22 h at pasture. The lack of treatment effect on grazing time reported by Chilibroste et al. (2012) and Perez-Prieto et al. (2011) could be due to their observation of grazing being over three different herbage allowances (low, medium, and high), and in both studies the low/medium herbage allowances offered was comparable to the high herbage mass offered in this study. Additionally, Chilibroste et al. (2012) and Perez-Prieto et al. (2011) conducted their studies in winter in contrast to the current experiment and that of Perez-Ramirez et al. (2009) which were conducted in the summer and spring respectively. As season can influence dairy cow preference to be at pasture (Krohn et al., 1992), it may also influence the proportion of time spent grazing when dairy cows had a choice to be indoors.

Lying and ruminating time were affected by distance to pasture, with cows spending more time lying and ruminating when offered pasture at the near vs. the far distance. As rumination time is more frequent during periods of lying (Schirmann et al., 2012) it is logical that cows spent more time ruminating when offered the near distance as they also spent more time lying. It should be noted that both rumination and lying behaviour were not recorded at night and 24 h time budgets may be different from time budgets during the day. Free-choice cows at the far pasture may have been altering their time budget during the day when compared to free-choice cows at the near pasture. Cows at the far pasture spent less time lying and subsequently less time ruminating as they spent less time at pasture during the day, and spent more time indoors eating TMR. However, at night when they actively chose to access pasture, they may have made up the difference.

Previous research has reported that Holstein – Friesian dairy cattle are particularly susceptible to heat stress (Blackshaw and Blackshaw, 1994) and are highly motivated to seek shade to reduce the negative consequences of heat stress (Schütz et al., 2008). If given a choice, dairy cows will move indoors during the day as thermal comfort decreases with increasing THI at pasture (Legrand et al., 2009 and Falk et al., 2012). In the current study the THI did not influence cow preference to be indoors or at pasture which has also been reported by Charlton et al. (2013). Both the current study and that of Charlton et al. (2013) reported similar average values for THI at pasture (60.2 and 59.6 respectively) and the maximum THI value of 65 in the current study did not exceed the reported upper critical limit for lactating dairy cows of 72 (corresponding to 25 º C and 50 % RH) (Tucker et al, 2008). Although Legrand et al. (2009) reported a mean THI value of 60.5 which was similar to the current study, the THI range was much wider (49.9 – 74.6) than that of the current study (56-65) making it more likely for changes in pasture use to become apparent, and the maximum value exceeded the upper critical limit of 72. This may be why in the current study THI did not appear to affect preference for pasture. Rainfall has also been reported to affect preference (Legrand et al., 2009 and Charlton et al., 2011a), but as rainfall was negligible in the current study when compared to both Charlton et al. (2011a) and Legrand et al. (2009) (0.04 vs. 0.60 vs. 5.4 mm/d) it did not influence where cows spent their time.

When comparing housed cows vs. free-choice cows, the main differences occurred when free-choice cows had access to the near pasture. Housed cows however, spent more time eating TMR than free-choice cows at either distance but had similar daily intakes, with all cows eating approximately 22 kg DM/d. This indicates that free-choice cows re-allocated their time budget compared to housed cows but were still able to maintain a high intake. Additionally, free-choice cows at either distance produced more milk than housed cows. This is potentially a function of both herbage intake and increased cow comfort. The average herbage intake (kg DM/d) of 1.22 would account for about 3 kg of the 7 kg increase in milk yield if the ME of the grass was assumed to be between 10.0-12.0 MJ/kg DM and that the ME of milk was 5.2 MJ (McDonald et al., 2011). There is the potential that some of the increase may also be accounted for by lying time. Overall lying time in free-choice cows with access to the near pasture was greater than housed cows, and there is some evidence to suggest that mammary blood flow increases during periods of lying (Metcalf et al., 1992) which may increase milk yield. Further study is necessary however, to quantify exactly how lying time affects milk yield. The increase in lying time among free-choice cows is a particularly interesting finding because although overall time spent lying was more than housed cows, free choice cows did not spend more time lying on pasture than indoors (during the hours where manual observations of lying was carried out), a finding that contradicts Ketelaar-de Lauwere et al., (2000) and Spörndly and Wredle (2004). This suggests that allowing dairy cows to have control over their environment by offering them a free-choice promotes daytime lying behaviour in dairy cows, even if the majority of time is not spent lying on pasture (however it unclear whether lying time over 24 h is more at pasture vs. indoors). It is also possible that lower stress levels contributed to this increase in milk yield as agonistic encounters are more easily avoidable at pasture because of the increased inter-cow distance outdoors (Philips, 2010).

2.5 CONCLUSIONS

Herbage mass is not a driving factor in dairy cow preference for pasture, but it does influence grass intake which is likely to have contributed to the increase in milk yield. Distance affects pasture-use, but only during the day suggesting that dairy cows are more highly motivated to access pasture at night. Finally, continuously housed cows spend less time lying down than cows given access to a near pasture and produce less milk than cows with free access to pasture. Giving dairy cows control over their own environment seems to have both welfare and production benefits, and as such should be seen as a gold-standard to strive towards. Further research is warranted to investigate how to achieve this under practical conditions.

CHAPTER 3: Effect of previous experience of pasture on non-lactating Holstein-Friesian dairy heifer preference to be indoors or at pasture

3.1 INTRODUCTION

Following the domestication of cattle, between 8000 – 10,000 years ago (Clutton-Brock, 1999), cattle have come under increasing control of humans (Rutter, 2010). Although cattle have been traditionally housed during the winter and grazed in the summer in temperate regions like Northern Europe, ruminant livestock are increasingly being housed year-round (Haskell et al., 2013) and fed a TMR rather than the majority of their intake being achieved from grazing (Rutter, 2010). As a result, modern dairy cows may never have the opportunity to be at pasture or to engage in grazing behaviour. Pasture is generally seen as an environment that encourages natural behaviour including grazing (Albright and Arave, 1997, Philips, 2002) and lying (Krohn and Munksgaard, 1993).

As discussed in Chapter 2, the ability to graze isn't necessarily the key driving factor influencing preference for pasture in high-yielding dairy cows, but cows appear to supplement their feed intake with grass which can positively affect milk yield and individual cows may be using grass as a way to fulfil nutritional deficiencies. Particularly for growing cattle, Atwood et al. (2001) reported that heifers given the opportunity to select their own diet were better able to meet individual nutrient needs than when limited to a single mixed diet (even though the mixed diet was nutritionally balanced). In addition to this, there are several health benefits to providing dairy cows with access to pasture including decreased levels of lameness (Haskell et al., 2006, Olmos et al., 2009), mastitis (Washburn et al., 2002), and abnormal behaviours often seen in confinement are eliminated at pasture (Redbo, 1990).

It was also reported in Chapter 2, that when given a free-choice between being at pasture or cubicle housing, lactating dairy cows prefer to be at pasture. This is in agreement with other research (Charlton et al., 2011b, Charlton et al., 2013), but this preference is complex and influenced by a number of factors (Legrand et al., 2009, Charlton et al., 2011a, Charlton et al., 2013). As part of research looking into dairy cow preference to be indoors or at pasture, a study by Charlton et al. (2011a) reported that when given a free choice to be in cubicle housing or at pasture dairy cows spent 90 % of their time indoors. The follow-up study to this reported the opposite: when given a free choice, dairy cows spent 70 % of their time at pasture (Charlton et al., 2011b). One of the main differences between these two studies was that the cows that chose to spend the majority of their time indoors had limited experience of being at pasture while the ones that spent the majority of their time at pasture had experience of pasture during rearing. As previous experience can influence preference (see ***section 1.6.3***) this may explain the conflicting results, and warrants further investigation. In addition to previous experience influencing preference for pasture, it may also influence grazing behaviour (see ***section 1.6.3.1).***

The ancestors of domestic cattle have been exclusively grazing animals since the Plio-Pleistocene age and also evolved as specialized grazers (grass-dominated diet) during that time (Albright and Arave, 1997, Janis, 2008). Even today, Chillingham cattle (free-ranging, semi-wild cattle), the closest equivalent to a wild counterpart for domestic cattle still alive, have been shown to spend about 58% of their time engaged in ingesting behaviour (grazing and ruminating) during the summer and about 75% of their time during the winter (Hall, 1983). In a review of the behaviour of cattle kept on pasture with limited human intervention, grazing time over a 24 h period was as much as 13.0 h/day, and grazing was the most common behaviour reported of all behaviours in their repertoire (Kilgour, 2012).

Ekesbo (2011) stated that synchronized grazing in cattle is an innate behaviour. It is logical to suggest that the synchronization of this behaviour is indeed innate. As dairy cows are prey animals, the synchronization of grazing would have evolved over millions of years as an anti-predator behaviour. It is well documented that grouping behaviour is utilized by prey species in order to decrease the risk of predation among individuals within a group and to maintain group vigilance (the many-eyes hypothesis) (Lima, 1995) so that the group as a whole will be able to spend a longer time grazing without fear of being killed (Lima and Dill, 1990). It was this grouping behaviour that helped make cattle a good candidate for domestication (Albright and Arave, 1997). However, the assumption that grazing behaviour itself is innate is less clear, as it is likely that previous experience influences grazing behaviour: although this has been extensively researched in sheep and goats, few studies have researched the effect of previous experience in cattle (see ***section 1.6.3.2***).

In part, the assumption that grazing is a natural and genetically pre-determined behaviour, has contributed to legislation in Sweden, Finland, and the Netherlands mandating that cattle must be kept at pasture for some part of the year (Pol-van Dasselaar et al., 2008). Additionally, EFSA (2009) recommendations from their scientific panel on animal health and welfare stated that cattle should be on pasture during the summer and any period of dry weather. The reasoning behind these mandates seem to hinge upon the fundamental question of whether or not grazing behaviour is an innate genetically pre-determined response—if it is, then impeding the ability to graze would cause the animal to suffer making it a reasonable animal welfare concern. In addition to this, heifer rearing is variable (Philips, 2010) and often heifers without prior exposure to pasture are turned out to pasture without buffer feed (Garnsworthy, 2005). If dairy heifers do require a period of adaptation to grass, and they are not immediately able to graze—this management practice may be of concern. Although recent work by Lopes et al. (2013) reported that an adaptation to grazing existed for dairy cattle when forced out at pasture, it is unclear how preference for pasture and adaptation to grazing will be affected when Holstein-Friesian cows have free access to pasture with ad lib access to TMR indoors.

Therefore, the objectives of the current study were to determine if exposure to pasture during rearing influenced preference for pasture in non-lactating heifers, and if there was any effect on grazing behaviour. It was hypothesized that heifers without experience of pasture would spend less overall time at pasture, and less time grazing than heifers with experience of pasture.

3.2 MATERIALS AND METHODS

3.2.1 Animals and management

Ethical approval for this study was provided by the HAU Research Ethics Committee

3.2.1.1 Rearing. Holstein-Friesian dairy heifers (n=46), born between December 2010 to May 2011, were reared in 2 groups from May 2011 to July 2012. The initial sample size of 46 heifers was chosen to ensure that we would be able to account for the 13-16% losses that typically occur on UK dairy farms before heifers reach first-calving (Brickell et al., 2009), and the 20-25% culling rate before their third lactation (Bell et al., 2010) and still be able to provide for a robust 3 cows x 2 groups x 4 study periods design in 2012 and a 3 cow x 3 groups x 4 study periods design in 2013 (Chapter 4).

Heifers were randomly allocated to treatment group P1 (to be reared with maximum exposure to pasture post-weaning for the first and second grazing seasons, n = 14) and treatment group P2 (to be reared without exposure to pasture in their first grazing season post-weaning and then maximum exposure to pasture in their second grazing season (first exposure to pasture was during the experiment in 2012, n=16). The remaining 16 heifers (treatment group P3) were to be reared with no exposure to pasture for their first two grazing seasons post-weaning and were used for the final experiment presented in this thesis where the preference of lactating cows with no experience, experience of pasture in their second year of life, or experience of pasture in both their first and second year of life

(Chapter 4) was tested. When heifers were approximately 130 d old and weighed 100 kg they were allocated to group P2 or P1 by weight. All heifers were weighed monthly and had health checks on a daily basis.

Group P2 heifers had no exposure to pasture until being tested in 2012. They were kept in an open-air (3 walls, roofed, 1 open face side) straw-yard (18.48 m x 10.1 m) divided into 2 bays (to minimize mixing stress between the older heifers and the newly allocated ones—these partitions were removed when heifers became more equal in size, Figure 5a). Four feed troughs (4.7 m x 0.75 m x 0.49 m, Figure 5a) were placed at the front of the bays and attached to the open-faced side of the building in order to encourage heifers to access an area with concrete flooring (18.6 m x 4.5 m, Figure 5b) to help maintain foot/claw health, and to provide increased ventilation to help minimize respiratory illnesses. Treatment P2 heifers were reared with the sixteen heifers that were required for the study in 2013 (treatment group P3, Chapter 4). Both groups were fed *ad libitum* haylage and 2 kg of concentrate/animal/day (Wynnstay Heifer Rearer 600+:105 g/kg DM NDF, 200 g/kg DM CP, composed of: distillers, palm kernel, wheat feed, soya, wheat, rapeseed meal, soya hulls, molasses, sugar beet pulp, calcium carbonate, maize gluten, fat, salt, vitamins). All heifers had access to water *ad lib* from 2 troughs (0.34 m x 0.69 m x 0.58 m) at either side of the pen. Fresh straw was added on a weekly basis, and the concrete area was scraped down as required.

a

b

Figure 5. Heifer housing during rearing for treatment groups P2 and P3 (**a**) and concrete flooring area within the indoor housing facility (**b**).

Heifers allocated to treatment group P1 were given the maximum possible exposure to pasture (depending on weather, grass availability, and date of birth) and were rotated among four paddocks, from May to November 2011. The sward was predominantly perennial ryegrass and white clover. Throughout their rearing, the heifers were supplemented with 1.5 – 2.5 kg concentrate (based on age and weight) and haylage at pasture to achieve a similar target growth rate as P2 heifers. The nutrient composition of the haylage and concentrate was the same as that given to group P2. Water was available on an *ad libitum* basis in each paddock.

