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Effects of Physical Enrichment and Social Housing on Calves' Growth, Behaviour, Affective State and Cognitive Ability

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School of Agriculture, Policy and Development

Chenyu Zhang

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Declaration of Original Authorship

Declaration:

I confirm that this is my own work and the use of all materials from other sources has been properly and fully acknowledged.

Chenyu Zhang

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Abstract

Dairy calves are commonly reared in artificial environments in the dairy industry. It is essential to understand and satisfy their motivations so as to improve their welfare, promote their behavioural and cognitive development and increase their growth and adaptability to environmental changes. The first aim of this dissertation was to study effective ways to complicate calves' housing environments and understand how the environment complexity impacts behaviour and improves calves' welfare. We investigated the effectiveness of physical enrichment protocols and determined calves' preferences for and ways of interacting with various physical enrichment items. A fixed multi-item enrichment presentation schedule was considered an effective protocol. Nets filled with scented hay seemed to be the most attractive item tested, which stimulated calves to show five types of interaction behaviours towards them. We then determined how the provision of the physical enrichment items in the effective protocol identified, pair housing, and the combination of both components in calves' pre-weaning period affect pre- and post-weaning weight gain, pre- and post-weaning behaviours, fear responses to novelty and memory ability. We found that adding complexity to pre-weaning calves' housing environments using the three methods appeared likely to improve their welfare by providing outlets for natural behaviours, mitigating undesirable behaviours, promoting growth, increasing behavioural flexibility or improving memory ability. In addition, since calves' fear responses in repeated fear tests are inconsistent, this dissertation also aimed to investigate the potential mechanisms resulting in this uncertain reliability. We investigated whether calves have visual lateralization in

processing fear in novel object tests and if initial monocular presentation of novel objects affects calves' fear responses. The results suggested that the poor test-retest reliability in repeated novel object tests could not be explained by visual lateralization in processing fear, and eye laterality might not be considered a useful measure of fear at this age.

Chapter 1

Literature Review

1. Literature Review

1.1 Introduction

In natural conditions, newborn calves are reared by the dam (Von Keyserlingk and Weary, 2007), including getting nourishment, being protected from potential predators and building strong bonds through a series of maternal behaviours such as licking, grooming and grunting vocalisation (Hermann and Stenum, 1982; Grandinson, 2005). Neonates usually hide in grasses or bushes for the first few days after parturition (Langbein and Raasch, 2000), followed by integrating into herds (Rørvang et al., 2018), interacting with peers (Sato et al., 1987) and exploring complex physical environments (Whalin et al., 2021). Natural environments provide calves with opportunities to show their natural behaviours and allow them to engage in positive experiences and behavioural development (Špinka, 2006).

In the dairy industry, calves are commonly separated from the dam within hours after parturition and housed individually. For instance, 60% and 63% of farms rear calves in individual housing conditions before weaning in Europe and the United States respectively (Marcé et al., 2010; USDA, 2016). The early separation of cows and calves and individual housing of calves is suggested to increase cow productivity (Mikuš and Mikuš, 2020), increase convenience in managing calves (Ventura et al., 2013), reduce feeding costs (Mandel et al., 2016) and limit or prevent the horizontal spread of diseases such as diarrhea and respiratory infections. However, since conventional individual housing usually refers to housing calves in individual pens or hutches with limited stimuli, this housing method restricts calves' opportunities of getting maternal care from the dam and interacting with other conspecifics and complex environments (Cantor et al., 2019). These deprivations may have short- and long-term effects on calves' physical, behavioural and cognitive development. For instance, when housing environments are small or lack appropriate stimuli, calves' behavioural needs cannot be fulfilled and their abilities to show functional or adaptive behaviours are restricted (Mason and Burn, 2011). It thus may lead to persistent frustration for calves and result in the development of abnormal behaviours (Mandel et al., 2016). In addition, individually housed calves have poor adaptive skills when facing novel social situations and novel environments (Vieira et al., 2012). Moreover, individually housed calves express learning and memory deficits, demonstrated by poor performance in the tasks of reversal learning and recognition of novel objects (Gaillard et al., 2014). Consequently, when calves who experienced deprivations grow to adult cattle, they may not be able to fully achieve their biological potential, showing compromised emotion and ability to express their natural behaviour through their lives (Cantor et al., 2019).

Dairy calves are usually weaned between six and nine months old in nature, with characteristics of reduced suckling, increased solid feed intake and social interaction (Weary et al., 2008). In contrast, calves reared on farms are normally weaned at younger ages than in the wild and then they may be mixed with unfamiliar conspecifics in novel post-weaning pens (Weary et al., 2008). These changes are considered stressors, which may result in increased social stress and aggression, and reduced feed intake and growth rate (Bøe and Færevik, 2003; Weary et al., 2008).

Since housing environments during the infancy period play a crucial role in the short- and long-term development of mammals (Bornstein, 1989), the aim of this review is to summarise the effective ways to improve calves' barren housing environment and promote their development. I begin with a description of the effects of social housing on calves' growth, behaviour, affective states and cognition. I then describe the effects of physical environmental enrichment on these outcomes. Because of the limited literature on physical environmental enrichment on calves, I also review the literature on other species (especially pigs) describing the effects of physical environmental enrichment. As an affective state commonly affected by housing and management, fear has been widely assessed in calves using various methods such as novel object tests. However, calves' responses to the test of fearfulness are inconsistent (Meagher et al., 2016), which may result from brain lateralization in processing certain emotions. Therefore, I review the known functions of each hemisphere related to asymmetric emotion processing in a range of species. Finally, I include the overall objective of this dissertation.

1.2 Effects of Social Housing on Dairy Calves

Social housing, meaning calves are reared in pairs or groups of more than two animals, is prevalent in the last few years (Cobb et al., 2014) because of the increased welfare concerns among stakeholders in the dairy industry as well as in public (Ventura et al., 2013). For farmers, social housing provides easier management, especially with the use of automated feeding systems (Hötzel et al., 2014). For calves, social housing provides a chance for them to gain access to full social contact. Since calves are motivated to physically interact with conspecifics (Costa et al., 2016) and can form long-lasting social relationships from a young age (Raussi et al., 2010), social housing provides a variety of benefits for them.

1.2.1 Growth

Gregarious animals are deemed to benefit from social learning (Warnick et al., 1977) or social facilitation (Galef, 1981). When a calf shows approaching, manipulating and feeding behaviours, their pen-mates may learn how to consume feeds or imitate these behaviours while observing the calf engaged in these behaviours. Therefore, social housing is suggested to result in calves consuming more solid feed (e.g. pair housing: Vieira et al., 2010; group housing: Babu et al., 2004), particularly when calves receive an enhanced milk allowance. Jensen et al. (2015) reported that enhanced milk feeding reduced calves' concentrate intake, but pair housing could stimulate concentrate intake in these calves. As an important digestive organ, the rumen plays a key role in growth, production performance and health (Diao et al., 2019). Since the postnatal period is a sensitive window for rumen development, providing readily fermentable carbohydrates to calves enhances VFA production in the rumen, induces changes in the microbial community and stimulates the development of rumen epithelium (Mentschel et al., 2001; Guzman et al., 2016). Therefore, largely as a result of increased feed intake, social housing can benefit calves' growth (Bernal-Rigoli et al., 2012), evidenced by increased feed intake and greater body weight gain in pair housed environment (Jensen et al., 2015) and group housed environment (Tapki, 2007). Costa et al. (2015) also found that pairing calves at one week of age increased calves' starter intake and daily weight gain compared to pairing calves at six weeks of age and individual housing, which may indicate that social housing at an early age promotes social facilitation, social learning, or some combination.

For post-weaning calves, they are usually mixed with unfamiliar conspecifics at a similar age in novel group pens with novel diets (Bolt et al., 2017). Calves previously reared in conventional environments experience growth checks in this phase since the changes are considered stressors, compromising their growth (Vieira et al., 2010). In contrast, calves previously reared in pair pens experience reduced growth checks in the weaning and post-weaning phase than those previously reared in individual pens (Chua et al., 2002; Vieira et al., 2010) as the calves previously reared in pair pens are better adapted to solid feed in the pre-weaning period (Babu et al., 2004). Duve et al. (2012) demonstrated that calves previously reared in pair pens spent more time eating concentrates than those previously reared in individual pens in a post-weaning feed competition test. Therefore, calves reared in pair or group pens before weaning continually get benefits from the pre-weaning social experience after weaning and regrouping.

1.2.2 Behaviours

Many studies have investigated the associations between social housing and calves' positive behaviours such as play and allogrooming. Play behaviour is considered an indicator of positive welfare (Held and Špinka, 2011) because animals show play behaviour when their basic requirements such as feed, health and safety are fulfilled or they are in a positive experience (Jensen et al., 2015). Duve et al. (2012) found that pair housing increased calves' play behaviour compared to individual housing. Jensen et al. (1998) observed the expression of social play in group pens and suggested that social housing might stimulate locomotor play. Allogrooming is suggested to help calves build and maintain strong bonds with conspecifics (Færevik et al., 2007), and reduce the heart rate of the receivers, which may indicate the experience of positive emotions (Laister et al., 2011). Allogrooming may also remove debris or ectoparasites from the body of animals and thus maintain their hygiene (Rich, 1973). Tapki (2007) indicated that social housing increased the expression of allogrooming. Therefore, pre-weaning social housing fulfils calves' needs for social contact with conspecifics, which may stimulate their motivations to express positive behaviours.

Cross-sucking has been defined as a non-nutritive suckling of one calf towards the body of another calf (Jensen, 2003). Although cross-sucking can be reduced by using nipple milk feeders, reducing the speed of milk flow through the nipple, using automatic milk feeders, better designing the milk feeding area to reduce competition, increasing milk allowance, providing dry artificial teats, feeding fibre after milk feeding and feeding starter from specialized starter teat bottle (Hammell et al. 1988; Lodberg and Lidfors 2001; Jung and Lidfors 2001; Weber and Wechsler 2001; Ude et al. 2011; Salter et al., 2021), frequent cross-sucking in socially reared calves is still reported on many farms (e.g. Lidfors and Isberg, 2003). Cross-sucking is considered an abnormal behaviour, which stems from a redirection of natural sucking behaviour and may lead to calves' health issues (Jensen, 2003). For instance, the sucking calves may ingest a large amount of hair, which can enter the rumen, form into hairballs and thus result in digestive disorders (Größbacher, 2013). Calves may suck the penis of conspecifics and drink their urine, which may reduce their feed intake and induce liver disorders (Größbacher, 2013). For the sucked calf, cross-sucking may lead to hair loss and inflammation (Day et al., 1987). The higher occurrence of teat injuries and *C. pyogenes* mastitis has also been reported in farms with cross-sucking calves (Lidfors and Isberg, 2003). In addition to cross-sucking, competition and aggression among calves are other problems of social housing (Costa et al., 2016). For group housed calves with only one single automatic milk feeder, most aggressive interactions happened near the feeder (Herrmann and Knierim, 1999), which can reduce calves' milk consumption (Von Keyserlingk et al., 2004). Therefore, although social housing benefits calves, the expression of the detrimental behaviours may make farmers concerned about this type of housing environment.

1.2.3 Response to Novelty

Calves are often exposed to novel situations such as changes in housing environment, changes in milk feeding procedure and regrouping with novel conspecifics. However, individually housed calves may feel fearful or stressful during these novel events and show reduced ability to cope with the stressors (Mandel et al., 2016). Pre-weaning social housing has been studied as an effective way to improve calves' adaptability to novel events, reduce weaning distress and improve performance in post-weaning housing environments (Vieira et al., 2010; Vieira et al., 2012). For example, group housing reduced calves' latency to enter an open-field arena than individual housing in an open-field test, which might suggest that group housed calves were less fear of novel environments than individually housed calves (Jensen et al., 1999). Pair housed calves showed a shorter latency to sniff an unfamiliar calf compared to those housed individually in a social test, which might suggest that pair housing reduced calves' fear of novel conspecifics compared to individually housing (Jensen and Larsen, 2014). Pair housed calves vocalised less during the weaning period compared to individually housed calves, which might suggest reduced weaning stress for pair housed calves (Vieira et al., 2010). When mixing calves into a group pen, calves with previous social experience showed fewer agonistic behaviours and more non-agonistic behaviours than those without social experience (Veissier et al., 1994). Group housed calves spent more time staying with conspecifics than individually housed calves after being introduced to a new group (Broom and Leaver, 1978). Therefore, these studies indicate that pre-weaning social experience reduces calves' anxiety-related reactions, develops their social skills and improves their adaptability to environmental changes (Mandel et al., 2016).

1.2.4 Cognition

Individual housing has been suggested to impair calves' cognitive ability. Gaillard et al. (2014) reported that calves with social deprivation made lots of mistakes in a reversal-learning task. Since social deprivation has been suggested to have negative influences on neural development and brain function (e.g. Martin et al., 1991; Poletto et al., 2006), calves' early life may be an important period for their brain development, which may affect their behavioural flexibility and adaptive capacity (Meagher et al., 2015). In order to eliminate the learning deficit and improve brain development, researchers studied the effect of social housing. For instance, Meagher et al. (2015) showed that calves in a complex social environment and simple pair pens performed better in the reversal learning phase of a colour discrimination task compared to those in individual pens. It may indicate that early social housing benefits calves' cognitive development.

1.3 Effects of Physical Environmental Enrichment on Farm Animals

Physical environmental enrichment refers to expanding the size of animals' enclosure, adding accessories to the enclosure or altering the complexity of the enclosure (Bloomsmith et al., 1991), so as to provide appropriate stimuli to promote an animal's normal behaviours and improve its biological functioning and welfare (Newberry, 1995; Mandel et al., 2016). Most farm animals, such as sheep, pigs and beef cattle, are housed with the dam or allowed to have social contact with conspecifics during the pre-weaning period (Costa et al., 2016). However, although the housing environment provides animals with the chance of maternal or social contact, they receive limited stimuli from external environments and are not able to cope with their captive environments very well (Poole, 1992). The loss of adaptability may increase animals' stress and reduce their welfare (Van De Weerd and Day, 2009). In order to improve the negative status, physical environmental enrichment has been studied in farm animals (e.g. Veissier et al., 1997; Kutzer et al., 2009; Miranda-de La Lama et al., 2013). In this section, I will mainly use pigs as an example to describe the effects of physical environmental enrichment because previous research has produced a wealth of physical environmental enrichment knowledge on pigs.

In intensive housing systems, pigs are often reared within simple and invariant environments that provide little stimulus to accommodate their highly motivated species-specific behaviours (Van De Weerd and Day, 2009). For instance, the housing environments are usually barren with concrete (slatted) floors and there is no substrate for pigs to root. The restriction of motivated behaviour can lead to psychological distress and is associated with the manifestation of undesirable behaviours such as ear and tail biting (Wood-Gush and Beilharz, 1983; Fraser et al., 1991). In order to improve pigs' welfare, effective physical environmental enrichment needs to be studied.

1.3.1 Characteristics of Effective Physical Environmental Enrichment

For pigs, functional and effective physical environmental enrichment is suggested to meet a series of characteristics, which stimulate pigs to show certain behaviours while interacting with the enrichment (Van De Weerd and Ison, 2019). Council Directive 2008/120/EC (European Commission, 2016) described the characteristics of materials that may be used to improve animal welfare as investigable, retaining pigs' interest and stimulating them to explore the materials through their snout; manipulable, allowing pigs to change the materials' location, appearance and structure; chewable, allowing pigs to deform or destroy the materials by biting or chewing; edible, attracting pigs through its interesting texture, flavour or smell. Investigability may be considered an important characteristic as it is believed that pigs have an intrinsic need to engage in inquisitive exploratory behaviour for the purpose of acquiring and processing information and thus, efficacious materials should elicit and reinforce the expression of exploratory behaviour, including snout manipulatory elements (Fraser et al., 1991; Wood-Gush and Vestergaard, 1991). Feddes and Fraser (1994) reported that objects were used more when they can be altered by chewing. Van De Weerd et al. (2003) found that the materials with characteristics of ingestible, destructible, and contained could sustainably attract attention. According to these characteristics, physical environmental enrichment can be divided into three categories. If items meet all of the criteria mentioned above, they can be considered optimal physical environmental enrichment (Godyń et al., 2019). In addition, if items meet part of the characteristics, they can be considered suboptimal physical environmental enrichment (Godyń et al., 2019). Since a suboptimal item is an essential component of the pigs' enrichment, it needs to be used with other items together to fulfil pigs' needs (European Commission, 2016). Moreover, the worthless physical environmental enrichment is an item of marginal interest, which should not be considered fulfilling the essential requirements of the pigs although they can distract pigs (Godyń et al., 2019). Most types of substrates including straw incorporate all characteristics when used as bedding, and thus are regarded as optimal physical environmental enrichment (Bulens et al., 2015). The items such as fresh wood, peanut shells, natural ropes, corn cobs and compressed straw cylinders are considered suboptimal physical environmental enrichment because they have most but not all of the characteristics of an effective enrichment (European Commission, 2016). The effects of other items including chains, rubber, soft plastic, pipes, hardwoods, and balls are marginal because pigs' interactions with these items are short-lived (Godyń et al., 2019). Therefore, optimal items should be preferred to enrich pigs' environments. When optimal physical environmental enrichment cannot be provided (e.g. due to incompatibility with slatted floor or unsuitable climate conditions), suboptimal items and items of marginal interest can be used in combination.

1.3.2 Growth

As the optimal physical environmental enrichment, straw-based systems have been suggested to effectively improve pigs' growth. Guy et al. (2002) reported that pigs from straw-based systems, including straw yards and outdoor paddocks, showed higher daily feed intake and daily weight gain than those from fully slatted pens. Van De Weerd et al. (2005) also reported that pigs from straw-bedded systems expressed higher daily feed intake and daily weight gain as well as feed conversion efficiency than those from part-slatted systems. The better growth in straw-based systems may be attributed to greater levels of activity and exploratory behaviour (Morgan et al., 1998). In addition, pigs may get extra energy extracted from consuming straw. In pigs with restricted feed provision, straw can be considered a source of feed (Tuyttens, 2005). The straw provision may mitigate the adverse effects of the low feed level on pigs' weight gain (Spoolder, 1998). Moreover, the straw provision may keep pigs warm and save their energy, which reflects that straw may increase pigs' ability to control their micro-environment (Fraser, 1985; Morgan et al., 1998). Therefore, straw can be considered an external stimulus with multiple characteristics to give them the ability to control things about their environments and lives so as to satisfy their various requirements. The satisfaction of these requirements finally promotes their growth.

In contrast to the positive effects of straw-based systems on pigs' growth, the effects of point source enrichment items on pigs' growth are not consistent. Pearce and Paterson (1993) found that providing toys of chains, lifters bars, rubber tyres, cloth strips, swivel wheels and dustbin lids did not affect pigs' daily feed intake and daily weight gain. Similarly, Blackshaw et al. (1997) did not find any effect of the provision of sow neck tethers on the growth of weaner pigs. Hill et al. (1998), however, had different findings. They found a positive effect on daily weight gain and feed conversion efficiency in one of two genetic lines of pigs provided with items of rubber hoses on chains and spring-loaded chains. Similarly, Rodarte et al. (2004) reported that the provision of rubber tyre tubes and hanging ropes increased piglets' daily weight gain. Schaefer et al. (1990) also reported a higher daily weight gain in young pigs when providing them with hanging car tyres, suspended sugar-mineral blocks or hanging rubber belts. The contrasting findings among studies may result from the different housing and management procedures. For instance, in Pearce and Paterson (1993), pigs with toys were reared in crowded pens, which might restrict pigs' interaction behaviours towards the toys. Another potential reason for the contrasting findings is that in some studies the toys do not have the effective characteristics to attract pigs. For example, in Blackshaw et al. (1997), the toy was a shaped, solid metal sow neck-tethers covered with plastic piping, which might have been implemented on an intuitive basis without considering the requirements of the animals.

Some studies compared the effects of optimal items and other items used in combination. Van De Weerd et al. (2006) found that pigs housed in a straw-bedded pen had a significantly higher daily weight gain than those provided with a chewable liquid dispenser providing flavoured water, a substrate dispenser providing straw or a Bite Rite Tail Chew enrichment item (consisted of a plastic cone with four protruding sticks). In addition, a similar effect on pigs' growth when they were 13 to 18 weeks of age was found between pigs housed in a straw-bedded pen and pigs housed in a partly slatted pen with a substrate rooting box or a liquid dispenser, whereas there was no difference of daily weight gain between pigs from the diverse physically enriched pens when they were 10 to 13 and after 18 weeks of age (Van De Weerd et al., 2005).

1.3.3 Behaviours

Animals have strong motivations to meet their behavioural requirements including exploring their surroundings and expressing natural behaviours (Broom, 2011). Rooting is considered a positive behaviour in pigs, which is performed to forage and build a nest and may have the functions of thermoregulation (Burne et al., 2001; Olsen, 2001). However, a majority of pigs are reared in barren environments with slatted floors (Guy et al., 2013), which lack materials for manipulating, foraging or rooting (Kelly et al., 2000; Studnitz et al., 2003). The lack of rooting materials and restricted environmental stimulation in intensive housing systems may cause the redirection of the pig's attention to other conspecifics and lead to increased incidents of undesirable behaviours such as aggression, stereotypies, cannibalism and tail biting (Beattie et al., 1995; Cox and Cooper, 2001; Scott et al., 2006).

Brajon et al. (2017) reported that straw bedding promoted pre-weaning piglets' exploration and increased their time spent lying down, which was suggested to improve animal comfort. Martin et al. (2015) compared the behaviours of piglets housed in standard pre-weaning pens and pre-weaning pens with larger size and straw provision and found that piglets in the enriched pens played more pre-weaning and expressed less chronic aggression post-weaning as indicated by lower lesion scores. Hirt and Wechsler (1994) compared the diversity of pigs' behaviours in three housing environments, including a straw-free pen with an unstructured outside yard, an open front deep litter pen and a straw-bedded pen with an enriched outside yard and found that pigs expressed the highest and lowest behavioural diversity in the straw-bedded pen and straw-free pen separately. However, in the studies mentioned above, the positive effects cannot be attributed to straw because its effects cannot be separated from other resources, such as the size expansion of pigs' enclosures.

The effect of straw alone on pigs' behaviours has also been studied. For instance, Fraser et al. (1991) studied the effects of straw bedding and straw rack on the behaviour of post-weaning piglets and found that the straw provision led to reduced chewing and rooting behaviours towards their pen-mates. They also suggested that straw could attract piglets and provide an outlet for them to express exploratory and manipulative behaviours. The effects of straw provision on reducing undesirable behaviours such as tail biting, aggression and stereotypy, has also been reported by a number of studies (Burbidge et al., 1994; Spoolder et al., 1995; Day et al., 2002). However, some researchers hold the opposite opinion on the relationship between straw and undesirable behaviours. They found the provision of straw promoted pigs' aggressive interactions and indicated that this phenomenon might result from the high general level of activity in pens with straw bedding (Morgan et al., 1998; Whittaker et al., 1999).

In addition to straw, the positive effects of other resources are also observed. Pigs appear to prefer to point source enrichment items in which they can nibble, chew or bite (Tuyttens, 2005). Pre-weaning piglets having access to a box with wood bark or hanging objects showed more play behaviours than those kept in farrowing pens without any improvement (Yang et al., 2018). Providing chewable materials of sisal ropes and pieces of newspapers is suggested to induce the pre-weaning piglets' activity, as well as reduce their oral-nasal manipulation directed towards pen-mates (Telkänranta et al., 2014). The pre-weaning enrichment also causes reduced severe tail damage after weaning and regrouping, indicated as less loss of tails or fewer swollen and infected wounds. Some positive aspects of belly nosing reduction were found when providing black foam rubber mattings on pen walls (Bench and Gonyou, 2006). Providing wood shavings to finishing pigs can effectively reduce their ear and tail biting and increase their exploratory behaviours (Telkänranta et al., 2014).

1.3.4 Response to Novelty

Providing pigs with physical environmental enrichment in their home pens may enhance or promote positive affective states and mitigate how they perceive potentially negative or novel stimuli (Backus et al., 2017). Compared to regular commercial conditions, marginal enrichment (an additional feeder type, a larger number of conspecifics and more toys) increases pigs' contact frequency and manipulates time duration towards a novel object in a novel object test, which indicates that enriched pigs show more interest in or lower fear of the novel object (Tönepöhl et al., 2012). The enrichment combination of pen size and straw bedding also increases the expression of positive indicators in a novel environment (Geverink et al., 1999). The authors found that pigs from straw-bedded pens with bigger areas expressed more sniffing, chewing, rooting and biting in a novel lairage pen. Point source enrichment items including chains, tyres and bars are suggested to reduce pigs' fear towards humans in a human interaction test, evidenced by pigs housed in enriched pens taking less time to enter within 0.5 m of the human than those housed in barren pens (Pearce et al., 1989). In contrast to these findings, Pearce and Paterson (1993) reported that pigs with toys (i.e. tyres, bars or chains) spent less time interacting with a novel object than those without toys. This may indicate that due to the lack of stimulation in the barren environment, pigs compensated for their requirements for activity in the test situation. Pigs from barren pens are reported to spend more time examining a novel area and performed more sparring and scampering behaviours than those from pens with point source enrichment items including branches, logs, stones, chains and hanging branches (Wood-Gush et al., 1990). The barren housed pigs also spend more time examining the novel objects added to their home pens (Wood-Gush et al., 1990).

1.3.5 Cognition

Given the demands on animal health and behaviour and the nature of current farming practices, assessments of the learning and memory functions of pigs are relevant to their welfare. Cognition studies, including problem-solving, spatial memory and object recognition, have demonstrated the high cognitive ability of pigs (Gieling et al., 2011). However, intensive housing environments may impair pigs' cognitive development (Sneddon et al., 2000). The impairment of animal cognitive ability can trigger stress responses and negatively affect productivity and capacity to cope with their housing conditions (Held et al., 2002; Wechsler and Lea, 2007). In order to improve pigs' cognitive ability, researchers studied physical environmental enrichment. For example, Martin et al. (2015) reported that piglets from PigSAFE pens (an enriched farrowing and housing environment involving a nesting area and a dunning area, providing straw and light, and allowing the animals to have visual and some physical interaction with neighbours through the barred windows; more details are described in Edwards et al. (2012)) spent more time interacting with a novel object than those from standard pens on a cognitive spontaneous object recognition test. Sneddon et al. (2000) reported that exposure of pigs to an environment with extra space and peat and straw in a rack has been shown to improve performance on a maze test that involved spatial learning and an operant task that involved learning to push a panel for a reward. De Jong et al. (2000) also studied the effects of straw bedding on pigs' memory. They found that pigs from straw-bedded pens made fewer mistakes in a maze test than those from straw-free pens, which might suggest the impairment of long-term spatial memory for those barren housed pigs.

1.4 Effects of Physical Environmental Enrichment on Dairy Calves

For pigs housed in barren environments, physical environmental enrichment has been suggested to effectively improve their growth, increase positive behaviours, mitigate undesirable behaviours, reduce fear-related reactions and improve cognitions. Calves, as another ungulate livestock species, are also commonly reared in barren environments and deprived of external stimuli. So, physical environmental enrichment may similarly have the potential to improve their welfare. According to the studies on pigs, physical environmental enrichment can be divided into diverse categories based on characteristics. For functional and effective physical environmental enrichment, the characteristics can stimulate animals to show certain behaviours. In calves, physical environmental enrichment includes adding accessories to the enclosure, expanding the size of the enclosure and altering the complexity of the enclosure (Mandel et al., 2016).

1.4.1 Enclosure Expanding or Dividing

Studies regarding space allowance and space division suggest expanding the size of calves' enclosure and altering the complexity of their enclosure can benefit their behaviour. Jensen et al. (2015) found that increased space allowance can elevate the level of playing behaviour, which indicated that sufficient space is essential for the expression of play behaviour (Jensen et al., 1998). Ninomiya and Sato (2009) studied the expression of calves' agonistic behaviours, including escaping, chasing and head butting, in standard pens and in physically enriched pens divided by a wooden wall and found that the physical enrichment reduced stronger calves' motivation to chase weaker calves. It might suggest that blocking visual contact between calves could mitigate their aggressive motivation. However, in this study, the decreased agonistic behaviour could not be attributed to the wooden wall as its influence cannot be separated from other materials, such as the wood log and brush provided in the physically enriched pens.

