A behavioural, epidemiological study to evaluate positive welfare in dairy cows.

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Abstract

Animal welfare has historically been centred on the prevention of suboptimal conditions which may prevent suffering and allow animals to cope within their environment. However, this view of welfare does not provide the opportunity for positive experiences. There is currently a high level of societal interest and concern for dairy cow welfare and there is a demand for policy to push beyond solely the avoidance of negative welfare, to ensure that farm animals experience an acceptable quality of life, which is defined by the balance of experiences over an animals' lifetime being positive. There is an evident gap in the literature regarding the identification and facilitation of positive welfare for dairy cattle. The aims of this thesis were to evaluate the utility of environmental enrichment as an opportunity for positive experiences for housed dairy cows. Subsequent objectives were to evaluate the impact that enrichment has on specific behavioural indicators of positive and negative affect, the longevity of use of enrichment, cows' preferences for different environmental resources and the impact of this on cows' affective states.

The initial literature review identified the lack of validated positive welfare indicators needed to enable interpretation of animals' affective states. Furthermore, it highlighted a particular gap in the literature regarding strategies for the provision of positive welfare in dairy cows. Chapter three evaluated the use of a novel enrichment resource for housed dairy cows. Results showed a high level of interest and use of the enrichment by two separate groups of cows, with 82.86% and 83.33% of the groups still using the enrichment during the third week of provision. Behaviours expressed by cows in response to the

provision of enrichment inferred this to be a positive experience. Chapter four evaluated whether behaviours related to boredom were impacted by the provision of enrichment. ’Idling’ and ‘refusals’, two boredom associated behaviours, were significantly reduced when cows had access to enrichment when compared to standard housing conditions in two separate groups of cows. In addition to this, self-grooming, a behaviour tentatively linked to positive affective states in animals, was found to increase in both groups of cows when cows had access to enrichment compared to standard housing conditions. Chapter 5 evaluated cows’ use of enrichment over a prolonged period and found that 87.18% and 86.11% of two groups of cows, still used novel enrichment two months following installation. This chapter also evaluated cow preferences between two different resources, access to an outdoor concrete yard and novel indoor enrichment. Cows expressed a clear preference for access to the outdoor yard spending 55.67 ± 32.11 (Group 1) and 102.26 ± 59.92 (Group 2) minutes per day using it compared to 6.34 ± 4.62 (Group 1) and 10.13 ± 8.66 (Group 2) minutes per day using indoor enrichment. In Chapter 6 Qualitative Behavioural Assessment was used as a potential proxy for cows’ affective states in varying environmental conditions. Significant differences in QBA results were identified between treatment (enrichment) periods indicative of cows having improved affective states when access to enrichment resources were present compared to standard housing conditions.

The results of this thesis have shown that environmental enrichment is valued by housed dairy cows, has the ability to facilitate positive experiences and therefore, would be a beneficial management strategy for producers to enhance dairy cows’ quality of life. The provision of environmental enrichment for housed dairy cows has also shown to decrease behaviours associated with boredom, a negative affective state. Cows continued to utilise enrichment resources over a prolonged period of time and showed a clear preference for the ability to spend time outside on a small concrete pad, over resources available indoors, showing that even without pasture the ability to have access to an outdoor environment is important.

This thesis has contributed to the knowledge gap on positive welfare for dairy cows and provides industry relevant insights on the topic which have the potential to positively impact welfare. Practical avenues of positive welfare provision for dairy cows have been demonstrated in the commercial environment and appeared to provide, not just positive experiences, but also the reduction of suboptimal welfare. In light of these new findings, the provisional of additional environmental resources, most importantly access to alternative forms of outdoor exercise areas, should be seriously considered by producers. This thesis has made a start in addressing the knowledge gap on positive welfare for dairy cows and has suggested key areas where further research should be directed, based on the current findings.

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Publications

Chapter 4: The work in this chapter has been submitted to Nature Scientific reports and is currently under review.

Ethics Approval

The study was granted ethical approval by The University of Nottingham, School of Veterinary Medicine and Science Ethical Review Committee, approval number 2697-190221.

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Chapter 1: Introduction and Literature Review

1.1 Introduction

The current literature review will discuss the need for the provision of positive welfare experiences to be incorporated within welfare consideration for dairy cows in context with the background to this subject and the wider literature. First of all, a brief overview of some of the current key welfare issues present in dairy cows and public opinion on the industry will be provided. How animal welfare is defined, regulated and assessed will be discussed before explanation of the emergence of the topic of positive animal welfare. A discussion of the research to date exploring potential positive welfare indicators will be provided. The concepts of interest and boredom in animals will be discussed and how these affective experiences tie into environmental enrichment strategies will be provided. Finally, the literature regarding positive welfare provision for dairy cows, highlighting the research gap, is discussed before outlining the objectives of this thesis.

1.1.1 The dairy industry and key welfare issues

The 2020 figures for dairy cow numbers in the UK sits at 1.9 million, a 27% reduction from the 2.6 million in 1996 (Uberoi, 2020). Despite this decline, average herd size and individual cow yield have increased, with the highest annual production of milk from the UK being seen in 2020, since 1990 (Uberoi, 2020). These changes reflect the marked increase in milk production per cow, driven by genetic selection (Oltenacu & Algers, 2005), which is commonly considered to come hand in hand with a trade off in health and welfare (Barkema et al., 2005; Oltenacu & Broom, 2010). Likely a result of the increased strain high producing cows are under, they may be considered more at risk to production related disease and physiological complications

(Rauw et al., 1998). Associations have been shown between higher producing cows and increased risk of mastitis infection (Fleischer et al., 2001; Ingvartsen et al., 2003), lower body condition scores (Green et al., 2014), lameness (Green et al., 2014; Green et al., 2010) and reduced longevity (González-Recio & Alenda, 2007; Miller et al., 2008). This is one of many profound changes to the dairy industry over recent decades, with implications for dairy cattle health and welfare (Barkema et al., 2015).

Dairy cows face substantial and diverse health challenges throughout their lives (von Keyserlingk et al., 2009; Wells et al., 1998), likely one factor predisposing the discord between their natural and productive longevity. Despite having a life expectancy of approximately 20 years (De Vries & Marcondes, 2020), high producing dairy cows survive on average, three to four lactations (De Vries & Marcondes, 2020; Pritchard et al., 2013). Lameness and mastitis are just two examples of health challenges, however are commonly considered to be the biggest welfare challenges for dairy cattle (Barkema et al., 2015; Petersson-Wolfe et al., 2018), as well as being the most economically detrimental production diseases to the industry (Kossaibati & Esslemont, 1997). Both conditions are widely described as painful experiences for cows (Leslie & Peterrson-Wolfe, 2012; Whay & Shearer, 2017). The mean within farm lameness prevalence in England and Wales has been reported at 31.6% (Griffiths et al., 2018). Of the 14,700 cows mobility scored by Griffiths et al. (2018), 28.2% were identified as lame – reflecting over 4,000 cows suffering this painful condition in as small a sample of 61 farms. If this prevalence were extrapolated to the entire UK dairy cow population, this would suggest over half a million dairy cows within the UK alone to be afflicted. Similarly, clinical mastitis incidence has been reported at between 47/71 cases per 100 cows per year, in England and Wales (Bradley et al., 2007), is a significant risk factor for involuntary culling (Bell et al., 2010; Chiumia et al., 2012) and causes suffering expressed physically (Bhosale et al., 2014), behaviourally (Fogsgaard et al., 2012) and emotionally (Des Roches et al., 2018).

In addition to the chronic physical challenges that dairy cows face, they are also subject to a frequent range of psychological stressors, as a result of modern commercial management. Some examples include calf removal (Stěhulová et al., 2008), pain and discomfort associated with artificial insemination and resultant follow up pregnancy checks (Cingi et al., 2012), constantly changing social groups (Hubbard et al., 2021), and close proximity and handling by people (Pas et al., 1998). Another challenge is the potential long term negative affective states associated with behaviourally restrictive housing (Crump et al., 2019). This highlights another development in the dairy industry, the shift to zero grazed systems, which are increasing in Great Britain (March et al., 2014) and more widely, in the US, where less than 5% of lactating cows are provided access to pasture (USDA, 2007).

Historically research into dairy cattle welfare has been dominated by objectives to improve biological success and health related production issues, which are pinnacle concerns for dairy cattle welfare. More recently however, a growing area of science, with the critical goal of evaluating animals' mental state, specifically regarding the subjective evaluation of their environment, has emerged (Yeates & Main, 2008), as an important additional element of an animals' overall welfare. Alongside this, there is now a general consensus that the prevention of suboptimal welfare alone, does not provide for good animal welfare (FAWC, 2009; Green & Mellor, 2011), but that the provision of opportunities for positive experiences may offer a balance to negative experiences, enhancing overall welfare and quality of life. This, in combination with the evidently challenging conditions that dairy cows face, has extended the question as to how dairy cattle housing and management can not only prevent suboptimal welfare, but in addition provide opportunity for positive experiences.

1.1.2 Consumer opinion and the need for further research

Demand on food production is continually increasing, with rises in population (Delgado et al., 2001; FAO, 2009) and income growth, particularly in developing countries (Henchion et al., 2014; Schroeder & Barkley, 1996). These drivers pioneered the shift to intensified animal production systems over the past 60 years. Whilst this shift has resulted in significant advances for agriculture and economical food production, it has also been met with increased concern for animal welfare (Perry, 1983; Woods, 2012). A significant aspect of intensification has been the production of livestock indoors, with less space, requiring less time and labour. There is concern that these production systems may not be adequately meeting animal needs, and so present a lower than acceptable standard of animal welfare (Harrison, 1964). A wide consumer focus, has now developed, regarding the background to purchased animal products (Hughes, 1995) and results from a nationwide telephone survey showed that 95% of people felt it was important how farm animals were cared for, with 76% stating that animal welfare was more important to them than low meat prices (Lusk et al., 2007). A relatively modern trend, a vegan diet, has also been gaining in popularity, with a recent study by Janssen et al. (2016), showing that the motives driving this diet choice for 89.4% of people are related to animal welfare, animal agriculture or animal rights. More than half of the participants who took part in a survey in the US expressed concern for dairy cattle welfare (Wolf et al., 2016) and animal welfare was again highlighted to be the primary issue raised in another US survey, specifically in regard to space, naturalness and quality of life (Cardoso et al., 2016). Jackson et al. (2020) found access to grazing, cow health, welfare and comfort to rank equally of highest importance, in a public questionnaire ascertaining specific aspects of concern.

This high perceived importance of access to grazing and naturalness, presents further discord about dairy cattle management and welfare, paired with the growing number of dairy cows being kept in zero grazed systems. As public policy and industry standards are shaped by societal influence, this continued concern around livestock welfare, highlights a need for further investigation into how current production systems can continue to improve their animal welfare standards.

1.2 Defining animal welfare

1.2.1 Definition of animal welfare and the five freedoms

There is no unified definition for animal welfare, a multitude of interpretations are available in the literature (Carenzi & Verga, 2009). Historically, welfare was judged on physical health and an animal thriving biologically could be seen as having good welfare (Hewson, 2003). As animal welfare science has developed, it is now widely accepted that animals are sentient (Duncan, 2006) and so capable of feeling emotions (Boissy et al., 2007). Emotions are short lived feelings, often psychological adaptations to environmental demands (Levenson, 1999). Affective states are components of emotions and mood, which are more prolonged (Ekkekakis, 2012). Affective states motivate behaviour (McDougall, 1926), and can be expressed on a continuum from negative to positive (Fraser & Duncan, 1998). It is now evident that physical health alone is not conducive to good welfare, assessment of mental state is also essential. A third component to welfare is the notion of naturalness, stemming from concern that animals should be able to perform natural behaviour, in an environment not far removed from the natural setting (Brambell, 1965). An all-encompassing description can be seen as three overlapping areas; biological functioning, affective state and natural living, which together make up the overall welfare state (Fraser et al., 1997).

Animal welfare assessment has historically focused on the alleviation of negative welfare states. A pinnacle framework for managing animal welfare emerged following the The Brambell Report (1965) (Table 1.1), based on a government enquiry into the welfare of intensively farmed animals. This was later refined by The Farm Animal Welfare Council (FAWC) in 1993 (Webster, 2016). The framework proposed 5 areas, which should be met in order to provide for the physical and psychological needs of animals, to safeguard their welfare. It has achieved public recognition worldwide and underpins legislation, policy and farm assurance scheme standards.

Table 1.1. *The 5 Freedoms and 5 Provisions FAWC (2009).*

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

1.2.2 Regulation and farm assurance schemes

Farm animal welfare in the UK is regulated by legislation, such as the Animal Welfare Act (2006) and the Welfare of Farmed Animals (England) Regulations (2007). Detailed species-specific codes of practice are also available in support of legislation. Farm assurance schemes provide a further level of regulation, the key objective for many being to assure consumers about animal welfare (Main et al., 2014). Farm assurance schemes stipulate animal welfare standards, often over and above that afforded by legislation, and are audited by independent certification bodies. Although voluntary, the schemes may be mandatory for entry into large retailer supply chains. Kilbride et al. (2011) investigated the relationship between farm assurance schemes and meeting animal welfare legislation and found that farms certified to a scheme were more likely to be compliant compared to uncertified enterprises.

1.2.3 Current farm animal welfare assessment

The safeguarding of animal welfare and adherence to welfare legislation and farm assurance regulations requires competent assessment of the welfare state of animals on farms. Identification of the welfare status of either individual animals or groups of animals on farms enables assessors to decide whether welfare is of a satisfactory level or whether welfare needs improving which then requires appropriate intervention. There is no single gold standard assessment for farm animal welfare, objective assessment requires a multitude of both environmental and animal-based measures (Spoolder et al., 2003). The suitability of system inputs can be evaluated, such as bedding, housing and feeding. The resultant physiological, behavioural and health status of the animal is also measured, which tends to be more challenging in terms of ambiguity, feasibility and cost (Johnsen et al., 2001). Animal outcomes are often linked with environmental management, for example hock injury and cubicle surface in dairy cattle (Wechsler et al., 2000). Scoring systems are frequently used to categorise and severity grade a specific welfare indicator, for example generally accepted in the UK, is a 4 point mobility scale for lameness identification (Reader et al., 2011; Whay et al., 2003). These grading systems tend to have agreed threshold values, an objective cut off for where this parameter is representing unacceptable welfare and therefore intervention is required (WelfareQualityNetwork, 2018). Figure 1.1 illustrates an example of a scoring system for body condition, an animal-based measure, audited through the Welfare Quality protocol. Some systems also provide benchmarking tools, for comparison of farm scores against industry statistics, such as AssureWel, one provider of farm animal welfare outcome assessments. Assessment tools vary, having been developed for different purposes, such as legislation compliance, scheme certification or system comparisons (Winckler, 2006). Generally, the assessment process is followed by identification of risk factors and interventions for improvement (Whay, 2007). The Welfare Quality protocol is perhaps the most well-known (Blokhuis et al., 2010) providing a multidimensional assessment, incorporating both animal and environmental measures. The comprehensive assessment collects data on four key areas; good feeding, good housing, good health and appropriate behaviour. Other assessments currently utilised for cattle welfare assessment, are the Austrian Animal Needs Index (Bartussek, 1999) and AssureWel, which is conducted on RSPCA Assured and Soil Association Organic certified farms (Schmid & Knutti, 2012). In line with the historical concept of animal welfare, farm animal welfare assessment has predominantly focussed on the physical health of animals. For example, through animal-based measures such as mobility scoring or evaluating inputs such as bedding, again linked to the physical condition. It is now accepted that animals' psychological health plays an inherent part in their overall welfare, highlighting a clear gap in current assessment of this for farm animals.

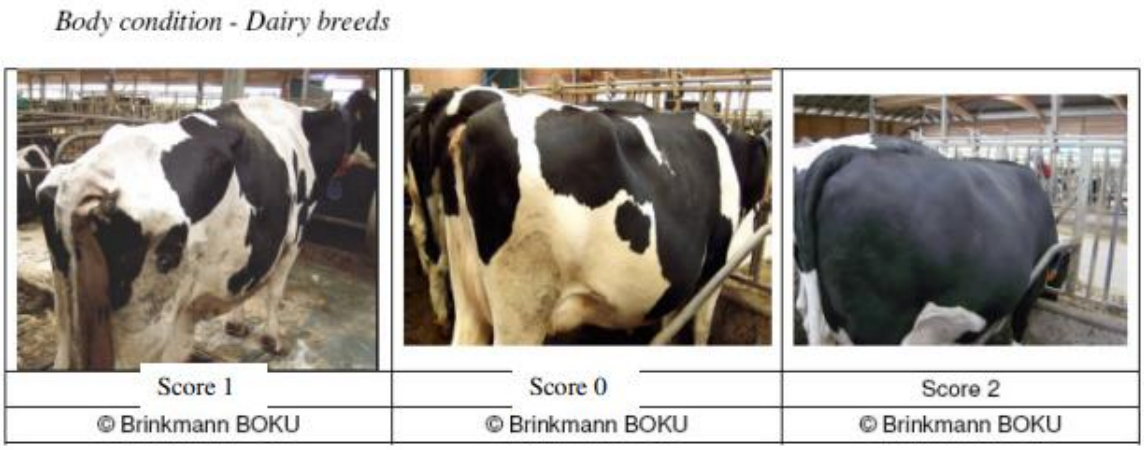


Figure 1.1. *Example of body condition scoring classifications taken from the Welfare Quality Assessment protocol for cattle (WelfareQualityNetwork, 2018).*

1.3 Advancing animal welfare

1.3.1 Quality of life and positive welfare

Despite the significant achievement the five freedoms framework has made to welfare, it has more recently come under criticism for focussing solely on negative states of welfare (McCulloch, 2013). Preventing negative states is essential and would allow animals to cope with the environment (Maple & Bloomsmith, 2018), but not necessarily provide good welfare and experiences. To address this, Mellor (2016) reviewed and developed the framework into a ‘Five Provisions and Aligned Animal Welfare Aims Paradigm’ (Table 1.2). The underlying concept of the five freedoms was retained but alongside avoiding negative states, it provides detail on how these can be linked to providing positive opportunities for pleasure, interest, comfort and control.

Table 1.2. *The updated Five Provisions and Aligned Animal Welfare Aims (Mellor, 2016).*

Provisions [1]	**Animal Welfare Aims** [2]
1. *Good nutrition:* Provide ready access to fresh water and a diet to maintain full health and vigour	Minimise thirst and hunger and enable eating to be a pleasurable experience
2. *Good environment:* Provide shade/shelter or suitable housing, good air quality and comfortable resting areas	Minimise discomfort and exposure and promote thermal, physical and other comforts
3. *Good health:* Prevent or rapidly diagnose and treat disease and injury, and foster good muscle tone, posture and cardiorespiratory function	Minimise breathlessness, nausea, pain and other aversive experiences and promote the pleasures of robustness, vigour, strength and well co-ordinated physical activity
4. *Appropriate behaviour:* Provide sufficient space, proper facilities, congenial company and appropriately varied conditions	Minimise threats and unpleasant restrictions on behaviour and promote engagement in rewarding activities
5. *Positive mental experiences:* Provide safe, congenial and species-appropriate opportunities to have pleasurable experiences	Promote various forms of comfort, pleasure, interest, confidence and a sense of control

With the acceptance of animals being sentient (Duncan, 2006; Proctor, 2012) and capable of experiencing affective states such as fear and stress (Boissy, 1995; Forkman et al., 2007), it is assumed that they are equally capable of experiencing positive feelings such as pleasure (Duncan, 2006). As such, outlining minimum welfare standards, where an animal should cope and survive, is now seen as setting the bar too low (Maple & Perdue, 2013). This highlights the gap in the five freedoms and current legislation by focusing on just the alleviation of negative states of welfare. Positive welfare refers to the interest in positive subjective feelings in animals. Positive affective engagement, another common term, refers to emotions that may be linked when animals carry out highly motivated, reward directed or rewarding behaviour (Mellor, 2015). A recent review by Lawrence et al. (2019), explored the decade worth of literature on the topic, showing that positive welfare encompasses a number of concepts including positive emotions, positive affective engagement, quality of life and happiness, which reflects the complex nature of the subject.

The FAWC (2009) suggested that policy focus should extend to animal needs and that animals should experience an acceptable quality of life. They defined this in three categories: a life not worth living, a life worth living and a good life. As with the definition of welfare, there is debate over the exact meaning of 'quality of life', but the predominant difference is that quality of life is a subjective perception by the animal over its lifetime, of positive and negative experiences (Scott et al., 2007). Compliance with legislation should afford a life worth living, which FAWC (2009) categorised as one where good welfare outweighs poor welfare over its lifetime. A life not worth living is one where poor welfare dominates the balance over the animals' lifetime. There is a clear distinction to achieving a good life. A good life would include environmental choice and opportunities for an animal's interest, curiosity, pleasure, comfort and confidence (FAWC, 2009). One of the aims of this shift, is to reassure British consumers that at the minimum, all farm animals have had a life worth living and aim to increase the number of those that experience a good life. It is accepted that farm animals will have to experience unavoidable and sometimes routine pain and suffering, through farm management and procedures. For dairy cows in particular, over their lifetime they will experience the stress associated with calf removal on an annual basis (Stěhulová et al., 2008), pain and discomfort associated with artificial insemination and resultant follow up pregnancy checks (Cingi et al., 2012). Moreover, stress associated with regular social mixing (Hubbard et al., 2021), potential long term boredom and negative subjective states associated with barren housing (Crump et al., 2019) and a continued physiological burden of combatting high prevalence production diseases such as lameness and mastitis (Petersson-Wolfe et al., 2018; Whay & Shearer, 2017). Aside from the provision of basic maintenance needs to prevent suffering, such as appropriate feeding and provision of comfortable resting spaces and veterinary treatment, it is unclear what specific 'positives' are also currently being offered, with one exception of brushes, which have been implemented solely for the behavioural benefit of cows. Therefore, when this is considered in line with the basic threshold that the UK is trying to achieve, with assurance that all animals' positives have outweighed the negatives throughout their lifetime, it seems unlikely that this balance is currently being achieved. The psychological state of the animal is considered to be as important as the physiological state, in terms of ensuring welfare (FAWC, 2009), yet the psychological states of commercially produced animals have been relatively overlooked until more recently. This might be explained by the complexity of measuring animals' affective states and also more specifically the unclear links to productivity or physical health (FAWC, 2009).

As well as affective states being available on a continuum from positive to negative (valence), they can also differ in level of arousal (Mendl et al., 2010). Describing discrete emotions, such as fear and anxiety, doesn't address the full expanse of possible emotional states that may be experienced by animals (Mendl et al., 2010). Mendl et al. (2010) adapted upon work by Russell and Barrett (1999) and Burgdorf & Panksepp (2006) to visualise a two-dimensional framework, displaying core affect with varying degree of arousal, plotted against a negative to positive scale. Within this matrix, previously investigated discrete emotions can be placed (Figure 1.2).

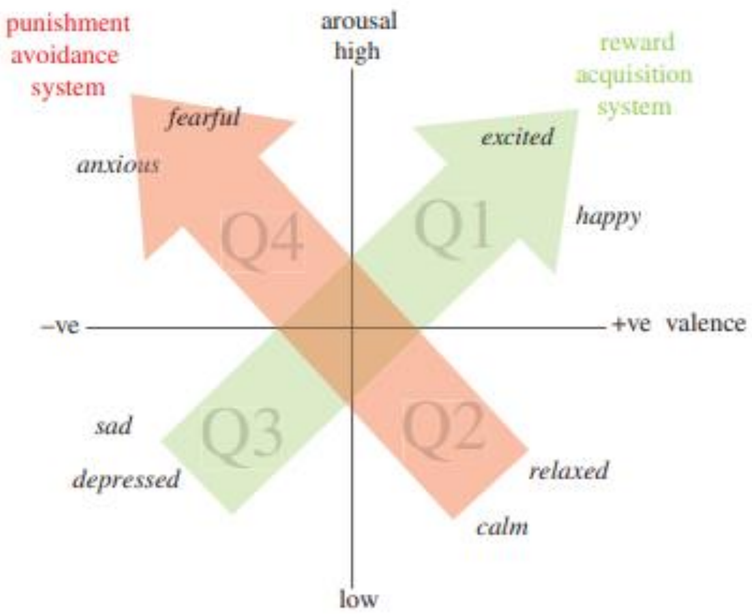


Figure 1.2. *Model of possible animal affect and mood adapted by Mendl et al. (2010) from Russell and Barrett (1999) and Burgdorf and Panksepp (2006).*

1.4 Identifying positive welfare

1.4.1 Behavioural and physiological parameters of positive welfare

To promote positive affective states, we need reliable methodologies to assess it (Yeates & Main, 2008). As we cannot evaluate the emotional state of animals directly, we need to look to behavioural and physiological markers which may act as a proxy. In human psychology, mental health assessment has relied predominantly on self-report and questionnaire style methods (Haberer et al., 2013). Numerous reliable and valid self-report assessments are utilised in health screening, providing a fast and direct insight into the subjective experience of the patient (Haberer et al., 2013). This direct evaluation is not possible in animals, which presents a significant challenge to assessing affective states. Several different avenues have started to be investigated in the search for indicators of positive welfare in animals.

One such avenue is using physiological indicators as a potential inference on animals' affective wellbeing. Physiology is inherently linked to mental state, via the nervous system and internal affect can alter physiological response (Cabanac, 1971). A selection of physiological parameters have started to be explored as potential indicators of positive affect, but the area is underdeveloped, with yet no gold standard (Vigors & Lawrence, 2019; Whittaker & Marsh, 2019). Heart rate (Feh & de Mazierès, 1993; Zupan et al., 2016), heart rate variability (Reefmann et al., 2009; Zupan et al., 2016), skin temperature (Chotard et al., 2018; Proctor & Carder, 2015; Travain et al., 2016) and neurotransmitters (Chen & Sato, 2017; Lansade et al., 2018), have shown ambiguous results and all require further research. Physiological responses may be limited as a method of assessing affective state as they are often an indicator of arousal, but it is difficult to differentiate the valence of the affect (Ede et al., 2019). With a lack of validated physiological parameters of positive affect, behavioural measures may be more appropriate proxies at present (Whittaker & Marsh, 2019).

Another avenue used for measurement of affective states is behaviour. To provide valuable resources and experiences to animals, we need to identify what they want. Preference testing is widely established as a method to assess preferences and choice in animals. An animal is presented with a choice of simultaneously available alternatives and it is assumed that the choice made, provides either a positive experience to the animal or an improvement in their situation and therefore welfare. These tests tend to be used to ascertain preferences over environmental conditions, such as bedding (Norring et al., 2010; Tucker et al., 2003), lighting (Greenwood et al., 2004; Mendes et al., 2013) and resources (Beattie et al., 1998; Struelens et al., 2008). Although providing invaluable insight into animal preference, there are multiple pitfalls to the method. Choices are only relative to one another, choices made may depend upon previous experience and it is likely that choices offered will only represent a small number of eventualities. For reviews to the method and pitfalls see; Duncan (1992); Duncan (2005); Fraser and Matthews (1997); Kirkden and Pajor (2006).

The main shortfall to preference tests is that they don't provide information on the power of the preference. Motivation tests have developed from preference tests and use consumer demand theory, by attaching a cost to offered choices, allowing researchers to ascertain the level of motivation behind choices (Duncan, 1992; Kirkden & Pajor, 2006). Often, the cost to the animal is incrementally increased until the animal will no longer work for the resource. Common examples of costs assigned to access to choices, are weighted gates (Olsson & Keeling, 2002), long walking distances (Duncan & Kite, 1987) and aversive physical barriers (Cooper & Appleby, 1994). Motivation testing offers an insight into what can be regarded as essential maintenance resources, such as food, or highly valued resources, for which a high price will be paid and fleeting preferences, where preference will decrease as 'cost' increases, known as inelastic demand. A fundamental example of consumer demand theory applied in animal welfare, investigated housing conditions of fur farmed mink (Mason et al., 2001). Mink were housed in standard commercial cages, connected to 7 compartments, offering an array of resources from a water pool, to novel objects, an additional nest box and an extra space compartment acting as a control. Incremental one-way weights were placed on the doors and activity was measured continuously over a week. Results showed the water pool had the greatest total expenditure, highest reservation price, greatest consumer and the most inelastic demand. Urine cortisol was measured following removal of a high, intermediate and low value choice and as a benchmark, an essential resource, food. Cortisol was unchanged for the low and intermediate resources, yet it rose by 33.8% upon removal of the water pool compared to 16.1% over baseline upon removal of food. Authors concluded that against arguments that mink are well adapted to captivity, based on health and breeding success (Joergensen, 1985), they may suffer and experience frustration by being deprived of resources that they could access in the wild. These results are particularly significant, considering the generations spent domesticating farmed mink, which spend their entire lifetime in captivity and will never have experienced a water pool before (Mason et al., 2001). One line of thought observed regarding captive animal welfare, is that animals shouldn't be able to miss what they have never experienced (Duncan, 1992; Te Velde et al., 2002). The study by Mason et al. (2001) refutes this misconception, showing that the deprivation of highly motivated biologically relevant resources, in captive animals, with no prior exposure, negatively impacts welfare.

Cognitive bias testing has emerged as another methodology to assess affective states in animals. This concept has been adapted from human psychology, where changes in information processing can reflect emotional state (Schachter & Singer, 1962). Optimists generally expect more positive outcomes and optimism has been linked with better mental and physical health and happiness (Alarcon et al., 2013; Scheier & Carver, 1992). The general method involves training the animal through negative reinforcement to one stimulus and positive reinforcement to another. Ambiguous cues are then offered and how the animal perceives these cues (positive or negative) is evaluated, for reviews see (Baciadonna & Mcelligott, 2015; Bethell, 2015; Mendl et al., 2009). Therefore, these tests hypothesise that animals that interpret ambiguous stimuli as positive, to have more positive affective states. The concept is hugely promising in the field of animal emotion, however given the individuality of the testing, there are significant drawbacks in terms of training time and its practical applicability to groups of animals.

1.4.2 Preference testing and consumer demand in cattle

Preference testing has been utilised in cattle to explore different avenues of management such as housing structure (Teixeira et al., 2006; Tucker et al., 2005) and shade (Gaughan et al., 1998; Schütz et al., 2011). Cows spent longer lying on soft rubber matting, than conventional matting and concrete flooring (Herlin, 1997), preferred straw to soft rubber mats in winter, but not in summer and would choose to lie on straw or rubber matting over sand (Manninen et al., 2002). Conversely, in a study investigating flooring preference at calving, Campler et al. (2014) found no significant difference between sand, rubber mats or concrete to calve on, albeit there was a slight preference for sand. All choices were covered in 15cm of straw, which could have been visually ambiguous for the cows. Although likely that the comfort differed between these choices, a cow might have been unlikely to change their choice once down, to avoid the physical effort of getting back up, particularly this close to parturition. Norring et al. (2008) investigated cow preference to sand or straw bedding, alongside hock injuries and hoof health. Cows bedded on straw spent longer lying than those on sand, however cows on sand were cleaner, had less severe hock lesions and showed a greater improvement in overall hoof health. During a preference test, cows previously housed on straw showed a preference for straw cubicles however cows previously kept on sand showed no difference. Although there was a behavioural preference for straw cubicles, there were clear health benefits from sand bedding, which highlights an important limitation of preference testing, that animals will not always choose the option that is best for their welfare. One explanation for this complication to preference testing is discussed by Fraser &

Matthews (1997), who discuss that it is plausible that animals may not have the capacity to evaluate potential consequences of decisions made and so preferences made are relevant to their immediate subjective experience. This means that animals may make choices that have an immediate benefit but that have detrimental long-term consequences. For example, one labour requirement for farms that use robotic milking systems, is the collection of cows to be guided through the robot that have not already voluntarily visited. Unal et al. (2017) hypothesised that cows that required collection for milking preferred freely available roughage over the concentrate available during milking, which may not represent a long term behaviourally beneficial strategy for welfare. Similarly, Rioja-Lang et al. (2009) found that cows would choose to eat low quality food by themselves when given the choice of high quality food in the proximity of a more dominant cow. This choice reflects an immediate benefit of avoiding social conflict, however not the long term benefits of food quality. A measure of the strength of cow preferences could be used as a trade-off to other welfare improvements, enabling producers to identify which bedding for example, provides the net welfare gain and overall best choice. If an animal showed an extremely strong motivation for a resource, teamed with indicators of reduced welfare on removal, yet the alternative option showed only minor health improvements, there may be argument that the initial option would provide for a higher overall level of welfare than the latter. This scenario would suggest that the resource chosen, is highly valued to the animal, potentially for reasons that are not obvious the observer. This limitation of preference testing could be controlled for by only offering options that would not have a detrimental impact on welfare.

Dairy cows without access to pasture provokes substantial public concern (Cardoso et al., 2016; Kuhl et al., 2019; Schuppli et al., 2014), yet the housed environment does offer an array of welfare benefits not provided on pasture (Laven & Holmes, 2008). Preference and motivation testing has been utilised in an effort to evaluate cow preferences. Legrand et al. (2009) found a strong preference for access to pasture, particularly at night. This preference was sensitive to temperature and humidity, with time on pasture declining during days with greater temperature and humidity and during the night with increased rainfall. A preference for pasture has also been shown in feedlot cattle, who spent a mean proportion of 75% of their time on pasture in comparison to a feedlot, indicating pasture had wider appeal than solely outdoor access (Lee et al., 2013). Smid et al. (2018) offered a choice of outdoor environments and found cattle spent considerably more time using an outdoor pasture than a small outdoor sand pack. The specifics of these preferences could offer producers management options, forming a compromise of welfare and economical concerns. For example, allowing seasonal pasture access at night where constant pasture access is not feasible. Von Keyserlingk et al. (2017) found that 59% of cows would push just as hard or harder to access pasture as they did to access TMR, using an incrementally weighted gate. These results are concurrent with results from an earlier study that instead used walking distance as the cost for pasture access (Charlton et al., 2013). Distance did not alter the amount of time spent outside at night, however during the day cows spent less than half of the time at pasture when they had to walk 260m to access it rather than 60m.

Motivation testing offers invaluable insights into highly valued resources, which was unobtainable before this method was developed. One flaw to assigning physical costs, is that it may not be equally attainable for all individuals, for example animals having recently undergone surgical procedures, calved or with subclinical disease. The same resource may need to be tested using different types of costs to improve accuracy of results. The gold standard in this behavioural evaluation, is to combine preference and motivation tests alongside physiological parameters (Mason et al., 2001). The fundamental limit to what this methodology can offer in terms of insights into what cattle value is knowing what choices to put on offer.

1.4.3 Ear position and eye white as possible parameters of positive welfare in cattle

There are now multiple review papers available that provide an overall consensus on the research to date on possible positive welfare indicators for cows (Keeling et al., 2021; Mattiello et al. 2019; Napolitano et al., 2009). Several specific behaviours have started to receive research interest as possible indicators of positive affective states in cows, such as self-grooming, allogrooming, tail position and play behaviour (Keeling et al., 2021; Mattiello et al. 2019; Napolitano et al., 2009). Often these potential indicators have emerged because they have been inadvertently linked to situations or environments which are hypothesised to be better for welfare, for example, increased self-grooming in enriched versus non-enriched environments (De Rosa et al., 2009). A small selection of studies have specifically attempted to investigate whether ear positions and eye whites in cows may provide information on positive affective states and so these will be discussed further within this section.

Facial expressions in people have been shown to be universally and reliably associated with emotional states (Tomkins & McCarter, 1964) and detailed coding of anatomical facial activity, provides a credible mechanism for pain quantification (Craig, 1992). This research has been extended to animals, with grimace scales established for pain identification for a variety of species (Mogil et al., 2020). Ear position is categorised within facial expressions as the ear position is controlled by facial muscles and ears exhibit a wide range of movement in ruminants which has made it an avenue of investigation as a potential indicator of affective states.

A small number of studies have carried out initial investigation into the value of ear position as an indicator of affective state in cattle. Proctor and Carder (2014) evaluated ear positions in cattle during stroking by people, assumed to elicit a low arousal, positive emotional state. They found both a backward and loose hanging ear posture was presented significantly more during the stroking period, compared to an upright or forward position. Authors suggested these ear positions may be indicative of a low arousal, positive affective state. Stroking was chosen following literature indicating it being a relaxing experience for cattle, however frustration or stress of human contact, common in cattle should also be considered.

Figure 1.3. *Four defined ear positions (Battini et al., 2019), previously defined by Proctor and Carder (2014) and used by Lambert and Carder (2019).*

Lambert and Carder (2019) measured ear position combined with heart rate, in assumed situations of positive and negative high arousal. Provision of unexpected high value feed and unexpected inedible food both resulted in significant increases in heart rates and significantly different ear postures. During the assumed positive context, an upward ear posture, with a forward facing or sideways pinna, was most commonly displayed, compared to a forward-facing ear position on the horizontal plane, which was expressed significantly more in response to the assumed negative condition. Most studies aiming to find outcomes of positive affect are unable to control for and separate out arousal and valence, this study attempted to match arousal but in opposing forms of valence, inferring the resultant ear postures were influenced by affective state. Oliveira and Keeling (2018) also found situational differences in ear postures, with ears in a back and up position during feeding, axial and forward whilst queuing for an automatic milking machine and backwards and asymmetric during brushing. Incorporating these results into a multidimensional model of core affect, authors theoretically deduced how postures may change in situations of varying valence and arousal (Figure 1.4). Battini et al. (2019) explored ear positions defined previously by Proctor and Carder (2014) (Figure 1.3), alongside eye white percentage, in different contexts and on farms with different management systems. At pasture, 39.4% of ear postures were loosely hung down and 37.9% backwards, following previous work indicating a low arousal positive state, which is in keeping with the welfare benefits to pasture access. Nearly all showed a horizontal forward facing stance during a human approach test and in farms with high competition for cubicle access most had ears held forward upright. Nearly half were in a forward facing position in a tie stall system, which has shown to be less comfortable than others and the highest percentage of hanging ears were displayed in a system which had a positive cubicle to cow ratio and additional access to outdoors.

The limited research to date suggests that ear position is situation dependent and may indicate state of arousal and valence. This one example of the use of specific behaviours as potential indicators of affective states, displays the limitations of current identification of affective states and the need for further research, particularly using a combination of indicators in unison for validation and investigating consistency between individuals.

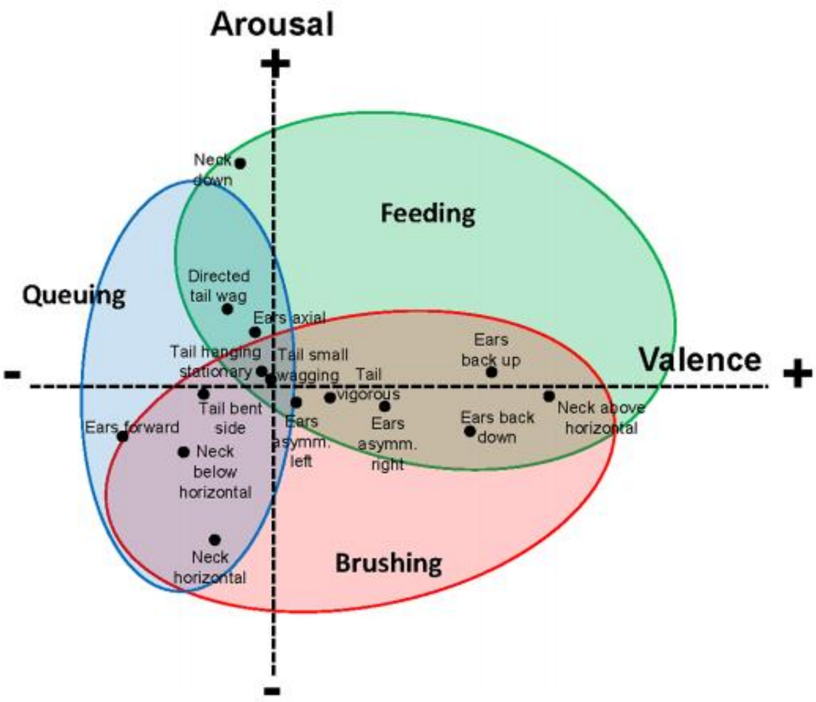


Figure 1.4. *"Hypothetical representation of the core affect diagram in two-dimensional space, illustrating ear, neck and tail postures of dairy cows during brushing, feeding and queuing in a loose housing system" (Oliveira & Keeling, 2018).*

Visible eye white has also been explored as an indicator of emotional state in cattle. Sandem et al. (2006) found that percentage of eye white significantly increased whilst anticipating food delivery, followed by a decrease upon arrival and during consumption of food. Authors suggest that a large percentage of eye white may indicate a strong emotional response, but no valence was indicated. The increased eye white during anticipation could represent either excitement or frustration. Food acquisition was chosen as a positive stimulus, however it should be questioned whether basic hunger needs being met should actually be perceived as a situation inducing a positive emotion, particularly when preluded by a period of potential frustration. The cows used were kept in tie stalls, with no autonomy over food acquisition. Sandem et al. (2002) previously found eye white percentage to increase significantly when given visual and olfactory stimuli but not direct access to food, reinforcing the suggestion that this parameter indicates a negative rather than positive emotion. Proctor and Carder (2017) again found increased eye whites during an assumed frustrating situation of being given inedible food but also found increases during provision of a highly valued concentrate feed, arguably a positive experience. These results support the suggestion of eyes being opened more widely during high arousal (Sandem et al., 2006) and increased eye white percentages as an expression of frustration have been tentatively validated using the anxiolytic, diazepam (Sandem et al., 2006). As yet, eye white doesn't appear to offer clear insight into identification of positive affective states. Even though suggested by Proctor and Carder (2017) in reference to concentrate feed, the generality of the parameter, means it is unlikely this indicator could be used to infer either positive or negative emotions in ambiguous situations. Proctor and Carder (2015) also reported the percentage of eye white to reduce in dairy cows during periods of stroking, deemed to elicit a positive low arousal state. Again, here the parameter seems to indicate arousal and some potential to infer emotional states, but not valence; more research would be required in order to establish whether low arousal negative states such as apathy or boredom could be distinguished.

1.4.4 Self-grooming behaviour and its relation to affective state

Self-grooming is another behaviour which has been identified as a potential indicator of positive affective states in cows (Keeling et al., 2021; Mattiello et al., 2019; Napolitano et al., 2009), however the behaviour is poorly understood. The literature on the occurrence of the behaviour is contradictory. The primary function of self-grooming is to control hygiene and parasites, however it also occurs in response to behavioural conflict or following stress or arousal (Spruijt et al., 1992). The last function is thought to be linked to hormones such as opioids (Niesink & Van Ree, 1989), which are released following stress, as a relaxation mechanism (Spruijt et al., 1992), which ultimately is positive. Reduced self-grooming has been demonstrated by treatment with opioid antagonists (Aloyo et al., 1983; Willemse et al., 1994). The physiological basis predisposes this behaviour to becoming overused, to self sooth or elicit a positive experience. On the contrary, behaviours not immediately required for survival, which reduce in times of constraint, such as sickness or stress are described as 'luxury behaviours' (Dawkins, 1990). The occurrence of these behaviours therefore indicate that all immediate needs of the animal are being met, implying a better state of welfare (Broom et al., 1993). More self-grooming has been displayed by dairy cows in stressful situations, such as restraint (Bolinger et al., 1997), social isolation and deprivation of lying down (Munksgaard & Simonsen, 1996). Increased self-grooming has also been displayed in more barren environments, such as caged calves (Kerr & Wood-Gush, 1987), tethered compared to loose housed dairy cows (Krohn, 1994) and in fully housed as opposed to cows with daily access to pasture (Di Grigoli, 2019). Self-grooming reduces in dairy cows suffering with mastitis (Fogsgaard et al., 2012), yet lame dairy cows have been shown to self-groom more than non-lame animals (Almeida et al., 2008). Conversely, self-grooming in other species is more widely linked to positive experiences. Hens show increased self-grooming when anticipating a positive reward (Zimmerman et al., 2011), self-grooming is positively correlated with a reduction in stocking density in intensively housed meat production horses (Raspa et al., 2020) and buffalo housed in enriched environments with outdoor access showed more self-grooming than standard indoor housed buffalo (De Rosa et al., 2009). Increased self-grooming has also been shown in cows in response to a different pen with unknown cows (Herskin et al., 2004) and novel food (Herskin et al., 2004).

1.4.5 Qualitative behavioural assessment

Another methodology that has emerged for assessment of affective states in animals is qualitative behavioural assessment (QBA). The nature of QBA is dissimilar to other quantitative behavioural and physiological indicators of affect, which identify and evaluate divergence in specifically identified parameters, such as ear position or heart rate. It uses human's intellectual judgement, or put simply, common sense, in complex interpretation of how animals interact with their environment, based on behavioural descriptive qualities, linked to emotional expression. Animals participate in a range of behaviours, but the way they conduct these behaviours can be done differently (Fagan et al., 1997). QBA is regarded as more than just this body language but as a psychological dimension allowing judgement to be made over the quality of an animal's experience (Wemelsfelder, 2007).

The general method for QBA assessments involves observing either individual or groups of animals, for a set period of time and then rating their expressive demeanour on a visual analogue scale, consisting of a number of qualitative terminologies, for example, "tense" or "content". Fixed list QBA uses a predefined list of terms, whereas the free choice profiling approach allows observers to generate their own descriptions following observation (Wemelsfelder et al., 2000). A score for each behavioural descriptive is then obtained by its location on a 125mm line. Terms can be analysed individually, however, generally QBA results are analysed using a multivariate technique, Principal Component Analysis, which evaluates data consisting of correlated quantitative dependent variables, to produce principal components; new variables which summarise the important information (Abdi & Williams, 2010).

Due to its qualitative and unique nature, QBA could be seen as anthropomorphic, subjective or unreliable, by scoring animals using behavioural terms with emotional connotations (Bokkers et al., 2012; Kennedy, 1992), as attempting to infer animals' affective experiences through behavioural expression is seen as anthropomorphic (Watanabe, 2007). Concerns of this issue are commonly described regarding the method of QBA (Wemelsfelder et al., 2001). Interpretation of affective states using behaviourally descriptive terms however, is not new and is utilised in pain assessment of animals. Assessments of terms such as restless (Mich & Hellyer, 2008) and general mood (Merola & Mills, 2016) are used to gauge the pain level of an animal, an affective experience in itself, which is used as a facilitation to veterinary decision making. With no accurate objective measures of pain (Conzemius et al., 1997), behaviour becomes the most informative parameter available, in line with the current state of evaluation of animals' wider affective states. Even when farmers are asked to discuss what positive welfare means to their animals, the terms happy, content and stressed are frequently used (Vigors, 2019), again showing the importance of human interpretation of behavioural expression of animals, in different settings, where an accurate objective measure is unavailable.

QBA has been widely used in an array of different species (Arena et al., 2019; Fleming et al., 2013; Phythian et al., 2013; Rutherford et al., 2012) and detected perceived changes in emotional state, from different conditions such as housing, surgical treatments and feeding regime (Grosso et al., 2016; Mialon et al., 2021; Vindevoghel et al., 2019). Des Roches et al. (2018), used QBA to see if behavioural changes could be detected in cows that had been infected with mastitis. Mastitis is a painful experience in cows (Leslie & Peterrson-Wolfe, 2012), however, how this impacts cows emotionally is unknown. QBA scores differed with stage of infection, with cows showing an increased suffering/dejected/lethargic expressivity during the acute stage of infection and remission. Cows in remission also showed an increased expressivity described as confident, calm and relaxed. Clinical and physiological welfare indicators were also assessed and were related to the QBA scores, with higher udder severity scores, body temperature and concentrations of cortisol, consistently linked to cows with higher scores of suffering, lethargic, dejected. Authors concluded that these results reflect negative emotional states in dairy cows, as a result of mastitis infection. Rousing and Wemelsfelder (2006) explored whether QBA would correlate with quantitative ethogram measures of social behaviour in dairy cows. The ethogram of behaviours assessed included social licking, social sniffing, pushing, head butting and fighting. An initial QBA component with the most positive loading adjectives of relaxed and calm, compared to the most negative loading adjectives of aggressive and bullying, was significantly positively correlated with social licking. This component was also negatively correlated with the quantitative scores for head butting and pushing. An additional QBA component, composed of highest loading adjectives of passive and indifferent, compared to the most negative loading adjectives of playful and social, was negatively correlated with the level of social licking. The research to date suggests that QBA can provide distinct insights into not only differences in dairy cows' behaviour in varying contexts, but its link to associated affective states. With the inherent challenges in the assessment of affective states in animals, and limited research available on other potential indicators, QBA represents the most widely tested and validated indicator of emotional states available currently.

QBA has now been incorporated into practical farm assurance assessments for dairy cattle, as a measure of positive emotional state (WelfareQualityNetwork, 2018). The Welfare Quality protocol for assessment of dairy cow welfare, incorporates a wide range of both animal and resource-based outcome measures, resulting in a time-consuming assessment to complete (Andreason et al., 2013). Andreason et al. (2013), explored the use of QBA as a potential screening tool, to pre identify poorly scoring farms, which would be followed up with a full Welfare Quality Assessment. Full Welfare Quality and QBA assessments were completed on 43 Danish dairy farms. Two significant correlations were found between QBA scores and specific categories of the Welfare Quality assessments, however overall, the QBA scores did not correlate with the overall Welfare Quality assessment results. Authors concluded that QBA is not capable of being used as a single assessment predictive of wider Welfare Quality results. One significant limitation of the study was that the assessments were completed at different times of day. Cows' activity budgets differ throughout the day (Mattachini et al., 2011) and it's likely for behavioural expressive demeanour to be impacted by interaction events such as cleaning, feeding, milking and veterinary intervention. Therefore, completing QBA's at different times on different farms, likely with different management routines, would not give an equal insight into the affective experiences of cows across farms, despite differences in welfare implied by other resource based and animal based measured outcomes.

The unique capacity of QBA is its potential to offer insight into the affective experiences of animals, which is an inherent scientific challenge. Therefore, it is a potential tool for making assessments on quality of life and positive welfare, which may be particularly useful when evaluating the success of interventions, aimed at improving the affective experience of animals. That being the case, in addition to its use in regulatory farm assurance, it is frequently used as a tool for gaining information about affective states, usually between treatments, such as different housing condition, in experimental research trials. Carreras et al. (2016) used QBA to evaluate whether pigs housed in enriched conditions – increased space allowance, straw and concrete flooring, differed from that of pigs housed in unenriched conditions – decreased space allowance, no straw and slatted floors. Not only did pigs in enriched housing have better QBA scores, but these were concurrent with lower cortisol concentrations and lower carcass wounds. Similar findings have been shown by Temple et al. (2011), who conducted QBA on 11 extensive and 10 intensive Iberian pig farms. Housing and management was variable between the systems, but 'intensive' was mainly indoor housed pigs whereas 'extensive' was pigs with free access to pasture. Production had a significant effect on QBA scores, with extensively housed pigs being assessed as happier, more content, enjoying and positively occupied, compared to intensively reared pigs. Again, this methodology has been used to show differences in affective states in dairy goats kept in varying housing conditions, with goats with access to an open outdoor range scoring higher on two PCA components, indicative of higher expressions of the attributes content, calm, curious and attentive, compared to fully housed goats (Grosso et al., 2016). One potential complication of QBA, is that assessors observe not just the isolated animals but the complete environmental situation that the animal is in. This introduces potential observer bias, which is heightened where moral connotations are involved – for example whether people have views on whether a certain housing condition is better for animal welfare compared to another. Evidence of this contextual bias has previously been observed whilst using QBA (Fleming et al., 2015; Tuyttens et al., 2014; Wemelsfelder et al., 2009).

Despite drawbacks, one distinct benefit that QBA has above other behavioural and physiological tests is the relatively small amount of time needed to complete the assessments and no resources are required, with farm assessments being conducted in the standard living conditions of the animals. Therefore, it provides a very accessible method for measurement of affective states on farms.

1.5 Interest and curiosity in animals

Providing opportunities for animals' interest is one suggested way of providing positive experiences (FAWC, 2009). Curiosity has been described with a variety of explanations. In human psychology, one theory is that it is an innate desire to acquire new knowledge which then stimulates exploratory behaviour. It serves as a function when presented with a knowledge gap, in filling that gap which results in knowledge acquisition and pleasure (Reio et al., 2006). Berlyne (1966) likens it to a state of discomfort, due to inadequacy of information, that motivates specific exploration. Most prominently, and particularly in animals, it may be seen as a motivation to interact with novelty in the environment. It has been described synonymously with interest (Peterson & Hidi, 2019) and motivates exploratory behaviour (Berlyne, 1966).

