Dairy calves' preference for rearing substrate

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This thesis is dedicated to my mum

Sue Worth

Thank you for all of your support and encouragement.

Abstract

Rearing substrate is an important component of the pre-weaning environment of dairy calves. Traditional substrate types, such as sawdust, are becoming difficult and/or expensive for farmers to obtain in New Zealand. Therefore, there is a need to evaluate alternative rearing substrates for dairy calves that that are economically viable for farmers, readily available and provide an acceptable level of animal welfare. The preference of dairy calves for four different rearing substrates and the effects on behaviour and physiology were evaluated. At 1 wk of age, 24 calves were housed in groups of four, in pens which were evenly divided into four rearing substrates: sawdust, rubber, sand and stones. During the first 3 d calves were given free access to all four substrates. Calves were then restricted to each substrate type for 48 h. In order to rank preference, calves were subsequently exposed to two surfaces simultaneously for 48 h until calves experienced all six treatment combinations. Finally, calves were given free access to all four substrates simultaneously for 48 h. Lying behaviour and location in the pen was recorded for 24 h at the end of each experimental period using handycams and accelerometers. Preference was determined based on lying times on each substrate. The insulating properties of each substrate were assessed using iButtons®.

During the initial free choice period, the proportion of time spent standing ($p < .001$) and lying ($p < .001$) was influenced by substrate. Calves spent a higher proportion of time on sawdust (88%) than all other substrates (rubber: 6%, sand: 4% and stones: 3%).

When restricted to each substrate, calves spent more ($p < .05$) time running on sawdust, rubber and sand compared to calves on stones. There were no effects

($p > .05$) of rearing substrate on the frequency of jumps, buck/kicks, head to object and mount/frontal pushing. Calves spent more ($p < .05$) time lying on sawdust and rubber in comparison to sand and stones. There were no effects ($p > .05$) of rearing substrate on the number and duration of lying bouts. We detected no effect ($p > .05$) of rearing substrate on concentrations of cortisol, lactate, glucose, or white blood cell, neutrophil and lymphocyte count or the neutrophil:lymphocyte ratio. The insulating properties were greatest for sawdust and lowest for sand.

During the pairwise choice period, calves had a strong preference for one substrate over another, spending on average, 89% of their time on the preferred surface. Calves preference ranking was for sawdust, rubber, sand then stones as determined by the proportion of time spent on each surface. At the end of the study, when given free access to all rearing substrates again, calves spent a higher proportion of time on sawdust (85%) than all other substrates (rubber: 5%, sand: 7% and stones: 3%).

In conclusion, dairy calves showed a clear preference for sawdust over rubber, sand and stones. This preference remained consistent over the course of the study. The calves' preference for sawdust may be associated with the physical and thermal properties in comparison to the alternative substrates. However, factors such as cost to the farmer, availability and practicality of alternative substrates need to be considered along with animal preferences before any recommendations can be made.

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Table of Contents

Dedication ii

Abstract iii

Acknowledgements v

Chapter 1: General Introduction 1

Animal welfare 1

Management and housing 5

Preference testing 12

Play Behaviour 18

Chapter 2: Dairy calves' preference for rearing substrate 25

Introduction 25

2. Materials and Methods 30

2.1. Animals and husbandry 30

2.2. Treatments and rearing substrates 32

2.3. Experimental design 34

2.4. Behavioural measurements 35

2.5. Lying and standing behaviour 36

2.6. Physiological measurements 40

2.7. Environmental conditions 41

2.8. Statistical analysis 41

3. Results 44

First free choice period 44

3.1. Proportion of time on each surface 44

Restriction period 45

3.2. Play behaviour 45

3.3. Lying time 48

3.4. Physiological Responses 52

Pairwise choice period 53

Second free choice period 55

3.5. Proportion of time on each surface 55

Environmental Conditions 56
3.6. Temperature of rearing substrate.. 56
4. Discussion 57

Chapter 3: Conclusions and recommendations.. 66
Implications for animal welfare and final conclusions 66
Future Research 67

References 69
Appendix A 85
Appendix B 86

List of Figures

Figure 2.1. Experimental facility design and positioning of pens with different substrates. 33

Figure 2.2. Example of one of the free choice pens, showing all four rearing substrates. 35

Figure 2.3. Onset Pendant G data logger used to record standing and lying events. 37

Figure 2.4. Calf fitted with the Onset Pendant G data logger to record standing and lying events. 38

Figure 2.5. Proportion of time calves spent lying and standing on each substrate (n=6 groups, 4 calves/group) over a continuous 24 h observation period. 44

Figure 2.6. The total time calves spent running when restricted to each rearing substrate (n=6 groups, 4 calves/group) over a continuous 12 h observation period.. 45

Figure 2.7. Number of running bouts performed by calves (n=6 groups, 4 calves/group) when restricted to each substrate during a 12 h continuous observation period.. 46

Figure 2.8. The total time calves spent lying (min) when restricted to each rearing substrate (n=6 groups, 4 calves/group) over a 24 h recording period.. 48

Figure 2.9. Number of calves (n=6 groups, 4 calves/group) lying when restricted to each substrate over a 24 h recording period... 49

Figure 2.10. Number of calves (n=6 groups, 4 calves/group) lying when restricted to rubber (a), sand (b), sawdust (c) or stones (d) over a 24 h recording period in relation to hourly average temperature within the experimental facility .... 50 - 51

Figure 2.11. The proportion of time calves spent lying and standing on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period.. 53

Figure 2.12. The proportion of time calves spent standing (a) and lying (b) on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period.. 54

Figure 2.13. Proportion of time calves spent on each surface (n=5 groups, 4 calves/group) over a 24 h observation period.. 55

List of Tables

Table 2.1. Description of behaviour's recorded for free choice, pairwise choice and restriction periods by continuous sampling over 12 h and 24 h. 39

Table 2.2. Frequency of running and play events performed by calves (n=6 groups, 4 calves/group) during a 12 h continuous observation period when restricted to each rearing substrate.. 47

Table 2.3. Average number of lying bouts and lying bout duration performed by calves (n=6 groups, 4 calves/group) when restricted to each rearing substrate for 24 h. 49

Table 2.4. Blood chemistry and haematological measures of calves (n=6 groups, 4 calves/group) when restricted to each rearing substrate for 24 h. 52

Table 2.5. Summary of the mean internal and surface temperatures of the different rearing substrates at the different ambient temperatures tested. 56

Chapter 1

General introduction

Animal welfare

Public awareness regarding the welfare of animals under the care of humans is increasing, with particular reference to modern farming techniques and the use of intensive husbandry practises (Krachun, Rushen, & de Passillé, 2010; Rushen, de Passillé, von Keyserlingk, & Weary, 2008). In the early 1980's the United Kingdom Farm Animal Welfare Council (FAWC) established what is now known as the 'five freedoms' (Mellor & Stafford, 2001). These are freedom from thirst and hunger, discomfort, pain, injury or disease, fear and distress and freedom to express normal behaviour (Gonyou, 1994; Rushen et al., 2008). The five freedoms are formulated as ideals of animal welfare. They are a useful guide to help identify welfare problems across numerous species and indicate the direction to follow in order to improve animal welfare (Broom, 1988; McCulloch, 2012; Rushen et al., 2008). The five freedoms are well known throughout farming, policy making and academic circles. They form the foundation for legislation and codes of recommendations and are still widely referenced today (Green & Mellor, 2011; McCulloch, 2012). Four of the five freedoms are derived from negative outcomes and the major concern for animal welfare is focused on indicators of poor welfare and negative outcomes. More recently, focus has shifted to include the presence of positive experiences in welfare assessment (Boissy, Manteuffel, Jensen, Moe, Spruijy, Keeling Winckler, Forkman, Dimitrov, Langbein, Bakken, Veissier, & Aubert, 2007; Green & Mellor, 2011; Yeates & Main, 2008).

Much research in animal welfare has been undertaken in the last few decades, and there has been huge progress in assessing how animals perceive their environment (Boissy et al., 2007; Veissier, Aubert, & Boissy, 2012). The scientific study of animal welfare is multi-disciplinary and includes fields such as ethology, physiology, biochemistry, genetics, immunology and nutrition. What constitutes animal welfare has been influenced by what society deems as acceptable and unacceptable ways of treating animals (Green & Mellor, 2011) and has led to vague and often contradictory views on how welfare can be assessed (Green & Mellor, 2011; Fraser et al., 1997). As a result, there is no universal view of what constitutes good animal welfare (Green & Mellor, 2011) and this lack of agreement has led to contrasting research methods and interpretations of results (Fraser et al., 1997; Rushen & de Passillé, 1992). Animal welfare can be characterised as state within an animal, therefore, the animal must be both sentient and conscious as welfare relates to the experienced sensations that may be negative, neutral or positive. The welfare status of an animal will vary as the balance of these inputs change. Indirect indices of these negative, neutral or positive experiences rely on physiological and behavioural responses that may be evaluated to reflect the overall welfare status of an animal (Mellor & Stafford, 2008).

Individuals have different concerns when judging how animals should be treated and what constitutes a good life. For example, those that are concerned with the biological functioning of the animal, those concerned with how the animal is feeling, such as ‘affective state’ and those that judge good welfare as allowing the animal to live a natural life (Fraser, 2003). These three views of animal welfare are closely related to the individual’s world view and beliefs, and have contributed to a more comprehensive understanding of animal welfare (Fraser, 2003; Green & Mellor, 2011). The first view of biological functioning is held by people involved in animal production, such as veterinarians and farmers. Providing an animal grows well, is free from stress, disease, injury and reproduces successfully its welfare is deemed to be good. The second view emphasises suffering and the affective state or emotions of animals with focus centred on how the animal is feeling. Negative feelings are likely to include pain, fear, hunger and anxiety. Positive feelings include contentment, curiosity, exploration and play. An animal in a positive affective state can adapt to emotional experiences without negative experiences occurring during interactions with humans, other animals and their environment. Finally, the third view emphasises natural living. This view was developed in parallel with the other two views. Natural living comprises of the animal having the ability to express normal, natural behaviours and that the animal should be living away from the constraints of modern society (Fraser et al., 1997; Fraser, 2003; Green & Mellor, 2011; Rushen et al., 2008). The view of natural living is difficult to incorporate into animal welfare assessment as aspects of this view are prevented in modern production systems and may not be adequate in terms of its full welfare implications (Broom, 2011; Green & Mellor, 2011). Natural living can highlight negative consequences within modern systems such as restrictive housing and has relevance when considered alongside biological function and affective state (Green & Mellor, 2011).

The idea that animal welfare is linked to stress (the physiological response to stressors) has grown since the 1960’s, when it was recognised that stressed animals were experiencing ‘poor’ welfare conditions and non-stressed animals were experiencing good welfare conditions (Volpato, Giaquinto, Castilho, Barreto, & Freitas, 2009). Indicators of poor welfare can be caused by disease or injury and can be straightforward to recognise and quantify e.g., gait scoring for signs of lameness. However, the effects of a suppressed immune system or of a reduction in food intake are less obvious, but they can be assessed to indicate that something is amiss before clinical symptoms are noticed (Dawkins, 2006). Physiological measures commonly used in welfare assessment include sympathetic responses such as, changes in heart rate, respiratory rate, body temperature, the secretion of corticosteroids (e.g., cortisol) and elevated plasma glucose and lactate concentrations. These measures can potentially be difficult to interpret and can also be invasive (e.g., blood sampling) which may cause a 'stress' response confounding the measurement of interest (Dawkins, 2003, 2006; Stewart, Webster, Schaefer, Cook, & Scott, 2005). The use of behaviour in animal welfare research has the advantage that it is non-invasive, non-intrusive, results in the animal making its own decisions and serves as a clinical symptom in its own right. Through the study of behaviour, animals can indicate their preference or aversion to certain environments giving direct insight into how an animal perceives its situation (Dawkins, 2003, 2004). Today, animal welfare science is recognised as multi-disciplinary, where no one measure can indicate whether welfare is good or bad (Nicol, Caplen, Edgar, Richards, & Browne, 2011). Incorporating both behavioural and physiological measures into the study of animal welfare can provide an accurate and robust evaluation (Lane, 2006).

In summary, societal concerns towards the treatment of intensively farmed animals and the increased demand for 'animal welfare friendly' products has been the driving force behind the scientific study of animal welfare. Animal welfare science is centred on questions regarding animal consciousness, health and emotion. While these cannot be measured directly, different techniques and approaches from other scientific fields are incorporated to make the science of animal welfare truly multi-disciplinary. When examining animal welfare it is beneficial to consider the three overlapping views: biological functioning, affective state and natural living to allow for a more comprehensive approach to the concerns prompted by the public. Whilst there is no single measure to indicate whether welfare is good or bad, using both behavioural and physiological measures allows researchers to ascertain what matters to the animals themselves.

Management and housing

All intensively farmed animals are species that are social and live within a group or interact with individuals of their own species. However, living within a social group does have its own set of advantages and disadvantages. The advantages of group living include a lower predation risk, companionship and a reduction in fear to novel situations. Costs are typically associated with competition for resources that are valuable to an individual's fitness (i.e., food and mates). The cost of group living is much the same for farmed animals. Individuals will compete for the resources made available to them, including food, water, lying spaces or the ability to move in a restricted space (Estevez, Andersen & Nævdal, 2007). Typically in dairy farming, animals are grouped for a number of reasons (i.e., age, days in milk, feed requirements and health status), each grouping exposes an animal to new individuals and changing group dynamics which can create social instability (Schirmann, Chapinal, Weary, Heuwieser, & von Keyserlingk, 2011). A newly created social group can result in some individuals having fewer opportunities to access resources and may result in increased aggression within the group. Group size and density of animals in the wild is determined by the resources available, which fluctuate as the conditions vary. However, consideration of what is best for individual animals is often forgotten as farmers increase their stocking densities to maximise their production (Estevez et al., 2007).

Trends in intensive animal production are a result of economic pressure to increase capital, the number of animals per farm and reduce manual labour. There is concern that as a result large numbers of animals are then housed at high stocking densities, with a minimum labour input from farmers. Farmers typically do not have sufficient time per animal or opportunity to modify the environment to best accommodate their livestock requirements (Baxter, 1983). As the New Zealand dairy farming industry continues to grow, so does the average herd size. During the 2012/2013 season, the average herd size was 402, this number has tripled in the last 30 seasons, increasing by 117 cows in the last 10 seasons (LIC & DairyNZ., 2013). Group size and density are key components associated with the costs and benefits of group living, the wellbeing of an animal may be compromised if these factors are not considered when making on-farm decisions. If animals are housed within large groups, potentially members are unable to maintain a stable hierarchy which could result in increased aggression and stress responses and a reduction in live weights. Subordinate animals may also lack opportunities to gain access to resources (Estevez et al., 2007).

Calves in New Zealand are typically housed indoors, at group sizes averaging 5 to 15 calves per pen, with little or no outdoor access until weaning (Capel, n.d.; Rushen et al., 2008). The implication for cattle husbandry is that early social relationships between calves are beneficial for them. Calves that have been together since 2 wk of age develop preferential relationships that last for at least 1.5 years (Raussi, Niskanen, Siivonen, Hänninen, Hepola, Jauhiainen, & Veissier, 2010). When regrouped at 2 months of age, dairy calves were found to displace unfamiliar calves from feed more often than familiar calves (Færevik, Andersen, Jensen, & Bøe, 2007).