All heifers were housed together from mid-November 2012 in heifer cubicle housing (1 stall/head). Heifers were offered a TMR (DM-428 g/kg, 129 g/kg DM CP, and 519 g/kg DM NDF consisting of 5.25 kg DM/hd grass silage, 0.87 kg DM/hd chopped wheat straw, 0.66 kg DM/hd protein blend, 0.68 kg DM/hd dairy minerals) daily during November 2011 to February 2012. From February 2012 to April 2012 they were offered a TMR composed of 2.64 kg DM/hd Lucerne silage, 1.92 kg DM/hd maize silage, 2.16 kg DM/hd chopped wheat straw, 0.66 kg DM/hd protein blend, 0.68 kg DM/hd soya hulls, 0.07 kg DM/hd limestone flour, 0.07 kg DM/hd dairy minerals, 0.03 kg DM/hd feed grade urea (DM- 453 g/kg, 135 g/kg DM CP, and 549 g/kg DM NDF). The heifers also had *ad libitum* access to water from 2 (1.7 m x 0.5 m x 0.5 m) water troughs at either end of the cubicle housing.

Heifers in group P1 were turned out to pasture in April 2012 with similar aged heifers, and heifers in group P2 (as well as the additional 16 indoor-reared heifers) were moved back into the straw yard before the start of the experiment.

As heifers reached 13-15 months of age, they were synchronized for oestrus in groups of six to help promote block calving: this would ensure that heifers on trial in 2012 would be pregnant so oestrus behaviour would not affect their preference to be indoors or at pasture, and that they would be lactating in time for the trial in the summer of 2013 (Chapter 4).

The synchronization protocol was developed by the Leonard, Lambert and May veterinary group which services the HAU dairy and was as follows:

Day 0: insert intra-vaginal progesterone releasing device (PRID-delta), (MSD animal health, Milton Keynes, UK)

Day 7: Intramuscular injection of 2 ml Estrumate (MSD animal health, Milton Keynes) to promote regression of the corpus luteum and subsequently oestrus.

Day 8: Remove PRID Delta. Intramuscular injection of 500IU Syncrostim (CEVA Animal Health Ltd., Amersham,UK) to promote induction and synchronization of oestrus and ovulation.

Day 10: 1st fixed time Artificial Insemination (AI), 48 hours after PRID Delta removal.

Day 11: 2nd fixed time AI, 72 hours after PRID Delta removal.

Trans-rectal ultrasound was used to detect pregnancy 35 days after heifers were artificially inseminated. If heifers were not pregnant, and a corpus luteum was present, they were injected with Estrumate (2 ml, intramuscular) and observed for oestrus behaviour within the next 3-6 days. If oestrus behaviour was observed they were served again with sexed semen. If oestrus behaviour was not observed during that time, a second 2 ml dose of Estrumate was given 11 days after the first dose and they were inseminated 72 hours after the second injection as advised by the veterinarian. For heifers that did not become pregnant after the second service, manual observations of oestrus behaviour was carried out 3x/d for 20 minutes/d and a third service with un-sexed semen was performed.

3.2.2 Experimental design

Twenty four, Holstein–Friesian heifers (reared in treatment groups previously described) were selected for trial during July to September 2012. Heifers had an average weight of

398 ± 50.2 kg (mean ± SD, age of 16.4 ± 1.24 months, BCS of 2.6 ±0.20 (Edmonson et al., 1989), and LS of 1.2 ± 0.37 (mean ± SD) (Flower and Weary, 2006) at the beginning of the study. All heifers used on the study were diagnosed pregnant by a veterinarian using trans-rectal ultrasound before the start of the study.

Heifers were randomly chosen from treatment groups P1 and P2 for one of four study periods (three heifers per group) (June to September 2012, Table 3) and balanced by age and weight (at the beginning of each study period).   Each study period (during which time free access to pasture was allowed and the effect of previous experience of pasture on preference for pasture was tested) lasted 12 days with a 2 day training period (where heifers were locked inside the housing area) for the heifers to get used to the housing area. Following the first 2 study periods, there was a 20 day period during which the sward was allowed to recover.

Table 3. Group allocations (P1-maximum exposure to pasture in the first year of life or P2-no exposure to pasture in the first year of life) to right or left side of indoor housing and days (d) spent in training or on a measurement period per study period

	Pasture Side			
Study period	Right	Left	Training period	Measurement period
1	P1	P2	d 1-2	d 3-12
2	P2	P1	d 1-2	d 3-12
RECOVERY	RECOVERY	RECOVERY	RECOVERY	RECOVERY
3	P1	P2	d 1-2	d 3-12
4	P2	P1	d 1-2	d 3-12

*RECOVERY indicates a 20 d period during which the sward was allowed to recover

3.2.2.1 Indoor housing. The indoor area (85.1 m^2 in addition to 8, 2.7 m x 1.2 m free stalls with 3 cm thick rubber mattresses, bedded with lime as 3x/week) allocated to the study heifers was located at the south end of one of the dairy cow housing facilities at the HAU dairy unit (Figure 6). The indoor area was divided into two equal areas (3.2 m x 13.3 m, with 1.3 free stalls/heifer) by a swing gate. Three, 5 m gates were erected to divide the trial heifers from the main herd, however they could still maintain visual, auditory, and limited physical contact. Hay or haylage (depending on availability) was available *ad libitum* from two wooden troughs (3.47 m x 0.650 m x 0.480 m) attached to a feed-face (1.6 head spaces/heifer) on the exterior of the building. At approximately 08:00 h each morning 2.5 kg concentrate/ animal was placed in individual buckets on top of the haylage for heifers in treatment groups P1 and P2. Water was also available on an *ad libitum* basis from four, 1.70 m x 0.500 m x 0.500 m water troughs (two on each side) in the indoor area. During the two training days, the heifers were confined to the indoor facility. During the 10 measurement days, the gates of the indoor area were opened at 06:00 h on day one to allow free-access to two paddocks, both 38m from the indoor facility, accessed via a concrete track. Heifers in treatment groups P1 and P2 were placed on either the right or left side of the housing area for alternating replicates, to eliminate any side biases that may have developed, using a Latin square design (Table 3).

3.2.2.2 Pasture. A 1.8 ha field (Figure 6) with a sward consisting predominately of perennial ryegrass and white clover was used (nutrient values shown in Table 4). Prior to replicates 1 and 3, the field was topped and 20 kg/ha nitrogen (N) (Lithan 34.5% N) was applied. The field was sub-divided using electric fencing and a back-fence was used which could be moved to provide a larger or smaller area depending on grass consumption and availability. Study heifers were allocated approximately 0.22 ha per group on either the right or left sides of the field. Additional heifers were kept on the opposite side of the fencing (0.66 ha on each side), to help manage the sward as well as ensure that heifers were not being influenced to stay in or out based on conspecifics in either area. Heifers from both groups that had just completed the study were moved to the opposite side of the fence to join the "spare" heifers. Study heifers could maintain auditory, visual, and limited physical contact with these spare heifers on pasture as well as with the main cow herd indoors.

The portion of the field used for the study was maintained at 2500 ± 200 kg DM/ha. Measurements of compressed sward height were taken five times (every other day) during each measurement period at approximately 11:00 using an electronic rising plate meter with a built in regression equation (Herbage mass = [sward height (cm) x 125] +640, Farmworks, F200, New Zealand, plate meter reading). The plate meter was placed randomly around the field 30 times in a W pattern, with 5 paces between each measurement.

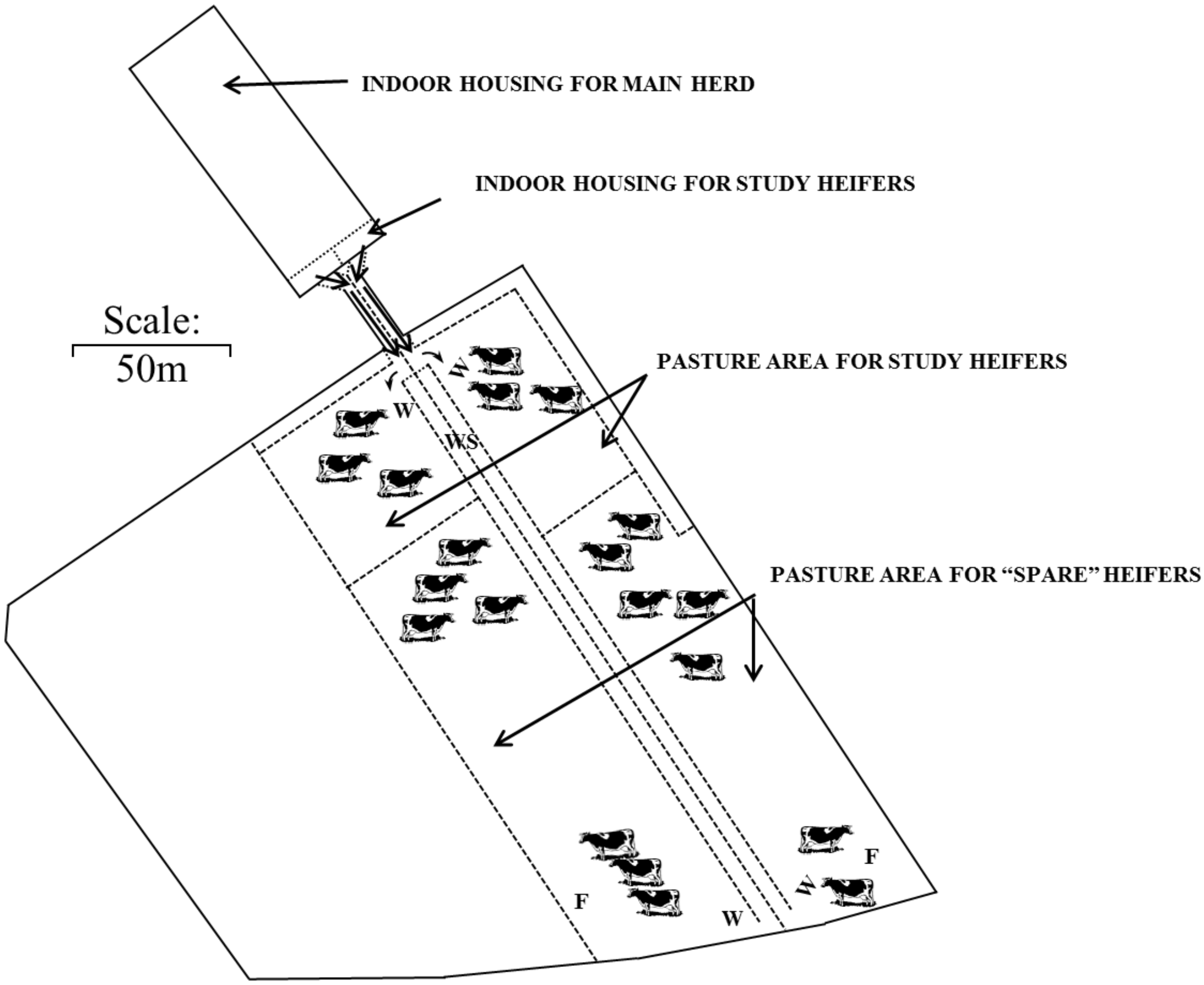


Figure 6. Position of the indoor housing facility in relation to the pasture. The pasture area closest to the indoor housing was used for groups P1 (maximum exposure to pasture during the first year of life) and P2 (no exposure to pasture in the first year of life). Each trial area was 0.22 ha. The larger pasture area (2 x 0.66 ha) was allotted for spare heifers from groups P1 and P2. W indicates the placement of water troughs in the pasture area, while F indicates the placement of ring feeders for haylage in the pasture area, and WS indicates the placement of the outdoor weather sensor.

3.2.3 Measurements

3.2.3.1. Total time spent outdoors vs. indoors. A Voltek night vision video camera (KT&C Co Ltd, Seoul, South Korea) connected to a digital video recorder was set up to continuously record heifer movement from indoor housing to pasture.

3.2.3.2 Manual behavioural observations. All behavioural activities and location were recorded for approximately 16 h/d for each heifer from 06:00 to 22:00 h (during daylight hours) on days 1, 3, 6, and 10 of each measurement period. Manual observations of behaviour occurred every five minutes during each observational period. During each 5-minute observation period the observer recorded the following activities for each heifer: (1) location: indoors, track, pasture; (2) position: lying, standing, or walking; (3) jaw activity: eating concentrate, eating hay, grazing, ruminating, drinking, idling; (4) investigatory grazing behaviour: sniffing, licking, exploring and nosing grass. This behaviour differs from fully formed grazing behaviour which is characterized by an upward jerk motion of the head in order to sever the plant (Philips, 2002). Two observers recorded behavioural activity for the duration of the study and inter-observer reliability was 100 %.

3.2.3.3 Weather conditions. Weather conditions were recorded automatically every 15 min, 24 h/d during the measurement period using a Davis Vantage PRO2 weather recorder (Davis Instruments Corp., Hayward, CA). Ambient temperature (°C) and relative humidity (%) were recorded indoors and ambient temperature (°C), relative humidity (%), wind speed (m/s), wind direction, and rainfall (mm) were recorded at pasture (outdoor weather sensor; Figure 6). The temperature-humidity index (THI) was calculated as THI= (1.8T + 32) – [(0.55 – 0.0055RH) x (1.8T – 26)] (NOAA, 1976), with T = ambient temperature (°C) and RH = relative humidity (%).

3.2.4. Feed sampling and nutrient analysis

3.2.4.1. Grass and hay/haylage sampling. Grass and hay/haylage samples were collected on day 3 of each measurement period for subsequent analysis of DM, CP, water soluble carbohydrates (**WSC**), and NDF (Table 4). Thirty random snip samples (to represent the grazed area) at a height of approximately 5 cm above the soil (Taweel et al., 2005) were taken from both the right and left side of the trial pasture in a W pattern. Haylage/hay samples were randomly taken from a fresh bale provided to the heifers on day 3.

3.2.4.2 Nutrient analysis. For DM determination, samples were weighed directly after collection and immediately oven dried to constant weight at 105 °C. Crude protein concentration in the samples was measured by combustion using a LECO FP 528 N analyser (Leco Corporation, St Joseph, MI, USA, AOAC 2000). Water soluble carbohydrate concentration in the samples was measured as described by Arthur (1977). NDF concentration analysis was carried out according to the methods of Van Soest et al., (1991).