In an evolutionary sense, curiosity is vital for survival, an innate drive to be able to adapt and survive (Gazzaniga, 2005). Voss and Keller (1983) stressed that curiosity and the exploratory behaviour it elicits, are vitally important because these traits help individuals flexibly adapt to continual environmental change. Exploratory behaviour provides opportunity to refine motor skills for tactile investigation of the environment (Glickman & Sroges, 1966). Glickman and Sroges (1966) suggest that animal reactivity should be related to the demands of their natural environment, their extent of daily challenge in their search for food and protection from predators. Curiosity was investigated in over 200 zoo animals, finding differences in reactivity between taxonomic groups, specifically an increase in sheer quantity of response to various novel objects, moving through reptiles to primitive mammals and rodents to carnivores and primates (Glickman & Sroges, 1966).

Curiosity is also a key driver in the search behaviour in foraging (Calhoun & Hayden, 2015). Investigation of novelty comes with possibility of reward, an animal that comes upon food during exploration will be encouraged to repeat this behaviour. Kang et al. (2009) investigated curiosity as anticipation of rewarding information using a combination of self-reporting and fMRI in people's completion of trivia questions and found that level of curiosity when reading questions was correlated with activity in caudate regions of the brain, previously suggested to be involved in anticipated reward. This reflects the information-gap theory of curiosity, where unknown information is anticipated to be rewarding (Loewenstein, 1994).

There is a certain survival risk in engaging in curiosity driven exploratory behaviour, in terms of unknown food or territories. It has been shown that wild orangutans will avoid novelty whereas orangutans in zoos or previously cared for in a captive setting will seek novelty and show a curious, exploratory response to a novel object (Damerius et al., 2017). This influence, termed the captivity effect, is likely due to captive animals developing a decreased vigilance for danger, living in a protected environment, having more time budget to expend and learning that provided novel items usually result in reward. Another factor could be that some captive animals are possibly bored, stimulus deprived which could increase their magnitude of reaction to novelty (Glickman & Sroges, 1966).

Exploration seems to appear frequently in the positive welfare literature. Livestock farmers describe exploration as a behaviour valued by animals, when describing how autonomy can enable positive affect, as animals have the choice to take part in these behaviours (Vigors & Lawrence, 2019). Boissy et al. (2007) directly refers to exploratory behaviour as active behavioural expression of positive emotion. Providing straw to pigs, to allow exploratory behaviour, is situationally described as ‘high’ welfare (Rius et al., 2018). The welfare importance of this behaviour in pigs has resulted in legislation now making provision of manipulatory material for investigation mandatory for all pigs (Council Directive 2008/120/EC). The fact that this legislation is only applicable to pigs, could be due to the more evident expression of frustration through maladaptive behaviours, such as tail biting, that cause immense health and economical losses to the industry (Sonoda et al., 2013). Exploration may even be incorporated into positive welfare experience through nutrition, by allowing food choice and variety through foraging (Mattiello et al., 2019). A recent paper by Mellor (2017), outlined opportunities for enhancement of animal welfare and described a low level of enhancement to offer limited opportunities for positively motivated exploration whereas a high level would provide diverse opportunities for this behaviour. The FAWC released a report in 2009, which discussed how an animal’s welfare over its lifetime could be classified into different categories of quality of life. A ‘life worth living’ was defined as one where the balance of an animal’s experiences over its lifetime were positive and that this should be achieved through full compliance with the law. For an animal to have experienced a ‘good life’, means that its welfare would have been distinctly better than that afforded to an animal which had experienced a 'life worth living'. To meet the criteria of a 'good life', in addition to full adherence to all legal requirements, animals should be provided with additional life opportunities. Opportunities that could contribute to an animal having a 'good life' were described as opportunities that an animal would not require for biological fitness but that are valued. Suggested examples were provided for what these opportunities could be and these were classified into four key areas – with opportunities for an animal's interest, forming one. This quality of life classification system was used to formulate a resource tier framework which stipulates how farms may provide different levels of quality of life for laying hens (Edgar et al., 2013). Authors suggested that the provision of varying levels of opportunity for interest could be provided through a positively enriched environment or by providing positive experiences within the outdoor environment. This concept has also been used to develop a positive welfare framework for dairy cows which suggests potential opportunities for cows' interest (Stokes et al., 2022).

Suggestions as to how exploration is connected to positive welfare, are that exploration is a highly motivated behaviour (Boissy et al., 2007), or ethological need (Hughes & Duncan, 1988), it is a pleasurable activity in itself (Boissy et al., 2007) and allows some agency over the environment (Wood-Gush & Vestergaard, 1993). As well as its links to positive welfare, deprivation of exploratory behaviour is described to be aversive, leading to boredom, apathy and stereotypical behaviours (Wood-Gush & Vestergaard, 1989), which is of particular concern for animals kept in monotonous environments. The restriction of exploratory behaviour likely causes behaviour to be redirected into stereotypical behaviour (Homeyer, 1969; Hughes & Duncan, 1988). Exploratory behaviour is usually tested in terms of activity (Hughes, 1997), space covered within an environment (Graham et al., 2018) and latency to approach new stimuli (Isabelle Veissier & le Neindre, 1992). This is carried out during tests allowing access to new or increased areas, or novel objects, aiming at facilitating exploration. The benefit that novelty provides is confusing, as many studies have shown that animals respond to novelty with a neophobic response (Mackay et al., 2014) and novel object tests are frequently used as a fear test (Forkman et al., 2007).

Increased exploration and response to novelty tends to be shown in animals from barren compared to enriched environments (Backus et al., 2017; Bracke & Spoolder, 2008). A number of suggestions have been made regarding the motivational basis behind this, but largely it is thought that animals kept in barren environments are deprived of opportunity to explore and so show a rebound in this behaviour when given the opportunity (Jensen, 1999; Vestergaard, 1982), with their sensitivity to reward from captive stress being altered (Burman et al., 2008; Van Der Harst et al., 2003). In light of this, Westerath et al. (2009) attempted to use exploration as an indicator for positive welfare, in an on farm welfare assessment of beef bulls. Authors hypothesised that animals kept on slats would investigate a novel object more than animals kept in larger littered pens, suggesting the test could highlight animals with less exploratory opportunities in their daily life and so poorer affective states. Barren housed bulls did spend more time in occupation with the novel object within the first 45 minutes of presentation, but differences were only slight. Behavioural testing was done by pens of animals, however testing at the individual level may allow better comparisons between groups and the level of variation between individual use of novel objects.

1.6 Animal boredom

1.6.1 What is boredom in animals

Boredom in animals has until recently been overlooked, primarily because it is deemed inferior to more severe welfare issues to be tackled, such as pain and disease, and secondly due to a wide opinion that boredom in animals can be seen as anthropomorphic (Svendsen, 2019; Wemelsfelder, 2005). The development of concern for animal boredom originally stems from intensified farming systems, where conventionally, animals are housed in restrictive and stimulus-lacking environments. This, in combination with the advances in scientific knowledge regarding the capabilities of animals to experience a range of affective states (Boissy et al., 2007; Paul et al., 2020), indicates that boredom could pose a welfare problem. Short term boredom may be negligible, but chronic boredom in people has serious health implications and is associated with psychological disorders such as anxiety and depression (Goldberg et al., 2011; Sommers & Vodanovich, 2000).

There is no unified definition of boredom (Goldberg et al., 2011). According to Wemelsfelder (1993), boredom in animals suggests a potential to suffer from a chronic lack of opportunities to interact with the environment, and this is specifically linked to the lack of species-specific behavioural opportunities available. Meagher (2018) suggests a resultant symptom of boredom to be motivation for general stimulation and Burn (2017) suggests behavioural indicators to include drowsiness, restlessness, avoidance and sensation seeking behaviour.

It is reasonable to expect that intensively kept animals, such as farm, laboratory and zoo species, to be particularly vulnerable to experiencing a boredom-like state, because they tend to be kept in stimulus lacking environments, with little opportunity to express species-specific behaviour. Whilst the direct causation of stereotypical behaviour is still debated (Rose et al., 2017), a widely acknowledged explanation is that it could be a coping mechanism, associated with inadequate environments (Cooper & Nicol, 1991; Mason, 1991). Common examples, are cribbing and wind sucking in horses (Cooper & Mason, 1998) and bar biting in pigs (Lawrence & Terlouw, 1993). The other driver contributing to the impact that barren housing has on psychological wellbeing, is the change in behavioural time budgets from the wild or more extensive management to smaller, usually indoor housing. Roca-Fernandez et al. (2013) found that grazed cattle spend 68% of their time grazing, whereas housed cattle spend only 22% of their time feeding. Although resting and ruminating are both important and along with grazing make up 90-95% of the animal's day, these two behaviours still fall behind grazing in frequency (Kilgour, 2012). This reduction in time budget, combined with a reduced need for food-directed exploratory behaviour or predator vigilance presents captive animals with a significant time void.

Boredom is accepted to be a negative affective state (Burn, 2017; Meagher, 2019a), is a common adjective used when people are asked to give examples of poor welfare (Duncan, 2002), and animals will actively choose to avoid it (Latham & Mason, 2010; Mason et al., 2001). Boredom induced, enhanced sensation seeking, may even provide motivation for aversive experiences, as preferable over monotony (Bench & Lench, 2013). Meagher and Mason (2012) found that unenriched mink showed lower latencies to approach aversive stimuli, such as air puffs and significantly more time in contact with stimuli with an ambiguous valence. Rats have also been shown to voluntarily chose toxic food over a previously enforced monotonous diet (Galef & Whiskin, 2003). This enhanced sensation seeking has also been expressed by increased psychoactive drug consumption and polyphagia (Meagher & Mason, 2012; Þorvaldsdóttir, 2014).

1.6.2 Inactivity as an indicator of boredom and negative affective states

Inactivity and idling behaviour have emerged as potential behavioural parameters for measuring boredom and negative affective states. Again, interest in this as a potential indicator of affective states, comes from human psychology where reduced activity is observed in patients suffering with depression (Bonnet et al., 2005; Van Gool et al., 2003). Burn et al. (2020) found that ferrets without a daily exercise session, lay awake with their eyes open significantly more, hypothesised to be an indicator of suboptimal arousal, compared to ferrets with an hourly daily exercise session. Meagher and Mason (2012) used lying but awake, as an indicator of boredom and found increased levels of this behaviour in unenriched compared to enriched housing conditions. Hintze et al. (2019), developed an inactivity ethogram for fattening cattle and found number of animals inactive per group and time spent inactive by focal animals, to increase from what was defined as a pasture based system, to a 'semi' intensive and then 'intensive' system. De Rosa et al. (2009) investigated idling behaviour, defined as open or closed eyes but no other overt activity, on two groups of buffalo. One group was housed in a standard open sided barn and the other had access to pasture, with wallows, vegetation and reduced stocking density. Proportion of idling animals was higher in the barren housed groups and authors concluded this to indicate a negative state of welfare. It's possible that there are different states of inactivity that could represent different affective states, for example, inactivity through boredom, or content resting (Fureix & Meagher, 2015).

Therefore, although this indicator is not yet validated, as is the case with many other potential indicators of affective state in animals, the research to date shows potential.

1.7 Enrichment

1.7.1 Environmental enrichment and animal welfare

Environmental enrichment involves modifications to a captive environment, which tend to be limited in stimuli, with an aim to increase health (Newberry, 1995). Environmental enrichment has been widely employed across zoos, aquaria and laboratory testing facilities, for the purpose of animal welfare improvement. Appropriate diversification of a barren environment can offer opportunities for choice and control over the environment and exploration (van de Weerd & Day, 2009), expression of highly motivated behaviours and improved coping abilities (Mason et al, 2001; Smith et al., 2007). The research documents a wealth of behavioural and physiological improvements including but not limited to; reduced stereotypical behaviours (Carlstead et al., 1991; Hetts et al., 1992), reduced stress hormones (Belz et al., 2003; Coppola et al., 2006; Rubin & Rhodes, 2003), increased weight gains (Aguayo-Ulloa et al., 2014; Flint & Murray, 2001), reduced self-harm (Bechard et al., 2011) and reduced inactivity through increased natural behaviours (Celli et al., 2003).

The literature also shows an inherent link between more complex environments and increased psychological functioning. Simpson and Kelly (2011) reviewed behavioural and neurochemical impacts of environmental enrichment on laboratory rats. Their collation of literature on anxiety and depressive-like behaviour testing reflect consistent improvements in animals housed in enriched conditions. The elevated plus maze, a validated test of anxiety, places an animal within a four-armed maze, two arms are completely enclosed and two remain open. The test is based upon rodent's aversion to open spaces and preference for enclosed, dark spaces. An increase in movement or occupation of open arms is indicative of decreased anxiety. Several studies have found increased open arm entries for animals housed in enriched environments compared to standard (Hellemans et al., 2004; Peña et al., 2006). Similarly, the literature shows a trend towards increased performance in forced swim tests (González-Pardo et al., 2019; Llorens-Martin et al., 2007; Singhal et al., 2019), used to assess depressive like states in animals. Rodents are dropped into water and decreased latencies to give up swimming are indicative of animals experiencing more depressive states. Brenes et al. (2008) found that rats housed in enriched cages from weaning, compared to standard cages and social isolation, showed the highest levels of swimming and climbing. Increased serotonin was also found in the prefrontal cortex in enrichment animals, consistent with wider literature showing a positive relationship between enrichment and neurological plasticity (Baroncelli et al., 2010; Brenes et al., 2016).

As well as the indication of decreased depressive and anxiolytic behaviours, cognitive bias testing has also shown a link between enriched environments and optimistic bias indicative of positive affective states. Rats showed a pessimistic bias towards ambiguous stimuli, after a period of unpredictable housing (Harding et al., 2004). Since then, these tests have been used to show optimistic bias following enriched housing conditions in rats (Brydges et al., 2011), pigs (Douglas et al., 2012), hamsters (Bethell & Koyama, 2015) and starlings (Matheson et al., 2008).

Enrichment for farmed animals has had the least uptake previously, but with the combination of farming intensification and societal pressure, there is reason to explore its use in this setting. It has been widely researched in the pig industry (van de Weerd & Day, 2009), where legally a sufficient quantity of manipulatable materials to enable proper investigation and manipulation must be permanently provided to all pigs in the European Union (Council Directive 2008/120/EC).

1.7.2 Enrichment for cattle

There is limited literature available on enrichment for cattle. Automatic brushes, first established in the 1980's (Georg & Totschek, 2001) are now widely used as enrichment on dairy farms. Brush access facilitates grooming (DeVries et al., 2007; Ninomiya, 2019), with other tentative benefits including, increased milk yield (Keeling et al., 2016; Schukken & Young, 2009), food intake (Keeling et al., 2016; Velasquez-Munoz et al., 2007) and decreased agonistic behaviour (Park et al., 2019; Park, Jennings et al. 2019). Grooming is a species-specific behaviour and provision of a brush may act as an artificial replicate for trees and hedging, which serve a similar purpose for grazed cattle (Kohari et al., 2007). The behaviour may arise as a control for hygiene and parasites. Moncada et al., (2020) found that severity of mange didn't alter cows brush use, however brush use did decline after application of an antiparasitic drug, indicating a wider contribution of factors behind motivation to use the brush. Grooming has also been suggested as a potential coping mechanism for stress (Krohn, 1994; Spruijt et al., 1992). Therefore, it could also be used to alleviate boredom, (Georg & Totschek, 2001), which is described as a distressing experience (Burn, 2017). Newby et al. (2013) investigated brush use around parturition and found that regardless of whether a brush was available, cows increased time spent auto grooming and scratching following removal of the calf, potentially a coping response to the stress of calf separation. Cows with access to a brush also spent more time licking their calves one-hour post calving compared to the non-brush group, authors suggested a possible explanation for this being increased oxytocin release with use of the brush. Conversely, Mandel et al. (2013) found brushing to reduce by approximately 50% on days of artificial insemination compared with the preceding and following 3 days. Similarly, decreases in use were found when the brush was located further away from food and during higher temperatures. Not supportive of the stress coping hypothesis, Mandel et al. (2013) suggested brush use to be a low resilience activity and a potential health indicator. Mandel et al. (2018) investigated this link and found that lame and severely lame cows would not use a brush located away from the feed alley but would continue to use one nearby. Mandel et al. (2017) previously found a 50% reduction in cows with metritis and a lower proportion of sick animals using it during the first week of diagnosis and treatment, highlighting that brushes could pose a potential advantage to producers as a proxy health indicator. McConnachie et al. (2018) investigated the value an automatic brush provides to cows, through assessing their motivation to access it. They trained cattle to push open a weighted gate for access to either fresh feed or a brush and found that cattle pushed similar weights to access both resources and significantly less weight to access an empty space used as a control. These results indicate that a brush is a valued resource for cattle, suggesting that access to one could facilitate positive affect.

Alternative enrichment has been largely unexplored in dairy cattle. Enrichment interventions for calves have shown to decrease oral stereotypical behaviour (Veissier et al., 1997), reduce cross sucking (Ude et al., 2011), decrease agonistic and increase affiliative behaviour (Ninomiya & Sato, 2009) and induce play (Bulens et al., 2014). Pelley et al. (1995) provided mounted brushes, salt/mineral licks and a bale of straw to steers and found that although they interacted most with the straw, likely because it allowed natural foraging behaviour, this also had the highest frequency of agonistic behaviour. Authors concluded there was little reason to believe that welfare was improved. Conversely, no differences in agonistic behaviour were found when beef cattle were enriched with a drum can containing hay (Ishiwata et al., 2006) and aggressive behaviour was reduced with a grooming brush and salt blocks (Šimić et al., 2018). The study by Pelley et al. (1995) placed straw in a corner, offering restricted access and promoting opportunity for competition, with no mention as to how frequently straw was replaced. These factors could understandably enhance agonistic interaction, hence, structural provision to avoid such effects need to be considered when designing enrichment strategies.

Auditory enrichment is another possible avenue for investigation. Kıyıcı et al. (2013) observed higher milk let down speeds in dairy cattle, with the addition of classical music during milking and Uetake et al. (1997) found music to stimulate cows to enter an automatic milking system. Although cows can react to sound, these results do not infer benefits to welfare. For example, heifers will avoid the playback of a milking facility if given the choice, which suggests it to be an aversive experience.

A recent study by Crouch et al. (2019) did find less tongue rolling behaviour, when dairy cattle were exposed to classical music, country music and an audiobook and specifically more positive social interactions with classical music and an audiobook than to the control and country music. Dairy farms tend to be a noisy environment, with farm machinery and traditional milking systems and so reducing noise could be more relevant, as auditory enrichment would be unavoidable for individuals adverse to it.

It has been suggested that providing animals a way of using their cognitive abilities is valuable for their welfare (Clark, 2017). Boissy and Lee (2014) reviewed the literature on cognition and welfare and outlined three processes which may elicit a positive emotion; by the signalling of a reward in advance, receiving a greater reward than expected and having control over a positive outcome. Occupational or cognitive enrichment, encouraging both exercise and cognitive work (Mandel et al., 2016) has only started to be explored in cattle. Hagen and Broom (2004) investigated the emotional reactions of cattle to learning an operant task. Heifers were trained to press a panel, to open a gateway to a 15m raceway, at the end of which was a food reward. Cows in the control group did not have to press the panel, with the gate opening automatically matched to the same time as the experimental individual. Heifers from the experimental group, tended to have higher heart rates and a faster raceway speed, when they made an improvement in learning the operant task. Behavioural differences were observed between groups, with jumping, bucking and kicking occurring 9 times in experimental heifers and not at all in control heifers. The study indicates an emotional reaction, but not necessarily a positive one. Wredle et al. (2006) taught 10 commercial dairy cows to approach an automatic milking unit when an auditory signal was played through a collar. Cows were able to learn the association but no inferences to welfare were made. As most farmed animals are housed in large groups with restricted space, practically implementing cognitive enrichment without risk of injury and competition would be challenging. However, automatic milking systems, which are becoming more popular on dairy farms, may already be offering some of the benefits of cognitive enrichment. Cattle learn to associate entry and milking with a reward, usually concentrate feed, this allows a period of positive anticipation upon entrance to the machine. It also provides cattle with a large level of control in accessing this positive outcome. Positive anticipation and control are both suggested as avenues for positive experience (Boissy et al., 2007).

A review on environmental enrichment opportunities for housed dairy cattle, suggested different categories of enrichment; social – with conspecifics or humans, occupational – including exercise or cognitive work, physical and sensory – including auditory, visual, olfactory, tactile and nutritional (Mandel et al., 2016). Burn (2017) suggests toys, novel objects, puzzle feeders and exercise apparatus as potential boredom reducing enrichment opportunities. Products such as puzzle feeders that release food slowly when moved (Young et al., 1994), or filling manipulatory objects with food for zoo animals are used widely as enrichment (Swaisgood & Shepherdson, 2005), but nothing in line with this is currently available for group-housed cattle. It has been argued that enrichment may be of no benefit if it has no functional significance to the animal, or serves no specific function for an outlet of behaviour (Newberry, 1995). Therefore, tentative suggestions such as toys that may serve no behavioural benefit need to be evaluated. It is possible that there may be other valued forms of enrichment for dairy cows, with the potential to enhance welfare. Given the evident welfare benefits in other species, this underexplored area demands further research for dairy cows.

1.8 Research gap

As outlined in the literature review, there is currently a lack of validated behavioural and physiological indicators of positive welfare in cattle. In addition to this gap, in the ability to identify whether cattle perceive events as positive, there is a lack of knowledge on what situations may offer this potential, and more specifically, how this could be practically and feasibly implemented in current production systems. The affective lives of dairy cows have more recently come under scrutiny from the public, with boredom and the lack of provision of opportunities for positive life experiences being key, reflecting the need for research to address these gaps.

1.9 Thesis aims

This research aims to further the current knowledge base on positive welfare in dairy cattle, by establishing whether providing opportunity for interest and exploration, through enrichment in the housed environment, may have welfare benefits. Specifically, the aims of the thesis are to evaluate:

- The potential utility of novel environmental enrichment for housed dairy cows, through assessing the level of interest and utilisation of novel enrichment by two separate groups of commercially managed cows (Chapter 3).
- Whether the provision of environmental enrichment affects boredom associated behaviours in groups of commercially-housed dairy cows (Chapter 4).
- The long-term use of enrichment by housed dairy cows to establish whether cows habituate to enrichment over time (Chapter 5).
- Housed dairy cow preferences between two different forms of enrichment; indoor novel enrichment and access to an outdoor concrete yard (Chapter 5).
- Whether the affective states of housed dairy cows are impacted by the provision of enrichment, through use of a positive welfare indictor – Qualitative Behaviour Assessment (Chapter 6).

Chapter 2: Methods

2.1 Overview

The results reported in Chapter 3 and Chapter 4 of this thesis were gained from one experimental trial. The methods for this trial are detailed in the current chapter. The experimental trial investigated different aspects of cow behaviour and the analysis and interpretation of these results have been split between Chapter 3 and Chapter 4. These methods solely relate to Chapter 3 and Chapter 4 and a separate methods section is provided for Chapter 5 and Chapter 6.

2.2 Cows and treatment

2.2.1 Study herd

The experimental trial was replicated on two groups of cows. Group 1 consisted of 37 Holstein cows averaging (mean±SD) 160.3±59.9 days in milk (median: 179, IQR: 91, range: 65-279), producing on average 49.2±11.8L of milk/day, of parity 2.6±1.4, proportion of parity groups were parity 1: 0.26, parity 2: 0.34, parity 3: 0.17, parity 4+: 0.23 and weighing 767.9±85kg at the start of the study (Table 2.1). Group 2 consisted of 37 Holstein cows averaging 141.4±41.3 days in milk (median: 130, IQR: 67, range: 45-231), producing on average 41.6±9.7L of milk/day, parity 2.3±1.3, proportion of parity groups were parity 1: 0.32, parity 2: 0.35, parity 3: 0.14, parity 4+: 0.19 and weighing 748.2±96.9kg at the start of the study (Table 2.2). Cows were selected on the basis of including a variety of animals in different lactation groups and stage of lactation. A stable experimental group of cows were required for the study, therefore only cows with a drying off or calving date of after the study end date were selected. Both cohorts of cows were moved into the test environment at least one week before data collection started to allow the cows to habituate to a different building and their new social group. The study housing provided the same facilities, management and structure as the other adult dairy cow housing on the farm and therefore the trial housing conditions would not have been novel for the cows.

Table 2.1. *Cows enrolled in first replicate of study (Group 1).*

No.	Ear tag (UK)	Date of birth	Lactation	DIM CL	Last calving date	Expected calving date	Expected dry off	Status	Paint number
520	141797602430	31/03/2016	3	50	03/05/2020	19/05/2021	24/03/2021	Inseminated	1
876	141797602759	17/02/2018	1	143	31/01/2020	10/05/2021	15/03/2021	Inseminated	2
1890	141797402883	17/08/2018	1		23/07/2020			Open	3
633	141797302203	16/02/2015	4	104	10/03/2020	09/03/2021	12/01/2021	Pregnant	4
248	141797502415	05/03/2016	3	81	02/04/2020	03/04/2021	06/02/2021	Pregnant	5
912	141797202832	10/05/2018	1	13	09/06/2020	17/05/2021	22/03/2021	Inseminated	6
651	141797302252	10/06/2015	4	28	25/05/2020	05/05/2021	10/03/2021	Inseminated	7
784	141797102586	04/04/2017	2	12	10/06/2020	20/05/2021	25/03/2021	Inseminated	8
563	141797702515	21/11/2016	2	134	09/02/2020	25/02/2021	31/12/2020	Pregnant	9
894	141797702844	30/05/2018	1		11/07/2020			Open	10
730	141797102649	12/08/2017	2	40	13/05/2020	24/04/2021	27/02/2021	Inseminated	11
792	141797202594	11/04/2017	2	179	26/12/2019	25/02/2021	31/12/2020	Pregnant	12
109	141797202314	01/10/2015	3	83	31/03/2020	03/04/2021	06/02/2021	Pregnant	13
905	141797202790	22/03/2018	1	43	10/05/2020	14/05/2021	19/03/2021	Inseminated	14
761	141797702613	16/05/2017	2	75	08/04/2020	22/04/2021	25/02/2021	Inseminated	15
20	141797402561	12/02/2017	2	16	06/06/2020	11/05/2021	16/03/2021	Inseminated	16
530	141797102425	28/03/2016	3	85	29/03/2020	22/03/2021	25/01/2021	Pregnant	17
718	141797702620	28/05/2017	2		25/06/2020	17/05/2021	22/03/2021	Inseminated	18
72	141797501841	20/05/2013	6	16	06/06/2020	18/05/2021	23/03/2021	Inseminated	19
736	141797702641	18/07/2017	2	84	30/03/2020	11/05/2021	16/03/2021	Inseminated	20
410	141797102075	23/07/2014	4	155	19/01/2020	06/02/2021	12/12/2020	Pregnant	21
760	141797202636	15/07/2017	2		06/07/2020			Open	22
751	141797602556	04/02/2017	2	87	27/03/2020	17/03/2021	20/01/2021	Pregnant	23
785	141797402589	08/04/2017	2		15/07/2020			Open	24
734	141797602661	25/08/2017	2	28	25/05/2020	17/04/2021	20/02/2021	Pregnant	25
56	141797202769	27/02/2018	1	137	06/02/2020	05/03/2021	08/01/2021	Pregnant	26
384	141797701689	25/07/2012	6	147	27/01/2020	15/02/2021	21/12/2020	Pregnant	27
80	141797301874	11/07/2013	5	181	24/12/2019	27/04/2021	02/03/2021	Inseminated	28
629	141797502226	03/04/2015	3	87	27/03/2020	23/03/2021	26/01/2021	Pregnant	29
412	141797102040	31/05/2014	4	105	09/03/2020	31/01/2021	06/12/2020	Pregnant	30
38	141797702578	22/03/2017	2	3	19/06/2020	16/05/2021	21/03/2021	Inseminated	31
879	141797202727	06/01/2018	1	87	27/03/2020	31/03/2021	03/02/2021	Pregnant	32
230	141797301972	01/01/2014	5	45	08/05/2020	25/05/2021	30/03/2021	Inseminated	33
206	141797601884	30/07/2013	5		27/06/2020			Open	34
314	141797302791	24/03/2018	1	83	31/03/2020	14/03/2021	17/01/2021	Pregnant	35
130	141797702389	26/01/2016	3		25/07/2020			Open	36
817	141797302721	30/12/2017	1	101	13/03/2020	10/05/2021	15/03/2021	Inseminated	37

Two subjects had to be removed from the first replicate of the study to be treated for lameness. Cow 20 was removed on 30/09/2020 and cow 206 was removed on 10/10/2020, these cows remained absent for the remainder of the study.

Table 2.2. *Cows enrolled in second replicate of study (Group 2).*

No.	Ear tag (UK)	Date of birth	Lactation	DIM CL	Last calving date	Expected calving date	Expected dry off	Status	Paint number
1	141797602696	13/11/2017	2	21	15/01/2021			Open	unmarked
6	141797102089	08/08/2014	5	158	13/07/2020	22/07/2021	27/05/2021	Pregnant	6
7	141797402239	10/05/2015	4	52	27/10/2020	23/10/2021	28/08/2021	Inseminated	7
22	141797302238	10/05/2015	4	114	26/08/2020	01/08/2021	06/06/2021	Pregnant	2
34	141797402946	19/11/2018	1	29	19/11/2020			Open	unmarked
39	141797301769	20/12/2012	6	113	27/08/2020	01/11/2021	06/09/2021	Inseminated	14
48	141797202923	01/11/2018	1	31	17/11/2020	15/10/2021	20/08/2021	Inseminated	4
49	141797602521	30/11/2016	3	6	12/12/2020			Open	22
57	141797302973	31/12/2018	1	29	19/11/2020	08/10/2021	13/08/2021	Pregnant	5
140	141797702410	28/02/2016	3	90	19/09/2020	02/10/2021	07/08/2021	Pregnant	34
165	141797202363	17/12/2015	3	92	17/09/2020	16/09/2021	22/07/2021	Pregnant	12
423	141797402092	15/08/2014	5	24	24/11/2020	09/11/2021	14/09/2021	Inseminated	35
450	141797602157	25/11/2014	4	120	20/08/2020	29/09/2021	04/08/2021	Pregnant	13
524	141797302441	26/04/2016	3	115	25/08/2020	15/08/2021	20/06/2021	Pregnant	27
583	141797602486	08/09/2016	3	121	19/08/2020	16/09/2021	22/07/2021	Pregnant	21
656	141797702249	05/06/2015	4	51	28/10/2020	12/09/2021	18/07/2021	Pregnant	28
675	141797202888	30/08/2018	1	49	30/10/2020	12/11/2021	17/09/2021	Inseminated	1
692	141797602864	04/07/2018	1	56	23/10/2020	25/10/2021	30/08/2021	Inseminated	16
714	141797602626	13/06/2017	2	57	22/10/2020	27/09/2021	02/08/2021	Pregnant	31
756	141797102628	19/06/2017	2	101	08/09/2020	08/08/2021	13/06/2021	Pregnant	10
804	141797302672	13/09/2017	2	115	25/08/2020	13/07/2021	18/05/2021	Pregnant	30
809	141797202706	07/12/2017	2	43	05/11/2020	23/10/2021	28/08/2021	Inseminated	18
813	141797602682	11/10/2017	2	98	11/09/2020	17/10/2021	22/08/2021	Inseminated	26
814	141797302665	04/09/2017	2	90	19/09/2020	07/11/2021	12/09/2021	Inseminated	37
825	141797602689	17/10/2017	2	88	21/09/2020	20/08/2021	25/06/2021	Pregnant	36
831	141797702655	16/08/2017	2	134	06/08/2020	01/08/2021	06/06/2021	Pregnant	33
841	141797402680	07/10/2017	2	85	24/09/2020	23/10/2021	28/08/2021	Inseminated	3
845	141797502702	25/11/2017	2	54	25/10/2020	31/10/2021	05/09/2021	Inseminated	20
857	141797302735	15/01/2018	2	5	13/12/2020	12/11/2021	17/09/2021	Inseminated	24
875	141797302749	31/01/2018	2	33	15/11/2020	22/10/2021	27/08/2021	Inseminated	32
895	141797102845	01/06/2018	1	51	28/10/2020	07/11/2021	12/09/2021	Inseminated	25
937	141797102964	24/12/2018	1	104	05/09/2020	31/08/2021	06/07/2021	Pregnant	23
941	141797102950	24/11/2018	1	61	18/10/2020	17/09/2021	23/07/2021	Pregnant	17
961	141797403016	15/02/2019	1	42	06/11/2020	08/11/2021	13/09/2021	Inseminated	15
962	141797503010	11/02/2019	1	22	26/11/2020	09/11/2021	14/09/2021	Inseminated	29
965	141797603004	05/02/2019	1	38	10/11/2020	06/10/2021	11/08/2021	Pregnant	11
3005	141797703005	07/02/2019	1	89	20/09/2020	26/09/2021	01/08/2021	Pregnant	19

Two subjects had to be removed from the second replicate of the study for veterinary intervention. Cow 714 was removed on 10/04/2021 and cow 656 was removed on 18/04/2021, these cows remained absent for the remainder of the study.

2.2.2 Treatment

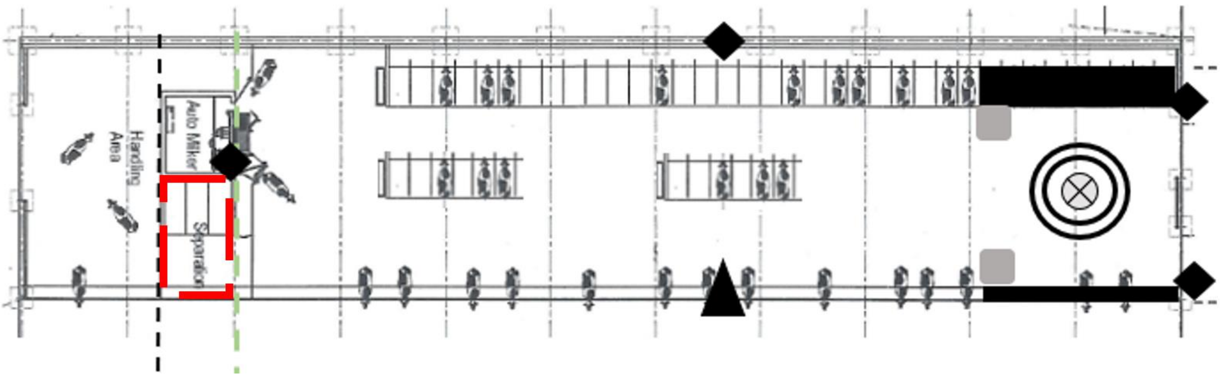
The trial ran from 28.09.2020 - 08.11.2020 (Group 1) and 01.03.2021 – 11.04.2021 (Group 2). Each six week trial period consisted of one initial week of no interventions ('baseline week 1'), three weeks of continuous 24 hour access to a novel object in the home environment ('intervention weeks 1 to 3'), 1 washout week with no interventions and a final week with no interventions ('baseline week 2').

The novel object consisted of an orange inflatable sailing buoy measuring 575mm in length by 460mm diameter (Figure 2.1). This was suspended by rope at one end of the housing, in an area of extended loafing space, at approximately cow shoulder height. The buoy was suspended from the roof by rope, and therefore moved when cows touched it. Circles of 1m and 2m radius were marked out on the floor surrounding the buoy using white paint. Plastic water filled barriers were used to mark the entry to a loafing area within the building that provided no facilities to the cows, apart from the novel enrichment during treatment weeks; there were no lying, drinking or feeding resources in the loafing space (Figure 2.2). Forty cubicles were available as lying areas for the 37 trial cows throughout both study periods, providing 15% additional cubicle space.

Standard housing conditions for this building included a water trough situated on the end wall of the building within this area of extended loafing space, where the novel object was placed during the treatment period. This was emptied one week before the trial started, to allow a 7-day habituation period for the herd and remained empty throughout the rest of the trial. Access to 9 cubicles were blocked using metal gates secured to the cubicle infrastructure. The equivalent length of feed space on the opposite side of the building was blocked from use and not used for TMR provision by secured metal gates to the feed face infrastructure. The cubicles and feed space were located within the enrichment area (Figure 2.1; 2.2) and were therefore removed to ensure this part of the housing provided no other resource than the novel object.

The novel object used in this study was chosen based on the assumption that the cows would not have previously seen such an object, so it would provide a novel experience for them.

Figure 2.1. *Novel object suspended in centre of extended loafing space.*



Start of housing	- - - - -	Movable barriers marking start of novel area	■
Start of camera view	– – – –	Novel object	⊗
Camera locations	◆	1m and 2m painted lines surrounding buoy	○
Automatic brush	▲	Blocked off facilities	━━━
Blind spot			

Figure 2.2. *Layout of the barn and the novel object area.*

2.2.3 Housing, management, diet

The study was conducted at the Centre for Dairy Science Innovation, University of Nottingham, a 300-cow research dairy herd producing milk commercially. Cows in the experimental groups were continually housed in one 774.9m^2 building, containing 51 sand bedded cubicles with concrete slatted flooring, scraped automatically daily. Subjects received ad libitum access to fresh water via 3 water troughs and were fed a total mixed ration (TMR) ad libitum which was replenished daily at 09:00. Subjects were milked robotically via a Lely automatic milking system where they received additional concentrate feed.

2.3 Data sources

All video footage was collected using 4 fixed Axis M1065 IP cameras. These were held in place by movable clamps secured to the barn infrastructure. Cameras were positioned in 4 separate locations of the building (Figure 2.2), aimed to give full coverage of the herd’s living space. One small area of the building remained out of view to the cameras due to the building design. This area contained: 3 sand bedded cubicles and a small area of loafing and feed space. Given the size of the area it was considered acceptable to not include this section of the building in the video analysis. Figures 2.3-2.6 display the camera view provided by each of the cameras used within the study. Cameras were connected to a laptop situated within the barn (Figure 2.7), video files were instantly saved using 'MediaRecorder' (VideoLAN). Power over ethernet cabling was used to create a closed system requiring a constant power source to run the cameras. Video footage was collected continuously throughout the study period in 24-hour slots. Slots of 24 hours were chosen as manageable files for data transfer and earlier trialling of the equipment had shown technical faults when attempting to leave camera's recording for more prolonged periods of time. Footage clips were collected manually by setting MediaRecorder to start recording on a Monday morning at 07:00 and then daily throughout the rest of the week until Saturday mornings. Manual changeover of MediaRecorder each day involved stopping recording for a period of a few seconds and commencing recording again, resulting in loss of a few seconds of footage each day. Video footage was not collected over weekends throughout the study period except for Saturday mornings which made up part of Fridays 24-hour recordings. All video files were named with a date and time stamp identifying the start time of recording. Video files were transferred to, and stored on, separate laptop computers for remote analysis using an external hard drive.

Figure 2.3. *Camera view 1.*

Figure 2.4. *Camera view 2.*

Figure 2.5. *Camera view 3.*

Figure 2.6. *Camera view 4.*

Figure 2.7. *Central hub, all cameras connected to single laptop located above the Lely robotic milker.*

Individual cow use of the robotic milking machine was recorded. Records from the Lely milking robot were collected throughout both trials detailing; animal ID, date, time of milking, milking time, milk yield, whether it was an unsuccessful milking attempt (refusal), milk temperature and cow weight.

Cows were marked to enable identification via video recordings, as neither collar tags or freeze brands were large enough to be visible on the recordings. Cows were marked with a green or yellow painted number on either side of their back using Fil Detail Tail Paint. Cows were painted during feeding times on Monday and Wednesday mornings (09:00-10:30), whilst locked in yokes, a management practise the cows were used to for regular veterinary visits. During the first replicate of the study, it was identified that some predominantly white cows were more sensitive to paint application than cows with black hair. Due to this, for the second replicate of the study, two cows with predominantly white hair and lighter skin were identified by their coat appearance instead of by applying paint. These cows continued to be locked in head yokes with the rest of the herd whilst applying paint numbers.

2.4 Behaviour recording

2.4.1 Overview of behaviour recording throughout the trial

Table 2.3 provides an overview of the different behaviour sampling that was carried out throughout the course of this trial and when it was conducted. A detailed description of each of the different behaviour sampling methods is provided in sections 2.4.2 to 2.4.6.

Table 2.3. *Overview of when all behaviour sampling was carried out throughout the course of the trial.*

		Day					
		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Week	Baseline	Scan sampling of herd behaviour (11:00-07:00)			24-hour continuous recording of self-grooming (Group 1 and 2)	Scan sampling of herd behaviour (11:00-07:00)	
	1	Cows first interaction with object (ethogram) 24-hour following object provided Scan sampling of herd behaviour (11:00-07:00)	24-hour continuous recording of novel object use and self-grooming (Group 2)		24-hour continuous recording of novel object use and self-grooming (Group 1)	Scan sampling of herd behaviour (11:00-07:00)	
	2	Scan sampling of herd behaviour (11:00-07:00)			24-hour continuous recording of novel object use and self-grooming (Group 1 and 2)	Scan sampling of herd behaviour (11:00-07:00)	
	3	Scan sampling of herd behaviour (11:00-07:00)	24-hour continuous recording of novel object use and self-grooming (Group 2)		24-hour continuous recording of novel object use and self-grooming (Group 1)	Scan sampling of herd behaviour (11:00-07:00)	
	Baseline	Scan sampling of herd behaviour (11:00-07:00)				Scan sampling of herd behaviour (11:00-07:00)	

2.4.2 Use of novel object

Interactions with the novel object (sailing buoy) were evaluated using a single 24 continuous hours of footage each week, a total of 72 continuous hours of footage analysis per study group. The 24-hour period of continuous footage was selected such that no routine or unexpected farm interventions occurred (e.g. no routine foot trimming or veterinary examinations); the 24-hour period represented a 'normal' day for the cows. For group 1 this was Thursdays (08.10.2020, 15.10.2020, 22.10.2020) starting from 00:00 and for study group 2 were: Tuesday 09.03.2021 starting from 09:00, Thursday 18.03.2021 from 00:00 and Tuesday 23.03.2021 starting from 09:00. The days evaluated for study group 2 differed to study group 1 to avoid interference from routine herd hoof trimming visits. In terms of day of experiment, the recorded days for group 1 were day 4, day 11 and day 18. For group 2 recorded days were day 2, day 11 and day 16. Day 1 of the experiment represented the day the novel object was provided. A continuous 24-hour period of footage was chosen for analysis per week instead of other sampling periods, in order to gain a clearer insight into the patterns of individual cow use and how the use of enrichment varies throughout time of day. In addition to this, this sampling method was chosen to provide more reliable information on the amount of time cows spent using the novel object and the amount of different cows using it than would have been acquired from sampling smaller periods of time, chosen based on when cows are likely to be more active.

A cow interaction with the novel object was defined as physical contact with the novel object. An interaction started when any part of a cows' body came into contact with the buoy. If physical contact stopped for longer than five seconds, then the interaction was deemed to have ended. If the cow then contacted the object again, this was defined as a new interaction. Where the cow was not in contact very briefly (less than five seconds) during an interaction, the interaction was deemed to be continuing. All occurrences sampling was used to record interaction with the novel object throughout the recorded 24-hour recorded periods. For every interaction, cow ID and length of interaction were recorded. Specific outcomes of interest, were the total time the buoy was in physical use (i.e. cows were interacting with the buoy) and the proportion of hour slots throughout the continuous 24-hour recording during which one or more cows interacted with the buoy. All interactions were recorded using Noldus Observer XT behaviour recording software.

2.4.3 Herd level behaviour

Herd level behaviour was evaluated to investigate whether the addition of the novel object had any impact on the wider behavioural budgets of the herd. Herd activity was evaluated using a scan sampling procedure. Scan sampling comprised evaluation of video footage at specific points in time from all four cameras covering the whole housed area, with an interval between scan samples of 60 minutes. The scan sampling procedure was carried out on Mondays and Fridays each week (excluding the washout week) between 11:00-07:00, to avoid routine management procedures, meaning a total of 42 scans each week. For each scan, all 4 camera views were used to place every cow in one of the behaviour categories listed in Table 2.3, to give a total number of cows performing every behaviour at each scan. Results were accumulated to provide a sum for the numbers of each behaviour exhibited by all cows each day and this was divided by the numbers of cows present (to adjust for cows removed from the study due to veterinary intervention). We also provide the results as the mean total daily number of cows scored within each behavioural category.

Table 2.4. *Behaviours and descriptions used for herd level scanning.*

Inactive standing/Idling	Cow is standing stationary. May be looking around or changing position but with no other overt activity (De Rosa et al., 2009; Webb et al., 2017). For further clarification on active or inactive standing behaviour see (Hintze et al., 2019). Cow may be ruminating. Excludes cows queuing at the milking robot.
Positive social interactions	Social licking (tactile oral contact directed to the body except the anal region, udder or claws), horning (rubbing of foreheads or horn bases against the head or neck of another animal) (Winckler et al., 2015) and social sniffing (muzzle closer than 10cm from head or body of another cow, sniffing) (Krohn, 1994).
Negative social interactions	Any form of agonistic interaction - bite, kick, butting, displacing, or chasing where one cow forces another to either remove themselves from the activity (for example using the brush) or take 2 steps away.
Lying in cubicle	Cow is lying in cubicle.
Standing in cubicle	Cow is stood either fully in cubicle or with front two feet in cubicle.
Cow at feed face or water trough	Cow has head fully through feed face, is at a water trough actively drinking or using the salt licks.
Cow moving	Cow is walking through the barn.
Brush	Cow is in physical contact with brush.
Interacting with enrichment	Cows are within the enrichment area – 2 hooves over approach line, may be in physical contact with enrichment.
Novel area of barn	Cow is within the end area of the barn (past motorway barriers) but is not within the zones.
Out of sight	Cow at top end of barn. There is a small visual field inaccessible to the cameras, which includes access to a treatment pen and the robotic milker. If part of cow is visible but unable to classify which behaviour category the cow falls into then to be classed as out of sight. This also applies to cows penned off receiving veterinary investigation.

2.4.4 Ethogram

Every individual cows' first approach and interaction with the novel object was recorded. This was evaluated for every cow from day 1 of installation of the novel object. A 60-minute period was omitted following the installation of the novel object, this was to avoid a crowding of cows that occurred following initial exposure of the object. Following this omitted 60-minute period, every individual cows' first interaction was recorded, from the moment they first approached the novel object. A cows first approach was defined as the first occasion when a cow put one hoof over the 2m marked out approach line surrounding the novel object. A detailed ethogram of cow behaviour was created and used to evaluate the behavioural response of the cows to the novel object (Table 2.4). The behavioural categories in the ethogram were coded for the entire initial approach session. Focal sampling was used to evaluate every individual cows behaviour using the ethogram. Social behaviours were only recorded if another cow entered the 2m radius of the novel object whilst the behaviour of a focal cow was being recorded within the zone. The first approach session ended when the entire cow exited the 2m marked out radius surrounding the object.

Table 2.5. *Ethogram of behaviours, with definitions used to measure cows' initial response to the novel object.*

Behavioural category	**Behavioural parameter**	**Description**
Occupying 2m radius of novel object (individual cow).		
Chewing/ licking	Cow chewing or licking object (duration)	Cows tongue is in contact with the object or the cow is attempting to chew the object. Any appearance of the behaviour is recorded. If there is more than a five second gap between bouts of this behaviour, then the behaviour is classed as ended.
Other physical contact (more than five second gap in contact) new bout	Head/nose/shoulder contact (duration)	Cows head is in physical contact with object, may be pushing, butting or attempting to scratch head. This includes the ears, the neck or the shoulders of the cow. If there is more than a 5 second gap between bouts of this behaviour, then the behaviour is classed as ended.
	Other body part (duration)	Other body part of cow is in physical contact with object.
Stationary	Stationary orientated towards object (duration)	Cow is stationary and is orientated towards the object. Behaviour stopped being scored when cow orientates away from object.
	Stationary orientated away from (duration)	Cow is stationary and is orientated away from the object. Behaviour stopped being scored when gaze changes to being diverted at object.
	Submissive posture (duration)	Cow stationary with head lowered outstretched to ground.
Locomotion	Walking (duration)	Cow moves all four legs and covers ground (more than 2 continuous steps to be taken to be recorded).
	Fast locomotion (duration)	Cow covers distance in faster pace than a walk (trotting/running), may include jumping or bucking 2

		or more hooves off floor including hopping and kicking.
	Startle	The animal flinches, jumps or bucks in response to the stimulus (Gibbons et al., 2009) and takes 2 or more steps back.
Other	Tail shaking (duration)	Tail is vigorously wagging in any direction (tail naturally swinging as a result of movement of the cow not included).
	Head shaking (count)	The head is shaken or rotated.
	Urination/Defecation (count)	Cow is urinating or defecating.
	Self-grooming (duration)	Cow is licking any part of its own body.
Social interaction	Allogrooming (duration)	Any form of licking or grooming with the mouth from one cow to another.
	Agonistic interaction (count)	Any form of agonistic interaction - Bite, kick, butting, pushing, fast approach to another cow or displacing where one cow approaches novel object and forces another to retreat (receiving cow takes more than 2 steps to move away from initiating cow).

2.4.5 Self-grooming

For this experimental trial, self-grooming was defined as any licking, chewing or scratching carried out by the cow either by mouth or by hoof directed at the cows own body. To evaluate the occurrence of self-grooming events that were specifically linked to an interaction with the novel object during intervention weeks 1 – 3, all self-grooming events that occurred within a 2m radius of the novel object (and following an interaction with the object) were identified and recorded during the 24 continuous hours of footage each week (Figure 2.8). To provide a comparison with the occurrence of self-grooming in the baseline week, all instances of self-grooming that occurred within the 2m novel object area (but with no novel object present) during baseline week 1 were recorded during the 24hr period of continuous footage.

Figure 2.8. *Footage images showing physical interaction with novel object (a) and self-grooming occurring following interaction with object (b).*

2.4.6 Refusals

Data were recorded continuously from the Lely robotic milking system for the entirety of both experimental replicates. Records included; animal number, date, time of each visit, milk yield and number of milking refusals. A refusal was defined as when a cow entered the milking robot but was immediately released; this would occur when a cow entered the robot before a minimum amount of time had elapsed since the cows last visit. Minimum between visit time periods are detailed in Table 2.5. The maximum and minimum number of milkings apply to a 24-hour period. Cows in the 0-40 days post calving category, would have a refusal if they attempted to be milked within 4.8 hours of a previous milking.

Table 2.6. *Lely T4C settings controlling access to be milked.*

	0-40 days post calving	**40 days post calving – 30 days before dry off**	**30 days before dry off - end of lactation**
Maximum number of milking's	5	5	3
Optimum expected yield per miking	8.5	9.5	10.5
Minimum number of milking's	4	2	2

2.5 Data handling and statistical analysis

2.5.1 Chapter 3: Evaluation of the use of novel enrichment by commercially-housed dairy cows

2.5.1.1 Statistical analysis

All statistical analyses were performed using RStudio version 4.0.3 using packages dplyr (Wickham et al., 2022), tidyverse (Wickham et al., 2019) and lme4 (Bates et al., 2015). The data presented in Figures 3.3 to 3.17 were plotted using the package ggplot2 (Wickham, 2016). Details of each element of analysis are provided in the sections below.

2.5.1.2 Herd use of buoy across treatment weeks

Results have been reported as;

- The percentage of time the novel object was in use in total across the intervention period (out of 72 sampled hours) and by week (out of 24 sampled hours).
- The percentage of the herd that used the novel object per day each treatment week (out of a continuously monitored 24-hour slot taken each treatment week).
- The mean minutes per day that cows spent using the novel object across treatment weeks (out of a continuously monitored 24-hour slot taken each treatment week).
- The number of separate interactions cows made with the novel object per day each week (out of a continuously monitored 24-hour slot taken each treatment week).

2.5.1.3 Time of day

Results have been reported as the number of hour slots, out of 24, in which the novel object was in use and the number of different cows that used it within each hour slot.

2.5.1.4 Ethogram

Results have been reported as;

- The duration (minutes) of each cows' initial interaction (see 2.4.3) with the novel object.
- The most frequently expressed behaviours during the initial interactions.
- The duration of expression of each behaviour (minutes) that occurred during each cows' initial interaction.

2.5.2 Chapter 4: Novel enrichment reduces boredom associated behaviour in housed dairy cows

2.5.2.1 Statistical analysis

All statistical analyses were performed using RStudio version 4.0.3 using packages dplyr (Wickham et al., 2022), tidyverse (Wickham et al., 2019) and lme4 (Bates et al., 2015). The data presented in Figures 4.2 and 4.3 were plotted using the package ggplot2 (Wickham, 2016). Final inference was made from linear models including significant explanatory variables. Details of each element of analysis are provided in section (4.3), inference was conducted through assessment of model parameter confidence intervals with a significance threshold set at $P<0.05$.