Access to comfortable resting places can be a source of competition even in situations where enough physical space is provided, possibly due to bedding conditions, drafts or the availability of a wall (Færevik, Tjentland, Løvik, Andersen, & Bøe, 2008). The preference for lying against a wall rather than in an open space has been shown in both sheep (Bøe, Berg, & Andersen, 2006), goats (Andersen & Bøe, 2007) and bulls (Gygax, Siegwart, & Wechsler, 2007). The same experiments showed that a reduced lying space allowance resulted in a reduction in total lying time, less synchronous resting and more aggressive interactions. The European Union legislation states that group housed animals should have the ability to lie simultaneously in order to synchronise their behaviour. Calves should be provided with access to a comfortable lying area, with a minimum space allowance of 1.5 m^2 /calf at a live weight of less than 150 kg (Færevik et al., 2008; Jensen & Kyhn, 2000). In New Zealand, there are no requirements or guidelines on space allowance for rearing dairy calves however, the industry standard is 1.0 m^2 /calf and 1.5 m^2 /calf is recommended (On-Farm Research, 2014). Space allowance during the pre-weaning phase has been shown to influence calf behaviour. The total time spent lying was similar when calves were reared at space allowances ranging from 0.75 to 4 m^2 (Færevik et al., 2008; Tapkı, Şahin, & Önal, 2006) however, synchronous resting reduced with smaller space allowances (Færevik et al., 2008). Calves reared at 2.0 m^2 /calf spent less time lying and more time standing and walking compared to calves reared at a space allowance of 1.0 or 1.5 m^2 /calf (Sutherland, Worth, & Stewart, 2014). These results suggest that an increase in space allowance provides calves with more opportunity to perform active behaviours and to synchronise their resting behaviour and should be taken into account when designing housing systems for dairy calves.

The early pre-weaning phase of a dairy calf's life is crucial. Calves are separated from their dams within 24 h of birth (as within dairying systems, milk is the product to be sold) and placed into an environment where they are highly susceptible to the spread of disease as their immune systems are almost non-existent and the digestive system is immature (National Animal Welfare Advisory Committee, 2010; Panivivat, Kegley, Pennington, Kellog, & Krumpelman, 2004; On-Farm Research, 2014). Calves are vulnerable to temperature fluctuations (both hot and cold) and require access to some form of shelter or shade (Panivivat et al., 2004; Rushen et al., 2008). The type of substrate used in calf rearing facilities can provide warmth and comfort, but can also affect calf cleanliness (Panivivat et al., 2004), weight gain, the incidence of scouring (Hill, Bateman, Aldrich, & Schlotterbeck, 2011) and skin surface temperature (Sutherland, Stewart, & Schütz, 2013). Rearing substrates can vary in dry matter content, production of ammonia, substrate surface temperature and bacteriology, and therefore can have a differential impact on the health and welfare of calves (Camiloti, Fregonesi, von Keyserlingk, & Weary, 2012; Hill et al., 2011; Panivivat et al., 2004). Adequate volumes of clean and dry rearing substrates are essential for the health of calves (Capel, n.d); providing a deep volume of substrate that allow calves to nestle within (i.e., straw) has been shown to reduce the prevalence of respiratory disease (Lago, McGuirk, Bennett, Cook, & Nordlund, 2006). Traditionally, organic materials (e.g., straw and wood shavings) have been used as rearing substrates for farm animals. Recently the trend is to move away from traditional bedding materials, due to hygiene concerns, and labour and transportation costs, which can affect the total on-farm price and use (Kartal & Yanar, 2011; Panivivat et al., 2004). Substrates recently investigated include sand, rice hulls (rice husks), rubber mats and concrete (Hänninen, de Passillé, & Rushen, 2005; Hill et al., 2011; Panivivat et al., 2004). To improve a calf's welfare, it is important to select clean, dry, comfortable and warm materials for bedding (Kartal & Yanar, 2011; Panivivat et al., 2004; Rushen et al., 2008).

Panivivat et al. (2004) investigated the effects of different bedding materials (sand, straw, wood shavings, rice hulls and granite fines (by-product of crushing syenite granite rock)) on the growth performance, health, and welfare of dairy calves. Calves were individually housed on 1 of the 5 substrates, with growth rates, health, cleanliness and behaviour measured. Results showed no significant differences in calf growth rates irrespective of the bedding material used, however, calves reared on the firmer surfaces (granite fines and sand), were treated with antibiotics for scours more often and calves on granite fines were scored the dirtiest overall (Panivivat et al., 2004). Calves reared on rice hulls and sand spent more time self-grooming than calves reared on straw, but no other behavioural differences were found (Panivivat et al., 2004).

The quality and quantity of substrate has been noted to influence the behaviour and comfort of dairy cows. Cows were found to spend more time lying on softer substrates, when given the choice. Sawdust and sand were chosen in preference to a rubber-filled geotextile mattress (Tucker, Weary, & Fraser, 2003) and cows spent more time lying in stalls with greater quantities of substrate (Tucker, Weary, von Keyserlingk, & Beauchemin, 2009). Time spent lying down can provide valuable information about how comfortable an animal finds a surface (Tucker et al., 2003). Dairy cows have also been shown to spend more time lying on straw when given the choice between straw and sand in a free stall (Norring, Manninen, de Passillé, Rushen, & Saloniemi, 2010). Adequate rest is also essential for the welfare of young calves (Yanar, Kartal, Aydin, Kocyigit, & Diler, 2010); calves lie down to rest for typically 18 h/d (Panivivat et al., 2004) and a reduction in lying times can influence growth rates (Hänninen et al., 2005). Little is known about the effects of rearing substrate on the lying behaviour of calves. However, it has been shown that when given a choice between concrete and sawdust, 2 wk old calves prefer lying on sawdust (Camiloti et al., 2012). This suggests that softer surfaces are comfortable and important to young calves.

Within New Zealand, organic materials for rearing substrates are becoming difficult and/or expensive to obtain, which has driven farmers to seek appropriate alternatives. The use of stones as an alternative rearing substrate for calves is increasing in popularity. Stones are used by some farmers due to cost effectiveness, availability and perceived health benefits. The type of stones farmers use are also found in gardens for decorative purposes or used for drainage. Sutherland et al. (2013) found that calves reared on stones spent less time performing locomotor play, preformed a less complex repertoire of play behaviours and had reduced lying times at 5 wk of age compared to calves reared on sawdust. There were no differences in lying times when calves were 1 wk of age, which may be due to young calves having a high motivation to lie down, irrespective of substrate type. Reduced lying behaviour for calves at 5 wk of age, reared on stones may be due to the differences in compressibility or softness between the two substrates. Preference testing can be used as a tool to assess how an animal perceives the substrate it is reared on. When feedlot lambs were given the choice of 4 substrates: sawdust, waste paper, straw and rice husk, the total time spent lying and lying time on each substrate were significantly different (Teixeira, Miranda-de la Lama, Pascual-Alonso, Aguayo-Ulloa, Villarroel, & María, 2013). Lambs spent more time on sawdust and of that time, 80% was spent lying down. Straw and rice husks were the least preferred substrates, with 6% and 7% of the lambs time spent on these surfaces respectively. The distinct preference for sawdust may be related to its physical and thermal properties compared to that of the other substrates (Teixeira et al., 2013).

As already mentioned, the pre-weaning phase of a dairy calves' life is crucial as they are highly susceptible to the spread of disease and vulnerable to changes within their environment; dry and clean surfaces are important. Throughout the literature rearing substrates have been shown to impact calf physiology and production, such as weight gain and body temperature and to impact their behaviour through changes in lying times and the occurrence of play. Adequate rest is essential for the welfare of calves, therefore, lying times can provide valuable information about how comfortable an animal finds a surface. One way to assess how an animal perceives its environment is to use preference testing to assess their preference for alternative substrates.

Preference testing

The decisions that animals make are influenced by their previous experiences, either good or bad, and can provide information regarding their welfare. The welfare of captive animals is enhanced if they are provided with access to environments, objects and activities that they prefer (Dawkins, 1983; Temple & Foster, 1980). However, most assessments of animal welfare are based on what an animal does rather than what it perceives or feels (Gonyou, 1994). The behavioural assessment of an animal's welfare should involve both what an animal likes and dislikes (Dawkins, 1983). In order to facilitate this, it is necessary to establish what animals rank as important in their environments (Beattie, Walker & Sneddon, 1998). Preference testing has been used since the 1970's to identify how animals perceive certain aspects of their environment (Beattie et al., 1998; Fraser & Matthews, 1997; Nicol, Caplen, Edgar, & Browne, 2009). The principal assumption is that animals will make choices that are in their own best interest. Knowledge of the preferences shown by animals under different conditions can be used to make recommendations regarding animal husbandry to ultimately improve welfare (Bateson, 2004; Beattie et al., 1998; Fraser & Matthews, 1997; Rushen et al., 2008).

The first experiment using preference testing to understand a farm animal welfare issue arose from recommendations made by the Brambell Committee (Fraser & Matthews, 1997), a technical committee appointed by the British government to enquire into the welfare of intensively farmed animals (Gonyou, 1994). The Brambell Committee recommended that 'chicken wire' (thin, hexagonal netting) was unsatisfactory as a flooring material in hen cages because hen's feet are not well adapted to grip. The committee suggested that a heavygauge metal mesh in a rectangular shape would be more comfortable. However, this recommendation was based upon assumption rather than experimental evidence (Fraser & Matthews, 1997; Gonyou, 1994; Hughes & Black, 1973). To gain a scientific understanding from the hen's point of view, Hughes and Black (1973) tested hen's preference for four different types of flooring. The hens were housed in cages with two sections that were floored with different materials. The materials were offered as pair-wise comparison and the time hens spent on each option was observed to determine preference. The results showed that hens had no strong preference or aversion for the different materials however, their overall preference was for the material that had previously been considered unsuitable by the Brambell Committee (Fraser & Matthews, 1997; Gonyou, 1994; Hughes & Black, 1973). The key finding from this study highlights the point that humans and other animals differ in their view about what is important (Foster, Temple & Poling, 1997). Since this early experiment, preference testing has been widely used in animal welfare research (Fraser, 1993; Fraser & Matthews, 1997).

There are three main approaches to assess the preferences of animals: 1) motivational or operant conditioning, 2) T-maze procedures and 3) choice tests (Sumpter, Foster, & Temple, 2002). Operant conditioning tests involve giving an animal access to one environment or resource at a time, where animals are required to perform a response in order to obtain access to the resource, e.g., pressing a lever. If the animal makes the required response, the resource becomes available intermittently, demonstrating motivation for the resource (Foster et al., 1997; Kirkden & Pajor, 2006). Additionally, the greater the number of responses the stronger the motivation is said to be (Krikden & Pajor, 2006). This method can be used to determine a preference over two resources offered one after the other.

More commonly, it is used to test the strength of an animal's preference for a single resource both before and after there has been a change in the environment (Kirkden & Pajor, 2006). For example, the effect of feeding motivation of pigs was assessed by diluting nutrient restricted food with straw. Pigs were found to increase their response rate when their meal was diluted with straw compared to pigs receiving a maintenance or rationed meal (Lawrence, Appleby, Illius, & MacLeod, 1989).

The second approach, known as a T-maze, requires the animal to turn left or right within a T-maze in order to access one of two environments. Preference for an environment is assessed on the number of times one arm of the T-maze is chosen and/or the amount of time it takes for the animal to make its choice (Sumpter et al., 2002). The T-maze procedure has been used as a measure of choice behaviour in dairy cows to assess noise avoidance (Arnold, Ng, Jongman, & Hemsworth, 2008), various handling treatments including shouting, hitting and electric shocks (Pajor, Rushen, & de Passillé, 2003) and restraint procedures in sheep (Rushen,1986), deer (Pollard, Littlejohn, & Suttie, 1994) and beef cattle (Grandin, Odde, Schutz, & Behrns, 1994). However, results can be difficult to interpret. If the preferred alternative is deemed from the larger percentage of animals choosing it, it remains unclear how to interpret the percentage of animals that select the other alternative (Sumpter et al., 2002).

Choice tests are the third approach and are useful when establishing an animal's environmental preference as they focus on the decisions animals make between resources or stimuli (Kirkden & Pajor, 2006). The measure of preference is taken from the amount of time an animal spends on an option, the number of times it chooses one option over another, or consumes in a greater quantity

(Kirkden & Pajor, 2006; Nicol et al., 2009). An early example of this method is the Hughes and Black (1973) study. Choice tests enable researchers to ascertain whether an animal is motivated to obtain or avoid a resource and can also identify preference between two or more resources (Kirkden & Pajor, 2006). As a measure of preference, choice tests are relatively easy to conduct as they require a simple response from the animal and provide researchers with rapid results (Sumpter et al., 2002). There are limitations associated with choice tests. An animals' choice is restricted to the available options and having a preference for one environment or resource does not necessarily indicate the value of the alternative therefore, it cannot be suggested that it is ideal for the animal (Rushen et al., 2008; Sumpter et al., 2002).

Preference depends on previous experience, an animal may spend more time in an environment because it is familiar with it rather than because it is preferred and avoid the unfamiliar environment (Dawkins, 1977; Fraser & Matthews, 1997; Kirkden & Pajor, 2006). We presume that when given a choice, animals will actively seek out environments that they find comfortable and avoid others they find less comfortable however, animals do not always choose what is in their best interest. While choice tests indicate an animal's immediate response, they may not reflect long-term preference (Dawkins, 1983; Beattie et al., 1998; Rushen et al., 2008). Also, the resource that is chosen more often may not necessarily be the preferred option and in some cases, preference can alter with a change in an external variable (Kirkden & Pajor, 2006). For example, dairy cows preference for bedding types can differ depending on the time of year in which the tests are performed. Cows were found to prefer straw to rubber mats in winter which may be due to the thermal properties of the bedding materials (Manninen, de Passillé, Rushen, Norring, & Saloniemi, 2002).

Ultimately, the use of preference and motivational testing to investigate how an animal feels is very important in animal welfare research. These tests allow us to infer that an animal prefers one environment over another but cannot allow us to conclude that the animal likes or dislikes both environments or that the animal is suffering in the environment that is least preferred (Dawkins, 1977). All three approaches have strengths and limitations. Providing that the preference and motivational tests are well designed and the results from these tests are carefully interpreted, they hold merit as ultimately they allow animals to express what is most important to them (Kirkden & Pajor, 2006; Rushen et al., 2008). “Animals may not be able to talk, but they can vote with their feet and express some of what they are feeling by where they choose to go” (Dawkins, 1980, p. 91).

Four directions and challenges for future research on animal preference have been proposed by Fraser and Matthews (1997). Firstly, preference tests need to identify the factors that influence the preferences that animals show. For instance, when comparing alternatives, the preference or preference ranking is established among the options. For example, the use of shade and sprinklers at the milking parlour during the afternoon is common practice during the New Zealand summer and is an effective way to reduce the heat load of dairy cows. The use of sprinklers during milking can reduce body temperature for up to 4 hours post milking (Kendell, Verkerk, Webster, & Tucker, 2007). To assess preference for cooling options during summer, dairy cows were assessed with different paired choices: 1) shade or sprinklers, 2) shade or ambient conditions and 3) sprinklers or ambient conditions. Dairy cows preferred to use shade over both sprinklers and ambient conditions and did not show a preference between sprinklers or ambient conditions despite sprinklers being more efficient at reducing heat load (Schütz, Rogers, Cox, Webster, & Tucker, 2011).

Secondly, preference research has typically been conducted separately from other types of animal welfare research. By incorporating physiological measures, a clearer picture can be provided as to how an animal perceives its environment. Animals kept within an environment they prefer should have lower stress levels and perhaps greater health. For example, when hens chose an environment they preferred, their levels of blood glucose, heterophil:lymphocyte ratio and body temperature were lower than when in environments they found more aversive, in addition hens had an increase in feed digestibility and self-grooming behaviour in the preferred environment (Nicol et al., 2009).