1.4.2 Adding Accessories to Enclosure

Adding accessories to the enclosure may also be beneficial for calves. Scratching items (e.g. brushes) are commonly used by calves and cattle (Horvath and Miller-Cushon, 2019a; Park et al., 2020), since this type of items may help them meet their natural grooming behavioural requirements through scratching hard to reach places (DeVries et al., 2007). In addition, ropes are routinely used by calves to satisfy their oral manipulation and discourage fly activity (Zobel et al., 2017). Moreover, bucket-fed calves are motivated to suck dry teats after milk feeding to release their strong sucking motivation and redirect their non-nutritive oral behaviour (Veissier et al., 2002). Finally, providing hay is suggested to promote calves' foraging behaviour (Horvath and Miller-Cushon, 2019b), as well as improve their rumen environment (Khan et al., 2011) and increase growth (Coverdale et al., 2004; Castells et al., 2012). To ensure adequate effective forage intake, an odour is recommended because animals can use their sensorial perceptions to develop preferences and avoidance for certain feedstuffs (Baumont, 1996; Abd Rahim et al., 2020). Another potential way to promote forage intake is putting hay in a net to increase the naturalness of foraging behaviour since calves often prefer to work for a reward (Mandel et al., 2016).

Some studies with calves have found that point source enrichment items can benefit calves, but others remain inconclusive. The provision of dry teat can divert calves' sucking behaviour from their pen-mates to the item (De Passillé et al., 2011), and thus reduce the expression of the non-nutritive oral behaviour (De Passillé and Caza, 1997). In addition, calves from group pens are encouraged to express more play and social behaviours when the enrichment items of brushes or balls are provided (Bulens et al., 2014). Furthermore, for individually housed calves, access to a stationary brush can increase their grooming behaviour, reduce non-nutritive sucking towards pens, and improve coat cleanliness during weaning (Horvath et al., 2020). Moreover, providing hay as an additional feed can promote group housed calves' total feed intake and reduce their non-nutritive sucking towards pens before weaning, as well as tending to increase their average daily gain during weaning (Horvath and Miller-Cushon, 2019b). Finally, Horvath et al. (2017) studied the effect of consuming milk via teats instead of buckets and providing hay before weaning on calves' cognition and responses to environmental changes. They used a T-maze test with a reward placed in one arm to assess initial spatial learning and reversal learning where the reward location was changed to the opposite arm. They also placed a novel object (a coloured ball) in the maze between the start position and reward to assess the calves' response to an intra-maze change. The results showed that enriched calves completed the task faster in the reversal stage and found the reward faster in the novel object session than non-enriched calves, which might indicate that providing simple enrichments could improve pre-weaning calves' cognition and their responses to environmental changes. However, Pempek et al. (2017) suggested that providing point source enrichment items to standard individual housing had no significant benefits for calves. They studied the effects of artificial teats, stationary brushes, calf ‘lollies’ (i.e. pipes containing molasses) and rubber chains, and found that although calves from enriched environments interacted with all of the items offered and showed more play behaviour than those from standard environments, the provision of the items did not affect calf feed intake, weight gain, non-nutritive sucking behaviour nor behavioural response to environmental and social novelty after weaning. The differences between studies may result from the limited understanding of the characteristics, presentation methods and management of various potential enrichment items.

1.5 Emotional Lateralization in Visual Modality in Various Species

Fear has been considered an important welfare issue in farm animals, which may result in reduced productivity and management challenges (e.g. Hemsworth et al., 2000). In calves, fear is commonly assessed using various methods such as human approach and novel object tests. However, calves’ responses to the tests of fearfulness are inconsistent (Meagher et al., 2016), which may indicate reduced reliability. Since a calf’s initial responses to restraint affect their subsequent responses (Bench and Gonyou, 2006), the low repeatability of novelty tests in calves may result from their diverse initial responses. Given that many animals have brain lateralization in processing certain emotions, the functions of each hemisphere related to asymmetric emotion processing may have an effect on their initial responses and lateralised responses may be a useful measure of fear.

Ungulates have a relatively small binocular visual field (Murphy et al., 2009) and a high degree of decussation (a midline crossing of nerves inside the brain) in their optical fibres (e.g. cattle: 82.9%, Herron et al., 1978; horses: 90%, Cummings and Lahunta, 1969). Since the high degree of decussation of optical fibres ensures that input from the used eye is predominantly processed in the contra-lateral brain hemisphere (Leliveld, 2019), eye preference can indicate lateralized processes of the brain hemisphere. In practice, because of the laterally placed eyes, it is easy to exclude possible input to the other eye, making it easier to reliably assess eye preferences.

The majority of the evidence suggests that left and right brain hemispheres are specialised to process different information (Rogers, 2010). The left-hemisphere is suggested to specialise to feed related information (Rogers, 2010). For example, in a pebble-grain task, chicks used the right eye (left-hemisphere) to identify the grain and avoid pecking at the pebbles (Rogers, 1997). In addition, Robins and Rogers (2004) studied the responses of toads to prey stimuli. The experiment was designed with a toad placed in a glass cylinder and cricket or other prey species rotating around the outside of the cylinder in the clockwise or anticlockwise direction (seen from overhead). The results showed that toads frequently struck at the prey when it was moved in the clockwise direction and crossed the animal’s midline into the right visual hemifield, whereas toads were inclined to disregard the prey when it was moved in the anticlockwise direction and crossed the animal's midline into the left visual hemifield. Using the left-hemisphere in the task of finding feed is also shown in wild stilts (Ventolini et al., 2005) and pigeons (Güntürkün and Kesch, 1987).

In contrast to the left-hemisphere, the right-hemisphere is dedicated to detecting and responding to novel stimuli and controlling avoidance responses when necessary (Rogers, 2010). When the right-hemisphere is active, animals show fear or aggression (Leliveld, 2019). For fear contexts, in chicks during feeding, when placing a silhouette of a predatory bird overhead, the chicks observe the predator with shorter latency and make more distress calls when the predator approaches in the left visual field compared to the right visual field (Rogers, 2000; Dharmaretnam and Rogers, 2005). The findings indicate that, if chicks see the predator with the left eye, they show a stronger fear response. In reptiles, lateralized processing of fear is also observed with the wall lizard, evidenced by their left eye preference during predator inspection (Bonati et al., 2010; Martín et al., 2010). In amphibians, Lippolis et al. (2002) studied lateralization in emotional processing in toads by moving a model snake towards the left or right visual field of a toad. Compared to when the snake was on their right side, stronger avoidance responses were observed with toads when the snake was on their left side. In mammals, studies also show right-hemisphere dominance for the visual processing of fear (e.g. cattle: Robins and Phillips, 2010; horses: Austin and Rogers, 2007). However, Des Roches et al. (2008) reported a left eye bias in horses for observing a white short-sleeved shirt worn by the farm vet, but a right eye bias for observing an orange plastic cone, although both are associated with fear-inducing situations. For aggressive contexts, in amphibians, the right-hemisphere is considered to dominate agonistic interactions. For instance, frogs and toads usually use their left eye to guide their tongue strikes (Robins et al., 1998). In reptiles, Hews et al. (2004) found that lizards showed a left eye bias for conspecific aggression. In chickens, McKenzie et al. (1998) found pecking at social partners was restrained when the animals used their right but not left eyes. Howard et al. (1980) also reported an increase in aggressive behaviour following glutamate treatment of the left-hemisphere in chicks. Given that glutamate modifies neural pathways and results in the suppression of the normal function of the hemispheres (Howard et al., 1980), this phenomenon suggests that aggressive behaviour is normally inhibited by the left-hemisphere. In mammals, Casperd and Dunbar (1996), using photographic sequences of aggressive interactions and records of facial injuries, studied the visual field preferences of baboons during agonistic and post-conflict behaviour and found that baboons used their left visual field significantly more frequently than their right during fights, threats and approaches, which indicated a right-hemisphere dominance in the processing of the negative emotional information.

Therefore, since feed-related contexts may be tighter associated with positive emotions, while aggression and fear with negative emotions, the majority of the evidence appears to suggest that positive emotions are processed with left hemispheric dominance and negative emotions with right hemispheric dominance (Leliveld, 2019).

1.6 Conclusions

Calf housing environments are typically associated with various challenges such as social deprivation and lack of stimuli, which have been suggested to have negative effects on their productivity, behavioural expression and affective state, and lead to deficient social skills, difficulties in coping with novel situations, and poorer learning abilities. The literature review reported two methods, including social housing and physical environmental enrichment, to help calves prevent frustration, increase the fulfilment of behavioural needs, cope with stressors and improve cognitive abilities. Because of the limited studies carried out on physical environmental enrichment in calves, the literature review also reported the benefits of physical environmental enrichment on pigs, on which previous research has produced a wealth of physical environmental enrichment knowledge, including the characteristics of functional and effective physical environmental enrichment for pigs. In addition, since calves' responses to the tests of fearfulness are inconsistent and this phenomenon may relate to brain lateralization in processing certain emotions, the literature review reported the known functions of each hemisphere related to asymmetric emotion processing in a range of species.

1.7 Dissertation Aims and Outline

Since providing physical enrichment items to calves can be done in a way that does not significantly increase costs and labour costs on dairy farms, it may be an acceptable way for farmers to improve calves' welfare. Compared to the range of studies of physical environmental enrichment in pigs, studies of physical environmental enrichment in calves are limited and the items used in the different studies are not consistent. Therefore, this dissertation firstly aimed to investigate an effective physical enrichment protocol to add complexity to calf housing environments and to determine how this method could improve calf welfare. In addition, social housing, which has been determined to effectively improve calf welfare and development, is becoming more and more popular in the dairy industry. The second aim of this dissertation was to repeat the study of the effects of social housing on calf welfare and compare it to the effective physical enrichment protocol. Moreover, since physical enrichment and social housing improve animal welfare in different ways, by increasing environmental complexity (Bloomsmith et al., 1991) and providing social contact (Costa et al., 2016), the third aim of this dissertation was to investigate the effects of the combination of the physical enrichment and social housing on calves' welfare. Finally, fear has been considered an important welfare problem for calves that is potentially affected by these housing types. However, although this is most often studied in calves using fear tests such as the novel object test and human approach test, their responses in these tests are inconsistent (Meagher et al., 2016). The fourth aim of this dissertation was to investigate the potential mechanisms resulting in the uncertain reliability of calves' fear tests.

To achieve the aims, Chapter 2 compared the use of potential enrichment items between a fixed multi-item potential enrichment presentation schedule with a presentation schedule in which a single enrichment item was provided at a time. Chapter 2 also studied the characteristics of the physical enrichment items by determining calves' preference for and ways of interacting with the physical enrichment items in the effective physical enrichment protocol and investigated the effects of pair housing on calf use of the physical enrichment items in the effective physical enrichment protocol. Chapter 3 studied the effects of providing the physical enrichment items in the effective physical enrichment protocol, pair housing and the combination of both components before weaning on pre-weaning calves' growth, behaviour and response to novelty. Chapter 4 studied the effects of providing the physical enrichment items in the effective physical enrichment protocol, pair housing and the combination of both components before weaning on the growth, behaviour and cognitive ability of calves after weaning and regrouping. Chapter 5 assessed the test-retest reliability of calves' responses in novel object tests, determined if calves had visual lateralization in processing fear and investigated if calves' initial monocular presentation toward fear-inducing stimuli would affect calves' fear responses.

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Chapter 2

Holstein Calves' Preference for Potential Physical Enrichment Items on Different Presentation Schedules

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2. Holstein Calves' Preference for Potential Physical Enrichment Items on Different Presentation Schedules

Statement

I contributed to 70% of the content in this chapter, including formulating hypotheses for the second experiment, designing and conducting the second experiment, compiling and analysing data from the first and second experiments and writing this published manuscript. Other authors formulated hypotheses for the first experiment and designed and conducted the first experiment.

Abstract

Impoverished housing environments are thought to prevent motivated behaviours and may result in frustration. We first aimed to investigate an effective physical enrichment protocol to improve dairy calves' welfare and initially determine their use of various items. Thereafter, we aimed to determine dairy calves' preference for and ways of interacting with various items, and whether this was influenced by social housing. In experiment 1, at 21 ± 3 d of age, 27 individually housed calves were assigned alternately into 1 of 3 treatments: control (CON, no additional items), rotating enrichment (RE, one item each week on a rotating presentation schedule), and fixed enrichment (FE, 4 types of item at the same time). The items were stationary brushes, ropes, springs, nets filled with strawberry-scented hay, and dry teats. Calves' behaviours were observed from 4 to 7 wk of age using focal observations after feeding, followed by instantaneous scan sampling. Their behavioural responses to a novel object were assessed at 43 ± 1 d of age. In the instantaneous scans, calves in FE tended to interact with items more often than calves in RE. Calves in RE and FE expressed less non-nutritive oral behaviour than those in CON. Latency to touch novel objects did not differ significantly between treatments. Calves in RE and FE interacted with nets filled with strawberry-scented hay more often than with other items in instantaneous scans. In experiment 2, 24 calves were assigned alternately into 8 individual pens and 8 pair pens at 2 d of age. All pens were provided with a stationary brush, plastic chain, net filled with strawberry-scented hay, and dry teat. Calves' behaviours were collected from 2 to 5 wk of age using instantaneous scan sampling. Calves interacted with nets filled with strawberry-scented hay more often than with other items. Pair housing reduced calves' interactions with items compared with individual housing. Individually and pair housed calves' frequencies of overall interaction with items varied with time of day, with frequencies increasing to peaks at 0700, 1500, and 1900 h. Calves showed scratching, sniffing, sucking, butting, and hay intake toward nets filled with strawberry-scented hay and showed the first 3 behaviours toward stationary brushes, plastic chains, and dry teats. In conclusion, dairy calves are likely to prefer a fixed multi-item enrichment presentation schedule over a rotating schedule with a single enrichment item presented at one time. For the fixed multi-item enrichment presentation schedule, items were used more in individual pens than in pair pens, and a diurnal pattern was observed for use of the items. Nets filled with scented hay might be the most multifunctional and attractive item of the items tested.

Key words: animal welfare, dairy calf, social housing, environmental enrichment, behaviour

2.1 Introduction

It is common to individually rear newborn dairy calves in relatively barren environments (Horvath et al., 2020). Such impoverished housing environments can restrict the expression of calves' natural behaviour, which may lead to frustration (Mason and Burn, 2011). Environmental enrichment has been suggested to satisfy animals' species-relevant motivations and provide behavioural opportunities to control their environments (Van De Weerd et al., 2006).

Among the different environmental enrichment methods, adding items to animal enclosures has been implemented in many farm animals. Effective physical enrichment items are thought to have functional utilities (Newberry, 1995) and to facilitate the use of animals' behavioural skills (Mench, 1998). For example, in dairy cattle, mechanical brushes are effectively used to groom their bodies, particularly in places that are hard to reach. Use of brushes can keep animals clean and stimulate their grooming motivation and may reduce stress or frustration resulting from boredom (DeVries et al., 2007). For calves, brushes are consistently used to satisfy their natural grooming behavioural motivations (Pempek et al., 2017; Horvath and Miller-Cushon, 2019), which may help to compensate for the frequent grooming they would receive from their dam in natural conditions (Johnsen et al., 2015). Ropes may satisfy calves' oral manipulation (Zobel et al., 2017). Teats are used to release calves' sucking motivation and reduce their non-nutritive oral behaviours, including sucking peers or fixtures (Rushen and De Passillé, 1995; Jung and Lidfors, 2001; Veissier et al., 2002), as well as stimulate them to secrete more hormones related to satiety (insulin and cholecystokinin), which may help them relax (De Passillé et al., 1993). Providing part of the feed ration through a feed net is thought to promote calves to engage in feed collection and serve as a measure to increase the naturalness of feeding behaviour (Mandel et al., 2016). Because calves can use their sensorial perceptions to choose palatable feeds (Baumont, 1996; Miller-Cushon et al., 2014), spraying heifers' preferred aroma of red berry (Meagher et al., 2017) on the feed ration may further stimulate their interest in the feed. However, it is still not clear which of the items mentioned above provide the most stimulation. Because social housing is growing in popularity (James and Machado, 2013) and animals housed in the same environment may mimic each other's behaviours (Galef, 1988), it is worth studying whether social housing can further increase the use of physical enrichment items.

In addition to satisfying these motivations, another characteristic of effective physical enrichment items is reliably attracting and sustaining an animal's interest (Jones et al., 1991). Ways of presenting items (e.g., alternately or simultaneously) have been suggested to play an important role in item use. Renewing items (replacing familiar items with novel items) maintains the novelty of items and has been shown to sustain animals' interest in enrichment for a protracted period (e.g., Trickett et al., 2009; Roy et al., 2019); thus, providing items to calves on a rotating presentation schedule may maintain their interest and provide experience with exposure to novel, harmless stimuli. However, some items can consistently satisfy animals' motivation to perform certain behaviours. For instance, heifers and adult cattle do not habituate to scratching enrichment devices and ropes (Wilson et al., 2002; Stanford et al., 2009). Therefore, calves may not habituate to these types of items and may use them intensively over long periods. Providing enrichments on a rotating schedule may not be a practical way to satisfy these motivations, given that farmers are unlikely to have several different items to target each and providing multiple items on a rotating schedule would increase labour. We therefore wished to compare the effects of multiple, fixed enrichments with a rotating single item enrichment protocol.

The first aim of the present study (experiment 1) was to investigate an effective physical enrichment protocol to promote dairy calves' use of the items (stationary brushes, ropes, springs, dry teats, and nets filled with strawberry-scented hay), to improve their welfare and initially determine dairy calves' use of various items. In experiment 1, we hypothesized that (1) compared with providing a single item on a rotating presentation schedule, providing the items in combination throughout the period would stimulate more interactions with items and result in a greater reduction of non-nutritive sucking because items eliciting oral manipulation would always be present; (2) the rotating presentation schedule of a single enrichment item would be most effective in reducing fear of novelty through more frequent exposure to novel stimuli; (3) nets filled with strawberry-scented hay would be used more often than other items because they might offer more complex stimulation and provide extrinsic reinforcement (hay consumption). The second aim of the study (experiment 2) was based on the first aim to determine dairy calves' preference for and ways of interacting with various items in the effective physical enrichment protocol, and whether this was influenced by social housing. It would contribute to our understanding of mechanisms underlying the improvement of dairy calves' welfare by these items. For experiment 2, we hypothesized that (1) nets filled with strawberry-scented hay would be used more often than other items in both individual and pair pens; (2) compared with individual housing, pair housing would promote calves' interactions with items because it could promote social facilitation; (3) calves' interactions with items would show a circadian rhythm associated with feeding patterns because they might be more active around feeding times; and (4) calves would show different interaction behaviours toward individual items to satisfy their diverse behavioural motivations.

2.2 Materials and Methods

Experiments 1 and 2 were conducted at the Centre for Dairy Research, University of Reading (Reading, UK). Both experiments were approved by the ethics administrator at the university and the departmental ethics coordinator. All procedures complied with the guidelines for the Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research (Sherwin et al., 2017).

2.2.1 Experiment 1

Animals, Housing, and Feeding

Twenty-seven female calves (pure registered Holsteins) with birth weights (mean ± SD) of 40.43 ± 5.94 kg were individually housed from 3 ± 3 d of age to 49 ± 3 d of age in individual pens (2.0 m^2 each). Calves could have limited tactile contact with their neighbours above the panels and through the gaps between the panels. At 21 ± 3 d of age, calves were assigned alternately into 1 of 3 treatments: (1) control (CON), providing no additional items for the full study period; (2) rotating enrichment (RE), providing one type of item each week on a rotating presentation schedule for 4 wk; or (3) fixed enrichment (FE), providing 4 types of item at the same time for 4 wk. These protocols were chosen as practical ways that farms might implement enrichment. The items given in this experiment were stationary brushes (170 mm long, 65 mm wide, a combination of horse hair and pig bristles; Robinsons Equestrian), either ropes (nylon rope, 20 mm in diameter) or springs (flexible nylon tubing, 5 mm in diameter; Altec Extrusions Ltd.), dry teats (black rubber teat, 25 mm in diameter, 100 mm long; Tanner Trading Ltd.), and nets (Fine Mesh Haynet, 760 mm long, 4 kg capacity; Robinsons Equestrian) filled with strawberry-scented hay; the strawberry flavouring (Sainsbury's) was sprayed on ryegrass hay in nets every 2 d. Items were secured on the bars of the front or side panel, 800 mm away from the floor (Figure 1a). In RE, all 9 calves received stationary brushes, dry teats, and nets filled with strawberry-scented hay; 4 received ropes and 5 received springs as their fourth enrichment. In FE, all 9 calves received stationary brushes, dry teats, and nets filled with strawberry-scented hay; 5 received ropes and 4 received springs. Ropes and springs were hung vertically and were of similar length when hanging untouched; these last enrichments were not considered to have any specific biological relevance but could be orally manipulated and were tested as potential practical items that might provide general enrichment. All items were checked daily and cleaned if needed; hay nets were refilled if substantial amounts of hay were missing. The concrete floor of every pen was bedded with deep straw. Calves were fed milk replacer twice per day at 0800 and 1500 h using teat buckets. Calves were fed 6 L of milk replacer per day until 7 ± 3 d of age and after 28 ± 3 d of age. Calves were fed 8 L of milk replacer per day between 7 ± 3 d of age and 28 ± 3 d of age. Calves also had ad libitum access to concentrate, plain hay, and water throughout the study period.

Home Pen Behaviours

Calves' behavioural interactions with items and non-nutritive oral behaviours (defined in Table 1) were directly observed and recorded by observers when calves were 4, 5, 6, and 7 wk of age. Calves were observed 3 times per week for 2.5 h during an undisturbed period of the afternoon (1200 to 1425 h) using instantaneous scan sampling at 5-min intervals, with observers slowly walking down the aisle and recording the behaviour before they reached the individual's pen. Calves were also continuously observed twice per week for 10 min immediately after morning milk feeding using focal sampling, with feeding being staggered such that one observer watched only 1 or 2 calves at a time.

Novel Object Tests

Fear can be elicited by events' characteristics of novelty and presentation method (Forkman et al., 2007). Currently, fear is often assessed through response to novelty (neophobia) in novel object test (Meagher et al., 2016). In this experiment, novel object tests were carried out when calves were 43 ± 1 d of age. Calves were tested sequentially in birth order. One calf at a time was walked to a testing pen (3.33 m^2) with solid sides such that calves were visually isolated from other subjects but still had some auditory contact. After 5 min to habituate to this environment, a novel object (black and white rubber disks hanging from a string at approximately calf eye level or just above) was then extended into the pen on a rod. The duration of latency to touch the object and the frequencies of vocalizations and retreats from the object over a 10-min test period were video recorded as indicators of fear.

2.2.2 Experiment 2

Animals, Housing, and Feeding

Twenty-four male calves (pure registered Holsteins) with birth weights (mean ± SD) of 43.90 ± 4.80 kg were included in this experiment from 2 to 42 d of age. At 2 d of age, calves were assigned alternately into 1 of 2 treatments: (1) physically enriched individual pens (IP; n = 8, 2.4 m^2 each), 1 calf in each pen with 1 stationary brush (330 mm long, 72 mm wide, plastic bristles; O'Donovan Engineering Co. Ltd.), 1 plastic chain (25 mm diameter), 1 dry teat (white rubber teat, 25 mm diameter, 100 mm long;

Tanner Trading Ltd.), and 1 net filled with strawberry-scented hay; or (2) physically enriched pair pens (PP; n = 8, 4.8 m^2 each), 2 calves in each pen with 2 stationary brushes, 2 plastic chains, 2 dry teats, and 1 net filled with strawberry-scented hay. Items were secured on the bars of the side or back panel, 800 mm away from the floor (Figure 1b). All items were checked daily and cleaned if needed; hay nets were refilled if substantial amounts of hay were missing. Calves could have limited tactile contact with their neighbours through the gaps between the panel bars. The concrete floor of every pen was bedded with deep straw. Calves were fed milk replacer twice a day at 0700 and 1500 h using teat buckets. Calves were fed 5 L of milk replacer per day until 14 d of age, followed by 6 L of milk replacer per day between 15 and 42 d of age. Calves also had ad libitum access to concentrate, plain hay, and water throughout the study period.

Home Pen Behaviours

Calves' behavioural interactions with items (defined in Table 1) were recorded by closed circuit cameras (Transit-PTZ, Revader Security Ltd.) for 14 h (0600 to 2000 h) twice weekly when calves were 2, 3, 4, and 5 wk of age. Calves were observed during daytime because they are more active during this time period (e.g., Zobel et al., 2017). Video recordings were played using Windows Media Player (Microsoft Corp.) and data recorded using Excel (version 16.53, Microsoft Corp.) by one observer watching the video recordings using instantaneous scan sampling at 5-min intervals.

2.2.3 Statistical Analysis

Statistical analysis was performed using SPSS Statistics (version 27.0.1.0, IBM Corp.). Significant differences were declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

Experiment 1

Use ratios (UR; defined as frequency or duration of calves' interaction behaviours toward items divided by frequency or duration of all behaviours) of overall items, UR of individual items, and ratios of non-nutritive oral behaviours for every calf collected using instantaneous scans. Continuous focal observations were first calculated by averaging UR of overall items, UR of individual items, and ratios of non-nutritive oral behaviours across the 12 testing days due to the very large number of zeroes.

Generalised linear mixed models were used to compare UR of overall items in different physical enrichment protocols. For both instantaneous scans and continuous focal observations, the fixed factors were enrichment treatments (RE and FE) and calves' birth weight. The random factor was age span (i.e., how many weeks of life were included in the data for that calf). The Satterthwaite method was used to calculate degrees of freedom.

Generalised linear mixed models were used to compare UR of stationary brushes, ropes, springs, dry teats, and nets filled with strawberry-scented hay. For instantaneous scans, the subject was calves' ID number; the repeated measure was individual items. The fixed factors were individual items, enrichment treatments (RE and FE), interactions between both factors, and calves' birth weight. The random factor was calves' ID number. For continuous focal observations, the subject was calves' ID number; the repeated measure was individual items. The fixed factors were individual items, enrichment treatments (RE and FE), interactions between both factors, and calves' birth weight. The random factor was calves' ID number and age span. The Satterthwaite method was used to calculate degrees of freedom, and a post hoc test of least significant difference (LSD) was carried out to identify differences among individual items.

Generalised linear mixed models were used to compare ratios of non-nutritive oral behaviours in enriched and nonenriched environments. For instantaneous scans, the fixed factors were all treatments (CON, RE, and FE) and calves' birth weight. For continuous focal observations, the fixed factors were all treatments (CON, RE, and FE) and calves' birth weight. The random factor was age span. The Sattherwaite method was used to calculate degrees of freedom, and LSD was used to identify differences among all treatments (CON, RE, and FE).

Seven of the 27 calves (2 from CON, 3 from RE, 2 from FE) could not be observed for the full period in continuous focal observations. To determine the interobserver reliability, 2 observers (O; O1 and O2) observed 22 calves together for 1 hour using instantaneous scan sampling at 5-min intervals. Another observer (O3) also observed the calves with the 2 observers in the first 30 min of the observation. The reliability between every pair of observers was compared using Cohen's kappa (κ). According to Landis and Koch (1977), O1 and O2 had substantial reliability (κ = 71.97%; $P < 0.001$), O1 and O3 had almost perfect reliability (κ = 87.27%; $P < 0.001$), and O2 and O3 had substantial reliability (κ = 78.18%; $P < 0.001$).

Latencies to touch the novel object were non-normal, and a log transformation was therefore applied so that the assumptions of parametric tests were met. Data were then analysed for differences between housing treatments using ANOVA. One calf from the FE treatment was not recorded because of a recording error with the camera. The details for vocalizations and retreats from the object are reported in the Supplemental Material.

Experiment 2

The UR of individual items for every calf in every testing week was collected using instantaneous scans. A generalised linear mixed model was used to compare UR of stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay and to determine the effect of pair housing. The subjects were pen number and calves' ID number; the repeated measures were calves' week of age and individual items. The fixed factors were individual items, pair housing (IP or PP), interactions between both factors, calves' birth weight, milk refusal during the testing days, and average temperature of the barn during the testing days. The random factors were pen number, calves' ID number, and calves' week of age. The Satterthwaite method was used to calculate degrees of freedom, and the least significant difference (LSD) test was used to identify differences among individual items.

The UR of overall items across hours for every testing day was collected using instantaneous scans. The data of every calf between 0600 and 1959 h were categorized into fourteen 1-h periods: 0600 h (i.e., 0600 to 0659 h), 0700 h (i.e., 0700 to 0759 h), and so on. Descriptive statistics were run to calculate means of UR of overall items for every 1-h period for the 8 testing days.

Calves' behavioural ratios toward stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay were defined as times of calves' interaction behaviours toward these individual items divided by total number of times observed. Descriptive statistics were run to calculate means of behavioural ratios and coefficient of variations for the 8 testing days.