2.5.2.2 Self-grooming

The probability that a cow would self-groom given that she was in the 2m zone was calculated and compared between weeks. Cows were also categorised as either having self-groomed (on one or more occasion) or not during each one hour period of the 24 continuous hours of footage; this was coded as 1 (self-groomed) or 0 (did not groom) for all cows that were eligible having entered the 2m zone. Final inference on the probability of self-grooming was made from a conventional mixed effect logistic regression model (Wickham, 2016) that incorporated a random effect for cow, to account for the repeated measurements of self-grooming over time within cow and therefore ensured a robust estimate of the conditional probability of self-grooming.

2.5.2.3 Refusals

Data were recorded continuously from the Lely robotic milking system for the entirety of both experimental trials. Records included; animal number, date, time of each visit, milk yield and number of milking refusals. A refusal was defined as when a cow entered the milking robot but was immediately released; this would occur when a cow entered the robot before a minimum amount of time had elapsed since the cows last visit (Table 2.5).

Data were recoded as a total number of refusals per cow per day and final inference made from a mixed effect linear model with number of refusals per cow per day as the outcome variable and a random term for cow to account for repeated measurements of refusals over time within cow (Wickham, 2016). Since initial exploratory models revealed that model residuals displayed overdispersion (non-normality), a transformed outcome variable was used ($\log_{10}$ (number of refusals per day+1)) to ensure models met the required underlying assumptions.

2.5.2.4 Herd level behaviour

Results were accumulated to provide a sum for the numbers of each behaviour exhibited by all cows each day and this was divided by the numbers of cows present (to adjust for cows removed from the study due to veterinary intervention). Final inference was made from a linear model with the outcome variable as the number of recorded behavioural events per cow per day. The results mean total daily number of cows scored within each behavioural category is also provided.

Chapter 3: Evaluation of the use of novel enrichment by commercially-housed dairy cows

3.1 Introduction

Animal welfare has historically focussed on negative experiences such as pain, suffering and disease (Yeates & Main, 2008). Substantial developments in knowledge of animal welfare and animal intelligence have occurred over the last decade, resulting in a wide acceptance that animals are sentient (Duncan, 2006; Proctor, 2012) and capable of experiencing emotions and affective states (Bekoff, 2000; Boissy et al., 2007; Machado & Da Silva, 2020). This transition in knowledge brings with it moral questions regarding the affective lives of animals, specifically animals managed in intensive commercial environments, which are frequently associated with negative outcomes for animal welfare (Perry, 1983; Woods, 2012).

This has driven the emergence of the science of positive animal welfare, animals' experience of positive affective states (Yeates & Main, 2008). When considering the historical concept of animal welfare, which involved preventing negative states as much as possible, given that agricultural production of animals involves frequent unavoidable aversive experiences (FAWC, 2009; Nordquist et al., 2017), it is clear to see that prevention of severe negative states is not conducive to 'good' animal welfare. As an example, the provision of sufficient food, would prevent animals from experiencing the negative state of hunger, but may not provide an enjoyable experience, due to the monotony of feed provided or social competition during feeding times. The FAWC (2009) urge policy makers to ensure that animals have experienced a life worth living, defined by the balance of experiences being positive over the entirety of an animal's lifetime. One suggested way of offering captive animals positive experiences has been suggested to be providing opportunities for their interest, pleasure and control (FAWC 2009; Edgar et al., 2013; Mellor, 2015), which can be created through diversification of the environment to facilitate exploration and agency.

Environmental enrichment has been widely incorporated in animal management, particularly the pig industry (van de Weerd & Day, 2009). Enrichment has been shown to offer a breadth of welfare improvements for pigs (Mkwanazi et al., 2019) with the ability to make significant positive impacts on both health (Gentry et al., 2002; Lyons et al., 1995) and behaviour (Beattie et al., 1995; Mkwanazi et al., 2019). Most notably, the provision of enrichment has been shown to significantly reduce tail biting (Buijs & Muns, 2019; Lahrmann et al., 2018), a severely problematic behaviour related to stress and boredom (Benard et al., 2014; Paul et al., 2007; Schrøder-Petersen & Simonsen, 2001), which is economically detrimental (D'Eath et al., 2016; van Staaveren et al., 2021) and a challenging welfare issue for the industry. Asides from these already significant impacts, research has also indicated that enrichment strategies can positively impact pigs' emotional lives (Carreras et al., 2016; Douglas et al., 2012; Ocepek et al., 2020;). The significant impact that enrichment strategies have made in enhancing pig welfare, is reflected in legislation requiring provision of manipulatable materials to enable proper investigation and manipulation, to be permanently provided to all pigs in the European Union (Council Directive 2008/120/EC). The magnitude of impact that environmental diversification and modifications have made, to not just overall welfare, but the elusive goal of improving animals' affective states, critically questions why this topic of research is almost non-existent for dairy cows. Similarly, this is further questioned when considering that commercially managed pigs and dairy cows are kept in systems not completely different from one another, particularly when comparing pigs and cows that are fully or partly housed. Evidently, the natural behavioural needs and motivations of the two species are diverse and therefore identifying resources that may offer the opportunity for cows' interest, pleasure and exploration is an initial question to address.

The literature review in chapter one highlighted a clear gap in research evaluating housing and management modifications, particularly regarding environmental enrichment strategies, for adult dairy cows. A large body of research has been directed at evaluating the health and welfare benefits of access to pasture (Arnott et al., 2016; Charlton & Rutter, 2017) and to cows' use of automatic brushes (Mandel et al., 2016), a resource that has become popular on dairy farms. Asides from this, limited studies have briefly explored the use of music (Kıyıcı et al., 2013), gentle tactile contact by people (Waiblinger et al., 2004), cognitive exercise (Hagen & Broom, 2004) and alternative outdoor areas (Smid et al., 2018) on the behaviour of dairy cows, yet to our knowledge the use of physical enrichment through housing modifications and additional resource objects has not been explored. A particular need for research in this area has been driven by the general societal concern regarding dairy cow welfare (Weary & von Keyserlingk, 2017; Wolf et al., 2016). One such example, is that cows having access to grazing is an important concern of consumers (Jackson et al., 2020), yet zero grazed systems are increasing in Great Britain (March et al., 2014).

In response to the vast research gap that has been identified regarding the affective lives of commercially managed adult housed dairy cows, with a particular reference to the potential of environmental enrichment as a possible avenue for enhancing welfare, the primary objective of this experiment was to evaluate the interest and uptake by two groups of commercially-housed adult dairy cows, to a novel object provided in the home environment, proposed to offer environmental diversity and an opportunity for interest and exploration. Further objectives were to evaluate the variability of use amongst cows, of varying ages and stages of production and behaviourally how cows interacted with the object. This first study aimed to address whether the provision of a novel environmental enrichment may be a valuable way to provide positive experiences to dairy cows and in turn provide initial information on whether this would be a valuable area of future research for the improvement of dairy cow welfare.

3.2 Materials and methods

Detailed methods for this study are described in Chapter 2.

3.2.1 Statistical Analysis

All statistical analyses were performed using RStudio version 4.1.2 using packages readr (Wickham et al., 2022), dplyr (Wickham et al., 2022), tidyverse (Wickham et al., 2019), stats (R Core Team, 2021). Results are reported as the mean ± standard deviation.

3.3 Results

3.3.1 Herd use of buoy across treatment weeks

Trial 1 cows used the buoy for a total of 776.31 minutes out of the 72 sampled hours (17.97% of the observed time). During the 24-hour period in week 1, the buoy was in use for 390.7 minutes (27.13% of observed time), this dropped to 216.39 minutes during week 2 (15.03% of observed time) and 169.22 minutes during week 3 (11.75% of observed time) (Figure 3.1 and 3.2). Herd physical interaction with the object decreased by 44.61% in week 2 of presentation compared to week 1. A lower reduction was observed in week 3 compared to week 2 of 21.8%. Out of the herd of

36 cows, 34 interacted with the buoy at least once throughout the 72 sampled hours (94.44% of the herd). During week 1, 32 cows interacted with the buoy (88.89% of herd), 30 cows during week 2 (85.71% of herd) and 29 cows (82.86% of herd) during week 3.

Trial 2 cows used the buoy for a total of 773.89 minutes out of the 72 sampled hours (17.91% of the observed time). During the 24-hour period in week 1, the buoy was in use for 433.7 minutes (30.11% of observed time), this dropped to 235.94 minutes during week 2 (16.38% of observed time) and 104.25 minutes during week 3 (7.24% of observed time) (Figure 3.1 and 3.2). Herd physical interaction with the object decreased by 45.6% in week 2 of presentation compared to week 1. Interaction decreased by 55.82% from week 2 to week 3. During week 1, 35 cows interacted with the buoy (94.59% of herd), during week 2 this was 33 cows (89.19% of herd) and during week 3 this was 30 cows (83.33% of herd). All cows interacted with the buoy at least once throughout the 72 sampled hours.

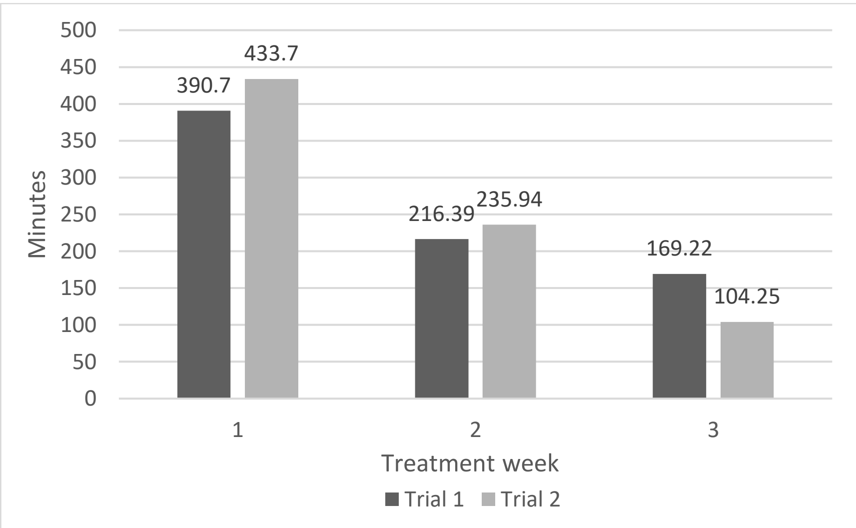


Figure 3.1. *Total minutes Trial 1 and Trial 2 cows spent in physical contact with the buoy, during the 24-hour period, each treatment week.*

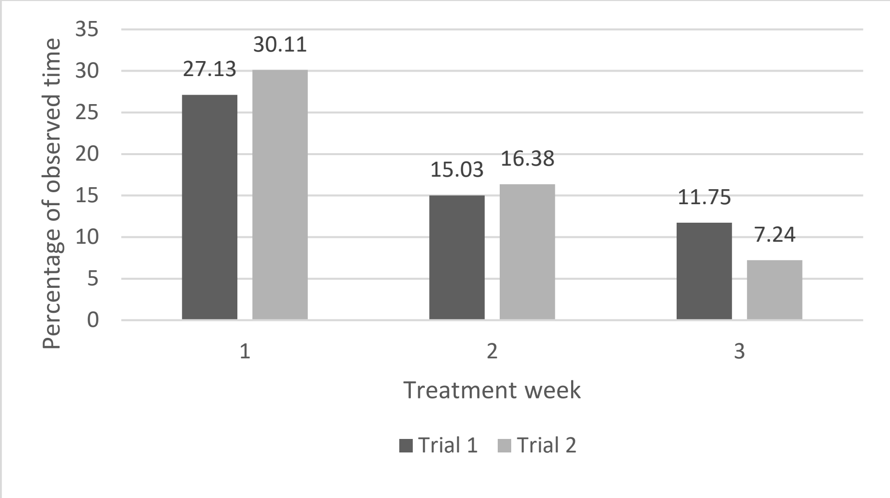


Figure 3.2. *Percentage of observable time the buoy was in use, during the 24-hour period, each treatment week (Trial 1 and Trial 2 cows).*

Trial 1 cows spent 10.85 ± 10.29 (range: 0 – 38.95) minutes per day interacting with the buoy during week 1; 6.18 ± 7.54 (range: 0 – 35.99) minutes per day during week 2 and 4.83 ± 4.97 (range: 0 – 20.57) minutes per day week 3. Trial 2 cows spent 11.72 ± 9.69 (range: 0 – 51.60) minutes per day interacting with the buoy during week 1; 6.38 ± 7.42 (range: 0 – 31.42) minutes per day during week 2 and 2.90 ± 4.31 (range: 0 – 24.59) minutes per day week 3 (Figure 3.3). Mean individual interaction time with the novel object decreased by 43.04% in week 2 compared to week 1 and 21.84% in week 3 compared to week 2 for trial 1 cows. For trial 1 cows, 75% of the cows that used the buoy during week 1 reduced the amount of time they used it during the second week with 68.8% reducing their use in the third week compared to the first week of presentation. For trial 2 cows, 80% of the cows that used the buoy during week 1 reduced the amount of time they used it during the second week, with 94.1% reducing their use in the third week compared to the first week of presentation. Both groups of cows spent significantly less time interacting with the buoy during the second and third week of presentation compared to the first week it was present ($P<0.05$).

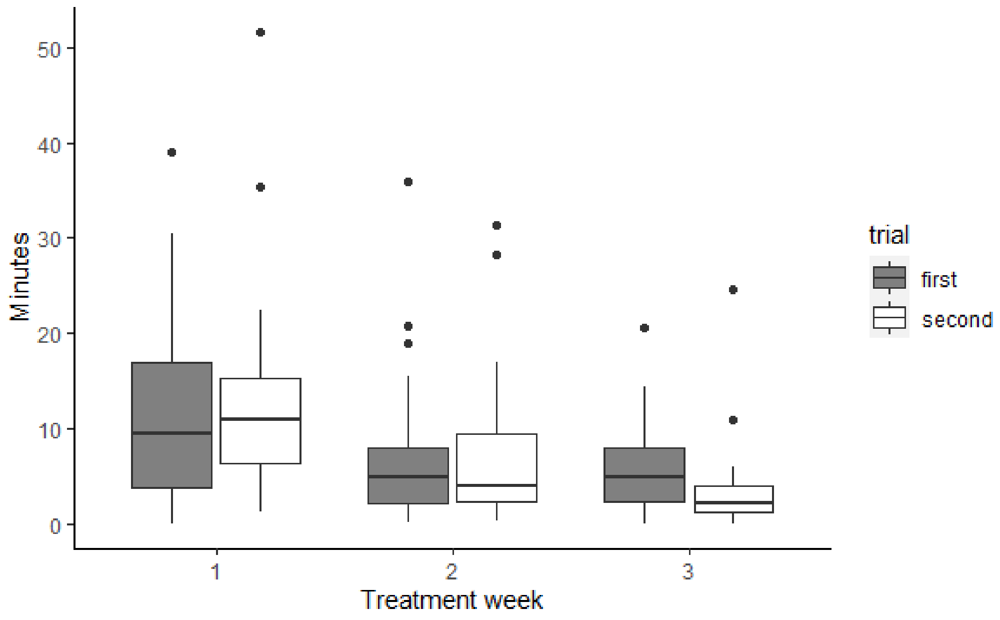


Figure 3.3. *Distribution of minutes Trial 1 and Trial 2 cows spent interacting with the novel object per day each week the object was present.*

Trial 1 cows made 735 and Trial 2 cows made 720 separate interactions with the novel object throughout the 72 samples hours across the three weeks the object was present (Figure 3.4).

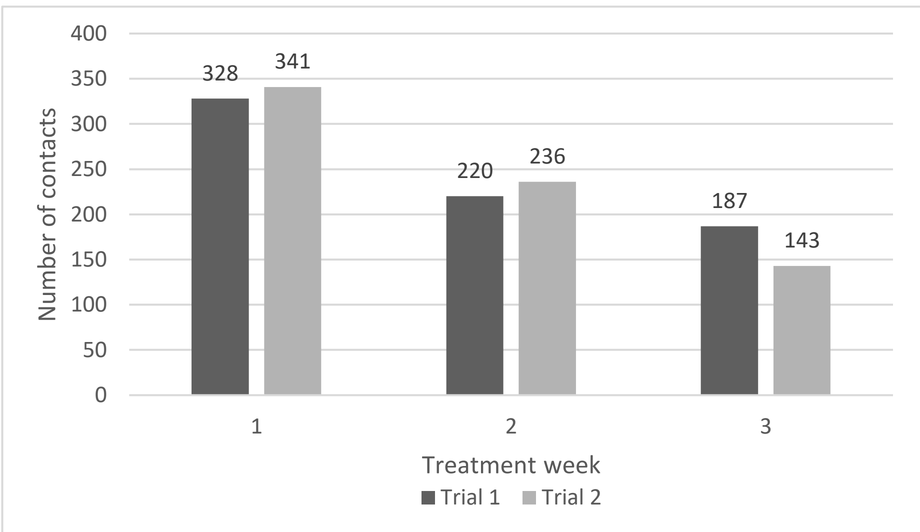


Figure 3.4. *Total physical contact interactions with the buoy per day each treatment week (Trial 1 and Trial 2 cows).*

Trial 1 cows made 9.11 ± 7.20 (range: 0 - 33) separate interactions with the buoy per day during week 1, 6.29 ± 5.34 (range: 0 - 22) interactions per day during week 2 and 5.34 ± 4.14 (range: 0 - 14) interactions per day during week 3. For trial 2 cows this was; 9.22 ± 6.33 (range: 0 - 27) interactions per day during week 1, 6.38 ± 4.95 (range: 0 - 17) interactions per day in week 2 and 3.97 ± 3.6 (range: 0 - 14) interactions per day in week 3 (Figure 3.5). Mean individual number of interactions with the novel object decreased by 30.96% per day in week 2 compared to week 1 and 15.1% per day in week 3 compared to week 2 for trial 1 cows. For trial 2 cows, mean individual number of interactions the novel object decreased by 30.8% per day in week 2 compared to week 1 and by 37.77% per day in week 3 compared to week 2. Both groups of cows made significantly fewer interactions with the buoy during week 2 and 3 compared to the first week it was present (P<0.05).

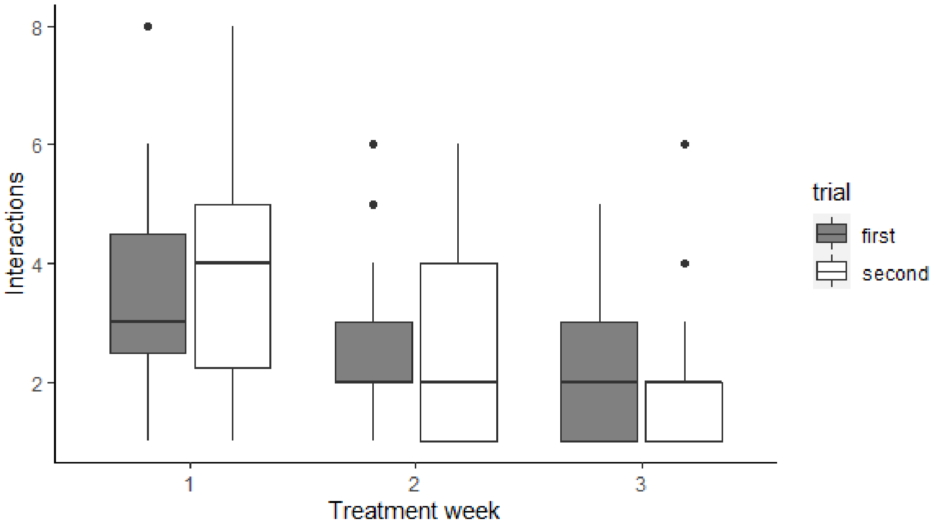


Figure 3.5. *Distribution of the number of physical interactions made by Trial 1 and Trial 2 cows with the buoy per day across treatment weeks.*

3.3.2 Time of day

Trial 1 cows interacted with the buoy throughout 63 of the 72 hours sampled. Hour slots when the buoy was not visited during week 1 were; 03:00-04:00 and 13:00-14:00 (Figure 3.6). During week 2 the buoy was not visited on 3 out of the 24 sampled hour slots: 02:00-03:00, 03:00-04:00, 05:00-06:00 (Figure 3.7). During week

3 the buoy was not visited during 4 of the 24 hour sampled hours: 01:00-02:00, 04:00-05:00, 13:00-14:00, 14:00-15:00 (Figure 3.8). Trial 2 cows interacted with the buoy throughout 70 of the 72 hours sampled. Hour slots when the buoy was not visited were 02:00-03:00 during week 1 (Figure 3.6) and 01:00-02:00 during week 3 (Figure 3.8).

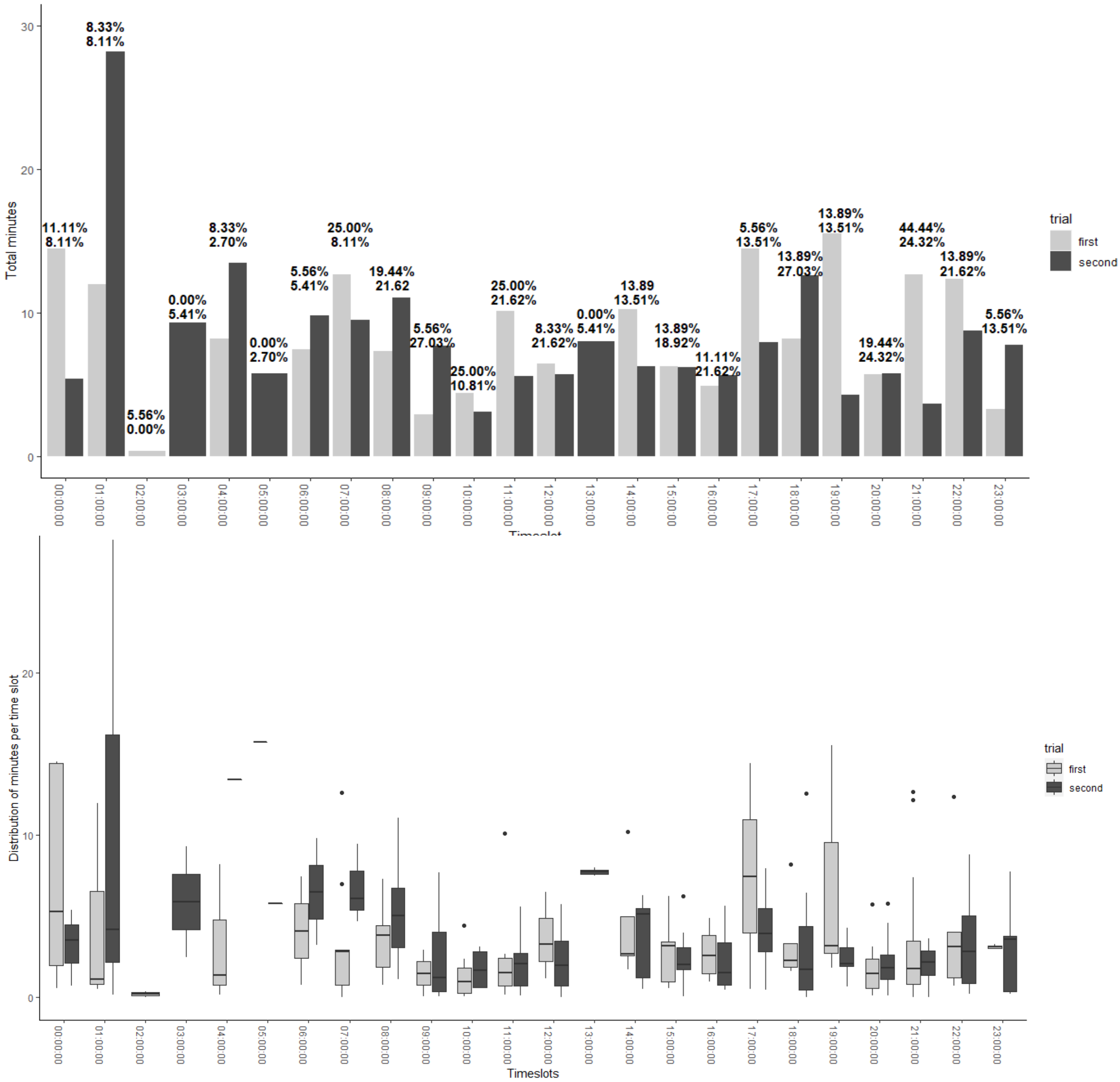


Figure 3.6. *Total minutes spent using the buoy, across the 24 hours sampled throughout week 1, including percentage of the group that used it within each hour slot (top) and the distribution of minutes cows spent using the buoy during each time slot in week 1 (bottom). The first percentage displayed for any associated time slot refers to the first trial and the second percentage refers to the second trial.*

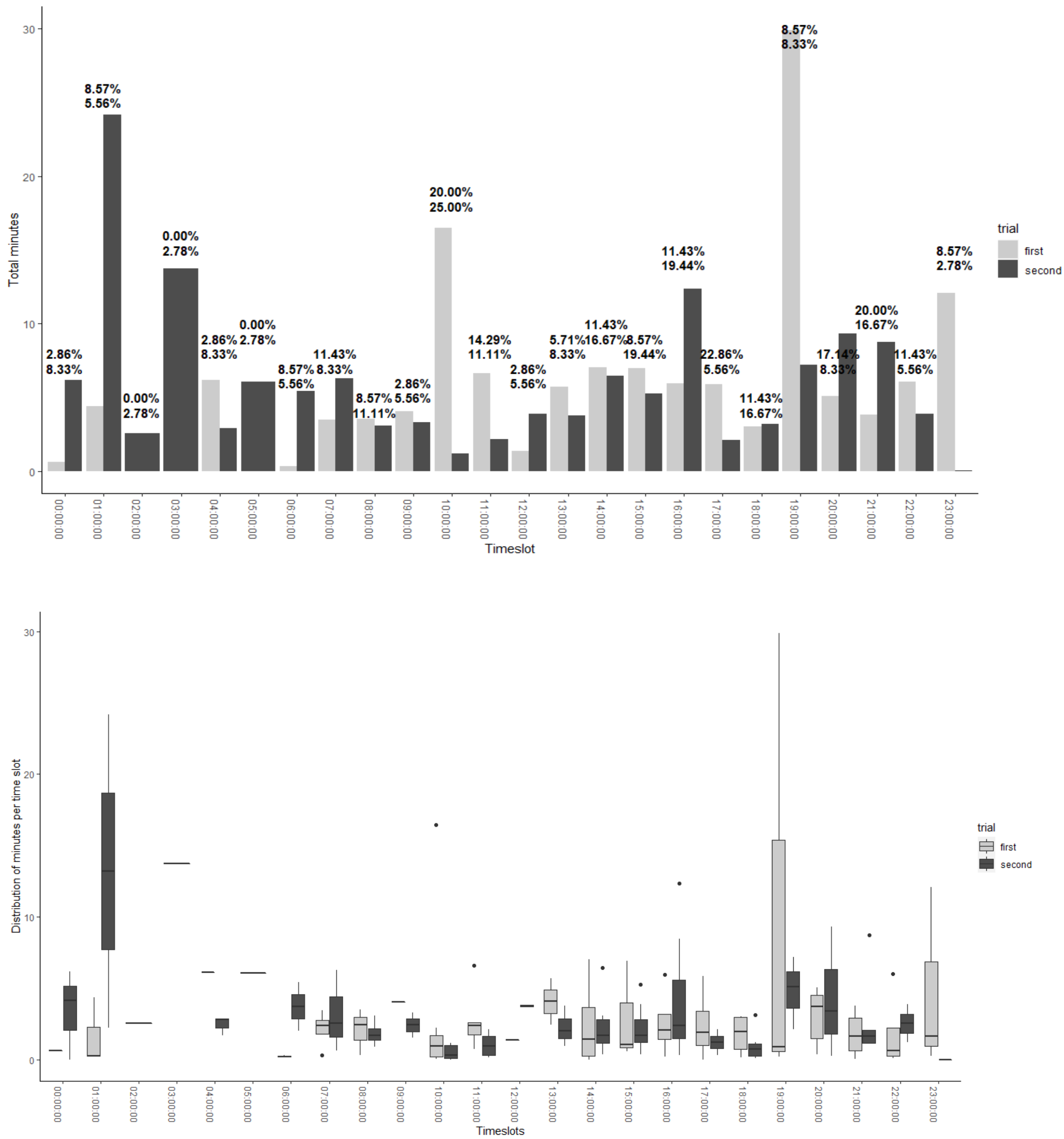


Figure 3.7. *Total minutes spent using the buoy, across the 24 hours sampled throughout week 2, including percentage of the group that used it within each hour slot (top) and the distribution of minutes cows spent using the buoy during each time slot in week 2 (bottom). The first percentage displayed for any associated time slot refers to the first trial and the second percentage refers to the second trial.*

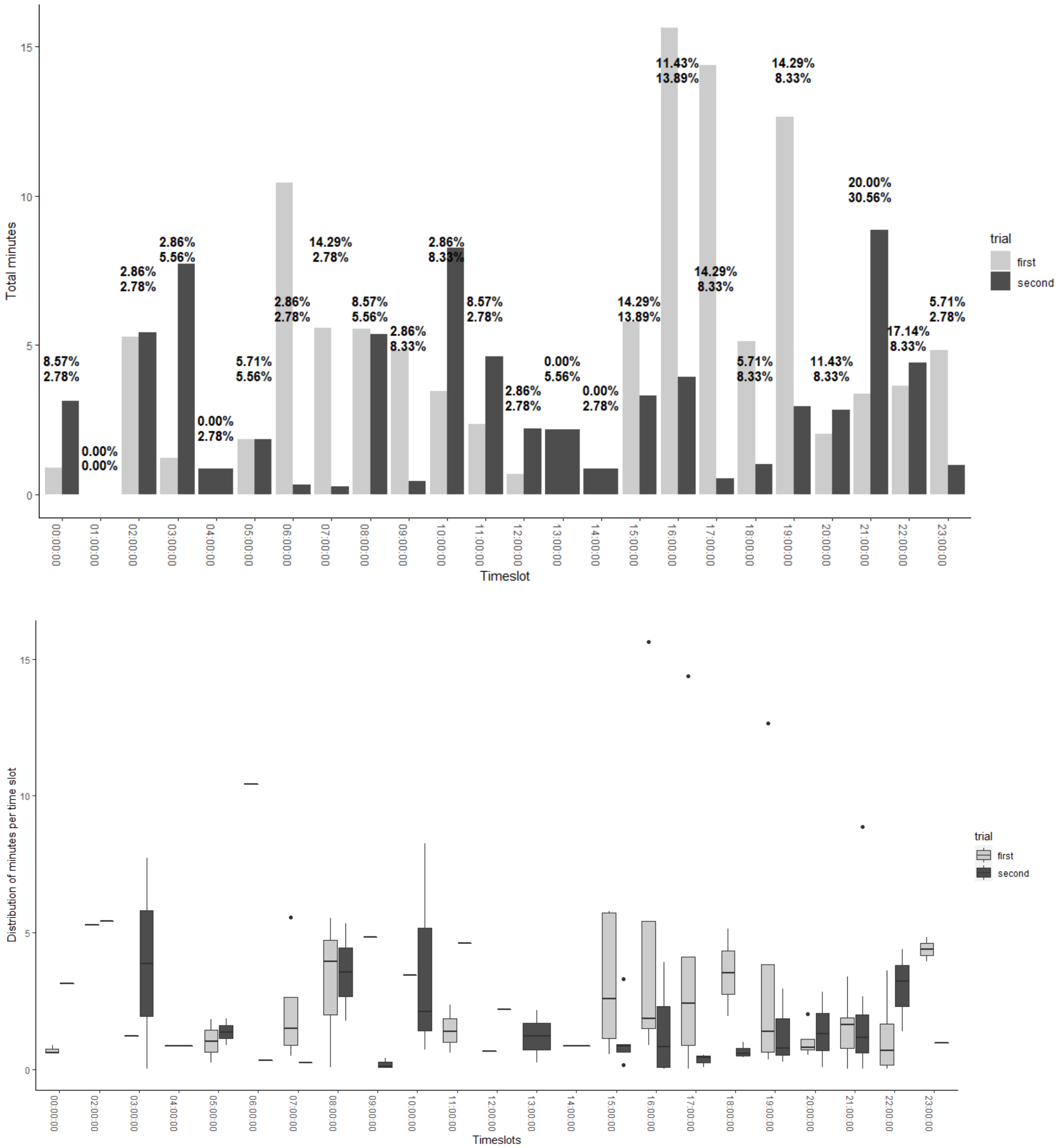


Figure 3.8. *Total minutes spent using the buoy, across the 24 hours sampled throughout week 3, including percentage of the group that used it within each hour slot (top) and the distribution of minutes cows spent using the buoy during each time slot in week 3 (bottom). The first percentage displayed for any associated time slot refers to the first trial and the second percentage refers to the second trial.*

Across the entire study period, the largest number of different cows used the novel object between 21:00-22:00, with the least number of cows using it between 03:00-04:00 (Trial 1 cows) and 02:00-03:00 (Trial 2 cows) (Figure 3.9).

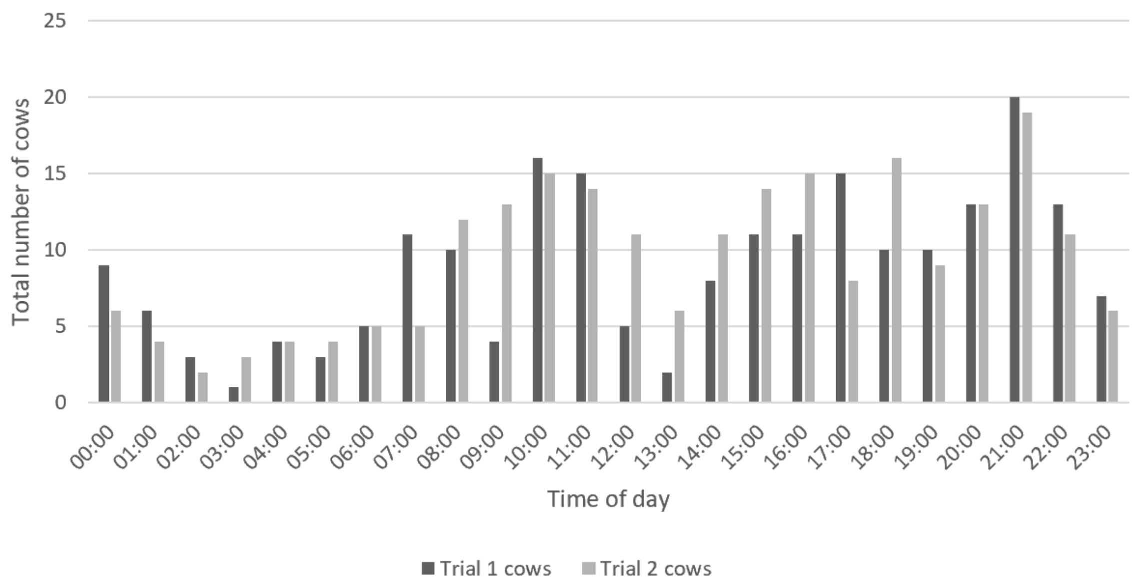


Figure 3.9. *Total number of different Trial 1 and Trial 2 cows interacting with the buoy throughout different time slots of the day across the complete study period.*

Trial 1 cows spent 6.16 ± 8.78 minutes (range: 0.25 – 30.31) using the buoy between the hours of 00:00 – 06:00 (am1), 7.08 ± 6.34 minutes (range: 0.07 – 20.55) between 06:00 – 12:00 (am2), 6.80 ± 5.42 minutes (range: 0.43 – 20.69) between 12:00 – 18:00 (pm1) and 9.32 ± 8.19 minutes (range: 0.27 – 31.85) between 18:00 – 00:00 (pm2) (Figure 3.10). Trial 2 cows spent 11.41 ± 10.69 (range: 0.73 – 32.64) minutes using the buoy during am1, 5.84 ± 5.90 minutes (range: 0.07 – 28.18) during 'am2', 6.04 ± 4.03 minutes (range: 0.25 – 14.29) during 'am2' and 6.52 ± 6.05 minutes (range: 0.15 – 25.17) during 'am4' (Figure 3.10).

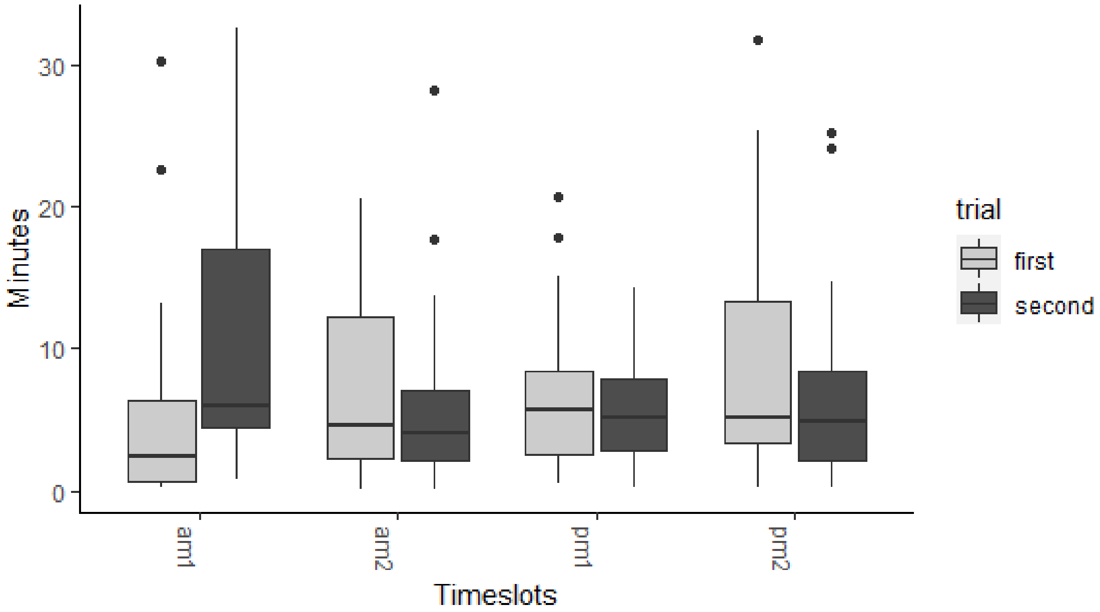


Figure 3.10. *Distribution of total minutes Trial 1 and Trial 2 cows spent using the buoy during different periods of the day (am1: 00:00 – 06:00, am2: 06:00 – 12:00, pm1: 12:00 – 18:00, pm2: 18:00 – 00:00) across the entire study period.*

3.3.3 Individual cow variation in buoy use and herd level characteristics

There was a large amount of individual variation in how much cows used the buoy, which was reflected across both groups (Figure 3.11). Trial 1 cows spent a mean of 22.61 ± 15.82 minutes (range: 0.27 - 66.12) using the buoy in total per cow, across the study period (intervention weeks 1-3). This was 21.26 ± 16.16 minutes for trial 2 cows (range: 1.57 - 83.24). Trial 1 cows had a mean individual bout interaction time of 3.0 ± 3.74 minutes (range: 0.01 – 29.88) using the buoy, across the study period (intervention weeks 1-3). This was 2.84 ± 3.74 minutes for trial 2 cows (range: 0.02 – 28.33) (Figure 3.11). Trial 1 cows made a mean of 20.74 ± 13.45 separate individual contacts with the buoy (range: 0 - 60) during intervention weeks 1-3. For trial 2 cows, this was 19.78 ± 11.5 (range: 3 - 47). Trial 1 cows visited the buoy on 6.8 ± 3.68 hour slots out of the 72 sampled (range: 0 - 18). Trial 2 cows visited the buoy on 7.58 ± 3.61 hour slots out of the 72 sampled (range: 1 - 15) (Figure 3.12).

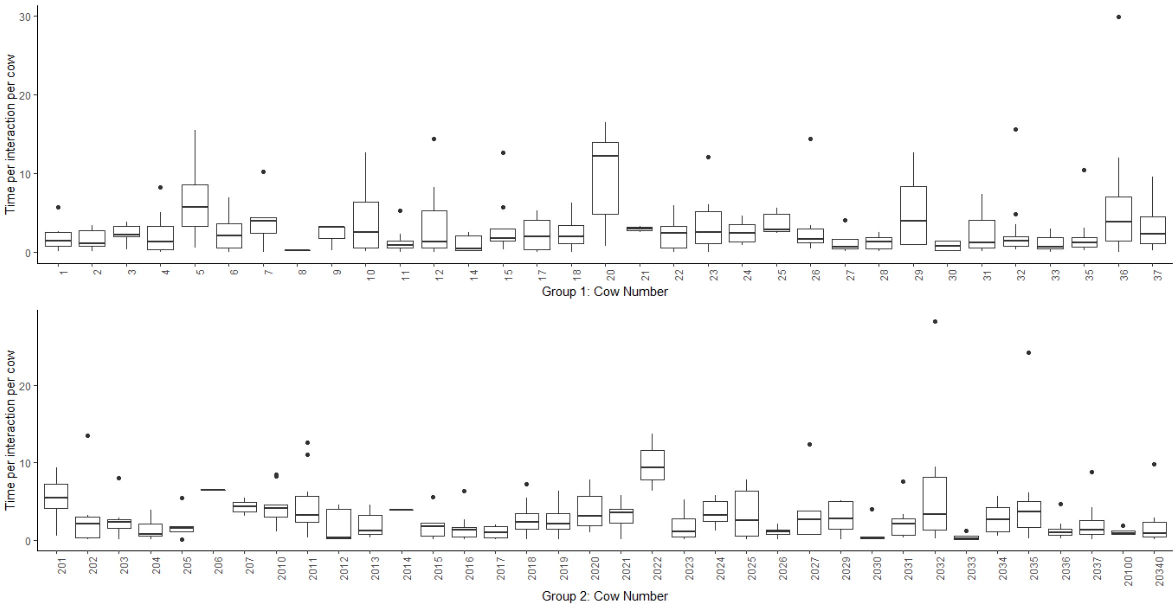


Figure 3.11. *Distribution of the time per interaction per cow with the buoy for Trial 1 and Trial 2, across study period (intervention weeks 1-3).*

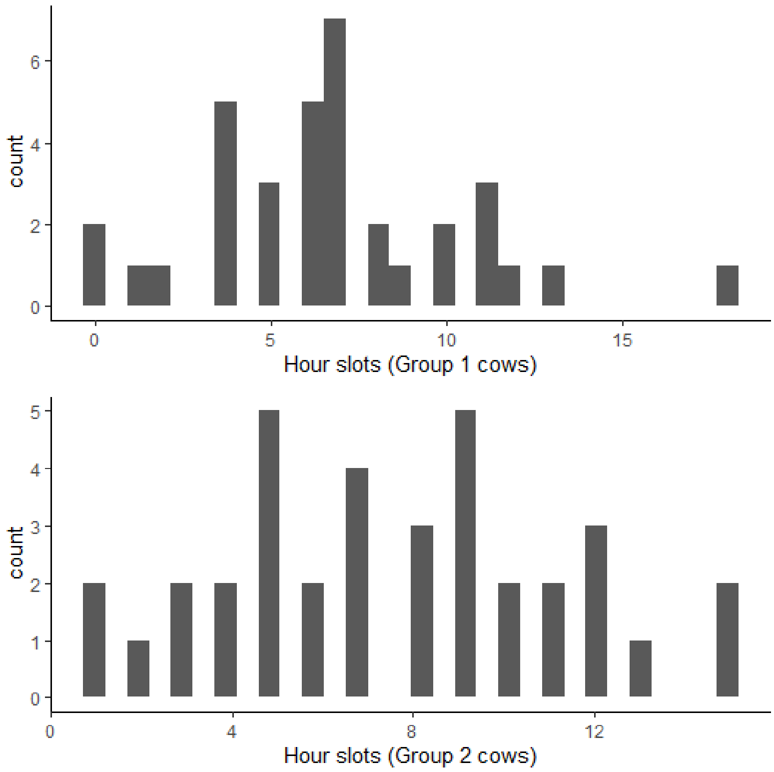


Figure 3.12. *Histogram illustrating the distribution frequency of the total number of hour slots the novel object was visited by Trial 1 and Trial 2 cows.*

For Trial 1 cows, parity 3 cows spent the most time interacting with the novel object across the treatment period (intervention weeks 1-3); 27.62 ± 21.97 minutes (range: 0

- 66.12) (Figure 3.13). This was followed by cows in their second lactation 24.84 ± 15.25 minutes (range: 0.27 - 58.63) and then cows in their first lactation 22.79 ± 11.58 (range: 5.38 - 39.36). For Trial 2 cows, cows in their second lactation, spent the most time interacting with the novel object; 22.91 ± 20.37 (range: 1.57 - 83.24). This was followed by cows in their first lactation 22.5 ± 14.18 (range: 4.01 - 47.25) and then cows in their third lactation 18.91 ± 5.68 (range: 12.95 - 29.3) (Figure 3.13). Cows within their fourth parity or higher, spent the least time interacting with the novel object in both study groups (trial 1 cows: 11.94 ± 10.36 minutes (range: 0 - 30.31); trial 2 cows, 15.94 ± 12.32 minutes (range: 3.9 - 41.17) (Figure 3.13).

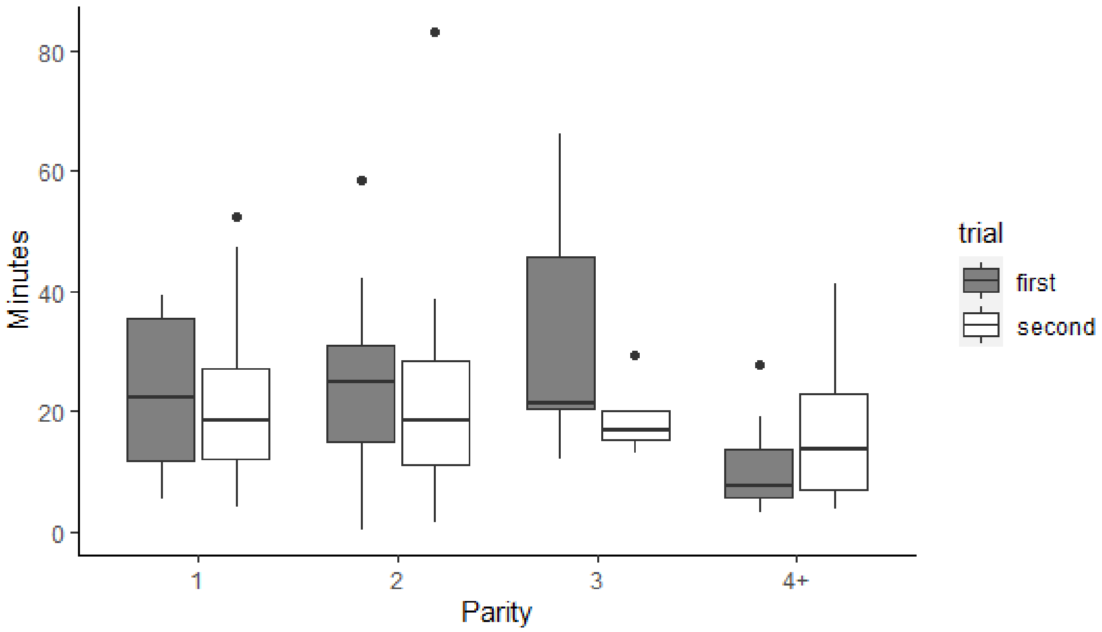


Figure 3.13. *Distribution of the total minutes spent using the buoy across the study period (intervention weeks 1-3) for Trial 1 and Trial 2 cows by parity.*

For trial 1 cows, cows within their second lactation made the most separate interactions with the novel object, mean = 25.08 ± 14.42 (range: 2 - 60) (Figure 3.14). For trial 2 cows, cows in their first lactation made the most separate interactions with the novel object, 23.58 ± 11.72 (range: 11 - 44) (Figure 3.14). Parity 4 or above cows made the least separate interactions with the novel object in both groups (trial 1 cows, 13.67 ± 8.79 (range: 0 - 27); trial 2 cows, 12.86 ± 9.05 (range: 3 - 26) (Figure 3.14).

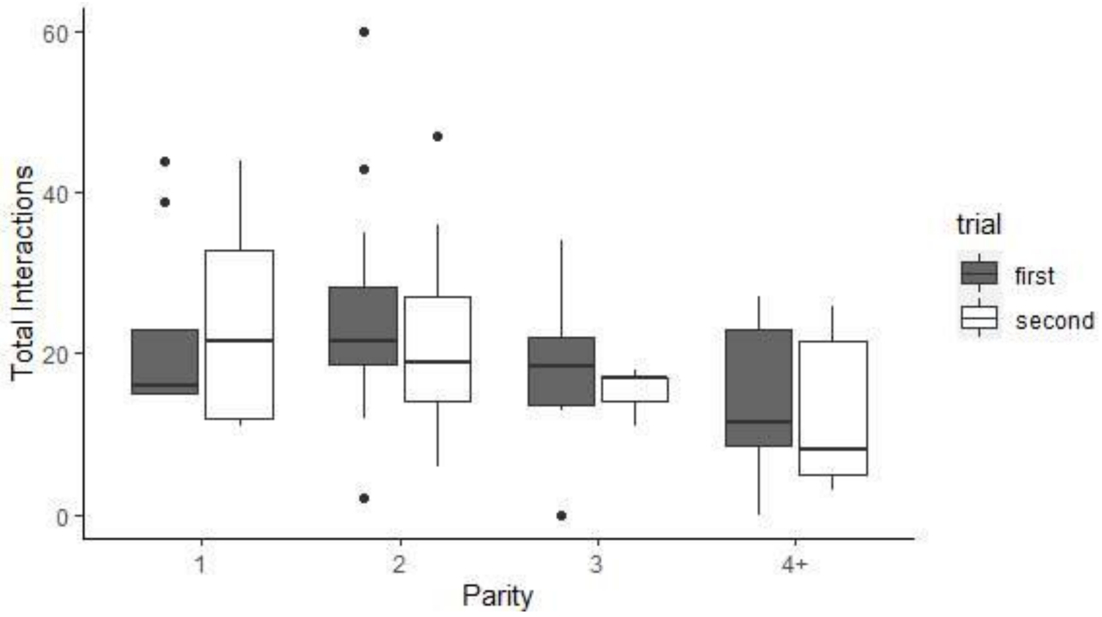


Figure 3.14. *Distribution of the total number of separate interactions with the buoy across the study period (intervention weeks 1-3) for Trial 1 and Trial 2 cows by parity.*

Cows in late lactation (200-300 days in milk) spent the least time using the buoy in both trials, compared to cows in early (0 – 100 days) to mid (100 – 200 days) lactation during the study period (intervention weeks 1-3) (Figure 3.15). Trial 1 cows in early lactation spent 37.31 ± 20.12 (range: 11.58 – 66.12) minutes using the buoy throughout the study period, compared to 22.53 ± 14.14 (range: 0.27 – 58.63) for cows in their mid-lactation and 15.38 ± 12.43 (range: 3.12 – 36.40) for cows later in lactation. This pattern was reflected for trial 2 cows. Trial 2 cows in early lactation spent 18.57 ± 12.16 (range: 5.36 – 29.30) minutes using the buoy throughout the study period (intervention weeks 1-3), compared to 23.02 ± 17.00 (range: 3.90 – 83.24) for cows in their mid-lactation and 10.58 ± 7.98 (range: 1.57 – 18.89) for cows later in lactation (Figure 3.15).

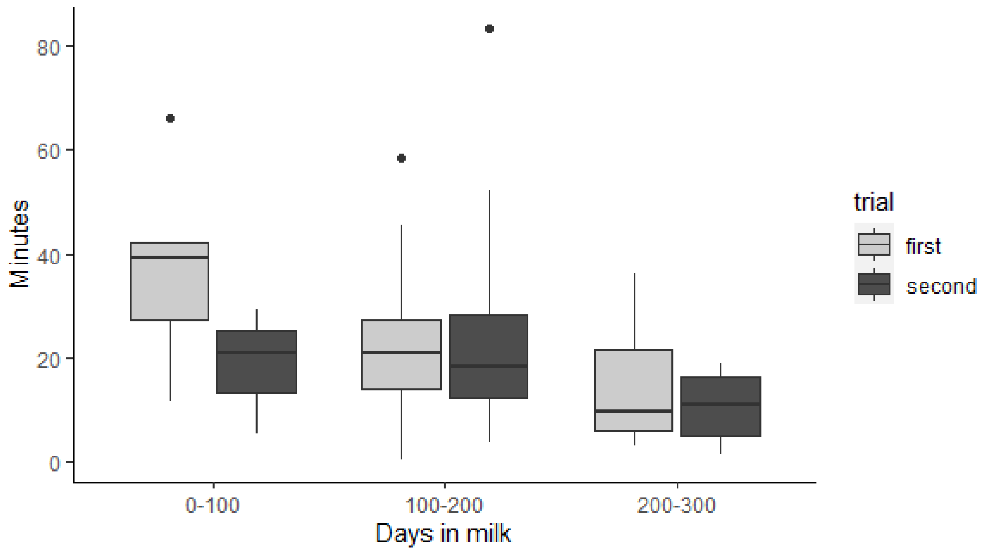


Figure 3.15. *Distribution of the total minutes Trial 1 and Trial 2 cows from different stages of lactation spent using the buoy across the study period (intervention weeks 1-3).*

Similarly, trial 1 cows in early lactation made more separate interactions with the buoy than cows in their mid or late lactation (Figure 3.16). Trial 2 cows in mid lactation made the most separate total interactions during the study period (intervention weeks 1-3), followed by cows in early lactation and then cows in late lactation (Figure 3.16).

