The Effects of Different Milk Allowances on the Behaviour and Liveweights of Holstein-Friesian Bull Calves

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Abstract

Despite the importance of milk for young calves, it is often still provided in restricted amounts on farms. The aim of this study was to determine the effects of high and low milk allowance on the behaviour and liveweights of calves from approximately one week of age until one year old. I predicted that calves offered a lower milk allowance would show signs of negative affective state and therefore demonstrate behaviours associated with negative welfare, as well as have lower liveweights, compared to calves offered a greater milk allowance who would sustain a more positive affective state, subsequently expressing behaviours associated with positive welfare and have larger liveweights. Twenty-two Holstein-Friesian bull calves were offered 5 L or 10 L of milk replacer/calf/day from approximately one week of age. Calves were weighed weekly until weaned off milk which then became monthly until one year of age. At three time periods throughout the trial (at four weeks of age, the first time out on pasture, weaning period) the behaviours of the calves were recorded using validated accelerometers and either live observations or video analysis.

From one to five weeks of age, calves offered 5 L of milk/day were observed visiting the meal feeder, milk feeder and hay feeder more often ($P<0.001$, $P<0.001$ and $P=0.028$, respectively), were less involved in self and allo-grooming ($P=0.011$ and $P=0.012$) and spent less time lying ($P=0.024$) compared to calves on the 10 L milk allowance. Furthermore, calves offered the higher milk allowance, were 5.7 kg heavier ($P<0.001$), at five weeks of age compared to the calves offered the lower milk allowance. During the initial period on pasture, the calves offered 5 L of milk/day were observed conducting less ruminating and sternal lying and during the pre-weaning period were observed grazing and standing more often, compared to calves offered 10 L of milk/day who were observed resting and grooming other calves more often. The liveweight differences from five to eleven and a half weeks of age were significantly different, however over the weaning period from twelve to thirteen weeks of age this liveweight difference began to shrink and by fourteen weeks of age, the difference was 5.55 kg. There was no weight difference at one year of age.

The milk allowance significantly affected the behaviours and liveweights demonstrated by the calves. This study confirmed this study's hypothesis that prior to weaning, calves offered more milk demonstrate behaviours associated with positive welfare indicating satiety, comfort and grooming in comparison to calves offered less milk who demonstrate behaviours associated with negative welfare such as hunger and restlessness (higher activity). Creating change in the dairy industry enhancing positive animal welfare gives calves the best opportunity to have a good life.

Key words: Positive and negative welfare indicators, behaviour, milk allowance, calves, weight gain, pasture and weaning

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Chapter 1
Introduction

1.1 Early life of a dairy calf

The natural life of a dairy calf (*Bos taurus*) begins when a female cow is either artificially inseminated or naturally conceived by the mating of a bull, where the cow will then have a gestation length of approximately 282 days (~10 months) (LIC, 2023). During this period the calf grows and develops and is typically born in New Zealand from July or August (Adler *et al.*, 2015). Good practice on farms is to collect cows and their newborn calves twice per day (DairyNZ, 2023a). Once the calf is removed from the mother, the cow is milked so her colostrum can be stored and fed to the new-born calf, which is subsequently now dependent on humans for its health and welfare needs. Calves in New Zealand should receive 4 L or more of gold colostrum (>22 % on a Brix refractometer) within the first 6-12 hours of its life (DairyNZ, 2023b). This process varies to the life a calf, in nature (with no human contact) experiences. Calves in this scenario attempt to stand within 20 minutes of birth and within two hours are suckling colostrum from their mother's udder (Whalin *et al.*, 2021). These calves also tend to feed up to 12 times/day (NZAGBIZ, 2023). There are a series of pathways for calves in New Zealand and in 2020 approximately 28 % of them were reared as dairy replacement heifers, 27 % went to the beef industry, 35 % were sold as bobby calves and the remaining 10 % were either born dead, died, euthanised or classified as other (Edwards *et al.*, 2021). Calves that are reared on farms are then typically fed twice/day for approximately one month, to grow and develop sufficiently and are generally not weaned off milk any earlier than six weeks of age (DairyNZ, 2023e). The way dairy farmers rear their calves in New Zealand varies, which leads to discussions and development protocols around best animal welfare practises.