The third challenge is to have an understanding of the animal's behaviour. Having this information helps to identify variables that are of importance to the species studied. For example, calves are highly social animals, however, throughout Europe individual housing is common practise. Holm, Lopes, de Oliveira and Guidoni (2002) assessed the motivational priority of calves for social contact. Calves were more motivated to seek social contact and utilised more of the reward period for direct social interactions. These results indicate the value of social behaviour for calves and suggest that individual housing impairs their full social behaviour repertoire. Finally, the fourth challenge is to use environmental preference testing in the design of animal environments. Having a grasp of what an animal's environmental preferences are would allow for better designed environments that are tailored to the priorities of the animal. For example, when given the choice of four substrates: sawdust, waste paper, straw and rice husk, lambs had a distinct preference for sawdust with a 47% occupancy rate. The preference for sawdust may be attributed to the physical and thermal properties in comparison to the other substrates offered (Teixeira et al., 2013).

Evaluating animal welfare requires an understanding of how animals react to different environments. Preference tests are a tool in the assessment of animal welfare as they examine how an animal perceives its environment. Allowing an animal to express its own behavioural priorities provides insight into what is important to them and the value of an environment. There are limitations with the use of preference tests, but these problems are considered to be outweighed by the benefits of allowing an animal to choose. Providing results are interpreted carefully and used in conjunction with other measures of animal welfare assessment, preference tests provide scientists with a basis to form recommendations regarding animal husbandry and to ultimately improve welfare.

Play Behaviour

The measurement of an animal's behaviour has a number of major advantages in terms of welfare assessment. It is non-invasive, does not disturb the animal, and allows the animal to express its normal behaviour (Dawkins, 2004). Play has been identified as a useful behavioural measure of animal welfare as it is generally expressed when an animal's basic needs are met and drops from the behavioural repertoire when conditions become challenging (Dawkins, 2006; Held & Špinka, 2011; Jensen, Vestergaard, & Krohn, 1998; Krachun et al., 2010). Within animal welfare, data from the measurement of play has the potential to contribute to the development of standards, policies and practices (Fraser, 2009). The amount of play can highlight conditions when an animal's welfare may be compromised or when conditions are favourable (Held & Špinka, 2011). Play is thought to have a key role in terms of an animal's development as it is more commonly observed in young animals. It can shape development and help juveniles to adapt to the environment by exploring and experimenting, creating effective strategies for potential changes within their environment (Donaldson, Newberry, Špinka, & Cloutier, 2002; Pellegrini, Dupuis, & Smith, 2007; Špinka, Newberry, & Bekoff, 2001). However, assessment of play behaviour is not without its limitations. Play includes vast behavioural categories, it varies between and within species and it can be difficult to define due to its lack of obvious consequence in comparison to behaviours such as aggression, communication and predatory behaviour (Bekoff, 1984; Held & Špinka, 2011; Holloway & Suter, 2003; Martin & Caro, 1985). Regardless of these issues, observers can generally agree that an animal is playing. Outwardly, play is a 'purposeless' and 'non-serious' behaviour, however, animals will not do something unless the benefits exceed the costs. If there is no benefit to play, it could be predicted that play will not occur. One theory behind why animals play is that it has a wide variety of benefits to the animal that may be small or undetectable to the observer (Martin & Caro, 1985).

Play is typically characterised into three different forms; object, social and locomotor play. Object play refers to play with an inanimate object either occurring naturally within the environment or a human-provided object. Object play is thought to relate to the development of motor skills and can be solitary or social. Social play refers to playing with others, preferred play mates are generally of similar age and size. Behaviours such as play fighting are thought to provide the animal with skills needed for hunting, fighting or mating. Social play enables animals to develop the necessary social skills for integration into group living. Locomotor play refers to activities such as running, jumping and kicking. These activities are thought to provide the animal with exercise, training and physical development which aid in the strengthening of bones, muscles and the refinement motor skills. Both social and locomotor play can occur together as animals often perform these behaviours as a group (Dugatkin, 2008; Jensen & Kyhn, 2000; Oliveria, Rossi, Silva, Lau, & Barreto, 2010; Pellegrini et al., 2007). As play behaviours are complex in their development, with different movement patterns occurring at different stages in the animal's development, there is a possibility that diverse benefits are received at each of these stages (Barber, 1991).

Many hypotheses have been proposed as to why play occurs, (1) the enhancement of physical development, (2) the formation of social bonds and (3) the development of cognitive abilities (Thompson, 1996). The period in an animal's life when the majority of play occurs also coincides with the period of physical, hormonal and social development, suggesting that play behaviour assists in juvenile development (Oliveria et al., 2010). The long-term benefits of play include stronger bone, muscle, cardiovascular condition, learning and acquiring social skills which enhance the animals' actions so they are better equipped for future situations (Oliveria et al., 2010; Rushen et al., 2008). Certain aspects of play, for example social play, can be innovative, for example, where juveniles learn the behaviours and strategies of adults and then develop them into novel situations for play (Pellegrini et al., 2007). Play fighting may also serve in the development of cognitive and social skills, where animals reverse dominant and subordinate roles. Larger and more dominant individuals handicap themselves in play fights with smaller and weaker individuals, allowing them the opportunity to act as if they are dominant. Play fighting may also enable the juvenile to interpret the intentions of others, such as lying, which may be more beneficial than physical skills (Dugatkin, 2008; Goodenough, McGuire & Wallace, 2001). Locomotor play has also been found to increase an animal's agility, assist in the fine-tuning of responses to novel situations, facilitate the ability to recover quickly from collisions with others or objects and to help maintain composure while running (Oliveria et al., 2010; Špinka et al., 2001). Juvenile animals have the highest play drive which appears to arise spontaneously, without prior experience (Holloway & Suter, 2003). Play may benefit an individual physically with the formation of connections between neurons in the brain, especially in the cerebellum, which is important in motor coordination and memory of motor patterns (Dugatkin, 2008; Goodenough et al., 2001).

Play behaviour arises early in an animal's life and has been observed to occur at high frequencies shortly after birth and slowly decline thereafter (Jensen et al., 1998). All forms of play follow an inverted-U curve in relation to development, where the peak period of play is observed during the juvenile period, relating to the availability of resources and protection from adults and then tapering off around puberty (Pellegrini et al., 2007; Trezza, Baarendse, & Vanderschuren, 2010). In some instances it can extend throughout adolescence and into adult life however, at these stages in the life cycle, play would be more of a direct strategy rather than learning a new skill (Burghardt, 2005; Donaldson et al., 2002; Pellegrini et al., 2007). A reduction in the amount of play can be a valuable indicator of an animal's physical and mental wellbeing and can result from changes in environmental and housing conditions, or food availability. Play is easily interrupted by threats to an individual's fitness (Held & Spinka, 2011).

For example, in dairy calves, a reduced milk allowance and early weaning reduces the duration of play running (Krachun et al., 2010). The duration of play running was also found to decrease by 70% in response to hot-iron dehorning in dairy calves (Rushen & de Passillé, 2012). This type of research has led to the belief that the occurrence of play indicates that the animal is relaxed in the sense that it is free from sickness, hunger, injury and pain (Held & Spinka, 2011).

Play is typically a low cost and low risk way for animals to learn new behaviours in an environment where resources are abundant (Pellegrini et al., 2007). In most situations, the occurrence of play will only account for 10% of an animal's day to day activity (Martin & Caro, 1985) and play has been reported to be as low as 0.2 to 2% of the day (Jensen & Kyhn, 2000; Vilá, 1994) which is minimal in terms of time and energy budgets (Bekoff & Byers, 1992). Therefore, observing play naturally can involve long periods of observation which is not practical for on-farm welfare assessment (Krachun et al., 2010). Another way to ensure that animals will play during the set observation period is to induce the behaviour. This has been used in dairy cattle as a result of prior confinement (Jensen et al., 1998; Sutherland , Worth, Schütz, & Stewart, 2014), through the addition of fresh bedding (Špinka, et al., 2001) or through using an arena or open-field test (Jensen & Kyhn, 2000; Mintline, Wood, de Passillé, Rushen, & Tucker, 2012; Sutherland et al., 2014). The use of arena tests, for research purposes, is increasing as it is an efficient way to measure animal's propensity to play such tests are used to assess how different procedures or housing conditions affect an animal's motivation to perform locomotor play (Mintline et al., 2012).

The effect of rearing substrate in the home environment on the motivation of dairy calves to play in an arena test was examined by Sutherland et al. (2014). Calves were reared on either sawdust or stones and remained in these pens from 1 wk of age up until 6 wk of age. Calves were tested individually in an arena, at both 3 and 6 wk of age for 20 min. Results show that calves reared on stones spent more time performing locomotary play at both 3 and 6 wk of age than calves reared on sawdust. These results indicate that housing conditions can affect calves motivation to play in an arena test, which may reflect a rebound effect associated with reduced activity from the rearing substrates provided (Sutherland et al., 2014). Furthermore, the motivation to perform locomotor play behaviours was assessed in dairy calves by Jensen (2001) using an arena test. Calves denied access to the arena for 3 days performed higher durations of locomotor play and less time standing still than calves that had 1 or 0 days deprivation. This increase in play behaviour after confinement has also been reported in other species and has been suggested to be a release of built-up emotional tension (Brownlee, 1984).

In summary, play behaviour has a role in the assessment of animal welfare as it can be influenced by physical and environmental conditions. Play has long-term physical and social benefits for juvenile animals. An absence or reduction in play can be a valuable indicator that the animals' welfare is compromised as play is removed from the behavioural repertoire under challenging situations, for example, dehorning and weaning. Therefore, play behaviour can be used as a welfare tool to assess housing and management practises for rearing juvenile animals.

In conclusion, animal welfare can be assessed using the biological function, affective state and naturalness model. One important animal welfare issue is the management of rearing dairy calves, in particular rearing substrates. Initial work has predominantly focused on biological function. However, assessing calves' preference as well as affective state, through behaviour such as play may help us understand how calves perceive their rearing environment.

Chapter 2

Dairy calves' preference for rearing substrate.

Introduction

The rearing and management techniques used during the pre-weaning period of a dairy calf's life need to reduce stress and to minimise the risk of disease (Panivivat et al., 2004; Sutherland et al., 2013). Calves are vulnerable to temperature fluctuations (both hot and cold) and require access to some form of shelter or shade (Panivivat et al., 2004; Rushen et al., 2008). Traditionally, organic materials such as sawdust and wood shavings were used as rearing substrates for dairy calves. As previously discussed, the recent trend is to move away from such materials, due to hygiene concerns, labour and transportation costs which affect the total on-farm price and use (Kartal & Yanar, 2011; Panivivat et al., 2004). Therefore, there is a need to evaluate alternative rearing substrates for dairy calves that are economically viable for farmers, readily available and provide an acceptable level of animal welfare.

Rearing substrates evaluated throughout the literature include concrete, straw, stones, granite fines, rice hulls, rubber mats, wooden slats, sand and wood shavings or sawdust (Camiloti et al., 2012; Hänninen et al., 2005; Panivivat et al., 2004; Sutherland et al., 2013; Yanar, Kartal, Aydin, Kocyigit, & Diler, 2010). The type of substrate used in calf rearing facilities can provide warmth and comfort, but can also affect calf cleanliness (Panivivat et al., 2004), weight gain, scouring (Hill et al., 2011) and skin surface temperature (Sutherland et al., 2013). Adequate levels of clean and dry rearing substrates are essential for the health of calves (Capel, n.d). Providing a deep volume of substrate to allow calves to nest within (i.e., straw) has been shown to reduce the prevalence of respiratory disease (Lago et al., 2006). Rearing substrates have also been found to influence the behaviour of calves, for example Panivivat et al. (2004) found that calves reared on sand or rice hulls, spent more time self-grooming than calves reared on long wheat straw. In addition, when given a choice between concrete and sawdust, 2 wk old calves showed a preference for lying on sawdust and an aversion to concrete (Camiloti et al., 2012).

As discussed in the previous chapter, the choices that animals make and how this relates to animal welfare has been studied since the 1970's with the use of preference tests (Fraser & Matthews, 1997; Nicol et al., 2009). Preference tests appear to be a direct and simple way of assessing how an animal perceives its environment (Rushen et al., 2008) and can be used to examine the choices animals make between resources (Kirkden & Pajor, 2006). This technique assumes that animals will be highly motivated to interact with and/or have access to resources they need or recognise as important (Teixeira et al., 2013). How animals perceive the substrates they are housed on has been used to contribute to recommendations on how to improve comfort and efficiency of management techniques for dairy cows, lambs, sheep and goats (Bøe, Andersen, Buisson, Simensen, & Jeksrud, 2007; Færevik Andersen, & Bøe, 2005; Teixeira et al., 2013; Tucker et al., 2003). Preference tests allow us to infer that an animal prefers one environment over another but do not allow us to conclude that the animal likes or dislikes both environments or that the animal is suffering in the environment that is least preferred (Dawkins, 1977). Incorporating information on an animal's physiological response to the environment might assist in the interpretation of anomalous choices. However, to date there is minimal information on how behavioural and physiological responses inter-relate (Nicol et al., 2009). Mason, Cooper and Clarebrough (2001) assessed the demand of mink for different resources and their physiological response when the access to different resources was blocked. The strength of demand was correlated with the degree of physiological stress when access to food and a water pool was subsequently prevented, suggesting that these two resources are of high value to mink. Animals unable to express and perform actions that they are motivated to do, due to restrictions within the environment, can result in impaired health, reduced survival and reduced productivity (Fraser et al., 1997). An aversive environment may prompt a stress response as a means for the animal to cope with or avoid potentially harmful situations.

Animals have both physiological and behavioural mechanisms to cope with stress (Moberg, 2000). Stress can be defined as a state that occurs when an animal is required to make abnormal or extreme adjustments, in either its behaviour or physiology, to cope with aspects of its environment (Fraser et al., 1975). Welfare problems and stress reactions can result from factors such as feeding, housing and poor management practises (Merlot et al., 2011). While stress is a natural part of life, experiencing severe stress can cause animals to succumb to disease, reduced reproductive success, impaired development and a suppressed immune system. Therefore, if there is a biological cost associated with stress, an animal's welfare is negatively impacted (Moberg, 2000). A range of physiological measures are commonly used to assess stress responses, such as heart rate, blood pressure and blood hormones (Moberg, 2000). The physiological measures used in the present study were plasma cortisol, glucose, lactate, white blood cell (WBC), neutrophils, lymphocytes and the neutrophil to lymphocyte

(N:L) ratio as commonly used indicators of stress, anaerobic metabolism, inflammation and the immune response. These physiological measurements typically involve the use of blood sampling which can involve invasive procedures and cause a stress response in itself. One of the greatest problems with measuring stress is the inter-animal variation. Animals within the same species may react differently when faced with the same stressor depending on how the animal perceives the stimulus. Factors such as age, genetics and social relationships have also been found to influence the stress response (Moberg, 2000).

The use of behaviour to assess the effects of stress has the advantage of being non-invasive. Behaviour can show displacement and abnormal patterns which can be an indicator of when animals are deprived of their behavioural needs. Healthy and content animals can be identified by the presence of certain behaviours (e.g., grooming, stretching on rising and taking interest in novel stimuli within the environment) and the absence of other behaviours (e.g., a decrease in general activity and abnormal stance). It is now widely accepted that incorporating both behavioural and physiological measures provides a more comprehensive assessment of animal welfare (Ewbank, 1985; Lane, 2006) than either alone.