Because of navel inflammation in one calf from PP, behavioural interactions with the items at 3, 4, and 5 wk of age for this calf were discarded before analysis. Due to a technical problem, behavioural interactions with the items for 2 calves (1 from IP, 1 from PP) at 2 wk of age were not recorded. To determine the interobserver reliability, another observer who was blind to the hypothesis under test watched the video recordings of 4 calves by randomly choosing 1 wk of data from 2, 3, 4, and 5 wk of age for each calf. The reliability between the 2 observers was compared using Cohen's κ, which indicated substantial reliability (κ = 71.80%; $P < 0.001$).

2.3 Results

2.3.1 Experiment 1

Use of Overall Items in RE and FE

In the instantaneous scans, calves in FE tended to spend more time interacting with overall items than those in RE ($F_{1,15} = 3.51$, $P = 0.081$; Figure 2a). In the continuous focal observations after feeding, no significant differences in interacting with overall items were found between calves in RE and FE ($F_{1,15} = 0.356$, $P = 0.560$; Figure 2b).

Non-nutritive Oral Behaviours

In the instantaneous scans, calves in RE and FE spent less time expressing non-nutritive oral behaviours than those in CON ($F_{2,23} = 8.34$, $P = 0.002$; Figure 3a). In the continuous focal observations post-feeding, calves in RE and FE tended to spend less time expressing non-nutritive oral behaviours than those in CON ($F_{2,23}$ = 2.69, P = 0.089; Figure 3b).

Response to Novelty

Latencies (in seconds) to make contact with the novel object did not differ significantly between treatments. The back-transformed means (95% CI) were as follows: CON 45.1 s (19.8–103.6), FE 40.3 s (16.7–97.4), and RE 33.2 s (14.4–76.2) ($F_{2,23}$ = 0.15, P = 0.863; n = 26). The results for vocalizations and retreats from the object are reported in the Supplemental Material.

Calves' Preference for Individual Items

In the instantaneous scans, calves in RE and FE interacted with nets filled with strawberry-scented hay more often than with stationary brushes, ropes, springs, or dry teats ($F_{4,16}$ = 4.97, P = 0.008; Figure 4a). In contrast, in the continuous focal observations post-feeding, calves in RE and FE spent similar amounts of time interacting with the individual items ($F_{4,15}$ = 1.22, P = 0.343; Figure 4b).

2.3.2 Experiment 2

Calves' Preference for Individual Items and Effect of Pair Housing

There were no interactions between items and pair housing with respect to the incidence of uses of individual items ($F_{3,90}$ = 2.01, P = 0.119). Calves interacted with nets filled with strawberry-scented hay more often than with stationary brushes, plastic chains, or dry teats, and calves interacted with stationary brushes more often than with plastic chains ($F_{3,92}$ = 35.81, $P < 0.001$; Figure 5). Pair housing (PP) reduced calves' interactions with overall items compared with individual housing ($F_{1,84}$ = 6.14, $P = 0.015$; Figure 6).

Hourly Distributions of Calves' Interaction with Overall Items

The frequency of calves' interaction behaviours with overall items changed throughout the day (Figure 7), peaking in the hours beginning at 0700, 1500, and 1900 h, and falling to the lowest incidences at 1200 and 1600 h.

Calves' Interaction Behaviours toward Individual Items

For stationary brushes (Table 2), calves spent the longest time scratching on them, with the rest of the time spent sucking and sniffing them. Calves sucked plastic chains for the longest time, followed by sniffing and scratching them. For nets filled with strawberry-scented hay, calves spent the longest time consuming hay from them, with the rest of the time sniffing, sucking, scratching, and butting the net. Calves sucked dry teats for the longest time, followed by sniffing and scratching them.

2.4 Discussion

Our results suggest that multi-item FE and RE of a single item at a time were both effective protocols to reduce calves' non-nutritive oral behaviours, although FE tended to attract more single-item interactions than RE. Calves had a preference for nets filled with strawberry-scented hay, which seemed to be the most multifunctional item and stimulated a larger range of behaviour types. Compared with IP, the PP treatment reduced calves' interactions with the items. Calves had a diurnal pattern of interaction with items in 3 peak periods (0700, 1500, 1900 h) every day.

2.4.1 Enrichment Protocols

In experiment 1, all items provided were used no matter whether they were available alternately or simultaneously, which might indicate that both enrichment protocols are valuable for calves. Although neither protocol significantly reduced fear of novelty according to our measure, latencies were numerically lower in both enrichment protocols compared with controls, so there may be a welfare benefit that the present study did not have adequate power to detect. Because calves in FE tended to spend more time interacting with items than calves in RE, some properties of the enrichment protocol other than novelty may be more effective in maintaining calves' attention. This is in agreement with Trickett et al. (2009), who found that providing ropes and wood together for pigs elicited higher item interaction than providing the items in rotation. This may be explained as various items having different properties, which may be attractive in different and additive ways; by having all available at once, the calves make use of all or several of them throughout the day. In experiment 2, calves showed different principal behaviours toward stationary brushes, plastic chains, dry teats, and nets filled with strawberry-scented hay. The items might satisfy their intrinsic behavioural motivations of grooming, sucking, and feed intake, which is restricted or redirected in barren housing conditions (De Passillé, 2001; Khan et al., 2011; Zobel et al., 2017). Thus, compared with providing an individual item, providing those items in combination may lead to a cumulative effect. Moreover, calves' behavioural motivations of grooming, sucking, and feed intake may not be affected by the novelty of stimuli. For example, Horvath and Miller-Cushon (2019) suggested that brushes were consistently used by calves across weeks (4–7 wk of age). Hammell et al. (1988) indicated that calves having access to dry teats usually sucked them after milk feeding. Horvath and Miller-Cushon (2017) showed that calves consumed increasing amounts of hay with increasing age. Therefore, calves are likely to continue using these items and not lose interest due to habituation. Although we cannot exclude the possibility that having multiple items in the pen increases the chance a calf will interact with one at any given moment regardless of the item time (i.e., that providing 4 brushes would also increase interaction compared with a one-at-a-time rotating schedule), it seems likely that the increased overall use of enrichments in this treatment is due to their ability to satisfy these different, ongoing motivations.

Non-nutritive oral behaviours, including non-nutritive and cross-sucking, are nonfunctional and potentially harmful for calves (Le Neindre, 1993; Jensen, 2003) and are considered detrimental to calves' health and welfare (Babu et al., 2004). In experiment 1, calves in FE and RE expressed less non-nutritive oral behaviour than calves in CON, which might indicate that the items used in both enrichment protocols could attract calves' attention and effectively reduce their undesirable behaviours. Because calves with FE and RE showed similar frequencies of non-nutritive oral behaviour, FE did not show a cumulative effect on reducing non-nutritive oral behaviours. The finding is consistent with previous studies. Horvath et al. (2020) found that the provision of brushes, hay, or brushes and hay all reduced teat-directed sucking, but all treatments showed similar pen-directed non-nutritive oral behaviours. Haley et al. (1998) also indicated that hay provision reduced the duration of teat-directed sucking. The results may indicate that the provision of an alternative outlet for oral behaviours to accommodate a greater range of behavioural expression can only partly satisfy calves' sucking motivations and cannot fully eliminate non-nutritive oral behaviours (Horvath et al., 2020). Future research should study other ways such as milk feeding methods to further reduce calves' undesirable behaviours.

Because calves fed ad libitum milk can drink around 9 L of milk per day (Jasper and Weary, 2002), the amount of milk replacer provided in both experiments was restricted to some degree. Limited milk provision has been reported to negatively affect calves' behaviour. For instance, calves fed limited milk spent less time on locomotor play than calves fed more milk (Krachun et al., 2010; Jensen et al., 2015). However, the amount of milk consumed per se does not necessarily affect non-nutritive sucking; Rushen and De Passillé (1995) reported that halving the amount of milk that calves drink during a meal did not increase the amount of non-nutritive sucking that occurs after the meal. The performance of sucking behaviour itself is more effective in reducing the underlying sucking motivation (De Passillé, 2001). When calves take longer to suck their milk; for example, due to use of teats with reduced milk flow rates (Haley et al., 1998), they do less non-nutritive sucking (Haley et al., 1998; Jongman et al., 2020). Because limited milk provision may lead to shorter milk durations, which can reduce time for secretion of cholecystokinin and other hormones to provide negative feedback during a meal, calves with limited milk provision may finish their meal before negative feedback occurs and thus show increased non-nutritive sucking (De Paula Vieira et al., 2008). Therefore, the calves in the current studies may have performed more oral behaviours toward the enrichments than calves on ad libitum schedules would; however, there was no obvious difference in their use between the calves in these 2 studies despite having different milk allowances.

The results of UR of overall items in continuous focal observations are inconsistent with the results in instantaneous scans in experiment 1. This may be because of the special testing time of the continuous focal observations. The test of continuous focal observations was implemented for 10 min immediately after morning milk feeding for every calf. Calves have a strong sucking motivation during this period (Loberg and Lidfors, 2001), which may suppress other behavioural motivations. Because calves with FE and RE were fed identical amounts of milk replacer through teat buckets, they might spend similar amounts of time interacting with items in their pens after milk feeding to release sucking motivation.

2.4.2 Calves' Interaction Behaviours toward Individual Items

The intensity of behavioural interactions with items reveals their significance to an animal's key motivations (Van De Weerd and Day, 2009). In experiment 2, as predicted, calves expressed behaviours reflecting different key motivations toward individual items, spending the highest proportion of time scratching stationary brushes, sucking plastic chains and dry teats, and consuming hay from nets filled with strawberry-scented hay. The findings are in agreement with previous studies. Toaff-Rosenstein et al. (2017) reported that weaned heifers were motivated to use brushes to scratch their head and body. Veissier et al. (2002) showed that bucket-fed and teat-fed calves were motivated to suck dry teats after milk feeding. The circadian pattern found in experiment 2 also fits with this reported pattern of sucking motivation. Mandel et al. (2016) suggested that providing part of feed rations through feed nets could prolong feeding behaviour. In addition to these key behaviours toward specific items, the same behaviours were performed to some degree using other items. For instance, calves showed scratching behaviour toward plastic chains, dry teats, and nets filled with strawberry-scented hay. Calves expressed sucking behaviour toward stationary brushes and nets filled with strawberry-scented hay. Calves also sniffed all the items. This may be partly due to calves' exploratory motivation, which can be stimulated when animal is in novel situations with restricted fear (Murphy, 1978). In experiment 2, calves spent the second highest proportion of time scratching nets filled with strawberry-scented hay (following stationary brushes), which may indicate that the hay net is an effective item to satisfy calves' scratching motivation. Calves spent a high proportion of time sucking stationary brushes and nets filled with strawberry-scented hay, in addition to plastic chains and dry teats. This may indicate that all the items were outlets for calves' sucking motivation and thus the items need to be kept clean to protect calf health and welfare. Calves spent more time sniffing nets filled with strawberry-scented hay than other items. This may indicate that besides exploratory motivation, calves' preferred aroma of red berry (Meagher et al., 2017) plays an important role in attracting their attention. Calves also showed butting behaviour toward nets filled with strawberry-scented hay. Because butting has been considered as a play behaviour (Jensen et al., 1998), its expression may indicate that nets filled with strawberry-scented hay can stimulate calves' play motivation. Therefore, the items used in experiment 2 may be multifunctional, which can satisfy multiple behavioural motivations in calves.

2.4.3 Calves' Preference for Individual Items

In experiments 1 and 2, as predicted, calves interacted with nets filled with strawberry-scented hay more often than with other items. According to Table 2, nets filled with strawberry-scented hay could stimulate 5 types of interaction behaviours, whereas other items could only stimulate 3 types of interaction behaviours. This finding may indicate that nets filled with strawberry-scented hay have more characteristics than other items to stimulate calves' behavioural motivations. As different characteristics of an item may be synergistic and capture more interests of animals (Bracke et al., 2006), the multiple characteristics of nets filled with strawberry-scented hay may explain the increased interaction. Another potential reason is that these hay nets provided extrinsic reinforcement, which occurs when the performance of behaviour leads to a consequence that is external to the behaviour itself and increases the likelihood that the behaviour will recur (Tarou and Bashaw, 2007). In dairy cattle, red berry flavouring was previously found to be a preferred aroma to increase the palatability of feeds (Meagher et al., 2017). Therefore, in experiment 2, spraying strawberry flavouring onto hay in nets may increase its palatability and promote an external outcome of hay consumption. It also suggested that compared with independently using aroma, in which cattle lose interest within days of using it (Wilson et al., 2002), using their favourite aroma for items that can lead to external outcomes may be a better presentation method. In contrast, stationary brushes, plastic chains, and dry teats did not result in external outcomes. Those items may be considered to provide intrinsic reinforcement, which occurs when simply performing a behaviour increases the probability that the behaviour will occur again (Hughes and Duncan, 1988). Tarou and Bashaw (2007) suggested that extrinsic reinforcement generally has a longer lasting attraction to animals than intrinsic reinforcement because the external outcome can increase the likelihood that the behaviour will be performed again. Therefore, nets filled with strawberry-scented hay were used more often than other items.

2.4.4 Effect of Pair Housing

Galef (1988) defined social facilitation as “the initiation of a particular response while observing others engaged in that behaviour”. In experiment 2, we predicted that in PP, when one calf interacted with an item, the other one could observe the process and initiate a particular response toward an identical item; thus, pair housed calves interacted with overall items more often than calves in the IP group. However, the results determined that pair housing reduced calves' interactions with overall items compared with individual housing, which is contrary to the prediction. It may be because the unrestricted social contact in pair pens takes up part of calves' active time and suppresses their interactions with overall items. Pre-weaning calves rest for large parts of the day (Horvath et al., 2020), and thus they may have limited time to show active behaviours. For pair housed calves, they are attracted to each other and show unrestricted social contact (Jensen and Larsen, 2014). Compared with individually housed calves, they may spend part of their active time expressing social behaviours and spend less active time interacting with items overall. Another potential explanation is that in pair pens, one calf was dominant over a preferred item. Although 2 sets of items were provided to calves housed in every pair pen in experiment 2 to ensure that both calves in the same pens could interact with every type of item at the same time, stationary brushes and dry teats were fixed on opposite panels of the pens. This suggests that when one calf observed the companion calf interacting with a dry teat or a stationary brush, the calf might not see the other available identical item and not be triggered to interact with it.

2.4.5 Hourly Distributions of Calves’ Interaction with Overall Items

In experiment 2, overall items were used most around milk feeding times, as well as at 1900 h. Similarly, Zobel et al. (2017) found that use bouts of rotating brushes and hanging ropes peaked around milk feeding times, and around 1800 and 1900 h. Miller-Cushon et al. (2013) showed increased hay consumption around milk feeding times. Pempek et al. (2017) reported that artificial teats, rubber chains, and calf lollies (pipes containing molasses) were used most in the 3 h following milk feeding. Therefore, milk feeding times are considered periods of increased activity for most behaviours (Horvath et al., 2020), such as sucking and nursing behaviours (Pempek et al., 2017). Because cattle are most active at sunrise and sunset (Albright, 1993), 1900 h may be another active time for calves. The patterns of use of overall items may be related to the redirection of motivations to engage in particular behaviours that cannot be satisfied in the environment.

2.5 Conclusions

Compared with RE, FE with multiple items might be a better protocol to improve dairy calves’ welfare because it promoted more total item interactions and reduced non-nutritive oral behaviours. Dairy calves had a diurnal pattern with 3 peak periods for interacting with the items every day, 2 of which coincided with feeding times (0700 and 1500 h). The net filled with scented hay might be the most multifunctional and attractive of the items tested, given that dairy calves showed the most types of behaviour toward it and had the most frequent interaction with it. Interactions with items were reduced by pair housing, suggesting that provision of the items to individually housed dairy calves may be more important for their welfare than to calves housed together.

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Table 1. Ethogram of behavioural interactions with items and non-nutritive oral behaviours

Category	Behaviour	Definition
Items including stationary brushes, ropes, springs, plastic chains, dry teats, and nets filled with strawberry-scented hay	Item scratching[1]	Putting head, neck, or body in contact with the items and slightly moving back and forth or up and down
	Item sniffing[1]	Putting muzzle in contact with or less than one muzzle length from the items with neck not relaxed
	Item sucking[1]	Licking, sucking, or biting the items
	Item butting[1]	Standing and butting head against the items in a playful manner
	Hay intake[1]	Chewing hay from nets filled with strawberry-scented hay
Non-nutritive oral behaviours	Non-nutritive sucking	Licking, sucking, or biting any fixture except the items
	Cross-sucking	Sucking or biting toward ear, mouth, navel, or other body parts of other calves

[1]Behaviours toward items were only recorded in experiment 2.

Table 2. Descriptive statistics for behavioural ratios toward enrichment items in 24 calves averaged over 8 testing days each from 0600 to 1959 h in experiment 2

Item	Behaviour	Ratio of all scans[1] (%)	CV[2] (%)
Stationary brushes	Scratching	0.3565	60.59
	Sniffing	0.1565	62.02
	Sucking	0.2427	66.31
Plastic chains	Scratching	0.0195	172.12
	Sniffing	0.1297	100.07
	Sucking	0.3475	83.31
Nets filled with strawberry-scented hay	Butting	0.0613	144.19
	Hay intake	2.5450	41.96
	Scratching	0.1885	76.40
	Sniffing	0.5885	66.06
	Sucking	0.2569	116.29
Dry teats	Scratching	0.0161	194.00
	Sniffing	0.0900	139.19
	Sucking	0.5850	91.07

[1]Ratio = times of an interaction behaviour/times of all behaviours in calves × 100%.

[2]Coefficient of variation provides a measure of the dispersion of the means of each calf over the 8 testing days (the higher the %, the higher the variance between individual calves).

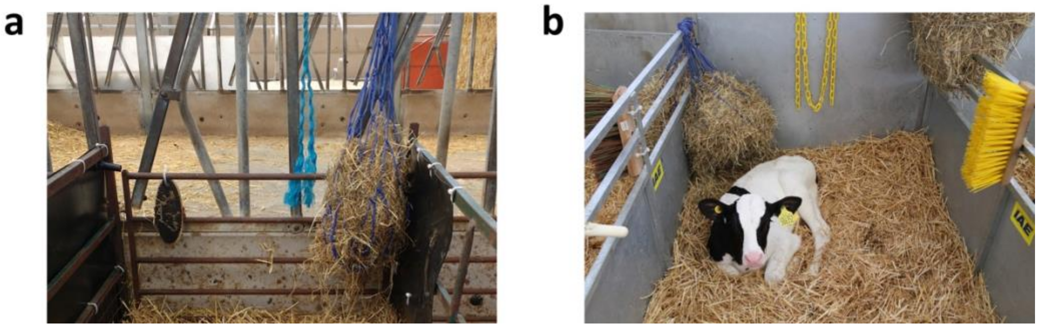

Figure 1. Physically enriched individual pen in (a) experiment 1, including stationary brush, rope, dry teat (black), and net filled with strawberry-scented hay, and (b) experiment 2, including stationary brush, plastic chain, dry teat (white), and net filled with strawberry-scented hay.

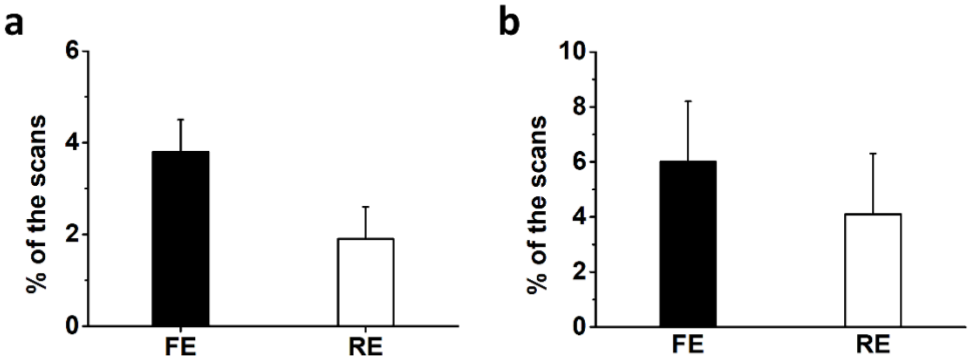

Figure 2. Least squares means (±SEM) of use ratios of overall items for calves in pens with fixed enrichment (FE, n = 9 pens) and rotating enrichment (RE, n = 9 pens) in experiment 1 collected using (a) instantaneous scans in the afternoon (30 times/d), and (b) continuous focal observations after morning feeding (10 min/d).

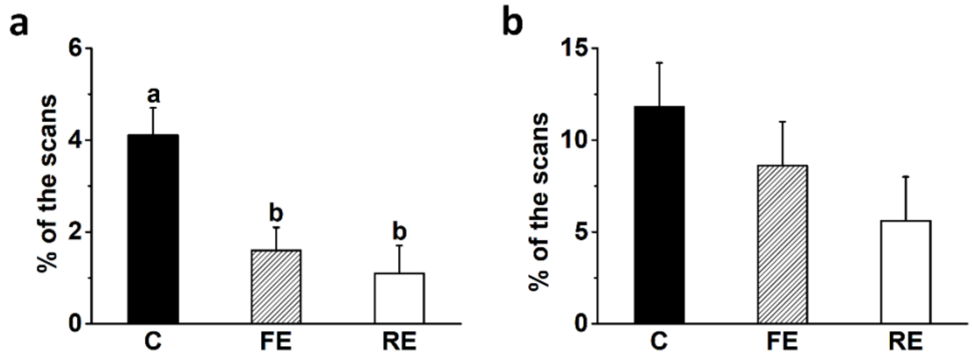

Figure 3. Least squares means (±SEM) of ratios of non-nutritive oral behaviours for calves in pens without additional enrichment (control, CON, n = 9 pens), with fixed enrichment (FE, n = 9 pens), and with rotating enrichment (RE, n = 9 pens) in experiment 1 collected using (a) instantaneous scans (30 times/d, afternoon) and (b) continuous focal observations (10 min/d, morning). Different letters (a, b) indicate significant differences between treatments at $P \leq 0.05$.

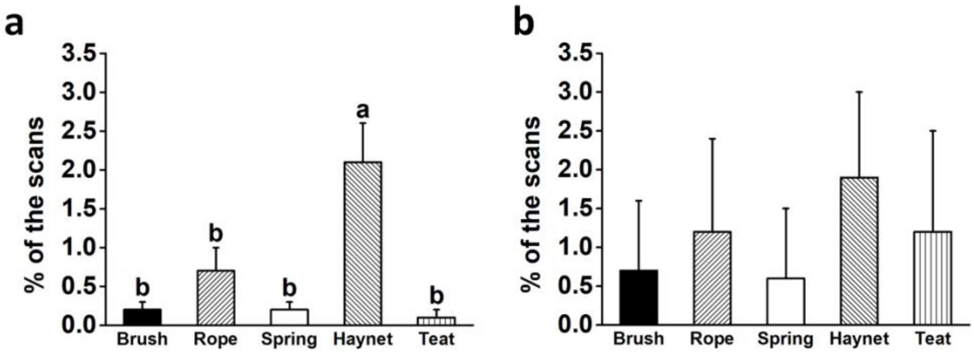

Figure 4. Least squares means (±SEM) of use ratios of stationary brushes, ropes, springs, dry teats, and net filled with strawberry-scented hay (haynet) for calves with rotating enrichment (n = 9 pens for stationary brushes, dry teats, and net filled with strawberry-scented hay; n = 4 pens for ropes; n = 5 pens for springs) and fixed enrichment (n = 9 pens for stationary brushes, dry teats, and net filled with strawberry-scented hay; n = 5 pens for ropes; n = 4 pens for springs) in experiment 1 collected using (a) instantaneous scans (30 times/d, afternoon) and (b) continuous focal observations (10 min/d, morning). Different letters (a, b) indicate significant differences between treatments at P ≤ 0.05.

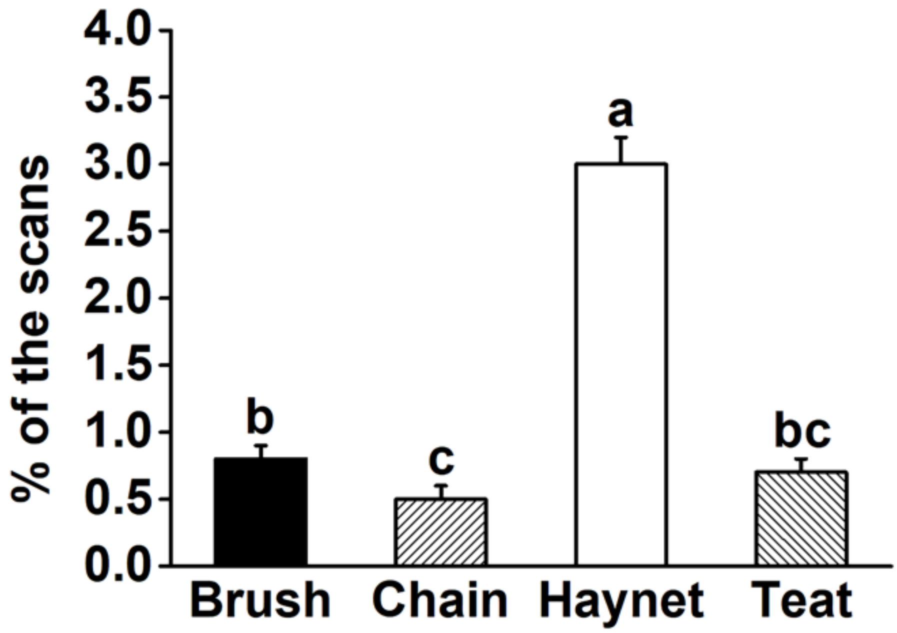

Figure 5. Least squares means (±SEM) of use ratios of stationary brushes, plastic chains, nets filled with strawberry-scented hay (haynet), and dry teats for calves in individual pens (n = 8 pens) and pair pens (n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Different letters (a–c) indicate significant differences between treatments at $P \leq 0.05$.

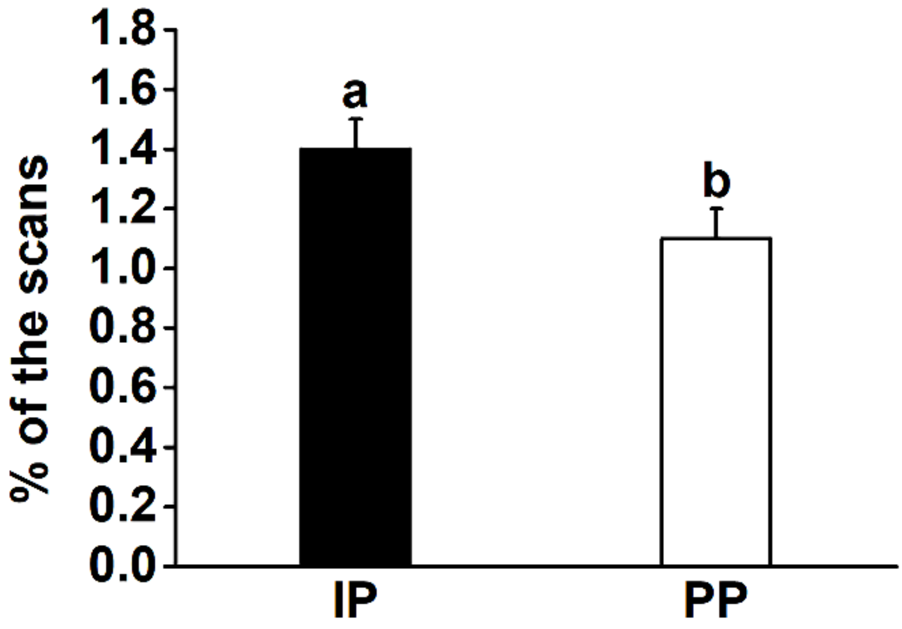

Figure 6. Least squares means (±SEM) of use ratios of overall items for calves in individual pens (IP, n = 8 pens) and pair pens (PP, n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Different letters (a, b) indicate significant differences between treatments at P ≤ 0.05.