1.2 Bobby calves and solutions to reduce their numbers

A series of options have been investigated to reduce the number of calves slaughtered at an early age. One option is to use sexed semen, where cows can be predominantly inseminated with female sperm, increasing the chance of a cow having a female calf to 90 % (Bolton, 2019). Sexed semen reduces the number of low-value male dairy-type bobby calves being born as well as accelerating genetic gain due to replacement heifers being born to top tier cows (De Vries *et al.*, 2008). Any surplus female calves can, therefore, be sold to other dairy farms as replacement heifers (Bolton, 2019). Limitations to this solution, however, are the cost of the semen and poor conception rates, which have been found to be reduced by 13.3 %, compared to standard frozen semen. Also, this is not a solution at scale. Since most dairy farms rear sufficient replacements themselves, there will still be a considerable number of surplus calves. An additional solution is using beef bulls instead of dairy bulls on dams not used for breeding replacements. This enables more calves to be raised as beef animals. However, the increased risk of dystocia (calving difficulty) has affected the success of this solution, particularly in heifers (Bolton, 2019). Clearly, there are still areas of research needing to be addressed, including the potential for rearing bobby calves for longer, to improve the ethical use of calves and their welfare. Although additional research is required in this area, there are new regulations and initiatives to improve the status and welfare of bobby calves that are being enforced. A number of these new regulations include needing to be four days old and physically fit before being transported, a maximum 12 h journey time, no calves are to be transported by sea across the Cooks Strait and no calves are to be killed via the use of blunt force trauma unless the calf is in a state of emergency (Guy, 2016).

Given that bobby calves continue to be a management challenge for farmers and a reputational risk to the industry, alternative solutions should be explored. Management systems need to be considering not only the growth and health of calves, but also their overall welfare. They also need to be practical and economical to the farmer. As these adjustments continue to be developed on farms and if it is financially viable for farmers, rearing “by-products” (bobby calves) for longer on dairy farms could be an option to potentially grow the New Zealand meat industry and meat exports and reduce the slaughter of bobby calves. A primary focus of this thesis is looking at management systems in terms of feed allowances and how it affects the overall welfare of the calves not only in terms of the health and growth of the animals but also on their behaviours.

1.3 Animal welfare

1.3.1 Positive and negative welfare indicators in calves

According to the Five Domains framework, there are five domains of animal welfare: nutrition, the environment, health, behaviour, and the mental state of the animal (Figure 1.1) (Mellor, 2012). The first four domains refer to the physical and survival critical characteristics an animal is faced with. Nutrition involves access to food and water, the environment refers to the challenges the animal experiences within its surroundings, health includes diseases and injury, and behaviour includes the interactions the animal has and what the individual animal demonstrates. The fifth component (mental or affective state) refers to the overall experience of the animal which includes emotions such as anxiety, distress, frustration, boredom, isolation or excitement, relaxation and calmness, as well as physical feelings of hunger, thirst, weakness, breathlessness or strength, energetic and fullness. Each domain can be graded via a non-numeric scale in terms of the severity that the animal is experiencing (Mellor, 2012). Each of the five domains equally contributes to an animal's overall health and welfare.

Historically, welfare research mainly focussed on negative welfare affective states demonstrated by animals. However, today the focus is also around positive welfare/affective states which means the calf is presented with positive experiences and sensations, therefore it is comfortable, well-nourished, healthy, it can express its natural behaviours and is not suffering from fear or pain (Mattiello *et al.*, 2019). The implementation of positive motivational affective states has evolved over time in environments where an opportunity has arisen, primarily due to the cost of implementing it declining (Fraser & Duncan, 1998). Positive behaviour indicators are motivated because of the pleasure the behaviour gives the animal (Fraser & Duncan, 1998). This is why positive affective states are now being utilised as health and welfare indicators as well as negative behavioural indicators. Negative behavioural indicators not only show what an animal needs more of but also what an animal needs less of for example pain related behaviour. Positive behavioural indicators on the other hand highlight what animals need to have sustained.