Table 4. Nutrient composition of grass available at either the right (R) or left (L) side of pasture, and forage available indoors (mean ± SD). DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, and WSC = water soluble carbohydrates. No significant differences were found in the nutrient quality of the R or L side of the pasture ($P > 0.05$).

Nutrient Composition	Grass R	Grass L	hay/haylage
DM (g/kg)	158 ± 15.6	152 ± 19.3	680 ± 11.1
CP (g/kg DM)	206 ± 41.5	249 ± 55.3	109 ± 27.7
NDF (g/kg DM)	480 ± 14.4	457 ± 49.4	595 ± 39.6
WSC (g/kg DM)	138 ± 38.3	153 ± 45.9	104 ± 7.6

3.2.5 Statistical analysis

Absolute preference (defined as within treatment preference) for cubicle housing vs. pasture was determined by comparing the time spent indoors for each group (n=12) with 0 % (choice to be at pasture), 50% (indifference) and 100% (choice to be indoors) using a one-sample t-test. Overall time spent indoors or at pasture is expressed as a percentage of the total time offered a choice between being indoors vs. at pasture (24 h). A one-way ANOVA was used to determine a treatment effect on overall time spent indoors or at pasture, day-time (06:00 to18:00 h) vs. night-time pasture use (18:00 to 06:00 h), and behavioural observations. Data was blocked by cow group (replicate). A repeated measures ANOVA was used to determine an effect of time point on both overall percentage of time spent at pasture as well as the percentage of time spent grazing. Linear regressions were used to examine development over time for total time spent at pasture, but not for grazing time as too few data points existed to ensure that a significant result was not just due to random chance. Linear regressions were also used to determine whether a relationship existed between weather conditions and time spent indoors or on pasture.

All analyses were carried out in GenStat (13^{th} edition; Lawes Agricultural Trust Co. Ltd., Rothamsted, UK) and descriptive data are presented as mean ± SEM unless otherwise stated. Significant differences were determined at $P \leq 0.05$. Analyses were conducted at the individual level in order to have a more powerful sample size and to account for individual rates of learning. Although group level analysis eliminates the effect of social facilitation, evidence exists that cows exhibit individual variation in their behaviour, particularly when they are allowed access to environments that encourage the expression of natural behaviour (Adamczyk et al., 2013). Additionally, behavioural synchronicity and social facilitation can be reduced when dairy cows are not exclusively at pasture (Miller and Wood-Gush, 1991).

3.3 RESULTS

3.3.1 Absolute preference

Heifers without previous exposure to pasture prior to being tested (P2) expressed a partial preference to be indoors (82.6 ± 3.45 % over a 24 h period) and this was different from 0 % (t = 23.98, P < 0.001), 50 % (t = 9.48, P < 0.001), and 100 % (t = -5.03, P < 0.001). Heifers with previous exposure to pasture (P1) did not show a clear preference to be indoors or at pasture with time indoors (55.6 ± 7.09 % over a 24 h period) differing from 0 % (t = 7.84, P < 0.001) and 100 % (t = -6.26, P < 0.001) but not from 50 % (t = 0.79, P = 0.448).

3.3.2 Treatment effect on total time spent indoors vs. at pasture.

When compared to group P1, group P2 heifers spent more time indoors (F = 15.42, P < 0.001, Table 5), and less time at pasture (F= 15.42, P < 0.001).

Table 5. Effect of treatment (P1- maximum exposure to pasture in their first year of life vs. P 2-no exposure to pasture in their first year of life) on 24 h time spent either indoors or at pasture, and on day-time vs. night-time choice to be at either location.

	Treatment			
Item (%)	P1	P2	SEM	*P*-value
Indoors	55.6	82.6	3.45	< 0.001
Pasture	44.4	17.4	3.45	< 0.001
In during day	53.6	83.4	1.81	< 0.001
Out during day	46.4	16.6	1.81	< 0.001
In during night	56.8	81.6	3.24	0.050
Out during night	43.2	18.4	3.24	0.050

3.3.3 Effect of time on 24 h time spent at pasture (over the 10 measurement d) on total time spent at pasture and effect of time (over the 4 manual observation days) spent grazing

An effect of time (F= 2.36, P = 0.049) was revealed for total time spent outdoors in P2 heifers. No effect of time was observed with P1 heifers. A time x treatment interaction was revealed (F = 3.39, P = 0.008). A linear regression revealed that as the study period progressed, time spent outdoors increased (and time spent indoors decreased) for heifers in group P2 (F= 6.82, P = 0.031, R^2 = 0.46, Figure 7). There was also an effect of time on grazing over the four manual observation days for P2 heifers (F = 2.98, P = 0.047, Figure 8).

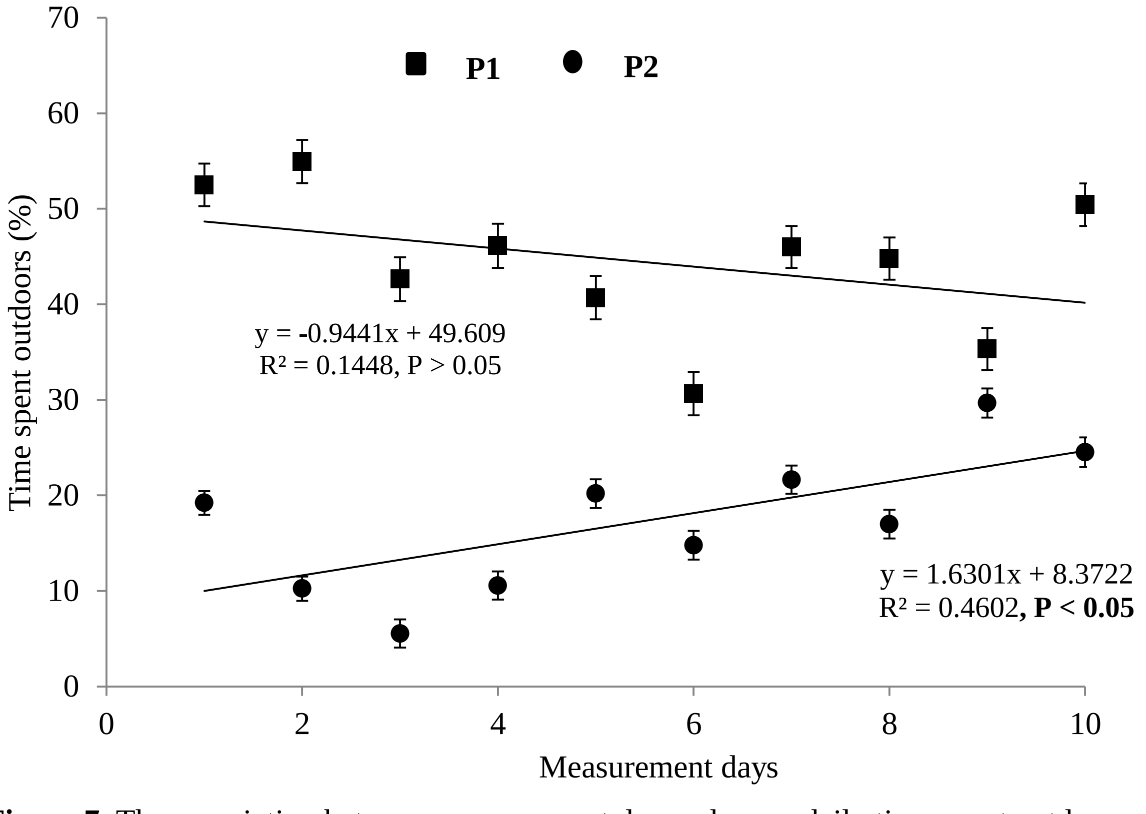


Figure 7. The association between measurement day and mean daily time spent outdoors for P1 heifers (maximum exposure to pasture during the first year of life) and P2 heifers (no exposure to pasture during the first year of life). Each data point represents the mean time spent at pasture on a particular measurement day for either group P1 or P2.

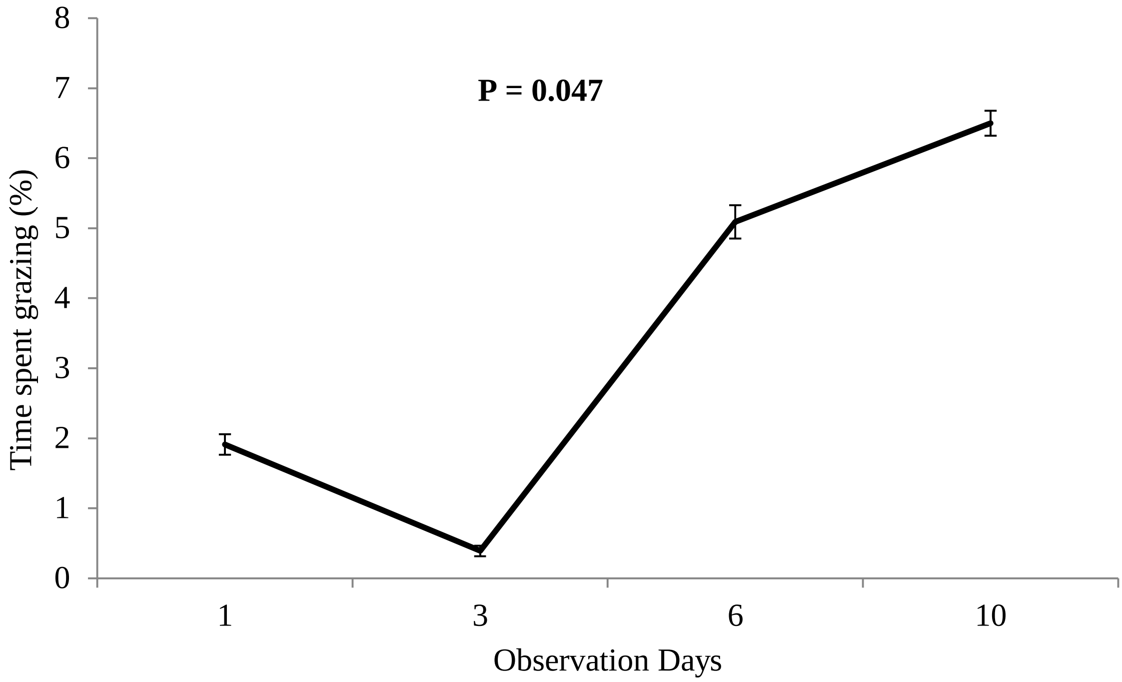


Figure 8. Effect of time on grazing for P2 heifers (no exposure to pasture in their first year of life) over the four manual observation days.

3.3.4 Time spent indoors or at pasture during the day vs. during the night

No absolute preference to be indoors or at pasture during either the day-time or night-time was shown for either group. Group P2 heifers spent more time indoors during the day ($F = 4.25$, $P < 0.001$, Table 5) and night ($F = 3.85$, $P = 0.05$) and less time outdoors during the day ($F = 17.28$, $P < 0.001$) than P1 heifers. Finally, P1 heifers spent more time outdoors during the night than P2 heifers ($F = 6.58$, $P = 0.005$).

3.3.5 Behavioural observations

Manual observations revealed that during the hours of 06:00 to 22:00 on days 1, 3, 6, and 10 of the measurement period heifers in treatment group P2 spent more time indoors, less time at pasture, more time eating haylage, less time grazing, and more time idling than heifers in group P1 (Table 6). No differences were observed with respect to percentage of time spent on the track, lying, standing, walking, eating concentrate, ruminating, or drinking (Table 6).

Table 6. Effect of treatment (P1- maximum exposure to pasture in their first year of life vs. P 2-no exposure to pasture in their first year of life) on location, posture, and jaw activity during 06:00 to 22:00 h.

	Treatment				
Item (%)	P1	P2	S.E.M.	*F*-value	P-value
Indoors	54.9	79.0	4.71	13.06	**0.001**
Track	8.10	7.43	0.844	0.31	0.580
Pasture	37.0	13.6	4.82	11.80	**0.002**
Lying	33.4	34.0	1.67	0.07	0.790
Standing	63.4	62.1	1.44	0.40	0.530
Walking	3.21	3.86	0.506	0.84	0.369
Eating concentrate	1.04	0.921	0.109	0.61	0.442
Eating haylage	17.0	24.2	1.36	14.00	< **0.001**
Grazing	18.1	3.5	2.04	25.56	< **0.001**
Ruminating	24.6	24.1	1.63	0.09	0.766
Drinking	0.90	1.24	0.149	2.56	0.121
Idling	39.4	46.1	1.89	16.51	< **0.001**

3.3.6 Investigatory grazing. Group P2 heifers spent more time engaged in investigatory grazing behaviour than P1 heifers (F = 7.70, P = 0.032, Figure 9).