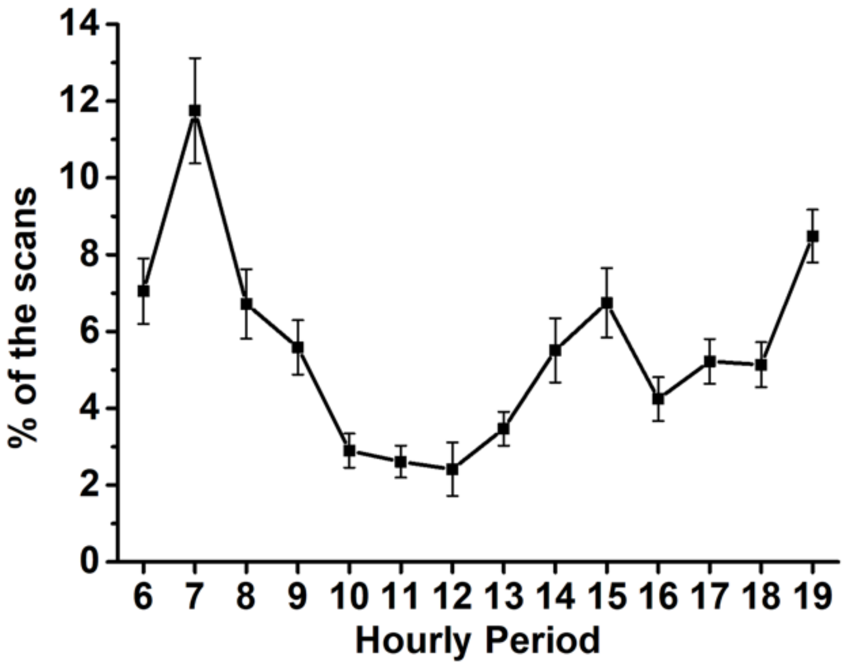

Figure 7. Means (±SEM) of use ratios of overall items by hourly period for calves in individual pens (n = 8 pens) and pair pens (n = 8 pens) in experiment 2 collected using instantaneous scans (168 times/d). Observation spanned from 0600 to 1959 h.

2.6 Supplementary Material

2.6.1 Statistical Analysis

Novel object tests

Vocalisations were analysed for differences between housing treatments using a general linear model. One calf from the FE treatment was not recorded because of a recording error with the camera. For retreats from the object, the behaviour was relatively infrequent, so the data were transformed to categorical: showed or did not show retreats from the object. Because some cell counts were less than 5, the treatments of CON and FE were pooled (CF) to meet the Fisher’s test requirements. After that, a Fisher’s test was conducted to analysed for differences between these treatments and RE, which was most strongly predicted to decrease fear of novelty due to the regular addition of new items to the pen. Two calves were not recorded due to recording errors.

2.6.2 Results

Response to novelty

Vocalisations did not differ significantly between treatments (means (95% CIs): Control 7.3 times (3.2 - 11.5), FE 5.5 times (1.1 - 9.9), RE 6.9 times (2.7 - 11.1); $F_{2,23} = 0.21$, $P = 0.813$; $n = 26$). Calves from RE and CF did not differ significantly in showing retreats from the object ($P = 0.156$).

Chapter 3

Effects of Physical Enrichment Items and Social Housing on Calves' Growth, Behaviour and Response to Novelty

A version of this chapter has been published as:

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3. Effects of Physical Enrichment Items and Social Housing on Calves' Growth, Behaviour and Response to Novelty

Statement

I confirm that this study was conducted for my dissertation research. My contributions included formulating hypotheses for the experiments, planning and conducting the experiments, compiling the data, conducting the statistical analysis and writing this published manuscript. All authors approved the final version prior to submission to Applied Animal Behaviour Science.

Abstract

The objective of this study was to determine the effects of social housing, the provision of physical enrichment, and the interaction between the two on calf growth, behaviour and fearfulness. Forty-eight calves were randomly allocated to either individual (IP, sixteen calves) or pair (PP, thirty-two calves) pens from 2 days to 8 weeks of age. Half of the calves in each housing treatment were provided with physical enrichment items (stationary brushes, plastic chains, rubber teats and haynets filled with strawberry-scented hay; PE). The remaining calves received no physical enrichment items (NPE). Concentrate consumption within each pen was measured daily and calves were weighed at birth and weekly thereafter. Concentrate feeding efficiency was then calculated by the ratio between average daily gain within each pen and daily concentrate consumption within each pen. When calves were 2-5 weeks of age, they were recorded by a camera between 06:00 h and 20:00 h twice weekly, and behavioural data were collected using instantaneous scan sampling at 5-min intervals. Their behavioural responses to a novel environment and a novel object were then assessed once each at 5 or 6 weeks of age. PE calves tended to have greater average daily gains than NPE calves (mean ± IQR; 610.6 ± 151.8 g/d vs. 568.8 ± 77.1 g/d; $p = 0.095$). PE calves spent more time consuming hay than NPE calves. Among calves in IP pens, PE calves consumed less concentrate but had better concentrate feeding efficiency than NPE calves. For home pen behaviours, PE calves showed less frequent non-nutritive sucking than NPE calves (0.802 ± 0.451 % vs. 1.897 ± 0.401 % of scans) and less frequent cross-sucking. Furthermore, PP increased or tended to increase the time spent on locomotor play, fixture sniffing, social sniffing, allogrooming and cross-sucking, but tended to decrease non-nutritive sucking compared to IP. No treatment effects were found on behaviour in the novelty tests. In conclusion, physical enrichment may improve calf growth more effectively than social housing does. Physical enrichment and social housing may satisfy diverse natural behaviours and reduce undesirable behaviour in different ways. However, these treatments had no effect on calf fear responses in novel environment and object tests. The combination of physical enrichment and social housing showed no further improvement in calf welfare.

Key words: dairy calf, environmental enrichment, average daily gain, behaviour, fear, welfare

3.1 Introduction

In the dairy industry, it is standard practice to raise calves in non-enriched individual pens after birth (Pempek et al., 2016). However, since this living environment fails to meet the needs of calves and restricts their natural behaviours (e.g. Jensen et al., 1998), social housing has been used to improve their welfare (Van De Weerd and Day, 2009). Many studies have shown that social housing provides benefits by promoting natural behaviours, reducing undesirable behaviours, and improving emotional states and production performance (e.g. Costa et al., 2015; Pempek et al., 2016). However, social housing has also been suggested to induce new welfare problems, such as greater risk of respiratory disease (Cobb et al., 2014) and increased cross-sucking behaviour (Lidfors and Isberg, 2003), which may cause health problems (Größbacher, 2013).

One further way of improving animal welfare is to provide physical enrichment (Boissy et al., 2007) through altering the complexity of animals' enclosure or adding physical enrichment items to the enclosure (Bloomsmith et al., 1991). For calves, enhancing the complexity of their enclosure can stimulate the expression of natural behaviour and reduce undesirable behaviour (e.g. Jensen et al., 1998). However, the effect of adding physical enrichment items to the enclosure on calves' behaviours is rarely studied. Pempek et al. (2017) indicated that adding physical enrichment items (teat, brush, “lollie” and chain) to calves’ hutches promoted the expression of locomotor play. Horvath et al. (2020) illustrated that the provision of a brush reduced total time engaged in non-nutritive oral behaviours but increased time engaged in grooming. Ude et al. (2011) found that after adding teats into standard pens, calves showed reduced non-nutritive oral behaviours.

As well as benefits to calves’ behaviours, physical enrichment items may also affect calves’ growth. For instance, providing food rations through a net instead of an open trough can enrich animals’ feeding experience since they often prefer to work for a reward (Mandel et al., 2016). For calves, putting hay in a net may enable calves to engage in hay collection (pulling hay from nets) and occupy more of their time than simply picking it up from feeders. It may increase the naturalness of calves’ feeding behaviour and improve feed consumption (Mandel et al., 2016).

Furthermore, physical enrichment items have effects on responses to novelty in many farm animals. Fear, which can increase risk of injury and decrease biological functioning (e.g. reducing reproductive performance or immune function; Morgan and Tromborg, 2007), is a common emotion for animals when they face novelty (Forkman et al., 2007). It can be expressed by behaviours such as active defence, passive avoidance, expressive movements and alarm calls in novelty tests (Erhard and Mendl, 1999; Forkman et al., 2007). Although a given behaviour can be attributed to multiple emotions, some behavioural variables such as latency to contact and time spent in contact with novel object in novelty tests may be true indicators of fear based on their high correlation with other fear-related measures indicating physiological arousal (Van Reenen et al., 2005). Other behaviours in novelty tests such as eliminative behaviour and vocalisation are also usually used to reflect fear as they are typically seen in contexts where we would expect animals to be frightened (Forkman et al., 2007). Recording of several of these behaviours simultaneously can therefore strengthen conclusions about the underlying state. Adding relevant items meeting animal's needs to the enclosure has been shown to affect such fear responses, as seen through reducing avoidance and freezing of a novel object in domestic chicks (Jones and Waddington, 1992) and reducing latency to approach a person in piglets (Rodarte et al., 2004). However, the effect of adding items to the enclosure on calves' emotional states is less well known.

Whilst the individual effects of social housing or physical enrichment items on the improvement in animal's welfare has been widely studied in many species, investigation into the combination of both components is still limited. However, a number of studies in laboratory rats have demonstrated that the application of both social housing and physical enrichment items had diverse and non-additive behavioural effects in open-field and novel object tests (e.g. Zimmermann et al., 2001; Schrijver et al., 2002), and improved the animals' ability to cope with social challenges (Pietropaolo et al., 2004). Although little is known in calves, since social housing and physical enrichment items improve animal welfare by providing social contact (Costa et al., 2016) and increasing environmental complexity (Bloomsmith et al., 1991) separately, it might be expected that calves' welfare may be further improved by the combination of both components.

The present study aimed to determine the effects of social housing, the provision of physical enrichment items to calf pens, and the interaction between both components on calf growth, behaviour and response to novelty. It was hypothesised that 1) physical enrichment items and social housing will separately stimulate calf growth, increase play, exploratory and grooming behaviours, reduce non-nutritive oral behaviours and reduce fear of novelty; 2) there will be an interaction between physical enrichment items and social housing in terms of their influence on calf growth, behavioural expression and response to novelty, with the combination of both components having a more profound influence than one of the single enrichments.

3.2 Materials and Methods

3.2.1 Ethics Statement

The study was performed at the Centre for Dairy Research, University of Reading (CEDAR), Reading, UK. All procedures complied with guidelines for the Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research (Sherwin et al., 2017), and UK and EU laws governing research in animals.

3.2.2 Animal, Housing and Feeding

Forty-eight male Holstein Friesian calves were included in this study from 2 days of age until 8 weeks of age. When calves were born, 6 L colostrum was offered to each calf three times within 24 h of birth. Birth weight, ID and date of birth of the newborn calves were recorded. Calves with birth weights below 35 or above 55 kg were excluded, as well as any calves that were not drinking milk on their own by day 4.

Calves were assigned into eight blocks (six calves in each) according to their date of birth. Within blocks, calves were allocated to either individual (IP, two calves per block) or pair (PP, four calves per block) pens. Half of the calves in each housing treatment were provided with sensory and physical enrichment items (PE): one stationary brush, one plastic chain, one rubber teat and one haynet filled with strawberry-scented ryegrass hay for IP; one haynet filled with strawberry-scented ryegrass hay and two of all other items for PP. Physical enrichment items were chosen based on the motivations hypothesised to be inadequately fulfilled in standard housing. Remaining calves received no additional physical enrichment items (NPE). The area of an IP and a PP was 2.4 m^2 and 4.8 m^2, respectively; the whole area of each pen was covered with deep straw and fresh straw were added daily into each pen after morning milk feeding.

This trial was completed in two cohorts (24 calves in each cohort). Within each cohort, pens were arranged in three rows, so that the calves' visual contact in between rows would be limited by the 2-metre wide passages; calves' physical contact with their neighbours within one row would be limited to the gap between the panel bars (120 mm, large enough for calves' muzzles to go through the gap). All calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets from 2 days of age to 49 days of age. 2.5 L per feeding (L/f) of milk was offered to each calf until 14 days of age, followed by 3 L/f from 15 to 42 days of age and 2.5 L/f between 43 and 49 days of age. During 50–56 days of age, 2.5 L/f milk was fed to each calf only in the morning. Calves had *ad libitum* access to concentrate (VITA concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and water throughout the study period.

3.2.3 Growth

The daily provision of concentrate and the daily collection of concentrate refusals were weighed in each pen until 8 weeks of age. All calves were weighed weekly until 8 weeks of age using a wheeled scale (Ritchie Agricultural, UK). Daily concentrate intake and average daily gain were calculated by averaging across the entire period. Weekly concentrate intake and weekly gain were calculated by summing every week. For pair housed calves, concentrate consumption of both calves in the pen was averaged to calculate daily and weekly concentrate intake of pair pens. Calves' concentrate feeding efficiency was calculated by the ratio between average daily gain within each pen and daily concentrate intake within each pen.

3.2.4 Home Pen Behaviours

Home pen behaviours were recorded by a CCTV (Transit-PTZ, Revader Security Ltd, UK) for 14 h (06:00 h-20:00 h) twice per week when calves were 2, 3, 4, and 5 weeks of age. Video recordings were watched using instantaneous scans at 5-min intervals. The frequencies of calves' behaviours as listed and defined in Table 1 were recorded.

3.2.5 Novelty Tests

Following home pen behavioural observations, an environmental novelty test was conducted one day before the novel object test. Both tests were conducted one calf at a time. A wheeled scale was used to move each test calf between its home pen and the test arena (4.0 × 4.0 m^2). The test arena was set up in different locations in the barn for the calves in the first and second cohorts, because of changing space needs in the facility. When arriving at the entry of the test arena, the calf was lightly tapped on the hindquarters to encourage it to enter the test arena, in which the calf could not see any other calves. Both tests were recorded by either CCTV or webcam (C525, Logitech International S.A, Switzerland). Video recordings were observed continuously to record the behaviours of interest as defined in Table 2. These were selected because calves feel fearful of the test arena or novel object, they are typically reluctant to touch the pen fixtures or object, defecate and vocalise more, and show sudden movements (Jensen et al., 1999).

The environmental novelty test started when the door of the test arena was fully closed. Each calf stayed in the test arena for 15-min. For the novel object test, once entry into the test arena calves were allowed to habituate for 5-min. Following the period of habituation a novel object (a white bucket or a traffic cone, used for alternate blocks of calves) was lowered to the centre of the test arena on a pulley. The calf remained in the pen with the novel object for 10 min.

3.2.6 Statistical Analysis

All data were analysed using Minitab 18 (Minitab, LLC, USA). Significant differences were declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.10$.

For growth, a general linear model (GLM) was used to determine the effect of forms of enrichment on daily concentrate intake, average daily gain and concentrate feeding efficiency across the pre-weaning and weaning periods. Tukey's multiple comparison test was used thereafter to do multiple comparisons. Factors in the model included physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between these two factors. Calves' birth weight was used as a covariate when determining average daily gain. Calves' averaged birth weight within each pen was used as a covariate when determining daily concentrate intake and concentrate feeding efficiency. The residuals of concentrate feeding efficiency were not normally distributed, and thus it was square root transformed before analysis.

A mixed effects model (MEM) was used to determine the effect of forms of enrichment on weekly concentrate intake and weekly gain across weeks of the pre-weaning and weaning periods. Tukey's multiple comparison test was used thereafter to do multiple comparisons. The fixed factors in the model included physical enrichment items (NPE or PE), social housing (IP or PP), calves' week of age and the interactions between these factors. When determining weekly concentrate intake, the random factor was pens' number, and the covariate was calves' averaged birth weight within each pen. When determining weekly gain, the random factor was calves' ID number, and the covariate was calves' birth weight. The residuals of weekly concentrate intake were not normally distributed, and thus it was square root transformed before analysis.

For home pen behaviours and both novelty tests, video recordings were played with Windows Media Player (Microsoft Corporation, US) and data recorded by one observer. In order to determine the inter-observer reliability, another observer watched the home pen behaviour videos of eight calves by randomly choosing one week from 2, 3, 4, and 5 weeks of age for each calf. For both novelty tests, eight calves' videos of environmental novelty test and eight calves' videos of novel object test were randomly selected and watched by another observer who was blind to the hypothesis under test. A Pearson correlation was used to compare the reliability between the two observers, which suggested strong positive relationships (home pen behaviour: $r = 0.995$, $p < 0.001$; environmental novelty test: $r = 0.999$, $p < 0.001$; novel object test: $r = 0.999$, $p < 0.001$) and good reliability. For novel object test, the videos were also watched for latency to contact by one of two other observers who were blind to treatment to ensure that data were reliable. MEM, GLM or binary logistic regression (BLR) were used to analyse the calves' behaviours in the three tests. For the behaviours analysed by BLR, in order to fit in the regression model, the data of the behaviours were converted to binary by coding any values greater than zero as "1". False discovery rate (FDR) was used to solve multiple testing issues by calculating adjusted p values (Jafari and Ansari-Pour, 2019).

For home pen behaviours, time spent consuming concentrate and hay, ruminating, fixture sniffing, non-nutritive sucking and social sniffing were analysed by MEM. The fixed factors were physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between the two factors. The random factor was calves' ID number. The covariates were calves' birth weight, age, average temperature of the barn during the testing days and milk refusal during the testing days. The residuals of time spent consuming concentrate and hay, ruminating, non-nutritive sucking and social sniffing were not normally distributed or did not meet the assumption of homogeneity of variance, and thus these variables were square root transformed before analysis. In addition, locomotor play, fixture scratching, tongue rolling, allogrooming, social play and cross-sucking were analysed by BLR. The categorical predictors were physical enrichment items (NPE or PE), social housing (IP or PP) and the interaction between the two factors. The continuous variables included in the analysis were calves' birth weight, age, average temperature of the barn during testing days, and milk refusal during testing days. Fixture play and straw play were not analysed because they were rarely expressed.

In the novelty tests, fixture touching and abrupt movement in the environmental novelty test, and object touching and latency to first contact with the object in the novel object test were analysed by GLM. Factors included physical enrichment items (NPE or PE), social housing (IP or PP), the interaction between the two factors, arena locations and objects; object was only included as a factor in the novel object test. The covariate was average temperature of the barn during the testing day. Variables, with the exception of latency to first contact with the object in the novel object test, were square root or logarithm transformed before analysis as residuals were not normally distributed. Defecation bout, sudden neck movement and vocalisation in the environmental test, and defecation bout, abrupt movement and vocalisation in the novel object test were analysed by BLR. Categorical predictors included physical enrichment items (NPE or PE), social housing (IP or PP), the interaction between the two factors, arena locations and objects; object was only included as a factor in the novel object test. The continuous variable was average temperature of the barn during the testing day. Sudden neck movement in the novel object test was not analysed because calves rarely showed this behaviour.

One calf's data for all measures was discarded due to an abscess on its tongue. In addition, on one occasion home pen behaviours for two calves were only recorded for 14 h due to a technological problem. As a result of navel inflammation in another calf, the home pen data from 3, 4, and 5 weeks of age for this calf were discarded before analysis. Moreover, the data from both novelty tests were discarded before analysis for another calf who was familiar with the test arena and the novel objects due to his pen location.

3.3 Results

3.3.1 Growth

Growth performance variables, including daily concentrate intake, average daily gain, and concentrate feeding efficiency are shown in Table 3. Physical enrichment items and social housing had interactions on daily concentrate intake and concentrate feeding efficiency, with calves in PE-IP pens showing reduced daily concentrate intake but having better concentrate feeding efficiency than those in NPE-IP pens. Calves in PE pens tended to have greater average daily gains when compared with those in NPE pens (mean ± IQR; 610.6 ± 151.8 g/d vs. 568.8 ± 77.1 g/d).

For calves' weekly concentrate intake and weekly gain, physical enrichment items, social housing and calves' week of age tended to have interactions on weekly concentrate intake ($F_{7,189}$ = 1.96, p = 0.063; Figure 1) and weekly gain ($F_{7,343}$ = 1.77, p = 0.093; Figure 2), with calves in PE-IP pens consuming less concentrate compared with those in NPE-IP pens at 6 weeks of age, but calves in different treatments showing similar rates of weekly gain at every week of age.

3.3.2 Home Pen Behaviour

Feed intake related behaviours, including hay intake, concentrate intake, and ruminating are shown in Table 4. Hay intake behaviour was significantly more frequent for calves in PE pens than calves in NPE pens. Physical enrichment items and social housing tended to have interactions on the time spent consuming concentrate ($F_{1,38.92}$ = 3.74, p = 0.061), with calves in PE-IP pens showed reduced time spent consuming concentrate compared with those in NPE-IP pens (0.738 ± 0.440 % vs. 1.512 ± 0.259 % of scans).

There were no interactions between physical enrichment items and social housing with respect to the incidence of the natural and undesirable behaviours. Calves in PP pens expressed more fixture sniffing (Table 4) than those in IP pens. Social sniffing was more frequent for calves in PP pens than calves in IP pens. In terms of non-nutritive sucking, calves in PE pens were observed to show less non-nutritive sucking than those in NPE pens, and calves in PP pens tended to show less non-nutritive sucking than those in IP pens.

PE tended to suppress the expression of fixture scratching (Table 5) compared with NPE. PP increased or tended to increase the expression of locomotor play and allogrooming in comparison with IP. For cross-sucking, PE suppressed the expression of this behaviour compared with NPE, while PP increased the frequency of this behaviour in comparison with IP.

3.3.3 Novelty Tests

In the environmental novelty test, calves in PE pens and NPE pens showed similar durations of fixture touching (316.3 ± 0.5 vs. 331.0 ± 1.0 s; $F_{1,40}$ = 0.17, adjusted p = 0.908) and similar frequencies of abrupt movement (0.312 ± 1.000 vs. 1.040 ± 3.000 bouts; $F_{1,40}$ = 3.41, adjusted p = 0.288), defecation (adjusted p = 1.000), sudden neck movement (adjusted p = 1.000) and vocalisation (adjusted p = 1.152). Calves in PP pens and IP pens showed similar durations of fixture touching (303.8 ± 0.6 vs. 344.6 ± 0.3 s; $F_{1,40}$ = 1.33, adjusted p = 0.640) and similar frequencies of abrupt movement (0.466 ± 1.000 vs. 0.803 ± 2.000 bouts; $F_{1,40}$ = 0.73, adjusted p = 0.498), defecation (adjusted p = 0.625), sudden neck movement (adjusted p = 1.220) and vocalisation (adjusted p = 0.752). In addition, physical enrichment items and social housing had no interactions on these behavioural responses.

In the novel object test, calves in PE pens and NPE pens showed similar durations of object touching (21.1 ± 19.7 vs. 12.8 ± 51.0 s; $F_{1,39}$ = 0.87, adjusted p = 0.446) and latency to first contact with the object (135.7 ± 139.0 vs. 256.8 ± 560.0 s; $F_{1,39}$ = 3.36, adjusted p = 0.375), and similar frequencies of abrupt movement (adjusted p = 0.295), defecation (adjusted p = 1.000) and vocalisation (adjusted p = 0.258). Calves in PP pens and IP pens showed similar durations of object touching (15.9 ± 62.1 vs. 17.0 ± 25.5 s; $F_{1,39}$ = 0.01, adjusted p = 1.131) and latency to first contact with the object (198.3 ± 534.5 vs. 194.1 ± 476.5 s; $F_{1,39}$ < 0.01, adjusted p = 0.950), and similar frequencies of abrupt movement (adjusted p = 0.828), defecation (adjusted p = 0.200) and vocalisation (adjusted p = 1.495). In addition, physical enrichment items and social housing had no interactions on these behavioural responses.

3.4 Discussion

Physical enrichment offered some benefits for growth and supressed non-nutritive oral behaviours. Social housing had no effect on calves' growth but promoted, or tended to promote some positive behaviours. Physical enrichment and social housing had non-additive effects on calves' growth and home pen behaviour. Physical enrichment, social housing and the interaction between these two factors had no effect on calves' behavioural responses in the novelty tests.

3.4.1 Growth and Feeding Effects

As observed, calves with physical and sensory enrichment tended to have higher average daily gains and lower daily concentrate intake, resulting in improved concentrate feeding efficiency among individually housed calves. This contrasts with the findings of Pempek et al. (2017), who reported that furnished pens had no effect on calves' concentrate intake and weight gain. The difference may be attributed to the provision of roughage to calves. According to Pempek et al. (2017), calves had no access to hay or other roughage, but in this study, ryegrass hay was provided *ad libitum* to calves. While calves without physical enrichment only consumed ryegrass hay from hay racks, strawberry-scented ryegrass hay was also provided to physically enriched calves from haynets. Since animals can use their sensorial perceptions to choose palatable feeds (Baumont, 1996) and some aromas can increase the palatability of hay (Cannas et al., 2009), the strawberry aroma in this study might have stimulated calves to eat more hay. Strawberry was chosen because red berry flavouring was previously found to be a preferred aroma for dairy cattle (Meagher et al., 2017). As observed, calves with physical enrichment items showed a higher frequency of hay intake, which may result in increased consumption of hay and increased average daily gain compared with non-physically enriched calves.

Altogether, the increase in roughage intake and better concentrate feeding efficiency in calves with physical enrichment items are likely to be economically beneficial on farms. Improved feed conversion efficiency is an important objective for profitable dairy operations (Bach et al., 2007). Oostindjer et al. (2010) also demonstrated that physical enrichment (straw, wood shavings, peat, and branches) positively affected the feed conversion efficiency for piglets. These results may be attributed to the reduced stress in physically enriched living environments (Barnett et al., 1983).

In contrast to the effect of physical enrichment, social housing had no effect on calves' daily concentrate intake, average daily gain and concentrate feeding efficiency in this study. However, previous studies found that social housing increased weight gain in calves (Tapki, 2007; Jensen et al., 2015; Pempek et al., 2016). This is likely owing to increased concentrate intake through social learning or social facilitation (Costa et al., 2015). In other words, the presence of other calves near the bucket or sight of them eating would increase the likelihood of calves paying attention to feed and perform similar behaviours; calves could also learn where to find concentrate and how to consume it by observing, or interacting, with calves showing those behaviours. One potential reason for the lack of treatment effect in this study is the different housing design. When researchers previously studied the effect of social housing on calves' growth, they compared calves in grouped environments with calves in individual environments with only auditory contact or auditory and visual contact. For instance, Jensen et al. (2015) positioned adjacent pens 1.5-metre apart to prevent physical contact between calves in different pens. However, in the current experiment, calves had auditory, visual and limited physical contact (calves' muzzles could go through the gap between the panel bars) with their neighbours. Therefore, calves in individual pens may imitate or learn how to consume concentrate from their pair housed neighbours. Jensen and Larsen (2014) similarly demonstrated that calves in individual pens with limited physical contact with their neighbours and calves in paired pens had similar daily concentrate intake and average daily gain.

3.4.2 Home Pen Behaviour

Expression of locomotor play tended to be higher in pair housed calves than in individually housed calves. As play behaviour typically reflects an absence of negative affective states, or indicates increased positive experience (Held and Špinka, 2011), social housing may provide a more pleasurable living environment for calves. Jensen et al. (1998) also showed that social stimulation might lead to the appearance of locomotor play. However, calves in individual and paired pens showed similarly low frequencies of social play. One reason for this phenomenon could be the later emergence of social play in the calves' life, with limited amounts occurring in the first few weeks (Jensen et al., 1998). In addition, physical enrichment had no effect on locomotor or social play. It may indicate that the play behaviours are not stimulated by these specific external objects, since animals with higher stimulation to play will show more play behaviour (Jensen et al., 1998). Hubrecht (1993) similarly demonstrated that physical enrichment items did not increase locomotor or social play in dogs. Therefore, other physical enrichment items need to be studied in the future to stimulate calves' play behaviour.

Sniffing is a type of exploratory behaviour which is motivated by the animals need gather environmental information (Westerath et al., 2009). The expression is perceived to be intrinsically pleasant or self-rewarding (Boissy et al., 2007). In the present study, calves in paired pens showed more fixture sniffing than those in individual pens. This result may indicate that housing calves in pairs may be an effective way to release calves' exploratory motivation and stimulate them to explore their living environment. The increased expression of social sniffing in pair housed calves may also corroborate this view. By contrast, physical enrichment had no effect on calves' exploratory behaviour, maybe because the additional items attracted the calves' attention so that they spent more time exploring additional items rather than the rest of the environment (e.g. Zobel et al., 2017).

Allogrooming and fixture scratching are body care behaviours (Kohari et al., 2007), which help maintain hygiene of the animal's body by removing debris or ectoparasites (Rich, 1973). Moreover, allogrooming is important in forming or maintaining social relationships between calves (Færevik et al., 2007). In this study, social housing tended to increase the expression of allogrooming, but physical enrichment had no effect on this behaviour. This result agreed with previous studies conducted by Tapki (2007), and Horvath and Miller-Cushon (2019). The former showed that social housing encouraged calves to express allogrooming voluntarily. The latter suggested that the provision of physical enrichment in the form of a brush had no effect on this natural behaviour. The result from this study may demonstrate that allogrooming does not relate to stimuli from external items. In addition to social body care behaviour, fixture scratching was expressed less in physically enriched calves, which might be a consequence of the existence of other more suitable scratching items in physically enriched pens.