Several authors have considered positive welfare indicators in calves managed under different circumstances. Napolitano *et al.* (2009) outlines several behaviours that are associated with positive affective states. One of these is play behaviour, where environmental stimulation in an enriched environment drives the animal to express positive feelings (Napolitano *et al.*, 2009). Play behaviour is a key indicator of positive welfare as it not only indicates an absence of fitness threats, but it also acts as a reward. Additionally it brings psychological benefits to the individual animal, as well as to surrounding animals as it is socially contagious (Held & Špinka, 2011). Calves tend to play when their primary needs are met but also when it is reinforced by their peers (Jensen *et al.*, 1998). Calves typically perform locomotor or social play. Locomotor occurs first which includes play such as galloping, bucking and kicking, whereas social occurs at a later age and refers to play-fighting or non-reproductive mounting. Both types of play can be regularly exhibited in calves when all needs are sufficiently met (Jensen *et al.*, 1998). Play is a critical behaviour to observe when assessing welfare as it not only indicates positive welfare, but it also acts as a tool to improve welfare (Held & Špinka, 2011).

In addition to these positive welfare indictors, there are many more behavioural and physiological aspects (Papageorgiou & Simitzis, 2022). One of these is exploration, which refers to the animal investigating and gathering information about its surrounding environment to feel in control of it. A second behaviour is feeding and sucking. Feeding in animals is a motivational yet also pleasurable behaviour that supports the survival of the animal and reduces stress as it enables the animal to express its natural behaviour (Papageorgiou & Simitzis, 2022). Ruminating is also related to feeding, but is used to instead assess welfare and is a positive indicator involving a process of regurgitation, salivation and swallowing (Schirmann *et al.*, 2009). Ruminating occurs so that cattle can further reduce the size of particles they have ingested and also better digest fibre (Schirmann *et al.*, 2009). Other behaviours indicating positive welfare outlined in Papageorgiou and Simitzis (2022) include lying and resting behaviours. Generally increased lying times are an indication of comfort, leading to positive affective states. The authors also noted the idea of behaviours being synchronized between individuals. Often animals will demonstrate the same lying behaviour as their close neighbours, which has been thought of as a symbol of positive social status. A series of other behaviours were evaluated such as pro-social behaviours, which are the behaviours calves indulge in to benefit their peers, and anticipation behaviours such as waiting for a reward, postures, vocalizations and facial expressions (Papageorgiou & Simitzis, 2022). Because of this wide range of positive behaviours, it is important to include measurements of multiple key behaviours as indicators of positive welfare in studies of calf behaviour, to understand how animals perceive their environment, and ultimately to maintain or improve welfare for animals on all farms.

Negative welfare indicators can also be exhibited by cattle. Two of these include ear position and tail position (Keeling *et al.*, 2021). Ears appearing backwards or hanging low demonstrate fear or unpleasant emotions. Certain tail positions such as raised high can indicate fear, less movement could indicate lack of pleasure and change in laterality could indicate negativity. An additional negative behavioural indicator is the lack of allo-grooming or self-grooming. Allo-grooming in particular enables the calf receiving the grooming to experience a calming effect and when this is absent it shows the animals are not in a state of relaxation. A further negative welfare behaviour is reduced exploration. Exploration can appear in two forms; inquisitive or inspective, with inquisitive involving the calf looking for novel objects versus inspective referring to a situation where the animal is confronted with an unusual stimulus (Keeling *et al.*, 2021). Both types of exploration indicate that the calves' primary needs are met, including being in a stable environment, to be able to demonstrate this type of behaviour. Therefore, a lack of social behaviour shows all five domains of welfare have not been sufficiently met.