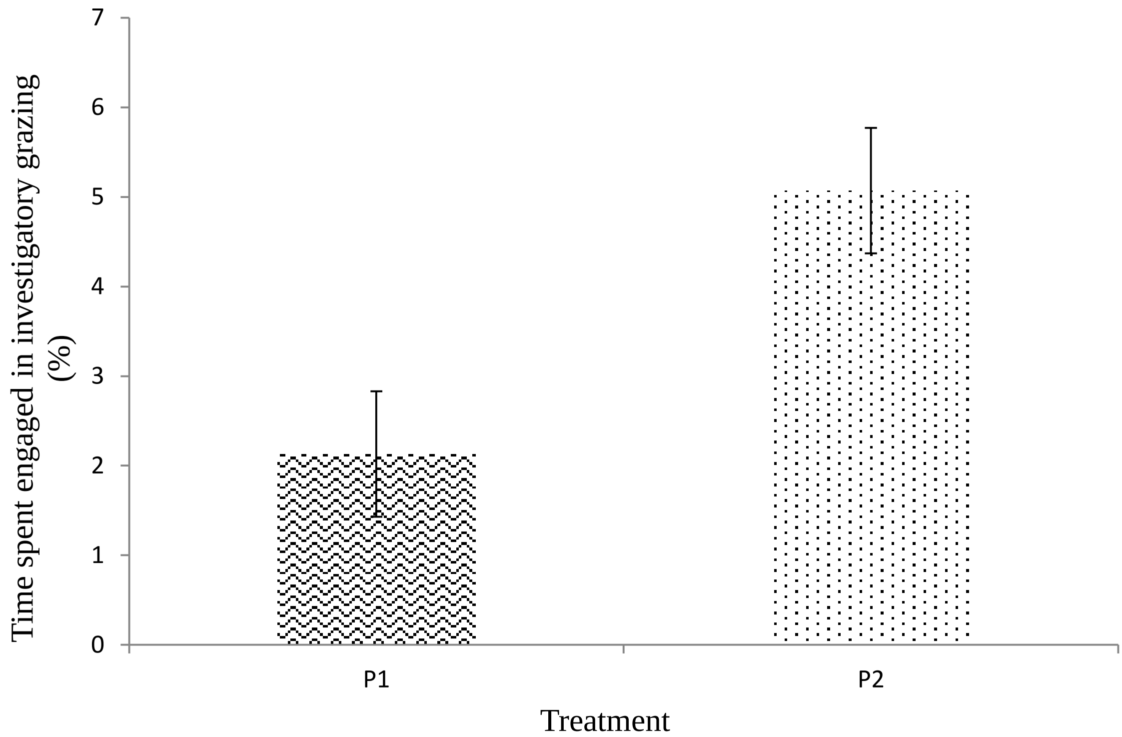


Figure 9. Effect of treatment (P1- maximum exposure to pasture in their first year of life vs. P2-no exposure to pasture in their first year of life) on percentage of time spent engaged in investigatory grazing behaviour.

3.3.7 Weather

3.3.7.1 Time spent indoors vs. outdoors and grazing time. The average temperature during the study was 15.4 ± 1.1°C outdoors and 16.9 ± 0.93 °C indoors. As the temperature increased outdoors, the time spent outdoors increased for P2 heifers (F = 21.23, P = 0.044, R^2 = 0.91). The THI was 59.9 ± 0.42 outdoors and 63.5 ± 0.39 indoors. As the THI outdoors increased, time spent outdoors also increased for P2 heifers (F = 21.02, P = 0.044, R^2 = 0.91). The average rainfall throughout the study was 3.69 ± 0.45 mm and this did not influence preference (P = 0.867) for either treatment group. Group P2 heifers tended to graze less when rainfall increased (F = 15.47, P = 0.059, R^2 = 0.88). As the THI outside increased P2 heifers tended to graze more (F= 16.40, P = 0.056, R^2 = 0.89). Treatment group P1 was not influenced by any of the measured weather conditions.

3.4 DISCUSSION

The results indicated that heifers reared without exposure to pasture had a partial preference to be indoors, spending over 80 % of their time in the cubicle housing. Charlton et al. (2011a) reported a similar result with lactating dairy cows that spent over 90 % of their time indoors. Since time spent at pasture increased over time for P2 heifers, their preference for it may have also changed given a longer study period, suggesting that preference for pasture may increase with increasing exposure to pasture. Although P1 heifers spent more overall time at pasture when compared to P2 heifers, they did not show a clear preference to be indoors or at pasture, spending approximately half their time in either location. Previous preference experiments have reported contradictory results to the current study with lactating cows with previous experience of pasture spending about 70 % of their time at pasture when given a choice (Charlton et al., 2011b, Krohn et al., 1992). Legrand et al., (2009) however, reported that when given a choice, lactating cows that were reared on pasture as growing heifers spent 46 % of their total time indoors which was not different from the their time on pasture. As none of these studies were conducted on non-lactating heifers it is unclear whether they are directly comparable as heifers have different behavioural motivations and growth requirements compared to lactating cows: in particular, nutrients are utilized for growth rather than milk production in heifers vs. multiparous cows (Philips, 2010).

Night-time data indicated that P2 heifers spent more time indoors when compared to P1 heifers, but P1 heifers did not have an absolute preference for pasture at night. Previous studies have shown that lactating cows may not show a preference to be at pasture during the day, but they do show a preference at night (Legrand et al., 2009) and they are willing to work to gain access to pasture at night suggesting it may be a necessary resource (Charlton et al., 2013). This is thought to be because the majority of lying behaviour occurs at night (Tucker, 2009) and pasture can be a more comfortable lying environment when compared with cubicle housing (Olmos et al., 2009). The cubicles used in the current study were designed for cows and not adapted to conform to the smaller size of heifers. Therefore, the cubicle design used may have allowed heifers more space (proportionate to their body size) to lie down comfortably and in more natural lying positions which cows may have been unable to do (Endres and Barberg, 2007). Weather may have also had an influence on their lack of preference as the summer of 2012 was particularly wet, with months June to August breaking records for average rainfall in the UK. Although the rainfall during the measurement periods was low, the above-average rainfall over the summer resulted in very wet ground conditions that would have reached their saturation capacity. Since dairy cows prefer to lie in dry vs. wet areas (Fregonesi et al., 2007), and lying time decreases when pastured cows are not given the opportunity to lie on well-drained surfaces (Fisher et al., 2003) pasture that would normally be seen as a more comfortable lying area may not be preferred in this instance, potentially explaining the lack of preference for pasture at night.

Heifers in group P1 spent approximately 18 % of their time grazing while heifers in group P2 spent just over 3 % of their time grazing in a 16 h period. Despite this, grazing times for both groups were low when compared to other studies conducted over the summer that have reported averages of about 30 % in heifers without previous exposure to pasture (Rutter et al., 2002, Forbes et al., 2000). Heifers in these studies were not provided with a choice between pasture and cubicle housing, and they were not supplemented at pasture. Both groups P1 and P2 spent approximately the same time eating (grazing + eating haylage + eating concentrate) as the heifers in previously reported studies, but since they were given a choice, they divided their eating time differently. Group P1 heifers spent about the same amount of time grazing as they did eating haylage, but group P2 heifers spent more time eating haylage than grazing. This may be due to early life experience with high energy food during rearing (TMR). It was reported that ruminants can develop a strong preference for high energy feed within 10 d (Provenza, 1995). The provision of concentrate and haylage indoors may have provided a higher energy feed than what was available to them via grass. Additionally, cattle with experience of pasture at an early age have been shown to graze more efficiently than animals that have not had exposure to pasture until later in life (Hodgson and Jamieson, 1981). This suggests that there might have been some combined effect of lack of grazing ability and preference for haylage/concentrate which influenced grazing time for heifers in group P2. Additionally, rainfall may have affected grazing time for group P2 since on average, days one and three had the most rainfall, and these were also the days where grazing time was the lowest. Previous research suggests that rainfall can influence grazing, with grazing time decreasing during rainy weather (Spörndly and Wredle, 2004). It should also be noted that grazing time was observed on days when the heifers happened to spend most of their time indoors, due to pre-determined observation days. If measurements had been taken on all 10 days, the low grazing time reported for both groups may have increased.

The higher percentage of time spent investigating grass observed in group P2 vs. P1 is likely to be indicative of individual learning, a method by which herbivores develop a preference for certain foods based on the positive or negative physiological, sensory, and nutritional consequences associated with each food (Arnold and Maller, 1977). Since heifers in group P1 had already acquired preferences associated with grass, and they had developed the skills required to graze efficiently, they did not have to spend time engaged in this investigatory grazing behaviour and they could spend more time engaged in fully formed grazing than the inexperienced group P2 heifers (Provenza and Balph, 1987). This result, along with the reported effect of time on grazing for P2 heifers indicates that grazing was being learnt. It also shows that grazing is not fully developed at first performance and seems to be refined with practice: two tenets of a learned behaviour vs. a behaviour that is innate (Manning and Dawkins, 1992). It is unclear how free-choice influenced learning however, and there is the potential that if P2 heifers were confined to pasture, the results may have been altered. In a similar study conducted by Lopes et al. (2013) investigating adaptation to grazing rather than preference for pasture, inexperienced heifers exhibited similar grazing times as experienced heifers after 1 day (although inexperienced heifers did not graze immediately) when they were forced to be exclusively at pasture (Lopes et al., 2013), but as discussed in ***section 1.6.3.2*** this rapid adaptation to pasture may have been due to a breed affect.

3.5 CONCLUSIONS

Heifers without exposure to pasture during rearing spend less time at pasture initially than heifers with exposure to pasture, but this changes as experience with pasture increases. Time at pasture is affected by weather for heifers without prior exposure to pasture. Both experienced and inexperienced heifers spend low amounts of time grazing when given a choice to feed indoors. Investigatory grazing behaviour as well as an effect of time on grazing for inexperienced heifers suggests a learnt component to grazing for Holstein-

Friesian dairy heifers. This result is particularly important for on-farm application as it may be useful to provide buffer feed during the adaptation period for inexperienced heifers to avoid poor welfare associated with hunger and frustration.

CHAPTER 4: Effect of previous experience of pasture on lactating dairy cow preference to be indoors or at pasture

4.1 INTRODUCTION

In Chapter 3 it was reported that non-lactating heifers without previous exposure to pasture chose to spend about 80 % of their time indoors. However, time spent at pasture increased over the observation period indicating that preference for pasture may develop with experience. Although previous experience may influence preference in general (Keeling and Jensen, 2009) and there is speculation that previous exposure to pasture influences preference in lactating dairy cows (Charlton et al., 2011a), there are no studies directly addressing this question. Additionally, as heifers have different metabolic demands than lactating cows, their preference and expression of behavior may not be the same as that of lactating cows.

Some studies have indicated that pasture may be more comfortable to lie down on than cubicles with rubber mattresses (Hernandez-Mendo et al., 2007, Cook et al., 2004) as it allows dairy cows to lie in natural lying positions (Krohn and Munksgaard, 1993). However, studies that have quantified lying time at pasture vs. in cubicle housing have yielded conflicting results with Krohn et al. (1992) and Ketelaar-de Lauwere et al. (1999) reporting a preference for pasture as a lying location while Charlton et al. (2011a) reported a preference to lie down indoors. Additionally, results reported by Charlton et al. (2011b) and results reported in Chapter 2 and 3 indicate no clear preference for lying location. As lying behavior is a useful indicator of dairy cow welfare (Fregonesi and Leaver, 2001) and as reported in Chapter 2 it may be linked to increased milk yield, it is important to understand where cows prefer to lie down to help optimize both welfare and production. Additionally, few studies have attempted the quantification of lying postures of cattle in free-stalls vs. pasture, and lying position may influence preference to lie at pasture. Lateral lying positions have been reported to be important for comfortable resting behavior and

REM sleep (Merrick and Scharp, 1971) which can have subsequent effects on animal welfare and production via altered hormone regulation and brain development (Hänninen et al., 2008).

The objectives of the current study therefore were to determine if exposure to pasture during the first year of life post-weaning vs. the second year of life vs. the third year of life influenced preference for pasture in dairy cows, and if there was any effect on grazing behaviour as seen in the experiment in 2012. A secondary objective was to determine where dairy cows prefer to lie down and if lying posture was influenced by lying location.

It was hypothesized that dairy cows without prior exposure to pasture would prefer to be indoors, but that this would change over time. Additionally it was hypothesized that dairy cows would prefer to lie down in stretched positions, and pasture would be their preferred environment to lie down on in order to achieve these positions.

4.2 MATERIALS AND METHODS

4.2.1 Animals and management

4.2.1.1 Rearing. Methods were similar to those reported in Chapter 3 with the following modifications. Some descriptions have been repeated here to aid clarity. Holstein-Friesian dairy heifers (n=46), born between December 2010 to May 2011, were reared in 3 groups from May 2011 to May 2013. Heifers were randomly allocated to treatment group P1 (to be reared with maximum exposure to pasture for two grazing seasons post-weaning, n=14), P2 (to be reared without exposure to pasture for their first grazing season post-weaning and with exposure to pasture for their second grazing season n=16), and treatment group P3 (to be reared with no exposure to pasture for two grazing seasons post-weaning, n = 16). When the heifers were approximately 130 d of age and weighed 100 kg they were allocated to group P1, P2, or P3 by weight. All heifers were weighed on a monthly basis and were health checked daily.

P2 heifers had first exposure to pasture while tested in 2012 at about 16 months of age (Chapter 3), while P3 heifers had no exposure to pasture until being tested in 2013 in the present study at about 26 months of age. Both groups were kept in an open-air (3 walls, roofed, 1 open face side) straw-yard (18.5 m x 10.1 m) divided into 2 bays (to minimize mixing stress between the older heifers and the newly allocated ones—these partitions were removed when heifers became more equal in size). Four feed troughs (4.7 m x 0.75 m x 0.49 m) were placed at the front of the bays and attached to the open-faced side of the building in order to encourage heifers to access an area with concrete flooring (18.6 m x 4.5 m) to help maintain foot/claw health, and to provide increased ventilation to help minimize respiratory illnesses. These heifers were fed *ad libitum* haylage and 2 kg of concentrate/animal/day (Wynnstay Heifer Rearer 600+: 200 g/kg CP and 105 g/kg NDF). All heifers had access to water *ad lib* from 2 troughs (0.34 m x 0.69 m x 0.58 m) at either side of the pen. Fresh straw was added on a weekly basis, and the concrete area was scraped down as required.

Heifers allocated to treatment group P1 were given the maximum possible exposure to pasture (depending on weather, grass availability, and date of birth) and were rotated among four paddocks. The sward was predominantly perennial ryegrass and white clover. Throughout their rearing, they were supplemented with 1.5 – 2.5 kg concentrate (based on age and weight) and haylage at pasture to achieve a similar target growth rate as P2 and P3 heifers. The nutrient composition of the haylage and concentrate was the same as that given to groups P2 and P3. Water was available on an *ad libitum* basis in each paddock.

All heifers were housed together from mid-November 2012 in heifer cubicle housing (1 stall/head), in order for them to become familiar with cubicle housing before the trial and before becoming integrated with the main HAU dairy herd. Heifers were offered a TMR daily during November 2011-February 2012 with 428 g/kg DM, 129 g/kg DM CP, and 519 g/kg DM NDF. From February 2012-April 2012 they were offered a TMR with 453 g/kg DM, 135 g/kg DM CP, and 549 g/kg DM NDF. The heifers also had *ad libitum* access to water from 2 (1.7 m x 0.5 m x 0.5 m) water troughs at either end of the cubicle housing.