Non-nutritive sucking, cross-sucking and tongue rolling are considered as non-nutritive oral behaviours, which are non-functional and harmful (Le Neindre, 1993; Jensen, 2003; Garner, 2005). Non-nutritive sucking may be considered as redirected sucking behaviour (De Passillé et al., 1992). Calves have a strong motivation for suckling. Therefore, in the absence of their dam or a teat, they may redirect this behaviour toward elements in their environment. This is different from calves' behaviour in nature and might be an indication of frustration (Leruste et al., 2014). Cross-sucking is an abnormal behaviour, which is a redirection from milk suckling behaviour toward the ear, tail, navel, prepuce, or other body parts of other calves (Leruste et al., 2014), and can lead to hair loss, inflammation and diseases in the receiver (Jensen, 2003). Tongue rolling is considered as a stereotypic behaviour indicating frustration or lack of stimulation (Leruste et al., 2014; Mason and Latham, 2004). In the present study, calves in paired pens tended to show less non-nutritive sucking but showed more cross-sucking than those in individual pens. This result agrees with that of Pempek et al. (2016), whose study showed that although non-nutritive sucking was observed more often among individually housed calves, calves housed in pairs appeared to redirect this behaviour to their companion as cross-sucking. Physically enriched calves show less non-nutritive sucking and cross-sucking than non-physically enriched calves. This was shown by Veissier et al. (2002), whose study suggested that providing a teat after milk feeding reduced non-nutritive sucking, while Newberry (1995) demonstrated that the occurrence of cross-sucking behaviour was reduced when calves were presented with dry rubber nipples following milk feeding. In addition, the expression of tongue rolling was not affected by physical enrichment or social housing. This may be because tongue rolling is directly related to feeding and ruminating behaviours (Webb et al., 2012). In this study, although the frequency of concentrate intake, hay intake and ruminating were affected by different treatments, the frequency of feed intake behaviours was similar across all treatments.

3.4.3 Response to Novelty

Neither physical enrichment nor pair housing were found to affect calves' behavioural responses in either novelty test. These findings agree with previous studies showing no effect of social housing (Jensen and Larsen, 2014) or the provision of physical complexity to a standard hutch (Pempek et al., 2017) on calves' behavioural responses to social and environmental novelty. This phenomenon may indicate the static environment created by providing additional objects was not complex enough to elicit emotional change in novel situations. Therefore, a more complex and dynamic environment is probably needed in future studies to reduce calves' fearfulness. In terms of the effect of social housing, Leruste et al. (2014) found that some behavioural responses of calves (e.g. vocalisation and exploratory behaviour) in individual pens with tactile contact were similar to those of pair housed calves, which may indicate that individual housing with tactile contact may result in similar fearfulness in pair housed calves.

3.5 Conclusion

Provision of physical enrichment improved calves' growth by promoting intake of roughage and increasing weight gain and concentrate feeding efficiency. In contrast, social housing was less effective at improving calves' growth. Provision of physical enrichment reduced calves' non-nutritive oral behaviours, while social housing had a positive impact on play, exploratory, and social behaviours. However, neither treatment affected calves' fear of novelty. In conclusion, physical enrichment and social housing may satisfy calves' needs in different ways, but the combination of both components did not further improve calves' welfare.

Declaration of Competing Interest

None.

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Table 1. Ethogram of the home pen behaviours

Category	Behaviour	Definition
Feeding & ruminating	Concentrate intake	Heading in or above the concentrate bucket and chewing
	Hay intake	Chewing hay from the hay rack or haynet
	Ruminating	Chewing without concentrate, straw or hay
Exploratory behaviour	Fixture sniffing	Putting muzzle in contact with or less than one muzzle length from any fixture in the pen with neck not relaxed
Play	Locomotor play	Engaging in a gallop, leap, Jump, buck-low, buck-high, buck-kick or turn.
	Straw play	Kneeling down on the two forelegs and butting straw, or rubbing head or neck in straw in a playful manner
	Fixture play	Standing and butting head against any fixture in the pen in a playful manner
Grooming	Fixture scratching	Putting head, neck or body in contact with any fixture in the pen and slightly moving back and forth or up and down
Non-nutritive oral behaviour	Non-nutritive sucking	Licking, sucking or biting any fixture of the pen
	Tongue rolling	Making a repeated rolling and stretching of the tongue outside or sometimes inside open mouth

	Cross-sucking	Sucking or biting toward ear, mouth, navel, scrotum, prepuce, or other body parts of other calves
Social behaviour	Social sniffing	Putting muzzle in contact with or less than one muzzle length from other calves with neck not relaxed
	Social play	Mounting other calves, running with other calves or butting head against head, neck or body of other calves in a playful manner
	Allogrooming	Putting tongue out of mouth and in contact with head, neck or body of other calves
Others	Other behaviours	Such as lying down, standing, walking and drinking water

Table 2. Ethogram of the recorded behaviours in the environmental novelty test (ENT) and the novel object test (NOT)

Test(s)	Behaviour	Definition
ENT	Fixture touching[a]	Sniffing, licking or sucking the testing arena while standing or walking
ENT, NOT	Defecation[b]	The bouts of defecation
ENT, NOT	Abrupt movement[b]	Showing an abrupt movement in a reverse direction from the area being explored
ENT, NOT	Sudden neck movement[b]	Showing a sudden neck movement such as a startle reflex while exploring
ENT, NOT	Vocalisation[b]	Vocalising with mouth opened or closed
NOT	Latency to first contact with the object[a]	Time interval from lowering the object to the centre of the test arena to touching the object
NOT	Object touching[a]	Sniffing, licking, sucking or butting the object while standing or walking

[a]The time duration of the behaviour was recorded.

[b]The frequency of the behaviour was recorded.

Table 3. Growth performance variables (mean ± IQR) analysed using general linear models (GLM)[a]. Sample sizes for average daily gain were physically enriched individual PE-IP, n = 7 calves; non-physically enriched individual NPE-IP, n = 8 calves; physically enriched pair PE-PP, n = 16 calves and non-physical enrichment pair NPE-PP, n = 16 calves; samples sizes for daily concentrate intake and concentrate feeding efficiency were physically enriched individual PE-IP, n = 7 pens; non-physically enriched individual NPE-IP, n = 8 pens; physically enriched pair PE-PP, n = 8 pens and non-physical enrichment pair NPE-PP, n = 8 pens

Variables	IP		PP		p-value		
	PE	NPE	PE	NPE	PE vs. NPE	PP vs. IP	Interaction
Daily concentrate intake (g/d)	177.5 ± 190.0	302.9 ± 165.0	276.1 ± 181.6	249.9 ± 133.9	0.149	0.500	**0.034**
Average daily gain (g/d)	591.8 ± 153.1	563.6 ± 49.7	629.5 ± 115.7	574.0 ± 90.4	**0.095**	0.331	0.585
Concentrate feeding efficiency[b]	3.826 ± 0.612	2.068 ± 0.141	2.449 ± 0.135	2.412 ± 0.216	0.016	0.109	**0.023**

[a]Significant difference was declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.10$ (shown in bold text).

[b]Square root transformation was applied to the variable. The values of mean ± IQR for the variables were back-transformed.

Table 4. Six home pen behaviours analysed using mixed effects models (MEM)[a]. Samples sizes were physical enrichment PE, n = 23 calves; non-physical enrichment NPE, n = 24 calves; pair PP, n = 32 calves and individual IP, n = 15 calves

Variables	Mean ± IQR		(adjusted) p-value[c]	Mean ± IQR		(adjusted) p-value[c]
	PE	NPE	PE vs. NPE	PP	IP	PP vs. IP
Concentrate intake (%)[b]	0.790 ± 0.200	1.237 ± 0.453	0.005	0.916 ± 1.776	1.090 ± 1.779	0.253
Hay intake (%)[b]	3.138 ± 2.142	2.202 ± 1.063	**0.006**	2.846 ± 2.041	2.459 ± 1.083	0.236
Ruminating (%)[b]	6.423 ± 3.452	6.310 ± 4.028	0.879	6.442 ± 3.912	6.292 ± 3.549	0.840
Fixture sniffing (%)	5.080 ± 2.663	4.947 ± 2.663	1.079	5.765 ± 2.643	4.263 ± 2.128	**< 0.001**
Social sniffing (%)[b]	0.410 ± 0.071	0.342 ± 0.071	0.794	0.686 ± 0.070	0.157 ± 0.045	**< 0.001**
Non-nutritive sucking (%)[b]	0.802 ± 0.451	1.897 ± 0.401	**< 0.001**	1.096 ± 0.452	1.503 ± 0.477	**0.098**

[a]Significant difference was declared at (adjusted) p ≤ 0.05 and a trend at 0.05 < (adjusted) p ≤ 0.10 (shown in bold text).

[b]Square root transformation was applied to the variables. The values of mean ± IQR for the variables were back-transformed.

[c]Adjusted p-values were calculated using false discovery rate (FDR) to fixture sniffing, social sniffing and non-nutritive sucking.

Table 5. Six home pen behaviours analysed using binary logistic regression (BLR)[a]. Samples sizes were physical enrichment PE, n = 23 calves; non-physical enrichment NPE, n = 24 calves; pair PP, n = 32 calves and individual IP, n = 15 calves

Variables	Coefficient		Adjusted p-value[b]		Effect[c]	
	PE vs. NPE	PP vs. IP	PE vs. NPE	PP vs. IP	PE vs. NPE	PP vs. IP
Fixture scratching (%)	-1.387	0.000	**0.078**	1.000	PE < NPE	No
Locomotor play (%)	1.154	1.108	0.198	**0.065**	No	PP > IP
Allogrooming (%)	0.048	0.924	0.933	**0.059**	No	PP > IP
Social play (%)	-0.082	0.794	1.093	0.150	No	No
Tongue rolling (%)	-0.693	0.239	0.298	0.626	No	No
Cross-sucking (%)	-1.594	3.067	**0.012**	**<0.001**	PE < NPE	PP > IP

[1]Significant difference was declared at adjusted p ≤ 0.05 and a trend at 0.05 < adjusted p ≤ 0.10 (shown in bold text).

[2]Adjusted p-values were calculated using false discovery rate (FDR).

[3]Whether enriched treatments (tend to) make each behaviour been expressed more likely or less likely.

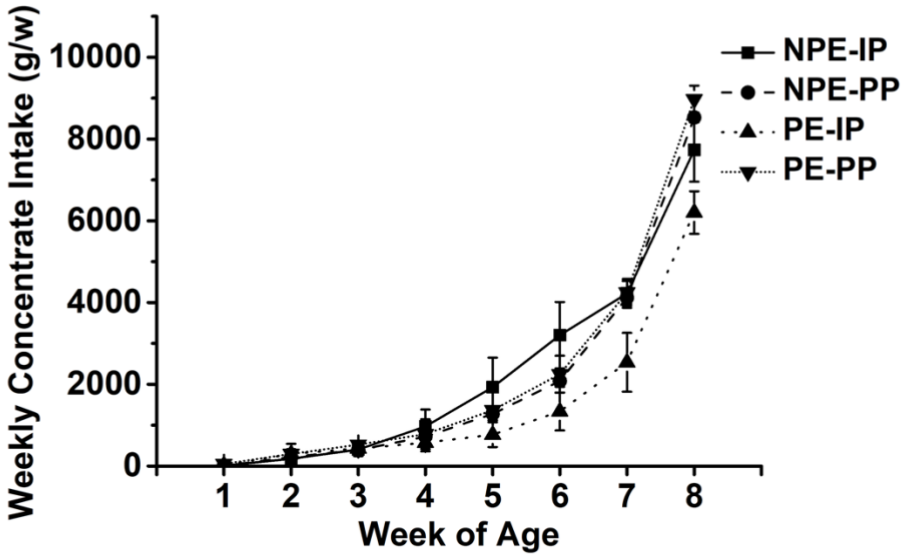

Figure 1. Back-transformed means (±IQR) of weekly concentrate intake across weeks of age for calves from non-physically enriched individual pens (NPE-IP; n = 8 pens), physically enriched individual pens (PE-IP; n = 7 pens), non-physically enriched paired pens (NPE-PP; n = 8 pens) and physically enriched paired pens (PE-PP; n = 8 pens).

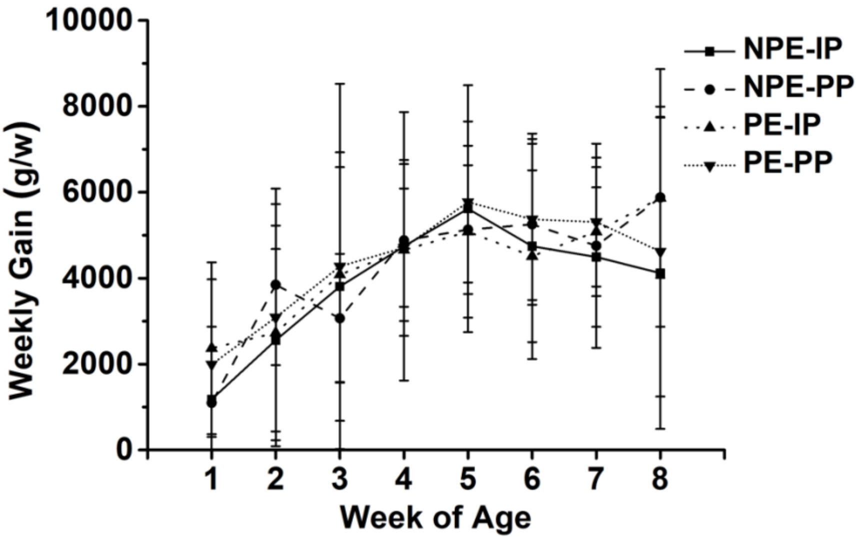

Figure 2. Means (±IQR) of weekly gain across weeks of age for calves from non-physically enriched individual pens (NPE-IP; n = 8 calves), physically enriched individual pens (PE-IP; n = 7 calves), non-physically enriched paired pens (NPE-PP; n = 16 calves) and physically enriched paired pens (PE-PP; n = 16 calves).

Chapter 4

Effects of Physical Enrichment and Pair Housing before Weaning on Growth, Behaviour and Cognitive Ability of Calves after Weaning and Regrouping

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4. Effects of Physical Enrichment and Pair Housing before Weaning on Growth, Behaviour and Cognitive Ability of Calves after Weaning and Regrouping

Statement

I confirm that this study was conducted for my dissertation research. My contributions included formulating hypotheses for the experiments, planning and conducting the experiments, compiling the data, conducting the statistical analysis, and writing this published manuscript. All authors approved the final version prior to submission to Applied Animal Behaviour Science.

Abstract

Housing unweaned calves individually in barren environments negatively affects their growth, cognitive ability, and adaptability to environmental changes in later life. Social housing has been shown to improve those aspects, whereas physical environmental enrichment has rarely been studied in calves. Little is known about whether the combination of both components offers further benefits. Furthermore, curiosity has been considered an intrinsic factor underpinning cognitive performance, which has yet to be determined in calves. The first objective was to compare the effects of providing physical enrichment items and pair housing calves before weaning, and their combination on the weight gain, behaviour and cognitive ability of calves once regrouped after weaning. The second objective was to investigate if calves' exploratory behaviour in a novel environment can predict their cognitive ability. Forty-eight Holstein calves were allocated to eight groups 2 days after birth. Within each group, two calves were assigned to individual pens and four to two pair pens. One individual pen and one pair pen within each group were provided with brushes, chains, teats, and nets filled with strawberry-scented hay as physical enrichment items. Remaining pens received no additional enrichment items. All calves from a group were introduced to one post-weaning pen when the youngest calf was 9 weeks of age. Calves were weighed on days 1 and 7 in post-weaning pens. They were video recorded on days 1, 3 and 11 and behavioural data were collected. Spontaneous object recognition tests were conducted within one week after behavioural data collection to assess cognitive ability in terms of how long after exposure calves recognised objects, indicated by differential expression of exploratory behaviour. Physical enrichment items and pair housing had interactions on average daily gain ($F_{1,33} = 5.460$, $p = 0.026$), with calves in physically enriched pair pens showing higher average daily gain than those in non-enriched pair pens and tending to show higher average daily gains than those in physically enriched individual pens. Physically enriched calves expressed more exploratory behaviour and social sniffing than non-enriched calves ($F_{1,111} = 20.691$, $p < 0.001$; $F_{1,111} = 14.433$, $p < 0.001$). Pair housed calves spent more time cross-sucking than individually housed calves ($F_{1,111} = 8.848$, $p = 0.008$). Compared with non-enriched calves, physically enriched calves were more inclined to explore the novel object than the object already presented 15-min ago ($\chi^2 = 3.282$, df = 1, $p = 0.070$). There was no association between exploratory behaviour upon initial introduction to post-weaning pens and performance in object recognition tests. In conclusion, the combination of physical enrichment and pair housing improves calves' average daily gain after weaning when compared with either component alone. Physical enrichment seemed to improve calves' memory and adaptability to change, whilst pair housing did not. Calves' exploratory behaviour in novel environments may not contribute to their cognitive performance.

Keywords: pre-weaning environmental enrichment, post-weaning regrouping, average daily gain, behaviour, cognitive ability, curiosity

4.1 Introduction

It is a common practice on dairy farms to separate newborn calves from their dams immediately or within hours of parturition and rear them in individual pens or hutches (Mikuš et al., 2020). Thereafter, calves are weaned and moved to group pens, which must happen no later than eight weeks of age in the European Union and the United Kingdom (Council Directive 97/2/EC). At this stage, calves experience diet changes and new social and physical environments (Bolt et al., 2017), all of which are considered stressors, compromising their performance and welfare; signs of this include a growth check (Chua et al., 2002; Vieira et al., 2010), increased undesirable social behaviours (Kerr and Wood-Gush, 1987) and increased distress responses (Weary et al., 2008).

Based on the reality of periodic changes in management and environments including those at regrouping after weaning, calves need to learn how to respond to and utilise complex environments (Horvath, 2019). Since calves with better cognitive abilities are able to show more flexible behaviour (Gaillard et al., 2014) and increased behavioural flexibility can help calves better adapt to environmental changes (Horvath, 2019), calves' adaptive capacity may depend on their cognitive ability. Social housing in the pre-weaning period has been widely studied as a means to improve calves' capacity to adapt to environmental changes since this may be a sensitive period in brain development and can impact later behavioural flexibility (Meagher et al., 2015), which measures an individual's ability to adjust their behaviour in response to environmental cues (Coppens et al., 2010). Calves who are socially housed before weaning show higher tolerance to unfamiliar animals later in life with less aggressive interactions but more non-agonistic interactions (Veissier et al., 1994). The feed intake behaviours in post-weaning home pens including latency to start feeding and time spent at a feeder, concentrate consumptions and weight gains are all improved by pre-weaning social housing (Vieira et al., 2010).

Physical enrichment is similarly suggested to alter social skills and abilities to cope with stressors (Weary et al., 2008). Studies in piglets have shown that physical enrichment improved piglets' performance in a cognitive ability test (Grimberg-Henrici et al., 2016), increased feed consumption in the first two days in post-weaning pens (Oostindjer et al., 2010) and might reduce aggression in post-weaning pens (Kutzer et al., 2009). However, in calves, the effect of pre-weaning physical enrichment on their ability to adapt to weaning and regrouping has not yet been explored. Since physical enrichment may enable pre-weaning calves to garner more experience dealing with external stimuli, it is expected to positively impact calves' adaptive capacity in later life.

In addition, research on the combined effect of social housing and physical enrichment on animals' adaptive capacity to environmental changes is growing. For example, physical enrichment and social housing mitigated piglets' weaning stress and reduced their post-weaning aggression (Ko et al., 2020). In calves, although combined methods have not been explored, it is expected that the combination of social housing and physical enrichment may further improve their cognitive ability and adaptability to environmental changes, since both components may stimulate calves in different ways (Mandel et al., 2016).

The effect of different emotions on cognitive functioning is an important research area in animals, which may contribute to understanding the mechanisms underlying individual variation in cognitive performance (Broom, 2010). Barren environments may lead to prolonged high levels of stress hormones (e.g. glucocorticoids) in livestock, which can affect neurons within the hippocampus (Lupien et al., 1998). For this reason, researchers have mainly focused on the impact of negative emotions induced by poor environmental conditions on cognitive ability (e.g. dairy calves: Gaillard et al., 2014; broiler chickens: Tahamtani et al., 2018). However, the positive emotion of curiosity is also considered an intrinsic factor underpinning cognitive performance (Kidd and Hayden, 2015). In orang-utans, exploratory behaviour is assessed as the indicator of curiosity to predict their problem-solving abilities (Damerius et al., 2017). In young horses, exploratory behaviour towards novel objects correlates with increased learning capability (Christensen et al., 2021). However, in calves, whether curiosity levels are correlated with cognitive ability is still unknown.

The first aim of the present study was to determine the effects of providing physical enrichment items to pre-weaning calf pens, pair housing pre-weaning calves and the combination of both components on post-weaning calves' behaviour, growth and cognitive ability. Since physical enrichment and social housing might improve calves' emotional states and cognition as well as promoting positive activities by providing different types of stimulation, it was hypothesised that 1) providing physical enrichment items and pair housing would both promote positive behaviours, such as exploratory, play, ruminating and social sniffing behaviours, and reduce undesirable behaviours, such as cross-sucking and agonistic behaviours, and also promote weight gain and performance in cognitive ability tests; 2) the combination of physical enrichment items and pair housing would have an additive effect when compared to either component alone. The second aim was to investigate associations between calves' exploratory behaviour in a novel environment and their cognitive ability. Since curiosity might underpin cognition, it was hypothesised that calves that showed more exploratory behaviour upon initial introduction to post-weaning home pens would have better performance in a cognitive ability test.

4.2 Materials and Methods

4.2.1 Animals, Housing and Feeding

The study was carried out at the Centre for Dairy Research, University of Reading, Reading, UK between May and November 2019 and was approved by the ethics administrator at the University and the departmental ethics coordinator. Forty-eight male registered pure Holstein calves were included from 2 days of age to 12 weeks of age. They had birth weights of 35-55 kg and were separated from dams between 24 and 36 h after birth. An additional eight male registered pure Holstein calves were used for a pilot study (for details, please see 2.4.2) and were housed in standard non-enriched group pens.

For the forty-eight calves in the main study, in the first eight weeks following birth, they were reared in one of four treatments: non-enriched individual housing (n = 8 calves), physically enriched individual housing (n = 8 calves), non-enriched pair housing (n = 16 calves) and physically enriched pair housing (n = 16 calves). Calves were allocated to pre-weaning treatments in blocks according to their date of birth such that each housing type was represented within each of eight groups (i.e. the six calves born first were assigned to the first group, the next six calves born were assigned to the second group, etc). Within each group, this meant that two calves were assigned into individual pens (2.4 m^2 each) and four calves were assigned into two pair pens (4.8 m^2 each). One individual pen and one pair pen within each group were provided with physical enrichment items: one net filled with strawberry-scented ryegrass hay, one rubber teat, one plastic chain, and one stationary brush for individual pens; one net filled with strawberry-scented ryegrass hay and two of all other items for pair pens to reduce competition for items (see Zhang et al., 2021 for more details). The aim of these items was to satisfy foraging, sucking, and grooming motivations of young calves (see Mandel et al., 2016). The rest of the individual and pair pens were not provided with the physical enrichment items. All pens were bedded with deep straw. All calves were offered milk replacer twice daily at 07:00 h and 15:00 h using teat buckets until 49 days of age. A total of 5 L/d of milk was offered to each calf until 14 days of age, followed by 6 L/d from 15 to 42 days of age and 5 L/d between 43 and 49 days of age. From 50–56 days of age, calves received 2.5 L milk at 07:00 h only. Thereafter, calves were weaned at 57 days of age. In the pre-weaning and weaning period, all calves had ad libitum access to concentrate (VITA concentrate, ForFarmers, Lochem, the Netherlands), ryegrass hay and water. Calves had auditory and visual contact with one another and limited tactile contact with neighbours through the gap between the panel bars (120 mm, large enough for calves' muzzles to go through the gap).

Calves within each pre-weaning group were introduced to eight post-weaning home pens (six calves in each) together following the weaning of the youngest calf in the group and were monitored for four weeks. The area of each post-weaning home pen is reported in Table1. The lying area was bedded with deep straw and the feeding area had a concrete floor. Calves had ad libitum access to total mixed ration (TMR; grass silage, maize silage and concentrate) and water throughout the period.

4.2.2 Growth

All calves were weighed on days 1 and 7 in post-weaning home pens by a wheeled scale (Ritchie Agricultural, UK). Average daily gain was calculated by averaging across the 6 days.

4.2.3 Behaviours in Post-weaning Home Pens

Calves' behaviours in post-weaning home pens were recorded by CCTV (Transit-PTZ, Revader Security Ltd, UK) for 24 h on days 1, 3 and 11 after initial introduction to post-weaning home pens (09:00 h ± 0.5 h). Behaviours, being considered as indicators of adaptive capacity, defined in Table 2 (adapted from Zhang et al., 2021), were recorded using instantaneous scans at 5-min intervals by watching video recordings. The behavioural frequencies in the time periods of 00:00-06:00 h and 20:00-24:00 h were not recorded since calves were less active during these periods.

4.2.4 Cognitive Ability Test

The spontaneous object recognition test was used to assess memory alterations by measuring the difference in exploration time of novel and familiar objects (Antunes and Biala, 2012). The test consisted of observing animals in the presence of two sample objects (sample phase) and the observations were repeated after a certain retention time with one of the sample objects replaced by a novel one (test phase). Retention time was defined as the interval between the sample phase and the test phase of the object recognition test; for example, 15 min retention time meant 15 min between the two phases for the calf being tested. The preference for novel object in test phase indicated that re-presentation of sample object existed in animal's memory since they have natural propensity to novelty (Baxter, 2010; Ennaceur, 2010).

Experimental Setup

In this experiment, exploratory behaviour included sniffing, sucking, licking, scratching and butting the objects. A square testing arena (16 m^2) was setup with the ground being covered by straw. The arena was equipped with a webcam (C525, Logitech International S.A, Switzerland) to record calves' behaviour. Test objects were placed on the opposite panels of the entrance. The left and right objects were both 50 cm away from the left and right corner and were 90 cm away from the ground. Two sets of objects were used in this experiment, with the first set of three green feeders (26 cm × 26 cm × 19 cm; GN1, GN2, GN3) and one blue bottle (12 cm × 12 cm × 28 cm; BE1) and the second set of three grey feeders (35 cm × 12 cm × 10 cm; GY1, GY2, GY3) and one brown pipe (11 cm × 11 cm × 38 cm; BN1). The testing arena and the objects were cleaned between phases to minimise olfactory traces.

Experimental Spatial and Object Bias

A pilot study was conducted on four consecutive days before the cognitive ability test to assess spatial bias in the arena and bias towards the two sets of objects. Eight male registered pure Holstein calves reared in group pens with birth weight between 35 and 55 kg were used when they were 10–12 weeks of age. All calves were individually handled to habituate to the empty arena for 20-min on day 1, followed by individually exploring two identical yellow brushes (11 cm × 13 cm × 32 cm) in the arena for 15-min after 5-min habituation on day 2. On days 3 and 4, calves were handled to individually explore the first set of objects (GN1 vs. BE1) and the second set of objects (GY1 vs. BN1) for 15-min separately after 5-min habituation. The differences of the ratio of exploratory duration towards the left and right yellow brushes, towards the two different objects in the first and second set were tested separately by conducting pair-samples t-tests. The results showed that calves did not have spatial bias ($t = -1.20$, $p = 0.270$) and bias towards the two sets of objects (set one: $t = 0.39$, $p = 0.708$; set two: $t = 0.98$, $p = 0.941$).

Experimental Procedures

Each calf was tested for 15-min and 60-min retention times on two consecutive testing days. Calves attended the test within one week after the recording of post-weaning home pen behaviours and took turns to be tested on same testing days.

Each calf was individually handled to habituate to the empty testing arena for 20-min one day before testing. On the first testing day, the following pattern was used: a testing calf habituated to the empty testing arena for 5-min. Then, in the sample phase, the calf was removed and two identical objects (GN1 and GN2 or GY1 and GY2; used for alternate calves at alternate retention time) were placed on the panels, followed by letting the calf back to the testing arena for 15-min to explore both objects. Thereafter, the calf was transported back to its post-weaning home pen. The test phase was initiated after 15-min retention time. Following habituation to the empty testing arena for 5-min (these 5-min were included in the total retention time) a novel object and a familiar object in the same set used in the sample phase (BE1 and GN3 or BN1 and GY3) was placed in the testing arena and the calf was allowed 15-min to explore both objects. For each set of objects, the novel object and familiar object were separately placed on the left and right side of the panel in one test and changed side in the next test. After the test phase, the calf was transported back to its post-weaning home pen. The procedure was repeated on the second testing day with 60-min retention time.