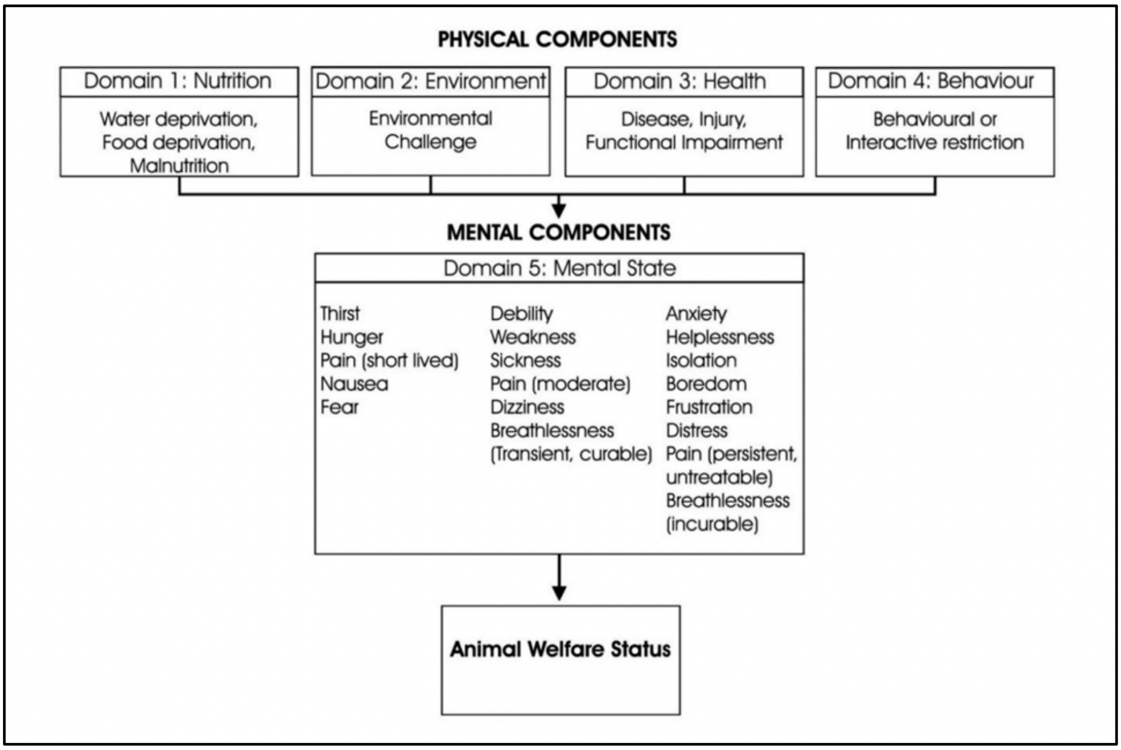

Figure 1.1. The five domains of animal welfare (Mellor, 2012)

1.4 Calf welfare around rearing practices

Numerous experiments have investigated different calf rearing practices, with a growing focus on the welfare of calves under various management systems (Stanley *et al*., 2002; Jensen & Holm, 2003; Wagenaar & Langhout, 2007; Lee *et al*., 2009; Veissier *et al*., 2013; Medrano-Galarza *et al*., 2017). Several themes emerge in terms of what is considered positive welfare and whether farmers are satisfying these criteria with regards to the growth, health and welfare of their animals. According to Fraser (2008), our understanding of animal welfare is a combination of both values and science. This is because people address different concerns when it comes to animal welfare including biological aspects (physical health and functioning of animals), affective states (feelings) or natural behaviours (aspects of natural living) being expressed by the animals. Because of these varying approaches, animal welfare can effectively be defined as the animal's overall wellbeing, based on a combination of values found within social and biophysical science (Fraser, 2008).