Play behaviour has been identified as a potential indicator of positive welfare as it is associated with positive experiences and is sensitive to both physical and environmental conditions (Dudlink, Simonse, Marks, de Jonge, & Spruijt, 2006; Held & Špinka, 2011). Play is generally expressed when an animal’s basic needs are met (Krachun et al., 2010). The motivation of dairy calves to perform play decreases in times of low nutrition (Krachun et al., 2010), following a painful procedure (Mintline, Stewart, Rogers, Cox, Verkerk, Stookey, Webster, & Tucker, 2013; Rushen & de Passillé, 2012) and when reared on substrates that limit the ability to play (Sutherland et al., 2013). Calves reared on sawdust spend more time performing locomotor behaviours, exhibited a more complex repertoire of play behaviours in the home pen and spent more time lying down at 1 and 5 wk of age in comparison to calves reared on stones (Sutherland et al., 2013). Calves reared on stones spent more time running during an arena test at 3 and 6 wk of age in comparison to calves reared on sawdust (Sutherland et al., 2014). These differences may be attributed to the characteristics (e.g., size, rigidity and instability) of stones inhibiting the performance of these behaviours. The use of stones as a rearing substrate is increasing due to cost effectiveness and availability. The type of stones farmers use are also found in gardens for decorative purposes or used for drainage (Sutherland et al., 2014). If farmers wish to continue using stones as a rearing substrate, it is important to understand how calves perceive them in comparison to alternatives.

The objectives of this study were to: 1) investigate the preference of dairy calves for different rearing substrates (rubber chip, sand, sawdust or stones) using choice tests, and 2) investigate the effect of rearing substrate on the behaviour and physiology of calves. The substrates used were chosen due to their current use on-farm and within the literature, and rubber chips because they are a novel alternative commonly used for horse arenas, landscaping and construction. Given the limitations of choice tests discussed in the previous chapter, we aimed to provide a more comprehensive picture by incorporating lying and play behaviour with physiological measures rather than simply assessing time spent on a substrate as the measure of preference. The preferences of calves were evaluated using four phases: 1) first free choice, 2) restriction, 3) pairwise choice and 4) second free choice. It was predicted that calves would spend more time on sawdust when presented with a choice, have increased occurrences of lying and play behaviour and increased concentrations of physiological indicators of stress such as cortisol when on less preferred substrates. This research addresses a gap in current knowledge as to how dairy calves perceive their environment and ultimately, to improve on-farm welfare.

2. Materials and Methods

2.1. Animals and husbandry

All procedures involving animals were approved by the AgResearch Ruakura (Protocol N° 12966) and University of Waikato Animal Ethics Committees (Protocol N° 897) under the New Zealand Animal Welfare act 1999. The study was conducted between July and September (southern hemisphere winter) 2013 at the AgResearch Ruakura research farm, Hamilton (latitude 37°47'S, longitude 175°19'E), New Zealand.

Twenty-four Friesian-cross dairy heifer calves were used in the study. Eight calves were sourced from a commercial farmer located within the Waikato region and were tested as two replicates. The remaining sixteen calves were sourced from the AgResearch dairy research farm, South Waikato, New Zealand and were tested as four replicates. The calves were separated from their dams within 24 h of birth and transported to the farm's calf rearing facility. Calves were kept in pens with floors covered in woodchip (*Pinus radiata,* 15-30 mm in length) prior to being transported to the study site at approximately 1 week of age (range 3 - 8 d old). Calves arrived at the study site in groups of 8 animals over a 2 month period, this staggered arrival allowed time to perform all possible treatments on the animals at the same age.

On arrival to the study site, calves were weighed and assigned to replicate groups identified by a coloured collar (yellow, blue, grey, purple, red or green) placed around their neck. Calves were also identifiable by ear tag. In addition, animal marking paint (Tell-tail paint, FIL New Zealand, Mount Maunganui, New Zealand) was used across the back in varying patterns to distinguish between individuals via video observations. Paint was refreshed fortnightly. Calves were individually fed 2.5 L of colostrum twice a day at 08:00 and 15:30 h for the first 4 d after birth. Thereafter, the equivalent amount of milk replacement was offered (Ancalf, Fonterra LTD, Auckland, New Zealand) using a round 20 teat (1100 mm diameter x 860 mm depth) milk feeder (Stallion Plastic Ltd, Palmerston North, New Zealand) placed in the middle of the pen to ensure calves could choose which surface they wanted to stand on to feed. The feeder was removed after each feeding. Additionally, calves were given *ad libitum* access to meal (NRM Moozlee, Auckland, New Zealand) consisting of 18% crude protein, 10% crude fibre and 5% crude fat. Water was provided *ad libitum*. Revive (Virbac LTD, Auckland, New Zealand) was given to calves that showed signs of scours. As a preventative measure, calves were injected subcutaneously with 1 mL/kg bodyweight of Bivatop® 200 (containing 200 mg oxytetracycline; Boehringer Ingelheim Ltd, Auckland, New Zealand) as a broad spectrum antibiotic, when calves were moved into the experimental pens. The same dose was repeated at 72 h for long-acting cover. All other health problems were addressed under the advice of the farm staff and/or veterinarians.

2.2. Treatments and rearing substrates

The study site had solid concrete flooring and walls on all four sides. Twelve equal sized (3.5 x 3.4 m) pens were constructed, 8 of these pens were used for both restriction (2.9 m^2/calf) and pairwise choice (5.9 m^2/calf) periods, by opening or closing the gate between pens. The remaining 4 pens were designated as free choice pens, whereby two adjoining pens were made into one large pen (5.9 m^2/calf) containing all four substrates (1.75 x 1.7 m/substrate). The experimental facility design is shown in *Figure 2.1.* Wooden boards were placed between each substrate and were raised 100 mm above the substrate to prevent the calves from lying on more than one surface at a time and to prevent the substrates from mixing. Each pen was separated by a steel gate, boarded with ply-wood to remove visual and tactile contact between animals in adjoining pens. All pens had plastic troughs (200 mm width x 650 mm length x 130 mm depth) for feed (Snack Bar, Milk Bar, McInnes Manufacturing Ltd, New Zealand) and steel troughs (150 mm width x 300 mm length x 150 mm depth) for water. Feed and water troughs were available on each substrate in the free choice pens.

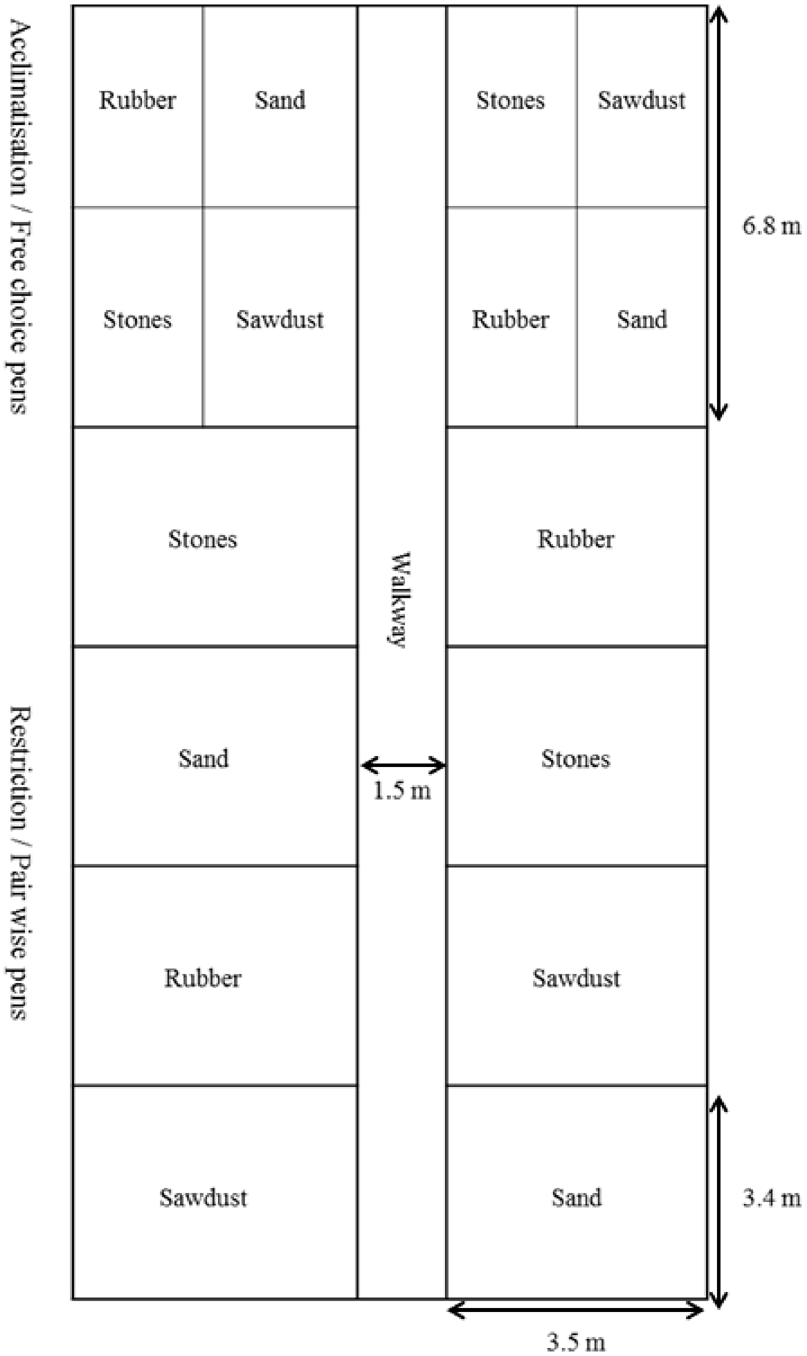


Figure 2.1. Experimental facility design and positioning of pens with different substrates.

Four materials were used as rearing substrates. Stones (Mangatangi River Rock Ltd, Auckland, New Zealand, www.mmrl.co.nz) with an approximate diameter of 20 to 40 mm, washed river sand (Daltons Ltd, Matamata, New Zealand) with a particle size of 0.1 to 0.6 mm, rubber chip (Pacific Rubber, Auckland, New Zealand) with a particle size of 4 to 7 mm and sawdust with an average particle size of 10 mm. Rearing substrates were laid to a depth of 40 cm. The treatments were randomly distributed throughout the experimental facility. Rearing substrates had not been used prior to the start of the study. Sawdust pens were replenished with dry, clean sawdust when pens became damp. Stones, rubber and sand were not topped up as these substrates did not become damp during the experimental period.

2.3. Experimental design

At 1 week of age, calves (34.9 kg, range: 22.5 - 47 kg) were allocated to one of six treatment groups (four calves per group) balanced for age and body weight and then moved into the experimental pens. The study was replicated six times, for each replicate, 4 calves were tested simultaneously. Each test consisted of 4 consecutive testing periods: first free choice, restriction, pair wise choice and second free choice. During the first free choice period, calves had access to all four substrates for 3 d, which allowed animals to acclimate to the facilities (*Figure 2.2*). During the restriction period, calves were allowed access to only a single substrate at a time, each for a 2 d period, with the order of access assigned randomly. The restriction period ensured that calves had short term experience with each substrate. During the pair wise choice period, calves were allowed access to two substrates at a time, for a 2 d period, with the order of pairings and access assigned randomly. During the second free choice period, calves were allowed free access to all four substrates for 2 d. Each group was video recorded continuously during the last 24 h period of both free choice and pair wise choice periods and during the last 12 h of the restriction period, for a total of 10 d of recording.

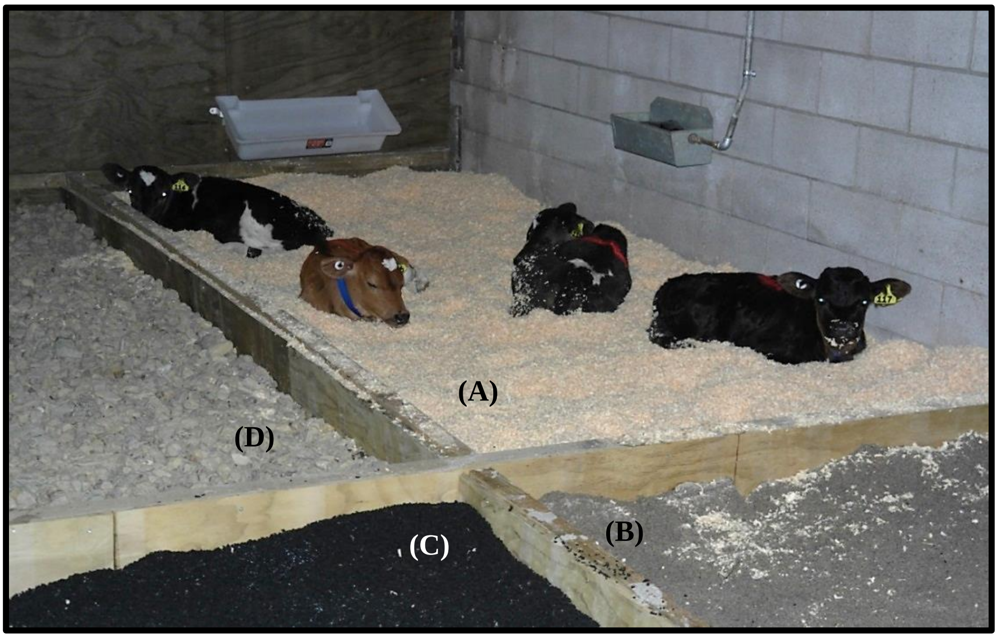


Figure 2.2. Example of one of the free choice pens, showing all four rearing substrates, sawdust (A), sand (B), rubber chip (C) and stones (D).

2.4. Behavioural measurements

Calf behaviour was recorded continuously in real time at 30 frames/s using overhead digital handycams (SONY Handycam® Camcorder DCR-SX65, Tokyo, Japan). The cameras were situated 2 m above the ground, fixed to stands that were attached to the side of the pens. Handycams were fitted with a fisheye lens (Raynox Digital 0.3x conversion lens, QC-303 “Snap-On”, Tokyo, Japan) to ensure the entire pen was in view. Two red lights (80 W), were hung 3 m above each pen to facilitate night observations with minimal effects to the calves’ behaviour. Video recordings were examined in two ways: firstly, continuous observations for each individual over a 24 h period to assess the duration of time calves spent on each available surface in the free choice and pair wise periods. Secondly, the calves' propensity to play was observed for each individual continuously over a 12 h period when restricted to one surface. The recorded behaviours are described in Table 2.1. Video recordings were analysed using Adobe Premiere Pro CS6 (version 6.0.5), results were then extracted using Premiere Extractor v0.2 (Psychology Department, University of Waikato, Hamilton, New Zealand) and input into Microsoft Excel 2010. Two trained observers recorded behaviour from all video recordings. Reliability was measured on two occasions, by having each observer watch four, 24 h videos twice. Inter and intra-observer reliability, as measured by percentage of agreement between observers, was between 98% and 100% for the duration calves spent on each surface. Play behaviours scored as frequencies were jumping, kicking, bucking, head to object, ground play, mounting and frontal pushing. Running was recorded as a duration with a start and end time. Running bouts separated by a pause of less than 1 s were considered the same event. All other play behaviours were recorded if they occurred on their own or within a running bout. Observer reliability for play behaviour was measured by percentage of agreement between observers. Inter-observer reliability was between 85% and 91% for all behaviours; intra-observer reliability was between 87% and 96% for all behaviours.