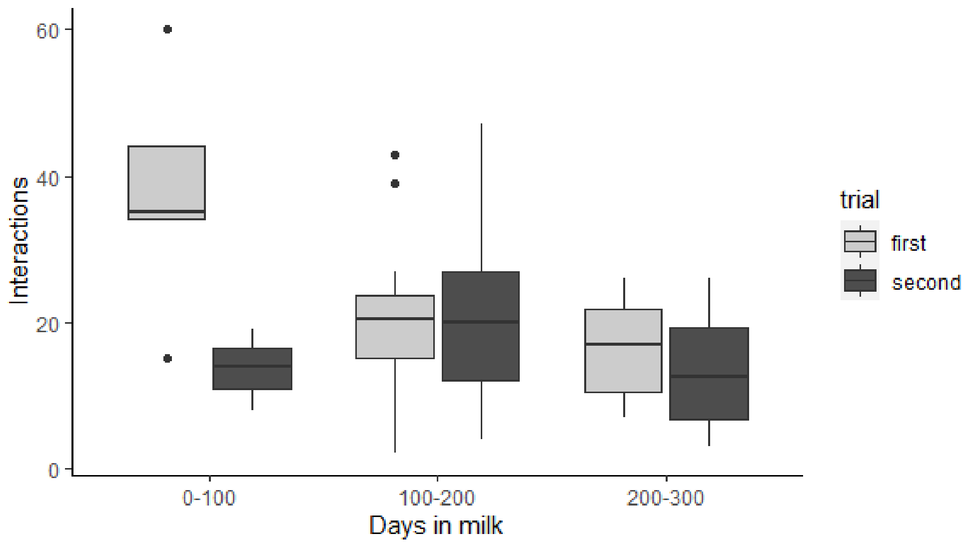


Figure 3.16. *Distribution of the total separate interactions Trial 1 and Trial 2 cows from different stages of lactation spent using the buoy across the study period (intervention weeks 1-3).*

Figure 3.17 illustrates the relationship between individual cows' days in milk with both the total minutes they spent using the buoy and the total separate interactions they made with it across the study period (intervention weeks 1-3). No relationship was observed between days in milk and either the total time cows spent using enrichment or the total separate interactions they made with it.

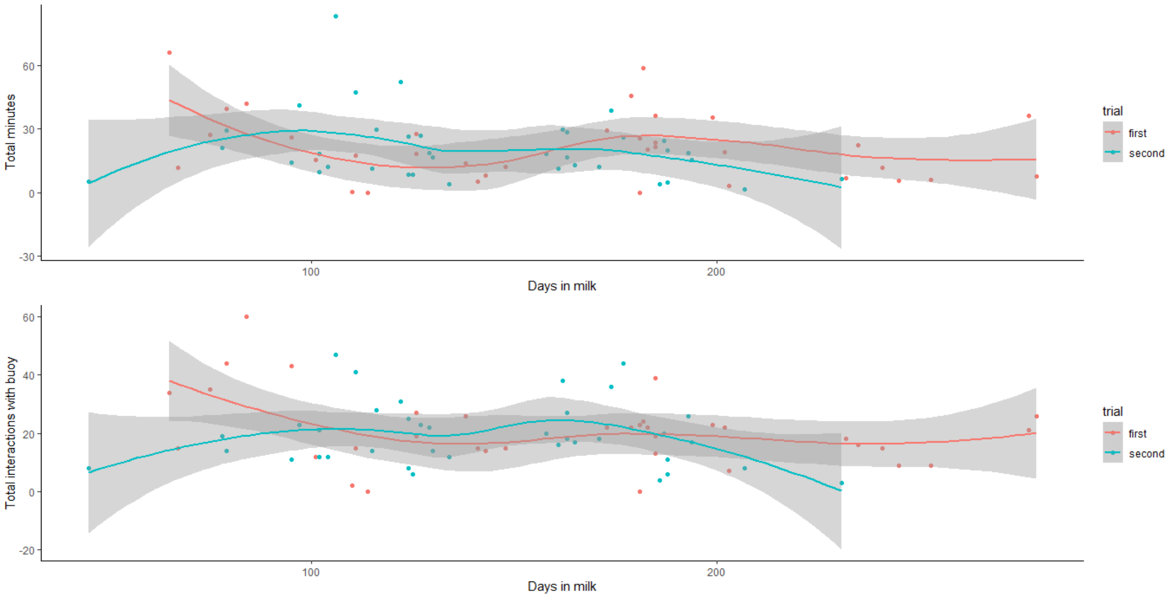


Figure 3.17. *Individual cows' days in milk at the start of the trial compared to their total minutes spent interacting with the buoy and total separate interactions with it across the study period (intervention weeks 1-3).*

3.3.4 Cows' initial response to novel object

For group 1, 34/36 (94.44%) of cows approached the novel object within the first 24 hours it was present. For group 2, this was 35/37 (94.59%) of cows. Trial 1 cows, spent 4.54 ± 4.82 (range: 0.07 - 18.27) minutes interacting with the buoy (within the 2m marked out approach line) on their first interaction with the buoy. For trial 2 cows this was 5.34 ± 4.03 (range: 0.07 - 16.72). The most frequently expressed behaviours during cows' first approach to the novel object for both trial 1 and trial 2 cows are illustrated in Figure 3.18. A cows first approach was defined as the first occasion when a cow put one hoof over the 2m marked out approach line surrounding the novel object, following the novel object being placed within the barn. The most frequently expressed behaviours for trial 1 cows were ‘tail wagging’ which occurred 5.65 ± 1.35 (range: 0 - 36) times during the first approach period, followed by ‘head and nose contact’ which occurred 5.54 ± 0.91 (range: 0 - 21) times (Figure 3.19). For Trial 2 cows, the most frequently expressed behaviours during first approach to the novel object were ‘head and nose contact’, occurring 9 ± 1.39 (range: 0 - 38) times, followed by ‘stationary attentive’ occurring 4.97 ± 0.71 (range: 0 - 18) (Figure 3.18) times. The least frequently expressed behaviours for trial 1 cows were ‘urination/defecation’, which did not occur at all, ‘submissive posture’ 0.03 ± 0.03 (range: 0 – 1) and ‘allogrooming’ 0.03 ± 0.03 (range 0 – 1). The least frequently expressed behaviours for trial 2 cows during the initial approach period were ‘urination/defecation’ 0.05 ± 0.32 (range: 0 - 2) and being ‘startled’ 0.08 ± 0.36 (range: 0 - 3) (Figure 3.18).

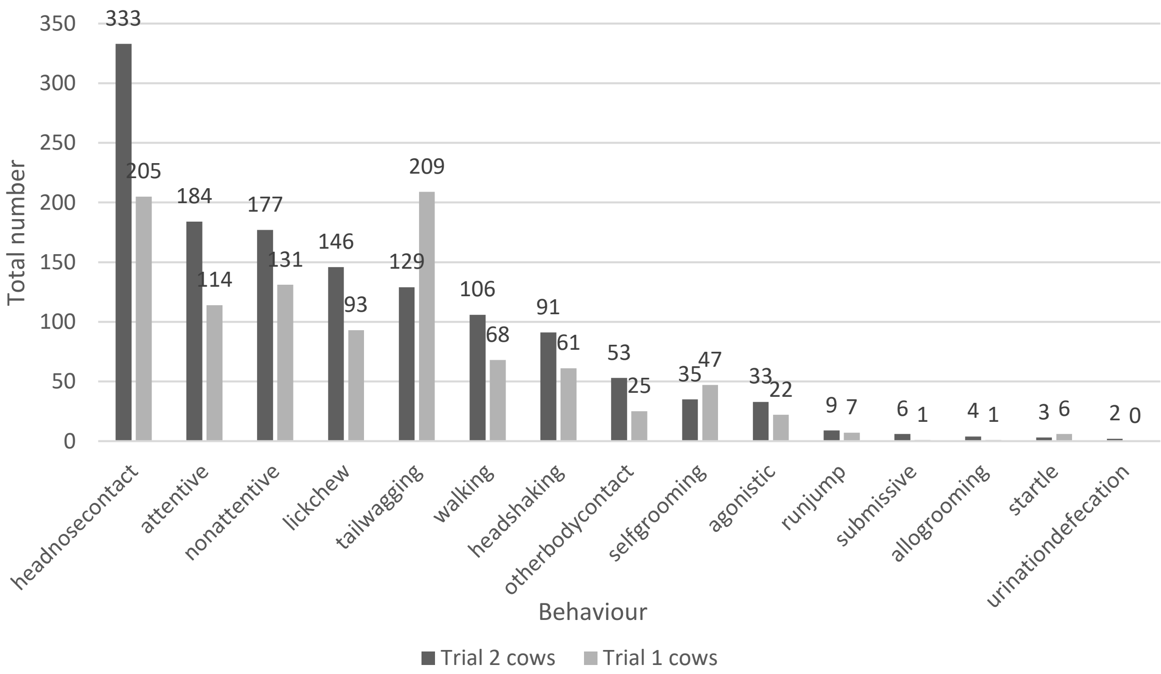


Figure 3.18. *Total number of ethogram behaviours observed in all cows during initial response to the novel object (Trial 1 and 2 cows).*

Behaviours that were expressed for the longest total duration in initial response to the novel object for both groups of cows were ‘head and nose contact’ 2.2 ± 0.07

(range: 0 - 7.72) minutes (trial 1 cows), 3.03 ± 0.4 (range: 0 - 7.57) minutes (trial 2 cows) and ‘non attentive’ 0.88 minutes ± 0.26 (range: 0 - 8.3) (trial 1 cows), 0.86 minutes ± 0.2 (range: 0 - 5.48) (trial 2 cows) (Figure 3.19). Behaviours expressed for the lowest duration during initial response to the novel object for both groups of cows were ‘submissive’ 0.001 ± 0.001 minutes (range: 0-0.05) (trial 1 cows), 0.01 ± 0.002 minutes (range: 0 - 0.07) (trial 2 cows) and ‘run and jump’ 0.01 ± 0.01 minutes (range: 0-0.15) (trial 1 cows), 0.01 ± 0.03 minutes (range: 0 - 0.18) (trial 2 cows) (Figure 3.19).

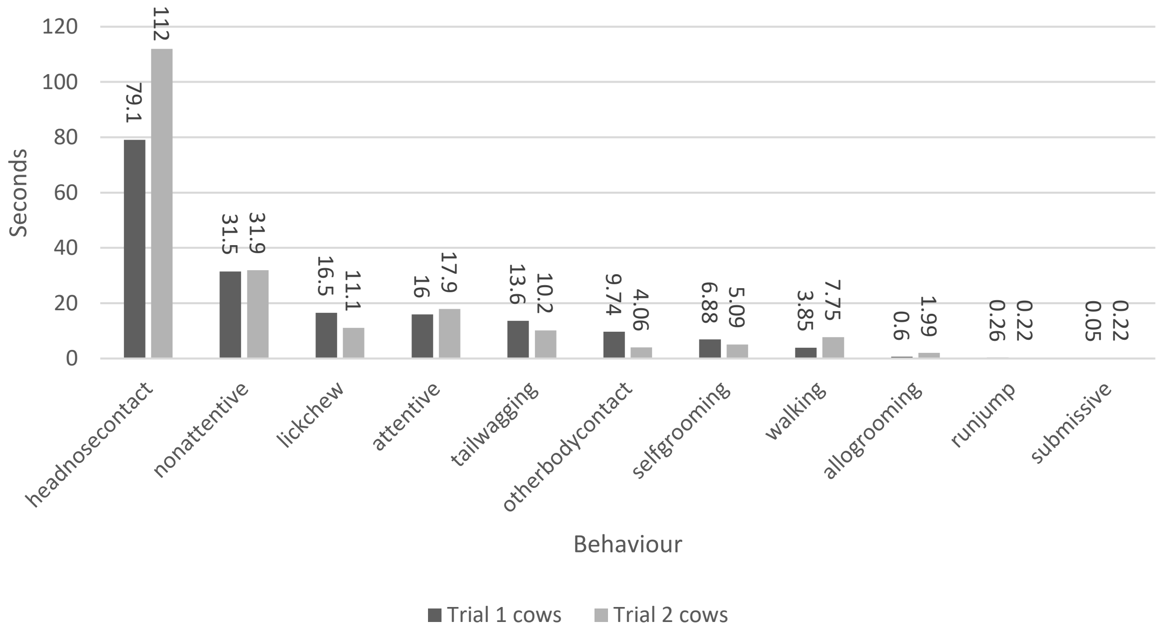


Figure 3.19. *Total duration of behaviours observed in all cows during initial response to novel object (Trial 1 and 2 cows).*

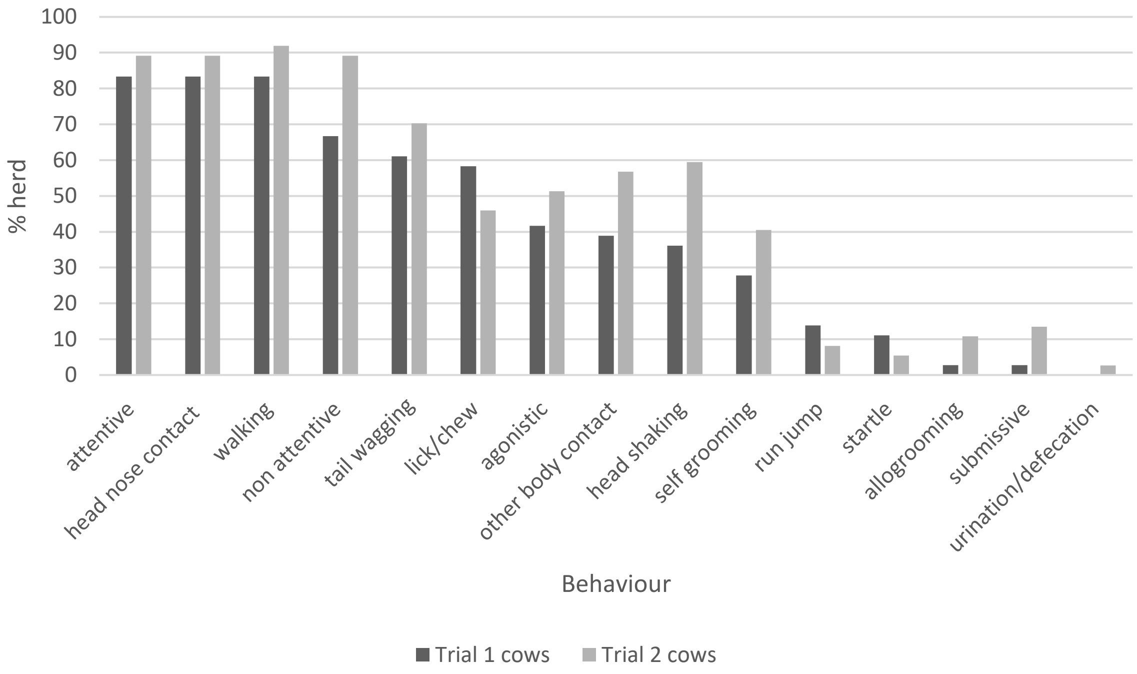


Figure 3.20. *Percentage of the group which displayed each ethogram behaviour during the first approach period (Trial 1 and 2 cows).*

The behaviours exhibited by the largest percentage of the herd for trial 1 cows were equally; ‘attentive’, ‘head and nose contact’, and ‘walking’, followed by ‘non attentive’. The behaviour exhibited by the largest percentage of the herd for trial 2 cows was ‘walking’, followed by ‘attentive’, ‘head and nose contact’ and ‘non attentive’ equally (Figure 3.20).

3.4 Discussion

3.4.1 Herd level use of the novel object

When provided with 24-hour free access to a novel object, trial 1 cows spent on average, 8.52 minutes per day interacting with it (trial 2 cows - 9.02), across the first two weeks it was present. This level of interaction is similar, yet slightly higher, to the 6.76 minutes per day that cows have been shown to use a mechanical brush for the first 2 weeks post installation (DeVries at al., 2007). The buoy used in this study was completely novel to the cows but DeVries et al. (2007) did not describe whether the cows used in their study had any prior experience of a mechanical brush. Although there is no quantifiable data available on how widely automatic brushes have been implemented on dairy farms within the UK, an increasing number of dairy farms now use them (Mandel et al., 2013), with several companies now manufacturing and selling them. A study evaluating management factors on 179 Dutch dairy farms found 74% of farms used some form of brush (DeVries et al., 2015). Brushes are widely regarded as beneficial to cow welfare, have potential use as a welfare indicator (Mandel et al., 2013) and using them is likely a positive behavioural activity in itself (McConnachie et al., 2018). McConnachie et al. (2018) showed that cows will work as hard to access an automatic brush as they will to access fresh feed, showing that this is a highly valued resource to them. In addition to the amount of time that cows spent using the novel object, the object provoked a surprisingly high level of interest in both trial groups of cows, which was sustained throughout the study, with 82.86% of trial 1 cows and 83.33% of trial 2 cows still using it by the third week. The novel object used in the current study, provided no species-specific behavioural outlet for behaviour, however its use was aimed to provide environmental diversity and an opportunity for exploration and interest. The combination of the amount of time cows spent using this novel resource, particularly when compared to an already industry wide established housing resource of a brush, combined with the high uptake by the herd, infers that this was a valued and positive behavioural outlet. These results show that offering opportunities for housed dairy cows' curiosity, interest and exploration may be a beneficial management strategy and worthy of further research. Understanding the underlying behavioural motivation for the high level of interest and use of the novel object, is beyond the scope of the current study, however some possible explanations could be that it provided a time filling activity, preferential to monotony, a segregation of the housing into another functional area, which may provide cows more control over avoiding certain conspecifics, as a distraction to the stressors of the housed environment or that the enjoyable experience of interacting with it in itself was a form of play.

The results from the current study showed that interaction with the buoy decreased across the 3 weeks it was present for both groups of cows, with trial 1 cows using it for only 43.3%, and trial 2 cows 24%, of the time that they used it during the original week it was present. This decrease in use of an environmental object is consistent with other research that has evaluated potential enrichment devices for cattle. Wilson et al. (2002) evaluated the use of two different scent diffusers and a stationary and mobile scratching device for feedlot cattle and found a decrease in use in the stationary scratching device, however this stabilised over time. There was a decline in time spent interacting with all of the scent devices as days progressed throughout the study. There were fluctuations in use of the movable scratching device, however, there was no difference in use of this resource at the end of the study, at day 22, compared to the second day after installation. Ninomiya and Sato (2009) found the average time that calves used a brush to decrease on day 51 compared to day 3 of the study. Ishiwata et al. (2006) provided fattening beef cattle with hay filled drum cans and found use to peak between months 1-3 of provision with a decline in month 4, however they didn’t describe whether the drums were continuously filled with hay 24 hours a day, or whether the cows were consuming the hay, which would have been influenced by season and weather. The current study evaluated cows’ level of use of a novel object, during the course of three weeks. To identify whether decline in use would eventually stabilise to a constant level, a more long-term study would be required. A decline in interaction with the object was expected, this could be explained by a short-term novelty effect of the object, prior to habituation (Dissegna et al., 2021).

An element of social competition could have played a factor in how much the cows in this study used the novel object, as cattle are known to compete for resources using aggressive behaviour (Val-Laillet et al., 2008). Housed cows will avoid more dominant cows or certain areas of the building (Miller & Wood-Gush, 1991), to avoid conflict and will trade-off access to high quality food, when given the choice between eating high quality food next to a dominant cow, compared to low quality food on their own (Rioja-Lang et al., 2009). More dominant ranking cows have been shown to use a brush more often and especially at peak feeding times (Foris et al., 2021). Stanford et al. (2009) provided feedlot cattle with ropes, predominantly for monitoring zoonotic bacteria and found that putting two ropes in a pen instead of one, increased use only in the first hour of provision and only by an average of one contact per animal. However, a similar study by Irwin et al. (2002), found a significantly greater percentage of cattle to interact with ropes in pens where 7 were provided instead of 3. Scott et al. (2007) investigated the ratio of pigs to enrichment and found no differences in object use between pens containing four enrichment objects as opposed to one. Larson et al. (2019) however found that providing pens of pigs with four instead of two wooden beams (a common source of enrichment for pigs), not only increased the frequency of bouts of interaction but also the duration of manipulation. The study was assessing pig to enrichment ratios stemming from the Danish implementation of the EU Directive 2008/120/EC, which specifies, that at least two beams of wood should be provided to pens of 10-18 finisher pigs. The differing results from the studies done in pigs by Scott et al. (2007) and Larson et al. (2019) likely reflects that the importance of the animal/enrichment ratio may be sensitive to how valued the resource is to the animals. Given that housed dairy cows have been shown to avoid more dominant individuals/areas of the building (Miller & Wood-Gush, 1991), it seems likely that social competition would play a role in access to any new resources. Therefore, varying availability, quantity and location of enrichment resources would be a valuable research question and may provide more accurate knowledge on how much dairy cows would utilise enrichment resources when unhindered by social dynamics.

3.4.2 Individual cow variation in buoy use and herd level characteristics

The current study observed a high level of variability between individual cows in how much time they spent interacting with the novel object. There are several possible explanations for this. Firstly, it's likely that personality differences played a role, as dairy cows show individual behavioural consistency, which is explained by underlying personality structures (Schrader & Müller, 2005). Two commonly evaluated personality traits in farm animals are 'boldness' and 'exploration' (Neave et al., 2020) which refer to risk taking in response to novelty and the latter, acquisition of environmental information. It is therefore likely, that individual cows varying in these key personality traits, will show altered responses to the addition of the novel object in the current study. It could be the case that individuals with greater personality tendencies for 'boldness' or 'exploration' to be the individuals that utilised the novel resource more, yet that was not evaluated in the current study. It may therefore be congruent for individuals of certain personality types that show more interest in enrichment strategies to benefit from them more, which may mean that enrichment strategies could be tailored to specific groups of animals, or personality types, if possible to identify them, where this may provide the most benefit. However, this is unlikely to be a feasible management strategy for dairy cows where social groups are already tightly controlled for production and management purposes. Similarly, personality traits may play a role in the level of interest specific environmental resources evoke, and therefore use of a different novel object may have attracted different levels of interest between individuals. Gibbons et al. (2009) evaluated the consistency of individual dairy cows' responses to a selection of human approach and novel object tests and found that some consistency was shown in individual behavioural responses to human approach tests over time, but that consistency was not shown between cows' responses to varying novel objects and their human approach scores, with individual behavioural reactions varying widely to all situations. Despite the variability in time spent using the novel resource between individual cows in the current study, the novel object elicited a substantial level of herd interest which persisted across the three weeks the groups of cows had access to the object, reflected with over 82% of both groups still using it by week 3. This high level of use was identified through one continuously monitored 24-hour sample throughout each week, providing a short snapshot of this behaviour over an entire week, likely presenting a smaller subset of information than the full picture of individual cows making use of it if continuous monitoring was available.

Another explanation for the individual variation in use of the buoy could be due to underlying health factors that we were not aware of. Subclinical disease is known to impact different areas of dairy cow behaviour such as feeding (Dollinger & Kaufmann, 2013), lying (Kaufman et al., 2016) and activity (Rutherford et al., 2016). Caplen and Held (2021) investigated the impact subclinical mastitis had on multiple behavioural indicators, including activity and 'luxury' behaviours, such as grooming and exploratory behaviours. Cows with subclinical mastitis showed reductions in activity, including distance moved, social exploration and grooming. Luxury behaviours are generally categorised as behaviours that have longer term survival benefits, compared to allowing immediate health needs to be met (Dawkins, 1990), which means they tend to decrease first in times of physiological energy challenge (Weary et al., 2009). Interacting with enrichment can be seen as a luxury behaviour, meeting no immediate health needs and therefore this is likely another behaviour to be one of the first to decrease in response to health challenge. Similarly, for the current study, only one novel object was added to the home pen, situated at the far end of the building, opposite to the Lely robot. This may have represented an additional barrier for use to cows showing reduced energy levels associated with subclinical disease. More widely, cows suffering with any health implications, particularly associated with lameness, may have had to trade off the benefits of moving to and using enrichment over other more immediate needs such as accessing feed, water and lying spaces, as lame cows show reduced activity through steps taken (Reader et al., 2011). Mandel et al. (2013) found brush use to be significantly reduced when the brush was located distantly from the feed bunk compared to when it was positioned next to it. Therefore, lower use by certain individuals may not have been due to a decreased behavioural motivation to use the object but due to competing behavioural priorities. To account for this, enrichment resources should be positioned in variable locations throughout the building to increase accessibility of use to all individuals.

As already discussed, social competition could have impacted the use of the novel object in the current study, but more specifically at an individual cow level. Social dominance was not measured in the current study, but it seems likely that as with the study by Foris et al. (2021), more dominant individuals could have utilised the resource more than more subordinate cows. To reduce the influence that social impact had on how much individual cows used the novel object, increased numbers of the resources could be provided at differing locations of the building.

There were no clear differences between parity groups in the time spent interacting with the buoy, however cows within their fourth parity or above, spent the least total time interacting with the buoy throughout the study in both groups. This trend was reflected for both groups when also evaluating separate interactions with the buoy, with parity 4 and above cows from the second group, making fewer separate interactions with the buoy than cows in their first or second lactations. It would be a biologically plausible hypothesis, for older cows in later lactations to have used the buoy less than younger cows, due to less expendable energy, resulting from higher physiological demands. It is sometimes considered, that the increased selection for milk yield in cows, is accompanied by increasing leg problems, metabolic issues and declining longevity (Oltenacu & Broom, 2010). The disease burden becomes more apparent with age, with incidence of health events increasing with increased parity (Pritchard et al., 2013). There is a significant relationship between parity and culling, with cows in their sixth or higher parity being four times as likely to be culled compared to a cow in its first lactation (Rajala-Schultz & Gröhn, 1999). Disease incidence, referring to mastitis, ketosis, digestive disorders and laminitis, has been shown to be higher in cows, even during their third lactation compared to their first (Ingvartsen et al., 2003). It has been described that ‘luxury’ behaviours will decline in times of energy or time constraint (Dawkins, 1983), one such example is play behaviour (Held & Spinka, 2011). Therefore, it is possible that environmental exploration and positively interacting with the environment could fit into this category, with older or more physiologically challenged animals, forgoing this behavioural activity to prioritise more immediate needs to maintain welfare such as accessing feed, water and lying areas. Despite this trend, the total time spent using the enrichment whilst it was present, was comparable across all parity groups showing that older cows valued it just as much as younger cows, reflecting that this type of resource would be widely relevant across adult herds despite varying age and stage of lactation and therefore has the potential to impact large numbers of cows.

3.4.3 Cows initial response to novel object

The ethogram used within the present study aimed to assess cows' initial behavioural response to the novel object. Tail wagging was identified as one of the most frequently expressed behaviours during this first interaction with the object, for trial 1 cows. Tail wagging in cows has received little research interest, however this behaviour is associated with positive emotions in other species such as dogs (McGowan et al., 2014; Travain et al., 2016) and pigs (Reimert et al., 2013; Rius et al., 2018). De Oliveira and Keeling (2018) evaluated dairy cows body postures during different activities; feeding, using an automatic brush and whilst queuing to access the robotic milker. Postures were situation dependent, with tail wags directed towards the body occurring whilst feeding, no tail movement whilst queuing and vigorous tail wagging whilst using the brush. The authors used principal component analysis to incorporate these results into a multidimensional model of core affect, to deduce how postures may change in situations of varying valence and arousal and tail wagging was placed as positive on a scale of valence. It is worth noting that increased tail wagging, albeit alongside an increase in general active behaviours, is linked to pain in calves, specifically surrounding more painful dehorning and castration procedures (Graf & Senn, 1999; Robertson et al., 1994). The current research available regarding tail movements in dairy cows, suggest that it could be a behavioural outlet of positive affective states, however more research is required to understand this link.

Head and nose contact was one of the top two most frequently expressed behaviours for both groups of cows. Cows are highly expressive with their heads, being a key facilitation of social communication (Reinhardt et al., 1986; Schmied et al., 2008), through head butting, shaking, rubbing and resting. Head butting of fixtures has also previously been considered as play behaviour (Jensen et al., 1998) in calves. Zhang et al. (2022) found head butting to be one of the behaviours expressed by calves in response to provision of hay nets, suggesting that the provision of this resource was able to stimulate calves' play motivation. It is therefore plausible that the behaviour expressed by the cows in the present study, in response to provision of a novel environmental resource, to similarly be explained by the stimulation of play behaviour, which is a widely considered behavioural expression of positive affective states (Boissy et al., 2007; Held & Spinka, 2011; Keeling et al., 2021). Licking and chewing of the object was the fourth most frequently occurring behaviour for both groups of cows and also the behaviour with the third longest duration for both groups. It seems likely that both using their head and oral manipulation by cows are likely ways for them to explore and interact with the environment.

The three behaviours which occurred the least frequently during initial response to the novel object were; urination/defecation, a submissive posture and being startled. Urination/defecation tends to be measured when trying to evaluate fear (Kilgour, 1975; Munksgaard et al., 1997) and a submissive posture is usually a result of social aggression and stress (Beilharz & Zeeb, 1982; Wagner et al., 2012). Therefore, all three of these behaviours can generally be regarded as being representative of a negative emotional valence – which further reinforces that the results from this study infer that interaction with the novel object was a positive experience for the cows.

Van de Weerd et al. (2003) evaluated the use of 74 different objects as potential enrichment for pigs. A list of definitions (for example odorous or deformable) were used to describe the varying characteristics of the different objects as an approach to identify what characteristics of enrichment would be the most successful, assessed by level of object interaction. A similar approach may be beneficial to get a better understanding of what resources could be used for impactful enrichment for dairy cows.

3.4.4 Limitations

One of the limitations of the current study was the length of time over which the study was conducted. Both groups of cows showed a significant decrease in time spent interacting with the novel object by week 2 and 3, compared to the initial week it was present, however, the majority of both groups sustained use in week 3, although at a decreased level. Results suggest that novel enrichment provided a rewarding opportunity to cows, yet to evaluate the longevity of this response, which would be important considering practical implementation, a more long-term study would be required. This poses questions as to whether interaction may be sustained for periods of time if the object is removed and then replaced, if the object is changed regularly or if different resources varying in qualities such as size, shape, location or functionality may elicit different levels of interest and sustained use.

As already discussed, the use of a single feature of novel enrichment may have impacted use by the cows in terms of social conflict and the potential of subclinical disease. These conflicts could have yielded data not truly representative to the time that housed dairy cows may be motivated to spent in occupation with novel enrichment features. It would be beneficial for future research to explore the use of multiple enrichment resources in varying locations to take this into account.

The current study did not evaluate cows' motivation to access the novel enrichment, this would be a valid research question for future work given the comparable level of interest and use in the novel object used this study compared to automatic brushes.

It is also worth mentioning that the results from the current study should only be extrapolated to housed dairy cows, in similar environmental management systems. Animals that are housed in stimulus lacking environments have been shown to display heightened stimulus seeking behaviour, likely a symptom of boredom (Meagher et al., 2018; Meagher & Mason, 2012). Therefore, whether cows in more diverse and stimulus rich environmental conditions, for example with access to pasture, additional space (whether that be inside or outside), and loose housing systems would show similar levels of interest and use of novel enrichment would be a valuable research question.

The depth of research on environmental enrichment in the pig industry has allowed classification of different types of environmental resource as optimal, sub-optimal and of marginal interest (Godyń et al., 2019), judged by the different impacts they have on different behaviours. It is likely that different resources and housing modifications, may have the potential to influence different behaviours and provide varying levels of interest for housed cows. Therefore, next stages of research should be directed to these key points of question.

3.5 Conclusions

Two separate herds of adult, commercially-housed, dairy cows repeatedly interacted with a novel source of enrichment over a three-week period. The novel enrichment, in the form of a suspended sailing buoy, was aimed to provide environmental diversity to the home pen and an opportunity for cows to express interest and exploratory behaviours. The majority of both groups, continued to use the novel object each week with 82.86% of trial 1 cows and 83.33% of trial 2 cows still using it by the third week. This high level of interest and use by two separate groups of commercially-housed dairy cows, suggests that interacting with the novel object represented a positive experience. Alongside this, the use of an ethogram revealed that, cows showed a positive behavioural response to the addition of the novel object within the home pen, with the highest frequency and duration of behaviours shown being exploratory (head and nose contact, licking/chewing, attentive) and tail wagging, a behaviour tentatively linked to positive affective states in other species. The exact motivational basis explaining the cows' use of the novel object in this research, is beyond the scope of the study, but these preliminary results suggest that provision of a simple novel enrichment provides opportunity for exploration and cows interest and likely a valued or rewarding subjective experience. The results of the current chapter show that environmental enrichment for housed adult dairy cows has the potential to provide positive welfare benefits and should be the subject of continued research.

Chapter 4: Novel enrichment reduces boredom associated behaviours in housed dairy cows

4.1 Introduction

Managing dairy cows indoors has increased in Great Britain, with only 1% of farms not housing their cows at any point throughout the year (March et al., 2014). Dairy cow housing is often associated with barren environments and reduced environmental resources which may lead to boredom (Wemelsfelder, 1993). Alongside this, housed cattle are presented with increased disposable time; cows at pasture have been shown to spend 68% of their time grazing, compared to housed cattle spending only 22% of their time feeding (Roca-Fernandez et al., 2013). Currently, there is a high level of societal concern regarding dairy cattle welfare (Wolf et al., 2016), with quality of life (Cardoso et al., 2016) and health/welfare (Jackson et al., 2020) being some of the most important aspects of concern for the public. Boredom is accepted to be a negative affective state (Burn, 2017; Meagher, 2018) and is a common adjective used when people are asked to give examples of poor welfare (Duncan, 2002). For housed cattle, boredom has been highlighted as a potential welfare concern (Crump et al., 2019), however there is a lack of evidence relating to how dairy cattle experience this affective state in the housed environment.

Boredom in animals describes the potential to suffer from a chronic lack of opportunities to interact with the environment (Wemelsfelder, 1993). Although short term boredom may have negligible impacts, chronic boredom in people has serious health implications and is associated with psychological disorders such as anxiety and depression (Goldberg et al., 2011; Sommers & Vodanovich, 2000). Animals will actively choose to avoid boredom (Latham & Mason, 2010; Mason et al., 2001), with some showing preference to aversive experiences over monotony (Galef & Whiskin, 2003; Meagher & Mason, 2012;). Environmental enrichment is usually the first approach for alleviating boredom in animals (Meagher, 2008), by providing opportunities for control and exploration (Fraser et al., 1991). However, scientific studies that evaluate the impact of enrichment on boredom behaviour of dairy cattle are lacking.

Inactivity and idling behaviour have emerged as potential behavioural expressions of boredom and negative affective states in animals (Fureix et al., 2012; Fureix et al., 2019; Harvey et al., 2019). This concept originates from human psychology where reduced activity is observed in patients suffering with depression (Bonnet et al., 2005; Van Gool et al., 2003). Animals housed in monotonous environments generally spend more time inactive than animals in more diverse, stimulus varied conditions (Burn et al., 2020; Ceballos et al., 2016; Webb et al., 2017). Hintze et al. (2019) developed an inactivity ethogram for fattening cattle, which showed the number of animals inactive and time spent inactive to increase from a pasture based system, to a 'semi' intensive and then 'intensive' system. Housed buffalos have been shown to display more idling behaviour, defined as having open or closed eyes but no other overt activity, compared to buffalos in similar housing but with access to an outdoor yard, a pool and extended space allowance (De Rosa et al., 2009; Tripaldi et al., 2004). Increased idling behaviour has been observed in housed dairy cows without access to daily grazing (Di Grigoli et al., 2019) and in aversive situations such as deprivation of lying down and isolation (Munksgaard & Simonsen, 1996). Conversely, self-grooming has been mentioned as a potential indicator of positive affective states in cows (Keeling et al., 2021; Mattiello et al., 2019; Napolitano et al., 2009). The action of self-grooming is linked to hormones such as opioids (Niesink & Van Ree, 1989), often released following stress, a relaxation mechanism (Spruijt et al., 1992), which may predispose it to being used to self sooth or elicit a positive experience. Increased self-grooming has been linked to anticipation of reward (Zimmerman et al., 2011), reduced stocking densities (Raspa et al., 2020) and housing with additional outdoor access (De Rosa et al., 2009). In dairy cows, however, it has been associated with negative states. Increased self-grooming has been displayed by dairy cows in stressful situations, such as restraint (Bolinger et al., 1997), social isolation (Munksgaard & Simonsen, 1996), deprivation of lying down (Munksgaard & Simonsen, 1996) and when offered unexpected, unusual food (Herskin et al., 2004). Increased self-grooming has also been displayed in more barren environments, such as caged calves (Kerr & Wood-Gush, 1987), tethered compared to loose housed dairy cows (Krohn, 1994) and in fully housed as opposed to cows with daily access to pasture (Di Grigoli et al., 2019). Assessment of idling and self-grooming behaviours both provide potential proxy behavioural measures for boredom and positive or negative affective states in dairy cattle, therefore offering opportunities to investigate boredom behaviours expressed within the housed environment.

Free stall housed dairy cows fill up their 'time budget' with the available activities of eating, drinking, resting, socialising in alleyways and standing in stalls (Gomez & Cook, 2010). Robotically milked cows have the additional pastime of choosing when to be milked throughout the day. Cows are conditioned to voluntarily enter the robot to be milked, as a result of receiving concentrate feed during milking. Based on specific selection criteria such as a minimum milking interval or individual cow milk yield, a cow may be immediately released by the robot (without the provision of food); these are classed as 'refusals'. Morita et al. (2017) found this type of visit to make up over 58% of the total visits to the robot over a 7-day period for one commercial dairy herd. One suggested behavioural indicator of boredom is the motivation for general stimulation (Meagher, 2018) and sensation seeking behaviours (Burn, 2017). We hypothesised that ‘refusals’ may be a behaviour associated with boredom, as it appears to be a sensation seeking activity. To our knowledge this is the first study which has assessed automatic robotic milking ‘refusals’ as a potential boredom associated activity.

The purpose of this research was to assess the use of novel enrichment in a commercial herd of fully housed dairy cattle. The primary objective was to assess whether novel enrichment (a novel object within the housed environment) impacted specific boredom-associated behaviours or self-grooming behaviour displayed by the herd.

4.2 Materials and methods

Detailed methods for this study are described in Chapter 2.

4.3 Results

4.3.1 Herd use of novel object

During intervention weeks 1-3, group 1 cows interacted with the buoy at least once during 63 of the 72 hours of continuous video footage and the buoy was in use for a total of 776.3 minutes out of the 72 sampled hours (18.0% of the observed time). During intervention week 1, the buoy was in use for 390.7 minutes (27.1% of observed time), this decreased to 216.4 minutes during week 2 (15.0% of observed time) and 169.2 minutes during week 3 (11.8% of observed time).

Group 2 cows interacted with the buoy at least once in 70 of the 72 hours of sampled video footage and the buoy was in use for a total of 773.9 minutes out of the 72 sampled hours (17.9% of the observed time). During intervention week 1, the buoy was in use for 433.7 minutes (30.1% of observed time), this decreased to 235.9 minutes during week 2 (16.4% of observed time) and 104.3 minutes during week 3 (7.2% of observed time).

The percentage of cows that interacted with the novel object at least once during the intervention weeks, is illustrated in Figure 4.1. In both study groups, fewer cows interacted with the buoy over time although in week 3 this remained >80% of cows.

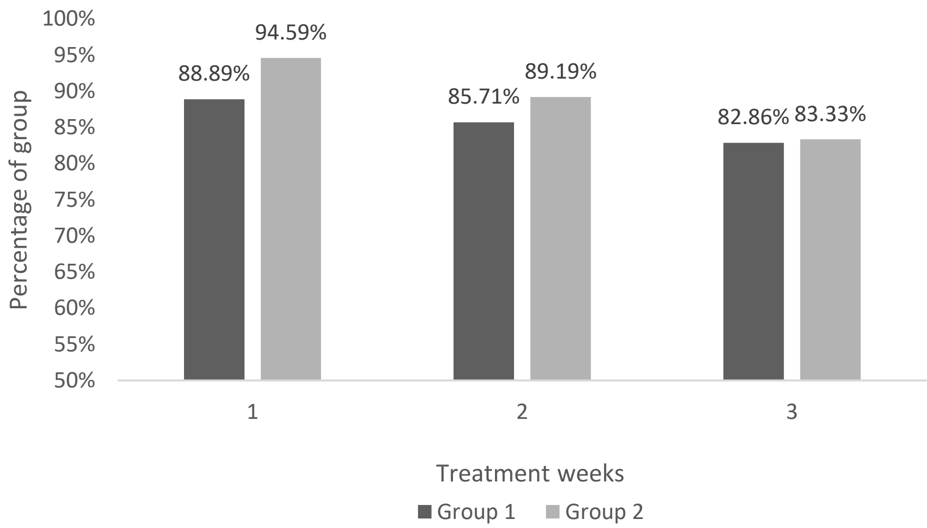


Figure 4.1. *Percentage of cows in each study group that interacted with the novel object (sailing buoy) by treatment week.*

4.3.2 Self-grooming

During the time of sampled video footage, in the baseline week for cows in group 1, 72 cows entered the 2m zone and 26 (36.1%) of these self-groomed at least once. During intervention weeks 1-3 respectively, the number (%) of cows that self-groomed of those eligible was 72/106 (67.9%), 41/77 (53.2%) and 39/62 (62.9%). For group 2, the equivalent values were 16/70 (22.9%) during the baseline week and

78/130 (60.0%), 56/84 (66.7%) and 40/61(65.6%) during intervention weeks 1-3 respectively.

Results of the mixed effects models revealed that for cows in group one, having accounted for repeated measurements of grooming within cow, the odds of a cow self-grooming were significantly increased in weeks 1-3 compared to the baseline week (odds ratio = 2.93, 95% CI 1.68-5.12; $P<0.05$). Based on this model, the calculated adjusted probability of self-grooming in the baseline week was 0.36 and during weeks 1-3 was 0.62.

Results of the mixed effects model for group 2 revealed that the odds of a cow self-grooming were significantly increased in weeks 1-3 compared to the baseline week (odds ratio = 6.21, 95% CI 3.28-11.7; $P<0.05$). For group 2 the adjusted probability of self-grooming in the baseline week was 0.22 and during weeks 1-3 was 0.64.

4.3.3 Unsuccessful milking attempts

The number of unsuccessful milking attempts (refusals) per cow per day followed an over dispersed right skewed distribution, with a small number of cows having relatively high numbers of refusals. A log (base 10) transformation was used to normalise the data and allow robust comparison between groups. The distributions of refusals per day (displayed as $\log_{10}$(refusals+1)) for both study groups are illustrated in Figure 4.2.

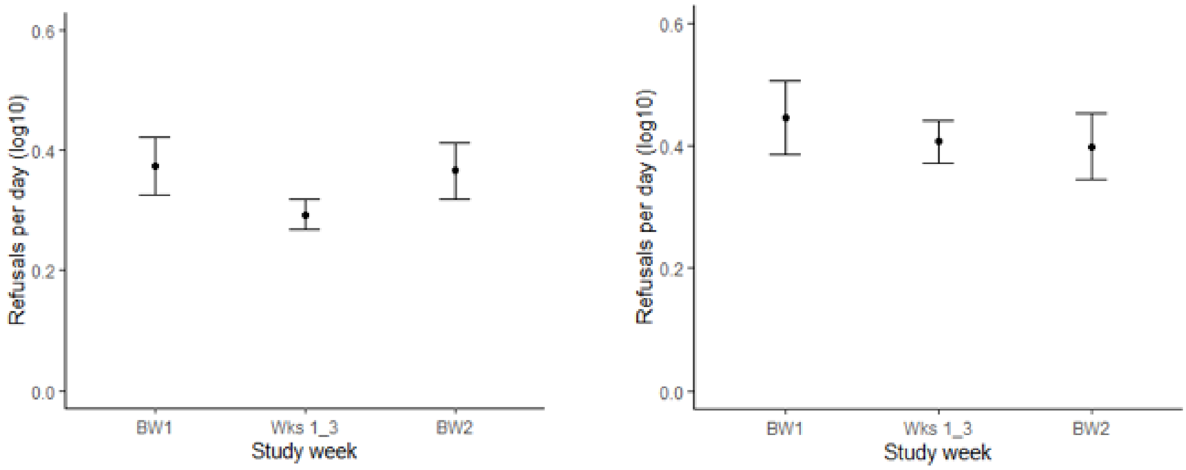


Figure 4.2. *Distributions of daily milking refusals (displayed as log_{10}(refusals+1)) throughout the trial periods for study groups 1 and 2. 'BW1' refers to the baseline week before cows had access to the novel object, 'Wks 1-3' refers to the treatment period of 3 weeks when the novel object was present, 'BW2' refers to the final baseline week following removal of the novel object.*

Results from the mixed effect linear model with log10(refusals per day+1) as the outcome are provided in Table 4.1. For study group 1, there was a significant reduction in daily cow refusals, displayed by a reduction of 0.08 log10 refusals, during the weeks when the novel object was present compared to the baseline week 1 ($P<0.05$) (which was similar to baseline week 2). For study group 2, the pattern was slightly different. Although there was a significant reduction in refusals per day when the novel object was present compared to the baseline week 1, displayed by a reduction of 0.04 log10 refusals ($P=0.05$), the level of refusals in baseline week 1 was greater than in group 1, the magnitude of the reduction in refusals was smaller during intervention weeks 1-3 and the level of refusals remained lower in baseline week 2.

Table 4.1. *Results of the mixed effects linear model with log10 (refusals per day) as the outcome variable.*

Model Terms	Coefficient	95% Confidence Interval
Group 1		
Intercept	0.37	
Baseline week 1	Reference	
Intervention weeks 1-3	-0.08	-0.04 - -0.12
Baseline week 2	-0.01	-0.03 - 0.01
Group 2		
Intercept	0.45	
Baseline week 1	Reference	
Intervention weeks 1-3	-0.04	0.00 - 0.08
Baseline week 2	-0.05	-0.08 - -0.03

4.3.4 Herd level behaviour

Daily mean numbers of cows scored for all behaviours described in Table 4.2. The only behaviour for which a clear difference was found when the novel object was present was idling behaviour and the mean number of idling events per cow per day is illustrated in Figure 5.

Table 4.2. *Mean (± standard deviation) number of cows scored for each behavioural category.*

Behaviour	Baseline week 1		Intervention weeks 1-3		Baseline week 2	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Inactive standing/Idling	51.00±4.24	58.00±9.90	43.17±2.99	49.83±7.03	55.50±4.95	54.00±2.83
Standing in cubicle	43.50±2.12	36.00±4.24	40.67±10.71	37.50±4.51	29.50±3.54	35.00±7.07
Cow at feed face or water trough	106.50±9.19	110.50±14.85	102.17±7.88	114.00±15.18	100.00±1.41	93.50±12.02
Lying in cubicle	480.00±8.49	452.50±21.92	469.33±17.72	466.50±10.95	473.±2.83	460.50±12.02
Brush	9.00±7.07	12.50±2.12	10.17±2.71	6.50±3.08	14.00±0.00	9.00±1.41
Cows within enrichment zone	3.50±3.54	3.50±5.00	8.33±7.39	10.67±5.72	2.50±2.12	5.00±1.41
Cow moving	8.00±0.00	18.00±7.07	9.83±2.99	12.83±5.27	9.50±0.71	10.50±0.71
Using extended loafing space	19.50±2.12	10.50±7.07	14.5±6.53	12.83±7.41	15.50±4.95	14.50±6.36

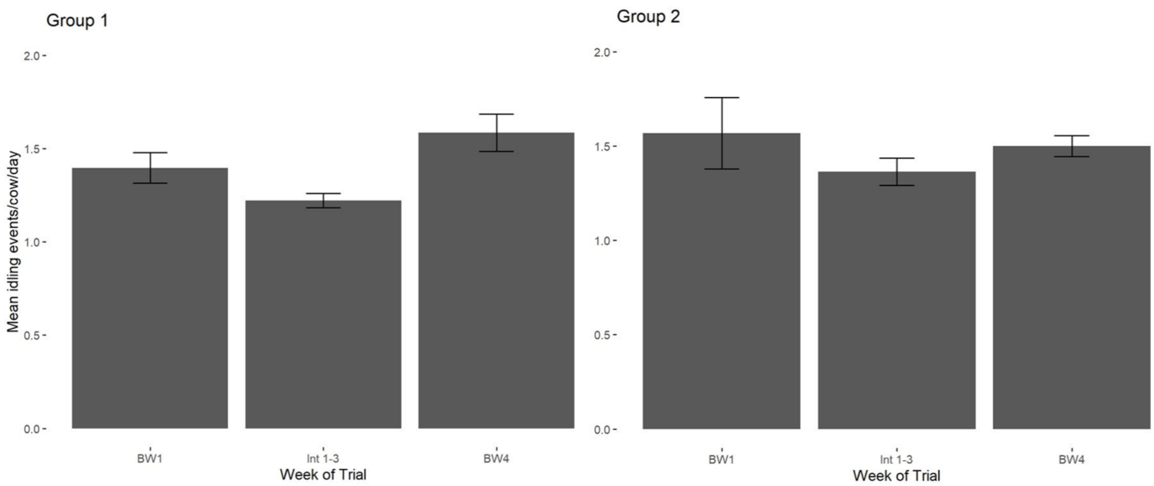


Figure 4.3. *Mean number of idling events per cow per day throughout the trial periods for study groups 1 and 2.*

Results of the final linear models are presented in Table 4.3. For group 1 cows there was a significant reduction in the number of idling events of 0.17 events per cow per day during intervention weeks 1-3 compared to the initial baseline week, ($P<0.05$). This trend was reflected in the second group of cows but there was more variability which meant that although the mean effect size was similar (a reduction of 0.2 idling events per cow per day as opposed to 0.17) the confidence interval spanned 0.

Table 4.3. *Results of the linear model with idling events per cow per day as the outcome variable.*

Model Terms	Coefficient	95% Confidence Interval
Group 1		
Intercept	1.40	
Baseline week 1	Reference	
Intervention weeks 1-3	- 0.17	-0.33 – -0.01
Baseline week 2	0.19	-0.01 – 0.39
Group 2		
Intercept	1.60	
Baseline week 1	Reference	
Intervention weeks 1-3	-0.20	-0.50 – 0.10
Baseline week 2	-0.07	-0.43 – 0.29

4.4 Discussion

4.4.1 Summary

The aim of this intervention study was to evaluate the behavioural impact of a basic, non-functional enrichment added to the housing environment of commercially-housed dairy cows. Findings demonstrated the vast majority of cows interacted with the novel object. Milking refusals and idling were both reduced in the presence of the novel object compared with baseline weeks, which may indicate a reduction in boredom.

Alongside this, there was a significant increase in self-grooming whilst the object was present, a potential indicator of positive affective states.