Heifers in group P1 were turned out to pasture in April 2012 with similar aged heifers, and heifers in groups P2 and P3 were moved back into the straw yard before the start of the experiment in 2012 (see Chapter 3). Following the experiment in 2012, heifers in group P2 and P1 were turned out to pasture on a staggered basis (July-September) depending on which experimental replicate they were allocated to in 2012. They were moved back into the HAU dairy no later than 42 d prior to parturition (Nov 2012-May 2013), depending on conception date) according to HAU protocol. All heifers in group P1 were exposed to pasture for approximately 13 months over 2011 and 2012.

4.2.2 Experimental design

Thirty-six, Holstein–Friesian dairy cows in their first lactation (reared in treatment groups P1, P2, and P3, previously described in ***section 4.2.1.1***) were selected for the study during May-August 2013. One cow was dropped from both groups P1 and P2 as they were not lactating due to teat papillomatosis, and one cow was dropped from group P3 as she went lame during the study which would influence her choice to be indoors or at pasture. The data from these cows were not used in analysis, but they were not taken off the study to maintain equal group sizes. The lame cow was treated for the early stages of digital dermatitis during milking time to avoid disruption of the study and influencing the behaviour of other cows in her group. Cows weighed 557 ± 55.6 kg (mean ± SD), had a BCS of 2.3 ± 0.276 (Edmonson et al., 1989), and a LS of 0.33 ± 0.534 (mean ± SD) (Flower and Weary, 2006) at the beginning of the study. All cows used on the study were either diagnosed pregnant by a veterinarian using trans-rectal ultrasound before the start of each study period or a PRID-Delta (Van Werven et al., 2013) was inserted to ensure that cows would not come into oestrus during the measurement period which may have disrupted the behaviour of each study group.

Cows were randomly chosen from treatment groups P1, P2, and P3 for one of six study periods, during which pair-wise comparisons (two groups of three cows each were tested at a time) took place using a Latin Square design, (May-August 2013, Table 7). Cows were balanced by DIM at the beginning of each study period. Mean DIM at the beginning of each study period was 109 ± 37.5. Each study period lasted 12 days (during which time free access to pasture was allowed and the effect of previous experience of pasture on preference for pasture was tested) including a 2 day training period (where heifers were locked inside the housing area) in order to get used to the housing area. Following the first, third and fifth replicate, there was a period during which the sward was allowed to recover.

Table 7. Group Allocations (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) to right or left side of indoor housing and days (d) spent in training or on a measurement period per study period

	Pasture Side			
Study period	Right	Left	Training period	Measurement period
1	P2	P3	d 1-2	d 3-12
RECOVERY	RECOVERY	RECOVERY	RECOVERY	RECOVERY
2	P3	P1	d 1-2	d 3-12
3	P1	P2	d 1-2	d 3-12
RECOVERY	RECOVERY	RECOVERY	RECOVERY	RECOVERY
4	P2	P1	d 1-2	d 3-12
5	P1	P3	d 1-2	d 3-12
RECOVERY	RECOVERY	RECOVERY	RECOVERY	RECOVERY
6	P3	P2	d 1-2	d 3-12

*RECOVERY indicates a period during which the sward was allowed to recover

4.2.2.1 Indoor housing. The indoor facility used for the experiment in 2013 is as described in Chapter 3 with the following modifications. Cows were offered a weighed amount (to allow for the recording of TMR intake) of a TMR composed of 7.84 kg DM/hd maize silage, 3.36 kg DM/hd Lucerne silage, 0.65 kg DM/hd chopped wheat straw, 2.64 kg DM/hd sweet starch, 2.00 kg DM/hd molassed beet pulp, 0.90 kg DM/hd spey syrup, 0.40 kg DM/hd ruminer fat, 0.15 kg DM/hd dairy minerals, 0.09 kg DM/hd Amaferm Provimi, 0.10 kg DM/hd feed grade urea, 0.10 kg DM/hd limestone flour, 0.07 kg DM/hd acid buffer, and 0.04 kg DM/hd Vistacell Ultra at 105 % of *ad libitum* intake between 7:30 and 8:00 each morning which they accessed via a feed-face (1.6 head spaces/cow) on the exterior of the building. Following the allocation of feed the gates of the indoor area were opened to allow free-access to pasture.

4.2.2.2 Pasture. Pasture used for the experiment in 2013 is as described in Chapter 3 (Figure 6) with the following modifications. Prior to the start of the study, 20 low yielding cows mob-grazed the experimental area to ensure appropriate herbage mass and 20kg/ha nitrogen (N) (Lithan 34.5% N) was applied. During the study the field was topped as required and additional N was applied during the recovery periods. Spare heifers and cows kept in the 0.66 ha pasture area were not offered supplementary haylage (as in 2012) because the herbage mass was sufficient for them in 2013. At the end of each study period the cows re-joined the main herd.

4.2.3 Measurements

4.2.3.1 Total time spent outdoors vs. indoors. Measurements as described in Chapter 3, ***section 3.2.3.1***.

4.2.3.2 Manual behavioural observations. Measurements as described in Chapter 3 with the following modifications. All behavioural activities and location were recorded for approximately 11.5 h/d for each cow from approximately 7:30 h – 14:30 h and 17:30 h – 22:00 h (during daylight, excluding milking time) on days 1, 2, 4, 6, 8 and 10 of each measurement period. In addition to the behaviours described in both Chapter 2 and 3, lying posture was recorded in order to quantify the different ways in which cows lie down as well as determine if lactating dairy cows lie down in different postures based on the environment (pasture vs. cubicle housing). After observing cows lying in both cubicle housing and pasture prior to the experiment as well as based on the postures recorded in studies discussed ***in section 1.6.4*** posture was divided into three categories: front legs, rear legs, and head, and then further divided into 10 sub-categories (see Table 8a-c). Finally, both investigatory grazing behaviour and general investigation (i.e. investigating anything except for pasture) were recorded.

Table 8a. Ethogram: front leg lying position.

Front Legs	Description	Example
Both under	Front legs bent underneath the knee joint, both the hoof and pastern are mostly hidden from view, and only the knee joint is clearly visible.	
1 under 1 out	One of the front legs is bent at the knee joint. The other leg is stretched out directly in front of the body with a clear view of the hoof, pastern, and knee joint. This leg may have a slight bend in the knee joint or may be completely straight.	
Both out	Front legs are stretched out directly in front of the body. Legs may be slightly bent at the knee joint or completely straight.	
Out sideways	Cow is lying laterally with front legs stretched out to the side of the body. Legs may be slightly bent at the knee joint.	

Table 8b. Ethogram: rear leg lying position.

Rear legs	Description	Example
Both under	Both legs pulled close to the udder. The fetlock of the outer leg must be in direct contact with the body.	
1 under 1 out	The innermost leg is in contact with the udder (either at the hock or the fetlock), while the fetlock of the outer leg is not touching the udder. It may be completely stretched away from the udder or slightly stretched away from the udder.	
Out sideways	Both legs are stretched towards the side of the body. Legs may be slightly bent at the hock. This most frequently occurs during lateral lying, but it does not have to. If the cow is not laterally lying, the hoof, pastern, and hock of both legs must be completely stretched and clearly visible.	

Table 8c. Ethogram: head lying position.

Head	Description	Example
Up	Head held above the floor and not resting on any surface.	
Rest front	Head resting on any surface either in front of the body or towards the side of the body. The head is not touching any other party of the body.	
Rest back	Head and neck curled towards the back of the body—either touching the body or a hand's length away from the body	

4.2.3.3 Weather conditions. Measured as described in Chapter 3, ***section 3.2.3.3***.

4.2.3.4 Grass and TMR sampling. Grass and TMR samples were collected on day 5 of each measurement period for subsequent analysis of DM, CP, and NDF (Table 9). Thirty random snip samples (to represent the grazed area) at a height of approximately 5 cm above the soil (Taweel et al., 2005) were taken from both the right and left side of the trial pasture in a W pattern. The TMR samples were randomly taken from fresh feed provided to the cows on day 5 of each measurement period.

4.2.3.5 Nutrient analysis of grass and TMR. Measured as described in Chapter 3, ***section 3.2.4.2***.

Table 9. Nutrient composition of grass available at either the right (R) or left (L) side of pasture, and TMR indoors (mean ± SD). DM = dry matter, CP = crude protein, NDF = neutral detergent fibre. No significant differences were found in the nutrient quality of the R or L side of the pasture (P > 0.05).

Nutrient Composition	R	L	TMR
DM (g/kg)	226 ± 7.38	205 ± 5.71	492 ± 9.16
CP (g/kg DM)	234 ± 32.6	222 ± 38.1	170 ± 44.3
NDF (g/kg DM)	461 ± 21.4	453 ± 15.8	344 ± 29.2

4.2.3.6 TMR intake and milk yield. Group TMR refusals were weighed and disposed of daily at approximately 5:30 h while the cows on trial were being milked. Milk yields for each animal on study were recorded at each milking by an automatic recording system (GEA Farm Technologies, Bönen, Germany) for ten days prior to each measurement period as well as for the ten day measurement period. This was in order to determine a treatment effect on milk yield as well as to investigate if access to pasture had an impact on milk yield as seen in Chapter 2.

4.2.4 Statistical Analysis

Analysis was as described in Chapter 3 with the following modifications. Absolute preference for cubicle housing vs. pasture was determined by comparing the time spent indoors for each group (n=11) with 0 % (choice to be at pasture), 50% (indifference) and 100% (choice to be indoors) using a one-sample t-test. Overall time spent indoors or at pasture was expressed as a percentage of the total time offered a choice (cows were offered a choice between being indoors or at pasture for approximately 19 h/d—this excludes time being milked). A one-way ANOVA followed by post-hoc Bonferroni tests was used to determine a treatment effect on overall time spent indoors or at pasture, behavioural observations during daylight hours, total time spent engaged in total investigation behaviour (investigatory grazing + general investigation), lying location, TMR intake, and milk yield.

A Kruskal-Wallis H –test followed by a post-hoc Mann Whitney U test with a Bonferroni correction was used to determine an effect of treatment on day-time (07:30-14:30 h) vs. night-time pasture use (17:30 – 04:30 h), time spent investigating grass and lying postures. An ANOVA with repeated measures was used to determine an effect of time point on both overall time spent at pasture as well as the percentage of time spent grazing and linear regressions were used to determine if associations exist between measurement day and overall time spent at pasture. Paired t-tests were used to determine differences in lying location as well as investigate whether there were differences in milk yield before the trial and during trial *within* each group. A Wilcoxon Matched-Pairs test was used to determine differences in lying posture at pasture vs. indoors within each group. Finally, linear regressions were used to determine whether a relationship existed between weather conditions and time spent indoors or on pasture.

All analyses were carried out using GenStat (15^{th} edition; Lawes Agricultural Trust Co. Ltd., Rothamsted, UK) and descriptive data from non-parametric tests are reported using medians and interquartile ranges to aid clarity, although tests were carried out using mean ranks.

4.3 RESULTS

4.3.1 Absolute preference

Cows without previous exposure to pasture (P3) expressed an overall partial preference to be indoors (97.5 ± 7.97 %) and this was different from 0 % (t = 114.49, P < 0.001), 50 % (t = 55.75, P < 0.001), and 100 % (t = -3.00, P = 0.013). Cows with previous exposure to pasture during the second grazing season (P2) expressed an overall partial preference to be at pasture (68.4 ± 2.45 %) and this was different from 0 % (t = 27.96, P < 0.001), 50 % (t = 7.52, P < 0.001), and 100 % (t = -12.92, P < 0.001). Cows with maximum exposure to pasture (P1) also expressed an overall partial preference to be at pasture with time at pasture (62.1 ± 7.17 %) differing from 0 % (t = 8.66, P < 0.001), 50 % (t = 7.55, P < 0.001), and 100 % (t = -5.29, P < 0.001).

4.3.2 Effect of treatment on overall time spent indoors vs. at pasture

Using 24 h video data, when compared to group P3, both P1 and P2 cows spent more time at pasture (F = 68.13, P < 0.001, Table 10). There was no difference in the time spent at pasture between P1 and P2 cows.

Table 10. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on 24 h time spent either indoors or at pasture. Means sharing the same superscript are not significantly different.

	Treatment				
Item (%)	P1	P2	P3	SEM	*P*-value
Indoors	37.9^{a}	31.6^{a}	97.5^{b}	4.40	< 0.001
Pasture	62.1^{a}	68.4^{a}	2.50^{b}	4.40	< 0.001

Cows in group P3 spent more time inside both during the day during the night than P2 or P1 cows (U = 0, P < 0.001, Table 11) and consequently spent less time outside both during the day night than P2 or P1 cows (U = 0.0, P < 0.001). There were no differences in time spent at pasture during the day or night when comparing P1 or P2 cows.

Table 11. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on overall time spent at indoors vs. at pasture during the day vs. the night. Medians sharing the same superscript are not significantly different.

	Treatment						
	P1		P2		P3		*P*-value
Item (%)		IR*		IR		IR	
In during day	48.3[a]	30.6	54.6[a]	3.01	97.7[b]	47.5	< 0.001
Out during day	52.7[a]	47.9	45.4[a]	30.6	2.15[b]	3.01	< 0.001
In during night	14.7[a]	41.0	16.1[a]	15.0	96.7[b]	8.33	< 0.001
Out during night	85.8[a]	38.2	85.6[a]	17.3	0[b]	7.38	< 0.001

*Interquartile Range

4.3.3 Effect of time on 24 h time spent at pasture (over the 10 measurement d) and time spent grazing (over the 6 manual observation days)

No effect of time was observed for time spent at pasture and time spent grazing with P1, P2, or P3 cows ($P > 0.05$), and no associations (Figure 10) were found between measurement day and time spent outdoors for P1, P2, or P3 cows ($R^2 = 0.016$, $P > 0.05$, $R^2 = 0.072$, $P > 0.05$ and $R^2 = 0.352$, $P > 0.05$ respectively).