Marian Stamp Dawkins considers good animal welfare to be a calf who is healthy and essentially has what it wants (Dawkins, 2021). Animal welfare can be expressed as a Venn diagram, indicating there are many different overlapping components contributing to animal welfare, essentially supporting the statement in Dawkins (2021), refer to Figure 1.2 (Fraser 2008). The environment a calf experiences during its early life affects the behavioural, physical and cognitive development of the animal, which generally persists into maturity. It is, therefore, evident that the type of calf you raise, is the type of cow you will produce (Costa *et al.*, 2019). Animal welfare in terms of colostrum management/feeding practises, thermal stress, ventilation and housing are some of the most important factors contributing to a calf's health and welfare (Costa *et al.*, 2019). Additionally, too big a group size and poor hygiene control can also contribute negatively to animal welfare (Boyle & Mee, 2021). A component that directly relates to rearing practises in New Zealand is the housing environment, particularly the type of surface the calves are kept on, for example river stone or woodchip and the type of surrounding environment (basic or enriched) (Neave *et al.*, 2021). Riverstone in particular has been found to reduce lying and play behaviour, as well as produce lower skin temperature indicating a lack of comfort, resulting in potential negative affective states when calves are housed on this surface (Sutherland *et al.*, 2013). Another factor contributing to the health and welfare of these animals is the removal of calves from cows (Neave *et al.*, 2022). Standard calf rearing farm practise in New Zealand is to remove the calf from the cow within 24 h of the birth, which can result in poor welfare, particularly if the calf misses out on adequate colostrum (Neave *et al.*, 2022). Colostrum intake in a new-born calf, provides the animal with passive immunity, where the calf receives maternal antibodies to infectious agents (Besser & Gay, 1994). Inadequate colostrum volume, method of feeding and the timing of this colostrum intake, are all risk factors associated with perinatal and postnatal mortality in New Zealand (Cuttance *et al.*, 2017). Therefore, optimal management of colostrum intake and reducing the rate of failure of passive transfer of immunity mitigates the risk of illnesses commonly found in calf rearing facilities (Boyle & Mee, 2021). A critical phase in preventing disease outbreaks among calves, and thereby improving their welfare, is to define the problem source and limit the exposure of this risk early on (McGuirk, 2008). Given the large number of factors associated with calf rearing practises that contribute to the animals' welfare, it is extremely important to provide calves with sufficient resources to mitigate detrimental effects on their health and welfare long term.

Much of the literature published around calf welfare concerns the more obvious treatment that these animals need to have the best opportunity to develop into healthy mature animals, rather than considering the intellectual or evolutionary components of calves that contribute substantially to their welfare. Costa *et al.* (2019) outline that animals should also be able to demonstrate their natural behaviours, such as suckling, to reduce the occurrence of abnormal behaviours, which can lead to negative welfare. Enabling an animal to express positive emotional states is a critical consideration associated with an animal's welfare (Duncan, 1996). In a natural environment, cattle will hide their calf away from the rest of the herd once it is born, to protect and bond with them, allowing the calf to feed as it requires without being disturbed (Costa *et al.*, 2019). Depending on the cow-calf pairing, a calf tends to suckle off its mother eight to twelve times per day, thereby optimising its readily available milk supply. Enabling a calf to express its natural behaviours in a rearing facility by having an optimal milk supply and opportunity to suckle for example, allows the animal to exhibit behaviours associated with positive welfare that stem from its natural environment, rather than exhibiting behaviours that are associated with negative welfare, such as cross-suckling or excessive vocalising (Costa *et al.*, 2019). de Passillé (2001) supports the idea that cross-suckling is evidence of negative welfare, as deprivation of milk intake causes frustration in animals and negatively affects digestive processes. Allo-grooming (calves grooming or licking one another) and playing are two positive behaviours that have been observed not only in semi-wild cattle (Costa *et al.*, 2019). These two behaviours are indicators of a tight social bond that has developed between animals and are natural behaviours that calves should be able to demonstrate because they are pleasurable and enhance biological functioning (Costa *et al.*, 2019). Animals not only have the basic needs, to function adequately and exhibit behaviours affiliated with positive welfare, but they also need to be given the opportunity to exercise natural psychological behaviours as well (Bracke & Hopster, 2006). Animals need to be positively motivated to perform their natural behaviours by satisfying their needs rather than becoming frustrated by them (Bracke & Hopster, 2006).