4.4.2 Herd use of enrichment and implications for affective states

Almost all cows from two separate trials, repeatedly interacted with the novel object throughout the three-week study period. It seems reasonable to interpret this interaction as a positive experience by the cows, given that cows will actively avoid situations they associate with negative events (Munksgaard et al., 1997; Pajor et al., 2000). Similarly, positive affective engagement, which describes the link between the resulting emotions of carrying out a highly motivated or rewarding activity, are regarded as positive (Mellor, 2015). These findings suggest that the provision of a simple environmental enrichment in the current study, was likely to provide a positive experience to the cows, which appears to support the hypothesis that environmental enrichment can promote positive affective states (Mandel et al., 2016). It has been argued that enrichment may be of no benefit if it has no functional significance to the animal or serves no specific function for an outlet of behaviour (Newberry, 1995). This concept was not reflected in the results of the current study, which used a novel object, that did not obviously offer any species-specific behavioural function. The underlying motivation for cows interacting with the novel object is unknown, however it seems plausible that it may have solely provided environmental diversity and some level of control and choice within their environment, all previously suggested benefits of enrichment (Mellor, 2015). The high level of interest and interaction in the simple novel object used in this study, indicates that boredom could be a potential welfare concern for housed dairy cows, however more work is still needed in this area. The potential of boredom to be a welfare concern for housed dairy cows has recently been highlighted by Crump et al (2019). Crump et al. (2021)

attempted to assess whether pasture access improves emotional states in dairy cows, using judgement bias, which monitors animal's responses to ambiguous situations to infer affective valence (Lagisz et al., 2020). These authors reported that cows with access to pasture, approached a known food reward slower than cows that were fully housed and proposed the explanation was a reduced reward anticipation, generally shown when higher or more frequent rewards are experienced in day-to-day life (Spruijt et al., 2001). The conclusion was that pasture access may facilitate more rewarding lives and therefore better welfare. The reduced reward anticipation expressed by cows with pasture access in the study by Crump et al. (2021) identifies a question of whether non or partly housed cows would show the same level of interest and uptake in enrichment resources as the cows used in the present study.

4.4.3 Unsuccessful milking attempts

Reduced unsuccessful milking attempts were made by both groups of cows when the buoy was present. There could be several reasons cows make repeated unsuccessful trips to the robotic milker, such as unsatisfactory diets or the general need to be milked (Kozłowska et al., 2017). This was the first study to identify 'refusals' as a potential behavioural indicator of boredom in dairy cows. It has been suggested that providing animals with a way of using their cognitive abilities is valuable for their welfare (Hagen & Broom, 2004; Meehan & Mench, 2007), most notably because it may help successful coping in restricted environments by offering some control over the environment alongside minimising boredom (Wemelsfelder, 1993). Boissy and Lee (2014) reviewed the literature on how cognition can improve welfare and outlined three processes which may elicit a positive emotion; by the signalling of a reward in advance, when receiving a greater reward than expected and having control over a positive outcome. Using the robot meets two of these criteria;

the anticipation of reward and the control over a positive outcome. More simply, it's a time filling activity which may provide cows with something to do. The need for a better understanding behind the motivation underlying these unsuccessful milking attempts has already been highlighted by Jacobs and Siegford (2012), to reduce the amount of time being wasted by robots for this activity. Our results suggest that the provision of environmental enrichment may have an impact on how cows use a robot milker, however further studies are required.

4.4.4 Idling

Our study identified that lower numbers of cows exhibited idling behaviours when the novel object was present compared to when it was not, however the results were only significant for one replicate. This is consistent with other studies which have shown decreased levels of inactivity in more stimulus diverse environments in dairy calves (Webb et al., 2017), fattening cattle (Hintze et al., 2019) and buffalo (De Rosa et al., 2009), among many other species (Bolhuis et al., 2006; Burn et al., 2020; Ceballos et al., 2016). These results suggest that idling could be an indicator of boredom in dairy cows, however further replication of these results is required. Further research may help support the hypothesis that idling may be an indicator of boredom in animals (Fureix & Meagher, 2015; Hintze et al., 2019; Meagher & Mason 2012). Idling behaviour could indicate that no available environmental opportunities are meeting the animals needs or that the animal is between activities with nothing to do. It seems plausible that increasing the available activities within an environment may lead to a reduction in 'empty time' as some of this is spent interacting with the environment. Since increased standing is a risk factor for lameness (Proudfoot et al., 2010; Sepúlveda-Varas et al., 2018), the additional enrichment in this study may have wider welfare implications than solely the promotion of positive welfare states; it is possible there could be subsequent health benefits associated with less unproductive use of the robotic milking machine. However, further research in this area is needed.

4.4.5 Self-grooming

The present study saw a marked increase in self-grooming behaviour following interaction with novel enrichment, compared to that observed in the same location during a baseline period. Although self-grooming has been mentioned as a possible indicator of positive affective states (Keeling et al., 2021; Mattiello et al., 2019; Napolitano et al., 2009), the majority of research on the behaviour in dairy cows has linked it to stressful or aversive experiences (Bolinger et al., 1997; Krohn, 1994; Munksgaard & Simonsen, 1996). The cows in this study could freely choose to interact or avoid the novel object, and high numbers of the herds repeatedly used it, suggesting it was unlikely to present a stressful situation, as cows are known to avoid situations they have previously found aversive (Munksgaard et al., 1997; Pajor et al., 2000). Self-grooming has previously been linked to novelty in dairy cows (Herskin et al., 2003, 2004, 2004), however the novelty used in these previous studies could be regarded as negative. Herskin et al (2004) and others, found dairy cows to self-groom more in response to novel feed. As dairy cows are highly feed motivated, a sudden, unexpected change in feed provision could be perceived to be stressful. Similarly, increased self-grooming has been observed in response to a novel object (Herskin et al., 2004) and novel environment (Herskin et al., 2003) but both studies used tethered cows, without the control over leaving the situation, again likely to be a stressful situation. Self-grooming is linked to hormones and displayed following stress or arousal (Niesink & Van Ree, 1989; Spruijt et al., 1992), which may help explain the conflicting literature on what self-grooming may actually represent. Affective states can occur on a continuum from positive to negative, described as valence, and can also differ in level of arousal and the association between them is usually expressed on a 2-dimensional figure, of two overlapping axis of valence and arousal (Mendl et al., 2010). Therefore, self-grooming may represent changes in stress or arousal which could be valenced in either direction. A review by Boissy et al. (2007) suggests one possible facilitation of positive emotions could be the ability to control the environment and successfully cope with challenge. The novel object used in this study and the way it was presented in the home environment, is assumed to be something the cows wouldn't have experienced before, meaning an initial reaction of stress or arousal would have been likely. Any initial stress response may have then subsided following cows successful coping with the situation, in terms of it not resulting in an aversive experience and having control over their interactions with it. It seems more appropriate that self-grooming may be a response to arousal, which could encompass both negative and positive experiences. Other validated indicators of positive states and stress could be used alongside self-grooming to allow better interpretation of behavioural responses to differently valenced situations. Further research on self-grooming in dairy cows in different situations, potentially alongside the use of anxiolytics, are required.

4.4.6 Limitations

Although very similar behavioural changes were shown by two independent groups of cows in the current study, the relatively small study size is worthy of note. Similar studies would be valuable using larger numbers of replicates. In addition, an inherent challenge in any research using behaviour is to make inferences about affective states, therefore we have been cautious in the interpretation of these behavioural changes. The literature suggests that idling is likely to be a behavioural representation of the negative experience of boredom, however, the reductions in idling observed could more simply, be a reflection of more cows engaged in other behavioural activities, without changes in affective states. Our research raises further questions including the extent to which behavioural responses to enrichment would vary between management systems and how long these behaviours and interest in a novel enrichment may persist if conducted over a longer period of time; these would be worthwhile subjects of future research.

4.5 Conclusions

This intervention study addressed an important research gap and observed notable changes to cow behaviour, suggestive of improved welfare. The changes in behaviour observed in the current study show that simple housing modifications may have an impact on the affective lives of cows in commercial settings. A small change to the housing conditions of commercial dairy cows via implementation of a practical, low cost enrichment resource, appeared to facilitate positive experiences, reflected in the continued and repeated use by the majority of cows from two study replicates. In addition to this, significant increases in self grooming were identified when cows had access to this additional resource compared to when housed in standard housing conditions. Two behaviours which have previously been associated with boredom in animals were reduced whilst cows had access to the novel enrichment compared to standard housing, however these results require further replication. These behavioural changes, paired with the high uptake from the herd, demonstrates the potential impact this type of housing modification could have on large numbers of housed dairy cows.

Chapter 5: Do housed dairy cows habituate to novel forms of enrichment over time? Do they show a preference between use of an outdoor concrete yard and an indoor novel object?

5.1 Introduction

One identified opportunity for providing commercially managed animals with positive experiences, is by offering opportunities for exploration through environmental enrichment (Mellor & Beausoleil 2015; Mellor, 2015). Enrichment has also been shown to offer a multitude of other welfare benefits (Carlstead et al., 1991; Coppola et al., 2006; Flint & Murray, 2001). One consideration of this method of welfare enhancement is the longevity of its benefits. For example, an enrichment intervention offering welfare benefits for a short period, is then redundant if the welfare benefits then cease, due to animals losing interest. Habituation is the progressive decrease in response to stimuli (Dissegna et al., 2021) and habituation to enrichment resources has shown to occur rapidly in pigs (Trickett et al., 2009; van De Weerd et al., 2003). One measure of the success of environmental enrichment could be the magnitude of welfare benefits, alongside a measure of the longevity of these benefits. Interventions providing more long-term benefits will primarily be more successful in enhancing welfare but may also help offset any financial or other barriers to implementation by producers. With the limited literature available on environmental enrichment for dairy cows, it is unknown if and how quickly, cows may habituate to housing modifications made to diversify the environment.

It has been argued that enrichment may be of no benefit if it has no functional significance to the animal or serves no specific function for an outlet of behaviour

(Newberry, 1995), with one example being novel objects such as 'toys'. In contrast, one aim of enrichment is to facilitate exploration within the environment, which could be argued to be achieved through both behaviourally functional and non-functional resources. As discussed by Newberry (1995), the gold standard of enrichment would be offering behaviourally relevant opportunities in environments mimicking a setting as natural as possible, however the fundamental complication is identifying resources and housing modifications that are practically achievable in commercial settings. Enrichment has emerged as a way of mitigating the behavioural and welfare implications of intensified production systems, which are far removed from what may be considered a 'natural' environment and therefore any resources that have a positive impact on behaviour or welfare should be considered. Van de Weerd et al. (2003) evaluated 74 different objects in an attempt to identify object characteristics which sustained the most interest in pigs and was able to define a small subset of resource characteristics, such as deformable and chewable, that were valuable to pigs. With the limited available literature regarding enrichment for housed dairy cows, new insights in this area would be of value.

One resource that dairy cows have been shown to highly value is access to pasture (von Keyserlingk et al., 2017). Access to pasture has also been associated with a number of both health (Hernandez-Mendo et al., 2007; Washburn et al., 2002) and behavioural benefits (Miller & Wood-Gush., 1991; Olmos et al., 2009;). One study by Crump et al. (2017), used a cognitive indicator of emotion to analyse potential differences in affective states, between cows in a housed and pasture-based environment. Crump et al. (2017) found cows with access to pasture to have a reduced reward anticipation, compared to cows without pasture access. Authors suggested this difference was due to pasture being a more rewarding environment for cows and therefore more likely to facilitate positive experiences. Despite the literature showing that pasture is a valued and beneficial resource, zero grazed systems have become a common management practise (March et al., 2014), with only 5% of lactating cows in the US being provided access to pasture (USDA, 2007). There are several barriers for farmers in offering access to pasture, ranging from profitability to general access to land (Winsten et al., 2011). This has directed research towards investigation of other potential forms of outdoor access for cows, such as sand bedded packs, which cows use but still show a clear preference for pasture (Smid et al., 2018) and short-term daily access to an outdoor concrete space, again not utilised as much as pasture (Haskell et al., 2013). Outdoor access for dairy cows is a socially contentious topic, with a general public consensus that cows should be allowed access to pasture (Schuppli et al., 2014), which again highlights the need for alternative outdoor areas to be evaluated. Although a strong preference is shown for outdoor space with pasture, facilitating the highly motivated behaviour to graze, little is known about how much cows value alternative outdoor environments and the general access to the outdoors.

The primary aim of the current chapter was to evaluate commercially-housed dairy cows' interest and level of habituation to novel enrichment resources over a prolonged period of time. The secondary objective was to assess if housed dairy cows would show a preference between two different resources as forms of enrichment. The two different resources used were designed to contrast in terms of behavioural functionality, with access to an outdoor concrete yard, providing cows the choice to be inside or outside and an indoor hanging novel object, aimed to provide opportunities for exploration. A better understanding of what environmental modifications dairy cows value will contribute to the lacking body of evidence regarding how positive experiences and therefore overall welfare, may practically be facilitated in commercial settings.

5.2 Materials and methods

5.2.1 Housing, management and diet

The trial was based at The University of Nottingham's Centre for Dairy Science Innovation. The study farm was chosen for its dairy housing facilities, the CIEL flexible housing unit allows reconfiguration of structure and space allowance enabling provision of unique resources.

The farm maintains a herd of 300 Holsteins, producing on average 12,800 litres of milk, per cow, annually. The cattle are housed all year round, in a sand bedded cubicle system. The slatted passageways provide increased loafing space and are scraped automatically via a robotic scraper machine. The closed herd calves all year round, with replacement heifers entering the milking herd. Cows are milked via robotic milking machine.

Cows were marked to enable identification via video recordings, as neither collar tags or freeze brands were large enough to be visible on the recordings. Cows were marked with a green or yellow painted number on either side of their back using "Fil Detail Tail Paint". Cows were painted during feeding times on Monday and Wednesday mornings (09:00-10:30), whilst locked in yokes, a management practise these cows were used to for regular veterinary visits. During the first replicate of the study, it was identified that some predominantly white cows were more sensitive to paint application than cows with black hair. Due to this, for the second replicate of the study, two cows with predominantly white hair and lighter skin were identified by their coat appearance instead of by applying paint. These cows continued to be locked in head yolkes with the rest of the herd whilst applying paint numbers.

5.2.2 Cows and treatment

Study herd

We selected 96 cows and assigned them to two separate groups. Cows were randomly selected to create two comparably distributed groups of cows in different lactation groups and stages of lactation. Cows were also selected subject to their drying off date being later than the end of the study period, to avoid removal of the cows from the study group. The two groups of cows were housed in identical buildings opposite one another (Figure 5.3). Nine cows were removed from Group 1 during the study for veterinary intervention or due to being regrouped unexpectedly for drying off (Table 5.2). Twelve cows were removed from Group 2 for the same intervention reasons (Table 5.4). Any cows that were removed from the study groups, remained absent for the remainder of the trial. Twenty-two cows were moved into the study buildings during the trial to maintain group size, these cows were not included in data collection. Nine cows entered Group 1 and 13 entered Group 2. Lactation and days in milk were matched for any new cows entering Group 1 and Group 2. Thirty-nine of the originally selected cows from Group 1 and 36 from Group 2, remained present for the entirety of trial (Table 5.1; Table 5.3). All results reported in this chapter are solely from these study cows because they experienced the environmental conditions throughout the entire study. Group 1 consisted of 39 Holstein cows averaging (mean±SD) 95.00 ± 50.28 days in milk (median: 83.00, IQR: 93.5, range: 26.00 – 180.00), producing on average 41.47 ± 9.79L of milk/day, of parity 2.15 ± 1.25, proportion of parity groups were parity 1: 0.38, parity 2: 0.28, parity 3: 0.21, parity 4+: 0.13 and weighing 681.5 ± 69.80kg at the start of the study. Group 2 consisted of 37 Holstein cows averaging 85.36 ± 44.92 days in milk (median: 76, IQR: 74, range: 27 - 169), producing on average 42.76 ± 9.82L of milk/day, parity 2.25 ± 1.25, proportion of parity groups were parity 1: 0.33, parity 2: 0.31, parity 3: 0.22, parity 4+: 0.14 and weighing 683.4 ± 88.8kg at the start of the study.

Table 5.1. *Details of Group 1 study cows present for entirety of study.*

Number	**Ear tag**	**Date of birth**	**Lactation**	**Days in milk at start of trial**
30	UK141797103160	19.07.2019	1	47
45	UK141797502835	19.05.2018	2	84
64	UK141797202762	21.02.2018	3	42
68	UK141797603088	06.05.2019	1	177
98	UK141797203217	09.10.2019	1	123
115	UK141797102313	01.10.2015	5	28
140	UK141797702410	28.02.2016	4	54
146	UK141797202391	30.01.2016	4	151
432	UK141797302091	14.08.2014	6	35
705	UK141797302658	21.08.2017	3	164
718	UK141797702620	28.05.2017	3	179
725	UK141797102656	16.08.2017	3	157
759	UK141797502611	07.05.2017	3	125
769	UK141797602605	01.05.2017	3	117
781	UK141797402547	19.01.2017	4	42
804	UK141797302672	13.09.2017	3	142
841	UK141797402680	07.10.2017	3	25
894	UK141797702844	30.05.2018	2	108
924	UK141797402925	03.11.2018	2	37
937	UK141797102964	24.12.2018	2	82
942	UK141797602927	05.11.2018	2	141
950	UK141797402918	27.10.2018	2	96
965	UK141797603004	05.02.2019	2	48
980	UK141797702956	09.12.2018	2	50
1890	UK141797402883	17.08.2018	2	167
3005	UK141797703005	07.02.2019	2	51
3168	UK141797203168	27.07.2019	1	48
3173	UK141797703173	30.07.2019	1	153
3174	UK141797103174	03.08.2019	1	60
3177	UK141797403177	06.08.2019	1	47
3181	UK141797103181	09.08.2019	1	72
3184	UK141797403184	19.08.2019	1	157
3188	UK141797103188	24.08.2019	1	166
3210	UK141797203210	28.09.2019	1	114
3213	UK141797503213	01.10.2019	1	65
3218	UK141797403128	08.06.2019	1	81
3222	UK141797703222	20.10.2019	1	77
3297	UK141797503297	13.01.2020	1	25
8941	UK141797602836	19.05.2018	2	129

Table 5.2. *Details of Group 2 study cows present for entirety of study.*

Number	**Ear tag**	**Date of birth**	**Lactation**	**Days in milk at start of trial**
16	UK141797402876	09.08.2018	2	61
85	UK141797302882	16.08.2018	2	137
86	UK141797502877	10.08.2018	2	26
441	UK141797702123	04.10.2014	5	107
459	UK141797502156	24.11.2014	6	37
520	UK141797602430	31.03.2016	4	134
547	UK141797502534	13.12.2016	4	61
583	UK141797602486	08.09.2016	4	65
601	UK141797402491	05.10.2016	3	160
671	UK141797203014	15.02.2019	2	28
702	UK141797502632	30.06.2017	3	168
735	UK141797702634	10.07.2017	3	137
756	UK141797102628	19.06.2017	3	108
785	UK141797402589	08.04.2017	3	119
813	UK141797602682	11.10.2017	3	34
846	UK141797702718	21.12.2017	3	27
873	UK141797402743	24.01.2018	3	46
906	UK141797602885	21.08.2018	2	93
910	UK141797502849	08.06.2018	2	161
925	UK141797102915	21.10.2018	2	100
927	UK141797702914	20.10.2018	2	60
931	UK141797402911	15.10.2018	2	89
934	UK141797302959	11.12.2018	2	38
938	UK141797302917	27.10.2018	2	140
3150	UK141797503150	11.07.2019	1	34
3186	UK141797603186	21.08.2019	1	48
3194	UK141797703194	04.09.2019	1	66
3197	UK141797303197	07.09.2019	1	165
3201	UK141797703201	17.09.2019	1	84
3212	UK141797403212	30.09.2019	1	120
3216	UK141797103216	04.10.2019	1	102
3226	UK141797403226	24.10.2019	1	91
3228	UK141797603228	26.10.2019	1	43
3230	UK141797103230	27.10.2019	1	39
3255	UK141797503255	22.11.2019	1	47
3257	UK141797703257	26.11.2019	1	62

Table 5.3. *Details of Group 1 study cows that were removed for veterinary intervention during the study.*

Number	Ear tag	Date of birth	Lactation	Days in milk at start of trial	Date removed
9	UK141797403219	18.10.2019	1	137	06.02.2022
28	UK141797602969	30.12.2018	2	107	27.02.2022
511	UK141797102411	29.02.2016	4	215	06.02.2022
749	UK141797202622	01.06.2017	3	109	13.01.2022
761	UK141797702613	16.05.2017	3	215	06.02.2022
764	UK141797402652	14.08.2017	3	215	16.02.2022
824	UK141797202692	09.11.2017	3	131	16.12.2021
981	UK141797703047	27.03.2019	1	237	22.03.2022
3238	UK141797203238	03.11.2019	1	76	06.02.2022

Table 5.4. *Details of Group 2 study cows that were removed for veterinary intervention during the study.*

Number	Ear tag	Date of birth	Lactation	Days in milk at start of trial	Date removed
87	UK141797102901	30.09.2018	2	159	10.12.2021
142	UK141797402344	18.11.2015	4	182	08.02.2022
715	UK141797302588	06.04.2017	3	206	14.01.2022
716	UK141797602584	02.04.2017	3	215	26.01.2022
750	UK141797702592	10.04.2017	3	213	28.02.2022
760	UK141797202636	15.07.2017	3	169	17.03.2022
3107	UK141797403107	21.05.2019	1	178	16.02.2022
3147	UK141797203147	06.07.2019	1	193	28.02.2022
3162	UK141797303162	22.07.2019	1	163	14.03.2022
3195	UK141797103195	05.09.2019	1	170	28.02.2022
3205	UK141797403205	20.09.2019	1	132	07.02.2022
3242	UK141797603242	05.11.2019	1	86	07.02.2022

Treatment

The intervention in this study consisted of two housing modifications to the standard living conditions of the cows. The first resource was a novel object (inflated sailing buoy), suspended within an area of loafing space within the cows building. This novel object was chosen following its suitability of use having been shown in an earlier trial, assessing novel enrichment in dairy cows (See Chapter 3).

The second resource was access to an outdoor yard with a concrete floor. Both groups were provided with an identical outdoor yard. The yard boundaries were constructed from 5 mobile steel gates which were secured in place by interlocking chains between gates and drop bolts. The initial gate was fixed to the building wall whilst the other gate was secured to the access gate to the housing. The outdoor yards measured approximately 54.8m^2. The outdoor yards for Group 1 and Group 2 were situated opposite one another (Figure 5.3). Due to the close proximity both yards provided almost identical outdoor views of the farms slurry collection area, an area of grassland used for storage and other farm buildings. However, the view from the right-hand side of the yard for Group 2 consisted of fields of pasture. The floor was scraped manually when required by both the PhD student and farm staff. A small covering of sand and grit was applied to the ground in icy weather conditions. The gate highlighted in Figure 5.1 shows the entry point to the outdoor yard, which was situated directly at one end of the building requiring no distance to be travelled by the cows to access it. Figure 5.2 displays the outdoor paddocks with the access gates open showing the close proximity to the indoor living space. All food and water were provided inside the building.

Figure 5.1. *Outdoor concrete paddocks provided as a housing modification in the trial. Group 1 (left) Group 2 (right). Access gate to cow housing highlighted in yellow.*

Figure 5.2. *Outdoor concrete paddocks provided as a housing modification in the trial, displaying area with housing gate open. Group 1 (left) Group 2 (right).*

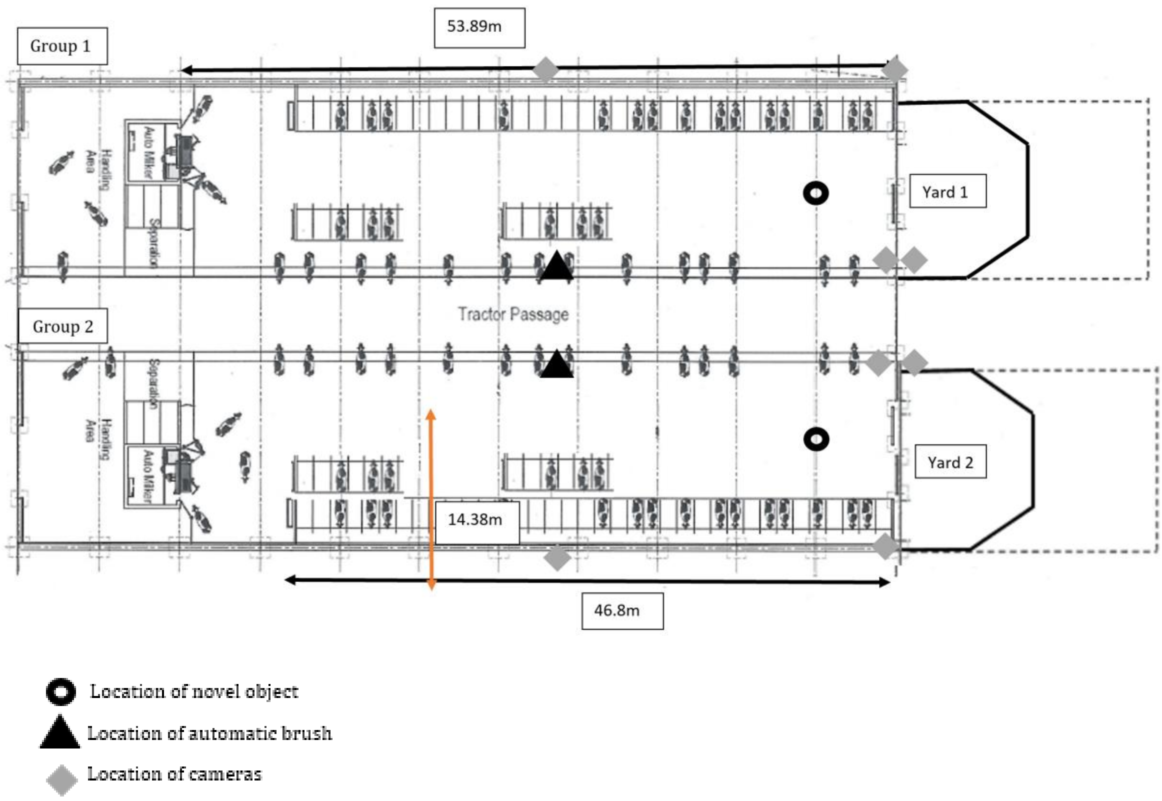


Figure 5.3. *Schematic diagram of experimental cow housing.*

The two groups of cows were tested simultaneously in adjacent buildings (Figure 5.3). Cows were moved into study groups 1 week before the start of the trial, so they had time to acclimatise to new social groups. Cows were housed in standard housing conditions for two weeks to allow baseline observations to be taken. Standard housing conditions were as described in Figure 5.3 but without additional enrichments of the outdoor yard and indoor novel object. Following this baseline period, both groups were given continuous 24-hour access to a different enrichment resource for a period of two weeks. Group 1 were given access to the outdoor concrete yard and Group 2 were given access to a novel object, an inflated sailing buoy suspended from the ceiling of the building. Following this two-week period, both resources were removed and cows remained in standard housing conditions for two weeks. Following this washout period, the initial treatment period was repeated but the resources were reversed, with Group 1 having access to the sailing buoy and Group 2 having access to an identical outdoor concrete paddock. At the end of this two-week period, access to resources was removed and cows were housed in standard conditions for a further two week washout period. Both groups of cows were then given continuous 24-hour access to both resources for a period of 9 weeks. The study ran between the dates: 22.11.2021 – 03.04.2022, the timeline is illustrated in Figure 5.4.

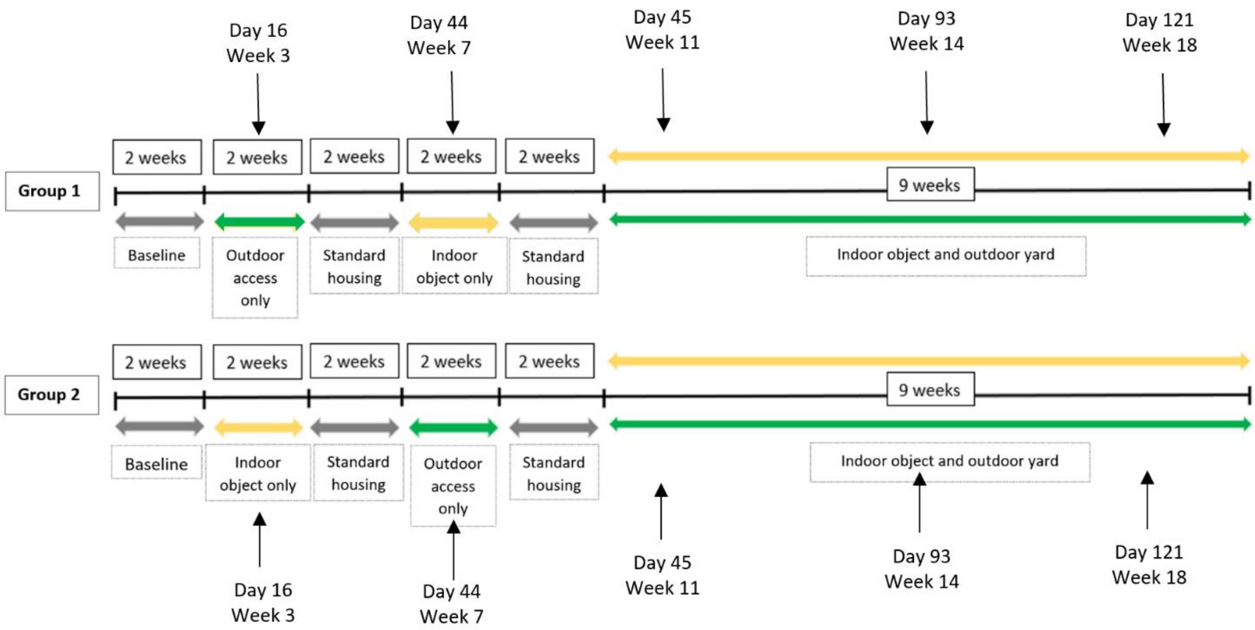


Figure 5.4. *Study timeline detailing intervention periods for Group 1 and Group 2 cows. The study ran for 19 weeks between the dates 22.11.2021 – 03.04.2022. Arrows indicate when behaviour was recorded from sampled video footage. Days indicate days passed since the start of the trial (22.11.2021 – day 1).*

5.2.3 Data sources

Video footage was collected using 4 fixed Axis M1065 IP cameras. These were held in place by movable clamps secured to the barn infrastructure (Figure 5.3.). Camera's were positioned in four separate locations of the building, aimed to give full coverage of the herd's living space. Camera's were connected to a laptop situated within the barn, video files were instantly saved using MediaRecorder. Power over ethernet cabling was used to create a closed system requiring a constant power source to run the cameras. Video footage was collected continuously throughout the study period in 24-hour slots; 24-hour slots were chosen as manageable files for data transfer and earlier trialling of the equipment had shown technical faults when attempting to leave camera's recording for more prolonged periods of time. Video files were removed from the farm laptop and stored on external hard drives.

5.2.4 Behaviour recording

5.2.4.1. Sampling

Use of the outdoor concrete yard and the novel object, were recorded throughout five separate, continuous 24-hour periods. One 24-hour period was sampled during period 1, when Group 1 cows had access to the outdoor area and Group 2 cows had access to the buoy. The same 24-hour period was sampled during period 2 when the treatments had been switched, with Group 1 cows having access to the buoy and Group 2 cows having access to the outdoor concrete yard only. Three 24-hour periods were sampled during the choice phase of the study. The first 24-hour period was taken on the 05.01.2022 (day 45, week 11) during the first week of the choice phase. The next two sampled 24-hour periods were taken on 23.02.2022 (day 93, week 14), during the fourth week of the choice phase and 23.03.2022 (day 121, week 18), during the eighth week of the choice phase. The 24-hour periods were recorded from 00:00-24:00 and chosen to avoid veterinary or husbandry intervention with the cows. All results reported are from these 24-hour clips.

Eleven 3rd and 4th year veterinary science students from The University of Nottingham were recruited and trained to assist with the PhD student coding the behavioural footage from this research trial. Recruited students had an initial in-person training session, followed by a period of coding practise footage reviewed by the PhD student.

5.2.4.2 Use of indoor enrichment

Physical interaction with the buoy was classed as any physical contact of the object, with any part of a cows body. Gaps of less than five seconds in physical contact were classified as the same bout of interaction. If a cow stopped touching the object for more than five seconds, then the next physical contact was classed as a new interaction. Cow ID and length of interaction were recorded for every contact made with the buoy throughout all 24-hour recording periods.

5.2.4.3 Use of the outdoor yard

Use of the outdoor area commenced when a cow put one hoof over the entry line to the outdoor yard (Figure 5.5). A cows time outside then ended the moment its entire body crossed over the entry line into the building. Cow ID and time spent outside were recorded for every visit made outside throughout all 24-hour recording periods.

Figure 5.5. *Classification of the start of a visit to the outdoor space – one hoof crossing over the entry line to the outdoor yard.*

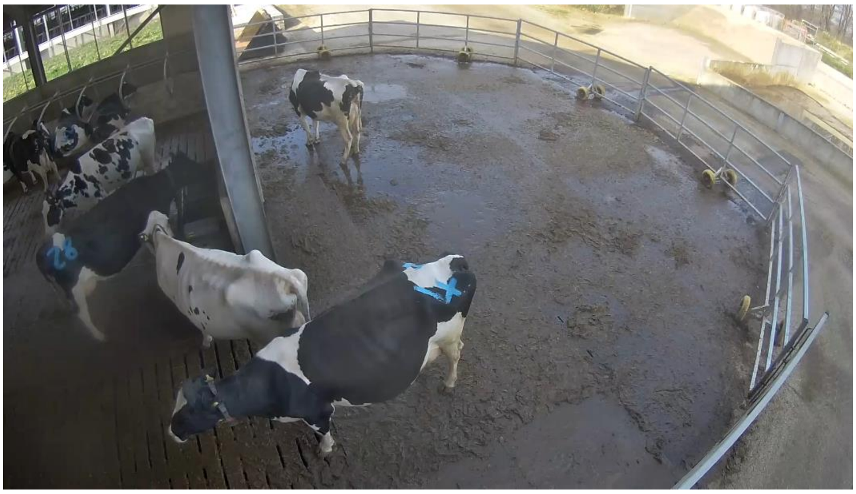


Figure 5.6. *Camera view used to monitor cow use of the outdoor paddock (Group 1), an identical setup was used for Group 2.*

5.2.4.4 Refusals

Data were recorded continuously from the Lely robotic milking system for the entirety of both experimental trials. Records included; animal number, date, time of each visit, milk yield and number of milking refusals. A milking refusal was defined as when a cow entered the robot but was immediately released; this would occur when a cow entered the robot within a minimum expected lapse time as detailed by criteria in Table 5.5. The maximum and minimum number of milking's apply to a 24-hour period. Cows in the 0-40 days post calving category, would have a refusal if they attempted to be milked within 4.8 hours of a previous milking.

Table 5.5. *Lely T4C settings controlling access to be milked.*

	0-40 days post calving	**40 days post calving – 30 days before dry off**	**30 days before dry off - end of lactation**
Maximum number of milking's	5	5	3
Optimum expected yield per miking	8.5	9.5	10.5
Minimum number of milking's	4	2	2

5.2.4.5 Weather recording

The weather was recorded throughout the study in two different ways. Readings were recorded from three "Immonit" weather sensors continuously throughout the study. Immonit sensors record the percentage humidity and temperature (°C), every two hours, throughout 24-hours, providing 12 data points per day. One sensor was positioned above the milking unit inside the building for Group 1 cows. Another sensor was positioned above the milking unit inside the building housing Group 2 cows. The third sensor was secured to the barn outside, within the outdoor paddock for Group 2. Given the small distance between the outdoor paddocks, this sensor was accepted to provide weather details for the overall outdoor area used by both groups of cows. Met Office weather data was also recorded from The University of Nottingham School of Biosciences Sutton Bonington Weather Database. Parameters recorded from this data were mean air temperature (°C), total rainfall (mm), mean wind speed (knots) and mean solar radiation W m-2.

5.2.5 Statistical Analysis

All statistical analyses were performed using RStudio version 4.1.2 using packages readr (Wickham, Hester and Bryan., 2022), dplyr (Wickham et al., 2022), tidyverse (Wickham et al., 2019) and lme4 (Bates et al., 2014). The data presented in Figures 5.7 – 5.17 were plotted using the package ggplot2 (Wickham, 2016). Results are reported as the total and mean time per cow, spent using enrichment resources. Linear models were used to identify statistical significance between mean time spent using resources per cow, between treatment weeks, using lme4 (Bates et al., 2014). Treatment weeks refer to the continuous 24-hour period of footage from which results were obtained for that week. Results are reported as the mean ± standard deviation. All results are reported with a significance threshold set at $P<0.05$.

5.3 Results

5.3.1 Preference between use of an outdoor yard or an indoor novel object

When cows were provided access to only one resource, both groups of cows spent significantly more time using the outdoor yard than the indoor enrichment. Group 1 cows spent 6.34 ± 4.62 (range: 0 – 17.99) minutes per day interacting with the indoor enrichment compared to 55.67 ± 32.11 (range: 0.00 – 148.62) minutes per day using the outdoor paddock ($t(72)=9.25$; $P<0.05$) (Figure 5.7). Cows spent 0.42% ± 0.33 (range: 0 – 1.25) of the 24-hours using the buoy and 3.67% ± 2.34 (range: 0 – 10.32) of the 24-hour period in the outdoor yard. Group 2 cows spent 10.13 ± 8.66 (range: 0.23 - 35.66) minutes per day interacting with the indoor enrichment compared to 102.26 ± 59.92 (range: 9.67 – 242.88) minutes per day using the outdoor yard ($t(70)=9.13$; $P<0.05$) (Figure 5.7). Group 2 cows spent 0.7% ± 0.60 (range: 0.02 – 2.48) of their day using the buoy and 7.10% ± 4.16 (range: 0.67 – 16.87) outside. For Group 1, 94.87% of the herd used both the buoy and the outdoor yard at least once during the 24-hour period. Every cow in Group 2 used both the buoy and the outdoor yard during the 24 hours.

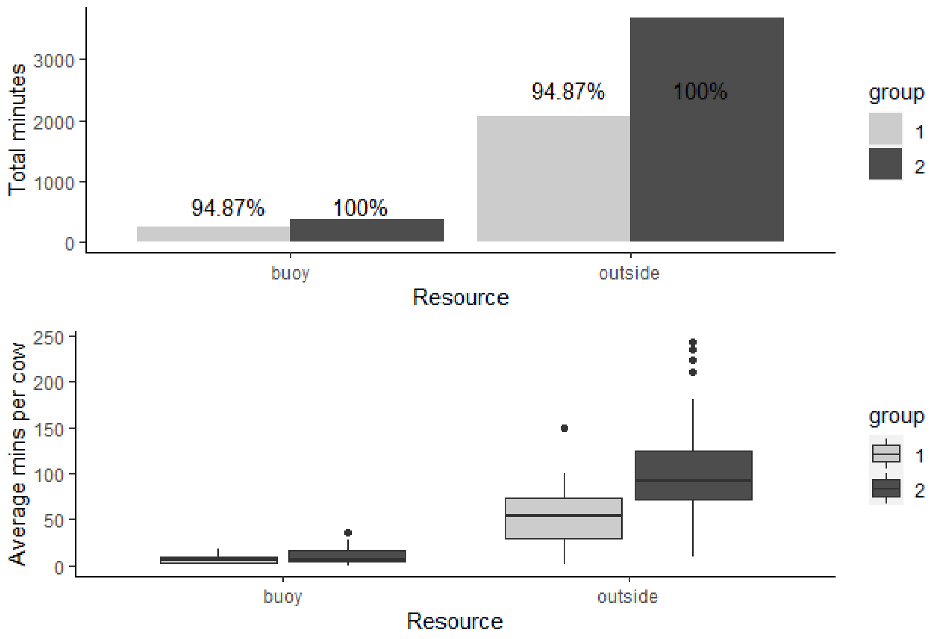


Figure 5.7. *Total minutes spent using the buoy compared to using the outdoor paddock when only one resource was available, with annotations indicating the percentage of the herd using the resource during the 24-hour period (top) and distribution of individual total cow minutes spent using the buoy compared to the outdoor paddock when only one resource was available (bottom).*

In contrast, both groups of cows made significantly more separate interactions with the buoy than visits outside. Group 1 cows made 9.38 ± 5.72 (range: 2 - 24) separate interactions per day with the indoor enrichment compared to 4.68 ± 2.15 (range: 1 - 9) separate visits outside ($t(72)=-4.69$; $P<0.05$) (Figure 5.8). Group 2 cows made 14.81 ± 10.76 (range: 1 - 41) separate interactions with the indoor enrichment compared to 5.94 ± 2.56 (range: 2 - 14) separate visits outside ($t(70)=-4.81$; $P<0.05$) (Figure 5.8).

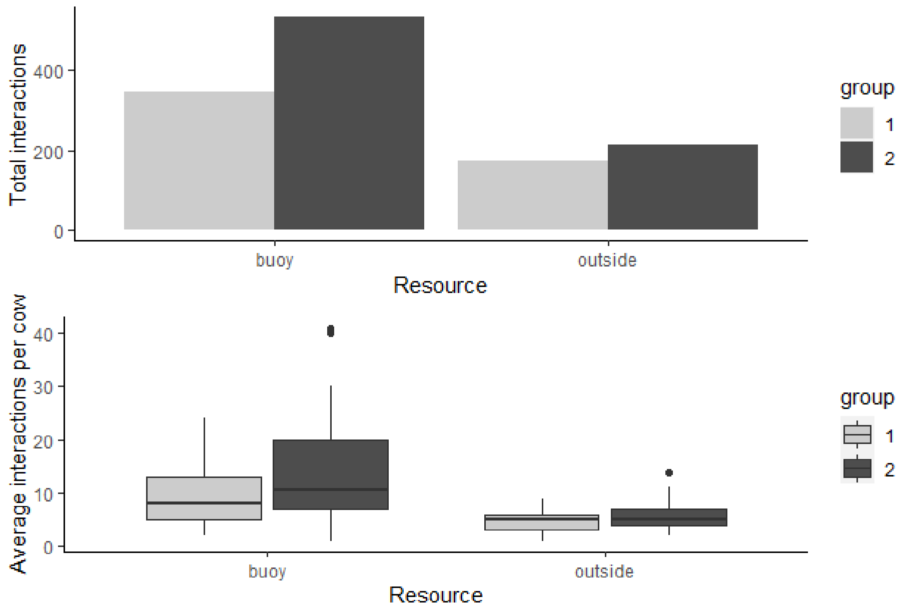


Figure 5.8. *Total individual interactions with the buoy compared to visits outside when only one resource was available (top) and distribution of individual total cow interactions with the buoy compared to visits outside when only one resource was available (bottom).*

5.3.2 Use of resources over time during the choice phase

When compared to the first treatment period when cows only had access to the buoy, Group 1 cows used the buoy significantly less by weeks 4 and 8 of the choice phase when they had access to both resources (t(141)=-3.27 (week 4), -3.20 (week 8); P<0.05) (Figure 5.9). There was no significant change in time spent using the buoy across the 3 sampled choice weeks. During week 1 of the choice phase Group 1 cows spent 4.91 ± 5.41 (range: 0.00 – 23.13) minutes per day using the buoy (0.32% ± 0.37 per day, range: 0 – 1.61), this decreased to 3.12 ± 3.15 (range: 0.00 – 14.40) minutes per day by choice week 4 (0.21% ± 0.22 per day, range: 0.00 – 0.83) and 3.12 ± 3.27 (range: 0.00 – 10.96) minutes per day by choice week 8 (0.20% ± 0.22 per day, range: 0.00 – 7.99) (Figure 5.9). This pattern was reflected in separate individual interactions, with significantly fewer being made by weeks 4 and 8 of the choice phase compared to the initial period when they only had access to the buoy (t(141)=-

3.63 (week 4), -3.84 (week 8); $P<0.05$). Percentage of the group using the buoy remained constant from the first week they had access to it to week 4 of the choice period, with 94.87% of cows using it, this decreased to 87.18% in the final week of the choice period.

Group 2 cows used the buoy significantly less during the last choice phase compared to the initial week when they just had access to the buoy ($t(134)=-4.81$; $P<0.05$) and made significantly less interactions with it ($t(134)=-4.81$; $P<0.05$) (Figure 5.9). During the choice phase, Group 2 cows used the buoy significantly less by week 8 compared to the first week ($t(99)=-5.10$; $P<0.05$). During week 1 of the choice phase Group 2 cows spent 9.6 ± 7.58 (range: 0.00 – 24.66) minutes per day using the buoy (0.44% ± 0.40 per day, range: 0.00 – 1.71), this decreased to 7.15 ± 6 (range: 0.00 – 19.52) minutes per day by choice week 4 (0.43% ± 0.37 per day, range: 0.00 – 1.36) and 2.3 ± 2.66 (range: 0.00 – 12.63) minutes per day by choice week 8 (0.14% ± 0.18 per day, range: 0.00 – 0.88). The percentage of Group 2 cows that used the buoy decreased each week, during the first week that cows had access to just the buoy, all cows used it, this decreased to 86.11% of the group by the final week of the choice phase (Figure 5.8).

Group 2 cows spent significantly more time interacting with the buoy than Group 1 cows, in both the initial period when cows only had access to the buoy ($t(71)=2.34$; $P<0.05$) and during the first and fourth choice week ($t(70)=3.04$ (week 1), 3.59 (week 4); $P<0.05$) (Figure 5.9).

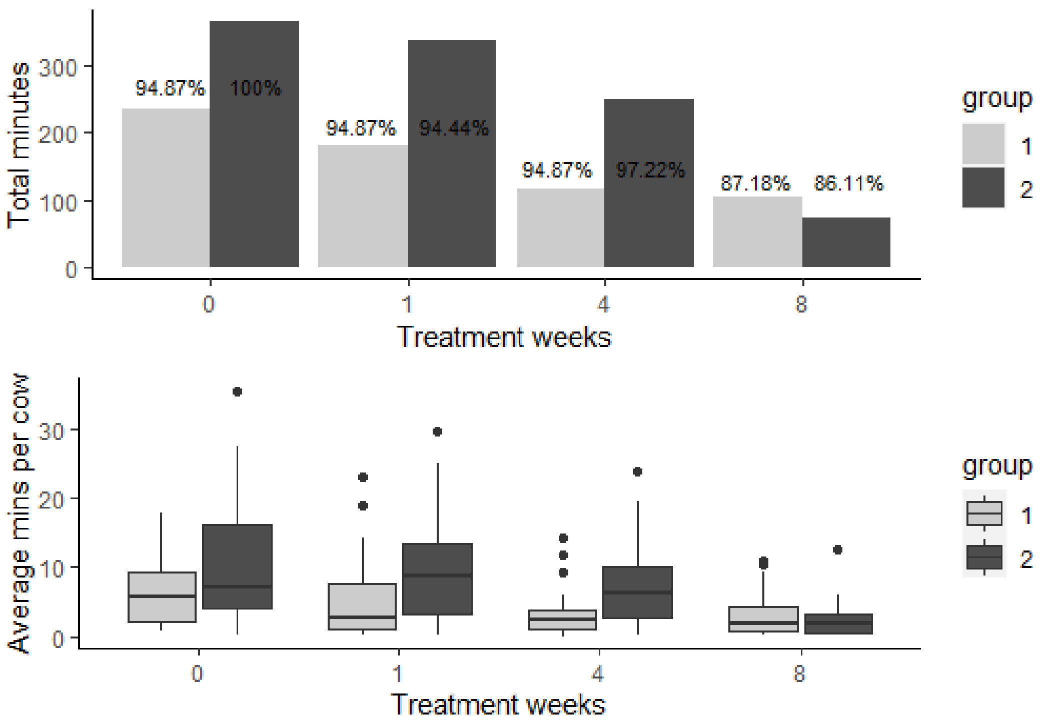


Figure 5.9. *Total minutes spent using the buoy across treatment weeks, with annotations indicating the percentage of the herd using the resource during that week (top) and distribution of individual total cow minutes spent using the buoy across treatment weeks (bottom). Week 0 represents the treatment period when cows only had access to the buoy, weeks 1, 4 and 8 represent the choice phase, where both resources were simultaneously available.*

Group 1 cows used the outdoor yard significantly more during choice weeks 1 and 4 compared to the initial period when they only had access to the outdoor yard (t(146)=3.92 (week 1), 5.29 (week 4); P<0.05) (Figure 5.10). Cows also made significantly more separate visits to the outdoor area during choice weeks 1 and 4 (t(146)=3.19 (week 1), 3.04 (week 4); P<0.05). There was no significant difference in use between how much time Group 1 cows spent outside between the initial treatment period when they only had access to outdoors and the final week of the choice phase. Group 1 cows spent significantly less time outside during week 8 of the choice phase compared to week 1 (t(110)=-3.67; P<0.05). During week 1 of the choice phase, Group 1 cows spent 98.37 ± 57.57 (range: 0.00 – 243.96) minutes outside per day, this increased to 113.26 ± 59.17 (range: 0.00 – 291.01) by choice week 4 and decreased to 55.06 ± 31.32 (range: 0.00 – 166.68) by choice week 8 (Figure 5.10). In terms of observable time throughout the 24-hour period, this was 6.66% ± 4.09 (range: 0.00 – 16.94) during choice week 1, 7.66% ± 4.25 (range: 0.00 – 20.21) during choice week 4 and 3.63% ± 2.28 (range: 0.00 – 8.81) during the final week of the choice phase. The percentage of Group 1 cows using the outdoor yard increased to 97.44% in choice week 1 and 4 compared to 94.87% in the initial access week, this decreased back to 94.87% in the final choice week.

Group 2 cows used the outdoor space for significantly less time and made significantly fewer visits during week 4 of the choice phase compared to both week 1 of the choice phase $(t(102)=-4.24; P<0.05)$ and the initial treatment period when they solely had access to the outdoor yard $(t(137)=-3.04; P<0.05)$. There was no significant difference in how much time cows spent outside during week 8 of the choice phase compared to week 1 of the choice phase or the initial treatment period where cows only had access to the outdoor yard. During week 1 of the choice phase, Group 2 cows spent 114.38 ± 55.28 (range: 0.00 – 235.18) minutes outside per day (7.72% ± 4.01 per day, range: 0.00 – 16.33), this decreased to 64.85 ± 43.54 (range: 0.00 – 154.57) by choice week 4 (4.38% ± 3.07 per day, range: 0.00 – 10.73) and increased to 91.46 ± 47.02 (range: 0.00 – 194.68) by choice week 8 (6.18% ± 3.39 per day, range: 0.00 – 13.52) (Figure 5.10). All cows used the outdoor yard during the initial week when they only had access to the yard, this decreased to 97.44% during the first choice week and remained constant throughout choice weeks 4 and 8.

When cows were given free access to both resources, both groups of cows showed a clear preference for using the outdoor paddock, with both groups spending significantly more time outside than using the buoy across choice weeks 1, 4 and 8;

Group 1: week 1 (t(73)=9.83; P<0.05), week 4 (t(73)=11.30; P<0.05), week 8 (t(69)= 9.61; P<0.05). Group 2: week 1 (t(68)=11.11; P<0.05), week 4 (t(68)=7.77; P<0.05), week 8 (t(65)= 10.70; P<0.05).

Group 2 cows spent significantly more time outside compared to Group 1 cows during the initial period when both groups only had access to the outdoor paddock (t(71)=4.16; P<0.05) (Figure 5.10). Group 1 cows then spent significantly more time outside compared to Group 2 cows during choice week 4 (t(71)=-3.95; P<0.05). Group 2 cows then spent significantly more time outside compared to Group 1 in the final week of the choice phase (t(70)=3.89; P<0.05) (Figure 5.10).

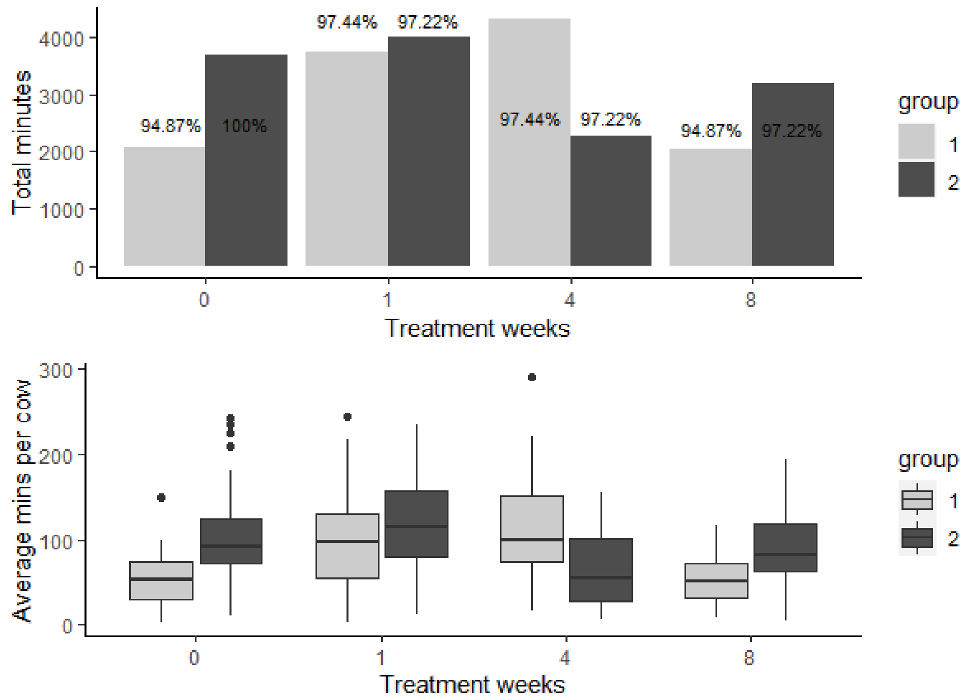


Figure 5.10. *Total minutes spent using the outdoor yard during treatment weeks, with annotations indicating the percentage of the herd using the resource during that week (top) and distribution of individual total cow minutes spent outside across treatment weeks (bottom). Week 0 represents the treatment period when cows only had access to the outdoor yard, weeks 1, 4 and 8 represent the choice phase, where both resources were simultaneously available.*

Figure 5.11 displays the variability of the distribution of total time spent using each resource as time progressed.