Eight calves' videos in the sample phase were randomly chosen and watched to measure the difference of the ratio of exploratory duration towards the left and right objects to ensure there was no spatial bias to the testing arena by conducting pair-samples t-tests. The results showed that calves did not have spatial bias ($t = -0.924$, $p = 0.386$). In the test phase, we determined discrimination index [= $(T_N - T_F) / (T_N + T_F)$] as the difference between the exploratory duration of the novel object (T_N) and the familiar object (T_F) divided by the sum of exploratory duration of the novel and familiar objects (Ennaceur and Delacour, 1988). A greater value of discrimination index indicated a better object recognition memory ability.

4.2.5 Associations between Exploratory Behaviour and Cognitive Performance

Calves' behavioural videos (recorded as described in Section 2.3.) were observed from initial introduction to post-weaning home pens until 20:00 h on that day and frequencies of exploratory behaviour (defined in Table 2) were recorded using instantaneous scans at 5-min intervals. The ratio of exploratory behaviour for every calf was expressed as the frequency of a calf's exploratory behaviour from initial introduction to post-weaning home pens until 20:00 h divided by the frequency of all behaviours observed during this period. Thereafter, the associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens, and discrimination indices for 15-min and 60-min retention times in cognitive ability test were measured.

4.2.6 Statistical Analyses

Data were analysed using SPSS Statistics (version 27.0.1.0, IBM) with individual calf as statistical unit. Significant differences were declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.10$.

Calves' average daily gains were analysed by a univariate general linear model, incorporating the fixed factors of physical enrichment items, pair housing and the interaction between these two factors and the covariates of calves' birth weight and days in pre-weaning pens after weaning. A post hoc test (LSD) was carried out thereafter to identify differences among treatment means.

Calves' behavioural variables in post-weaning home pens were collected by one observer and were expressed as proportions of total scans. All variables except play were analysed by generalised linear mixed models respectively. Play was not analysed because it was rarely expressed. For the data structure, the subjects were post-weaning home pen number and calves' ID number; the repeated measure was days in post-weaning home pens. The fixed effects were physical enrichment items, pair housing and the interaction between the two factors, post-weaning home pen number, days in post-weaning home pens, calves' birth weight and average temperature of the barn during the testing day. The random effects were calves' ID number and area of post-weaning home pens. LSD was used to undertake pairwise comparisons. Thereafter, to reduce the risk of chance significant results due to multiple testing, adjusted p-values were calculated to control the false discovery rate (Jafari and Ansari-Pour, 2019).

Calves' behavioural variables in cognitive ability test were collected by one observer. In the test phase, the variables of discrimination indices for 15-min and 60-min retention times were analysed by generalised linear models, incorporating the factors of physical enrichment items, pair housing, the interaction between these two factors, object set and location of the testing arena, and calves' birth weight and average temperature of the barn during the testing day were used as covariates in the model. Before conducting generalised linear models, calves that did not show exploratory behaviour towards novel and familiar objects in the test phase (seven calves for 15-min retention time and nine calves for 60-min retention time) were discarded from the analysis because those calves might not have learnt how to recognise the objects or they might not be motivated to explore the objects.

Associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens and discrimination indices for 15-min and 60-min retention times in the object recognition test were analysed by linear regressions, incorporating a dependent variable of discrimination indices for 15-min or 60-min retention time and independent variables of physical enrichment items, pair housing, the interaction between these two factors and the ratio of exploratory behaviour upon initial introduction to post-weaning home pens. Calves that were discarded from the analysis of cognitive ability test data were also discarded from this analysis.

To determine inter-observer reliability, another observer watched the post-weaning home pen behaviour videos of three pens by randomly choosing one pen from days 1, 3 and 11 separately. For the cognitive ability test, eight calves' test phase videos for 15-min retention time and eight calves' test phase videos for 60-min retention time were randomly selected and watched by another observer who was blind to the pre-weaning treatments. Pearson correlations were used to compare the reliability between the two observers. The results showed strong positive relationships between both observers for post-weaning home pen behaviour ($r = 0.994$, $p < 0.001$) and cognitive ability test ($r = 0.996$, $p < 0.001$).

One calf's data for all measures were discarded due to an abscess on its tongue. Another calf's data for average daily gain and behaviours in its post-weaning home pen were discarded due to diarrhoea. Owing to technical problems, the data of eight calves' post-weaning home pen behaviours on days 1 and 3 were discarded.

4.3 Results

4.3.1 Growth

Physical enrichment items and pair housing had interactions on average daily gain ($F_{1,33} = 5.433$, $p = 0.026$; Figure 1), with calves from physically enriched pair pens showing increased average daily gain compared to those from non-enriched pair pens ($p = 0.009$) and tending to show increased average daily gain than those from physically enriched individual pens ($p = 0.093$).

4.3.2 Behaviours in Post-weaning Home Pens

Physically enriched calves expressed more exploratory behaviour and social sniffing than non-enriched calves ($F_{1,111} = 20.691$, adjusted $p < 0.001$; $F_{1,111} = 14.433$, adjusted $p < 0.001$; Table 3). Pair housed calves showed increased time spent lying next to familiar calves than individually housed calves ($F_{1,111} = 8.812$, adjusted $p = 0.032$). Cross-sucking behaviour was more frequent in pair housed calves than in individually housed calves ($F_{1,111} = 8.848$, adjusted $p = 0.008$). There were no interactions between physical enrichment items and pair housing with respect to the incidence of natural and undesirable behaviours.

4.3.3 Cognitive Ability Test

For the 15-min retention time, physically enriched calves tended to show higher discrimination index compared to non-enriched calves ($\chi^2 = 3.282$, df = 1, $p = 0.070$, Table 4). Individually and pair housed calves showed similar discrimination indices ($\chi^2 = 0.060$, df = 1, $p = 0.806$). There were no interactions between physical enrichment items and pair housing with respect to discrimination index ($\chi^2 = 0.837$, df = 1, $p = 0.360$).

For the 60-min retention time, non-physically and physically enriched calves showed similar discrimination indices ($\chi^2 = 1.242$, df = 1, p = 0.265). Individually and pair housed calves showed similar discrimination indices (χ^2 = 1.130, df = 1, p = 0.288). There were no interactions between physical enrichment items and pair housing with respect to discrimination index (χ^2 = 0.706, df = 1, p = 0.401).

4.3.4 Associations between Exploratory Behaviour and Cognitive Performance

There were no associations between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens, and discrimination index for 15-min retention time in cognitive ability test (β coefficient ± SE: 0.092 ± 3.875; t = 0.237, p = 0.814; n = 40) and 60-min retention time in cognitive ability test (β coefficient ± SE: -2.894 ± 3.615; t = -0.801, p = 0.430; n = 38).

4.4 Discussion

Although this study could be considered as comparing enrichment types, we have not used the term ‘enrichment’ here for social housing. Environmental enrichment is a vague term in the way it is often applied in the field of applied ethology. The term implies improvements of the initial environment. In calves, environmental enrichment is intended to improve their biological functioning, fulfil behavioural requirements, help cope with stressors, reduce frustration, and promote positive emotions (Mandel et al., 2016). Although the initial environment varies between studies, since individual housing is widely used in the dairy industry, many researchers treat this as the baseline and suggest that social housing should be considered a type of environmental enrichment (e.g. Bloomsmith et al., 1991; Mandel et al., 2016). In contrast, others do not categorize social housing as a form of environmental enrichment (e.g. Costa et al., 2016) since calves are gregarious animals, housing them in groups is practice that satisfies their basic needs. In addition, the benefits of group housing have been gradually accepted by an increasing number of farmers, and housing pre-weaning calves in groups is becoming more popular in a number of countries. Therefore, as the dairy industry develops, we would argue that social housing should not be considered a type of environmental enrichment but rather a minimum standard of calves' early environment.

4.4.1 Growth

The combination of physical enrichment items and pair housing improved or tended to improve calves' average daily gain after weaning and regrouping when compared with either component alone. Although in the present study frequencies of TMR intake and rumination did not show statistical differences between treatments, the mean values for calves from physically enriched pair pens were numerically higher than those from the other treatments. Therefore, the combination of physical enrichment items and pair housing may reduce stress responses towards mixing in novel environments (Kutzer et al., 2009), thus increasing feed consumption and rumination resulting in improved weight gain. In contrast, neither providing physical enrichment items nor pair housing to pre-weaning calves affected their weight gain after weaning and regrouping. This is in agreement with studies in pigs (Oostindjer et al., 2010) and in calves (Duve and Jensen, 2012). These results may be attributed to the tremendous amount of stress that animals are subjected to during environmental change. The sudden transformation to novel environments with new feeds and mixing with unfamiliar peers may result in considerable stress for calves (Hulbert and Moisá, 2016), especially for those without experiencing both social and external stimuli before and thus, this overshadows the differences arising in their pre-weaning period.

4.4.2 Behaviours in Post-weaning Home Pens

Exploration is a process of information gathering for animals (Rojas-Ferrer et al., 2020), which may help animals to better control or predict new environments (Wood-Gush and Vestergaard, 1993). In the present study, the expression of exploratory behaviour in post-weaning home pens was promoted by pre-weaning physical enrichment. Since in pre-weaning pens, the provision of additional items may attract calves' attention to explore them (Zobel et al., 2017), the high exploratory motivation of physically enriched pre-weaning calves may persist in post-weaning group pens. The increased expression of exploratory behaviour towards peers (social sniffing) in the same post-weaning home pens for calves with pre-weaning physical enrichment may also corroborate this view. By contrast, pre-weaning pair housing had no effect on calves' exploratory behaviour towards post-weaning home pens and peers in the same pens. This is in contrast with the finding of Jensen et al. (1997), which indicated the lack of social housing early in life could delay exploratory behaviour. The difference may be because in the present study individually housed pre-weaning calves could have olfactory, visual and limited tactile interactions with neighbours in pair pens through the bars of panels and thus, they might have acquired some social experience like that of pair housed calves.

Social lying reflects the focal animal’s choice of having a social partner during rest (Duve and Jensen, 2012), which may indicate high social tolerance to peers in the same pens (Estevez et al., 2007). The timing of recording calves’ behaviours in their post-weaning home pens in the present study was selected to end on 11 after initial introduction to post-weaning home pens because new social relationships can be well established and other activities normally return to basic levels after this time (Bøe and Færevik, 2003; Færevik et al., 2007). Although pre-weaning pair housing increased the expression of social lying in post-weaning home pens towards the previously familiar calves throughout this period in the present study, this does not mean they have better tolerance. Since calves reared in the same pre-weaning social pens can establish strong bonds with each other (Raussi et al., 2010), the higher expression of social lying in post-weaning home pens towards previously familiar calves may indicate they are maintaining their strong relationships and increasing safety in novel environments (Grignard et al., 2000). Færevik et al. (2007) also found that introducing calves with their companions to a new group, they rested more with familiar companions in the first three days after grouping. Since in the present study pre-weaning pair housing and pre-weaning physical enrichment items did not affect social lying with unfamiliar calves in post-weaning home pens, both methods may not have effects on calves’ social capacity after weaning and regrouping.

Cross-sucking, as an undesirable behaviour in calves, may result in hair loss, inflammation and disease in receivers (Jensen, 2003). Since the present study illustrated that pre-weaning pair housing increased the expression of cross-sucking in post-weaning home pens, the higher expression of the undesirable behaviour may reflect frustration in the performing calf (Costa et al., 2016), and thus may indicate poor adaptability of calves with pre-weaning social experience to the weaning situation. In contrast, pre-weaning physical enrichment had no effect on the expression of cross-sucking in post-weaning home pens. Although providing physical enrichment items such as dry teats may redirect calves' cross-sucking motivation to the items and reduce their expression of the non-nutritive oral behaviour in the pre-weaning period (e.g. Newberry, 1995), yet the items obviously do not have a long-term impact on reducing cross-sucking. Appropriate physical enrichment items may be needed for post-weaning calves to redirect their cross-sucking motivation.

Agonistic behaviour usually refers to the negative side of social interactions in animals (Chaloupková et al., 2007), which may result in a tremendous cost to economic efficiency and animal welfare owing to stress and injury (Fraser and Rushen, 1987). In the present study, both pre-weaning pair housing and physical enrichment had no effect on the agonistic behaviour of post-weaning calves. Since calves from the different pre-weaning treatments rarely expressed agonistic behaviour in post-weaning home pens, it may indicate that cattle at a young age have limited motivation for resource monopolisation (Davies and Houston, 1984) and aggression (Veissier et al., 2001).

4.4.3 Cognitive Ability Test

In the present study, physically enriched calves tended to be better at discriminating familiar and novel objects than non-enriched calves for the 15-min retention time suggesting that physical enrichment might improve calves' object recognition memory ability. Pair housing had no effect on the discrimination indices for the 15-min and 60-min retention times indicating that pair housing might not improve calves' object recognition memory. The results of effect of physical enrichment on calves' memory ability in the current study agree with Martin et al. (2015) in piglets. However, the findings of pair housing not affecting calves' memory ability in the current study are in contrast to those reported by Gaillard et al. (2014). They demonstrated that pair housed calves showed reduced exploration in repeated object recognition test, but individually housed calves did not, which indicated that only pair housed calves learned to recognize the recurring object. The differences in the results of these studies may be attributed to the different housing designs. In the present study, individually housed pre-weaning calves had limited physical contact with neighbours, whilst in the Gaillard et al. (2014) individually housed calves did not have any physical contact with neighbours. Since limited physical contact between calves stimulates the expression of social behaviours and reduces their fear of novel situations (Jensen and Larsen, 2014), it is reasonable to deduce that individually housed calves with limited physical contact with neighbours may have acquired some social experience and improved their cognitive ability. Another potential explanation is that post-weaning group housing may reverse the deficits of brain development caused by pre-weaning individual housing. This stems from Bredy et al. (2003) who determined that the negative effects of low maternal care on rats' cognitive ability could be reversed by post-weaning environmental enrichment. Since calves can establish new social bonds with unfamiliar calves within 2 weeks after regrouping (Færevik et al., 2007) and may improve their learning ability within the short period (Lensink et al., 2006), calves may have become familiar with each other and may have improved cognitive ability before attending the cognitive ability test in the third or fourth week after regrouping.

4.4.4 Associations between Exploratory Behaviour and Cognitive Performance

In the present study, calves' exploratory behaviour after the initial introduction to post-weaning home pens might be considered as an indicator of curiosity. Curiosity refers to the motivation of information-seeking and is reflected in approaching and exploring novel stimuli (Damerius et al., 2017). The behaviour was only recorded from initial introduction to post-weaning home pens until 20:00 h that day because although animals have curiosity to novel information, it can be diminished when satiation occurs by continuing exposure (Kidd and Hayden, 2015). In the present study, there was no linear association between the ratio of exploratory behaviour upon initial introduction to post-weaning home pens and discrimination indices for 15-min and 60-min retention times in the cognitive ability test. Conversely, in horses, positive associations between exploratory behaviour towards novel objects and learning performance on the tasks of visual discrimination and pressure-release have, however, been reported (Christensen et al., 2021). One of the potential reasons for the different results is the different measures of curiosity. In Christensen et al. (2021), the testing duration of exploratory behaviour towards novelty for each animal was several minutes whereas in the present study the testing duration for each calf spanned several hours. Since exploratory motivation decreases over time as focal animals progressively habituate to the novelty (e.g. Van De Weerd and Day, 2009), the measure of curiosity in the present study may be less sensitive and not reflect calves' initial curiosity levels towards the novel environments. Moreover, the present study investigated the association between animal's exploratory behaviour and their object recognition memory, but Christensen et al. (2021) investigated the correlation between animal's exploratory behaviour and cognitive flexibility. Since cognitive flexibility is expressed as the ability to change behaviours according to the changes of environmental conditions (Nilsson et al., 2015), its fundamental process may include two executive functions, memory and inhibition, which enable individuals to adaptively control their thought and action (Buttelmann and Karbach, 2017). Therefore, it is reasonable to infer that compared with simple memory, flexibility in rule learning is a more complex form of learning. To understand the mechanisms underlying calves' cognitive performance, how exploratory behaviour affects their performance of tasks requiring cognitive flexibility needs to be further studied.

4.5 Conclusion

Pre-weaning physical enrichment may improve calves' memory and stimulate their exploration of new environments after weaning and regrouping. Pre-weaning pair housing, meanwhile, increased calves' sucking behaviour towards peers after weaning and regrouping. The combination of physical enrichment and social housing during pre-weaning period improved calves' growth after weaning and regrouping compared to either of these alone. Calves' exploratory behaviour in novel environments may not contribute to their cognitive performance, but this needs further confirmation by studies of associations between exploratory behaviour and more complex cognitive tasks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Area sizes (m^2) of eight post-weaning home pens

Sector	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7	Pen 8
Lying area	67.2	67.2	67.2	67.2	42.7	24.5	24.5	24.5
Feeding area	28.8	28.8	28.8	28.8	18.3	28.5	10.5	10.5
Whole area	96.0	96.0	96.0	96.0	61.0	53.0	35.0	35.0

Table 2. Ethogram of behaviours in post-weaning home pens (adapted from Zhang et al., 2021)

Behaviour	Definition
TMR intake	Heading through the feed barrier and chewing
Ruminating	Chewing without TMR and straw
Exploring	Sniffing, licking or sucking ground or any fixture in the pen
Play	Engaging in a gallop, leap, jump, buck, kick or turn, putting the forelegs on other calves' back or rubbing the forehead against other calves' forehead without pushing (Veissier et al., 1994; Jensen et al., 1998)
Fixture scratching	Putting head, neck or body in contact with any fixture in the pen and slightly moving back and forth or up and down
Social sniffing	Putting muzzle in contact with or less than one muzzle length from other calves with neck not relaxed
Allogrooming	Putting tongue out of mouth and in contact with head, neck or body of other calves
Lying next to familiar calves	Lying down with the head's distance to any lying calves who were companions or neighbours in pre-weaning pens being less than 30 cm (Færevik et al., 2007)
Lying next to unfamiliar calves	Lying down with the head's distance to any lying calves who were not companions and neighbours in pre-weaning pens being less than 30 cm (Færevik et al., 2007)

Cross-sucking	Sucking or biting toward ear, mouth, navel, scrotum, prepuce, or other body parts of other calves
Agonistic behaviour	Pushing, butting or chasing other calves, or displacing other calves from their feeding places or lying places (Færevik et al., 2007)
Other behaviours	Such as lying alone, standing, walking and drinking water

Table 3. Variables in post-weaning home pens (mean ± SE) analysed using generalised linear mixed models. Sample sizes were pre-weaning physical enrichment PE, n = 22 calves; pre-weaning non-physical enrichment NPE, n = 24 calves; pre-weaning pair housing PP, n = 31 calves and pre-weaning individual housing IP, n = 15 calves

Variables	Mean ± SE		Adjusted p-value[a]	Mean ± SE		Adjusted p-value[a]
	PE	NPE	PE vs. NPE	PP	IP	PP vs. IP
TMR intake (%[b])	21.3 ± 1.8	21.9 ± 1.7	0.775	22.2 ± 1.6	21.0 ± 1.8	1.104
Ruminating (%[b])	20.9 ± 1.4	18.9 ± 1.4	0.429	19.5 ± 1.3	20.3 ± 1.5	0.817
Exploring (%[b])	7.5 ± 0.3	5.5 ± 0.3	<0.001*	6.9 ± 0.3	6.1 ± 0.4	0.372
Social sniffing (%[b])	1.3 ± 0.2	0.6 ± 0.2	<0.001*	0.9 ± 0.1	1.0 ± 0.2	0.931
Fixture scratching (%[b])	0.2 ± 0.1	0.3 ± 0.1	0.691	0.2 ± 0.1	0.2 ± 0.1	1.152
Allogrooming (%[b])	0.3 ± 0.1	0.1 ± 0.1	0.414	0.2 ± 0.1	0.2 ± 0.1	0.991
Lying next to familiar calves (%[b])	10.5 ± 1.3	9.3 ± 1.3	0.632	12.0 ± 1.1	7.8 ± 1.4	0.032*
Lying next to unfamiliar calves (%[b])	6.0 ± 1.9	6.3 ± 1.9	0.854	6.6 ± 1.8	5.8 ± 2.0	0.947
Cross-sucking (%[b])	1.4 ± 0.5	1.4 ± 0.5	0.839	1.8 ± 0.4	1.0 ± 0.5	0.008*

Agonistic behaviour (%[b])	0.1 ± 0.1	0.1 ± 0.1	1.302	0.1 ± 0.1	0.1 ± 0.1	0.783

[a]Adjusted p-values were calculated using false discovery rate (FDR).

[b]% indicated percentage of observations.

*Indicated a significant difference.

Table 4. Discrimination indices (mean ± SE) of post-weaning calves for 15-min and 60-min retention times analysed using generalised linear mixed models. Sample sizes for 15-min retention time were pre-weaning physical enrichment PE, n = 21 calves; pre-weaning non-physical enrichment NPE, n = 19 calves; pre-weaning pair housing PP, n = 27 calves and pre-weaning individual housing IP, n = 13 calves; sample sizes for 60-min retention time were pre-weaning physical enrichment PE, n = 19 calves; pre-weaning non-physical enrichment NPE, n = 19 calves; pre-weaning pair housing PP, n = 25 calves and pre-weaning individual housing IP, n = 13 calves

Variables	Mean ± SE		p-value	Mean ± SE		p-value
	PE	NPE	PE vs. NPE	PP	IP	PP vs. IP
15-min	0.191 ± 0.189	-0.190 ± 0.206	0.070	0.026 ± 0.172	-0.025 ± 0.220	0.806
60-min	0.282 ± 0.145	0.052 ± 0.142	0.265	0.276 ± 0.122	0.059 ± 0.160	0.288

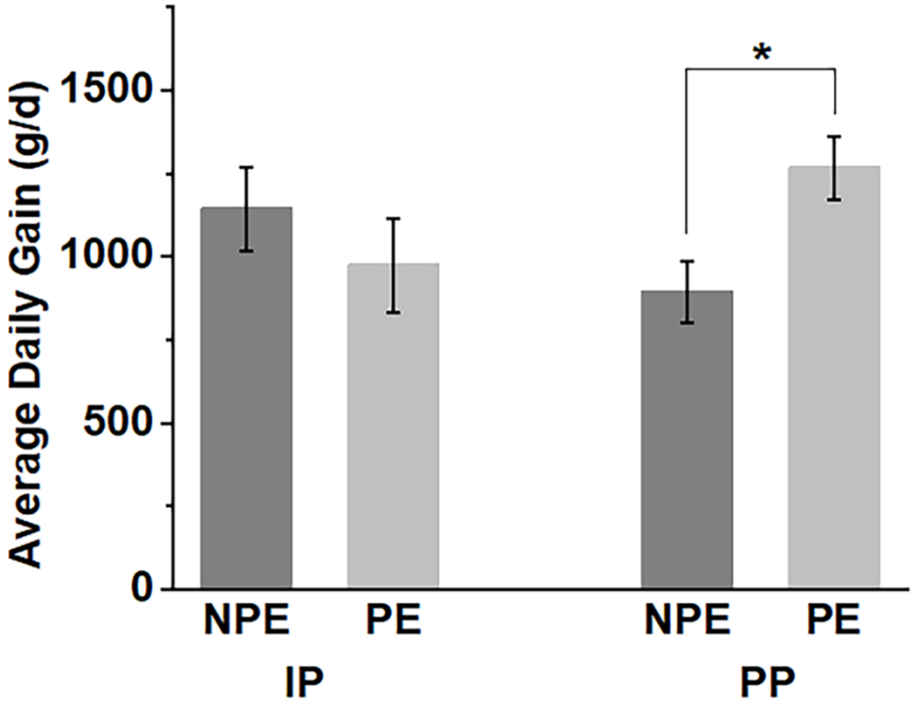

Figure 1. Means (± SE) of average daily gain measured on days 1 and 7 in post-weaning home pens for calves from pre-weaning non-enriched individual pens (NPE-IP; n = 8 calves), pre-weaning physically enriched individual pens (PE-IP; n = 7 calves), pre-weaning non-enriched pair pens (NPE-PP; n = 16 calves) and pre-weaning physically enriched pair pens (PE-PP; n = 15 calves). Asterisk (*) indicated a significant difference.

Chapter 5

Are Laterality Effects Present in Novel Object Responses of Calves?

5. Are Laterality Effects Present in Novel Object Responses of Calves?

Abstract

Many animals prefer to view fear-inducing stimuli with their left eyes owing to cerebral lateralisation in processing this emotion. Stimuli presented on the left visual field may also elicit greater responses. In novel object tests, objects are often presented in standardised spatial positions but positioning relative to the animal can vary. The first aim of the present study was to assess the test-retest reliability of calves' responses in novel object tests. The second aim was to determine if calves between 6 and 7 weeks of age had visual lateralization (cerebral lateralization) in processing fear and whether such lateralization could then be considered an effective indicator of fear. The third aim was to determine if calves' initial monocular presentation towards fear-inducing stimuli would affect calves' fear responses. To achieve these aims, two novel object tests were conducted, with the objects of a white bucket or a traffic cone. Thirty-six calves were tested when they were 6 or 7 weeks of age. Calves' behavioural responses including latency to make first contact with the novel objects and duration to view the novel objects with the left and right eyes were recorded along with first view towards the novel objects with the left, right or both eyes. There was no correlation between the first novel object test and the repeated novel object test for calves' latency to make first contact with the novel objects (r_S = -0.148, p = 0.403). No statistically significant differences were found in the proportion of time viewing the novel objects between the left and right eyes ($F_{1,99}$ = 0.025, p = 0.876).

There was no correlation between calves' proportion of time viewing the novel objects with the left eye and calves' latency to make first contact with the novel objects (r_s = -0.093, p = 0.503). The probability of contact with the novel objects did not differ depending on which eye the calf could view them with first (70.80 % with the left eye vs. 71.40 % with the right; Chi-squared p = 0.965). There were no statistically significant differences in the latency to make first contact with novel objects among calves' first view towards novel objects with left, right and both eyes ($F_{2,16}$ = 0.070, p = 0.933). To sum up, calves' fear response was inconsistent in repeated novel object tests. However, this inconsistency could not be explained by the novel objects being randomly presented to calves' different visual fields because calves might not have cerebral lateralization in processing fear and their initial monocular presentation towards fear-inducing stimuli did not clearly affect their fear responses. In addition, the absence of cerebral lateralization in processing fear might also indicate that laterality of visual response is unlikely to be a useful measure of fear in calves at this age.

Keywords: dairy calf, novel object test, repeatability, cerebral lateralization

5.1 Introduction

Fear has been considered a negative emotion resulting from a perception of danger (Ennaceur, 2014). It can be expressed through a series of physiological and behavioural reactions to a perceived threat (Forkman et al., 2007). In natural conditions, predators are an important source to stimulate fear. Although domestic animals do not commonly experience predations from natural predators, inappropriate housing and management procedures, such as dehorning, tail docking, castration, herding and transportation, can elicit fear-related responses (Hargreaves and Hutson, 1990; Wohlt et al., 1994), which may reduce animals' productivity, lead to chronic stress and thus alter fundamental behaviours (Forkman et al., 2007). For example, in sheep and cattle, fear-related reactions affect their social dominance ability and sexual and maternal behaviours (Bouissou et al., 2001; Fisher and Matthews, 2001). Therefore, fear may be a severe welfare problem. Although animals often feel fear when they have a perception of danger, finding effective ways to assess their fear levels and tendency to express fear (the personality trait of fearfulness (Wohlt et al., 1994)) is an important step to improve their welfare.

In calves, fear is often assessed through their responses to novelty and responses to humans (e.g. Forkman et al., 2007; Meagher et al., 2016). Reliability has been considered a criteria to assess the effectiveness of novelty tests, which means that an effective fear test can get the same result in repeated measures of the same construct (Forkman et al., 2007). However, Meagher et al. (2016) reported poor test-retest reliability in novel object tests, which indicated that it is difficult to draw strong inferences from a single test. Similar results are also found in older heifers and adult cows when tested using reactivity (Gibbons et al., 2009), avoidance (Van Reenen et al., 2013), time in proximity and number of interactions (Kilgour et al., 2006). Although the poor test-retest reliability in novel object tests has been widely reported, the mechanisms resulting in this result are rarely studied.