Improving the welfare of calves is an increasing priority for New Zealand agriculture. This focus not only applies to animals who are reared on farms for an extensive period, but also to animals, such as bobby calves, who are slaughtered early in life due to being considered as small, unprofitable and low priority (Van Dyke *et al.*, 2021). In 2019, 5.4 million dairy and beef calves were born in New Zealand and approximately 40 % of these were classed as by-products (i.e., bobby calves) (Van Dyke *et al.*, 2021). A calf deemed a by-product is not reared for beef or as a replacement dairy cow in New Zealand but is instead sent to an abattoir between the ages of four to seven days old. According to Van Dyke *et al.* (2021), the New Zealand Veterinary Association (NZVA) has emphasized that the Animal Welfare Act 1999 must not only ensure cattle are not exposed to cruelty, but must also be protected from physical and psychological abuse. What this means is that, the owner of the animals and person manging the calves must ensure that the physical health and behavioural needs of the animal, are met in accordance with good practise and scientific knowledge (Ministry for Primary Industries, 2023). There are still barriers related to animal welfare in terms of rearing practises, however, if these challenges are used as intervention strategies instead, animal welfare standards should continue to strengthen (Van Dyke *et al.*, 2021). Bobby calves are an ongoing issue and solutions to reduce the number of these animals being slaughtered at such an early age are being deliberated.

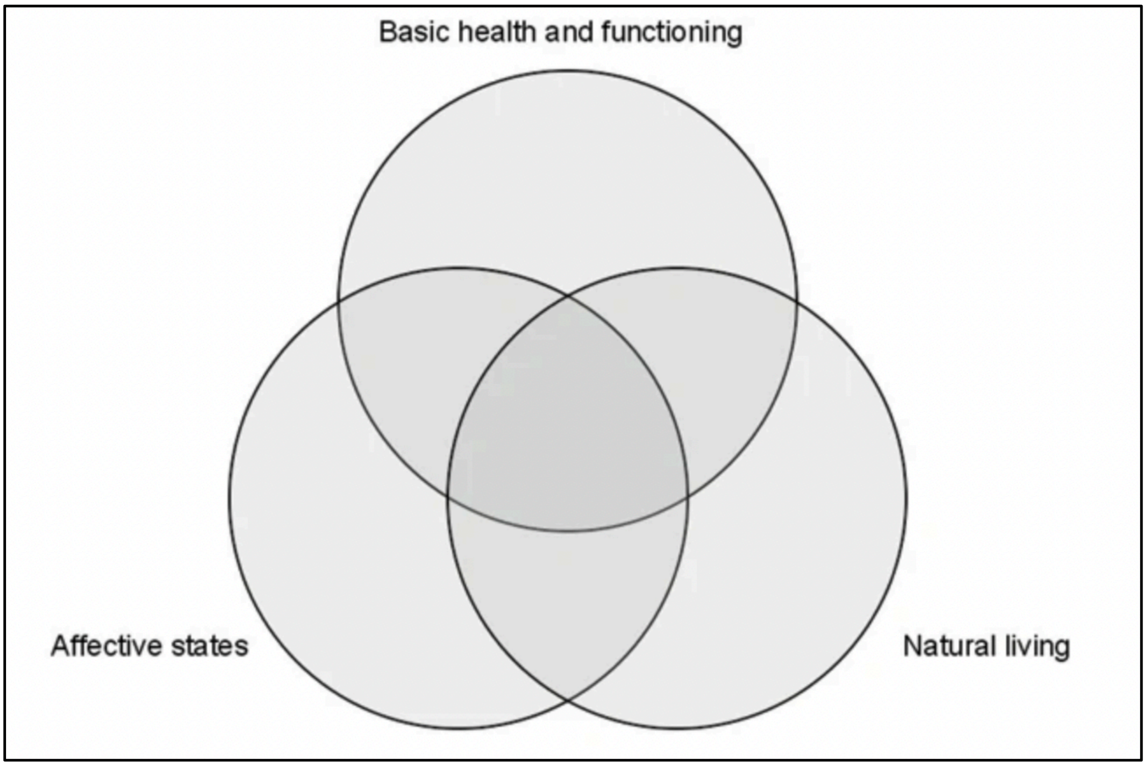

Figure 1.2. Ven diagram showing the three conceptions of animal welfare (Fraser, 2008)