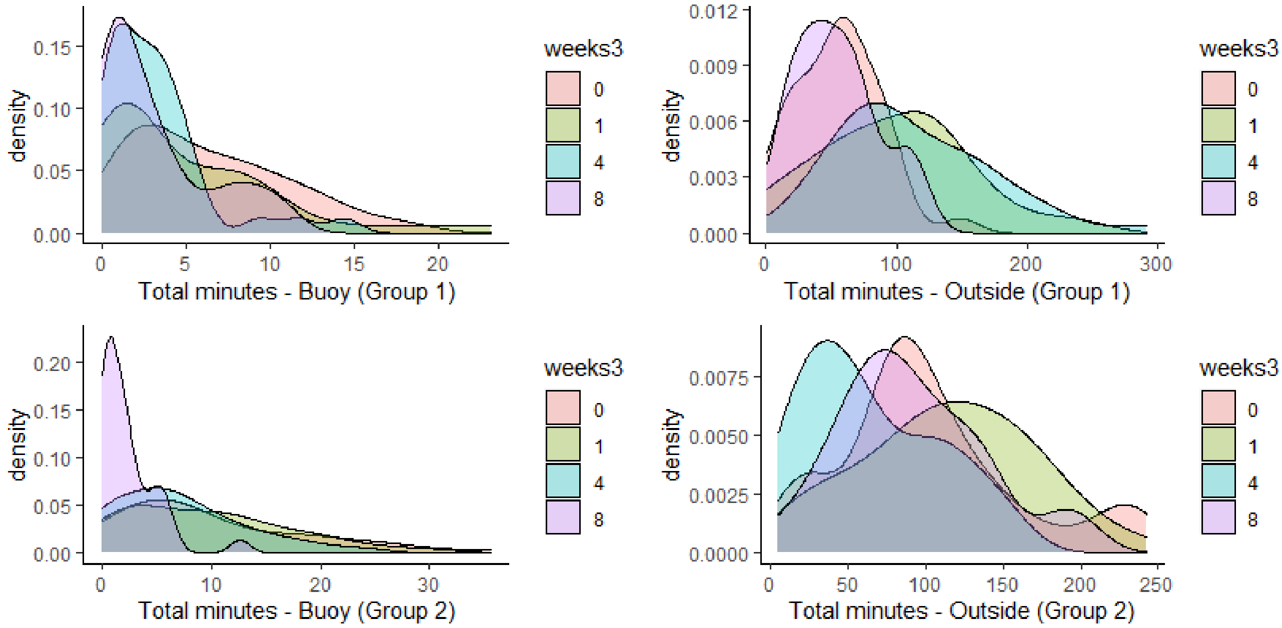


Figure 5.11. *Distributions of the total time cows spent using each resource across treatment weeks. Week 0 represents the treatment period when cows only had access to the outdoor yard, weeks 1, 4 and 8 represent the choice phase, where both resources were simultaneously available.*

Compared to when Group 1 only had access to outdoors, cows spent significantly more time occupied with enrichment when two resources were available, in choice weeks 1 and 4 (t(183)= week 1 (4.78), week 4 (6.12); P<0.05) (Figure 5.11). Total minutes the herd spent occupied with enrichment increased by 90.29% during choice week 1, 114.52% during choice week 4 and 4.03% during choice week 8 when two enrichment resources were available. Similarly, compared to when Group 1 only had access to the buoy, cows spent significantly more time occupied with enrichment every week of the choice phase (t(147)= week 1 (9.26), week 4 (10.52), week 8

(4.79); P<0.05) (Figure 5.12). Total enrichment occupation time increased by 1568.09% (choice week 1), 1780.43% (choice week 4) and 811.92% (choice week 8).

The same trend was displayed by Group 2 cows, which spent significantly more time occupied with enrichment across all choice weeks when two resources were present compared to when they just had access to the novel object (t(173)= week 1 (10.05), week 4 (5.32), week 8 (7.36); P<0.05) (Figure 5.12). Total enrichment occupation time increased by 1089.04% (choice week 1), 590.41% (choice week 2) and 797.26% (choice week 8). Compared to when they only had access to outdoors, Group 2 cows spent significantly less time occupied with enrichment in choice week 4 (t(138)=-2.57; P<0.05) (Figure 5.11). Total time spent occupied with enrichment increased in the first week cows had access to both resources (17.87%), however this decreased in the middle (-31.56%) and last choice week (-11.05%).

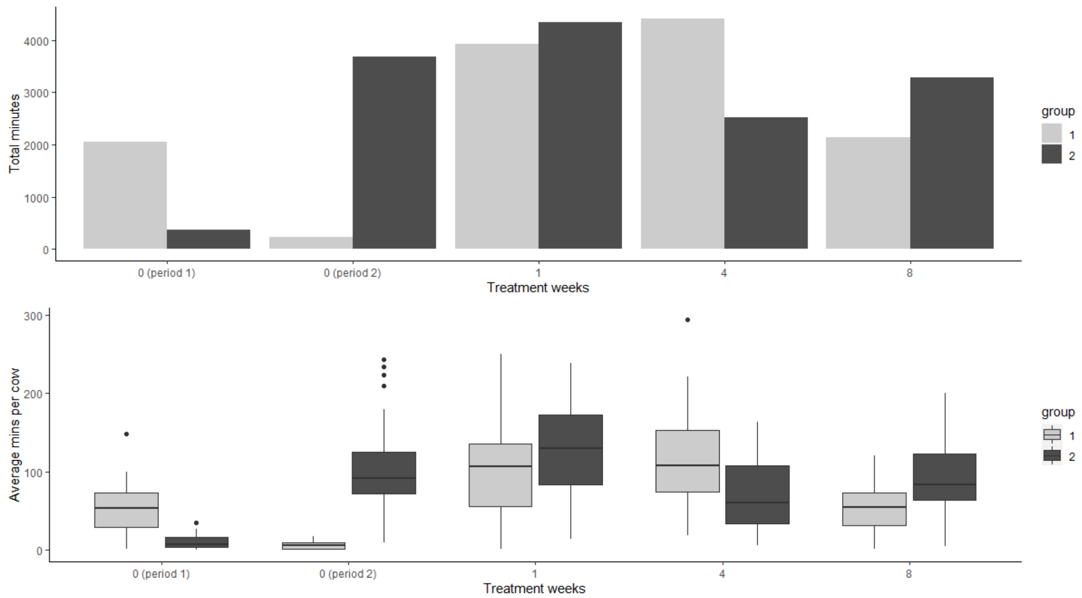


Figure 5.12. *Total minutes spent interacting with enrichment (either buoy or outdoors) during treatment weeks and distribution of individual total cow minutes spent interacting with enrichment across treatment weeks (bottom). Week 0 (period 1) represents the treatment period when Group 1 cows only had access to the outdoor yard and Group 2 cows only had access to indoor enrichment. Week 0 (period 2)*

represents the period when Group 1 cows only had access to the indoor enrichment and Group 2 cows only had access to the outdoor yard. Weeks 1, 4 and 8 represent the choice phase, where both resources were simultaneously available.

A positive linear relationship was shown between individual cows total time spent using the buoy and time spent outside for both groups (Group 1: t(34)=2.04, Group 2: t(34)=3.14 P<0.05) (Figure 5.13).

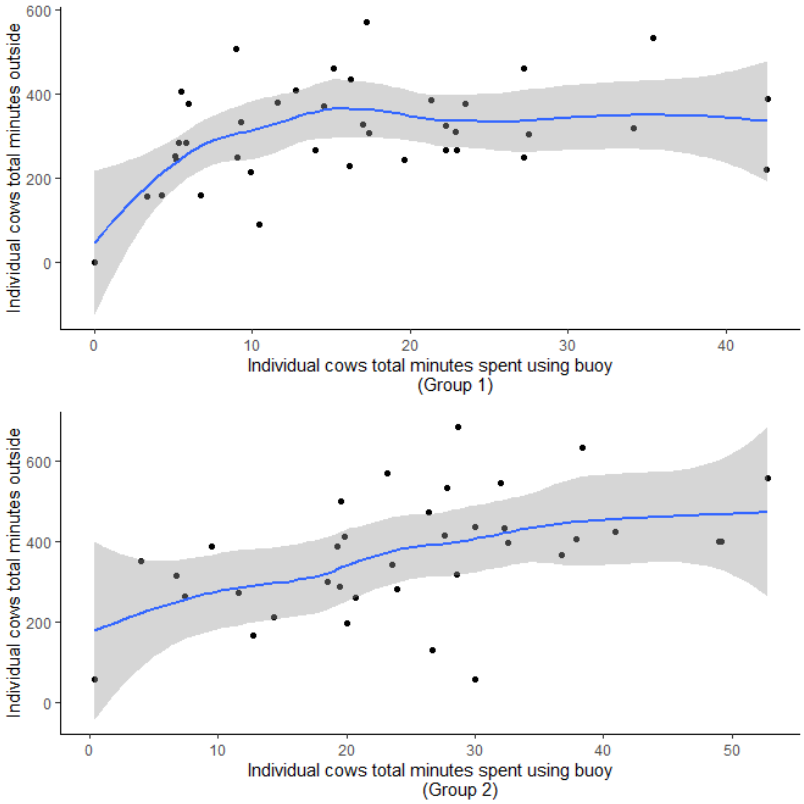


Figure 5.13. *Scatter graph displaying the relationship between individual cows' total time spent using the buoy and the outdoor paddock.*

5.3.3 Weather

During the outdoor access only period, the mean (±SD) temperature whilst Group 1 had access to outside was 5.21 ± 1.23°C. For Group 2, this was 1.90 ± 3.07°C. The mean temperature during the choice phase was; 9.58 ± 1.27°C choice week 1, 7.18 ± 3.12°C choice week 2 and 8.16 ± 6.66°C during choice week 3 (Figure 5.14). The temperature was significantly lower during the outdoor access only period for Group

2 cows compared to the outdoor access only period for Group 1 cows (t(55)=-2.63; P<0.05). The temperature was significantly higher during choice week 1 compared to the initial week that Group 1 cows had access to the outdoor yard (week 0 (period 1)) (t(55)=2.93; P<0.05). The difference in temperatures during choice weeks 4 and 8 compared to 0 (period 1) were non significant. The temperature was significantly higher during all choice weeks compared to week 0 (period 2), when only Group 2 cows had outdoor access (t(44)= choice week 1 (5.03), choice week 4 (3.57), choice week 8 (4.17); P<0.05). Differences in the percentage of humidity across the study period were non significant (Figure 5.14).

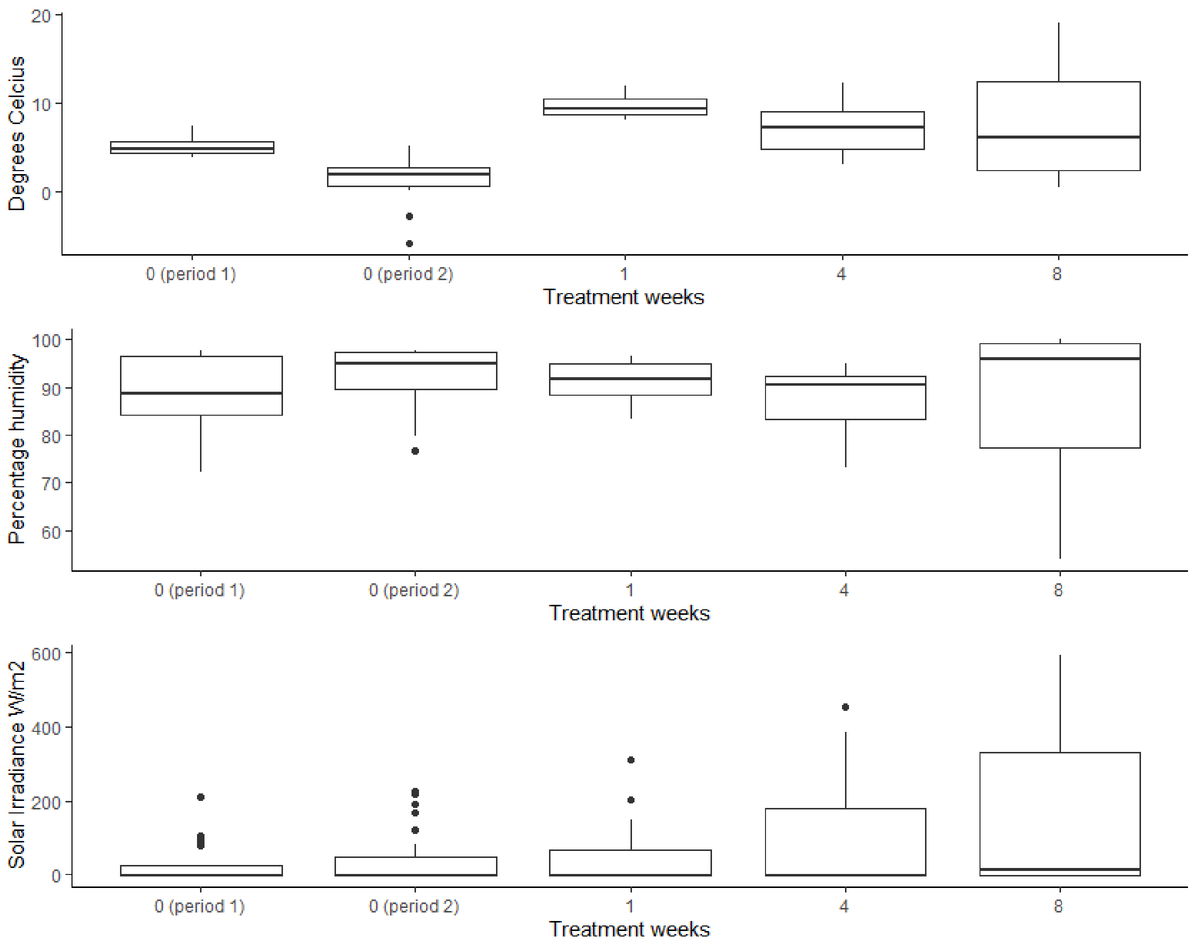


Figure 5.14. *Distribution of temperature, humidity and solar radiation values throughout treatment weeks. Week 0 (period 1) represents the treatment period when Group 1 cows only had access to the outdoor yard and Group 2 cows only had access to indoor enrichment. Week 0 (period 2) represents the period when Group 1 cows*

only had access to the indoor enrichment and Group 2 cows only had access to the outdoor yard. Weeks 1, 4 and 8 represent the choice phase, where both resources were simultaneously available.

During the outdoor access only period, the mean (±SD) solar irradiance whilst Group 1 had access to outside was 27.48 ± 54.12 W/m^2. For Group 2 this was 42.49 ± 79.25 W/m^2. The mean solar irradiance during the choice phase was; 44.36 ± 80.86 W/m^2 choice week 1, 100.36 ± 155.51 W/m^2 choice week 2 and 163.60 ± 225.79 W/m^2 during choice week 3 (Figure 5.14). The solar irradiance was significantly higher during the final choice week compared to both week 0 (period 1) when Group 1 cows only had access to the outdoor yard and week 0 (period 2) when Group 2 only had access to the outdoor yard (week 0 (period 1): $t(115)=3.50$; week 0 (period 2): $t(92)=2.83$ ($P<0.05$)).

5.3.4 Time of day

Group 1 cows spent significantly more time interacting with the buoy between the hours of 06:00 and 12:00 compared to the periods 00:00-06:00 ($t(94)=3.95$; $P<0.05$), 12:00-18:00 ($t(70)=-2.12$; $P<0.05$) and 18:00-24:00 ($t(70)=-2.56$; $P<0.05$), during the initial week they had access to just the buoy. There were no significant differences in buoy use across these 4 periods of the day in any of the other weeks (Figure 5.15). Group 2 cows spent significantly more time using the buoy between the hours of 18:00-24:00 ($t(84)=2.29$; $P<0.05$) compared to the period 06:00-12:00 during the first choice week. No differences were shown between how much Group 2 cows used the buoy throughout these different periods of the day during the other intervention weeks (Figure 5.15).

Group 1 cows spent most time outside between the hours of 06:00 and 12:00 compared to the periods 00:00-06:00 ($t(90)=3.20$; $P<0.05$) , 12:00-18:00 (t(84)=-

3.02; P<0.05) and 18:00-24:00 (t(84)=-2.12; P<0.05) during the middle choice week. They spent significantly more time outside during the hours 12:00-18:00 compared to the period 00:00-06:00 (t(74)=2.14; P<0.05) and significantly more time outside during the later half of the day (12:00-18:00 and 18:00-24:00) compared to the later half of the morning (06:00-12:00) (t(70)=3.39, 2.14; P<0.05), during the final week of the choice phase. Group 2 cows spent the least time outside during the night (00:00-06:00) compared to the rest of the day (t(86) 06:00-12:00 =2.29, 12:00-18:00=3.61, 18:00-00:00=3.52; P<0.05) during the initial week when cows only had access to the outdoor space. Group 2 cows also spent significantly more time outside between the hours of 06:00-12:00 compared to 00:00-06:00 and 12:00-18:00 in both choice weeks 1 (t(101)=2.71, t(91)=2.24; P<0.05) and 4 (t(72)=3.63, t(91)=-4.52; P<0.05). During the final choice week, Group 2 cows spent significantly more time outside during the afternoon (12:00-18:00) compared to the other parts of the day (00:00-06:00 t(81)=3.11, 06:00-12:00 t(76)=2.86, 18:00-00:00 t(52)=-2.23) (Figure 5.16).

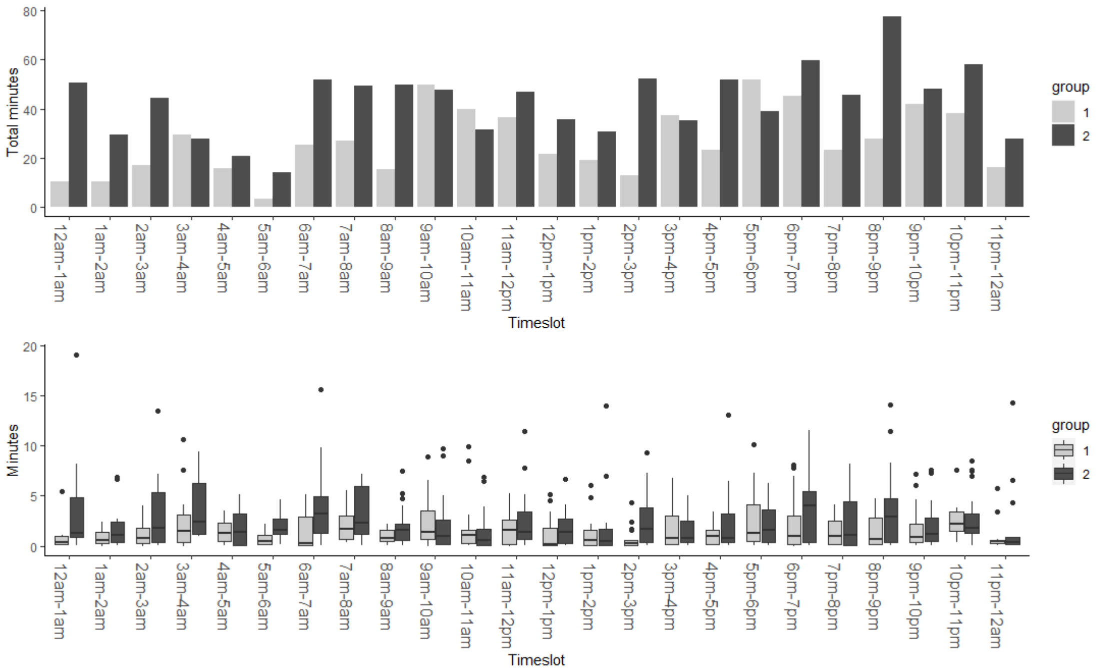


Figure 5.15. *Distribution of buoy use over time throughout all monitored 24-hour periods of the study. Total minutes (top) and mean minutes per cow (bottom).*

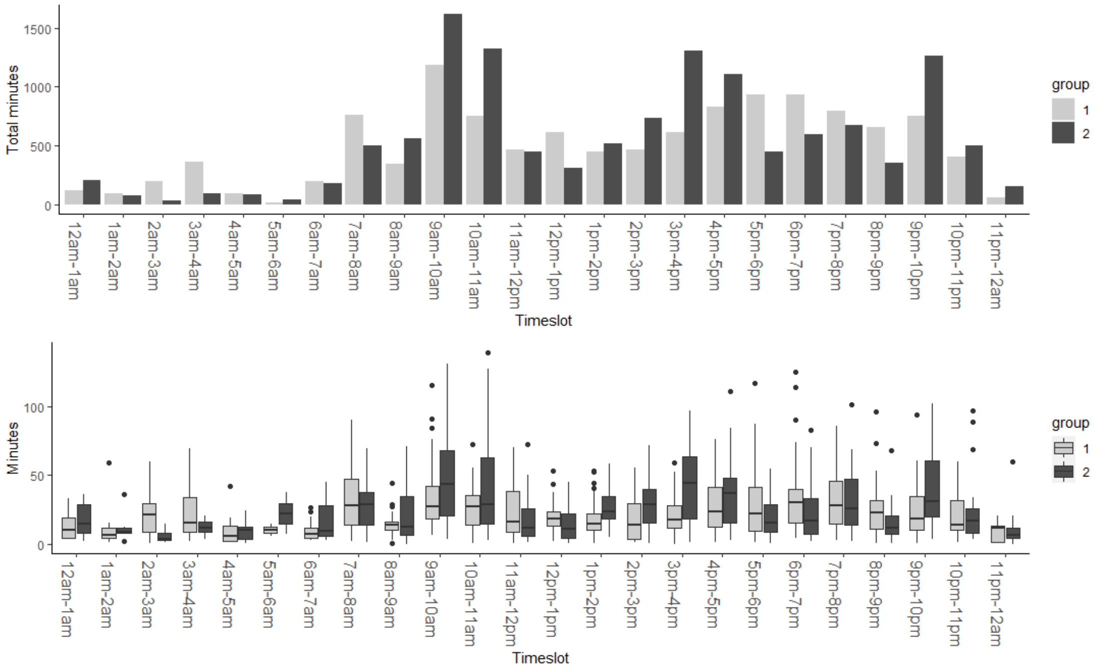


Figure 5.16. *Distribution of use of the outdoor yard throughout all monitored 24-hour periods of the study. Total minutes (top) and mean minutes per cow (bottom).*

Table 5.6 displays the hour slots throughout all monitored 24-hour periods, when at least one cow used one of the enrichment resources. For both groups of cows, during the first week they had access to the buoy, it was in use by at least one cow during every hour of the monitored 24-hour period. This decreased to 95.83% of the 24-hour period by the final week of the choice phase for Group 1 cows and to 91.67% for Group 2 cows. At least one cow from Group 1 used the outdoor yard throughout 91.67% of the 24-hour period in week 0 compared to 87.50% during the final week of the choice phase. Hour slots throughout 24 remained constant for Group 2 cows between the first week they had access to the outdoor yard and the final choice week at 95.83%.

Table 5.6. *Summary of the number of hour slots throughout the day when each resource was used by at least one cow.*

Treatment period	Robot 7, hour slots out of 24 buoy in use.	Robot 8, hour slots out of 24 buoy in use.	Robot 7, hours slots out of 24 outdoor area in use.	Robot 8, hours slots out of 24 outdoor area in use.
Week 0 (period 1)	-	24	22	-
Week 0 (period 2)	24	-	-	23
Week 1	24	24	24	24
Week 4	23	24	23	21
Week 8	23	22	21	23

5.3.5 Resource use by cows in different lactation groups

Use of both resources by cows from different lactation groups was relatively stable. There was no difference in use of the buoy between cows of different parities in Group 1 across the study. Cows in their third lactation from Group 2 used the buoy significantly less (21.52 ± 10.29 minutes) than cows in their first lactation (34.54 ± 14.94) ($t(32)=-2.21$; $P<0.05$) (Figure 5.17). There was no difference in Group 2 cows' use of the outdoor paddock across lactation groups, however for Group 1, cows in their second lactation used it significantly more (357.1 ± 110.72) than cows in their first lactation (271.9 ± 75.52) ($t(34)=2.04$; $P<0.05$) (Figure 5.17).

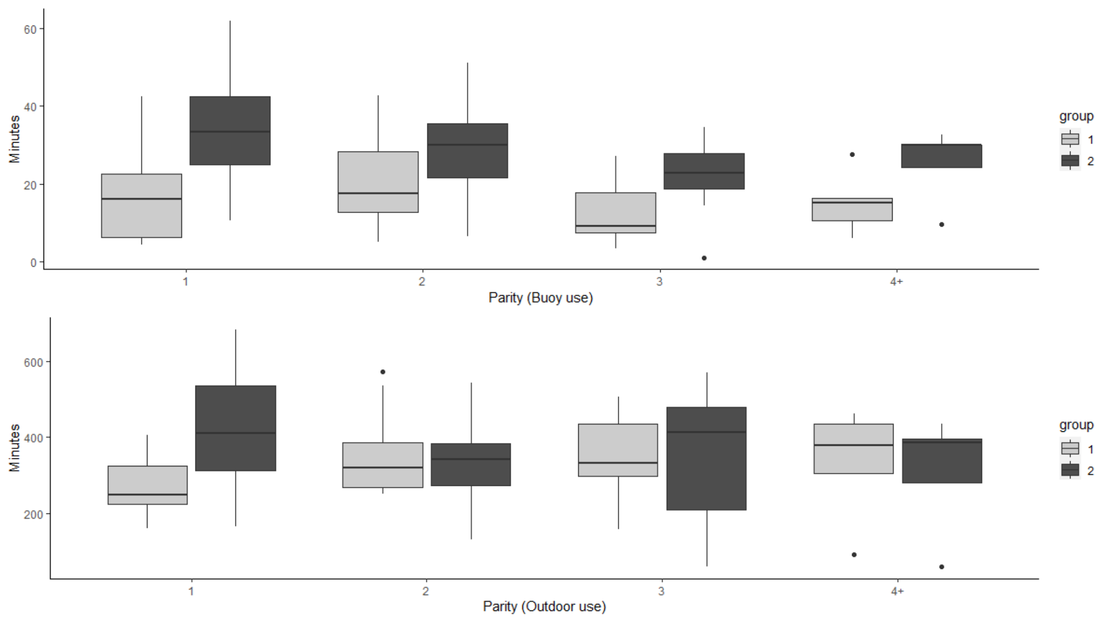


Figure 5.17. *Distribution of mean time spent using both resources between different lactation groups.*

5.4 Discussion

5.4.1 Use of enrichment over time

Time spent using the novel object in this study, was comparable to that of a brush during two weeks post installation, in a previous study (DeVries et al., 2007). When compared to how much time cows spent using a brush - 6.76 minutes per day (DeVries et al., 2007), time spent using the buoy during the initial week was comparable for Group 1, at 6.34 minutes per day, and higher for Group 2 cows at 10.12 minutes per day. Group 2 cows were still using the novel object for longer than that of the installed brush by DeVries et al. (2007) during the fourth week of the choice phase, at 7.15 minutes per day, with Group 1 having decreased their use to 3.12 minutes per day. The study by DeVries et al. (2007) only evaluated brush use during the initial 2 weeks following installation, likely representative of higher levels of use, before a certain level of habituation occurs. Keeling et al. (2016) evaluated use of an already established brush throughout 9 weeks and found first lactation cows to use it on average for 2.3 minutes a day compared to third and fourth lactation cows that used it for 4.47 minutes per day. These slightly lower durations of daily use compared to the study by DeVries et al. (2007) reflect a more accurate general daily use of brushes by dairy cows, again comparable to that shown by the cows in this study with the novel object, with Group 1 cows still using it for 3.12 minutes per day and Group 2 cows, 2.3 minutes per day by week 8 of the choice phase. A notable point is that the cows in the study by Keeling et al. (2016) only had access to one form of enrichment, the brush, whereas the cows in this study were given access to two additional sources of enrichment alongside having access to an already established brush. The cows in this study had substantially more time filling activities available to them, spending on average an hour outside per day alongside any buoy and brush use. This questions whether the cows in the study by Keeling et al. (2016) could have been using the brushes at a heightened level, compared to how cows were using the buoy in the present study. These results show that although there was a decline in time spent using the novel enrichment in this study over a two month period, it was still being used by the majority of both groups by the end of the study (Group 1: 87.18%, Group 2:86.11%).

5.4.2 Do cows show a preference between use of an outdoor yard and indoor novel enrichment?

When cows were given simultaneous free access to two different forms of enrichment, both groups of cows showed a distinct preference for an outdoor concrete yard, compared to a novel object hung inside the barn, in terms of how much time they spent using it. The outdoor yard may have represented a more species-specific behavioural opportunity to the cows than the indoor novel object, through offering the ability to be outside. Research has shown that dairy cows value access to outdoor pasture (Charlton et al., 2013; von Keyserlingk et al., 2017), however what specific qualities of outdoor pasture are the driving motivation for cows is relatively unknown, for example whether it is the specific ability to graze, thermoregulate, daylight or environmental diversity. Smid et al. (2018) explored this by evaluating dairy cows' preference to two different forms of outdoor access, pasture and an outdoor sand bedded pack. Cows initially had a period of access to only one outdoor area at a time and showed a strong preference for pasture. When given simultaneous access to both outdoor areas, this preference for pasture was further exemplified, with cows spending 90.5% of their time on pasture compared to 0.8% of their time on the outdoor pack.

Cows prefer outdoor access with pasture when compared to concrete floored outdoor areas (Langford et al., 2021). When given free choice between these areas, more lying behaviour was observed at pasture (69%) compared to concrete (0%) and this behaviour was weather dependent, with cows choosing to lie on pasture in dry conditions but in the house when the ground was wet, showing that having appropriate lying areas is a factor involved in cows motivation to be outside. Haskell et al. (2013) investigated dairy cows use of a roofed, concrete floored loafing area, specifically how dominance rank, weather and altering views affected this. Access to a view to surrounding fields did not affect how much time cows spent outside, however cows used the loafing area more in sunny weather compared to rain and increased their use when the temperature-humidity index rose inside, highlighting that thermoregulation is likely an important aspect of cows' choice to be indoors or outside. Alongside this, authors found more subordinate cows to be in the loafing area more frequently during feeding times, likely a way of avoiding agonistic interactions which occur most frequently at feeding times (Foris et al., 2019; Olofsson, 1999).

Providing different living areas is therefore likely beneficial to cows in reducing stress associated with social interactions.

The literature shows the complicated interplay of factors involved with outdoor use in dairy cows. The outdoor yard provided in this study offered no other resources than solely the experience of being outside, with no availability of feed, water or lying opportunities, suggesting that having the opportunity to be outdoors is a valuable experience to the cows. Von Keyserlingk et al. (2017) showed that dairy cows would work as hard to access outdoor pasture as they would to access fresh feed. It would be beneficial to investigate the level of motivation cows show for access to an outdoor concrete yard in comparison to pasture. This would provide more information on how important it is for cows to have the choice to go outside. Concrete yards may provide a more feasible avenue for providing dairy cows with outdoor access compared to providing outdoor access with pasture. Future research should evaluate cows' motivation to access different forms of outdoor areas offering different qualities.

On the converse to the significantly less time cows spent using the buoy compared to the outdoor yard, significantly more bouts of physical interactions were initiated with the buoy compared to visits made outside, showing that the resources were used differently, and likely provided very different opportunities for the cows. A wider range of resources should be explored to identify opportunities of the most value to housed cows.

Compared to the study by Smid et al. (2018), cows in the current study spent substantially less time outside when they only had access to the outdoor yard (5.49% of the available time) compared to when cows only had access to pasture (90%), or a sand bedded pack (44.4%). Conversely, when cows in the current study were given a choice between an outdoor yard and a novel form of enrichment inside, they spent on average, 6.22% of the available time outdoors, more than the 0.8% of available time that cows spent on the outdoor sand pack, when access to two different outdoor areas were available (Smid et al., 2018). This reduced time spent outdoors by cows in the current study, possibly reflects the lack of behavioural opportunities available to them, as opposed to pasture offering grazing or sand offering softer standing surfaces and lying areas. However, the study by Smid et al. (2018) only provided outdoor access during the night, compared to 24 hours a day in the current study, and for intermittent periods of two to three nights compared to a long-term continuous period in the current study. This could have seen higher levels of outdoor use by the cows, as a rebound effect, resulting from intermittent limited outdoor access. Similarly, Haskell et al. (2013) found cows spent 14% of the observation period in a roofed, open sided, concrete yard, again much higher than the 6.04% cows spent outdoors in the current study. The cows in the study by Haskell et al. (2013) had been at grass until one week prior to the trial starting, when they were moved indoors. Cows may have had an increased motivation to be outdoors, following confinement to housing in this scenario, for example, stereotypical behaviour has been shown to significantly increase following the grazing period compared to before, when cows are returned to housing (Redbo, 1990), possibly an indicator of frustration at the changed environmental situation. The cows in the present study were fully housed with no prior experience of outdoor access. Again, cows had restricted access to the loafing area, 7 hours per day compared to continuous 24-hour access in the current study, which may have increased cow use during these periods of availability. Haskell et al. (2010) also evaluated the variability of cow use of the same loafing space when additional enrichment objects were provided, over a 30-day period, but results were expressed as the proportion of animals which used it and not the time spent using it.

Despite the significant differences shown in time spent using the resources, it's worth noting that at its lowest, 86.11% and 87.18% of both groups of cows still utilised the novel object during any recorded 24-hour period. Enrichment that has not been designed to facilitate species-specific behaviours related to more natural behaviour or a more natural environment have been criticised as likely to be unsuccessful (Newberry, 1995). The novel object used in this study, was chosen on the basis it would have been something novel to cows and it offered no obvious species-specific behavioural function. Despite this, there was a high utilisation rate by both groups of cows which persisted over several months. Cows used the object by physically interacting with it using their head and mouth. The main stereotypical behaviours displayed by dairy cows are oral behaviours - tongue rolling and bar biting (Redbo, 1998; Sambraus, 1985; Wiepkema, 1987), which could be a redirected form of feed searching behaviour (Redbo, 1990). Research has shown increased incidences of stereotypical behaviour in dairy cows during feed restriction (Redbo & Nordblad, 1997; Redbo et al., 1996), housing (Corazzin et al., 2016) and tethering (Redbo, 1992), with zero occurrences whilst at pasture (Redbo, 1990). These associations, paired with the distinct reduction in time housed dairy cows spend eating (Roca-Fernandez et al., 2013), supports the hypothesis that this behaviour could be linked to reduced feeding and feed directed exploratory behaviour. Considering the way cows interacted with the novel object used in this study, it is plausible that it provided an alternative outlet for oral exploratory behaviour for the cows. In pigs, provision of objects which facilitate exploratory and manipulatory activities involving the snout and mouth, are considered valuable enrichment strategies (Van de Weerd et al., 2003), measured by reductions in adverse behaviours (Fraser et al., 1991). It is worth noting that the review by Newberry (1995) defined successful environmental enrichment as something that improves the biological functioning of an animal, specific examples being enhanced reproductive success, fitness or a health correlate. The impact enrichment may have on affective states was touched upon but not incorporated due to the challenges of identification. Given the development of positive animal welfare over the last decade (Lawrence et al., 2019), this should now be regarded as a justifiable inclusion in the measure of success of enrichment strategies. Artificial and novel devices should therefore not be disregarded as an avenue of enrichment, provided they can show enhancements in behaviour or welfare.

Asides from direct implications to cow welfare, availability of outdoor access for dairy cows is a socially contentious topic, with a strong discord between the importance the public places on cows having outdoor access (Cardoso et al., 2016; Schuppli et al., 2014; Ventura et al., 2016), to common dairy cow management practises. Kuhl et al. (2019) evaluated public acceptance of common dairy cow housing systems and found the acceptance of two forms of full housing to be as low as 4% and 17%. Access to an outdoor concrete paddock raised acceptance rates to 55%, with this being 96%, when pasture access was also provided. Words such as outdoor exercise, fresh air and naturalness commonly appear surrounding public perception of pasture access for cows, three qualities which alternative outdoor areas are able to provide (Cardoso et al., 2016; Schuppli et al., 2014; Weinrich et al., 2014). Incorporation of some form of outdoor access, likely to be more achievable to farms than pasture, may present a compromise in terms of offering outdoor access to dairy cows, which may facilitate public acceptance of different housing systems for dairy cows.

One unexpected finding from this study was the relatively consistent difference in use of both resources by the two groups of cows, with Group 2 tending to use both resources for more time across study weeks. Cows were randomly selected for the trial, to provide two matched groups of parity and stage of lactation, to limit group differences due to these factors. The final groups, whom the results are reported for within this study, did change due to cow losses due to veterinary intervention and drying off, however, the groups remained relatively well distributed in terms of age and lactation cycle. Dairy cows show individual behavioural consistency, which is explained by underlying personality structures (Schrader & Müller, 2005). Personality differences are most notably reflected in tests evaluating individuals' response to stress, usually elicited by novel object testing, human interaction, or social isolation (Neave et al., 2020; Neave et al., 2022; Van Reenen et al., 2004). Differences expressed in these situations have also now been linked to the day to day behaviour of cows, such as lying behaviour and robotic milker use (MacKay et al., 2014). It is therefore likely that personality differences played a role in the varying results from the two groups. As far as we're aware, how personality traits in animals affect their use of enrichment has not been explored. One explanation for these results could be that the groups differed in cows with different personality and coping styles.

Another surprising finding from the current study, was the clear preference that cows showed to use the outdoor paddock during the day compared to during the night. This contrasts with the preferences of dairy cows for outdoor pasture access, where a preference is shown to being outside at night (Charlton et al., 2011; Falk et al., 2012; Legrand et al., 2009). Dairy cows also show a strong motivation for pasture access at night and will expend more energy to achieve this, by continuing to walk up to 260 metres to get to pasture, but reducing their time spent at pasture during the day as the distance required to access it increased from 60 to 140 to 260 metres (Charlton et al., 2013). A selection of the studies which evaluated dairy cows use of alternative outdoor concrete loafing areas, either only provided access during the day, or did not mention how use differentiated between the day and night (Haskell et al., 2010; Haskell et al., 2013; Langford et al., 2021). However, Smid et al. (2020), who evaluated how dairy cows used an outdoor sand/wood chip bedded pack according to varying space allowances, still found cows to spend the majority of their time outside at night. Similarly, Smid et al. (2019), found the same results whilst evaluating cows use of an outdoor deep bedded pack across summer and winter, that cows spent more of their time outside at night. Dairy cows display diurnal rhythms and spend the majority of their time lying at night (Ketelaar-de Lauwere et al., 1999; O'Connell et al., 1989; Wierenga & Hopster, 1990; Winckler et al., 2015). As the outdoor space provided in this study offered no appropriate lying areas, it could be that cows motivation for lying down was stronger than their motivation to be outside during the night and therefore likely that if lying areas were provided outside, they would have spent more time outside. Alternatively, as a prey species, the outdoor space could have attracted more use during the day when other cows were also outside. Another possibility is that there was more environmental diversity occurring during the day with farm staff operating around the farm, which attracted the cows' interest to be outside.

5.4.3 Weather and time of day

Throughout the trial the temperature varied within a small range of 7.68°C and there was no significant difference in the percentage of humidity. Cows' use of the outdoor yard did not appear to be impacted by the slight variations in temperature. The temperature was significantly lower during the outdoor access only period for Group

2 cows compared to the same period for Group 1 cows, yet a higher percentage of Group 2 cows used the yard in this period compared to Group 1 cows and they also spent more time outside. Similarly, the temperature was significantly warmer during all choice weeks compared to the outdoor access only period for Group 2 cows, yet the percentage of cows going outside remained almost identical. Group 2 cows' use of the outdoor yard remained stable throughout the study apart from a decrease in use in the second choice week. Weather has been shown to impact cows choice between being inside or outdoors, however this has been observed in response to greater changes in temperature, or more adverse conditions in general, such as snow, strong winds or rain (Charlton et al., 2011; Legrand et al., 2009; Smid et al., 2019), which all appear to deter cows motivation to be outside. The temperature throughout the study remained well within the thermal comfort zone for dairy cows (Armstrong, 1994; Collier et al., 1982) and there were no clear adverse conditions. Given the previous literature showing how weather can impact cows' behaviour suggests that weather would likely be a factor in cows' use of alternative outdoor areas however not during minor deviations in temperature as experienced within the present study.

Smid et al. (2019) investigated dairy cows use of an outdoor sand bedded pack and found cows spent a quarter of their time outside during the summer (June-August) but only 1.8% of their time outside during the winter (October-February), comparably less than the 6.04% of time that cows spent outside in the present study, which ran throughout the winter months (December-March). Given the increase in use shown by Smid et al. (2019) during more favourable summer weather conditions, evaluation of the use of an outdoor concrete loafing paddock should be investigated throughout the remainder of the year.

5.4.4 Limitations

The current study ran from December to March, what might be considered the most aversive time of year for British weather conditions, a period when conventionally most dairy herds are housed. Despite this, the lowest the percentage of individuals within the herd using the outdoor yard dropped to, throughout any recorded period was 94.87%. The study should be replicated to evaluate use of an outdoor concrete loafing yard throughout the remainder of the year.

Another point of note, is that although the outdoor yards provided in this study were identical in terms of size, floor surface and environmental conditions, given their close proximity, the yards may have provided slightly differing views. The yard for Group 1 provided views to the wider farm yard, a grass covered storage area and other buildings. The yard for group 2 provided the same, however on one side, cows also had visual access to adjoining fields and countryside. Haskell et al. (2013) has investigated altering views on dairy cows' use of an outdoor loafing yard and found that cows didn't change how much time they spent using the loafing area between when they had visual access to surrounding pasture fields, or when screens were erected removing this visual access. It's unlikely the small divergence in view between groups impacted how they used the outdoor yards, however it cannot be ruled out. Behavioural coding for the two groups was completed by different individuals, however, all observers had the same training and were coding to the same procedures. This is a limitation of any behavioural study using more than one observer to assess behaviour and could be controlled for in future studies by matching observer coding of different groups. A step above this, would be the use of automated technology for future video analysis, eradicating any observer bias risk, this technique was not incorporated into the current research due to resource availability.

5.5 Conclusions

When dairy cows were provided access to a novel form of indoor enrichment and access to an outdoor concrete yard for a period of 9 weeks, use of the indoor enrichment significantly decreased over time, whereas the amount of time that cows spent outside, varied but remained constant. Cows showed a significant preference for the outdoor yard in terms of how much time they spent using it. When given simultaneous access to both resources, cows still showed a strong preference for use of the concrete outdoor yard. These results show that cows do habituate to novel forms of enrichment over time, reflected with continued but reduced use, but that the level of habituation varies between resource. These results also suggest that access to a simple outdoor area, is valued by dairy cows and therefore provision is likely linked to positive experiences and enhanced welfare. In addition, offering access to an outdoor space, is likely much more beneficial than offering novel forms of indoor enrichment.

Chapter 6: Does providing enrichment impact the affective states of commercially-housed dairy cows? Measured using a positive welfare indicator 'Qualitative Behavioural Assessment'

6.1 Introduction

The intensification of the dairy industry, marked by increased average herd sizes and individual cow yields (DairyCo, 2013) has required utilisation of efficient and modernised production systems, which are often described as intensive and generally associated with negative connotations for animal welfare (Perry, 1983; Woods, 2012). This shift in animal production, has been met with societal concern regarding the welfare of animals managed in these systems (Harrison, 1964). Concerns stem from decreased space allowances (Fraser, 2005; Harrison, 1964;) animals not having the environmental freedom to express a full range of behaviours (Spinka & Wemelsfelder, 2011; Wemelsfelder, 2005;), unavoidable management stressors (LeCorps et al., 2020; LeCorps et al., 2021), boredom and associated negative affective states (Crump et al., 2019; MacLellan et al., 2021) and the cumulative result that these influences have on animals' quality of life. More recently, the welfare concern for specifically, commercially managed dairy cows, has become apparent (Cardoso et al., 2016; Wolf et al., 2016), with as little as 4% of public respondents being accepting of common fully housed production systems (Kuhl et al., 2019). This heightened public interest, has coincided with the development of research regarding animal sentience and intelligence, with the current consensus that animals should not only be protected from suffering but should also have the opportunity for positive experiences throughout their life (Boissy et al., 2007; Harrison, 1964;), to be confident that they have had a life worth living (FAWC, 2009). To be able to address the concerns of the reality of animals' quality of lives and the associated public scrutiny, identification of management and housing conditions which can enhance welfare by promotion of positive states are required. This in turn, requires capable identification of changes in animals' affective states.

Environmental enrichment has been widely implemented in other settings such as pigs, zoo and laboratory animals, with the overarching objective of enrichment strategies being to improve some element of welfare (Newberry, 1995). Diversification of the environment to facilitate exploration and agency, has been suggested as one avenue for offering confined animals positive experiences (Mellor, 2017). Therefore, enrichment interventions are often implemented with the strategic goal of enhancing animals' affective states. This link has started to be explored, with indicators of more positive affective states following either a period of environmental diversity or compared to animals housed in more stimulus diverse conditions in chickens (Anderson et al., 2021; Zidar et al., 2018), pigs (Carreras et al., 2016; Douglas et al., 2012;) and rats (Brydges et al., 2011; Richter et al., 2012). The inverse effect has also been observed in starlings, with a pessimistic bias, indicative of poorer affective states being displayed following removal of enriched conditions (Bateson & Matheson, 2007) and increased negative behavioural decision making in pigs, that had previously spent time in enriched housing and then been transferred to barren housing, compared to pigs that had only ever experienced barren housing (Douglas et al., 2012). Crump et al. (2021) investigated whether pasture access improves emotional states in dairy cows, using judgement bias, which monitors animals' responses to ambiguous situations to infer affective valence (Lagisz et al., 2020). Cows with access to pasture, approached a known food reward slower than cows that were fully housed, authors proposed the explanation was a reduced reward anticipation, generally shown when higher or more frequent rewards are experienced in day-to-day life (Spruijt et al., 2001), concluding that pasture access may facilitate more rewarding lives and therefore better welfare.

Evaluation of animals' affective states is an ongoing complex challenge, yet to be able to assess the success of any interventions aimed at offering opportunities for positive welfare, evaluation of affective states is imperative. One promising potential measure of animals' affective states, when taking into consideration reliability, validity and feasibility, is Qualitative Behavioural Assessment (QBA) (Keeling et al., 2021). Perhaps reflective of the level of insight QBA can offer compared to other suggested positive welfare indicators, is reflected by it being the only measure of positive welfare that has so far been practically incorporated into on farm animal welfare assessments (WelfareQualityNetwork, 2018). The assessment uses observer evaluation and interpretation of animals' expressive demeanour, to formulate quantitative variables indicative of varying affective states (Wemelsfelder, 2007). The technique has been able to distinctly show differences in behavioural expression and therefore interpreted associative affective states in dairy cows infected with mastitis (Des Roches et al., 2018), in both positive and negative social situations (Rousing & Wemelsfelder, 2006) and between cows from tethered and loose housing systems (Popescu et al., 2014). The technique has previously been used to directly evaluate the success of enrichment intervention strategies, with results conducive of enhanced emotional welfare in extensive compared to intensive systems in pigs (Temple et al., 2011), similarly enriched compared to unenriched housing in pigs (Carreras et al., 2016) and dairy goats with access to pasture compared to without (Grosso et al., 2016).

The aim of the current chapter was to evaluate whether offering commercially-housed dairy cows a selection of enrichment opportunities, facilitating environmental diversity, exploration and behavioural agency, would have the achieved goal of positively impacting their affective states. This was assessed by QBA, what can be regarded as one of, at the present moment, the most promising indicators of affective states in animals.

6.2 Materials and methods

6.2.1 Cows and treatment

This study ran concurrently with the study outlined in chapter 5, therefore for exact details of the cows and treatment timeline in this study please see 5.2.2.

6.2.2 Housing, management and diet

This study ran concurrently with the study outlined in chapter 5, therefore for exact details of housing, management and diet, please see 5.2.1.

6.2.3 Data sources

6.2.3.1 Qualitative Behavioural Assessment

6.2.3.1.1 Qualitative Behavioural Assessment Protocol

One trained assessor completed one QBA for both groups of cows, three times per week, during every week of the trial (excluding washout weeks). One QBA assessment was completed for both groups of cows, on Mondays, Wednesdays and Fridays, between 12:30-13:30. These days were chosen to avoid days where any form of human disturbance occurred, such as routine vet or foot trimming visits. The timeslot used to perform the QBA assessments was chosen to avoid any routine management interference with the cows, such as feeding and cleaning. These days and times were therefore assumed to give the best snapshot of the herds natural behaviour.

The QBA assessment protocol and scoring sheet used was taken from the Welfare Quality Network Assessment Protocols for dairy cows (WelfareQualityNetwork, 2018). For each QBA, one viewpoint was used during periods when cows did not have access to outdoors, this was the platform above the Lely robot, which gave a complete and clear view of the entire herd. During the period where cows also had outdoor access, two viewpoints were used, the platform above the Lely robot and the far end of the building which gave visual access to the outdoor yard. The assessor observed the herd for 20 minutes in total, observing the expressive quality of group activity. If the cows were disturbed by the assessor's presence, the assessment would be started a few minutes later when cows had resumed normal activity, this occurred infrequently due to the distance of the viewing platform from the cows. The assessor then moved away from the herd and scored the 20 descriptive terms (Table 6.1), using a visual analogue scale (annexe). The visual analogue scoring system is explained by Welfare Quality Network (2018) – Each VAS is defined by its left 'minimum' and right 'maximum' point. 'Minimum' means that at this point, the expressive quality indicated by the term is entirely absent in any of the animals you have seen. 'Maximum' means that at this point this expressive quality is dominant across all observed animals. Note that it is possible to give more than one term a maximum score; animals could for example be both entirely calm and content. A score was then given for each term, by drawing a line on the assessment sheet on the visual analogue scale, at the point which best represented the level of that descriptive attribute to the herd. Each line point was manually measured in mm from the minimum mark to the given assessment line, resulting in a score between 0 – 125. Terms with positive connotations became more positive as the score increased and terms with negative connotations became more negative as the score became higher. To aid understanding of the terms used in the QBA assessment for dairy cattle from the Welfare Quality Assessment Protocols (WelfareQualityNetwork, 2018), definitions for each descriptor were checked via the Cambridge Dictionary online (Cambridge University Press, 2022). The QBA assessor spent two weeks conducting QBA assessments on cows housed in the experimental buildings as part of training. Following this, a table of behaviourally relevant descriptions were created to implement the QBA terms into the behavioural assessment for dairy cows (Table 6.1).

Table 6.1. *The 20 descriptive terms and their descriptions used in the QBA assessments.*

Terms	**Description**
Active	Cows are moving around the building. May be occupied by feeding, drinking, using the robotic milking machine, queuing at the robot, using the brush, using any other forms of enrichment, self-grooming, participating in social interactions both positive or negative.
Relaxed	Cows unalert and not surveying surroundings. All behaviour being conducted in a quiet and relaxed manner.
Fearful	Cows appear alert, startled, tense. Cows may be actively looking at surroundings particularly where people may be present. Cows may be flighty or jump in the presence of loud noises, sudden movements or appearance of people. Cows may be actively avoiding other cows or removing themselves from negative social interactions.
Agitated	Frequently changing position or activity. May appear uncomfortable. May be involved in negative social interactions.
Calm	Cows are quiet, activity is low, there is little disturbance. Behaviours are being carried out in a sedate manner.
Content	Cows appear in good physical condition, without any health concerns, satisfied with environmental situation, positively interacting with the environment and not displaying any behaviours included in the definition for 'bored'.
Indifferent	Cows do not appear interested in the environment or each other.
Frustrated	Frequently changing position or activity. May appear uncomfortable. May be involved in negative social interactions. Cows repeatedly vocalizing, pacing. Negative social interactions.
Friendly	Overall social interactions between cows are positive. Cows' response to any human sight is relaxed and inquisitive as opposed to alert and fearful.
Bored	Cows are idling (stood stationary, may be looking around or changing position but with no other overt activity). Cow may be ruminating. Excludes cows queuing at the milking robot. Cows are chewing or interacting with building infrastructure (e.g motorway barriers, gates). Cows are repeatedly going through the Lely robot or not entering the robot but interacting/disturbing cows queuing.
Playful	Any play behaviour such as running, hopping, jumping, skipping, head shaking or interacting with enrichment.