Growing studies have focused on non-human vertebrates' cerebral lateralization in emotional processing. In domestic species, many researchers have determined that animals dominate the processing of fear and anxiety through a right-hemisphere (Leliveld et al., 2013) expressed by a left visual preference because the high degree of decussation of optical fibres ensures that input from the used eye is processed predominantly in the contra-lateral hemisphere (Leliveld, 2019). For instance, chicks preferred to use their left eye to observe aerial predators (Dharmaretnam and Rogers, 2005) and adult chickens were reported to have the preference to scan the air for predators with their left eye after hearing alarm calls from a conspecific (Evans et al., 1993). Studies in horses and cattle also showed that the right-hemisphere dominated the processing of fear. Austin and Rogers (2007) reported that when a person walked toward a domestic horse while opening an umbrella, the horse moved further away when the person approached the horse from its left-side compared to from its right-side. Robins and Phillips (2010) investigated whether dairy and beef cattle had preferences to monitor challenging and found that cattle preferred to use the left eye viewing an experimenter walking to split the herd repeatedly through its centre. For altricial species, their brain is mainly developed after birth (Halley, 2017) and their specializations of the left- and right-hemispheres are slowly established during infancy (Rogers, 2014). For instance, in chicks, hemispheric laterality in charge of behaviour occurs quite precisely during early development post-hatching (Rogers, 2014). In contrast, the brain development of precocial species mainly takes place before birth (Halley, 2017) and thus their hemispheric laterality is assumed to be well developed after parturition. For example, neonatal lambs are reported to have strong behavioural biases (Versace et al., 2007). Since dairy calves are precocial, it is worth studying their development of emotional laterality.

Austin and Rogers (2007) reported that when the novel object was first presented on the right-side of a horse, it lowered the horse’s initial fear responses, as well as fear responses to subsequent presentations of the object, compared to those with an initial left-side presentation. Since the majority of evidence suggests that the left-hemisphere is in the dominance of processing positive emotions (e.g. Silberman and Weingartner, 1986), Austin and Rogers (2007) suggested that the initial right-side presentation might inhibit the fear response and allow horses to learn that the stimulus presented no threat, which could be transferred to the right-hemisphere. Therefore, animals’ responses to fear-inducing stimuli may be affected by the initial presentation of the stimuli.

The first aim of the present study was to assess the test-retest reliability of calves’ responses in novel object tests. It was hypothesised that calves’ latency to make first contact with the novel objects in the first novel object test and the repeated novel object test would be inconsistent. The second aim of the present study was to determine if calves had visual lateralization (cerebral lateralization) in processing fear and whether such lateralization could then be considered an effective indicator of fear. It was hypothesised that 1) due to brain development before birth, in novel object tests calves would prefer to view the novel objects with the left eye; 2) there was a positive relationship between calves' proportion of time viewing the novel objects with the left eye and their latency to make first contact with the novel objects. The third aim of the present study was to determine if calves' initial monocular presentation toward fear-inducing stimuli would affect calves' fear responses. It was hypothesised that in novel object tests, 1) fewer calves would make contact with the novel objects when they first viewed the novel objects with the left eye; 2) calves would spend longer latency to make first contact with the novel objects when they first viewed the novel objects with the left eye.

5.2 Materials and Methods

The study was conducted at the Centre for Dairy Research, University of Reading (CEDAR), Reading, UK.

5.2.1 Animal, Housing and Feeding

Thirty-six pure registered male Holsteins calves with birth weights (mean ± SD) of 44.67 ± 4.43 kg were included from 2 days to 49 days of age. At 2 days of age, calves were allocated to six groups (six calves in each) according to their date of birth. Within groups, calves were assigned into either individual (two calves per group) or pair (four calves per group) pens. Physical enrichment items were provided to half individual pens (one stationary brush, one plastic chain, one rubber teat and one haynet filled with strawberry-scented ryegrass hay for an individual pen) and half pair pens (two stationary brushes, two plastic chains, two rubber teats and one haynet filled with strawberry-scented ryegrass hay for a pair pen). The remaining individual and pair pens received no additional physical enrichment items. More details related to housing and feeding can be found in Zhang et al. (2021).

5.2.2 Novel Object Tests

When the calves reached 6 weeks of age, the first novel object tests were conducted with one calf at a time. Each test calf was moved between its home pen and the test arena (4.0 × 4.0 m^2) using a wheeled scale. Once arrived at the entry of the test arena, the test calf was gently tapped on the hindquarters to encourage him to enter the test arena, where no other calves could be seen by the test calf. After entering the test arena, the test calf was allowed to habituate for 5 min before a novel object (a traffic cone or a white bucket, used for alternate groups of calves) was lowered to the centre of the test arena on a pulley. The test calf stayed in the pen with the novel object for 10 min, while recorded by webcam (C525, Logitech International S.A, Switzerland) or CCTV (Transit-PTZ, Revader Security Ltd, UK) fixed to the ceiling. Video recordings were observed continuously to record the behaviours as defined in Table 1. Latency to make first contact with the novel object has been suggested as an indicator of fear in calves because it highly correlates with other fear-related measures indicating physiological arousal (Van Reenen et al., 2005).

Repeated novel object tests were conducted with the same procedure one week later for every calf. For every test calf, the novel object (a white bucket or a traffic cone) that had not been used in its first novel object test was used in its repeated novel object test.

5.2.3 Statistical Analysis

All data were analysed using SPSS Statistics (version 27.0.1.0, IBM). Significant differences were declared at $p \leq 0.05$.

Due to the non-normality of the data, a Spearman correlation was used to analyse the relationship between calves' latency to make first contact with the novel objects in the first novel object test and the repeated novel object test. Variables in the model included calves' latency to make first contact with the novel objects in the first novel object test and calves' latency to make first contact with the novel objects in the repeated novel object test.

The proportion of time viewing the novel objects with left or right eyes in the first and repeated novel object tests for every calf was calculated (= duration to view the novel objects with left or right eye/total duration to view the novel objects with left, right and both eyes). A generalized linear mixed model was used to compare calves' proportion of time viewing the novel objects with left and right eyes. The subjects were calves' ID number and left or right eye; the repeated measure was first novel object test or repeated novel object test. The fixed factors in the model included left or right eye, object, first novel object test or repeated novel object test, social housing and physical enrichment. The random factor was calves' ID number.

If calves at this age had the preference to view novel objects with the left eye, based on the design of this experiment, it was worth studying the effects of novels objects, first and repeated novel object tests and housing environments on calves' proportion of time viewing the novel objects with left eye. A generalized linear mixed model was used to analyse the data. The subject was calves' ID number; the repeated measure was first novel object test or repeated novel object test. The fixed factors in the model included object, first novel object test or repeated novel object test, social housing and physical enrichment. The random factor was calves' ID number.

Due to the non-normality of the data, a Spearman correlation was used to analyse the relationship between calves' proportion of time viewing the novel objects with left eye and their latency to make first contact with the novel objects to test whether left eye use correlated with an established indicator of fear. Before conducting the analysis, calves that did not make contact with the novel objects were discarded because those calves' fear levels could not be assessed through the indicator of latency to make first contact with the novel object.

A Chi-squared test was used to analyse the relationship between calves' first view towards the novel objects with the left or right eye and whether they made contact with the novel objects. Variables in the model included calves' first view towards the novel objects with left, right or both eyes and if calves made contact with the novel objects during the testing period (yes/no).

A generalized linear mixed model was used to compare calves' latency to make first contact with the novel objects when they first viewed the novel objects with left, right and both eyes. The subject was calves' ID number; the repeated measure was first novel object test or repeated novel object test. The fixed factors in the model included calves' first view towards the novel objects with left, right or both eyes, object, first novel object test or repeated novel object test, social housing and physical enrichment. The random factor was calves' ID number. A test of least significant difference was carried out to identify differences among different eyes. Before conducting the general linear model, calves that did not make contact with the novel objects were discarded from the analysis.

The data of two calves were discarded from all tests before analysis because one calf was familiar with the white bucket and traffic cone, and the other one caught his head in the handle of the white bucket in the repeated novel object test and could not remove it.

5.3 Results

There was a poor correlation between the first novel object test and the repeated novel object test for calves' latency to make first contact with the novel objects (r_s = -0.148, p = 0.403; Figure 1).

In the first novel object test and repeated novel object test, there were no statistically significant differences in the proportion of time viewing the novel objects with left and right eyes ($F_{1,99}$ = 0.045, p = 0.832; Figure 2). In addition, novel objects, first and repeated tests and social housing did not have significant effects on calves' proportion of time viewing the novel objects with left eye (objects: $F_{1,32}$ = 0.041, p = 0.842; tests: $F_{1,32}$ = 1.537, p = 0.224; social housing: $F_{1,31}$ = 0.437, p = 0.513), whilst calves from physically enriched pens spent reduced duration viewing the novel objects with left eye than those from non-physically enriched pens ($F_{1,31}$ = 6.864, p = 0.013).

There was a poor correlation between calves' proportion of time viewing the novel objects with the left eye and calves' latency to make first contact with the novel objects (r_s = -0.002, p = 0.503; Figure 3).

The probability of making contact with the novel objects in the first novel object tests and repeated novel object tests was 70.80 % if the calf could view the object first with the left eye vs. 71.40 % if the right, but this was not significant (Chi-squared p = 0.965). In the first novel object test and repeated novel object test, there were no statistically significant differences in the latency to make first contact with the novel objects among calves' first view towards the novel objects with left, right and both eyes ($F_{2,31} = 0.611$, $p = 0.549$; Figure 4).

5.4 Discussion

Since fear has been considered a welfare concern for many animals (Jones and Boissy, 2011), researchers are using different ways, such as novelty tests, to assess its levels (Forkman et al., 2007). However, the results in this study show poor test-retest reliability of calves' response to novel objects, which may make it difficult to draw inferences about welfare or personality from a single novel object test. The finding is consistent with some other studies on cattle (e.g. Kilgour et al., 2006; Van Reenen et al., 2013). For example, Gibbons et al. (2009) studied the consistency of cattle's reactivity responses to three novel stimuli and found low reliability among tests. Thereafter, we tried to find out the potential mechanisms resulting in the poor test-retest reliability.

Vertebrates have the preference to view fear-inducing stimuli using the left eye (Robins and Phillips, 2010), and the eye that first sees a stimulus may affect fear-related behaviours (e.g. Austin and Rogers, 2007). Inconsistency in calves' head orientation relative to fear-inducing stimuli may explain differences in responses on repeated tests. We, therefore, studied if calves had the preference to view fear-inducing stimuli using their left eyes and if calves' first view affected their fear responses. We assumed that if calves had cerebral lateralization in processing fear resulting from the novel objects, initially presenting the fear-inducing stimuli into calves' left visual field could stimulate increased fear responses. Since in the novel object tests, objects are randomly presented to calves' left, right or both eyes initially, it may lead to different levels of fear responses for every calf in repeated tests. However, our findings were inconsistent with the predictions.

No cerebral lateralization in processing fear resulting from novel objects is found in this study, whilst the opposite findings are reported in other species. For example, many bony fish species show a right-hemisphere dominance in the processing of predator stimuli, evidenced by lateralized motor responses (e.g. Lippolis et al., 2009) and lateralized visual perception (e.g. Andrew and Budaev, 2009). In reptiles, a left eye preference to inspect predators exists for a wall lizard (Martín et al., 2010). In chickens, Evans et al. (1993) reported that adult hens prefer to use the left eye to scan the air for predators after hearing an alarm calls from a conspecific. Even in cattle, the lateralized processing of fear is exhibited. Robins and Phillips (2010) found that cattle preferred to use their left eye to view an experimenter splitting the herd through its centre. The difference between this and other studies may result from the development of hemispheric specialization. In this study, calves attended the tests when they were six or seven weeks of age. Although brain development for ungulate livestock mainly takes place before birth (Halley, 2017), it can be hypothesised that hemispheric specialization may not be well developed in the neonatal period. The finding of a poor correlation between calves' proportion of time viewing the novel objects with the left eye and calves' latency to make first contact with the novel objects in this study supports this hypothesis and indicates that left eye observation towards fear-inducing stimuli may not be considered an effective fear indicator at this age.

Since calves may not have visual lateralization in processing novel stimuli in this study, there is no surprise to find that calves do not show a significant difference in fear-related responses to novel stimuli initially perceived in the left monocular field compared to the right monocular field. However, this finding is in contrast to Austin and Rogers (2007). They investigated if horses displayed greater reactivity to a novel stimulus initially perceived in the left monocular field compared to the right monocular field. The experiment was designed with a person opening an umbrella five metres away from a horse and then approaching the left- or right-side of the horse so as to measure the distance the horse moved away before stopping. They found that when the stimulus was initially presented in the left monocular field, the horses responded with greater left-side reactivity, whereas when the stimulus was initially presented in the right monocular field, horses did not show side differences in reactivity. It may suggest that due to interocular transfer (e.g. Hanggi, 1999), which occurs from the left-hemisphere to the right-hemisphere (Von Fersen et al., 2000), habituation to the stimulus occurs when the first presentation is to the right eye but not when it is to the left eye. However, for neonatal calves, their hemispheric specialization may not be well developed and thus, they may not have the interocular transfer. Therefore, calves' poor test-retest reliability may not result from cerebral lateralization.

In addition to calves not having well-developed their hemispheric specialization in processing fear at an early age, there is another potential explanation for the lack of lateralized responses seen in this study. When we conducted the tests, some calves lay down on the sawdust of ground several minutes after entering the test arena and showed limited behavioural responses to the objects. This may indicate that calves were not frightened enough in the novel object tests and thus, this test environment could not stimulate their lateralized responses. Future research needs to adjust the test environments to further stimulate calves' fear (e.g. providing a more frightening object to calves).

Animals' early experience is suggested to affect their development of hemispheric specialization (Rogers, 2010). In mammals, neonatal stimulation has a profound influence on the specialization of their brain (Denenberg, 1981). For instance, neonatal handling of rats can provide stimulation to give rise to right hemispheric dominance, which increases animals' emotionality, reactivity and the aggressive behaviour of muricide (Denenberg, 1981). Exposure of neonatal rats to novelty, meanwhile, modulates hand preference towards control by the right-hemisphere (Tang and Verstynen, 2002) and increases the right hippocampal volumetric dominance (Verstynen et al., 2001). In the current study, calves experienced differential levels of stimulation (i.e. social housing, physical enrichment) before attending the first and repeated novel object tests. Social housing seems unlikely to have affected calves' cerebral lateralization in processing fear, evidenced by calves from individual pens and pair pens spending similar amounts of time viewing novel objects with their left eyes. Although physical enrichment was found to reduce calves' time spent viewing novel objects with the left eye, this does not necessarily mean this stimulus promotes the development of cerebral lateralization in processing fear. Animals with environmental enrichment may be less likely to approach novel stimuli (e.g. Paterson and Pearce, 1992; Schütz et al., 2012). Since physically enriched calves had more experience with novel objects (e.g. stationary brushes, plastic chains, dry teats and nets filled with scented hay) in their home pens before attending the novel object tests, the objects in novel object tests might be less attractive for them and thus they spent less time viewing the objects, without being less fearful of the objects. The findings in Zhang et al. (2021) might also support this point of view, which indicated physical enrichment had no effects on calves' fear responses (e.g. latency to make first contact with novel objects) in novel object tests.

When we study the effects of cerebral lateralization on calves' test-retest reliability, we need to be careful to control for other factors that may affect the results. Meagher et al. (2016) reported that a long test-retest interval, combined with important management changes between tests such as weaning from milk, might have negative effects on test-retest reliability and they also found that reducing test-retest intervals from 20 days to 7 days with more consistent management of milk provision between tests might improve the reliability. In addition, increasing the test duration is suggested to improve the reliability of the latency measures because longer test durations can avoid an artificial upper limit in measures of latency (Fonio et al., 2012; Meagher et al., 2016). Moreover, animals' health status may be another factor to affect the reliability. For instance, Cramer and Stanton (2015) found that calves with fever or respiratory illness showed a decreased probability of approaching stationary humans and novel objects. Meagher et al. (2016) reported that excluding calves who had a cough on the day of testing could increase the reliability of calves' responses to humans. In this study, the test-retest interval for every calf was one week; milk was provided two times a week; the test duration for every calf was 10-min; calves' health status during the test periods was well managed. Therefore, these factors may have limited effects on test-retest reliability in this study.

5.5 Conclusion

There was a poor test-retest reliability of calves' response of latency to make first contact with the novel objects in novel object tests, which could not be explained by the hypothesis that fear responses would be stronger if calves first viewed the novel object with the left eye because calves' initial monocular presentation towards novel objects did not affect their expression of latency to make first contact with the novel objects. Since the present study also found that calves did not have visual lateralization in processing fear, it might suggest that calves of this age did not develop cerebral lateralization in processing fear, and thus monocular presentation was unlikely to have an effect on test repeatability. The absence of visual lateralization also suggests that using left eye to observe potentially fear-inducing stimuli is unlikely to be a useful measure of fear in calves at this age.

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Table 1. Ethogram of the recorded behaviours in the first novel object test and the repeated novel object test

Behaviour	Definition
First view	Which eye (left eye, right eye or both eyes) would have had a clear line of sight to the object when it came into the test calf's field of vision as it started moving towards the floor.
Left eye[a]	The object was to the left-side of the head's orientation of the test calf; the line of vision of the right eye was obscured; the object was not posterior to the abdomen.
Right eye[a]	The object was to the right-side of the head's orientation of the test calf; the line of vision of the left eye was obscured; the object was not posterior to the abdomen.
Both eyes[a]	The calf's head was orientated so that line of vision between both eyes and the object was unobstructed.
Latency to first contact with the novel object[a]	Time interval from the object been lowering to the floor to the test calf touching the object.

[a] The time duration of the behaviour was recorded.

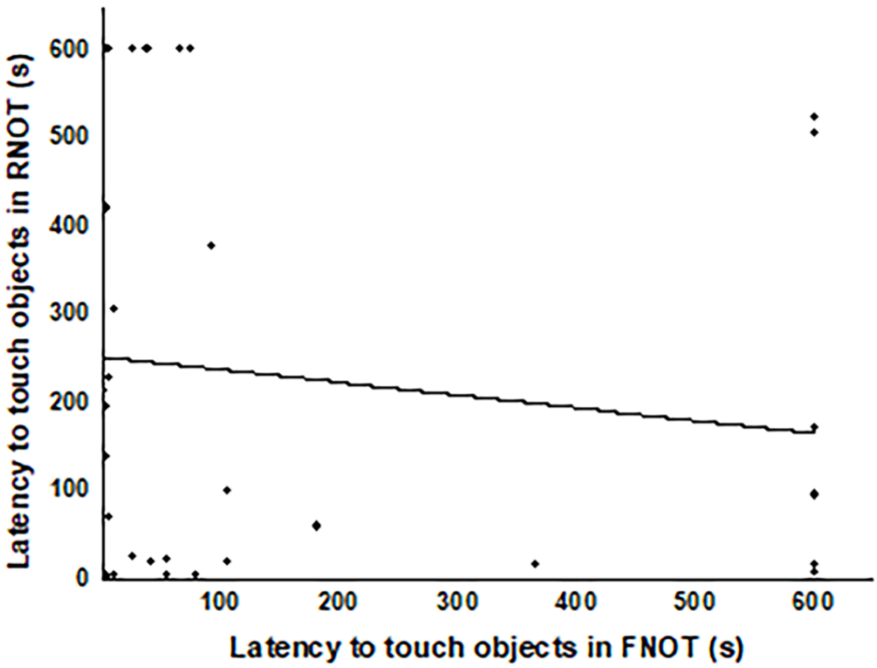

Figure 1. Correlation between calves' latency to touch the novel object across the first novel object tests (FNOT) and repeated novel object tests (RNOT).

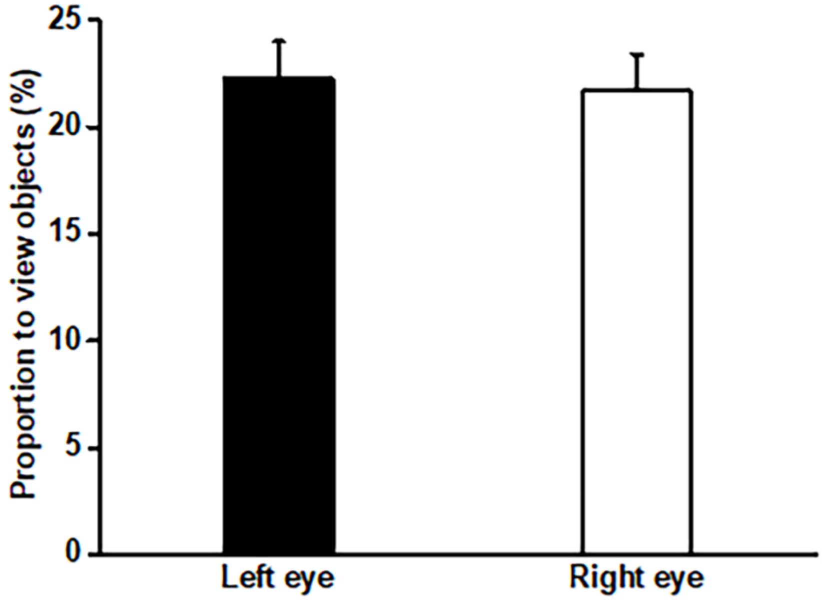

Figure 2. LSM (± SE) of the proportion of total viewing time using the left and right eyes (n=34 calves).

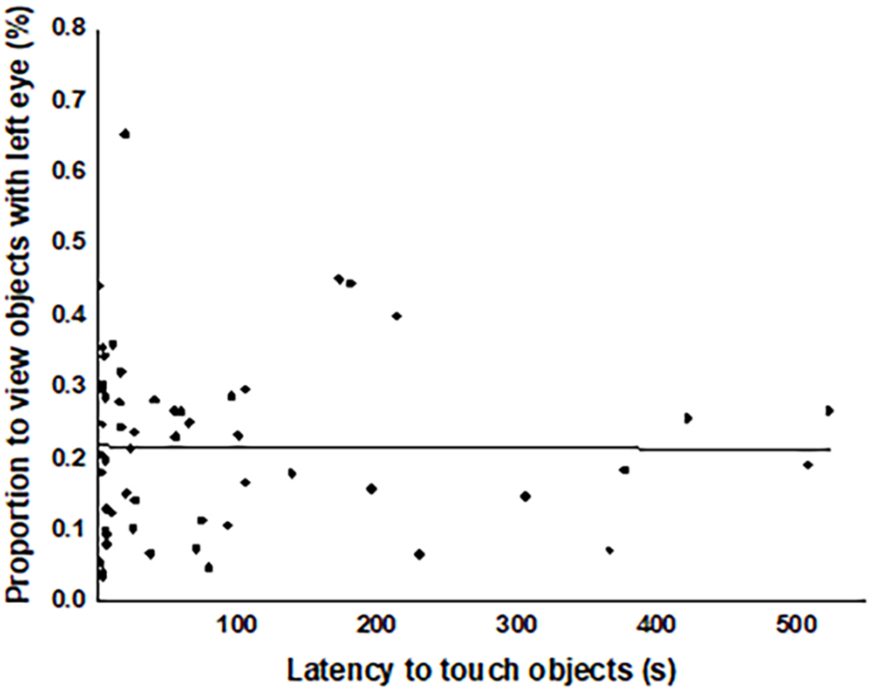

Figure 3. Correlation between calves' proportion of time viewing the novel objects with the left eye and calves' latency to touch the novel objects.

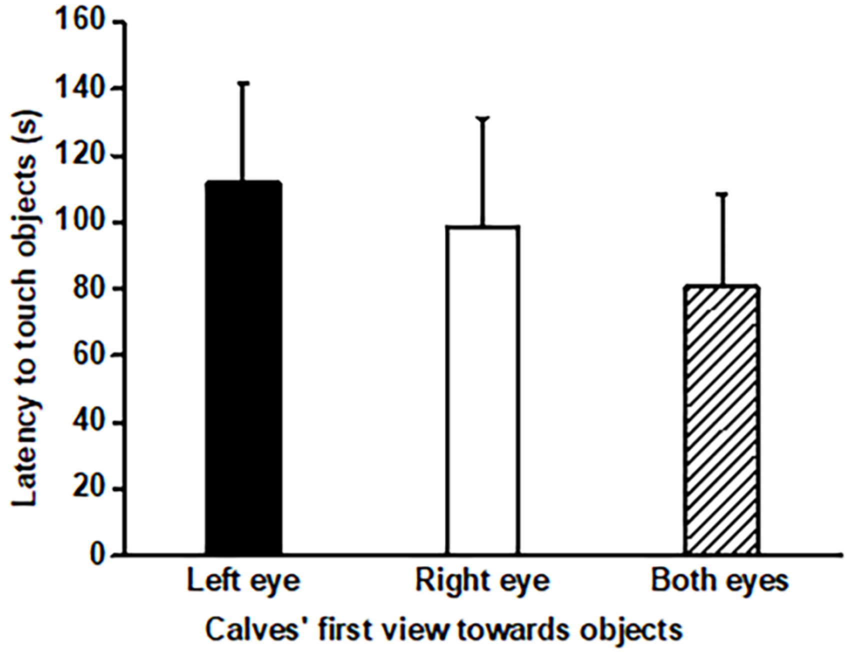

Figure 4. LSM (± SE) of the latency to touch the novel objects among calves' first view towards the novel objects with left, right and both eyes (n=34 calves).

Chapter 6

General Discussion

6. General Discussion

In the dairy industry, most calves are reared to satisfy their physiological requirements such as providing feed and water and offering protection from disease and environmental risks. However, calves' behavioural needs are less considered. As a procedure widely used in dairy production systems, calves experience maternal separation after birth and are individually reared in barren environments during infancy (Mikuš and Mikuš, 2020), which prevents them from expressing natural behaviours (e.g. Jensen et al., 1998) while developing undesirable behaviours (e.g. Bokkers and Koene, 2001). Since adding complexity to environments has been suggested as an effective method to improve the welfare of many animals, it is crucial to find effective ways to enrich calves' housing environment so as to meet their requirements.

This dissertation firstly aimed to investigate an effective physical enrichment protocol to complicate calves' housing environment and determine how this method could improve calves' welfare. To achieve the aim, the trial described in Chapter 2 of this dissertation investigated the effectiveness of potential physical enrichment protocols between a fixed multi-item enrichment presentation schedule and a rotating schedule with a single enrichment item presented one at a time through assessing calves' use of the items (stationary brush, dry teat, plastic chain, rope, spring, nets filled with scented hay) and determined calves' preference for and ways of interacting with various items in the effective physical enrichment protocol. Thereafter, Chapter 3 and Chapter 4 of this dissertation report the results of trials that determined how the effective physical enrichment protocol affected pre- and post-weaning calves' welfare. Specially, we studied the effects of the effective physical enrichment protocol on pre-weaning calves' growth, behaviour and response to novelty (Chapter 3). We also studied the effects of providing the effective physical enrichment protocol to pre-weaning calves on their behaviour, growth and cognitive ability after weaning and regrouping (Chapter 4).

The second aim of this dissertation was to repeat research on the effects of social housing on calves' welfare and compare it to physical enrichment. In Chapter 2, we report the effects of pair housing on calves' use of the items in the effective physical enrichment protocol. After that, we studied the effects of pre-weaning pair housing on pre-weaning calves' growth, behaviour and response to novelty (Chapter 3). We also studied the effects of pre-weaning pair housing on post-weaning calves' growth, behaviour and cognitive ability (Chapter 4).

Since physical enrichment and social housing have been suggested to improve animal welfare by providing social contact (Costa et al., 2016) and increasing environmental complexity (Bloomsmith et al., 1991) separately, the third aim of this dissertation was to investigate the effects of the combination of providing physical enrichment items through the effective physical enrichment protocol and pair housing on calves' welfare. To achieve the aim, in Chapter 3, we report our investigation of the effects of the combination of providing physical enrichment items through the effective physical enrichment protocol and pair housing on pre-weaning calves' growth, behaviour and response to novelty. In Chapter 4, we report our study of the effects of providing the effective physical enrichment protocol to pre-weaning calves and pre-weaning pair housing on their behaviour, growth and cognitive ability after weaning and regrouping.

Fear has been considered an important welfare problem for calves, whilst their responses in the common assessment methods including novel object test and human approach test are inconsistent (Meagher et al., 2016). The fourth aim of this dissertation was to investigate the potential mechanisms resulting in the uncertain reliability of calves' fear tests. To achieve the aim, in the research reported in Chapter 5, we firstly assessed the test-retest reliability of calves' responses in novel object tests. Then, we determined if calves had visual lateralization (cerebral lateralization) in processing fear and if calves' initial monocular presentation toward novel objects would affect calves' fear responses.