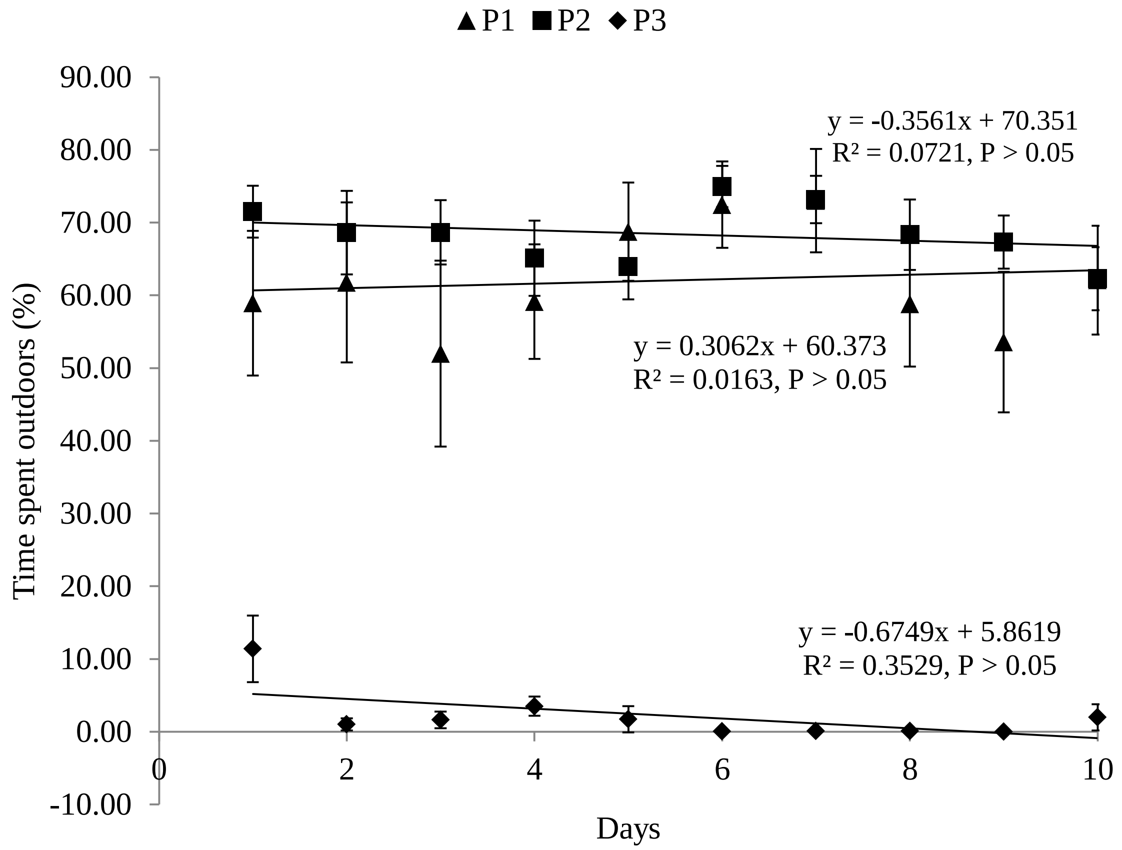


Figure 10. The association between measurement day and mean daily time spent outdoors for P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study. Each data point represents the mean time spent at pasture on a particular measurement day for each treatment group.

4.3.4 Behavioural observations

During the manual observation periods cows in treatment group P3 spent more time indoors, less time at pasture, less time walking, more time eating TMR, and less time grazing than cows in group P1 or P2 (Table 12). Group P2 cows spent more time on the track than group P3 cows, more time standing than P1 cows, and less time lying and ruminating than both P1 and P3 cows. No differences were observed with respect to percentage of time spent drinking (Table 12).

Table 12. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on location, posture, and jaw activity observed manually during daylight hours. Means sharing the same superscript are not significantly different.

	Treatment					
Item (%)	P1	P2	P3	S.E.M.	*F*-value	P-value
Indoors	44.8^{a}	45.9^{a}	94.2^{b}	5.43	26.98	**< 0.001**
Track	3.40^{ab}	5.50^{a}	3.00^{b}	0.666	3.99	**0.029**
Pasture	51.8^{a}	48.6^{a}	2.80^{b}	5.53	24.55	**<0.001**
Lying	40.8^{b}	33.4^{a}	41.4^{b}	1.73	6.52	**0.004**
Standing	55.5^{b}	61.9^{a}	57.1^{ab}	1.59	4.35	**0.022**
Walking	3.70^{a}	4.70^{a}	1.50^{b}	0.310	27.41	**<0.001**
Eating TMR	19.1^{a}	23.9^{a}	31.4^{b}	1.65	14.16	**< 0.001**
Grazing	21.6^{a}	21.5^{a}	1.19^{b}	2.14	30.10	**< 0.001**
Ruminating	30.5^{b}	25.3^{a}	31.3^{b}	0.970	11.32	**< 0.001**
Drinking	1.59^{a}	2.20^{a}	2.21^{a}	1.57	1.43	0.255
Idling	27.1^{a}	27.1^{a}	33.9^{b}	1.89	6.21	**0.006**

4.3.5 Investigatory behaviour

4.3.5.1 Total investigation (grass + general). Cows in treatment group P3 spent more time engaged in total investigation behaviour when compared with both P2 and P1 cows (2.45 ± 0.196 % vs. 1.50 ± 0.196 % and 1.58 ± 0.196 % respectively, F = 7.20, P = 0.003). No difference was observed between P1 and P2 cows.

4.3.5.2 Investigatory grazing (investigation of grass only). Although values for all treatment groups were low, P3 cows spent more time engaged in investigatory grazing than P1 cows (U = 22.0, P = 0.010), but no differences were found between P2 and P3 cows or P1 and P2 cows (Figure 11).

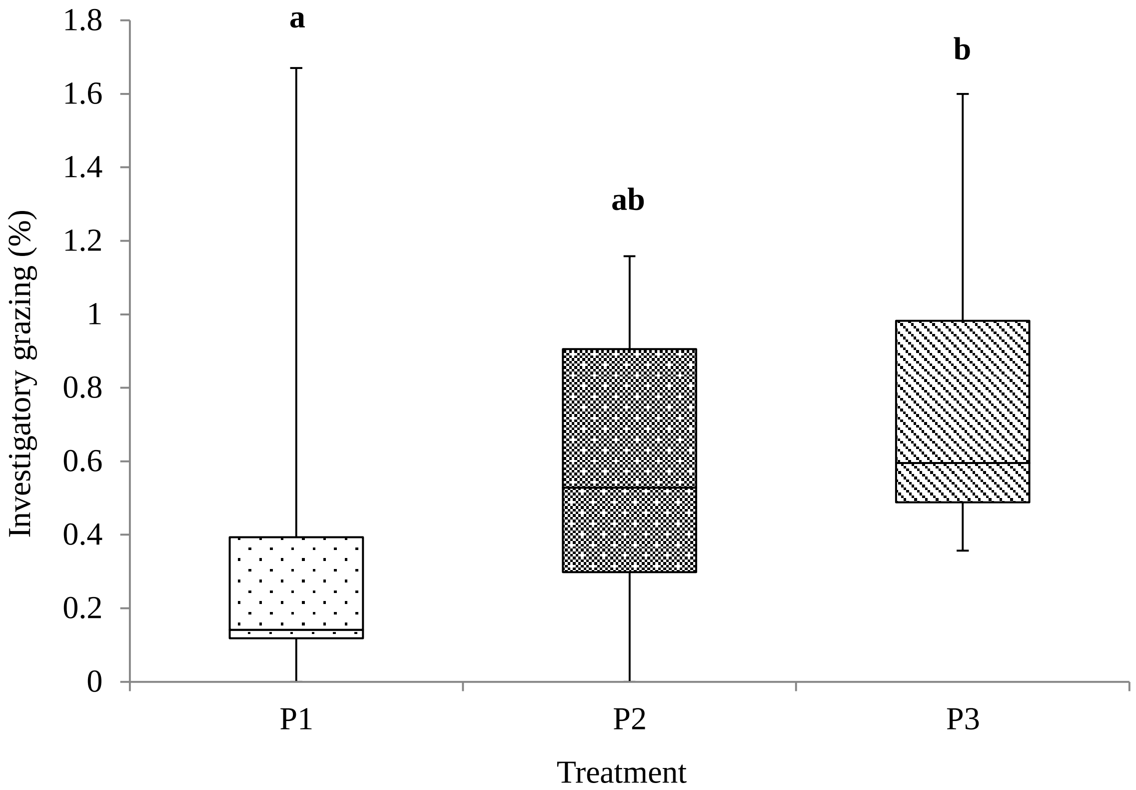


Figure 11. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on time spent engaged in investigatory grazing. Each box plot gives an indication of minimum and maximum values for each treatment group as well as the median and interquartile range. Post-hoc Mann Whitney U tests revealed that time spent engaged in investigatory grazing behaviour by group P3 was shown to be different from group P1 but not from P2. No difference was found between group P1 or P2. Differences are denoted by the letters a vs. b.

4.3.6 Effect of treatment on lying location and posture

Of the time spent lying, cows with no exposure to pasture (P3) spent 0% of their time lying at pasture which was different from both groups P2 and P1 (63.8 ± 7.74 % and 57.7 ± 7.74 % respectively, $F = 20.74$, $P < 0.01$). No difference was found between groups P2 and P1. Cows in treatment group P3 spent less time lying with their front legs and rear legs out sideways and their head resting to the front (lateral lying) when compared to P1 and P2 cows (Table 13a-c). No other differences were found between treatment groups in lying posture.

Table 13a. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on front leg lying posture during daylight hours. Medians sharing the same superscript are not significantly different.

Front leg posture (%)	Treatment P1	IR*	P2	IR	P3	IR	*P*-value
Both under	31.6[a]	27.8	31.2[a]	1.75	31.6[a]	26.0	0.832
One under one out	4.18[a]	5.19	2.13[a]	1.17	3.87[a]	4.27	0.119
Both out	0.08[a]	0.258	0[a]	0	0[a]	0.249	0.152
Out sideways	0.192[a]	0.547	0.270[a]	0.647	0[b]	0.06	0.001

*Interquartile Range

Table 13b. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on rear leg lying posture during daylight hours. Medians sharing the same superscript are not significantly different.

Rear leg posture (%)	Treatment P1	IR*	P2	IR	P3	IR	*P*-value
Both under	7.83[a]	4.94	6.79[a]	5.61	6.34[a]	6.44	0.611
One under one out	24.6[ab]	19.1	25.3[a]	5.66	28.9[b]	24.8	0.008
Out sideways	1.25[a]	1.78	0.958[a]	0.937	0.44[b]	0.335	0.009

*Interquartile Range

Table 13c. Effect of treatment (P1- maximum exposure to pasture in their first two years of life vs. P2 - no exposure to pasture in their first year of life, and maximum exposure in their second year of life vs. P3 - no exposure to pasture until being tested in 2013 during the current study) on head lying posture during daylight hours. Medians sharing the same superscript are not significantly different.

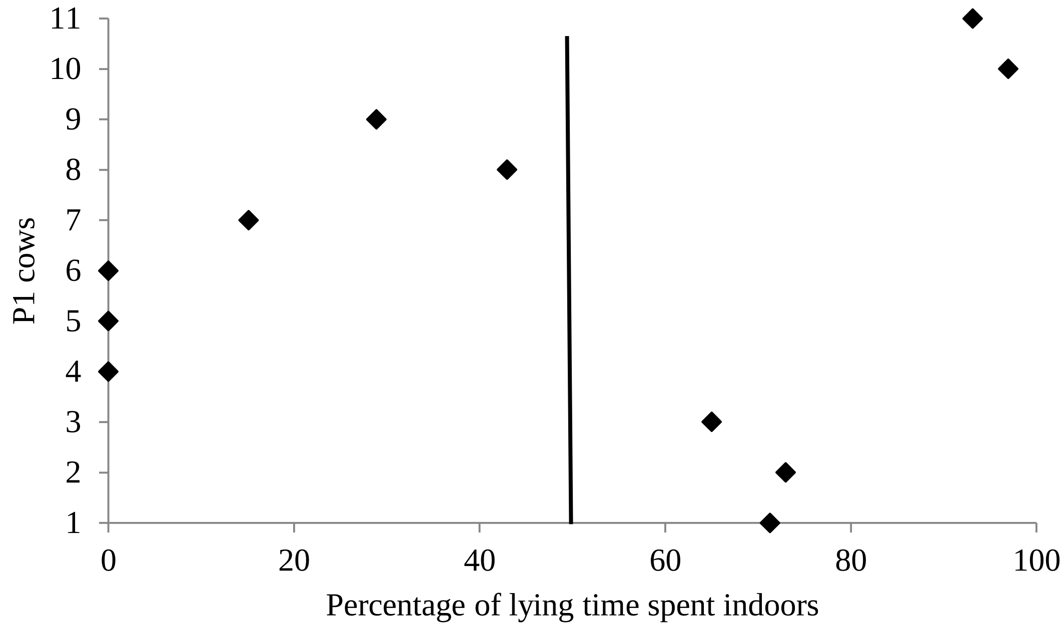
	Treatment						
Head posture (%)	P1	IR*	P2	IR	P3	IR	*P*-value
Up	30.4^{a}	21.1	30.2^{a}	1.88	31.0^{a}	24.5	0.148
Rest Front	1.08^{a}	1.07	0.796^{a}	1.15	0.206^{b}	0.273	0.008
Rest back	4.62^{a}	3.34	2.50^{a}	1.25	2.67^{a}	1.59	0.253

*Interquartile Range

4.3.7 Effect of location (either indoors or at pasture) on lying time and lying posture

A paired t-test revealed that there was no difference in time spent lying at pasture vs. indoors for either P2 cows (64.6 ± 6.60 % vs. 45.6 ± 6.65 %, t = 1.45, P = 0.178) or P1 cows (65.1 ± 9.23 % vs. 51.5 ± 11.1 %, t= 0.69, P = 0.503). Treatment group P3 spent 0% of their lying time at pasture so they were not included in this analysis. When examining the lying time each cow spent on average indoors, 3 cows in group P1 spent 0 % of their lying time indoors while 2 cows spent over 90 % of their lying time indoors and the remaining cows ranged between approximately 15 to 73 %. Four cows in group P2 spent over 50 % of their lying time indoors, while the remaining cows ranged between approximately 4 to 47 % (Figure 12a-b).