2.5. Lying and standing behaviour

Lying and standing times were recorded continuously using Onset Pendant G data loggers (64k, Onset Computer Corporation, Bourne, MA, USA). The devices are waterproof, 3-channel data loggers designed to measure acceleration and angular displacement in 1, 2 or 3 axes (*Figure 2.3*). The loggers were programmed to measure leg orientation via the y and z-axis at 1 min intervals as recommended by validation studies of lying and standing events in dairy cattle (Ledgerwood, Winckler, & Tucker, 2010) and dairy calves (Bonk, Burfeind, Suthar, & Heuwieser, 2013). The data loggers were placed in a durable fabric pouch and attached on the lateral side of the hind leg above the metatarsophalangeal joint (see *Figure 2.4*) 1 d before the start of data collection. The pouch was held in position using velcro patches, one sewn to the pouch, the other glued (KAMAR®, Livestock Improvement Corporation, Hamilton, New Zealand) to the leg of the calf. The pouch was further held in place by a strap around the leg of the calf.

Figure 2.3. Onset Pendant G data logger used to record standing and lying events.

Figure 2.4. Calf fitted with the Onset Pendant G data logger to record standing and lying events.

The loggers were positioned on the leg such that the y-axis was perpendicular to the ground pointing toward the calves back, and the z-axis parallel to the ground, pointing away from the sagittal plane. The data were downloaded using the Onset HOBOware Pro software (Onset Computer Corporation, version 3.4.1), which converted the *g*-force readings into degrees of tilt and further converted to daily summaries of lying behaviour using SAS software (SAS Institute Inc., Cary, NC, USA) code designed for this purpose (N. Chapinal, University of Guelph, Ontario, Canada, personal communication). Calf activity variables associated with the acceleration and angle displacement included total lying time, the number of lying bouts (the number of times an animal changes from lying to standing) and average bout duration.

Table 2.1. Description of behaviour's recorded for free choice, pairwise choice and restriction periods by continuous sampling over 12 h and 24 h.

Behaviour	Description
Location* (duration)	At least three hooves on one surface.
Jump (frequency)	The two forelegs are lifted off the ground; the front of the body is elevated. Movement is upwards but not forwards. The hind legs may be lifted off the ground.
Kick (frequency)	One or both hind legs are lifted off the ground and extended outwards from the body. The calf can be stationary or moving.
Buck (frequency)	While the calf is moving, the body ascends from front to back, and one or both hind legs are lifted off the ground in one rapid movement and extended outwards from the body. The hind hooves are raised as high as or higher than the front knees of the forelegs.
Head to object (frequency)	While at least two hooves are moving (front or hind) the calf touches (butts or rubs) its forehead, head or throat against the pen, feeder or another animal.
Running (duration)	Included trotting (two-beat gait), cantering (three-beat gait) and galloping (four-beat gait) with forwards or sideways movement. Lasting longer than 1 s in real time.
Social play only	
Mount (frequency)	A calf mounts another calf's body or head from front, side or back.
Frontal pushing (frequency)	Two calves are standing front to front, butting head against head/neck.

Note. *Free choice and pairwise choice periods only. Definitions based on the ethogram described by Jensen et al. (1998), Jensen & Kyhn (2000), and Mintline et al. (2012).

2.6. Physiological measurements

Calves were blood sampled as each group moved onto a new surface in the restriction period, before morning feeding. Blood samples were obtained by jugular venipuncture into evacuated tubes that contained sodium fluoride, EDTA, or no anticoagulant (BD Vacutainer, Franklin Lakes, NJ, USA). Samples containing sodium fluoride were placed immediately on ice and centrifuged within 60 min at 1,500 x g (~3,000 rpm) for 10 min. Samples in plain evacuated tubes were held at ambient temperature following collection for at least 2 h to allow serum to separate before centrifugation at 1,500 x g (~3,000 rpm) for 10 min. Following centrifugation, plasma and serum were aspirated and aliquots stored at -20°C until assayed for cortisol, glucose and lactate concentrations. Samples containing EDTA were placed immediately on ice and delivered within 3 h to New Zealand Veterinary Pathology laboratory (Hamilton, New Zealand). Blood smear slides were performed on a Sysmex XT-2000 iV using veterinary software and Sysmex reagents (Sysmex Corporation, Kobe, Japan) for estimation of plasma neutrophils, lymphocytes and total white blood cell counts. WBC differentials were performed using a standard 100 cell count and the N:L ratio was calculated by dividing the percent of neutrophils by the percent of lymphocytes. Cortisol concentrations were measured using a solid phase single antibody radioimmunoassay kit (Coat-a-Count® Cortisol; Siemens; Los Angeles, CA, USA). The minimum detectable level was 0.5 nmol/L, inter- and intra-assay coefficients of variation were 6.4% and 4.3% respectively.

2.7. Environmental conditions

The air temperature and the ambient temperature conditions in the calf barn were measured continuously during the trial period using weather stations (Vantage Pro2 Plus, Davis Instruments Corp, CA, USA). One weather station was located inside the barn and one located outside to measure air temperature and relative humidity. The average temperature inside the experimental facility was 11.7°C, (range: 2.1°C - 18.4°C) and humidity was 82.7% (range: 53% - 98%). The average temperature outside the experimental facility was 10.7°C, (range: -0.6°C - 20.1°C) and humidity was 89.1% (range: 60% - 99%).

To assess the insulating properties of the four different substrates, nine temperature data loggers (Thermochron ibutton, model DS1922L-F5#, range: -40 to +85°C, accuracy ± 0.5°C, Embedded Data Systems, Lawrenceburg, KY, USA) were placed within and on top of a sample of each substrate in three different temperature locations; an oven, refrigerator and ambient. Temperature data were recorded every 30 s over a 2 h period.

2.8. Statistical analysis

Analysis of preference was based on lying times as this behaviour provides a clear indicator that the animals are willing to use the rearing substrate. Lying time was used to rank each rearing substrate to assess which option was preferred by the calves. This study was replicated six times (four calves per replicate) with each replicate serving as an experimental unit. Data was analysed using GenStat (16^{th} edition, VSN International Ltd., Hemel Hempstead, UK). From the Onset Pendant G data loggers, lying and standing behaviours on each substrate were totaled over a 24 h period for the first and second free choice, restriction and pairwise periods to sum the proportion of time calves spent lying or standing on a given substrate.

The proportion of time spent on each surface during the both free choice periods were analysed from video results. Lying and standing data were analysed independently using an analysis of variance (ANOVA), the ANOVA model was blocked by replicate, with the rearing substrate as the treatment variable. One replicate were omitted from analysis of the second free choice period as lying and standing data were available from only 1 animal due to equipment failure.

Lying data from the restriction period were analysed using ANOVA. The ANOVA model was blocked by replicate and pen, with the rearing substrate as the treatment variable, to compare total lying time, number of lying bouts and average duration of lying bouts. Lying and standing data from 1 calf was excluded from analysis due to equipment failure. Due to the low incidences of social play behaviours, mounting and frontal pushing were combined for analysis, data was then analysed using ANOVA. All behavioural frequencies were log transformed prior to analysis to stabilize the variance. Physiological measures taken at the end of each restriction period were analysed using a residual maximum likelihood (REML) with random effects for the replicate and pen, and fixed effects for the substrate and previous treatment were used to compare the change from baseline for each available substrate.

For data from the pairwise choice period, the proportion of time each replicate spent on each surface (from video analysis) was compared to a null-hypothesis of .5 with a two-tailed Student's *t*-test. Each paired rearing substrate combination was analysed separately. The average proportion of time each replicate spent lying and standing on each substrate were conducted independently using ANOVA. Data from 4 calves during the pairwise choice period were excluded from analysis due to equipment failure.

The insulating properties of each rearing substrate were measured using ANOVA. If the null hypothesis of equal treatment means was rejected, Fisher's least significant difference (LSD) tests were used to compare treatment means. Statistical significance was determined at $p < .05$ for all tests.

3. Results

First free choice period

3.1. Proportion of time on each surface

During the first free choice period, the proportion of total time calves spent standing ($F_{3, 15} = 18.45$, $p < .001$) and lying ($F_{3, 15} = 1751.11$, $p < .001$) was influenced by substrate type as determined by one-way ANOVA's (Figure 2.5). Calves spent 88% of their time on sawdust, 77% of this time was spent lying. Numerically, calves spent a higher proportion of time standing than lying when on rubber, sand and stones.

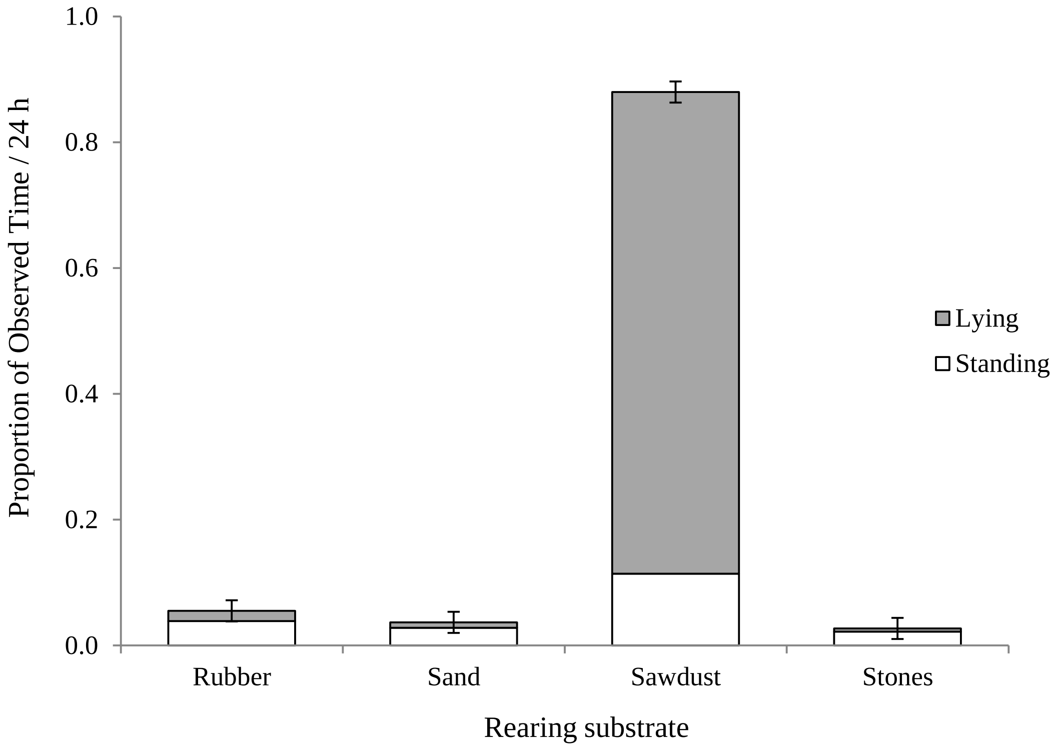


Figure 2.5. Proportion of time calves spent lying and standing on each substrate (n=6 groups, 4 calves/group) over a continuous 24 h observation period. Vertical lines depict the standard error of the mean.

Restriction period

3.2. Play behaviour

Rearing substrate influenced the average time calves spent running ($F_{3, 15}$ = 3.55, p = 0.040; Figure 2.6) as determined by a one-way ANOVA. Calves spent less (LSD_{15}: 1.100; $p < .05$) time on average running on stones in comparison to rubber and sawdust. There was no evidence that the average time calves spent running differed on rubber, sand or sawdust. The number of running bouts performed by calves was affected by time of day and rearing substrate (Figure 2.7). Calves ran more between 14:00 and 16:00 h and during this time, calves on sawdust, sand and rubber ran more than calves on stones. Furthermore, a one-way ANOVA determined that rearing substrate affected the frequency of running ($F_{3, 15}$ = 4.12, p = 0.026; Table 2.2). There was no evidence that rearing substrate affected the frequency of jumps, bucks/kicks, head to object and mount/frontal pushing performed by calves (Table 2.2).

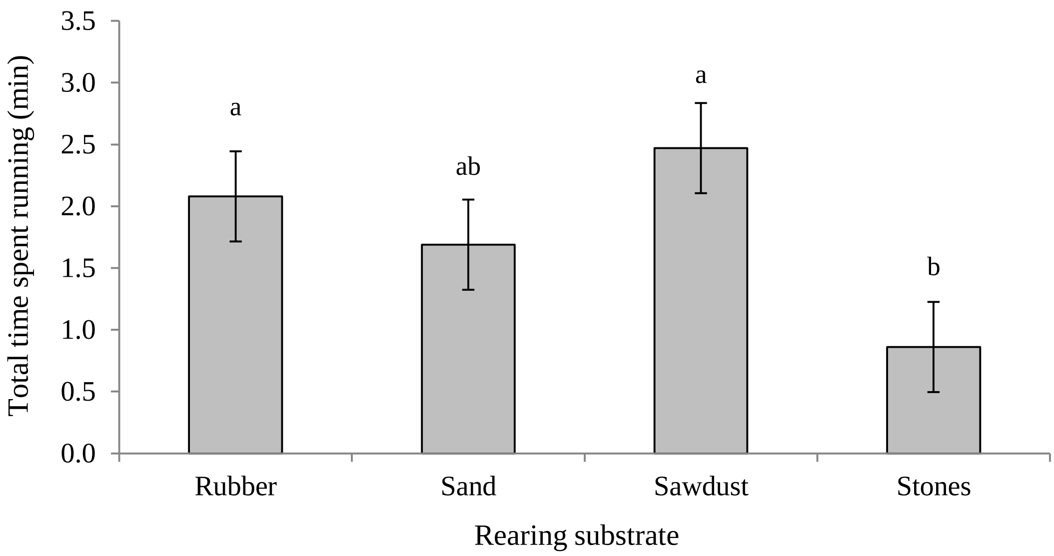


Figure 2.6. The total time calves spent running when restricted to each rearing substrate (n=6 groups, 4 calves/group) over a continuous 12 h observation period. Vertical lines depict standard error of the mean. Bars with different superscripts differ at $p < .05$.

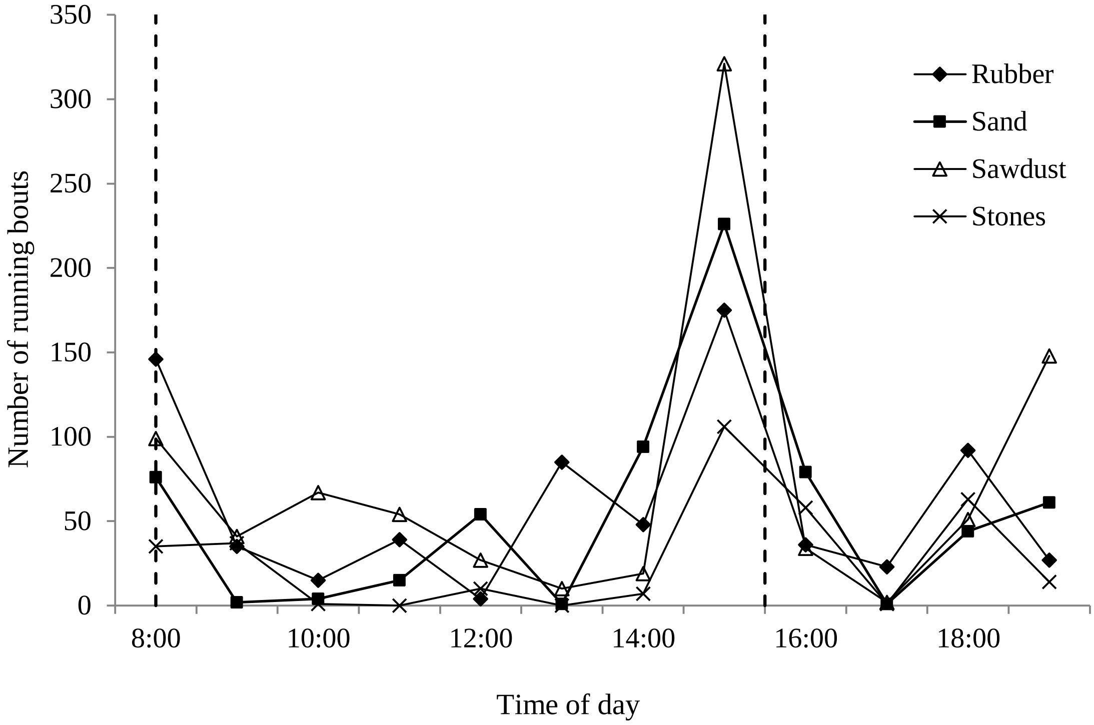


Figure 2.7. Number of running bouts performed by calves (n=6 groups, 4 calves/group) when restricted to each substrate during a 12 h continuous observation period. Vertical dashed lines indicate approximate feeding times.