Positively occupied	Cows are eating, drinking, lying in cubicles, using the brush, using any other form of enrichment, within or queuing for the Lely robot, moving between activities, involved in positive social interactions. Not included: cows idling, chewing or interacting with building infrastructure (e.g motorway barriers, gates), cows are repeatedly going through the Lely robot or not entering the robot but interacting/disturbing cows queuing and negative social interactions.
Lively	Behaviours are being conducted with a high level of activity. Cows may be moving quickly throughout the building at a quick paced walk or running, trotting, jumping or displaying any play behaviour. Cows may be actively responsive to anything occurring in the environment.
Inquisitive	Cows appear interested in the environment and looking for stimulation. Cows may be interacting with the brush or any other forms of enrichment. Cows may be interacting with building infrastructure (e.g motorway barriers, gates). Cows are repeatedly going through the Lely robot or not entering the robot but interacting/disturbing cows queuing. Cows are inquisitive to surroundings, attentive to anything happening in the environment.
Irritable	Predominant social situations are negative.
Calmless/Uneasy	Cows appear alert and vigilant to surroundings. Cows lying in cubicles are awake and not looking relaxed. Social situations appear tense.
Sociable	Participating in social interactions both positive and negative. Standing, feeding, drinking, loafing in close proximity of other cows.
Apathetic	Cows are idling (cow is standing stationary. May be looking around or changing position but with no other overt activity). Cows are chewing or interacting with building infrastructure (e.g motorway barriers, gates). Cows are lying but awake and not ruminating. Cows appear passive and lethargic. Cows appear unresponsive to environment.
Happy	Cows appear in good physical condition, without any health concerns, satisfied with environmental situation, positively interacting with the environment and not displaying any behaviours included in the definition for 'bored'. Social interactions are positive. Behaviour seems reflective of a positive affective state.
Distressed	Cows displaying any form of health concerns (e.g poor mobility or injury). Cows repeatedly vocalizing, pacing. Cows receiving negative social interactions.

6.2.3.1.2 Blinded Qualitative Behavioural Assessment

Twenty-four, 20 minute long video recordings were extracted for QBA analysis. Six recordings were taken from the baseline period of the study for both Group 1 and Group 2 cows and six were taken from the choice phase of the treatment period of the study for both Group 1 and Group 2 cows, when cows had access to both the outdoor yard and indoor novel object. The video clips taken for each group were matched to be the same day and same time slot. The video clips were also matched to the time periods when the on farm QBA had been completed. The dates used for baseline video clips were: 22.11.2021 (day 1 of trial), 24.11.2021 (day 3), 26.11.2021 (day 5), 29.11.2021 (day 8), 30.11.2021 (day 10), 01.12.2021 (day 12). The dates used for the choice phase blinded QBA clips were: 04.02.2022 (choice period day 32), 07.02.2022 (choice period day 35), 11.02.2022 (choice day 39), 14.02.2022 (choice day 42), 18.02.2022 (choice day 46), 21.02.2022 (choice day 49). The video clips taken displayed the main living area of the cows, excluding the top end of the building where cows had access to the Lely robotic milking machine. The view also excluded any visual access of the outdoor loafing yards or access gates leading to these. The view also excluded the far end of the building where novel enrichment could be viewed. The visual field displayed by the video clips is shown in Figure 6.1.

Figure 6.1. *Visual field displayed by the blinded QBA video clips.*

One trained assessor evaluated the 24 video clips and completed one QBA assessment for each clip using the same procedure used by the assessor completing the on farm QBA (described 6.2.3.1.1).

The assessor completing the blinded QBA was given no information regarding the research trial or the housing conditions of the cows, apart from what was visible in the video recordings and was therefore completely blinded to the study treatments.

6.2.3.1.3 Qualitative Behavioural Assessment assessors and training

The assessor completing the on farm QBA was a final year PhD student within the Ruminant Population Health Group, University of Nottingham. This assessor had been trained in QBA assessment, had prior experience of completing QBA on different dairy farms and had significant experience of dairy cow behaviour.

The assessor completing the blinded QBA was a first year Veterinary Medicine student at The University of Nottingham who had been trained in QBA assessment.

6.2.4 Statistical Analysis

All statistical analyses were performed using RStudio version 4.1.2 using packages readr (Wickham et al., 2022), dplyr (Wickham et al., 2022), tidyverse (Wickham et al., 2019), stats (R Core Team, 2021) and FactoExtra (Le et al., 2008). The data presented in Figures 6.2 – 6.13 were plotted using the package FactoExtra (Le et al., 2008). The raw QBA linear measurements were centred and standardised to create a normal distribution for further analysis. QBA data were analysed using a principal component analysis (PCA) using package stats (R Core Team, 2021). Principal Component Analysis is a multivariate technique which analyses data consisting of inter-correlated quantitative dependent variables, to produce principal components, new variables which summarise the important information (Abdi & Williams, 2010). The first two principal components, explaining the highest percentage of the variance of the data, with eigen values greater than 1.0, were used in the analysis. A linear model, using package stats (R Core Team, 2021) was then used to identify differences in mean PC1 and PC2 scores between treatment periods. Explanatory variables were retained in the models where p<0.05. QBA results are presented separately for Group 1 and Group 2 cows before the analysis of the combined QBA results for both groups. The axis titles labelled ‘Dim1’ and ‘Dim2’ illustrated in Figures 6.2-6.13 refer to the first and second principal component (PC1) and (PC2).

6.3 Results

6.3.1 On Farm QBA

6.3.1.1 Group 1

PCA of the QBA scores for Group 1 cows identified 5 principal components with eigen values greater than 1 (Table 6.2). The first 3 components explained 61.91% of the variance between treatment periods. The first principal component (PC1) accounted for 38.33% of the variance and displayed the highest loading adjectives of ‘relaxed’/‘content’, with the lowest loading adjectives of ‘fearful’/‘bored’. The second component (PC2) explained 13.29% of the variance and comprised of the highest loading adjectives of ‘apathetic’/’bored’ and lowest loading adjectives of ‘lively’/’playful’. Table 6.3 displays the full list of loading adjectives for both components with associated loading value. Figure 6.2 displays the relationship between all variables in PC1 and PC2.

Table 6.2. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	7.67	38.33
2	2.66	13.29
3	2.06	10.29
4	1.55	7.74
5	1.12	5.62

Table 6.3. *Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.*

Descriptor	PC1	PC2
Active	-0.20	-0.36
Relaxed	0.32	-0.04
Fearful	-0.32	-0.02
Agitated	-0.20	-0.20
Calm	0.21	0.11
Content	0.32	-0.13
Indifferent	-0.27	0.22
Frustrated	-0.16	-0.09
Friendly	0.10	0.07
Bored	-0.28	0.22
Playful	-0.07	-0.39
Positively occupied	0.29	-0.01
Lively	-0.13	-0.43
Inquisitive	-0.14	-0.27
Irritable	-0.13	-0.23
Calmless/uneasy	-0.24	-0.02
Sociable	-0.07	-0.28
Apathetic	-0.28	0.30
Happy	0.27	-0.23
Distressed	-0.20	0.09

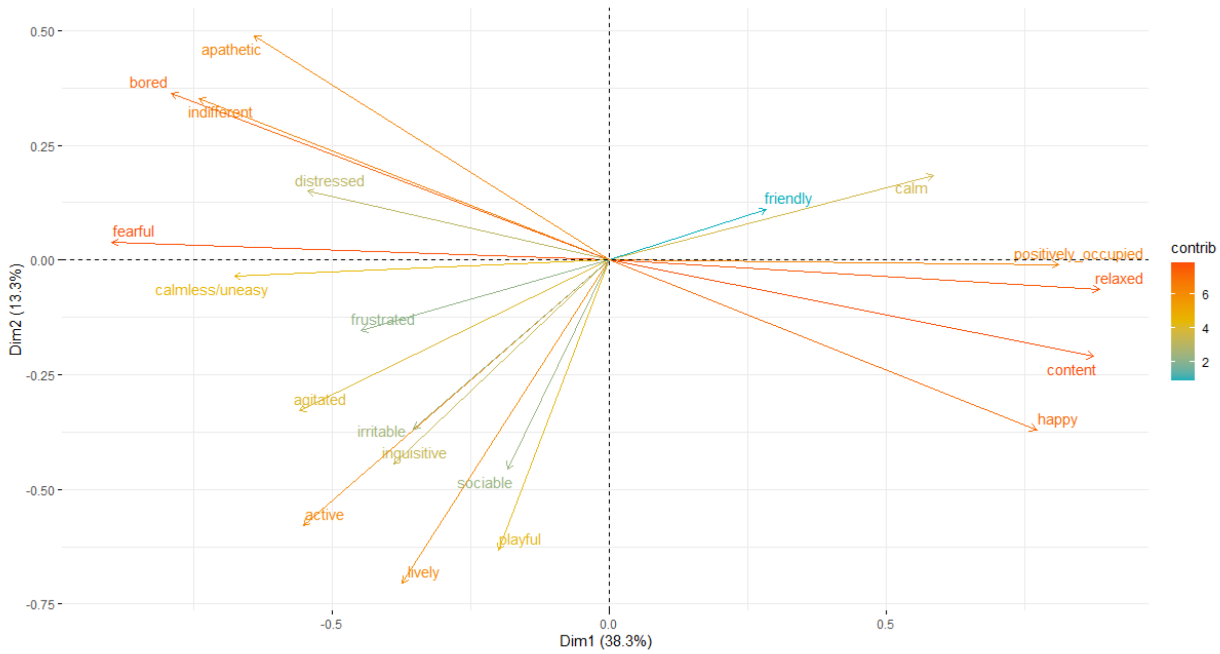


Figure 6.2. *Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each terms value figure in contribution to the principal component.*

Treatment period had a significant effect on PC1 ($P<0.05$), with cows scoring higher values on this component both during the choice period when cows had access to both the outdoor yard and the buoy and when they just had access to the buoy compared to the baseline period (Figure 6.3). Higher scores on PC1 reflected cows being assessed as more relaxed, content and positively occupied compared to apathetic, fearful and bored. The effect of treatment period on PC2 was non-significant.

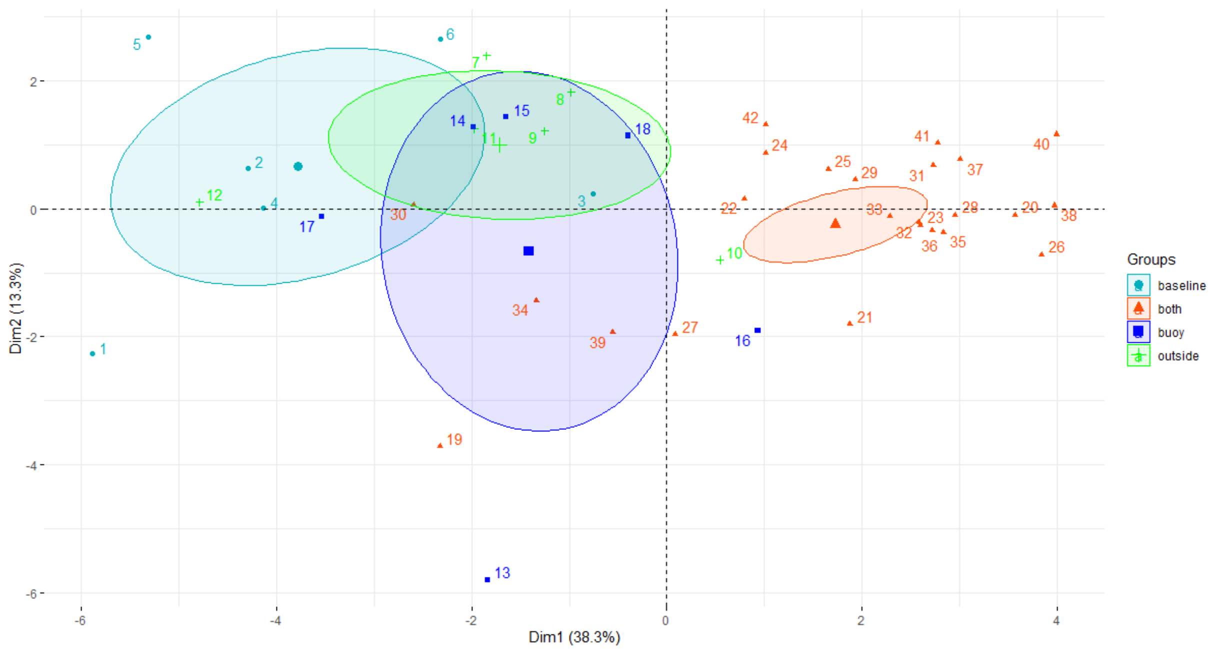


Figure 6.3. *Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.*

6.3.1.2 Group 2

The PCA of the QBA scores for Group 2 cows identified 5 principal components with eigen values greater than 1 (Table 6.4). The first 3 components explained 63.81% of the variance between treatment periods. Principal component 1 (PC1) accounted for 37.64% of the variance and displayed the highest positive loading adjectives of ‘inquisitive’/‘friendly’, with the most negative loading adjectives of ‘sociable’/‘content’. Principal component 2 (PC2) explained 15.73% of the variance and comprised of the highest positive loading adjectives of ‘bored’/’apathetic’ and most negative loading adjectives of ‘lively’/’inquisitive’. Table 6.5 displays the full list of loading adjectives for both components with associated loading value. Figure 6.4 displays the relationship between all variables in PC1 and PC2.

Table 6.4. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	7.53	37.64
2	3.15	15.73
3	2.09	10.43
4	1.41	7.05
5	1,22	6.11

Table 6.5. *Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.*

Descriptor	PC1	PC2
Active	2.43	-0.29
Relaxed	-3.11	-0.10
Fearful	2.89	-0.03
Agitated	2.13	-0.21
Calm	-3.06	0.01
Content	-3.12	-0.11
Indifferent	2.40	0.18
Frustrated	2.37	-0.29
Friendly	3.15	-0.22
Bored	2.87	0.22
Playful	1.74	-0.27
Positively occupied	-3.05	-0.16
Lively	1.22	-0.41
Inquisitive	6.26	-0.35
Irritable	3.13	-0.25
Calmless/uneasy	2.63	0.01
Sociable	-6.51	-0.28
Apathetic	2.48	0.19
Happy	-2.00	-0.21
Distressed	1.15	0.15

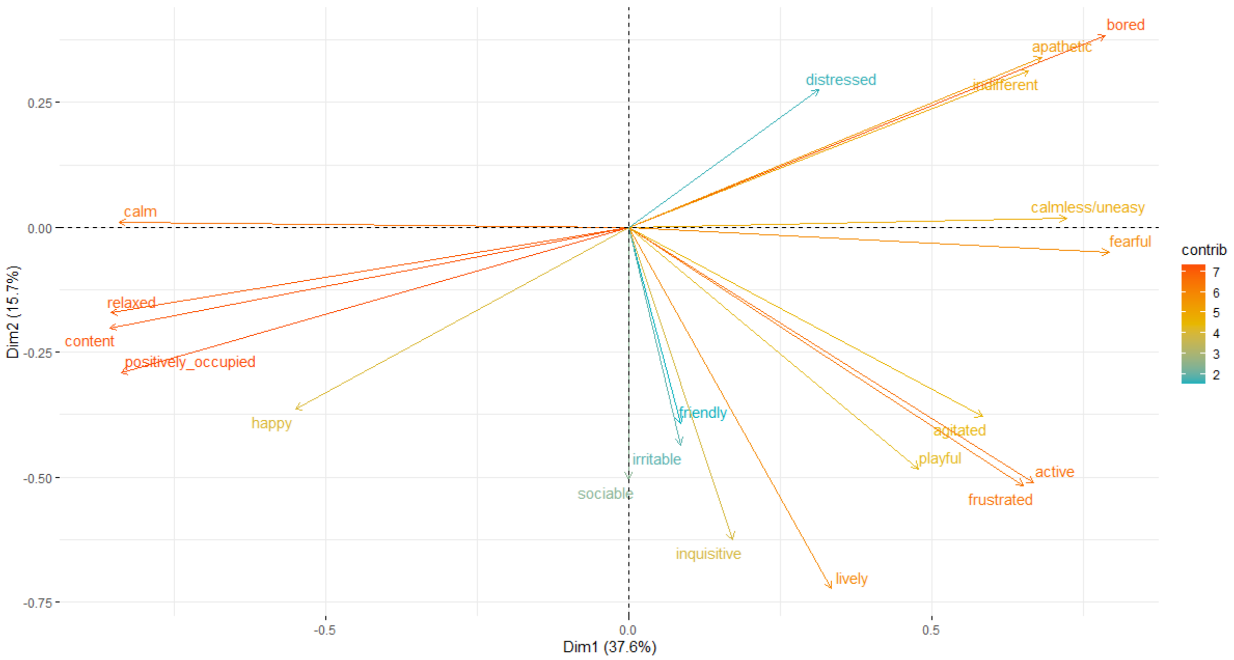


Figure 6.4. *Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each term's contribution to the principal component.*

Treatment period had a significant effect on PC1 (P<0.05), with cows scoring lower values on this component during the choice period compared to the baseline period (Figure 6.5). Lower scores on PC1 reflected cows being assessed as more content, sociable and relaxed compared to inquisitive, friendly and irritable. Significantly lower scores were attained on PC2 during all intervention periods; choice, buoy and outside compared to baseline (P0.05). Lower scores on PC2 were reflective of the cows being assessed as more inquisitive, lively and active compared to bored, apathetic and indifferent.

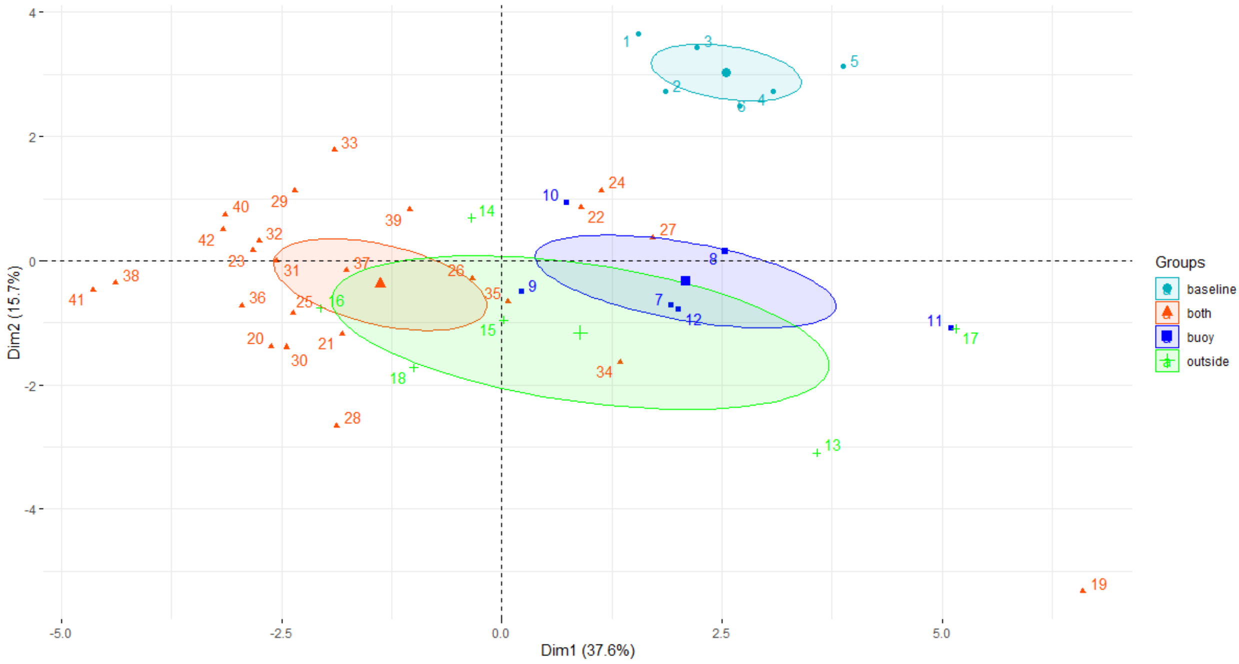


__Figure 6.5.__ Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.

6.3.1.3 Group 1 and 2 combined

PCA of the QBA scores for Group 1 and Group 2 combined identified 5 principal components with eigen values greater than 1 (Table 6.6). The first 3 components explained 60.43% of the variance between treatment periods. Principal component 1 accounted for 36.96% of the variance and displayed the highest positive loading adjectives of 'content'/'relaxed', with the most negative loading adjectives of 'fearful'/'bored'. Principal component 2 explained 13.54% of the variance and comprised of the highest positive loading adjectives of 'lively'/'active' and the most negative loading adjectives of 'apathetic'/'bored'. Table 6.7 displays the full list of loading adjectives for both components with associated loading value. Figure 6.6 displays the relationship between all variables in PC1 and PC2.

Table 6.6. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	7.39	36.96
2	2.71	13.54
3	1.99	9.94
4	1.27	6.37
5	0.95	4.77

Table 6.7. *Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.*

Descriptor	PC1	PC2
Active	-0.22	0.34
Relaxed	0.32	0.07
Fearful	-0.31	-0.01
Agitated	-0.20	0.23
Calm	0.26	-0.06
Content	0.32	0.12
Indifferent	-0.26	-0.21
Frustrated	-0.20	0.23
Friendly	0.03	0.11
Bored	-0.29	-0.23
Playful	-0.13	0.33
Positively occupied	0.30	0.09
Lively	-0.13	0.41
Inquisitive	-0.10	0.32
Irritable	-0.08	0.27
Calmless/uneasy	-0.25	0.01
Sociable	-0.04	0.28
Apathetic	-0.24	-0.26
Happy	0.25	0.22
Distressed	-0.16	-0.10

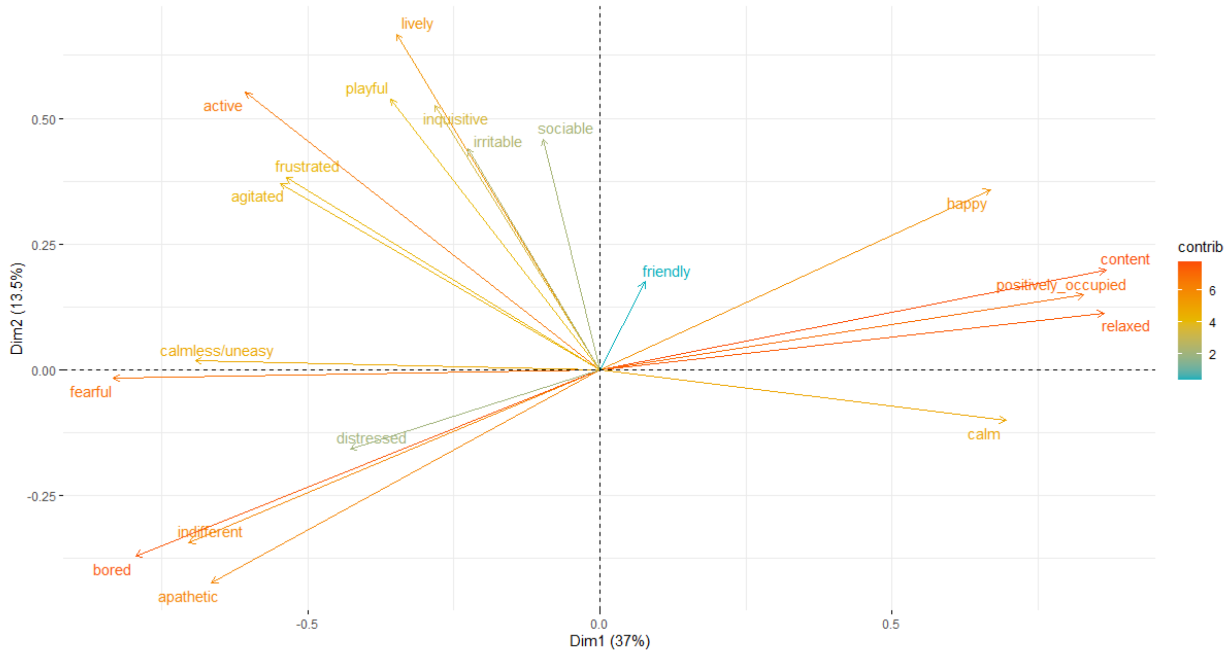


Figure 6.6. *Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each term's contribution to the principal component.*

Treatment period had a significant effect on PC1 (P<0.05), with cows attaining higher scores on this component during both the choice period and when cows had access to the outdoor yard only compared to the baseline period (Figure 6.7). Higher scores on PC1 reflected cows being assessed as more relaxed, content and positively occupied and less indifferent, bored and fearful. Cows scored significantly higher on PC2 during all intervention periods; choice, buoy and outside compared to baseline (P<0.05). Higher scores on PC2 were reflective of the cows being assessed as more lively, active and playful compared to indifferent, bored and apathetic.

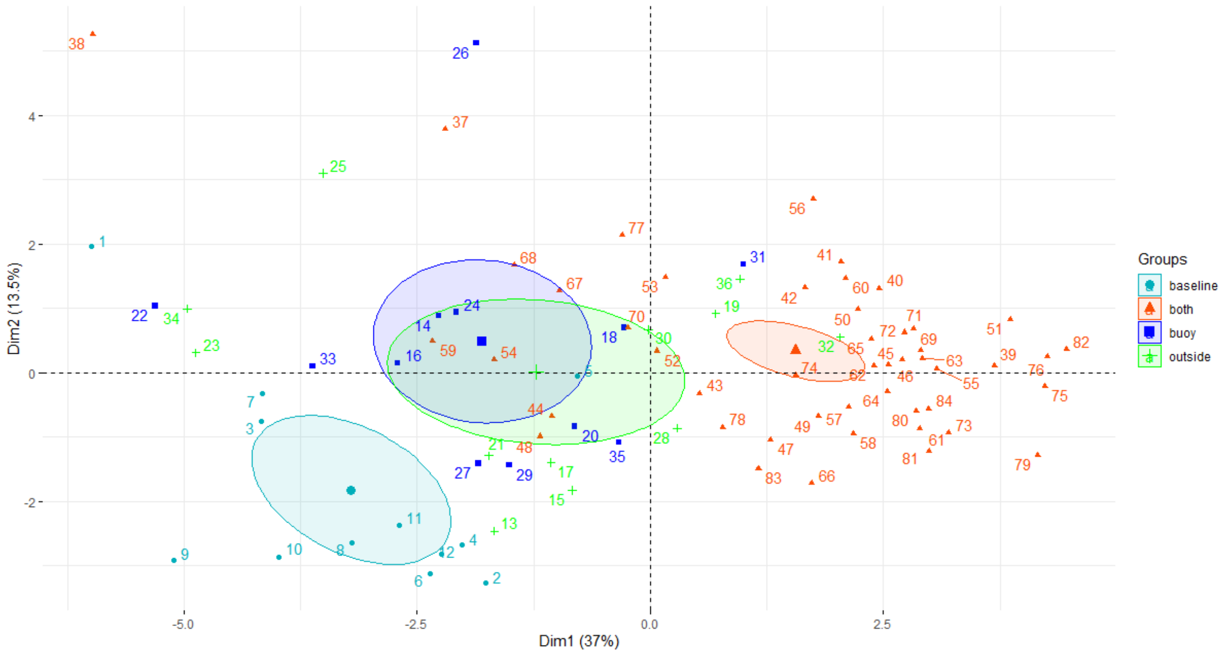


Figure 6.7. *Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.*

6.3.2 Blinded QBA

6.3.2.1 Group 1

PCA of the blinded QBA scores for Group 1 identified 5 principal components with eigen values greater than 1 (Table 6.8). The first 3 components explained 78.84% of the variance between treatment periods. Principal component 1 accounted for 55.30% of the variance and displayed the highest positive adjectives of ‘agitated’/‘lively’, with the most negative loading adjectives of ‘calm’/‘positively occupied’. Principal component 2 explained 13.23% of the variance and comprised of the highest positive loading adjectives of ‘calm’/’inquisitive’ and most negative loading adjectives of ‘frustrated’/’lively’. Table 6.9 displays the full list of loading adjectives for both components with associated loading value. Figure 6.8 displays the relationship between all variables in PC1 and PC2.

Table 6.8. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	1.11	55.30
2	2.65	13.23
3	2.06	10.31
4	1.66	8.32
5	1.10	5.51

Table 6.9. *Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.*

Descriptor	PC1	PC2
Active	0.25	2.09
Relaxed	-0.26	3.32
Fearful	0.08	-3.59
Agitated	0.29	-2.59
Calm	-0.29	8.82
Content	-0.24	-2.57
Indifferent	-0.14	4.91
Frustrated	0.26	-7.57
Friendly	0.04	-3.58
Bored	-0.00	4.06
Playful	0.25	4.93
Positively occupied	-0.26	5.69
Lively	0.27	-9.63
Inquisitive	0.27	6.01
Irritable	0.26	-7.18
Calmless/uneasy	0.24	1.41
Sociable	0.07	2.49
Apathetic	-0.15	3.66
Happy	-0.22	-3.51
Distressed	0.27	2.47

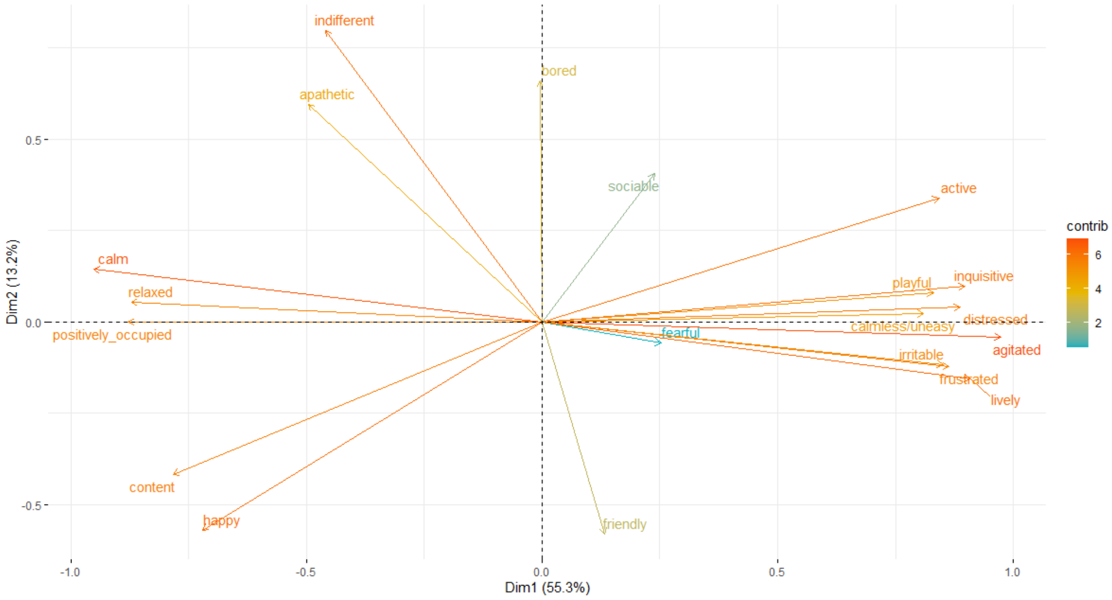


Figure 6.8. *Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each term's contribution to the principal component.*

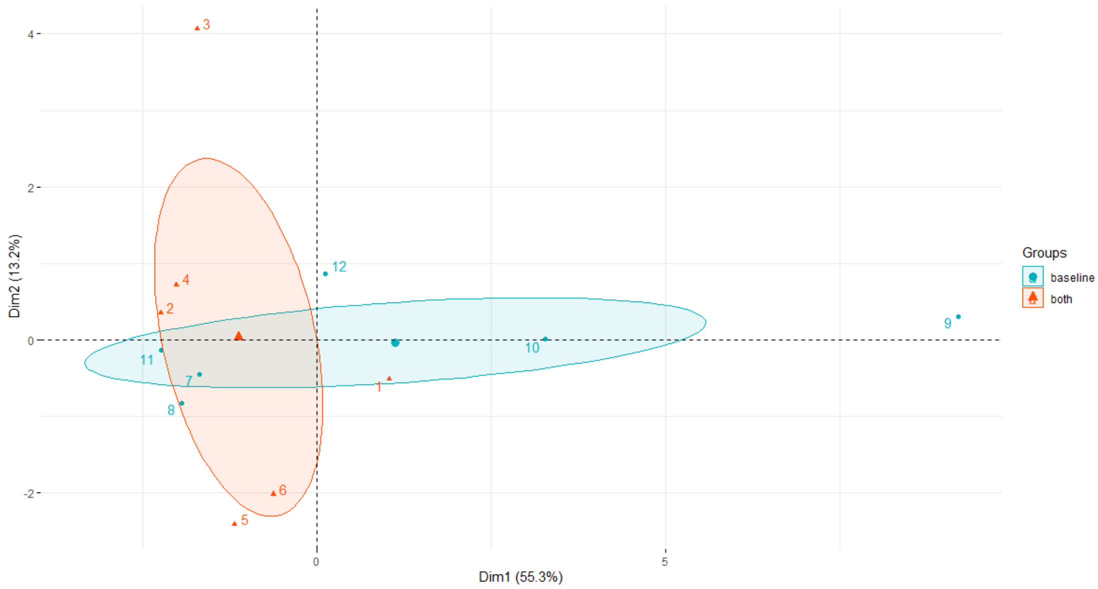


Figure 6.9. *Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.*

There were no significant differences between mean PCA scores between intervention periods. PC1 scores tended to be more negative during the intervention period when cows had access to both the outdoor yard and buoy compared to the baseline (Figure 6.9). This indicates that cows were assessed as more relaxed, positively occupied and calm compared to agitated, lively and inquisitive.

6.3.2.2 Group 2

PCA of the blinded QBA scores for Group 2 identified 5 principal components with eigen values greater than 1 (Table 6.10). The first 3 components explained 69.01% of the variance between treatment periods. Principal component 1 (PC1) accounted for 35.66% of the variance and displayed the highest positive loading adjectives of 'irritable'/'frustrated', with the most negative loading adjectives of 'calm'/'content'. Principal component 2 (PC2) explained 18.27% of the variance and comprised of the highest positive loading adjectives of 'happy'/'fearful' and most negative loading adjectives of 'indifferent'/'apathetic'. Table 6.11 displays the full list of loading adjectives for both components with associated loading value. Figure 6.10 displays the relationship between all variables in PC1 and PC2.

Table 6.10. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	7.13	35.66
2	3.65	18.27
3	3.02	15.08
4	2.12	10.61
5	1.21	6.04

__Table 6.11.__ Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.

Descriptor	PC1	PC2
Active	0.22	0.13
Relaxed	-0.28	0.15
Fearful	0.06	0.31
Agitated	0.33	0.03
Calm	-0.31	0.09
Content	-0.29	0.21
Indifferent	-0.03	-0.46
Frustrated	0.33	-0.01
Friendly	-0.15	0.20
Bored	-0.08	-0.20
Playful	-0.08	0.25
Positively occupied	-0.12	0.28
Lively	0.28	0.15
Inquisitive	0.30	0.04
Irritable	0.33	0.02
Calmless/uneasy	0.31	0.06
Sociable	0.17	0.20
Apathetic	-0.06	-0.24
Happy	-0.04	0.42
Distressed	0.08	0.26

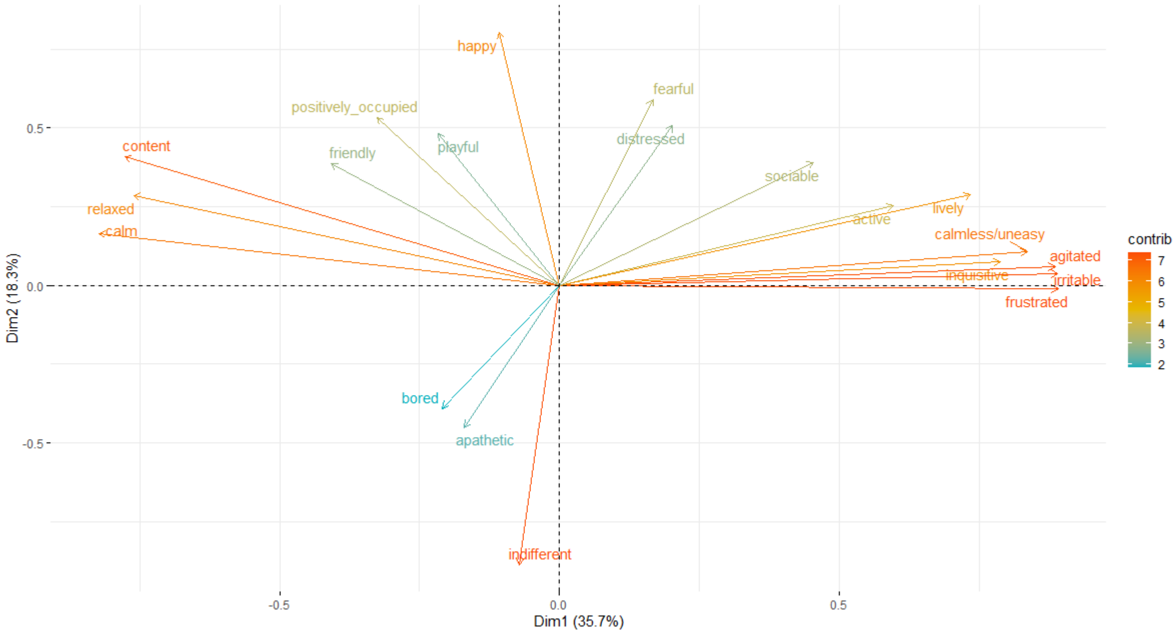


__Figure 6.10.__ Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each term's contribution to the principal component.

There were no significant effects of intervention period of either mean PC1 or PC2 scores. Figure 6.11 shows a slight trend of higher mean scores on both PC1 and PC2 during the choice intervention period compared to the baseline, reflective of cows being scored as more irritable, frustrated and agitated compared to relaxed, calm and content (PC1) and happy, positively occupied and fearful compared to bored, indifferent and apathetic (PC2).

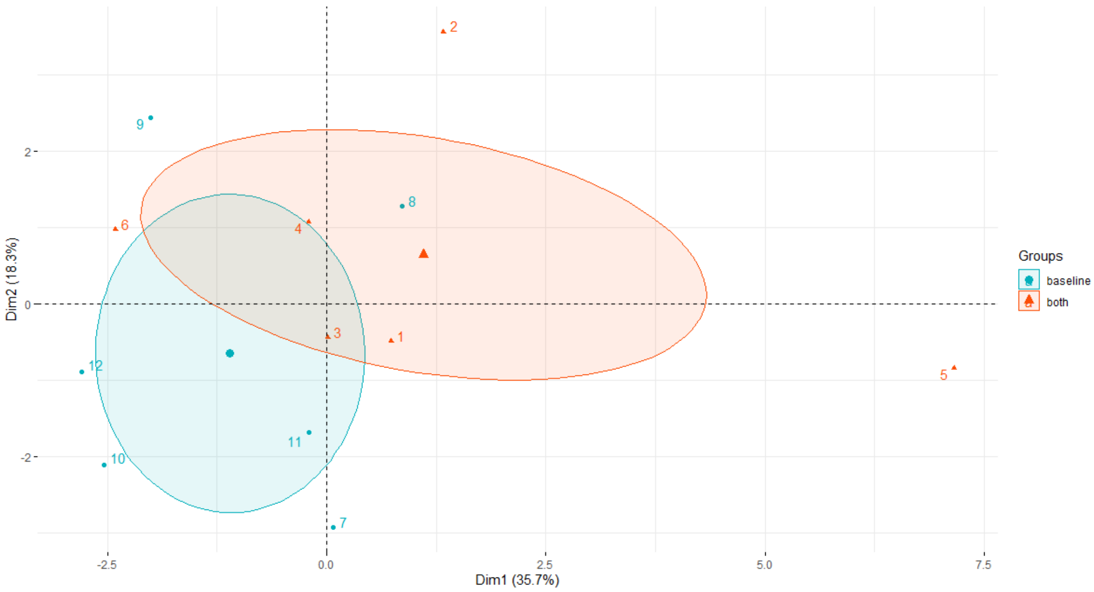


Figure 6.11. *Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.*

6.3.2.3 Group 1 and 2 combined

PCA of the blinded QBA scores for Group1 and Group 2 combined identified 5 principal components with eigen values greater than 1 (Table 6.12). The first 3 components explained 65.05% of the variance between treatment periods. Principal component 1 (PC1) accounted for 40.11% of the variance and displayed the highest positive loading adjectives of ‘calm’/‘relaxed’, with the most negative loading adjectives of ‘agitated’/‘inquisitive’. Principal component 2 (PC2) explained 13.47%

of the variance and comprised of the highest positive loading adjectives of 'happy'/'playful' and the most negative loading adjectives of 'indifferent'/'apathetic'. Table 6.13 displays the full list of loading adjectives for both components with associated loading value. Figure 6.12 displays the relationship between all variables in PC1 and PC2.

Table 6.12. *Top five dimensions identified by QBA, displaying associated eigen values and percentage of variance explained.*

Dimension	Eigen value	Percent variance explained
1	8.02	40.11
2	2.69	13.47
3	2.29	11.46
4	1.76	8.80
5	1.17	5.83

Table 6.13. *Principal components 1 and 2, displaying associated loadings of each behavioural descriptor.*

Descriptor	PC1	PC2
Active	-0.26	0.04
Relaxed	0.29	0.09
Fearful	-0.07	0.19
Agitated	-0.33	-0.02
Calm	0.32	0.01
Content	0.27	0.23
Indifferent	0.13	-0.46
Frustrated	-0.29	-0.04
Friendly	0.05	0.26
Bored	0.04	-0.17
Playful	-0.02	0.33
Positively occupied	0.19	0.27
Lively	-0.29	0.17
Inquisitive	-0.30	0.02
Irritable	-0.29	-0.01
Calmless/uneasy	-0.25	0.01
Sociable	-0.14	0.10
Apathetic	0.14	-0.38
Happy	0.07	0.47
Distressed	-0.23	-0.06

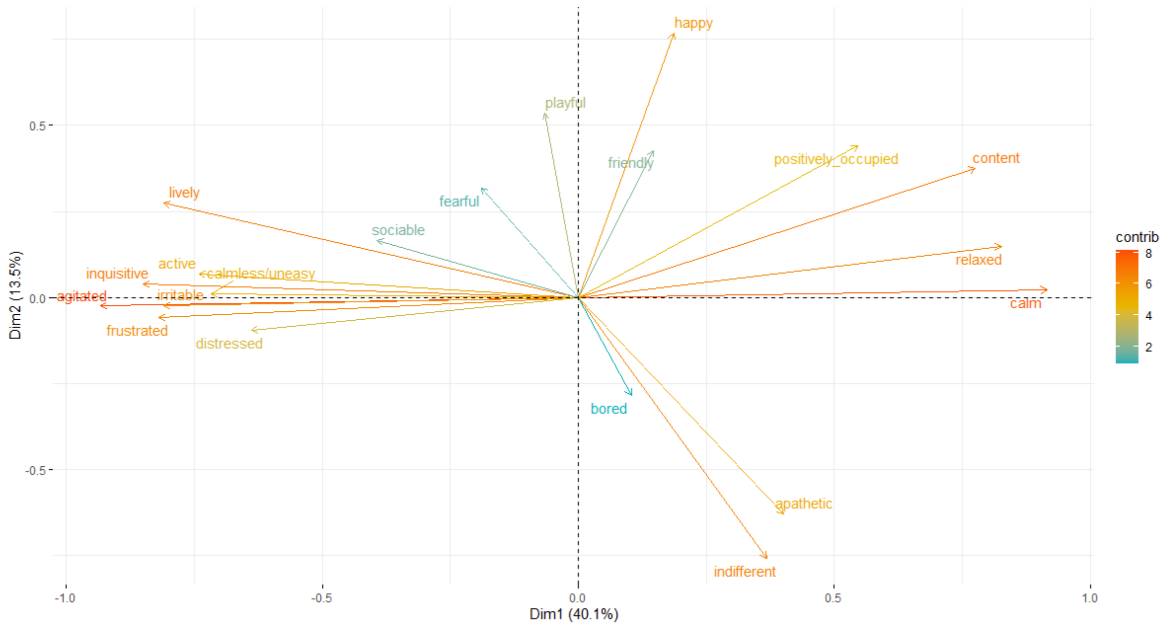


Figure 6.12. *Variable correlation plot displaying the relationship between all variables in PC1 and PC2 and each term's contribution to the principal component.*

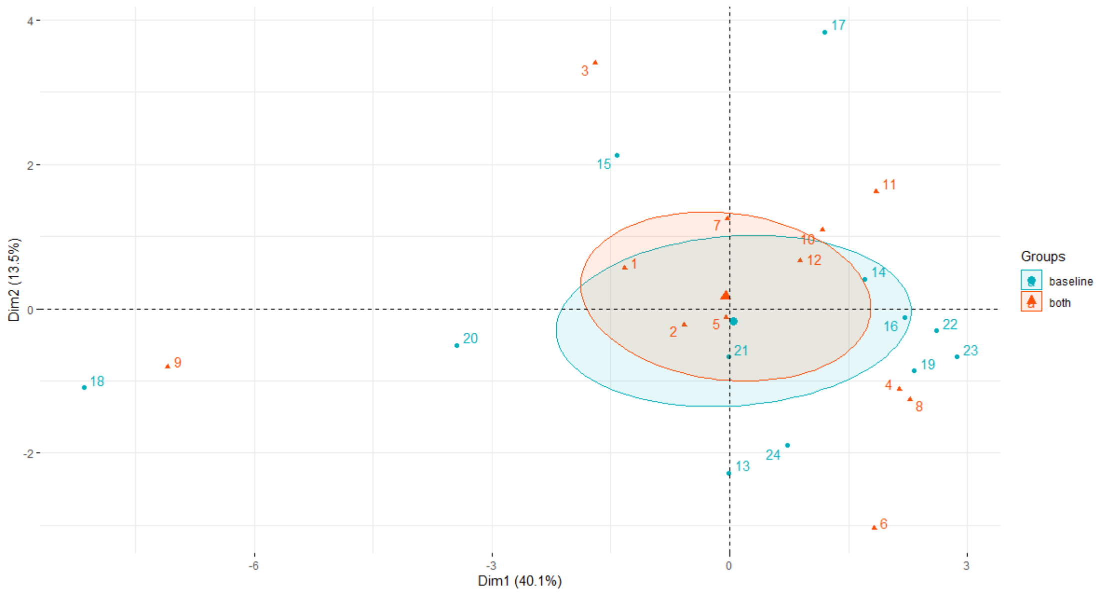


Figure 6.13. *Biplot displaying all QBA assessment scores in terms of PC1 and PC2. Each separate point displays one assessment date. Points are coded per treatment period (baseline, buoy, outside, both) as indicated on the plot. Group means are in bold and ellipses indicate the 95% confidence intervals.*

There were no differences identified in mean scores from PC1 or PC2 according to intervention period (Figure 6.13).

6.4 Discussion

6.4.1 On farm QBA

The current study is the first to utilise QBA to assess dairy cows' affective states in response to a positive welfare intervention. The intervention used in this study consisted of manipulating the standard housing conditions of commercially-housed dairy cows. Modifications consisted of providing cows with access to two different resources as forms of enrichment. One was an indoor novel object, with no obvious associated behavioural function and the other was access to an outdoor concrete yard, which offered cows the choice to be outdoors. Diversification of the environment through environmental enrichment which offers opportunities for exploration, control and choice within the environment, has been suggested as one way to offer confined animals positive experiences (Mellor, 2017). In line with this theory, the hypothesis of the study was that offering additional environmental resources would have a positive impact on cows' affective states. Results reported from the on farm QBA (as opposed to the video-based) support this hypothesis, with more positive affective states being indicated during the intervention periods when cows had access to enrichment.

Group 1 cows scored significantly higher on PC1 during both the choice period, when cows had access to both resources and when they just had access to the buoy compared to standard baseline conditions. The highest loading adjectives on PC1 were relaxed, content and positively occupied, all with positive emotional connotations and the lowest loading terms were apathetic, fearful and bored, all terms that could be used when describing negative affective states. Therefore, this principal component could be representative of general affective mood, on a scale from positive (higher scores) to negative (lower scores). These results suggest that when cows had access to both resources or the buoy, they were more relaxed, content and positively occupied than when in standard housed conditions. Although the behavioural descriptors were not analysed in isolation, as the nature of PCA, which is most commonly used to analyse QBA data, is to provide new variables from the correlated quantitative dependent variables, reference should be made to why cows were scoring higher on these terms during periods of access to enrichment. Positively occupied is the least complicated to interpret, the behavioural description for this term, defined in table 6.1, describes expression of this attribute to behaviourally be interacting with the environment in a positive way, for example, eating, drinking or interacting with enrichment. It also specifies that clear representations that do not fall into this category are cows that are interacting with the environment in what could be considered a negative way, for example, performing stereotypical behaviour which could be manipulating and chewing building infrastructure or in negative social interactions. It is therefore likely that increasing the behavioural options and activities within the cows' environment would increase the amount of time they spent being positively occupied. This has been shown in pigs, for example, where simply providing four instead of two wooden beams increases both the frequency and duration of manipulation bouts (Larson et al., 2019), similarly, increasing the amount of straw available to pigs increases both the time spent manipulating the straw and pigs' simultaneous straw manipulation (Jensen et al., 2015). Terms such as relaxed and content, are more challenging to interpret and explain. Content, although a term in itself, does have overlapping connotations with both positively occupied and bored, and the behavioural description for this study makes reference to cows showing a combination of being both positively occupied and not showing any behavioural indicators of boredom, amongst other things, which would imply a level of contentment with their environmental situation. Similarly, it could be that as animals spend more time positively occupied with the environment, they use more positively motivated energy which could be linked to being more relaxed/tired out.

The same results showed that Group 1 cows scored as less apathetic, fearful and bored when they had access to both enrichments or just the buoy. Again, boredom could be seen as a negative contrast to positively occupied, which would fit with the scientific literature, that it can be reduced by providing additional behavioural options through environmental enrichment (Meagher & Mason, 2012; Meagher, 2018; Polanco et al., 2021). Understanding how cows may be more behaviourally representative of negative affective states such as fear and apathy, between a simple change of environmental conditions, is again more challenging to interpret. In human psychology, the experience of boredom is described as extremely unpleasant and distressing (Martin et al., 2008). Given that it's an under researched area in animals (Burn, 2017), it is possible it is an equally aversive experience and links have been shown to support this, for example animals will choose aversive experiences over monotony (Bench & Lench, 2013). Apathy is linked to both boredom and depression in people (Goldberg et al., 2011; Greenson, 1949; LePera, 2011;). In animals, depression symptoms are linked to barren housing, which may develop from unavoidable chronic stressors in the environment (Maclellan et al., 2021). Proxies of low mood, one symptom of depression, such as negative information processing, have shown to be changed in pigs by environmental enrichment (Douglas et al., 2012). When pigs were moved from barren to enriched housing, they showed decreased negative information processing in cognitive bias tests. This result was exemplified in pigs that were transferred from enriched to barren housing, showing higher levels of negative information processing in cognitive bias tests when compared to that of pigs that had always been managed in barren housing (Douglas et al., 2012). This susceptibility in animals' emotional experiences between barren and enriched housing, suggests it may not be as surprising that subtle behavioural reflections of negative affective states such as apathy, could be detected in cows between different housing conditions. Boredom has also been correlated with anxiety and fear (LePera, 2011; Sommers & Vodanovich 1999; Vodanovich et al., 1991) and therefore, although not surprising that these interlinked negative affective states both scored lower, the underlying reason for this is not clear. Given the link between these states, reducing boredom could simply be paired with overall reductions in negative affective states such as fear, or providing more time filling environmental activities for the cows could act as some form of distraction from triggers of fear and anxiety. Increasing animals time in positively engaging behaviours, would likely decrease time spent in empty or boredom like situations, where cows are potentially more aware of surroundings and potential threats. Interestingly, anxiety behaviours in rats and mice have also been shown to be reduced through the use of environmental enrichment (Benaroya-Milshtein et al., 2004; Sampedro-Piquero et al., 2014; Vachon, 2014;) but this link has yet to be explored in dairy cows. Again, when compared to the field of human psychology, exercise is a known and widely used treatment for anxiety and depression (Carek et al., 2011; Long & Stavel, 1995). Dairy cows are frequently and routinely subject to stressful experiences likely to cause fear and anxiety, for example social regrouping (LeCorps et al., 2020; Von Keyserlingk et al., 2008) and handling by people (Hemsworth et al., 2000; Pas et al., 1998) and therefore if enrichment had the capability of providing some level of barrier to the negative experiences of environmental stressors, as well as its use as an opportunity for positive experiences, this could hugely reinforce the justification for its use in commercial settings and the overall welfare impact on cows. Finally, the on farm QBA results showing cows to be behaviourally expressive of more negative affective states, such as boredom and apathy, whilst housed in standard conditions, reinforces the concern surrounding affective states and the quality of lives of commercially-housed dairy cows.