1.5 Different feeding practises contribute to the overall welfare of calves

Much of the previous research into calf rearing practices has focussed on the effects of different milk allowances, milk types, feeding systems and feeding regimes. For example, a previous study investigated the effect of low and high milk allowances and flow rates on calves (Jensen & Holm, 2003). The low milk allowance in this study consisted of 4.8 L of milk/day and the high milk allowance consisted of 8.01 L of milk/day. Calves on the higher milk allowance entered the milk feeder less often and had fewer unrewarded visits, indicating the calves were more content and satisfied and showed fewer signs of hunger. This study also used a computer-operated system whereby the calves were offered a maximum of 12 meals per day, allowing calves to feed regularly and replicating a natural scenario where cows nurse their calves (Jensen & Holm, 2003). Similar results were reported from another study (Huuskonen & Khalili, 2008) where a computerised feeding system was used to provide calves with either restricted (6.0 L/day) or ad-libitum access to milk replacer. Here, the milk replacer intake of the calves on ad-libitum milk was 51 % greater and their supplement intake (silage) was less compared with calves in the restricted treatment group (Huuskonen & Khalili, 2008). Aligning with these previous studies, Rosenberger *et al.* (2017) allocated calves to four milk allowances (6, 8, 10 or 12 L/day). Calves fed less milk visited the feeder more (i.e. showed signs of being hungry) and had a smaller daily weight gain average compared to calves on 12 L/day (Rosenberger *et al.*, 2017). Furthermore, feeding calves high volumes of milk did not appear to cause any increased incidence of disease, but did increase lying time and the efficiency of the use of the feeder (Borderas *et al.*, 2009). In New Zealand it is advised to feed at least 10 % of a calf's body weight in milk (DairyNZ, 2023b). Burggraaf *et al.* (2020) specifically outlines this effect of restricted diets in terms of liveweights, with calves being offered four litres of milk/day and pasture from seven weeks of age, versus eight L of milk/day and as access to pasture after the first week. This resulted in a 31 kg liveweight difference, with the calves on the greater feed allowance growing twice as fast (Burggraaf *et al.*, 2020). An evident theme highlighted throughout most of this literature, is that calves fed greater milk allowances tend to be more content, satisfied and grow faster. This effectively means calves would spend more time lying therefore be less active and show fewer signs of hunger by visiting the feeder less.

Several different milk types are fed commonly to calves including colostrum, cow (whole) milk, pasteurized waste milk and milk replacers (Lee *et al.*, 2009). The timing and quality of colostrum and the different types of milk fed can affect the health and welfare of calves by contributing to their development. Calves fed whole milk had a greater average body weight at weaning and post-weaning than calves fed milk replacer (Lee *et al.*, 2009). Calves fed whole milk consumed a similar amount of dry matter but were always heavier, which is likely due to the improved bioavailability of whole milk diets (Lee *et al.*, 2009). Colostrum provided to calves during the first three days of life compared to milk replacer significantly increased blood concentrations of globulin, total protein, triglyceride, insulin and cholesterol concentrations, thereby positively impacting neonatal metabolism and growth rate (Kühne *et al.*, 2000). Results from (Zhang *et al.*, 2019) support these findings, showing the average daily weight gain was reduced for calves fed milk replacer compared with whole milk. Although milk replacer was found to result in slower growth weights, calves on this diet tended to have long term ruminal developments in terms of higher rumen pH and acetate/propionate ratio (Zhang *et al.*, 2019).