Table 2.2. Frequency of running and play events performed by calves (n=6 groups, 4 calves/group) during a 12 h continuous observation period when restricted to each rearing substrate. In order to normalise the data, the frequencies were log transformed (ln+1) prior to analysis.

	Rearing substrate						
Behaviour	Rubber	Sand	Sawdust	Stones	SED	$F_{3,15}$	*p*-value
Running (no.)	3.27[a]	3.10[a]	3.44[a]	2.16[b]	0.40	4.12	0.026
Jump (no.)	1.88	2.02	1.94	1.50	0.59	0.32	0.814
Buck/kick (no.)	1.48	1.51	1.77	1.12	0.33	1.38	0.288
Head to object (no.)	0.34	0.33	0.37	0.21	0.15	0.46	0.713
Social play							
Mount/frontal pushing (no.)	0.34	0.67	0.49	0.21	0.24	1.45	0.268

[a, b] Within rows, letters with different superscripts differ at $p < .05$

3.3. Lying time

A one-way ANOVA was conducted to compare the effect of rearing substrate on lying times (Figure 2.8). Rearing substrate influenced the time calves spent lying ($F_{3, 15}$= 4.62, p = 0.018). Calves spent a similar time lying on sawdust and rubber which was greater than sand or stones (Figure 2.8). There were no effects (Table 2.3) of rearing substrate on the number and duration of lying bouts over a 24 h period. The number of calves lying on each substrate was affected by time of day and rearing substrate (Figure 2.9). The time taken to lie down following afternoon feeding (15:30) was longer for calves housed on sand and stones than calves on sawdust and rubber. The number of calves lying was not affected by average temperature within the experimental facility irrespective of substrate type: rubber (Figure 2.10a), sand (2.10b), sawdust (2.10c) and stones (2.10d).

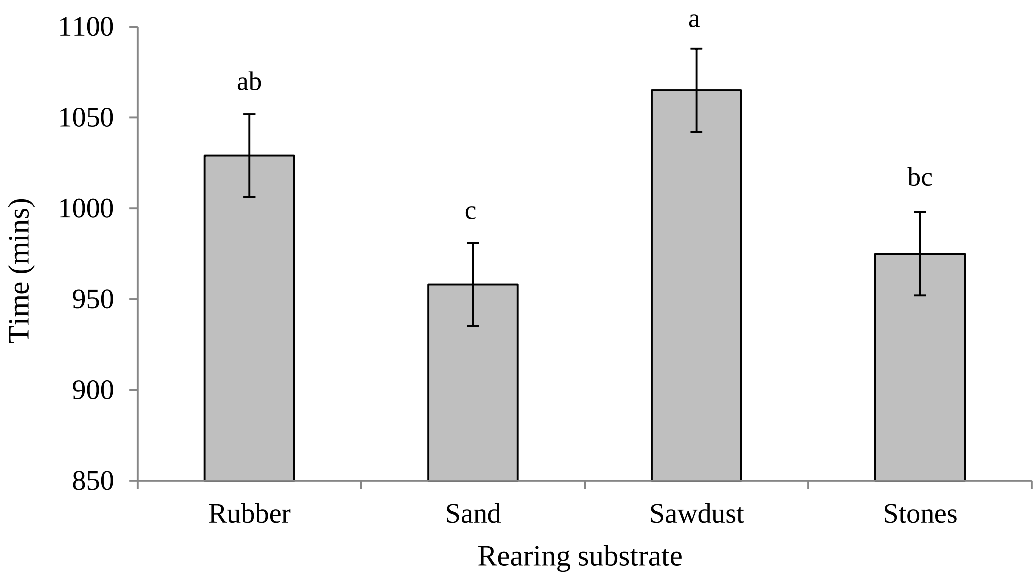


Figure 2.8. The total time calves spent lying (min) when restricted to each rearing substrate (n=6 groups, 4 calves/group) over a 24 h recording period. Vertical lines depict standard error of the means. Bars with different superscripts differ at p < .05.

Table 2.3. Average number of lying bouts and lying bout duration performed by calves (n=6 groups, 4 calves/group) when restricted to each rearing substrate for 24 h.

	Rearing substrate						
Behaviour	Rubber	Sand	Sawdust	Stones	SED	$F_{3,15}$	*p*-value
Number of Lying Bouts	22	18	22	22	1.8	2.16	0.136
Average Lying Bout Duration (min)	49	57	52	47	4.4	2.08	0.146

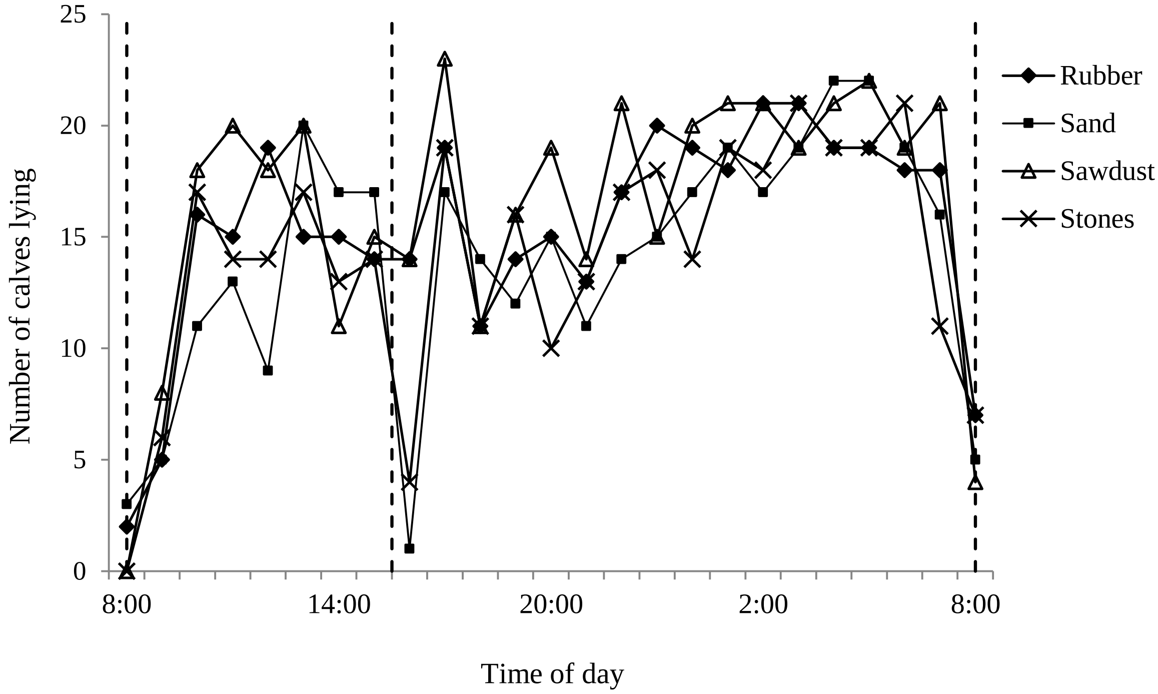
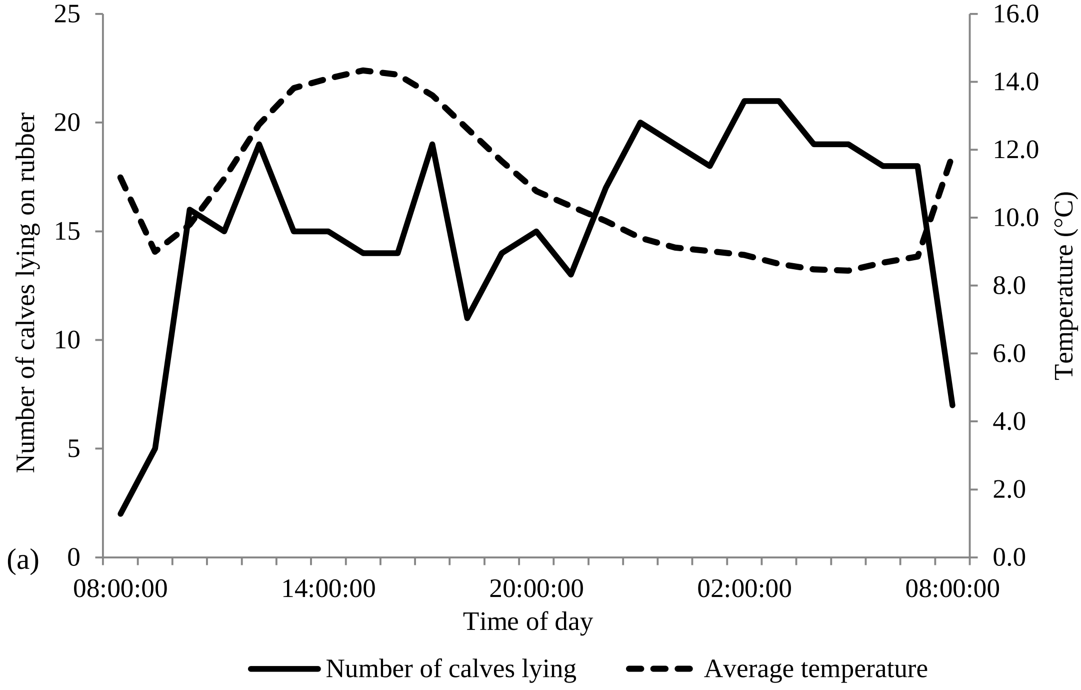


Figure 2.9. Number of calves (n=6 groups, 4 calves/group) lying when restricted to each substrate over a 24 h recording period. Vertical dashed lines indicate approximate feeding times.



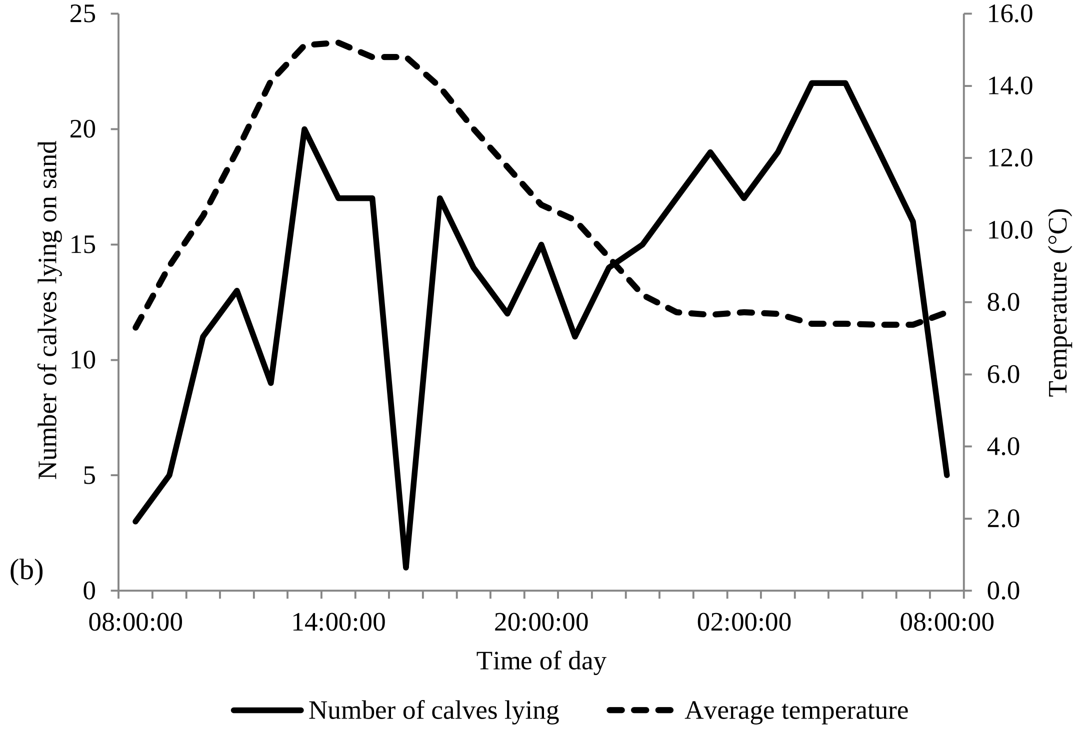
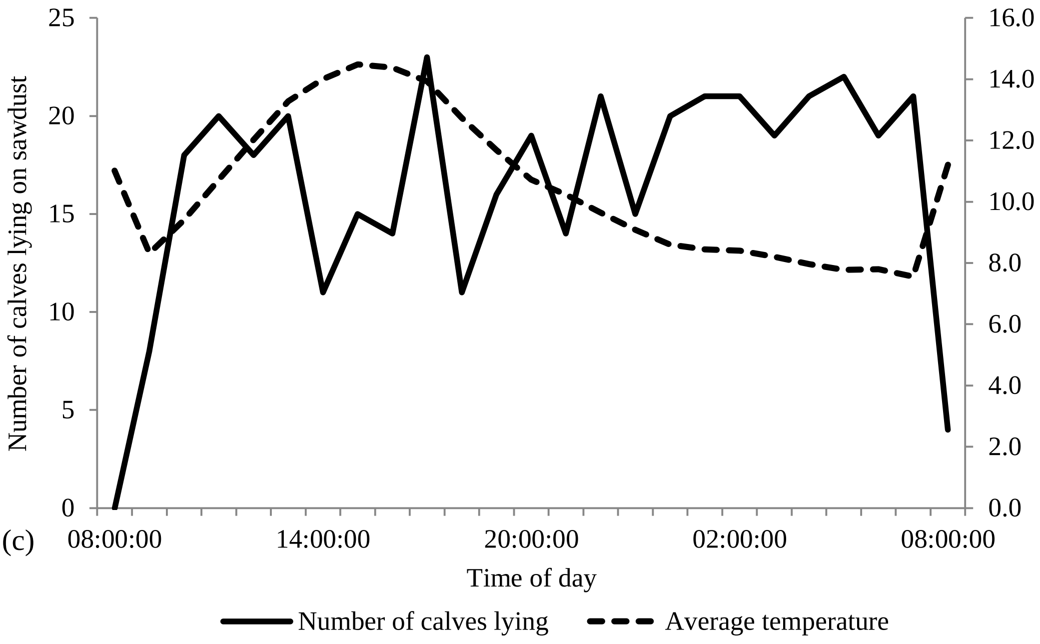
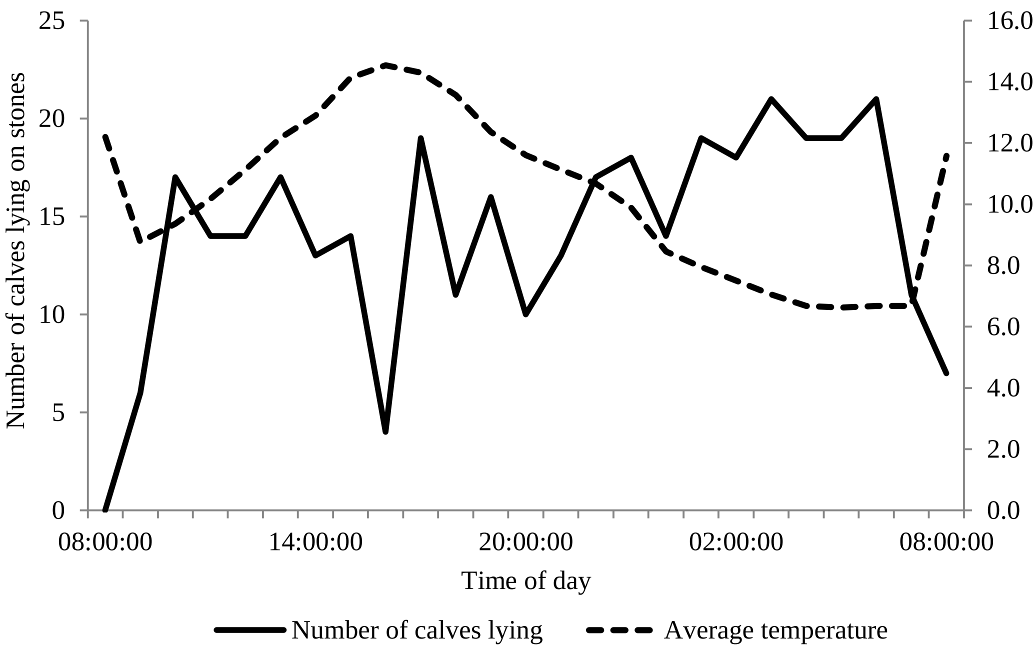


Figure 2.10. Number of calves (n=6 groups, 4 calves/group) lying when restricted to rubber (a), sand (b), sawdust (c) or stones (d) over a 24 h recording period in relation to hourly average temperature within the experimental facility.