a



b

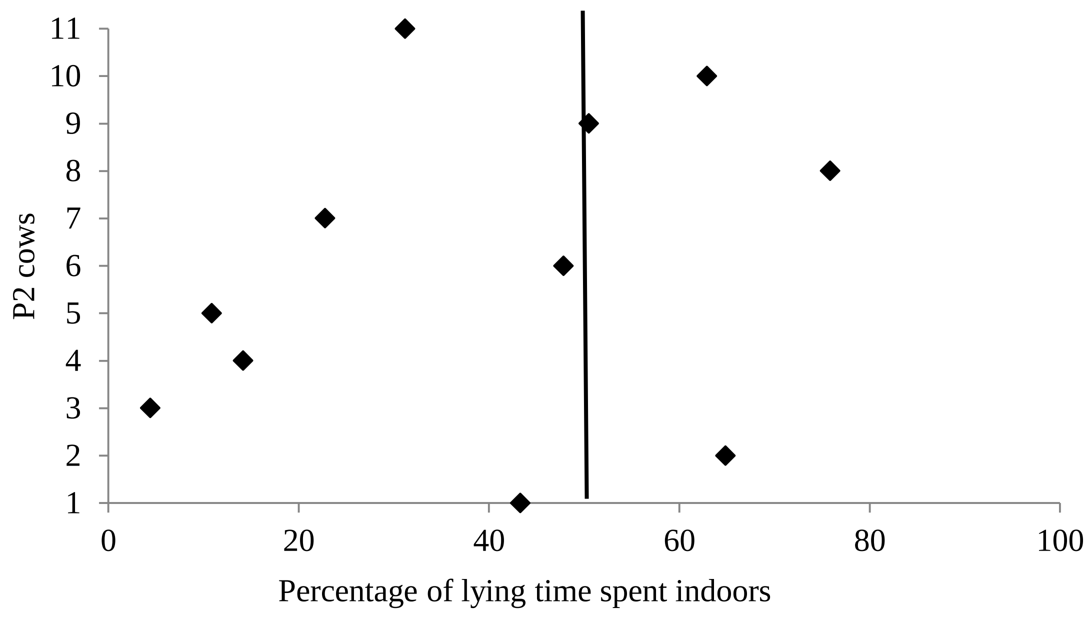


Figure 12. Mean total lying time spent indoors (%) for individual cows in treatment group P1 (reared with maximum exposure to pasture for the first two years of their life) (**a**) and group P2 (reared with maximum exposure to pasture for the first two years of their life) (**b**). The solid line indicates the 50 % mark.

At pasture a Wilcoxon Matched-Pairs test revealed that P1 cows spent more time with their front legs out sideways ($W = 0$, $P = 0.016$), rear legs out sideways ($W = 3$, $P = 0.010$), and head rest front ($W = 3$, $P = 0.010$) at pasture than when in housing. No lying posture differences at pasture vs. in cubicle housing were observed for P2 heifers.

4.3.8 Weather

4.3.8.1 Time spent indoors vs. at pasture and grazing time. Mean temperature during the study was 15.0 ± 1.53°C (mean ± SD) outdoors and 16.6 ± 1.69° C indoors. The THI over the course of the study was 59.2 ± 6.32 and rainfall was 0.14 mm. Neither THI ($P > 0.05$) nor rainfall ($P > 0.05$) influenced time spent indoors or at pasture for P1, P2, or P3 cows.

4.3.9 TMR intake and milk yield

No differences were found in group TMR intake ($P = 0.474$) or milk yield ($P = 0.529$) when comparing the three treatments. Cows consumed 59.7 ± 6.18 kg DM/d of TMR on a group basis and produced 26.7 ± 1.43 kg of milk on average per cow per day. In addition, there were no differences in average milk yield/d between the ten days before being on trial when cows were housed vs. the measurement period when cows had free access to pasture ($P > 0.05$) for any of the treatment groups.

4.4 DISCUSSION

The results indicated that heifers reared without exposure to pasture (P3) had an almost exclusive preference to be indoors, spending over 97 % of their time in cubicle housing. As discussed previously in Chapter 3, Charlton et al., (2011a) speculated that prior experience may have an effect on preference as cows with limited experience of pasture spent 92 % of their time in cubicle housing when given a choice. Additionally, P2 heifers in 2012 (who at that point were being exposed to pasture for the first time) also preferred being indoors spending about 80 % of their time in cubicle housing. P1 and P2 cows expressed a partial preference to be at pasture spending 62 and 68 % of their time at pasture respectively and these values were not different from eachother. Contrary to findings reported in Chapter 3 however, time spent at pasture did not increase over time for P3 cows suggesting that there is a specific learning period for dairy cows where exposure to pasture in their first two years of life post-weaning encourages a preference for pasture. Studies suggest that ruminants more readily accept a new environment and novel food choices during the first year of life while mature animals are less willing to accept novelty (Provenza and Balph, 1987, Lobato et al., 1980). Additionally, heifers appear to have an increased ability to learn when compared with both primiparous and multiparous cows (although multiparous cows learn better than primiparous cows) (Kovalcik and Kovalcik, 1986). As treatment group P3 were lactating cows when they were first exposed to pasture their ability to accept a new environment, increase their time spent on pasture as the study period progressed, as well as learn to graze may have been hindered when compared with groups P1 and P2 who were both exposed to pasture as non-lactating heifers. It does not seem to make a difference whether heifers are exposed to pasture in the first or second year of life post-weaning as both P1 and P2 cows spent a similar amount of time on pasture, spent the same amount of time grazing, and P2 cows actually changed their preference after a being exposed to pasture in the second year of their life.

During the night, both P1 and P2 cows spent 85% of their time at pasture. This result is similar to findings reported in Chapter 2, and by Legrand et al. (2009) and Falk et al. (2012) where dairy cows spent the majority of their time at pasture during the night. However, these results are contradicted by Charlton et al. (2011a) where dairy cows preferred to be indoors at night and findings reported in Chapter 3 where P2 heifers spent only 18% of the night at pasture and P1 heifers spent only 43 % of their time at pasture during the night. This change in night-time pasture use for P2 heifers is likely due to the change in overall preference for pasture, and the change in night-time pasture use for both groups is likely due to weather conditions. As explained in Chapter 3, cows prefer dry lying surfaces (Fregonesi et al., 2007) and decreased lying times have been reported when pasture is not well-drained (Fisher et al., 2003). Although the ground was saturated with water during the experiment in 2012, it was dry during 2013 which may have affected night-time pasture use. Additionally, as overall time spent indoors was 92 % in the Charlton et al., (2011a) study it is logical that night-time pasture use would be limited. It was also suggested by Charlton et al. (2011a) that temperature may have influenced preference to be indoors during the night-time while in the current study temperature did not exceed the upper critical limit for lactating cows (25º C) (Berman et al., 1985) and did not affect preference.

Manual behavioural observations revealed that P2 cows spent less time resting and ruminating than both P1 and P3 cows, but all groups spent the majority of their time engaged in resting and ingestion behaviour (ruminating + grazing/eating TMR) which is representative of a normal time-budget expressed by cattle with limited human intervention (Kilgour, 2012) and of cattle in conventional free-stall housing (Gomez and Cook, 2010). Treatment group P3 spent only 1.2 % of their time grazing, but this is likely because they only spent 2.5 % of their time at pasture. Lopes et al. (2013) reported that even when forced outside, cows introduced to pasture in their first lactation took longer to express behaviour similar to cows that had previous grazing experience as heifers.

Although P3 cows spent a limited amount of time at pasture, they mainly investigated grass and grazed when at pasture. Investigation behaviour in general was more prevalent in P3 cows than both P1 and P2 cows, and specifically investigating grass was more prevalent in P3 cows than P1 cows. This is similar to results reported in Chapter 3, where heifers with no previous experience of pasture spent more time investigating grass than heifers with previous experience of pasture suggesting an element of learning with respect to grazing.

Of the time spent lying, both P1 and P2 cows spent the same amount of time lying at pasture and in the cubicle housing facilities. This is similar to results found in Chapter 2 and by Charlton et al. (2011b), but contradicted by results reported by Ketelaar-de Lauwere (1999) and Krohn et al. (1992), who found that cows prefer to lie at pasture, and Charlton et al. (2011a) who reported that cows prefer to lie indoors. In the current study, observation of lying time was restricted to daylight hours vs. the study of (Ketelaar-de Lauwere et al., 1999) which accounted for 24 h lying time. This may explain why there was no effect of location on lying time in the current study. Additionally, individual cows showed great variation in where they preferred to lie down with some cows spending 0 % of their lying time indoors while others within the same treatment group spent almost all their lying time indoors—potentially accounting for the lack of effect. As has been discussed in Chapter 3, the majority of lying behaviour occurs at night, and dairy cows spent the majority of their time at pasture during the night in this study, so it would be logical to suggest that overall lying time at pasture (include time spent lying at night) was actually more than lying time in cubicle housing in the current study. The preference to lie indoors reported by Charlton et al. (2011a) was possibly due to the cows' overall preference to be indoors which was contradicted in the current study.

It has been suggested that cows prefer to lie down at pasture vs. cubicle housing because of the restrictions (neck bars, breast boards, cubicle partitions) present when compared to pasture (Falk et al., 2012) that may prevent cows from lying in a stretched position. Additionally, they have more choices as to where they lie down, who they lie down next to, and distance between neighbours (Falk et al., 2012). Holstein cattle, weighing approximately 600 kg (similar to the average weight of 557 kg reported in this study) were reported to use up to 3.00 m of longitudinal space and 1.09 m of lateral space when lying

(Ceballos et al., 2004). Although the width of the cubicles (1.2 cm) used in the current study would accommodate for the maximum lateral space used, the length of the cubicle (2.7 cm) would not account for the maximum longitudinal space used. Although cows in the current study did not express a preference to lie down at pasture during the day, both P1 and P2 cows spent more time lying laterally (front legs out sideways, rear legs out sideways, head rest front) than P3 cows. It has been previously reported that lateral lying is more common at pasture (Ketelaar-de Lauwere et al., 1999) or in loose-housing systems (Endres and Barberg, 2007) As lateral lying was only seen at pasture, in agreement with Krohn and Munksgaard (1993), it was likely that the cubicle housing prevented cows in the current study from assuming this posture indoors. In general however, lateral lying was uncommon with only about 1 % of their lying time spent in this position which is similar the 1.6 % reported by Krohn and Munksgaard (1993). The most common lying position (expressed as median percentages) was front legs both under (31 %), rear legs one under one out (26 %), and head up (30 %). Similar studies have also reported that head up is the most common position and that lateral lying is uncommon (Ketelaar-de Lauwere et al., 1999, Haley et al., 2000, Endres and Barberg, 2007). As night-time posture could not be recorded (due to lack of visibility and disruption to their normal behaviour), it is unclear whether the low percentage of time spent lying laterally was actually representative of 24 h lying time.

Weather did not influence preference which is in agreement with results reported in Chapter 2, but in contradiction to those reported in Chapter 3. The THI in the current study did not reach the upper critical limit for dairy cows and a wide temperature range was not seen over the course of the study. It rained on 31 of the 60 measurement days, but average rainfall appears to have been too low to have an effect on preference. Additionally no effect of weather on grazing time was seen. Although an effect of weather was reported in Chapter 3, preference and grazing time was only influenced for heifers that had no previous experience with pasture. In the current study, P3 cows spent almost no time at pasture, and as such it is difficult to determine whether there was an association between weather and time spent on pasture.

Lastly, TMR intake was maintained across all three groups, with each group intake being approximately 60 kg DM/d. Assuming individuals consumed an approximately equal share, this is a similar amount to individual TMR intakes reported in Chapter 2 (22 kg DM/d). No treatment effect on milk yield was found either suggesting that feed intake and milk yield may not be affected by exposure to pasture during rearing. In contrast to results reported in Chapter 2, when comparing the 10 days before the trial period when cows were continuously housed to the 10 days during the trial period no increase in milk yield occurred as a result of free access to pasture. Milk yield was maintained throughout the study period however and did not decrease. This is particularly interesting, because it suggests that access to pasture for late lactation cows (Chapter 2) may have a positive impact on milk yield, but does not affect cows in the beginning of mid-lactation in the same manner. However, the two experiments cannot be directly compared as there were a number of differing factors including the fact that continuously housed cows in Chapter 2 were compared with a different sample of cows while cows in the current study were compared against themselves at different time points and cows in the current study were all in their first lactation, which would impact yield.

4.5 CONCLUSIONS

Cows that are exposed to pasture within the first two years of their life post-weaning will express a preference for pasture while cows that are exposed to pasture after their second year of life prefer to remain indoors. Cows do not express a daytime preference to lie at pasture or in cubicle housing but it is likely that they spend the majority of 24 h lying time at pasture. Lying location affects lateral lying, but as time spent in this position was so low it is unclear if there is a negative impact on either welfare or production if cows are restricted from lying laterally.

5.1 ORIGINAL CONTRIBUTION TO KNOWLEDGE

The three experiments discussed in Chapters 2, 3, and 4 have revealed that preference for pasture is not influenced by herbage mass and although high-yielding dairy cows are motivated to access pasture, it is not to fundamentally express grazing behavior. In addition, previous experience of pasture plays a major role in determining dairy cow preference for pasture. The study discussed in chapter 2 also provided insight into the benefits of a free-choice system (free access to pasture and indoor cubicle housing) over a continuously housed system as dairy cows spent more overall time lying down when they had the choice between cubicles and pasture as a lying area and a large difference in milk yield between the two groups was recorded.