6.1 Major Findings

6.1.1 Physical Enrichment

In Chapter 2, we reported that a fixed multi-item enrichment presentation schedule and a rotating schedule with a single enrichment item presented one at a time were both effective physical enrichment protocols because they both reduced calves' non-nutritive oral behaviour. Since calves were reported to interact more with the items in the fixed multi-item enrichment presentation schedule than in the rotating schedule with a single enrichment item presented one at a time in Chapter 2, the fixed multi-item enrichment presentation schedule may be a more effective physical enrichment protocol to attract calves. Thereafter, we studied how calves interacted with the items in a fixed multi-item enrichment presentation schedule to understand its underpinning mechanisms. In order to choose effective physical enrichment items for this project, it is imperative to consider the criteria, including the functional utility of the items, the ability to facilitate the expression of animals' behavioural skills, and the capacity to reliably attract and sustain the interest of animals (Jones et al., 1991; Newberry, 1995; Mench, 1998). We found that although calves showed several kinds of behaviours towards every type of item, they mainly showed hay intake behaviour towards nets filled with scented hay, scratching behaviour towards stationary brushes (also reported in Strappini et al., 2021) and sucking behaviour towards dry teats (also reported in Veissier et al., 2002) and plastic chains. This may indicate that the items used in the fixed multi-item enrichment presentation schedule satisfy calves’ different behavioural motivations, and the functions of the diverse items are not replaced by each other. Thus, providing the items simultaneously is more attractive and effective for calves than providing one type of item at a time. In addition to the main behaviour towards every type of item in the fixed multi-item enrichment presentation schedule, calves also showed butting, scratching, sniffing and sucking towards nets filled with scented hay, showed sniffing and sucking towards stationary brushes, and showed scratching and sniffing towards dry teats and plastic chains. This may suggest that each of these types of item has various characteristics to satisfy calves’ different behavioural motivations. It also indicates that the characteristics of effective physical enrichment items for calves may be investigable (retaining calves' interest in exploring the materials), manipulable (allowing calves to suck, bite or otherwise deform or move the materials), able to be rubbed (allowing calves to groom themselves on the materials) or edible (attracting calves to ingest the materials that have some nutritional value). Since a net filled with scented hay was reported to attract calves more than other items in Chapter 2, it may have more relevant characteristics than other items, with the additional characteristic being edible because, in Chapter 2, the use difference between use of nets filled with scented hay and other items mainly resulted from hay intake from nets filled with scented hay. Although a fixed multi-item enrichment presentation schedule is suggested as an effective presentation method to stimulate calves' interaction behaviours towards items, this point of view needs to be re-validated when items do not have the characteristics listed above or have the same main characteristics listed above. Different items with characteristics not listed above need to be studied whether having long-lasting attractions to calves. Different items with the same main characteristics may stimulate the same behavioural motivations and thus are not able to satisfy all their behavioural motivations.

We also studied the hourly distributions of calves' interaction with the overall items in the fixed multi-item enrichment presentation schedule. The overall items were used most around milk feeding times, as well as at 1900 h. Similarly, Horvath et al. (2020) reported that heifers used brushes mainly during daylight hours with peaks in use around sunrise, sunset, and throughout the afternoon. Zobel et al. (2017) also suggested that the use of hanging ropes and rotating brushes reached peaks around milk feeding times and around 1800 and 1900 h. Since individuals are active around feeding time (Mandel et al., 2013; Pempek et al., 2017) and at sunrise and sunset (Albright, 1993), the findings suggest that individuals may be more likely to use physical enrichment items during their active period.

Since providing physical enrichment items of stationary brushes, dry teats, plastic chains and nets filled with scented hay in a fixed multi-item enrichment presentation schedule was suggested as an effective way to stimulate calves' interaction behaviours towards items, we further studied the effects of providing the physical enrichment items simultaneously in calves' pre-weaning period on their welfare.

In Chapters 3 and 4, we reported that pre-weaning physical enrichment improved pre-weaning weight gain but had no effects on post-weaning weight gain. For pre-weaning calves from all treatments, plain hay was provided through hay racks because foraging is part of the natural development of calf feeding behaviour (Horvath and Miller-Cushon, 2019). The provision of plain hay may broadly benefit calves. For instance, hay provision has been reported to improve the rumen environment and rumen development (Castells et al., 2013; Pazoki et al., 2017) by increasing rumen pH (Khan et al., 2011), inducing changes in ruminal bacterial diversity and abundance (Kim et al., 2016), expanding the rumen volume (Castells et al., 2012), reducing the keratinization of rumen papillae (Beiranvand et al., 2014) and negativing correlating with the severity of subacute ruminal acidosis (Laarman and Oba, 2011). Previous studies also reported that hay provision might improve feed efficiency and growth (Coverdale et al., 2004; Castells et al., 2012), although Hill et al. (2008) and Kertz et al. (1979) reported that the inclusion of forage in the diets of pre-weaning calves with low quantities of milk led to their poorer weight gains. In some studies, increased milk allowances were provided to pre-weaning calves to improve feed efficiency and enhance growth rate (Diaz et al., 2001; Jasper and Weary, 2002; Brown et al., 2005). For these calves, increased milk consumption may decrease starter intake, delay rumen development and retard growth at weaning (Jasper and Weary, 2002), but providing hay to these calves is suggested to improve this situation by promoting solid feed intake and rumen development (Khan et al., 2011).

In this dissertation, to further promote hay consumption, nets filled with scented hay were provided to physically enriched calves. For herbivores, sensorial perceptions are integral in the process of feed selection and intake, and certain extracts and aromas are able to evoke positive sensorial perceptions when foraging (Cannas et al., 2009). Since strawberry aroma was reported in this dissertation and it is considered a preferred aroma for heifers (Meagher et al., 2017), the strawberry-scented hay may result in the increased expression of hay intake behaviour for physically enriched calves as reported in Chapter 3. However, the positive effects of strawberry aroma on hay intake could not be identified because, in this dissertation, strawberry aroma and hay nets were used in combination to promote calves’ hay intake behaviour and the latter may also stimulate hay intake by increasing the naturalness of foraging behaviour (Mandel et al., 2016).

The increased hay intake behaviour reported in Chapter 3 can be reasonably assumed to increase calves’ total hay consumption. Since roughage consumption is considered essential for rumen development (Moran, 2012), increased hay consumption may promote calves’ digestive ability (Khan et al., 2016). Therefore, nets filled with scented hay may result in the increased growth of pre-weaning physically enriched calves as reported in Chapter 3. However, since increased hay intake increases gut fill (Drackley, 2008), it may complicate the interpretation of the increased growth responses. Chapter 3 also reported that physical enrichment reduced pre-weaning calves’ concentrate intake, which corresponds with the findings by Horvath and Miller-Cushon (2017). They indicated that calves provided with hay consumed less concentrate than those without hay provision. Since hay contains lots of undigested fibrous material, which has a low fermentation rate in calves’ rumen (Hill et al., 2008), increased hay consumption can increase satiety, and thus suppresses calves’ concentrate intake (Drackley, 2008). However, some studies reported that the provision of forage stimulates concentrate intake (e.g. Phillips, 2004). The difference may result from the amount of hay selected in calves’ diet. According to Castells et al. (2012), calves offered alfalfa hay as a dietary option consumed 8% of hay in their diet and exhibited decreased concentrate intake, whilst calves offered ryegrass hay, oat hay, and barley straw as a dietary option consumed 3 - 6% of hay in their diet and had higher concentrate intake compared with calves that were not provided with hay. Therefore, providing a highly palatable forage source may stimulate greater intake and offset concentrate intake (Castells et al., 2012). In addition to the hay variety, the length of hay may also affect hay consumption. Wang et al. (2020) reported that chopping hay increased hay intake, improved the rumen bacterial community, altered rumen fermentation and eventually promoted rumen development. Future research should further study the combined effects of the variety of hay, the length of hay, the palatability of hay and the container for hay on calves' hay consumption, rumen development and growth.

At the end of 8 weeks of age, calves were weaned and moved to post-weaning group pens. Calves from all pre-weaning treatments were transformed into the novel post-weaning group pens with new feeds and mixing with unfamiliar peers. This change may lead to a tremendous amount of stress for calves (Hulbert and Moisá, 2016). Stress caused by the weaning and environmental change for ruminants may lead to irregularities in the functioning of the microbiome and pathway of the digestive system and may further decrease their productivity (Cholewińska et al., 2021). Thus, post-weaning calves may experience a growth check at this stage and the external stimuli that they experienced before weaning cannot help them cope with it. The similar rate of TMR intake behaviour for calves from different treatments (Chapter 4) also corroborated this view. To better manage weaning and regrouping, further research needs to find ways to reduce calves' stress, such as dividing weaning and regrouping into two independent phases. By doing this, calves can experience changes in feeds and housing environments at different times, which may make it easier for them to cope with the changes. Although the effects of dividing weaning and regrouping into two stages for individually and pair housed calves have been rarely studied, in dam-rearing systems, there is growing evidence that weaning and separation of calf and cow in two steps may reduce their stress (Johnsen et al., 2016). For instance, Haley et al. (2005) suggested that compared to calves nursed from their dams until separation, calves that were prevented from nursing their dam for a period before their separation were less distressed after weaning and separation.

In addition to calves' growth, the effects of pre-weaning physical enrichment on calves' behaviours were another focus of this dissertation. Undesirable behaviours, such as sucking, biting and agonistic interactions were generally measured as indicators of the adaptive capacity of farm animals, which suggests that animals' environmental adaptability is mainly assessed from the negative aspect. Since improved housing environments can stimulate the expression of positive behaviours (e.g. Pempek et al., 2017), play, grooming, social behaviour and exploratory behaviour may be considered indicators to measure animals' environmental adaptability from the positive aspect. Therefore, in this dissertation, we assessed calves' environmental adaptability from both aspects.

Pre-weaning physical enrichment reduced pre-weaning calves' non-nutritive oral behaviours (Chapter 3), whilst not affecting the cross-sucking of post-weaning calves (Chapter 4). Calves are reported to have strong sucking motivation before and after weaning (De Passillé, 2001; Strappini et al., 2021). For pre-weaning calves, their sucking motivation reaches peaks after milk feeding and thus they show a large amount of non-nutritive oral behaviours in barren housing environments since they do not have appropriate ways to release their sucking motivation (Veissier et al., 2002). When the pre-weaning calves are reared in physically enriched pens, Chapter 2 has reported that they spent lots of time sucking the physical enrichment items including dry teats, plastic chains, stationary brushes and nets filled with scented hay and these items were used most around milk feeding times. Therefore, the provision of the physical enrichment items may redirect pre-weaning calves' non-nutritive oral behaviours. Since in this dissertation, we did not measure the total frequency of non-nutritive sucking (pen-directed sucking, enrichment-directed sucking and cross-sucking) in the different treatments, we could not conclude whether the use of physical enrichment items redirected or reduced the non-nutritive oral behaviours. Although the increased hay intake for physically enriched calves reported in Chapter 3 might lead to reduced non-nutritive oral behaviours, the reduced concentrate intake for these calves might point in the opposite direction. Future study is needed to investigate the effects of the physical enrichment items on non-nutritive oral behaviours. For post-weaning calves, they may maintain strong motivation for sucking (Strappini et al., 2021), which can be linked to the lack of suckling associated with limited pre-weaning milk availability (Margerison et al., 2003). Due to the lack of appropriate ways to release this motivation in post-weaning group pens, calves from all pre-weaning treatments may redirect to sucking their pen-mates. Given that non-nutritive oral behaviours are typically considered redirected to engage in a particular behaviour that is not met within the environment, (Leruste et al., 2014), effective outlets such as items for oral manipulation need to be used to satisfy the requirements. Strappini et al. (2021) provided cowhide and ropes to post-weaning calves to satisfy their sucking motivation and they found that calves showed high oral interactions towards both items. In addition to satisfying post-weaning calves' sucking motivation, the expression of sucking behaviour for post-weaning calves may be because they are curious and have a high motivation to explore their environment to have a comprehensive map of their surroundings (Wood-Gush and Vestergaard, 1989; Wilson et al., 2002). However, since in this dissertation, the cross-sucking behaviour was mostly observed during the feeding times towards the ears of the pen-mates, it should not be considered as resulting from calves' exploratory motivation.

For the positive indicators, pre-weaning physical enrichment had no effect on the expression of play, sniffing and grooming of pre-weaning calves (Chapter 3), whilst it increased post-weaning calves' exploratory behaviour towards their environments and pen-mates (Chapter 4). According to the research reported in Chapter 2, pre-weaning physically enriched calves showed plenty of interaction behaviours towards physical enrichment items, including sniffing, scratching, sucking, butting and hay intake. The interaction behaviours may satisfy calves' motivations related to play, grooming and exploration. It may thus redirect their expression of positive behaviours. For pre-weaning non-physically enriched calves, their barren housing environment may restrict the expression of positive behaviours (Jensen et al., 1998; Mason and Burn, 2011). Therefore, calves in pre-weaning physically enriched pens and pre-weaning non-physically enriched pens were observed with similar expressions of play, sniffing and grooming. After weaning and regrouping, calves that experienced pre-weaning external stimuli may retain greater motivations related to the positive behaviours. Thus, they showed more exploratory behaviour towards the post-weaning group pens than pre-weaning non-physically enriched calves. To sum up, pre-weaning physical enrichment can effectively reduce calves' undesirable behaviours, and enhance calves' adaptability to environmental changes to some extent.

In addition to the positive and undesirable behaviours measured above, self-grooming has also been used as an indicator of animals' environmental adaptability. However, the interpretation of this behaviour is not straightforward. On the one hand, self-grooming is suggested as a behavioural need of calves, which can be impacted by environmental conditions (e.g. Panivivat et al., 2004) and health conditions (e.g. Borderas et al., 2008). Therefore, self-grooming is considered a positive indicator of calf welfare (Tapki, 2007; Horvath and Miller-Cushon, 2019). On the other hand, self-grooming may occur as a displacement behaviour in situations of motivational conflict (Herskin et al., 2004), thus a negative indicator. An effort has been made in other species to decide whether self-grooming should be regarded as positive or negative behaviour. As reported by Duncan and Wood-Gush (1972), fowls in a frustrating situation spent a shorter duration on preening behaviour every time and spent more time preening plumage areas that were easy to reach than those in the normal situation. Further studies are needed to determine the implication of different self-grooming behaviours in calves.

The research reported in Chapter 3 studied the effects of pre-weaning physical enrichment on calves’ fear through assessing fear indicators including active defence, passive avoidance, expressive movements and vocalisations in the environmental novelty test and novel object test. Recording several of these behaviours simultaneously can strengthen conclusions about the underlying state. We found that pre-weaning physical enrichment had no effects on calves’ responses to the novel environment and novel object. A potential explanation is the static environment created by providing additional items was not complex enough to elicit emotional change in novel situations. However, this explanation is debatable because, in Chapter 4, pre-weaning physically enriched calves were reported exploring their post-weaning group pens more often than pre-weaning non-physically enriched calves. It may indicate that pre-weaning physically enriched calves have better behavioural flexibility to adapt to novel environments. The cognitive ability test reported in Chapter 4 also supports this point of view, which reported that pre-weaning physical enrichment might improve calves’ object recognition memory ability. Calves’ better cognitive ability may improve their behavioural flexibility (Gaillard et al., 2014), and thus increase their adaptability to novel environments. Therefore, the fact that calves from different pre-weaning treatments showed similar fear responses in the novelty tests may be due to other reasons. When we conducted the environmental novelty test and novel object test, we found that some calves lay down on the ground after several minutes of entering the test arena. Calves' might not be frightened enough in both tests and thus, calves from different treatments showed similar behavioural responses in both tests. Future research needs to adjust the test environments to further stimulate calves' fear. In addition, since in the environmental novelty test and novel object test physically enriched calves showed a nonsignificant trend of less fear (e.g. numerically shorter latency to make first contact with the novel object), the other potential explanation is we did not have sufficient replication to get a significant result.

6.1.2 Social Housing

In Chapters 3 and 4, we reported that pre-weaning pair housing had no effect on pre- and post-weaning calves' growth. Socially housed calves experience social learning or social facilitation, which can promote them to consume more concentrate (Costa et al., 2015). According to the experiment design reported in Chapter 3, individually housed pre-weaning calves had auditory, visual and limited physical contact with their neighbours. This design gives pre-weaning individually housed calves a chance to imitate or learn how to consume concentrate from their pair housed neighbours. Therefore, calves from pre-weaning individual and pair pens were reported to show a similar concentrate consumption in the pre-weaning period (Chapter 3) and similar TMR intake behaviour after weaning and regrouping (Chapter 4).

In Chapter 2, we reported that pre-weaning pair housing reduced pre-weaning calves' interaction behaviours towards physical enrichment items.

Social contact satisfies calves' demand for conspecific presence as gregarious animals and stimulates their diverse natural behaviours (Chapter 3). Pre-weaning calves reared in pens or hutches rest for large parts (around 18 hours) of the day (Horvath et al., 2020; Xiao et al., 2022), and thus they may have limited time to show active behaviours. Pair housed calves are attracted to each other and show unrestricted social contact (Jensen and Larsen, 2014). For example, in Chapter 3, we reported that compared to pre-weaning individually housed calves, pre-weaning pair housed calves spent part of their active time expressing social behaviours and locomotor play. Therefore, for pair housed calves, social contact takes up part of their limited active time and thus they spend less active time interacting with overall items.

Chapter 3 reported that compared to pre-weaning individual housing, pre-weaning pair housing reduced calves' non-nutritive sucking while increasing their cross-sucking. It may indicate that calves housed in pre-weaning pair pens redirect non-nutritive sucking to their pen-mates as cross-sucking. The high sucking motivation for pre-weaning pair housed calves might be kept after weaning and regrouping. Chapter 4 reported that in post-weaning group pens, calves from pre-weaning pair pens showed more cross-sucking than those from pre-weaning individual pens. As a welfare problem, cross-sucking can be induced by social housing (Lidfors and Isberg, 2003). In this dissertation, we observed that calves from pre-weaning pair pens showed more of this undesirable behaviour. However, some studies reported that cross-sucking could be well managed in group pens (e.g. Chua et al., 2002; Mattiello et al., 2002). The diverse results may come from the management difference among studies. Cross-sucking is suggested to be a redirected behaviour from milk suckling (Leruste et al., 2014). In Chua et al. (2002), ad libitum milk was provided to pre-weaning calves through artificial teats. Thus, compared with providing limited milk, providing ad libitum milk may promote calves to spend more time sucking to consume milk. Since the expression of sucking behaviour can effectively reduce the underlying sucking motivation (De Passillé, 2001), the ad libitum provision of milk may satisfy calves' sucking motivation and reduce cross-sucking. In Mattiello et al. (2002), calves showed 4.7% of cross-sucking at 2 weeks of age. In Chapter 3, we reported that calves at 2 weeks of age showed a lower frequency of cross-sucking (2.4%) than those in Mattiello et al. (2002). It means that cross-sucking is managed better in our study than in Mattiello et al. (2002). In order to further reduce or eliminate the expression of cross-sucking, other methods are needed. For instance, calves reared in a dam-calf contact system do not show cross-sucking behaviour (Fröberg and Lidfors, 2009).

Regarding the effects of pair housing on calves' affective state and cognitive ability, Chapter 3 reported that calves from pre-weaning individual and pair pens showed similar fear responses to the novel environment and novel object. However, since the novel environment and novel object may not be frightened enough for calves (as discussed above), these results may not accurately reflect the effects of individual and pair housing on calves' fear responses. In addition, calves from pre-weaning individual and pair pens had a similar object recognition memory ability (Chapter 4). It may be because calves reared in individual pens could have auditory, visual and limited tactile contact with neighbours (Chapter 3). The contact among calves may promote them to learn from each other and thus improve the cognitive ability of individually housed calves.

6.1.3 Combination of Physical Enrichment and Social Housing

The combination of pre-weaning physical enrichment and pre-weaning social housing had positive effects on post-weaning calves' growth compared to either individual method, whilst having no effects on pre-weaning calves' growth and behaviour, post-weaning calves' behaviour, affective state and cognitive ability. As discussed above, calves may experience tremendous stress in the weaning and regrouping period. Since physical enrichment and social housing may improve animal welfare by providing external stimuli (Bloomsmith et al., 1991) and social contact (Costa et al., 2016) separately, calves experiencing both stimuli together in their pre-weaning period may further improve their later behavioural flexibility and better adapt to the post-weaning environmental changes. Although calves' behavioural flexibility is related to their cognitive abilities (Gaillard et al., 2014), calves with the combination of pre-weaning physical enrichment and pre-weaning social housing did not display improved performance in the spontaneous object recognition test. It may be because the spontaneous object recognition test measures a simple aspect of calves' cognitive ability - memory ability. Either pre-weaning physical enrichment or pre-weaning social housing alone may be able to improve calves' memory ability. More complex forms of cognitive ability need to be measured using different cognitive ability tests, such as using T-maze tests measuring calves' reversal learning ability (e.g. Meagher et al., 2015; Horvath et al., 2017). To sum up, since growth is an important indicator of calves' welfare and farm management, the combination of pre-weaning physical enrichment and pre-weaning social housing is valuable for the dairy industry.

6.1.4 Are Laterality Effects Present in Novel Object Responses of Calves?

Fear is a welfare concern for many animals, which may decrease biological functioning and increase the risk of injury. In calves, fear is commonly measured when they face novelty, such as the novel object test reported in Chapter 3. However, the repeatability of the novel object test is poor (Meagher et al., 2016), which makes it difficult to draw inferences about welfare or personality from a single novel object test. Therefore, as reported in Chapter 5, we retested the repeatability of the novel object test and studied if calves have visual lateralization in processing fear and if calves' initial monocular presentation towards fear-inducing stimuli would affect calves' fearful responses and thus result in poor test-retest reliability. We found that calves' fearful response in novel object test and repeated novel object test was inconsistent, whilst their initial monocular presentation towards fear-inducing stimuli did not affect their fear responses. Since we also found that calves did not have visual lateralization in processing fear emotion, it might suggest that calves of this age (6-7 weeks of age) did not have well-developed cerebral lateralization in processing fear. Thus, initial monocular presentation is unlikely to have an effect on test repeatability and eye laterality is not considered a useful measure of fear in calves at this age.

6.2 Limitations and Future Research

In the research reported in Chapter 2, we compared two physical enrichment protocols: a rotating schedule with a single enrichment item presented at a time and a fixed multi-item enrichment presentation schedule. In the rotating schedule, one type of item was provided to every calf at a time. In the fixed schedule, four types of item were provided to every calf at the same time. We found that calves are likely to prefer the fixed schedule rather than the rotating schedule. From the practical aspect, both protocols may be acceptable for farmers because they will not sharply increase costs and labour costs. However, from the research aspect, every calf received one item at a time in the rotating schedule, whilst every calf received four items at the same time in the fixed schedule. Although it seems likely that the increased overall use of physical enrichment items in the fixed schedule results from the four types of item satisfying calves' different, ongoing motivations, there is a possibility that having multiple items in the pen increases the chance a calf will interact with one at any given moment regardless of the specific item characteristics themselves. Therefore, it is worth comparing a rotating schedule with four identical enrichment items presented at a time and the fixed multi-item enrichment presentation schedule.

In the research reported in Chapter 2, two sets of the items were provided to calves housed in every pair pen to assure both calves in the same pen could interact with every type of item at the same time. However, stationary brushes and dry teats were fixed on the opposite panels of every pair pen. When one calf in pair pens was dominant over a dry teat or a stationary brush, the other one might observe him interact with the item while not looking at the other available identical item. In addition, when fixing physical enrichment items to animals’ enclosures, the maintenance convenience is usually considered an important factor, whilst ignoring the use convenience for animals. For example, for pigs, if physical enrichment items are suspended over the sleep areas, it will lead to disruption of their sleep patterns (Van De Weerd and Day, 2009). Therefore, in calves, especially group housed calves, the location of physical enrichment items needs careful consideration because it may affect calves’ access to the items, specifically group housed calves may synchronise their interactions with physical enrichment items.

In the research reported in Chapter 3, we studied the effects of social housing on calves’ behaviours and found that calves in pair pens expressed more sniffing, locomotor play and allogrooming behaviours than those in individual pens. Calves housed in pair pens had a bigger space allowance than those in individual pens (4.8 m^2 vs. while 2.4 m^2 per pen). Since bigger space allowance has been suggested to promote the expression of play behaviour in domestic calves (Jensen et al., 1998), there is a possibility that space allowance contributes to the expression of calves’ positive behaviours.

In the research reported in Chapter 3, we found that calves in physically enriched pens showed more hay intake behaviour than those in non-physically enriched pens. We then reasonably deduced that physically enriched calves consumed more hay, which might lead to increased weight gain. Since calves from physically enriched pens could consume hay from hay racks and nets filled with scented hay, whilst calves from non-physically enriched pens could only consume hay from hay racks, future work can study if nets are a preferred container and if strawberry-scented hay is a more palatable roughage for calves to promote their hay consumption and further affects their growth.

In the research reported in Chapters 3 and 4, calves' behavioural responses towards novel objects such as time spent in contact with novel objects were used as a fear indicator in the novel object test and as a curiosity indicator in the cognitive ability test, since fear is a common emotion when calves face novelty (Forkman et al., 2007) and calves have a natural propensity to explore novelty (Baxter, 2010; Ennaceur, 2010). The behaviour of time spent in contact with novel objects was used to assess two different emotions because a given behaviour can be attributed to multiple emotions (Berlyne, 1950). In the novel object test, behaviour is suggested to reflect the balance of fear and curiosity (Bulens et al., 2015): calves with higher fear levels and lower curiosity levels are suggested to spend less time in contact with novel objects because time spent in contact with novel objects is correlated with other fear-related measures suggesting physiological arousal (Van Reenen et al., 2005). In contrast, in the cognitive ability test, calves with lower fear levels and higher curiosity levels may spend more than in contact with novel objects. To the best of my knowledge, it is difficult to discriminate between the two emotions using the behaviour of time spent in contact with novel objects.

In the research reported in Chapter 5, calves' time duration to observe the novel object using left, right or both eyes in novel object tests were recorded by cameras and collected by watching the video recordings using QuickTime Player (Version 10.5, Apple Inc.). Since calves frequently changed their eyes to observe the novel object and the eye-changing behaviour often occurred very quickly, it is quite difficult to accurately record their eye behaviours by watching the video recordings using common video players because common video players can only be accurate to the second. It thus may increase the range of errors and result in inaccurate results. This type of data is suggested to be collected using professional software, such as Observer and BORIS, which can be accurate to the millisecond.

In the research reported in Chapters 3 and 4, we illustrated that pre-weaning social housing and pre-weaning physical enrichment affected post-weaning calves' growth, behaviour and cognitive ability. However, the treatment differences were measured before weaning and within 3 weeks after weaning and regrouping. Whether those differences last beyond this time point is unknown. A long-term study following calves will help determine if the effects of early environmental complexity can endure for a prolonged period, and influence the lifetime health, adaptability to environmental changes and productive and reproductive performance of adult cattle.

In this dissertation, we only studied calves' behaviours and behavioural indicators of affective state and cognitive ability. In future work, it may be beneficial to measure the physiological changes underlying the behaviours that we measured in this dissertation so as to understand the mechanism underpinning the behaviours. Investigating brain development and hormonal expression can help us further understand how environmental complexity alters physiology. In rats, for example, increased grooming and maternal licking increase brain-derived neurotrophic factor mRNA and cholinergic innervation of the hippocampus, as well as enhanced learning and spatial memory (Liu et al., 2000). In this dissertation, we only studied the different memory abilities of calves from different treatments through the behaviour indicator but did not know the physiological changes occurring in the brain and pathways eliciting those changes. Therefore, studying the relationships between physiology and behaviour can improve our ability to assess different housing conditions of calves.

6.3 Conclusion

Fixed multi-item enrichment presentation schedule is an effective presentation method, with nets filled with scented hay being suggested as having more beneficial characteristics than other physical enrichment items to attract calves. Providing the physical enrichment items to individually and pair housed calves before weaning using the effective presentation method was found to improve pre-weaning calves' growth.

The pre-weaning physical enrichment and pre-weaning pair housing seem likely to satisfy pre-weaning calves' diverse natural behaviours and reduce their undesirable behaviour in different ways, whilst having no effects on their fear responses to novelty. Since pre-weaning calves do not have cerebral lateralization in processing fear emotion, eye laterality is not considered a useful measure of fear in calves at this age and initial monocular presentation is unlikely to have an effect on the repeatability of the novel object test. When calves from the pre-weaning treatments are weaned and regrouped to post-weaning group housing environments, the pre-weaning environmental complexity method is still suggested to have positive effects on calves' growth, memory and adaptability to environmental changes. In future work, additional research needs to investigate the effects of the location of physical enrichment items on calves' use of the items, the long-term effects of pre-weaning physical enrichment and pre-weaning social housing and the relationships between calves' physiology and behaviour in order to improve our ability to assess and improve the environmental complexity.

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