(c)



(d)

Figure 2.11. Number of calves (n=6 groups, 4 calves/group) lying when restricted to rubber (a), sand (b), sawdust (c) or stones (d) over a 24 h recording period in relation to hourly average temperature within the experimental facility.

3.4. Physiological Responses

There was no effect (Table 2.4) of substrate type on lactate, glucose or cortisol concentrations or haematological values during a 24 h restriction period.

Table 2.4. Blood chemistry and haematological measures of calves (n=6 groups, 4 calves/group) when restricted to each rearing substrate for 24 h. Values represent the mean change from baseline.

	Rearing substrate							
	Rubber	Sand	Sawdust	Stones	*SEM	**d.d.f.	F-value	*p*-value
Lactate (mmol/L)	-0.09	-0.15	-0.03	-0.26	0.18	84.7	0.51	0.677
Glucose (mmol/L)	0.19	0.16	0.20	0.37	0.19	88.0	0.33	0.800
Cortisol (nmol/L)	-18.10	-11.72	-17.38	-12.14	6.97	83.6	0.42	0.740
White blood cells (x10^9^/L)	-2.11	-1.45	-1.37	-1.99	1.00	84.2	0.31	0.818
Neutrophils %	-8.31	-10.98	-8.84	-11.66	4.14	84.7	0.16	0.925
Lymphocytes %	13.43	7.10	13.62	14.02	4.37	84.7	0.70	0.556
N:L Ratio	-0.46	-0.51	-0.43	-0.53	0.16	85.5	0.08	0.970

*Maximum standard error of the mean. **Denominator degrees of freedom.

Pairwise choice period

For each pairwise choice period a two-tailed student's *t*-test was conducted to compare the proportion of time spent on each available surface. Calves spent a higher proportion of time on sawdust when provided with two options simultaneously: sawdust and rubber ($t_5 = 33.39$, p < .0001), sawdust and sand ($t_5 = 43.49$, p < .0001) or sawdust and stones ($t_5 = 37.48$, $p < .0001$). Likewise, calves spent a higher proportion of time on rubber than sand ($t_5 = 23.46$, $p < .0001$) or stones ($t_5 = 11.97$, $p = .0001$), and a higher proportion of time on sand than stones ($t_5 = 6.17$, $p = 0.0016$; Figure 2.11). For each pairwise choice period, a one-way ANOVA was conducted to compare the average proportion of time spent standing or lying on each available surface. On average, calves spent a higher proportion of time standing (Figure 2.12a) than lying (Figure 2.12b) on the non-preferred surface (Appendix A).

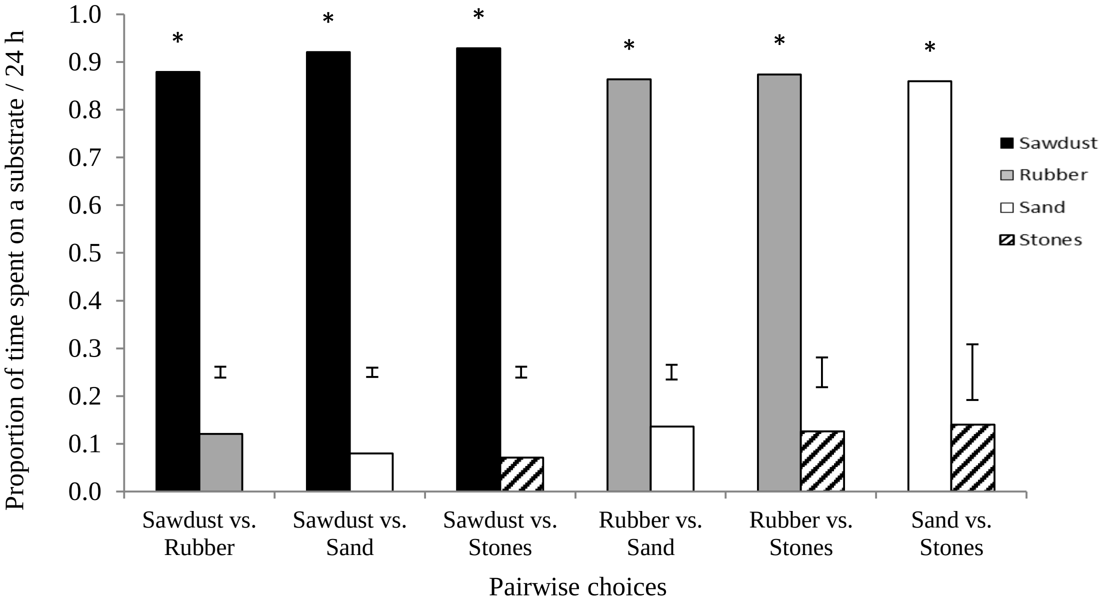


Figure 2.12. The proportion of time calves spent lying and standing on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period. Vertical lines depict the standard error of the mean for each pairwise choice. *Pairwise choices differ at $p < .001$.

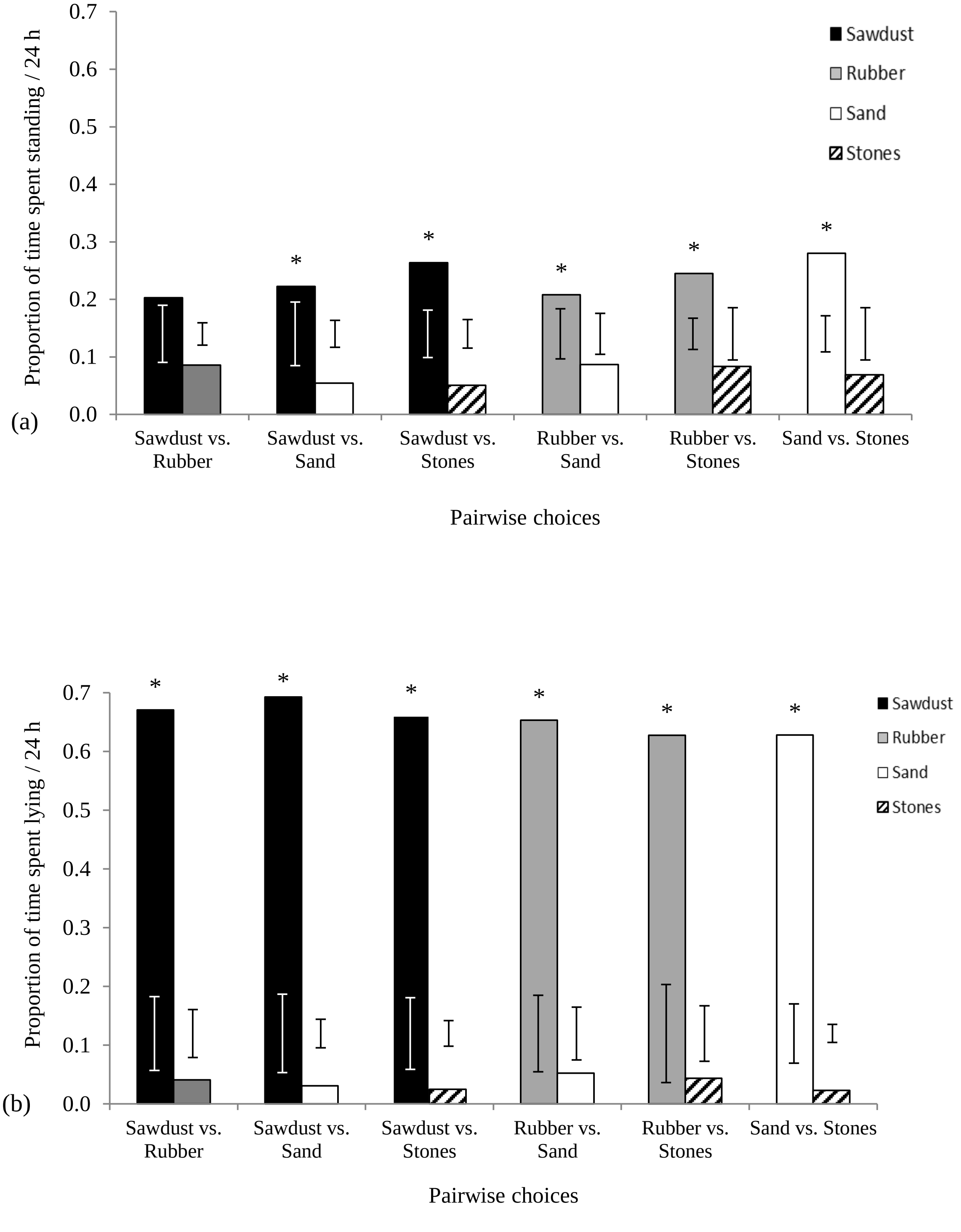


Figure 2.13. The proportion of time calves spent standing (a) and lying (b) on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period. Vertical lines depict the standard error of the mean for the proportion of time spent lying on each substrate. *Pairwise choices differ at $p < .001$.

Second free choice period

3.5. Proportion of time on each surface

During the second free choice period, the proportion of total time spent standing ($F_{3, 12} = 21.81$, $p < .001$) and lying ($F_{3, 12} = 184.77$, $p < .001$) was influenced by substrate type as determined by one-way ANOVA (Figure 2.13). Calves spent 85% of their time on sawdust, 67% of this time was spent lying. Numerically, calves spent a higher proportion of time standing than lying when on rubber, sand and stones.

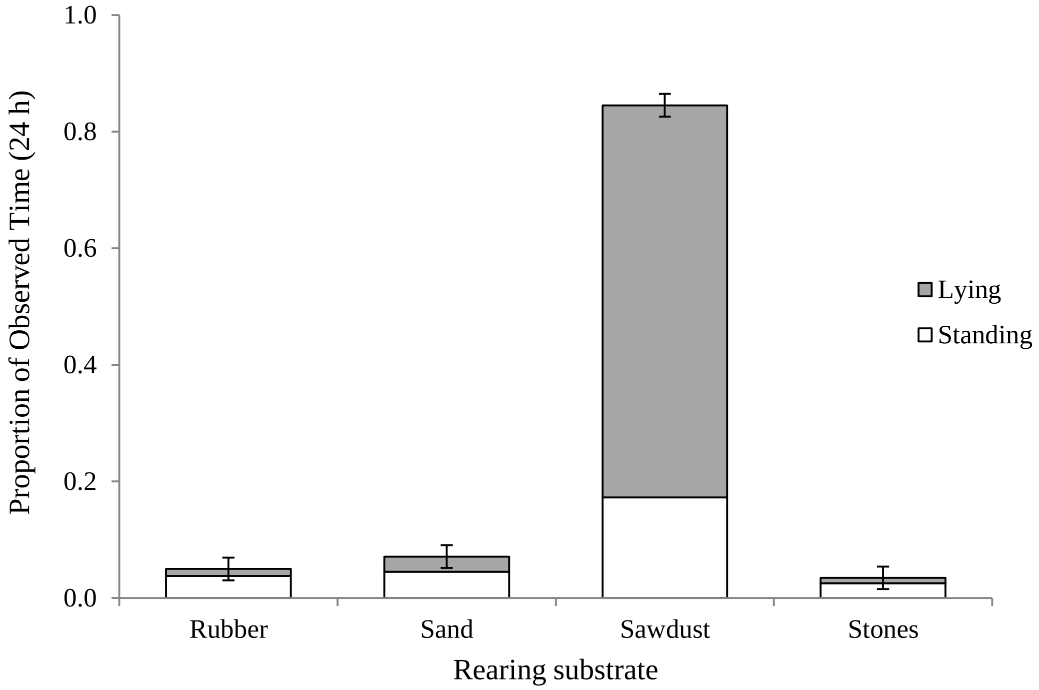


Figure 2.14. Proportion of time calves spent on each surface (n=5 groups, 4 calves/group) over a 24 h observation period. Vertical lines depict the standard error of the mean for the proportion of time spent on a substrate.

Environmental Conditions

3.6. Temperature of rearing substrate

A one-way ANOVA was used to compare the insulating properties of each rearing substrate, results are summarised in Table 2.5. Ambient temperature influenced the surface and internal temperature of the different substrates. The environments from which the temperatures were taken averaged 6.3 ± 0.4 and 62.0 ± 0.6 and 20.6 ± 0.0 for the refrigerator, oven and ambient temperatures respectively. Sawdust had the greatest insulating properties in all three locations in comparison to rubber, sand or stones.

Table 2.5. Summary of the mean internal and surface temperatures of the different rearing substrates at the different ambient temperatures tested.

	Rearing substrate										
	Rubber		Sand		Sawdust		Stones				
Location	Internal	Surface	Internal	Surface	Internal	Surface	Internal	Surface	SED	$F_{1, 3}$	*p*-value
Refrigerator	5.1	4.6	3.3	3.1	6.8	6.1	4.1	3.6	0.24	43.4	< .001
Oven	57.9	60.7	55.9	58.0	61.1	61.2	57.5	59.6	0.03	24.1	< .001
Ambient	17.5	19.5	17.0	18.0	18.7	20.4	18.1	19.2	0.04	178.7	< .001

4. Discussion

The results from this study support our prediction that calves would spend more time on sawdust when presented with a choice and have increased occurrences of lying and play behaviour when on preferred substrates. The prediction that physiological indicators of stress such as cortisol would decrease on preferred substrates was not supported. The objectives of this study were to, 1) investigate the preference of dairy calves for different rearing substrates (rubber chip, sand, sawdust or stones), and 2) investigate the effect of rearing substrate on the behaviour and physiology of calves. The preferences of calves were evaluated using four phases: 1) first free choice, 2) restriction, 3) pairwise choice and 4) second free choice. Calves spent more time on sawdust during both free choice phases. During the restriction period, calves spent more time lying and playing on sawdust and rubber in comparison to sand and stones. Calves blood chemistry and haematology measures were similar when restricted to each substrate type. In addition, calves spent a higher proportion of time on sawdust when provided with two substrates simultaneously. Calves preference ranking was for sawdust, followed by rubber chip, sand and lastly, stones.