Additionally, previous preference studies directly assessing preference to be indoors or at pasture (Legrand et al., 2009, Charlton et al., 2011a, Charlton et al., 2011b, Falk et al., 2012, Charlton et al., 2013) with the exception of Krohn et al. (1992), who did not report DIM, tested preference on cows in late lactation. In Chapter 4, preference tests were conducted on cows that ranged from early-mid lactation were on average 109 DIM. The results of the study in Chapter 4, suggested that on average, cows that were likely to be experiencing a negative energy balance (early lactation), or cows that have just achieved peak DM intake (mid-lactation) still prefer access to pasture (if they have had experience of it) just as cows in late lactation. In addition, access to pasture will not compromise feed intake or milk yield when a TMR is provided indoors for cows that are in a critical physiological state where maximum feed intake is not being achieved and body condition is being mobilised, or for cows that are in late lactation when metabolic demands have decreased (Chapter 2).

5.2 DEPRIVATION OF RESOURCES

The deprivation of the ability to perform certain types of behavior or behavioral patterns can cause animals to suffer (Dawkins, 1988). It has been previously argued that it is not possible to deprive animals of resources they have never had access to (Cooper and Appleby, 1995). Using motivation testing, it is possible to gain insight into which resources (that allow for the expression certain behaviors) are most important to animals, but results may be affected by previous experience of a resource as the expectations of the animal may differ when animals have not had exposure vs. when they have had exposure to the resource in question.

In Chapter 2 it was made apparent that dairy cows are highly motivated to gain access to pasture, and Chapters 3 and 4 indicated that this motivation stemmed from previous experience at pasture. Rutter (2013) poses the fundamental question of whether cow welfare would actually be compromised more if cows are exposed to pasture as young animals, develop a preference for it, and then become behaviorally frustrated as they are kept indoors most of their life or for long periods than if they were to be housed indoors year-round and never experience pasture at all. However, since the evidence shows that dairy cows value both indoor housing and pasture, and there are clearly times when cows want to be in either location, it can be argued that the behavioral frustration they may experience for part of the year does not outweigh the health and behavioral benefits of pasture if cows are given pasture-access when they are most likely to prefer to use it, and when a TMR is offered indoors (or at pasture). In other words, under conditions such as when the ground is dry, the temperature is optimal (between 5° to 25° C), appropriate cooling mechanisms (shade, sprinklers) are employed, or during the night-time.

The element of choice seems to be particularly important as cows have the ability to optimize their own welfare (i.e. they can choose to lie down indoors when the ground is wet, or lie down outside when conditions permit—increasing overall lying time) which in turn can optimize production as seen with the increased milk yield in the study conducted in Chapter 2.

5.3. ASPECTS OF LEARNING AND BEHAVIOURAL ACTIVITY

Heifers that learned to graze during the experiment reported in Chapter 3, grazed immediately when given access to pasture in the experiment reported in Chapter 4, indicating that once they learned to graze (even though they were housed for a period of time in between the experiments in 2012 and 2013) they retained this behavior in their repertoire. Previous research testing memory has indicated that cattle have an accurate spatial memory and can remember where they have foraged after an 8 h delay, with memory declining after 8 h, and an inaccurate memory after 12 h (Bailey et al., 1989). Similarly, long term memory (after 6 week delays) has been reported to be less stable when a foraging area was learnt as a heifer (Kovalcik and Kovalcik, 1986). The time spent at pasture and grazing time reported for cows with previous experience of pasture in Chapter 4 indicated that they remembered both how to graze and where the pasture was located in the experimental area (particularly as pasture use on d 1 was similar to pasture use on d 10) after a delay of up to 7 months. Similarly, Lopes et al. (2013) reported that heifers exposed to pasture at 6 months, and then confined for 18 months, exhibited normal grazing patterns immediately after being re-exposed to pasture. This suggests that retention in cattle may be more accurate than previously thought.

In Chapter 3, Figure 7 (which shows a change over time in time spent at pasture for P2 heifers) indicated that if the study period were to progress, both P1 and P2 heifers may eventually spend a similar amount of time at pasture, suggesting that the transition between "inexperienced" to "experienced" occurred in a short period. This was the result that was reported in Chapter 4, where P1 and P2 cows did not differ in time spent at pasture from d

1. Cows in treatment group P3 peaked in their time spent at pasture on d 1 (Figure 10) and then stabilised (spending almost all their time indoors over the rest of the measurement period), while P2 heifers in Chapter 3 spent the most time at pasture on d1 over the first four days, but then incrementally increased time spent at pasture after d 4. P3 cows may have been responding to novelty on d1, but upon becoming hungry, and potentially having a decreased desire to learn than heifers first introduced to pasture in 2012 (and having an increased metabolic demand than non-lactating P2 heifers) decided to spend time indoors where TMR was readily available (P3 cows did spend more time eating TMR than either P1 or P2 cows). P2 heifers may have had a similar issue in 2012 (which may be why there was decreased usage between d 1 – d 4), but as they did not have the same metabolic demands placed on them by lactation, heifers could afford to spend more time engaged in investigatory behavior and individual learning which would help them adapt to a system with free-access to pasture.

It is also interesting to note the differences in behavioral activity that occurred at pasture in Chapter 4 when cows had no prior experience with pasture. Although the percentage of time spent at pasture was very low for P3 cows, the majority of time at pasture was spent engaged in either investigatory grazing or grazing behavior. Of the time spent at pasture, both P1 and P2 cows spent about 65 % of the time lying. These results indicate that lying at pasture may also be encouraged by experience of pasture. Additionally, although dairy cows spent a very small percentage of lying time in a lateral position, it does not necessarily indicate that the ability to lie down in this position is not important. Although not clearly understood, Rapid-eye-movement (**REM**) sleep has been linked to the maintenance of normal brain processes including spatial learning ability (Smith, 1985). Hänninen et al. (2008) reported that REM sleep in dairy calves was most accurately recorded when the neck and head was in a relaxed position as opposed to an upright position and this particular head posture was observed more frequently when cows were lying on their side at pasture according to results reported in Chapter 4 and by Ketelaar-de Lauwere et al. (1999). Calves spent approximately 5 h out of 20 h sleeping (not simply lying or resting), and approximately half of that time was dedicated to REM sleep (Hänninen et al., 2008). As dairy cattle only sleep for about 4 h/d (Ruckebusch, 1972) , and young mammals tend to have more REM sleep than adult animals (to aid in brain development) (Siegel, 2005), it would make sense that lying postures that aid in REM sleep would occur in very low, but still important amounts. Additionally, the majority of REM sleep in adult cattle is experienced at night (Albright and Arave, 1997), when we were not able to record lying posture, which may indicate that the incidences of lateral lying may have been slightly higher over a 24 h period.

5.4 FURTHER RESEARCH

5.4.1 Housing type

Previous preference work has been conducted on dairy cows in cubicle housing with a variety of bedding options: deeply bedded sand, geo-textile mattresses, rubber mattresses with lime-ash or sawdust etc. Even in instances where deeply bedded sand was used, which is generally recognized as having many of the same health benefits as pasture over other types of cubicle bedding including decreased incidences of lameness (Cook, 2003), decreased instances of mastitis (Norring et al., 2008), and the provision of a soft substrate to lie on (Hernandez-Mendo et al., 2007) (which is often considered to be the reason why cows may prefer to lie down at pasture) dairy cows still preferred to be at pasture at least during the night (Legrand et al., 2009). Work presented as a conference paper reported that when given the opportunity to choose between cubicles and an outdoor lying paddock, cows preferred the outdoor lying paddock and actively chose the paddock to lie down in over the cubicles (Lanford et al. 2013). Cestari et al. (2013) reported in a conference paper that cows were willing to push a weighted push gate with up to 36 kg to gain access to

TMR which was not different from the 32 kg maximum they were willing to push to gain access to pasture—these cows also had access to free-stalls with deeply bedded sand.

Additional work presented as a conference paper, which provided dairy cows free-access between straw yards and pasture or cubicle housing and pasture (between 06:00-16:00) reported that cows with access to straw yards preferred to be at pasture more so than cows in cubicle housing (71 vs. 55 % of cows respectively) (Humphries, 2012). As the main argument with the important of pasture-access tends to be associated with lying behaviour, it is curious that cows with access to a comfortable and soft bedding material such as straw (which has been shown to actually increase the time cows spend lying (Norring et al., 2008)) and the opportunity to lie in open packs (which they prefer over free-stalls (Fregonesi et al., 2009)) show a stronger preference for pasture. It would be interesting to look at the difference between open-pack systems (straw-yards, compost-bedded yards etc.) over a 24 h period to see if this pattern continued at night.

5.4.2 Performance vs. function of behaviour

In Chapter 2 it became apparent that the function of grazing behaviour (to satiate hunger and maintain body condition) was not necessarily important to the average high-yielding cow provided a TMR. However, it is less clear whether the actual performance of this behaviour is important to dairy cows. In other species, certain behaviors are performed even in the absence of external cues: laying hens still perform “sham” dust-bathing in the absence of substrate (Mench, 1998) and the performance of wallowing behavior in pigs may be important even when the perceived main function of the behavior is fulfilled via temperature-controlled buildings (Bracke, 2011)—indicating that the performance of particular behaviors is important even without a functional result.

In Chapter 3 it was reported that there was an adaptation period to grazing, which occurs even when cows are forced onto pasture (Lopes et al., 2013) suggesting that there is not necessarily an intrinsic need to graze, and that environmental conditions (i.e. the presence of grass) stimulate grazing rather than solely internal cues. However, it would be interesting to pursue this further and characterize the behavioral sequences of grazing versus consuming TMR as oral stereotypies (which can sometimes be indicative of negative emotional states) are often a result of un-natural foraging conditions (Mason and Rushen, 2006). Additionally, there may be positive internal feedback received by the animal that occurs during grazing which may not occur during the consumption of TMR. Suckling behavior in dairy calves on non-nutritive substances (i.e. dry teats) following milk intake for example increases the release of cholecystokinin and insulin which aid in digestion (Mench, 1998): the behaviour itself has a positive impact on health and welfare. A similar occurrence (not necessarily related to digestion) is possible during the behavioral sequence of grazing, and warrants further investigation.

5.5 CONCLUSIONS

High yielding dairy cows prefer be at pasture when they have previous experience of it and are particularly motivated to access pasture at night. Pasture access does not appear to compromise feed intake or milk yields and had a positive impact on production and welfare. In addition, grazing has a learnt component. Modern-genotype Holstein-Friesians often do not access pasture to graze and as such the opportunity to graze may not be particularly important for these animals as long as nutritional demands are being met in another manner.

5.5.1 Recommendations for Practical Application

When turned out to pasture it is important to provide buffer feed for naive heifers to avoid welfare concerns associated with hunger and frustration. Additionally, where possible a choice between pasture and shelter (with TMR) should be provided. To ensure optimal usage of pasture (and the subsequent welfare and production benefits of pasture-access) it should be provided as close to the housing facility as possible.

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Initially, the extraction of n-alkanes from faeces and herbage was to be conducted at the Harper Adams University laboratory. As specific equipment was lacking, modified versions of this protocol was attempted multiple times at Harper Adams, but ultimately did not work. As such, extraction and preparation of fractions for analysis by gas chromatography (GC) (performed by me) and GC analysis (performed by the James Hutton Institute as the GC's were broken during the short time I was able to be placed at the Institute so they conducted this analysis after I left) was conducted at the James Hutton Institute using the following protocol. After receiving the GC data from the James Hutton institute all intake calculations were conducted by me.

Faeces and herbage were freeze-dried and samples were finely ground with a coffee grinder prior to extraction.

Faeces and herbage

Saponification and extraction of samples:

1. Duplicate 0.1 g samples of dried, ground faeces should be accurately weighed into 4 ml glass GC vials fitted with screw caps having PTFE-lined inserts
2. The following solutions are added by weight as the internal standards for alkanes: docosane (C_{22} and tetratriacontane (C_{34}) in decane (0.3 mg/g each alkane, 0.11 g solution to each vial))
3. Ethanolic KOH solution (1 M, 1.5 ml) is then added
4. The vials (Vial RAW) are capped and heated for 16 h at 90 °C in a dry-block heater. After partial cooling (to 50-60 °C) 1.5 ml n heptane is added and the tube is capped and shaken gently
5. Water (0.5 ml) is added and the tube is re-stoppered and shaken vigorously. After separation into two liquid layers, the top (non –aqueous) layer is transferred to a second 4 ml GC vial (Vial HALC) with a polyethylene Pasteur pipette
6. Another 1.5 ml of heptane is added to the tube and the extraction repeated, adding the top layer to the same vial. The solution in the vial is evaporated to dryness on a dry-block heater fitted with a sample concentrator blowing air into the vial.
7. The extracts are re-dissolved in 0.3 ml heptane, with warming, and applied to a small column containing silica gel (Keiselgel 60, 70-230 mesh) with a bed volume of 1 ml (equivalent to about 1.5 ml of a slurry of silica gel in heptane).
8. The hydrocarbons are eluted into a third 4 ml GC vial (Vial H) by the addition of 2 x 1.5 ml n-heptane to the column

For herbage, the protocol was similar with the following exceptions: larger samples are used (0.2 g) with greater quantities of liquid reagents (2 ml ethanolic KOH, 0.6 ml water, and 2 x 2 ml n-heptane). Screw capped culture tubes (16 mm x 100 mm) are used in place of the 4 ml GC vials for the initial saponification and extraction process. The clean-up of the crude extracts through silica-gel columns and subsequent analysis by gas chromatography are identical to the method used for faeces.

Preparation of fractions for analysis by gas chromatography

1. Dodecane (0.2 ml) is added to Vial H. The vials are then warmed and rolled to ensure dissolution of the hydrocarbons.
2. The contents are transferred to a 0.8 ml GC vial, which is then capped with a polyethylene insert prior to placement on a gas chromatograph.

Grass chromatography

As described by Mayes et al. (1986).

Herbage intake was calculated using the following equation:

$$\text{Herbage intake} = \frac{F_i/F_j\,(D_j + I_c \times C_j) - I_c \times C_i}{H_i - F_i/F_j \times H_j}$$

Where H_i, C_i, and F_i = concentrations (mg/kg DM) of the natural odd-chain alkane (C_{33}) in herbage, concentrate, and faeces.

H_j, C_j, and F_j = concentrations (mg/kg DM) of the even chain alkane (C_{32}) in herbage, concentrate and faeces

I_c = intake of concentrates (kg DM/day)

D_j = amount of dosed alkane (mg/d)