Similar results were found for Group 2 cows, with both components identified through PCA being impacted by the environmental intervention. The first component had the highest loading terms of inquisitive, friendly and irritable and the lowest loading terms of sociable, content and relaxed and cows scored significantly lower on this component during the choice phase compared to baseline. This component was not as distinctly separated in terms of positive and negative associated terms, with the higher loadings of inquisitive and friendly combined with irritable. Therefore, this component appeared more representative of arousal or activity level than affect. Group 2 cows scored significantly lower on the second component identified through PCA, which had the highest loading terms of bored, apathetic and indifferent, compared to the lower terms of active, lively and inquisitive. This component could have represented cows' positive engagement with their surroundings, on an affective scale from negative to positive. Enrichment is known to increase exploration and associated activity (Averós et al., 2010; Tarou & Bashaw, 2007) and therefore it would be understandable for cows to appear more active, lively or inquisitive when provided with enrichment resources.

QBA is utilised for its on-farm practicality, requiring little time to complete, when compared to other farm assurance assessments (Andreason et al., 2013) and requiring no resources or technical equipment, which is generally the case for other positive welfare indicators. Its practicality and ease of use, as an on-farm measure of welfare assessment, including aspects of positive welfare, was observed within the present study, however it should be noted how challenging some of the terms within the

Welfare Quality Dairy Protocol (WelfareQuality, 2018) were to assess. Some of the terms are relatively easily assessed due to being linked to clear expressions of behaviour, for example, terms such as positively occupied, which is linked to distinctly identifiable behaviours, such as eating, drinking or using an automatic brush. The number of animals within a group participating in these behaviours is also objective to assess. Generally, the terms fell within this category, for example, terms such as active and fearful have clearly expressed behavioural patterns in dairy cows (MacKay et al., 2014; Mattachini et al., 2013; Muller & Schrader 2003; Welp et al., 2004). A small number of terms were much more challenging to assess, with particular reference to the term happy. Although the concept of QBA is to use personal interpretation of animals' expressive demeanour to make the assessments, a certain level of knowledge as to how these affective descriptions would be behaviourally expressed is required. Very little is known about how animals behaviourally express happiness, which links into the challenges of the field of positive animal welfare. Play behaviour, is one currently accepted expression of positive affective expression in animals (Held & Spinka, 2011; Keeling et al., 2021) which is also a very specific behaviour, that is much reduced in adults (Held & Spinka, 2011; Oliveira et al., 2010) making it unlikely to play a large role in QBA assessments which are rapid snapshots in a moment of time on farm. Asides from this, making a visual judgement of an animals' happiness could be seen as an impossible question, as identification of positive affective states is currently an incredibly complex challenge, with a limited number of positive welfare indicators having started to be explored with mixed validity (Keeling et al., 2021). Considering this, caution should be taken when interpreting results where judgements have been made on very ambiguous terms such as these. QBA has proven itself as a reliable measure of making inferences of animals differing affective states (Keeling et al., 2021) yet the potential for it to be considered as anthropomorphic is frequently mentioned (Wemelsfelder & Lawrence, 2001; Wemelsfelder, 2007; Wemelsfelder, 2008). This criticism could potentially be controlled, by using careful consideration of the terms used for assessments, by removing highly ambiguous terms, as discussed for example ‘happy’ and including a variety of terms with more explicit species-specific behavioural representation. Similarly, using the free choice profiling approach in addition to fixed list QBA may strengthen the results by only assessing animals on terms that are easily interpreted by the assessor.

6.4.2 Blinded QBA

The results from the blinded QBA yielded less conclusive results. The first component identified for Group 1 cows, had the highest loading adjectives of agitated and lively and lowest of calm and positively occupied, potentially indicative of a general activity level with a more positive connotation with lower scores. Although cows tended to score lower on this component when they had access to both enrichment resources compared to the baseline period, there were no significant effects of treatment period and no difference in scores obtained from cows in different intervention periods on the second component. Similarly, there were no significant effects of treatment period on the principal component scores for Group 2 cows. The combined PCA from the two groups of cows showed similarly overlapping results from both the baseline period and from when cows had access to both results, not supporting the QBA results obtained from the on-farm QBA assessments.

The blinded QBA was incorporated into the present study as a way of validating the on-farm QBA results. The results from the blinded QBA did not support results from the on-farm QBA, however there are several possible explanations for this. One inherent difference between the on-farm QBA and the blinded QBA was that one was completed in person at the farm, with direct, close visual access to all of the cows. Completing the QBA assessment on the farm meant that the observer was able to see the entire group of cows during the assessment by use of two viewpoints. The assessor completing the blinded QBA had a restricted view compared to that of the on-farm QBA, with only one viewpoint, which did not give visual access to the entire group, therefore a much smaller number of cows could be observed per assessment. As one of the specifications of QBA, terms are rated depending on the level of a descriptive term, for example how frustrated an animal may look, but also when analysing groups of animals, how many animals express this particular attribute. Therefore, the blinded QBA likely presented a small sample of information compared to what the on-farm assessor could use. Asides from this, the on-farm assessor was visually closer to the cows than the located camera where the recordings came from, likely allowing a much more accurate quality of view. Another important point to note is the visual clarity that the on-farm observer would have had compared to the level of detail of the video recordings, making the on-farm assessor capable of identifying very subtle behavioural expressions, for example, cows gaze or ear positions which would have been challenging to pick up from the video recordings. Similarly, video recordings used in this study did not have sound, which would have blocked any information from cow vocalisations to the assessor. Cooke et al. (2022) investigated how completion of QBA using video recordings compared to real life assessments. Forty QBA assessments were completed on 2 groups of 30 beef cattle from the same farm. Two assessors were recruited for the live scoring and conducted the QBA from the video footage one month later. There was no statistical difference between the live QBA and the video QBA for 16 out of the 17 terms used and 12 out of the 17 terms showed positive significant correlations. The principal component analysis formed two components, one displaying no significant differences between the live and video QBA however the other did, but both correlated between the video analysis and the live assessments. The QBA results from the video footage tended to yield fewer extreme values from a component termed mood, with the highest loading adjectives of agitated and irritable and lowest loading terms, relaxed and content. Therefore, broad but not absolute agreement was found between the methods of QBA, and the authors concluded that until further validation is completed, the methods of QBA should be used independently. Video QBA was not an original objective of the current study and therefore the camera setup was not designed to achieve this. Validation through video recordings in future studies could be made more rigid, using a wider number of camera angles, including close distance, high clarity images of the animals to give a visual field as close as possible to that obtained from live QBAs. Alternatively, manipulation of video recording backgrounds as used by Wemelsfelder et al. (2009) could be used, which may be more complicated where analysis is required for groups of animals rather than individuals. Another alternative could be the removal of animals from differing housing conditions into a different area for QBA assessment, which would alter the animal's behaviour, but may be able to identify differences between animals from different treatment groups. Rutherford et al. (2012) used a similar method to validate QBA as a measure of affective states by using blinded observers to assess pigs that had been treated either with saline or a neuroleptic drug, in an open field or elevated plus maze test. Although open field or elevated plus maze tests are used to test and therefore elicit fear (Forkman et al., 2007), meaning a QBA assessment of manipulated behaviour, QBA was still able to detect significant differences between the pigs' emotional states between different treatment groups.

6.4.3 Limitations

One potential limitation of the on-farm QBA assessments in this study, was that it was not possible to blind the assessor to study treatments. The assessor was therefore aware of when the cows in the present study were housed in standard conditions and when they had access to other resources. This could introduce an element of unconscious assessor bias, particularly since the interventions could be considered to have moral connotations, for example one treatment being perceived as better for welfare than another. However, on the other hand, one of the underpinning concepts of QBA is to evaluate not just how animals are behaving but how they are interacting with their environment (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001) which clearly also requires access to the environmental situation. Knowledge of the animals' environment could also be seen as a prerequisite in being able to perform successful QBA assessments, for example when assessing the term positively occupied, which requires interpretation of what the animal is doing and therefore how the environment is providing opportunity for this. Wemelsfelder et al. (2009) investigated the impact of being contextually aware of the animals' environment on QBA results. Video recordings of 15 pigs interacting with a novel object were digitally extracted and applied to both an indoor and outdoor setting. The resultant 30 video clips were analysed by 16 blind observers. There was a strong correlation between the indoor and outdoor variants of video clips across both QBA components. Environmental background did have an effect on one of the QBA components (confident/content–cautious/nervous) but not the other (playful/active–bored/lethargic), inferring that outdoor pigs were more confident and content and less cautious and nervous.

Although slight shifts in assessors' scorings may be observed by contexts, the results indicated that the background environment did not contribute to large deviations in assessors' evaluation of the animals' body language. Although highlighted as a potential confounding issue with QBA, the overall concept of QBA relies on assessment of the whole animals' expressive demeanour within its setting, including how it interacts with its environment, therefore, the assessor's knowledge of the animals' environment does not seem unreasonable. We attempted to control for this risk, by recruiting a QBA assessor that had no knowledge of the research trial and conducted QBA assessments on anonymously labelled video clips, which did not provide the assessor with knowledge of study interventions. The results from the blinded QBA did not concur with the results from the on-farm QBA, however there were a number of possible causes for this, as discussed above (6.4.2). The gold standard of validation of the QBA results obtained in this study, would have been on-farm assessment, which was blinded to interventions, which was not possible in this study and would generally be very difficult to achieve in any studies evaluating changes in housing. Furthermore, QBA has been practically implemented in industry as an on-farm welfare assessment, with no reference of concern having been highlighted for potential bias by assessors, through also being aware of housing or management practises.

The results from the current study, could have been strengthened through use of a combination of positive welfare indicators, to assess differences in the cows' affective states through the varying environmental conditions. Wider health and physiological indicators of welfare have been used to validate differences in welfare as identified through QBA, in a number of studies, for example QBA detected adverse affective states in cows with a mastitis infection, with higher udder severity scores, body temperatures and concentrations of cortisol being positively associated with higher scores on a principal component analysis of the QBA results, indicative of cows with a more suffering, dejected and lethargic demeanour (Des Roches et al., 2018). Other examples of wider health indicators validating QBA results have been shown in broilers, with higher mortality and hock burn scores being positively correlated with decreased scores indicative of happy and active behavioural expressions (Rayner et al., 2020) and increased heart rates and levels of a stress leukogram, being linked to sheep scoring as more alert, anxious and aware which had never experienced road travel before compared to sheep habituated to it (Wickham et al., 2012). Validation studies such as these, support the use of QBA as a behavioural welfare assessment, however, not specifically as an indicator of positive welfare, as the health indicators tend to be indicators of poor welfare such as mortality (Rayner et al., 2020) and stress leukograms (Wickham et al., 2012), which correlate with scores indicative of poorer affective states. To validate QBA results indicative of enhanced affective states, a combination of suggested positive welfare indicators should be used in conjunction, for example studies using both QBA and cognitive bias testing, which appear to be more lacking, yet would provide a more confident validation for identification of positive states. A small selection of studies, have combined QBA with the analysis of objective, specific behaviours, considered to be positive. Rizzuto et al. (2020) evaluated calf welfare at rodeos and found both QBA and ear position to be indicative of more positive states during the recovery stage of the event. Similarly, Serrapica et al. (2017), found consistency between QBA results and ear positions, in evaluation of lambs' affective states, following habituation to people through stroking compared to no habituation. Consistencies between positive welfare indicators in intervention studies would provide increased confidence in our interpretation of the results and therefore our interpretation of the animals' affective states, but also, divergences may also highlight where studies making reference to changes in affective states require further replication or validation. A study by Carreras et al. (2016) aimed to evaluate whether affective states of pigs could be impacted by whether they were housed in an enriched (solid floor, straw and increased space allowance) or a barren (decreased space allowance, no straw, slatted floors) environment. A combination of both QBA and cognitive bias testing were used as measures of affective states, alongside two physiological indicators of welfare. Interestingly, the QBA results, cortisol concentrations and carcass wounds were all indicative of better welfare states in the enriched conditions, but no differences were detected in the cognitive bias testing. Vitali et al. (2021) evaluated the welfare status of pigs housed in mechanically compared to naturally ventilated housing, at three life time points and found that QBA identified pigs in mechanically ventilated buildings to be associated with more positive affective states. A significantly higher frequency of lesions were displayed in pigs in naturally ventilated buildings at the initial timepoint however no differences were observed at the later two time points. Interestingly despite the QBA indicating increased affective states in mechanically ventilated buildings, pigs in these buildings also performed higher levels of stereotypical and negative social behaviours and showed a higher general level of inactivity, all behaviours associated with negative affective states (Fureix & Meagher, 2015; Mason, 1991; Schrøder-Petersen & Simonsen, 2001). These inconsistent results highlight the importance of using QBA alongside other indicators of positive welfare to avoid making misinformed judgements about the affective states of animals. Although only one indicator of positive welfare was used in the present study, cows' preferences and uses of two environmental enrichment resources were measured as part of the wider study

(Chapter 5), which showed that the resources offered were valued and therefore likely to be a positive experience. For the current study, inclusion of a wider selection of positive welfare indicators was not possible due to time constraints, yet this approach should be used in any future studies using QBA on dairy cows. As an alternative, QBA could have been used in a wider selection of situations in the present study, with differing assumed affective valences, for example, QBA could have also been used during feeding times, cow movements and veterinary visits alongside quiet times without human intervention in both the baseline period and intervention periods, to further validate its use in this context for its ability to identify changes in affective states of the cows.

For the current study, QBA was conducted at regular multiple time points throughout the week, selected to give the best snapshot of the cow's behaviour, when undisturbed through external human or management factors. Despite this, within intervention periods, great variation was still shown between PCA scores, highlighting either inconsistency in QBA as a method, or that there may have been many unidentified variables which were not controlled for throughout the study which could have had an impact on the cow's behaviour. This variability does question its use as an element of farm assurance, which tends to be done irregularly (e.g., annually) and without matching of assessments to time of day or to avoid specific farms management procedures. Alongside any unidentified confounding factors, a number of other things could have made subtle changes to the herd behaviour throughout treatment periods such as subclinical disease, occasional cow movements and varying daily activity and energy budgets. This variability of QBA results over time, in fixed animal groups and settings requires further research.

6.5 Conclusions

Caution is always required in studies using results to interpret animals' affective states, and the limitations and potential confounding factors of the current study have been discussed, with direction for improved future research suggested. It is accepted that replication and validation of these results are required, however, the results reported here, are biologically plausible, both according to the scientific literature surrounding positive welfare, from a general common sense perspective and fit with the study hypothesis. QBA in the current study, was able to identify differences in cow behaviour and we hypothesise therefore associated affective state, between standard commercial housing conditions and modified housing offering variable enrichment resources. Although the results from the blinded QBA did not validate this, there were a number of possible explanations suggesting that this method of QBA was not as accurate in assessing the affective states of the cows. Results show that simple housing modifications are likely to positively impact the affective lives of commercially-housed dairy cows. One of the overarching challenges of offering animals' opportunities for positive experiences is knowing what we can offer to facilitate this. Although additional research is needed, the current study suggests that, some level of positive experience may be facilitated through simple modification to the housed environment using environmental enrichment strategies.

Chapter 7: Discussion and conclusions

7.1 Overview of thesis research findings

7.1.1 Chapter 1 – Introduction and literature review

The aim of this thesis was to use an epidemiological approach, to explore and evaluate, positive welfare in housed dairy cows. The literature review provided in Chapter 1, aimed to provide an in-depth collation and evaluation of the knowledge on positive welfare overall and specifically in dairy cows to date. An overview of the historic concept and assessment of animal welfare was provided, followed by discussion of the more recent addition to the scientific field, positive welfare, including the concept of its emergence and the current level of research which has been addressed to this area. The literature review highlighted that positive animal welfare research is very much split, between the search for robust indicators of animals' affective experiences and exploration into how animals' affective lives can be improved, with the provision of positive experiences. Despite the decade that has passed since the emergence of positive animal welfare as a research concept, the identification of animals' affective experiences is still a very complex challenge (Keeling et al., 2021). Knowledge is slowly being assimilated, with a diverse range of behavioural and physiological parameters having started to be explored as potential indicators of positive affective states (for reviews see; Boissy et al., 2007; Keeling et al., 2021; Matiello et al., 2019; Napolitano et al., 2009). There is no gold standard available to measure positive affective states, evidenced by the caution that is generally accompanied by reporting of results from studies, which have used potential indicators to make inferences on the affective lives of animals. The literature also suggests that behavioural measures are likely to have the best potential for identification of affective states, rather than physiological indicators, which lack the utility and sensitivity of being able to indicate affect not just arousal (Paul et al., 2005). To be able to identify and provide strategies to facilitate positive experiences in animals, validated positive welfare indicators are required. From the synthesis of knowledge on positive welfare provided in Chapter 1, further research directed at identifying accurate and robust indicators of positive affective states is clearly much needed.

At present, in terms of using positive welfare to enhance the quality of lives of farm animals, behavioural indicators appear to be the most informative and practical in terms of making judgements over situations animals prefer and value and for making inferences over their affective experiences in different situations. Investigation into how animals' affective lives can be improved, through the provision of positive experiences, has accumulated more impactful research to date, generally by evaluating animals' choices and preferred states, which are interpreted as providing the animal with a better or improved experience (Duncan, 2005; Fraser & Matthews, 1997). The outcomes of this research have been strongly taken up by multiple industries, such as animals kept in zoos, commercially produced pigs, poultry and laboratory animals and has, contributed to noticeable advancements in animal welfare (Buijs & Muns, 2019; Olsson & Dahlborn, 2002; Shyne, 2006; van Staaveren et al., 2021). The literature review in Chapter 1, identified an inherent gap in research evaluating positive welfare opportunities for dairy cattle. As with animals more widely, a number of potential positive welfare indicators have started to be explored for dairy cows, but with as-yet, no concrete validated measure (Keeling et al., 2021; Mattiello et al., 2019; Napolitano et al., 2009). The evaluation of dairy cows' affective lives, as a result of commercial management and exploration of how positive affective experiences may be able to be offered, is even more lacking. This overview of the scientific literature showed a clear gap in how positive experiences can be provided to dairy cows. This knowledge, therefore guided the research within this thesis, to address this gap. Specifically, the provision of possible positive welfare opportunities was chosen over exploration of positive welfare indicators, as more knowledge on this, compared to specific positive welfare indicators, would provide the maximum impact to industry and therefore potential to positively impact dairy cow welfare, through the short time of the project. In addition to this, the current research knowledge on positive welfare indicators in animals in general, appears equivalent across species, however, the research on positive welfare provision for dairy cows is almost non-existent, highlighting a more critical gap to be addressed.

7.1.2 Chapter 3 - Evaluation of the use of novel enrichment by commercially-housed dairy cows

In light of this, the objective of Chapter 3 was to evaluate the potential utility of novel environmental enrichment for housed dairy cows, a largely unaddressed question, through assessing the level of interest and utilisation of novel enrichment by two separate groups of commercially managed cows. The underlying concept behind the objective of the study was that environmental diversification and modification, with one such form being enrichment, may offer animals an opportunity for exploration, interest and control over their environment, which have been suggested as potential opportunities for positive affective states (Mellor, 2016; Wood-gush & Vestergaard, 1991; Yeates & Main, 2008). Every cow within two separate herds used the novel enrichment during the study. There was a surprisingly high level of interest and use of the enrichment, which was reflected across both groups, with on average 91.74% of the herds using the object during the first week it was present, but more interestingly, with still 83.10% of both groups still using it during the third week cows had access to it. Results showed that cows spent more time interacting with the novel enrichment during the first 2 weeks of presentation, compared to that of a brush during its first two weeks of presentation, in a study by (DeVries et al., 2007). Given that automatic brushes are a wildly established housing facility on dairy farms (DeVries et al., 2015), this comparable level of use suggests that other forms of novel enrichment may be just as valuable to cows.

Results from an ethogram showed that cows reacted to the initial presentation of the novel object with exploratory behaviours. Behaviours elicited the least were behaviours generally linked to stress or fear (Beilharz & Zeeb., 1982; Kilgour, 1975; Munksgaard et al., 1997; Wagner et al., 2012) indicating that the addition of the novel feature was not an aversive experience for the cows, but more generally reflected a motivation of the cows to approach and interact with it.

The results from this chapter revealed a high variability between individual cows, in terms of how much they used the enrichment, with older cows (parity 4 or above), using it the least in both groups, which could potentially be explained by a motivational trade off, due to energy constraints, stemming from increased health related physiological challenge (Pritchard et al., 2013; Rajala-Schultz & Gröhn, 1999). Another possibility could be reduced interest in the object, explained by altered affective states from conditions such as apathy or anhedonia, which are discussed in more detail in the next section. Generally, whether this reduced use by cows within later lactations could be explained by their physiological or psychological experience, or an interplay between the two, does pose concern regarding specifically the affective lives of older cows. Despite variability between cows, enrichment use was comparable between lactation groups and stage of lactation, again showing the utility of enrichment in this setting and its relevance to mixed cow groups at an individual cow level. The provision of enrichment therefore has the potential to positively impact large numbers of cows. These results provide confirmation that enrichment is likely to be one viable and practical way to facilitate opportunities for positive affective experiences in commercially-housed dairy cows and therefore an important area for further research.

During the intervention period, when the object was present, there appeared to be a substantial increase in play behaviour, exhibited by the cows when using the enrichment, a behaviour which had not been previously observed in this setting by the observer. Unfortunately, this behaviour was not formally measured during this study, and this is solely an observational remark. This was due to the time limitations of the study. Play behaviour is generally accepted to be a behavioural expression of positive affective states (Boissy et al., 2007; Held & Spinka, 2011) and would therefore be a valuable behavioural indicator to measure, to yield a true indication of how this behaviour was impacted by the provision of novel enrichment. If increased levels of play behaviour could be objectively assessed during the intervention period, this would markedly strengthen the inferences made from the study results, regarding the positive welfare impact that the novel enrichment had on cows' affective states.

Throughout referral to the literature within this thesis, frequent comparisons have been made to the pig industry, to clearly highlight the gap between the knowledge, perceived importance and industry uptake of enrichment. One reasoning behind the substantial research and implementation of enrichment within the pig industry could be pigs' strong underlying motivation for inquisitive exploration (Wood-Gush & Vestergaard, 1991; Wood-Gush & Vestergaard, 1993;) stemming from a natural need to root for food acquisition (Day et al., 1995; De Jonge et al., 2008; Studnitz et al., 2007). In more natural contexts, it's possible that dairy cows possess a similar underlying motivation, stemming from explorative and preferential grazing, in which cows will dynamically forage a range of areas (Hancock, 2009; Horadagoda et al., 2009). In housed settings, where this is no longer an opportunity, it could be possible that cows experience a heighted behavioural motivation for stimuli and associated opportunity for exploratory behaviour, as a rebound effect (Dawkins, 1988; Nicol, 1987;). Alternatively, and probably more likely, the successful industry implementation of enrichment, could be explained by the significant economic impact, brought by enrichment, as a mitigation for losses through injurious tail biting (D'Eath et al., 2014), which is linked to the behavioural deprivation and frustration of management of pigs in environments where they can't satisfy these behavioural motivations. Van de Weerd and Day (2009) produced a literature review on environmental enrichment for pigs housed in intensive systems and identified four criteria of success of which enrichment should meet, one of which being an improvement in the economics of the production system. It could be possible that dairy cows also suffer a similar level of frustration from the behavioural deprivation of their environmental conditions, but that this is not outwardly represented. Wood-gush and Vestergaard (1991) evaluated piglets' responses and preferences, over novel and familiar stimuli, by training them to either choose a pen with a known object in, or a pen with a novel object in. Piglets showed a strong preference to access the pen containing a novel stimulus, possessing no biological relevance. Authors concluded these results to show clear evidence of inquisitive exploration and curiosity. This evidenced motivation for environmental novelty, was followed by strong recommendations by authors for eradication of monotonous housing systems if welfare was to be considered seriously. Since cows are a different species, this initial question of whether cows would show inquisitive exploration in response to novel enrichment, needed to be addressed, however the results from this chapter, provide a strong body of evidence, that cows do possess a level of behavioural motivation for environmental stimuli and that diversifying the environment through environmental enrichment should be a serious welfare consideration for producers.

7.1.3 Chapter 4 - Novel enrichment reduces boredom associated behaviours in dairy cows

Alongside its facilitation for positive welfare opportunities, environmental enrichment is also usually the first strategy for boredom mitigation in animals in captive environments (Meagher, 2018). Boredom has recently been highlighted as a potential concern for the welfare of housed dairy cows (Crump et al., 2019), resulting from the monotony of housed environments (Wemelsfelder, 1993), paired with the increased disposable time dairy cows confined to housing have (Roca-Fernandez et al., 2013). Based on this knowledge, the objective of Chapter 4 was to evaluate the impact that the provision of novel enrichment had on behavioural indicators of boredom, in commercially-housed dairy cows. For this trial, we used ‘idling’, a form of inactivity, as a behavioural indicator of boredom. This behavioural indicator has shown promise as a marker of boredom and/or negative affective states in multiple species (Fureix & Meagher, 2015) but also dairy buffalo (Tripaldi et al., 2004), fattening cattle (Hintze et al., 2020) and dairy cows (Di Grigoli et al., 2019). We used unsuccessful repeated use of the robotic milking machine ‘refusals’, as another behavioural indicator of boredom. As far as we are aware, this behaviour has not previously been associated with boredom, but it was hypothesised to be a stimulus seeking and time filling activity which cows may use as way of coping with monotony. Results showed that both behaviours significantly decreased during the intervention period, whilst enrichment was present, compared to when it was not, indicating a reduction in boredom states. Boredom in dairy cows has received no research to date. Despite this, references to dairy cows experience of boredom occurs frequently within the literature, for example when discussing the underlying reasons cows use brushes (DeVries et al., 2007; Georg & Totschek, 2001; Meunier et al., 2017), as one suggested mechanism of rumination (Albright, 1983; Ewbank, 1978), as an explanation for increased salt block intake during low feed uptake (Doreau et al., 2004) and as a suggested likely experience of tethered cows (Krohn, 1994; Nalon & Stevenson, 2019). This study was the first to investigate the potential of boredom as a welfare concern for dairy cows, through the objective measurement of boredom associated behaviours. The results from this study were biologically plausible, with the provision of an additional time filling activity, reducing boredom associated behaviours. These results strongly support the suggestion that boredom is likely a welfare concern for housed dairy cows (Crump, 2019). In addition, the results of the study support the use of idling behaviour as a potential indicator of boredom or negative affective states in dairy cows. Results from this chapter have also contributed to the literature by identification of a new potential behavioural indicator of boredom 'refusals', which could be used in future research investigating boredom states in dairy cows. This behaviour was identified for this study as a potential indicator of boredom, as it appears to be a stimulus seeking activity, one behavioural motivation stemming from boredom (Burn, 2017).

Self-grooming was not an initially proposed measure for this trial, however it quickly became apparent that it was an important behaviour, with markedly increased levels being expressed when cows were both using and following use of the enrichment. As already discussed, self-grooming in dairy cows is a poorly understood behaviour, with it having been tentatively mentioned as a potential indicator of positive affective states (Keeling et al., 2021; Mattiello et al., 2019; Napolitano et al., 2009) but also being shown in diverse situations linked to stress and negative affective states in cows (Bolinger et al., 1997; Herskin et al., 2004; Munksgaard & Simonsen, 1996). The results from this study allowed us to provide a suggested explanation for the underlying mechanism of self-grooming, an arousal response, which could encompass both negative and positive experiences, adding to the literature and general understanding on the behaviour.

The potential use of environmental enrichment was initially explored in Chapter 3, as an avenue for the provision of environmental diversity and associated positive states in dairy cows. The results from this chapter, strengthen the findings from the previous chapter, that dairy cows value and will utilise environmental enrichment resources, by showing that in addition, this strategy also has the capacity to reduce negative affective states. The results from Chapter 4 offer an additional framing and motivation for the incorporation of some level of environmental enrichment strategies for housed dairy cows. Where the importance of positive welfare provision may not have reached its peak, or have the weight to influence industry and policy decisions, environmental enrichment as a strategy for mitigating negative affective states, could be focussed on in the interim, which fits with the more traditionally accepted concept of animal welfare management.

It should be mentioned that there are many different forms of inactivity, which are likely associated with different affective states and that further validation of the use of inactivity as an indicator of affective states in animals is still required (Fureix & Meagher, 2015). For example, idling was used as a behavioural indicator of boredom within this thesis, given the current literature available which suggests this (Burn, 2017; Fureix & Meagher, 2015; Meagher & Mason, 2012). Other negative yet different affective states, could also be represented by increased levels of inactivity, such as depression, anhedonia or learned helplessness, the hypothesis of which have all stemmed from analogies with humans (Fureix & Meagher, 2015). Given that these states are interlinked (Goldberg et al., 2011) and could all have root developments from barren or uncontrollably stressful environments, it may be difficult to decipher out the specific state attributable to the behavioural representation of inactivity. Meagher and Mason (2012) attempted to operationally validate the sensitivity of inactivity, as a behavioural representation for boredom, apathy (a lack of interest) and anhedonia (a symptom of depression, describing loss of pleasure) in mink. Authors hypothesised that boredom should be represented by increased interest in all stimuli, that apathy should be reflected with a decreased interest in all stimuli and that anhedonia should decrease interest in stimuli usually found rewarding. Mink managed in unenriched conditions showed reduced latencies and increased occupation times of all types of stimuli presented, whether positive, aversive or neutral. In addition to this, mink in unenriched conditions displayed higher levels of inactivity and showed no behavioural responses indicative of apathy or depression. Similarly, Webb et al. (2017) further investigated inactivity as a potential indicator of either boredom or apathy, in Holstein-Friesian veal calves, in simulated enriched or unenriched conditions, facilitated through level of feed availability and diversity. A combination of measurement of inactivity behaviours and results from a novel object test were used to further understand the association of these behaviours to boredom and apathy. No differences in inactivity were found in comparisons of feed restriction treatments, however, increased idling behaviour was displayed by calves that were fed more monotonous diets. Despite this, no corresponding link was shown through the results of the novel object tests, with no differences in latencies to touch the novel object being shown between treatment groups. Although increased levels of inactivity were shown in monotonous feed provision groups, authors concluded that no evidence was shown to support this behaviour as an indicator of boredom or apathy, in light of the novel object test results, where it would have been hypothesised that bored animals would have a heightened motivation for stimuli and should show shorter latencies to interact. One substantial criticism of this study, was that the novel object was presented for a period of three minutes, in one position of an approximately $10m^2$ enclosure, which inherently questions the opportunity that calves had to first of all notice this and secondly approach it. Given that both social competition and subclinical disease likely have large impacts of access of environmental resources, the very short time period that the novel object was presented for, likely does not reflect a true representation of the calves underlying behavioural motivation to interact with the object, which questions the validity of the authors conclusions. One strength of the study, was that multiple inactive behaviours were assessed, both lying idle and standing idle, with lying idle being the most able to distinguish between the different treatment groups. Within the study conducted in Chapter 4, only one inactive behaviour 'standing idle' was measured and this was directly due to the capabilities of our camera systems, in monitoring of an entire herd across a large area. The footage collected would not have provided the capability to distinguish between cows lying but awake, lying and ruminating and lying but asleep. Further research on inactivity in dairy cows should use a selection of subtypes of inactivity, to facilitate more accurate representation of this behaviour.

In human psychology, chronic boredom may be a precursor to depression (Todman, 2003), boredom and depression are generally linked (Goldberg et al., 2011) and the conditions which can cause development of these states (monotony/uncontrollable stress), are the same, which gives rise to the question of whether the idling results within this thesis could be attributed to boredom or depression. This infers the possibility of depression like states to also be a welfare concern for housed dairy cows. Drawing on the methodology by Meagher and Mason (2012), these concepts could be used in an attempt to validate inactivity as a behavioural indicator of boredom, or its potential to be reflective of different negative affective states, such as depression, through evaluation of dairy cows' responses to a range of stimuli, varying in valence and between dairy cows managed in what could be considered to be enriched and unenriched conditions. This, in combination with other behavioural indicators of boredom, such as stereotypical behaviour, indicators of affective states, for example, cognitive bias testing and motivation for stimuli, could be incorporated to further address these questions in dairy cows. Again, given the adaption of chronic boredom to depression, evaluation of inactivity and stimulus response at an individual level, instead of a group level, may yield more information regarding this association.

Although the results from this chapter strongly support the suggestion for boredom to be a welfare concern for housed dairy cows, the results have also provided a practical strategy for its mitigation. The results yielded from this preliminary research are promising, in terms of a practical solution for reducing the negative affective states, likely experienced by dairy cows as a direct result of housing conditions, however further validation is required across wider farm settings and to evaluate different or varied strategies for reduction of boredom.

7.1.4 Chapter 5 - Do housed dairy cows habituate to novel forms of enrichment over time? Do they show a preference between use of an outdoor concrete yard and an indoor novel object?

The results from Chapter 5 showed that two groups of housed dairy cows continually used two separate forms of enrichment over a period of 9 weeks. The time that cows spent using indoor enrichment significantly declined, during a period of 9 weeks, however the amount of time that cows spent outside remained relatively constant throughout. An evident preference was shown by cows to spend time outside, compared to interacting with indoor enrichment. The results from this chapter indicate that access to an outdoor loafing space, is valued and highly used by cows and does not show a decline in use over time. This resource was therefore identified in this chapter as another practical form of enrichment for housed dairy cows. The outdoor yard provided in the study, offered no other resources, such as feed or lying opportunities. This suggests that cows have a strong motivation to be able to go outside, which poses concern for cows when they are subject to solely indoor housing. The amount of time that cows spent outside, when they only had access to the outdoor yard, compared to when they had access to the outdoor yard in addition to indoor novel enrichment was comparable, which suggests that widening the behavioural opportunities available for cows within the building, was still not sufficient to demotivate their need to be outside.

The main limitation of this study was that it was conducted between the months of November to April only. This study requires replication throughout the full year to establish cows’ use of an outdoor concrete yard through different seasons. Alongside this, evaluating cows’ motivation to access an outdoor concrete space, in addition to measurement of other welfare indicators, may strengthen the potential for uptake on wider farms. A further limitation, was that only one form of enrichment was investigated as a comparison to cows' preference for being able to go outside, it is therefore possible that another indoor resource may warrant more interest, which could be comparable to the time cows spent outside.

A key point in this study was that it evaluated cows 24-hour free choice of use of an outdoor concrete yard, not the difference between indoor and outdoor environments. Therefore, recommendations stemming from these results are to offer cows the ability to have the choice of access to an outdoor area, one important aspect of welfare (Spinka, 2019; Spinka & Wemelsfelder, 2011). Interestingly, in a study exploring farmers perceptions of different elements of positive welfare (Vigors & Lawrence, 2019), although farmers were not familiar with the phrase positive animal welfare in itself, the concept of environmental agency arose primarily in reference to outdoor access. Providing animals with the individual choice to be inside or out, was seen as an important element of providing good welfare, which is promising in terms of encouragement for this to be taken up by the industry. Smid et al. (2021) explored the perceptions of Canadian dairy farmers on the provision of outdoor access for dairy cows. Five themes of reason were identified for why farmers would not provide outdoor access; adverse climate conditions, negative implications of outdoor access on cow welfare, concerns regarding decreases in profitability, unsuitable farm structure and a better ability or ease of managing animals solely indoors. The term 'outdoor access', was used in all discussions and this term was not specifically defined and so what type of outdoor access was being referred to was not specified. When looking at the descriptions given regarding reasons that discourage farmers from letting cows outside, it could be that alternative provision of outdoor areas may not present with the same issues, or may help mitigate these. First of all, for example, adverse weather conditions, would be unlikely to impact cows with the choice to come inside or go outside. Despite the provision of outdoor access being practically difficult, in a related study, farmers had positive perceptions about cows being outside, with reference being made to the enjoyment farmers feel when they see cows outside, their recognition that cows enjoy being outside and that letting cows outside has a positive impact on their personal wellbeing (Smid et al., 2022). Clearly providing cows with some level of choice between indoor and outdoor environments is positively perceived by cows themselves (Smid et al., 2018; von Keyserlingk et al., 2017), farmers (Smid et al., 2022; Vigors & Lawrence, 2019) and society (Cardoso et al., 2016; Schuppli et al., 2014). There are barriers which discourage farmers from allowing cows outside, but these have been mainly explored when discussing pasture (Smid et al., 2021; Smid et al., 2022). The outdoor space that was provided within this study, is likely to be much more achievable for farmers than allowing cows out to pasture and therefore research identifying the actual barriers to practical uptake of this and exploration of solutions would be very worthwhile. Alongside this, it could be possible that the phrase outdoor access is automatically assumed to mean access to grazing and therefore communication to the industry on practical alternative outdoor areas is critical.

Responses from farm staff during the trial when cows had outdoor access and afterwards were generally positive. Specific comments were made indicating that farm staff enjoyed seeing the cows outside and that cows appeared to really enjoy having access to it. There was one practical barrier in continuation of use of the outdoor yards following the end of the trial and that was the labour required to scrape the floor. The farm uses Lely automatic scrapers for slurry removal, which requires a concrete slatted system, where slurry is easily pushed through to an underground storage area. The outdoor concrete yards consisted of solid concrete flooring, not allowing use of the automatic Lely scraper and so required manual scraping by staff, a time and economic burden. The outdoor area provided to the cows in this study was small, and so the time taken to completely scrape the area was also negligible for one person, in consideration of a full working day. The real economic impact of this labour cost, required daily, could easily be calculated, and the possibility for this to be incorporated into the overall farm business plan considered. An alternative route, could be the discussion of this barrier with specialist industry manufacturers, such as Lely, who may have the potential to provide technical input, in an attempt to find a solution. Despite the discontinuation of use of this resource provision at the farm, it should be mentioned that the indoor enrichment used throughout this thesis has been retained.

Provision of access to an outdoor concrete exercise area, potentially a much more feasible compromise to letting cows out to pasture, was highly valued and used by cows, suggesting it offered them a positive experience and enhanced their welfare. These results offer another practical suggestion for how farmers can offer positive welfare opportunities to housed dairy cows.

7.1.5 Chapter 6 - Does providing enrichment impact the affective states of commercially-housed dairy cows?

Results from earlier chapters within this thesis, demonstrated that housed dairy cows showed a high level of interest and use of novel indoor enrichment and an outdoor concrete yard, suggesting that these were both valued resources and likely facilitative of a positive experience for the cows. The objective of Chapter 6, was to use a positive welfare indicator, to identify the possibility of whether cows' affective states may differ between housing conditions; standard housing conditions, compared to an intervention period consisting of provision of continual access to two different forms of environmental enrichment. In line with the strongly supportive literature, that provision of diverse stimulating environments are conducive to enhanced affective states in animals (Anderson et al., 2021; Brydges et al., 2011; Douglas et al., 2012), we hypothesised that cows would have improved affective states whilst they had access to additional environmental resources, compared to when they were housed in standard conditions. We chose QBA as the positive welfare indicator to evaluate this, due to it being regarded as one of the most promising indicators for measuring animals' affective states at present (Keeling et al., 2021) and being the only positive welfare indicator that has been practically implemented on farm welfare audits (WelfareQualityNetwork, 2018). The results that emerged from the on farm QBA results supported the hypothesis, with cows scoring higher on PCA components of positive affective terms and lower on PCA terms with negative emotional connotations, indicative of better affective states. These results are discussed and explained in depth in Chapter 6 (see 6.4.1).

We attempted to validate these results using blinded QBA assessment of video recordings, however results did not yield the same significant patterns. There were a number of valid explanations for this, which are discussed in detail in Chapter 6 (see 6.4.1). One of the main limitations of this study, was that only one positive welfare indicator was used to evaluate cows' affective states. The results of the current study could be further supported by use of a combination of positive welfare indicators in conjunction. QBA has been used widely in dairy cows as a general welfare assessment (Andreason et al., 2013; Des Roches et al., 2018; Popescu et al., 2014), but these results suggest it may be useful specifically for the evaluation of positive affective states. This trial was the first to utilise QBA to assess dairy cows' affective states in response to what was deemed a positive welfare intervention. Despite this, a small number of studies have used QBA to identify differences in cows' affective states in varying environmental conditions, which potentially reflects that cows' affective lives are sensitive to the everyday environments that they live in. These results have suggested potential environmental conditions that may provide for more positive affective states in cows. Ebinghaus et al. (2022) conducted a cross sectional exploration of the associations between QBA results and aspects of herd health, stock person and farm factors on 25 dairy farms. No associations were found between udder health and metabolic status and QBA results, a potentially surprising result, given the known painful condition that mastitis is (Boyer Des Roches et al., 2017; Leslie & Petersson-Wolfe, 2012;) and that QBA has been shown to reflect notable differences in behaviour and associated interpreted affective state in cows suffering with mastitis (Des Roches et al., 2018). Cows from farms which used deep bedded cubicles or straw yards, compared to cows from farms using raised cubicles, had QBA results indicative of more positive emotional states. In addition, cows from farms which had increased voluntary stockperson contact, were also reflected to have more positive affective states. A similar study by Brscic et al. (2019), conducted QBA on dairy calves across 49 different farms and found both organic farms and farms with a larger number of calves, to have significantly higher welfare quality criterion scores (an aggregated score derived from QBA results where 0 points = worst to 100 points = excellent situation). Ellingsen et al. (2014) used QBA to evaluate the handling style of farm workers on 110 dairy farms, in reference to calf handling. QBA was also conducted on calves to evaluate their response to different handling styles. Calves that were handled in a patient and calm manner had QBA scores indicative of higher levels of positive mood, compared to calves which were handled in a nervous or aggressive manner, which had higher scores indicative of poorer affective states. These results are perhaps unsurprising, however they highlight both the ability of QBA as a method of evaluation of affective states, to detect subtle differences in behaviour suggestive of different affective states, which repeatedly seem to fit with biologically plausible underlying explanations. As previously described, a potential criticism is the inability of observers to be blinded to treatments, highlighting risk of a subconscious bias, particularly in situations with moral connotations, for example observers assessing calves being handled aggressively may perceive the calves' behaviour to be more indicative of negative attitudes, such as fear or stress, compared to calves being handled in a patient and gentle manner. This could be controlled for to a certain extent, by providing clear, detailed descriptions of behaviourally relevant expression of each term used within the assessment, as was provided in accompaniment of the QBA assessment used in Chapter 6, but was not provided for this particular study. Overall, these results and the surrounding literature suggest that day to day environmental living conditions have an impact on the affective states of cows and calves and therefore should be a priority for research and welfare consideration. However, our results also showed a large amount of variability in QBA scores, within intervention periods, which means questions remain around how much it varies over short term periods and why such variation occurs when conditions are apparently unchanged.

Overall, the results attained through the use of QBA within this thesis, indicate that the provision of additional enrichment opportunities for housed cows and the provision of access to be able to go outside may have a positive impact on the affective lives of cows.

7.2 Recommendations for future direction of research

There are two main directions for future research following the research findings of the current thesis and the literature review provided in Chapter 1. The first line of direction, is the continuation of research into positive welfare in cows as a concept in general, including identification of valid indicators of affective states and evaluation of cows' behavioural choices to gauge housing and management modifications capable of enhancing welfare. The other line of direction, is strengthening the positive welfare concept to enhance practical and public uptake of positive welfare interventions.

The research conducted within this thesis, has shown that novel enrichment likely facilitates positive affective experiences in cows, paired with a reduction in boredom associated behaviours, indicating a reduction in negative affective states. Later research within the thesis, showed that providing cows with access to an outdoor concrete yard also provided an opportunity for positive experience and results indicative of improving cows' affective lives. From these behavioural results, it is clear to see that simple environmental modifications can enhance welfare, and so has provided relevant examples of how positive welfare experiences can be facilitated, a concept which has been urged for incorporation into animal welfare management by both policy (FAWC, 2009), animal welfare scientists (Mellor, 2016; Turner, 2019) and the general public (Vigors, 2019). I believe it would be worthwhile for the importance and weight of positive welfare to be further strengthened, to facilitate industry wide implementation. Measurable health or production benefits to positive welfare interventions, would add a greater incentive to uptake by farms as an extension to the face value findings of improved welfare. The research provided in this thesis, aimed to evaluate low cost, practical and feasible environmental modifications able to enhance welfare, with an aim to have the maximum real-life impact, however some form of return on cost may be required to enhance uptake.

The first and most obvious link to be explored, is the potential of any relationship between improving animals' affective lives, by offering opportunities for positive experiences and production. Tentative research already exists showing that there could be a possible connection between these factors. A recent study by Thompson et al. (2022) found that commercially-housed dairy cows which were provided with additional 'living space' ($6.5m^2$ vs $3m^2$ per animal), produced significantly more milk than cows housed in a lower living space group, however they did take longer to become pregnant after calving. The cows within the increased living space group, also spent more time lying in cubicles and less time in passageways. This was suggestive of better welfare as the increased space may have provided cows the opportunity to avoid certain conspecifics or negative social interactions in general, as passageways have been shown to be common areas for negative social interactions (DeVries et al., 2004). This could have facilitated unhindered agency over their environment, movement and activities and likely reduced stress associated with negative social interactions. Associations have also been shown between milk production and the use of brushes, a commonly implemented environmental feature for dairy cows. Schukken and Young (2009) provided two swinging brushes to 100 first lactation cows and 100 cows within their second lactation or higher and found that cows within their second lactation, produced approximately 3.5% more milk daily when compared to a matched control group, however there were no differences in milk production for cows in other parity groups. Keeling et al. (2016) evaluated brush use in 72 dairy cows, across a 9 week period and found a positive relationship between the frequency of brush use and milk yield. Results showed that each additional interaction with the brush was associated with an increased milk yield of 1.52kg per day. A study by Lin et al. (1998) evaluated different forms of cooling systems for cows and found that cows which were provided with a combination of both sprinklers within the feed alley and also misters over stalls, produced more milk than cows provided with one system or the other. In pigs, the provision of varying environmental enrichments has been linked with enhanced growth performance (Beattie et al., 2000; van de Weerd et al., 2006) and enhanced carcass quality (Beattie et al., 2000; Carreras et al., 2016), compared to pigs in unenriched environments. Economical production benefits have also been shown in broiler breeders with enrichment (Leone & Estévez, 2008; Nazareno et al., 2022) and feedlot housed lambs (Aguayo-Ulloa et al., 2014; Aguayo-Ulloa et al., 2015). A significant link was shown between environmental enrichment and a reduction in tail biting in pigs, which provided farmers with a substantial strategy for reducing economical losses. This may explain the large uptake of environmental enrichment within the pig industry. An alternative strategy to linking the provision of positive welfare opportunities with an economical benefit, could be either the incorporation of specific positive welfare opportunities into currently available farm assurance schemes or formulation of a new farm assurance scheme, where consumers could be given the choice of purchasing dairy products at a higher price, with a better return for farmers making positive welfare interventions. Although a complex way of offering producers higher financial returns, a strong demand for enhanced welfare is shown by the public (Alonso et al., 2020; Dransfield et al., 2005), particularly in regard to dairy cows (Cardoso et al., 2016; Ellis et al., 2009).

Another potentially valuable direction for research, is the evaluation of a possible link between the effects of provision of positive welfare opportunities and overall resilience, encompassing stress coping, physical and psychological recovery from traumatic events and health status. In human psychology, happiness, optimism and generally more positive affective lives, are often associated with increased resilience (Guest et al., 2015; Ovaska-Stafford & Maltby, 2021), stress coping (Khosla, 2006; Ong et al., 2006), recovery (Scheier et al., 1989; Scheier et al., 1999) and both psychological (Love & Holder, 2014; Rezaee et al., 2016) and physical health (Sabatini, 2014; Veenhoven, 2008). Reducing the perception of stressful experiences has also been shown to facilitate better psychological wellbeing (Ruiz-Aranda et al., 2014). More stimulus diverse and enriched environments, one such concept to facilitate positive experiences in animals, have also shown to be positively linked to recovery in people. Ulrich (1984) evaluated records of cholecystectomy recovery and found that 23 patients staying in rooms which provided access to a window with a view to natural green scenery, had shorter postoperative stays, required fewer analgesics and received less negative evaluation comments from staff compared to 23 matched pairs staying in similar rooms, with windows which faced a brick wall. A similar study by Illinois (2014), evaluated whether window views would impact stress recovery in students, through a combination of self-reporting and measurement of two physiological stress indicators. Window treatment had no effect on self-reporting stress scores, however physiological scores were significantly linked to window treatment, inferring that a window with a natural view had a positive impact on stress recovery, compared to a window with either a barren view or no window. The overall objective of the provision of positive welfare opportunities for animals, is to provide opportunity for them to experience positive affective states, in an attempt to increase the balance across their lifetime of positives to negatives. This concept is not far removed from people, where it is assumed that better affective lives represent happier people. Given this, it is credible to hypothesise that a similar connection could be possible in animals. Evidence of this has already started to emerge, a study by Wang et al. (2019) evaluated whether environmental enrichment may have an impact on pain sensitivity and depression like phenotypes associated with neuropathic pain in mice. Mice were housed in a sawdust bedded pen with access to food and water but the enriched group had access to frequently changed running wheels, toys, houses and maze systems. Mice in the environmental enrichment group showed attenuated pain threshold reductions and depressive like phenotype, as measured through behavioural tests of depression, following sciatic nerve injury. Pham et al. (2010) evaluated analgesic self-administration following a surgical procedure, in individually housed mice, separated into two groups, either living in enriched conditions or standard and also group housed mice, again separate into two groups of housed in enriched cages or unenriched cages. Socially enriched mice self-administered less analgesics in the recovery period than individually housed mice. In both the socially and individually housed groups, the cohort housed in also physically enriched environments also self-administered less analgesics compared to the non-physically enriched cohort. Similarly, pigs housed in environmentally enriched environments have shown to be less affected by stress in general (Reimert et al., 2014) and following stressful events such as regrouping (Ko et al., 2020). Links between the effects of positive welfare provision and overall resilience may have the potential to yield economic benefits from enhanced physical health parameters and recovery, alongside the further associated welfare benefits of this. In addition to this, managing animals in a way that promotes and enhances resilience could help buffer them to the negative psychological and physical experiences of routine aversive or stressful procedures that they are subject too. Either reducing the severity, or longevity of the aversion, would provide an additional function of offering positive experiences, not just the positive experiences in themselves, but the reduction in negative experiences, which would aid pushing towards the overall goal of balancing lifetime positive experiences to negatives. This may also bring with it social benefits, for example when being transparent with the public about socially contentious management issues, if producers could also show how they also provide positive opportunities, this may play a role in offsetting the negative impacts. These possible connections would aid industry wide acceptance and uptake of the provision of positive welfare opportunities.

7.3 Conclusions

This thesis has contributed to the literature regarding positive welfare in adult dairy cows. Results show that housed dairy cows possess a high level of interest in environmental stimuli and opportunities for environmental exploration. In addition, this research has shown that having access to a simple outdoor space, appears to be and remain of importance to cows, in that a high proportion continue to use it over a period of 9 weeks. The results suggest that offering opportunities for cows' interest and exploration through environmental enrichment, facilitates positive welfare experiences, something deemed important from both a social and political aspect. Alongside this, results showed that the provision of commercially practical, inexpensive, environmental enrichment resources, decreased boredom associated behaviours, which may be linked to negative affective states and therefore suboptimal welfare. Dairy cow housing can generally be regarded as monotonous, meeting basic needs of provision of feed, water and resting areas and therefore opportunities for cows' interest and exploration are currently limited, if present at all.

This research shows that environmental enrichment should be seriously considered as an important aspect of dairy cow housing and management. Furthermore, it has demonstrated two feasible and practical suggestions of how this can be achieved. Therefore, the inclusion of additional environmental enrichment resources or access to outdoor space, is worthy of consideration to enhance dairy cow welfare and for inclusion in basic welfare scheme requirements.

Further research should focus on finding robust, validated indicators of positive affective states, exploring different forms of environmental resources likely to have the greatest positive welfare impact on dairy cows and possible links between positive affective states in animals and wider benefits through links to resilience or production, to strengthen the concept of positive welfare and push industry uptake.

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