During the initial free choice period, calves spent a higher proportion of time lying and standing on sawdust than rubber, sand and stones. Preference can be affected by the animals' previous experience, where they may show avoidance or attraction to unfamiliar options (Fraser & Matthews, 1997; Tucker et al., 2003). The calves in our study had an average of 5 d previous contact with woodchip, but had not been in contact with any of the substrates used in this study. We ensured that calves had 3 d free access to all substrates prior to experimental testing to rule out novelty of the facility and substrates. Under commercial conditions, dirty and wet floors may be avoided irrespective of the type of substrate (Panagakis et al., 2004), therefore, in the present study the substrates were kept clean and dry throughout the experimental period. In social groups of animals, factors such as space allowance may influence the preference for lying area (Færevik et al., 2005). Each substrate area within the free choice pens gave $0.74m^2$ /calf, which is smaller than the New Zealand industry standard space allowance ($1.0m^2$/calf; Sutherland et al., 2014). Results shown by Færevik et al. (2008) suggest that when given more space, calves prefer to lie with distance to other calves to aid thermoregulation. In the present study, our results imply that while there was minimal space for all calves to lie together on one substrate, they adjusted to the decreased lying space by lying close together on sawdust rather than to spread out onto other substrates. Cattle are a social species that synchronise their grazing and resting patterns (Benham, 1982). Therefore, our calves' preference to lie together on one substrate suggests that their ability to perform natural behaviour and thermoregulate was not influenced by the space allowance provided. The calves in the present study were exposed to a second free choice period at 5 to 6 wk of age. The calves' preference for sawdust persisted, spending a higher proportion of time lying and standing on sawdust than rubber, sand and stones. As calves preference for sawdust did not change over time, the results obtained from the first free choice period were most likely not due to experience prior to the study, but because the calves had a strong preference for sawdust.

In the present study, calves restricted to sawdust, rubber and sand spent more time running, had a greater frequency of running events and performed a numerically more complex repertoire of play behaviours than calves restricted to stones. As discussed previously, it has been suggested that play behaviour is linked with good welfare as it is reduced during challenging conditions (Held & Špinka, 2011) such as dehorning (Mintline et al., 2013; Rushen & de Passillé, 2012), in response to low milk allowance (Krachun et al., 2010) and when reared on substrates that limit the ability to play (Sutherland et al., 2013). Sutherland et al. (2013) found that calves reared on stones spent less time performing play behaviour in comparison to calves reared on sawdust, which is consistent with the results obtained in the present study. Similarly, when removed from the home pen and put into an arena test area, calves reared on stones performed more play behaviour than calves reared on sawdust (Sutherland, et al., 2014). Suggesting that the rearing environment can affect calves motivation to play in an arena test. Therefore, stones appear to be a suboptimal rearing substrate as they reduce calves affective state and hence their motivation to play. Stones may inhibit calves' ability to play due to their consistency (e.g., stability or rigidity) making it difficult for calves to perform these behaviours. In the present study, calves performed the majority of locomotary play behaviour before and after feeding times. This result is similar to that of Jensen et al. (1998) where peaks in play behaviour occurred at morning and afternoon feeding times. External stimuli and enrichment appears to stimulate the occurrence of play behaviour. Brownlee (1954) discovered that the addition of novel objects into calf pens induced butting and pushing behaviours often followed by investigation. In addition, the announcement of enrichment increased play behaviour after weaning in piglets (Dudlink et al., 2006). In this study, no novel objects were provided and external stimulation was limited to normal feeding and management routines twice a day. The increased occurrence of play performed by calves on sawdust compared to stones suggests that sawdust is a more optimum rearing substrate for calves. However, as there were no differences in play between rubber, sand and sawdust, it is difficult to make further conclusions based on these results.

Lying behaviour has been used as an indicator of dairy cow comfort (Haley, de Passillé, & Rushen, 2001; Norring, Manninen, de Passillé, Rushen, Munksgaard, & Saloniemi, 2008; Tucker et al., 2009) and can be a useful indicator of dairy calf comfort in relation to different rearing substrates and how calves perceive them. Sufficient resting and sleeping time is important for young animals (Bonk et al., 2013), 2 wk old calves will typically spend about 17-18 h/d lying down (Panivivat et al., 2004). As the calf ages, the relationship between age and lying time has been shown to decrease (Hänninen et al., 2005). In the present study, lying times fluctuated for calves restricted to the different substrate types however, there were no differences in the number of lying bouts or the average duration of lying bouts. It has been hypothesised that in dairy cows, a reduced number of lying bouts is associated with discomfort during rising and lying down (Haley et al., 2001). Our results suggest that the substrates did not affect the ease with which calves got up and down. However, in the present study, calves restricted to stones and sand spent 6 to 8% (approximately 2 h) less time lying than calves on sawdust and 4 to 6% (approximately 1 h) less time lying than calves on rubber (over 24 h). These findings are similar to those of Sutherland et al. (2013; 2014) where calves reared on stones spent 3.5 to 4.5% less time lying than calves reared on sawdust between the ages of 4 to 6 wk. Panivivat et al. (2004) found similar lying times among calves reared on 5 different substrates, including granite fines, sand, rice hulls, long wheat straw and wood shavings at 2 to 6 wk of age. Furthermore, the resting behaviour was similar for calves reared on concrete or rubber mats over the first 20 wk of life (Hänninen et al., 2005). It is unclear whether the amount of lying time on sand and stones would be detrimental to the welfare of calves long-term as these values fall within the range of lying times reported in previous studies (Sutherland et al., 2013; 2014). The reduced lying time on sand and stones observed in the present study may be attributed to the softness or compressibility which is likely to impact behaviour and comfort.

Total lying times on sawdust and rubber observed in the current study are consistent with the values reported in this previous research however, the lying times on sand and stones were lower than these reports. Some data suggests that several physiological changes are associated with reduced lying time in cattle; these include a reduction in growth rates (Mogensen et al., 1997), a short-term increase in plasma cortisol levels (Ladewig & Smidt, 1989; Fisher, Verkerk, Morrow, & Matthews, 2002) and increased incidence of lameness (Leonard, O'Connell, & O'Farrell, 1994). However, to date there has been no evidence of these effects in calves. It has been well documented that adult dairy cows prefer to spend more time lying on soft, dry and well-bedded surfaces (Fregonesi, Veira, von Keyserlink, & Weary, 2007; Haley et al., 2001; Norring et al., 2010; Tucker et al., 2009). In the present study, the number of individual calves lying down was affected by the time of day and rearing substrate. The majority of rest occurred at night time or after feeding times however, when restricted to sawdust and rubber, calves were quicker to lay down following afternoon feeding than calves on sand or stones. The delay for calves on sand and stones to lie down following feeding may indicate reluctance of calves of calves to lie down on these substrates. Previous work in dairy cattle has shown that animals spend more time assessing their environment before lying down in confined lying spaces, such as in tie-stalls, compared with pasture or areas that have a deep layer of substrate (Krohn & Munksgaard, 1993; Müller, Ladewig, Thielscher, & Smidt, 1989).

To investigate the insulating properties of each substrate, the internal and surface temperature were measured. Temperatures were highest for sawdust and lowest for sand regardless of the location tested. The differences in temperatures could be attributed to the insulation properties of each substrate and may be associated with differences in the calves lying times. Sawdust has greater insulating properties than sand or stones, these substrates may also increase heat loss through conduction (Sutherland et al., 2014). This study was conducted during the Southern hemisphere spring, as this is the main calving period within New Zealand dairy systems. The temperatures within the experimental facility averaged 11.7°C. The number of calves lying on a substrate when restricted was not influenced by the temperature within the experimental facility. However, when given a choice, these low temperatures may have influenced calf preference for substrates with greater insulating properties rather than substrates with conductive properties (Sutherland et al., 2014). Similarly, preference may change for substrates with low insulation properties to keep calves cool if housed indoors during warmer months. Dairy calves have been reported to spent less time lying on cool or drafty floors and change their lying posture in response to cold temperatures, including resting on their sternums or with their legs contracted (Hänninen, Hepola, Rushen, de Passillé, Pursiainen, Tuure, Syrjälä-Qvist, Pyykkönen, & Saloniemi, 2003). Sutherland et al. (2013; 2014) found that calves reared on stones had lower skin temperatures compared to calves reared on sawdust. In addition, pigs reared on wood shavings had higher skin temperatures in comparison to pigs reared on partially slatted concrete floors (Hötzel et al., 2009). Further investigation into the thermodynamic properties of substrate types, calves skin temperature and lying postures in response to this is needed to fully understand this relationship and how it relates to the thermal comfort and welfare of dairy calves.

Rearing substrate did not affect concentrations of cortisol, glucose, lactate, WBC, neutrophils, lymphocytes or the N:L ratio. Similarly, both Panivivat et al. (2004) and Sutherland et al. (2014) found no effect of rearing substrate on cortisol concentrations in dairy calves reared on different substrates. Alsemgeest et al. (1995) found similar cortisol concentrations between calves reared on 2 different types of flooring (profiled durable plastic floor or a wooden floor with a rubber profile top layer). One of the most important roles of cortisol or corticosterone is to increase blood glucose levels in response to stress and is also known to influence the immune system, including neutrophils and lymphocytes (or heterophils and lymphocytes; Nicol et al., 2009). Nicol et al. (2009) found that corticosterone did not predict environmental choice in hens, however, lower blood glucose levels and the heterophil:lymphocyte ratio were associated with environmental preference. The rearing substrates used in the present study did not appear to differentially affect the stress or immune response however, if calves were reared on these surfaces for longer it is possible we may have found differences.

For all pairwise comparisons, calves had a strong preference for one substrate over another, spending on average, 89% of their time on one substrate (sawdust). When on their preferred substrate, calves spent a higher proportion of time lying than standing, there is evidence that when on the non-preferred substrate, calves had a tendency to stand rather than lie. When presented with two options simultaneously, calves spent a higher proportion of time on sawdust than rubber, sand and stones. Calves spent a higher proportion of time on rubber than sand and stones and a higher proportion of time on sand than stones. When on the preferred substrate of the pairings offered, calves spent approximately 66% of their time lying. Calves maintained a high proportion of time lying on one surface and rejected the less preferred surface. The rejection of sand and stones raises concern about the suitability of these as rearing substrates. Manninen et al. (2002) reported dairy cows having lower lying times on sand, but also found that additional experience with sand improved acceptance of this surface. This suggests that dairy cows require a period of adjustment when switching bedding. In the present study, for both the restriction and pairwise periods, we ensured calves had 24 h acclimatisation on each possible surface and choice before testing began. The question of how long an acclimatisation period is required remains unanswered, as data were not collected during this period we are unable to determine if preference would change over time. However, based on the results we can rank calves' preference as sawdust, followed by rubber chip, sand and lastly, stones.

One possible criticism of the present study is the use of simple choice tests as a measure to determine environmental preference. Preference assessments do have a number of methodological issues (Fraser & Matthews, 1997), which we attempted to address in our experimental design. It is important in preference testing to ensure that different surfaces are not confounded with location (Tucker et al., 2003). To minimise this, all experimental pens were constructed with each substrate in a different location and restriction and pairwise periods were presented in a different order for each replicate. Preferences are relative; for that reason, a non-preferred option may also be acceptable (Kirkden & Pajor, 2006; Tucker et al., 2003). By measuring the times spent standing, lying and playing when calves were restricted to each substrate enables assessment of whether the forced use of a less preferred substrate affects behaviour (Tucker et al., 2003). We accept that measures of demand provide a better overview of an animal's motivational strength, but due to the amount of time calves spent at rest we felt that assessing demand was not practical.

Our primary objective was to assess the preference of dairy calves for different rearing substrates. Overall, the results of this experiment highlight that calves prefer sawdust as a rearing substrate. By maintaining this preference for the duration of the study, sawdust has been reinforced as a 'gold' standard for lying surfaces. The clear preference shown by calves for sawdust may be associated with the physical and thermal properties compared to the alternative substrates. Other factors such as cost to the farmer, availability and practicality should be taken into account along with animal preference when assessing alternative materials for calf rearing. Further investigation into the long term management practices and calf health when reared on any alternative substrate is warranted before any recommendations can be made.

Chapter 3

Conclusions and recommendations

Implications for animal welfare and final conclusions

The dairy industry is crucial to New Zealand's export market with $13.7 billion dairy products exported in 2012. The government's Business Growth Agenda goal is set to increase the ratio of exports by 30-40% by 2025 (DairyNZ, 2013). Farmers have been known to partake in management practises that are focused on economic principles (Etim, Offiong, Eyoh, & Udo, 2013) as a result of a goal-orientated industry increasing pressure to maximise the number of animals per farm, labour and capital (Baxter, 1983; Gonyou, 1994). In the same instance, public awareness of farm animal welfare is increasing. Consumers are demanding 'welfare friendly' products and better living conditions for farmed animals, which places pressure on the livestock industry to improve welfare standards. Research into improving the rearing environments of young stock is important for the industry.

The present study found evidence to suggest that calves have a preference for some rearing substrates as measured by lying and standing times and play behaviour. However, we did not find evidence to suggest that rearing substrate influenced physiological indicators of stress. Research into the long term housing of calves on these substrates would be beneficial before making recommendations. If we could demonstrate that the rearing environment has positive effects on reducing stress and increasing production, it would have major implications for the dairy industry and animal welfare. Our results indicated that dairy calves had a clear preference for sawdust in comparison to rubber, sand or stones and this preference was maintained for the duration of the trial. We believe the information obtained from the present study is a first step for providing farmers with recommendations when preparing for calf rearing and for informing industry, policy and codes of practise for improving animal welfare.

Future Research

The labour intensive rearing of calves is a requirement of modern farming practices. Therefore, it is important that calves are given the best start in life through good management practices to ensure good welfare from birth and enhanced productivity at maturity. The following recommendations for future research are listed below:

- To investigate the effects of rearing calves on sawdust, rubber, sand and stones on their behaviour and physiology over a 6-8 week period to replicate on-farm practices.

- To investigate calves preferences for other wood products (e.g., wood chip and post peelings) currently available as rearing substrates in comparison to sawdust.

- Conduct a cost benefit analysis to assess sustainability, practicality and availability for each rearing substrate.

- Examine the cost effectiveness and long term feasibility of using rubber as an alternative rearing substrate, with reference on reusing it year to year.

- To examine whether the preferences and rearing environment as a calf follow through into maturity, with particular reference to indoor housed cows.

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Appendix A

AgResearch Ruakura (Protocal N° 12966) and University of Waikato (Protocal N° 897) Animal Ethics approval are attached on the accompanying CD.

Appendix B

Table B.1. Summary of the one-way ANOVA report showing the proportion of time calves spent standing on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period.

Pairwise choice	d.f.	ddf	F-value	*p*-value
Rubber vs Sand	1	10	27.78	< .001
Sand vs Sawdust	1	8	39.29	< .001
Sand vs Stones	1	8	72.91	< .001
Sawdust vs Rubber	1	8	24.17	0.001
Sawdust vs Stones	1	10	117.81	< .001
Stones vs Rubber	1	10	56.27	< .001

Table B.2. Summary of the one-way ANOVA report showing the proportion of time calves spent lying on each available substrate for all pairwise choices (n=6 groups, 4 calves/group) over a 24 h observation period.

Pairwise choice	d.f.	ddf	F-value	*p*-value
Rubber vs. Sand	1	10	344.61	< .001
Sand vs. Sawdust	1	8	434.31	< .001
Sand vs. Stones	1	8	656.3	< .001
Sawdust vs. Rubber	1	8	353.25	< .001
Sawdust vs. Stones	1	10	579.23	< .001
Stones vs. Rubber	1	10	221.81	< .001