Feeding calves on farms varies greatly depending on the management, goals, and money the farmer is willing to put into its supplies and processes. A feeding system is one of these factors that can be costly, but also cost effective in the long run if tied into the farms ambition. There are several feeding systems farmers can implement such as being fed by nurse cows, stay with the dam, buckets, artificial teats, or automatic feeders. In New Zealand the most common system appears to be artificial teat feeders.

In a study conducted by Appleby *et al.* (2001), calves were either bucket fed five percent of their body weight twice per day or fed ad-libitum from an artificial teat. Calves fed by bucket had 0.36 kg/day weight gain at two weeks of age compared with a 0.85 kg/day weight gain in calves fed ad-libitum on teats. Feeding calves ad-libitum on artificial teats allowed calves to control their own intake, as well as ensuring that all milk would pass into the abomasum whereas in bucket feeding milk can also pass into the reticulum. However, since the groups of calves were not fed the same milk allowance, it was uncertain to what extent the feeding method or the milk allowance impacted the growth weights (Appleby *et al.*, 2001). In contrast, Fallon & Harte (1980) assigned calves to either suckle feeding (nurse cow fed), bucket feeding or artificial teat feeding, but found no statistical difference in the overall calf performance in terms of daily liveweight gains or overall average weight gain between any of these methods (Fallon & Harte, 1980). Sinnott *et al.* (2021) also reported no significant differences between the daily liveweight gains or the average weight gain prior and post-weaning when comparing automatic and manual feeders. Feeding calves via artificial teats is better because it allows calves to undertake the natural behaviour of suckling, it also enhances the chance of milk entering the correct stomach within the calf which is likely to provide additive benefits to calf in terms of health and welfare (Wagenaar & Langhout, 2007).

According to a survey conducted in Canada, 16 % of farmers were using automatic feeders, compared with 84 % using manual feeders (Medrano-Galarza *et al.*, 2017). Although more farmers used manual feeders, there is a distinctive labour advantage when using automatic feeders compared to manual feeders (Sinnott *et al.*, 2021). Automatic feeders also enable the calves to experience more natural milk feeding volumes and frequencies (Medrano-Galarza *et al.*, 2017). When used correctly, automatic feeders can also reduce nutritional scours or non-infectious scours (DairyNZ, 2023e). Scours are associated with several diseases and are a form of diarrhea (Stoltenow & Vincent, 2003). Automatic feeders enhance animal health and welfare and could improve calf rearing in future, because they enable calves to feed without the social competitive pressure of other calves fighting to drink at the same time.

Along with different milk allowances, milk types and feeding systems, there are also different feeding regimes in terms of how often calves are fed each day. Ellingsen *et al.* (2016) fed six calves two L of milk three times/day. During three of the morning feeds, calves were offered more milk, where four out of the six calves drank more than 5 L in one meal (Ellingsen *et al.*, 2016). Another study indicated that blood insulin responses in Holstein and Jersey calves were better in calves fed twice per day, compared with calves fed once per day, which could help to improve overall health (Stanley *et al.*, 2002). There are a number of benefits to feeding calves twice per day compared to one per day including calves achieving higher initial daily gain as well as providing a routine that's somewhat closer to the natural environment a calf would experience if kept with its mother (AgriVantage, 2023). Generally, it is also easier to feed higher milk volumes on a twice a day feeding regime compared to once a day and it reduces instances of rejected volumes (AgriVantage, 2023). Feeding calves once per day during the first four weeks of age while they cannot fully digest solid feeds to support hunger, also does not fully support the animal's nutritional needs which can ultimately affect the overall welfare of the animal (DairyNZ, 2023e). A study using 46 Holstein cows and calves, had 24 of the dams separated from their calves, with the other 24 remaining together for 16 weeks (Veissier *et al.*, 2013). The study found that calves left on their dams suckled more often, than the other calves were seen on the calf feeder with artificial teats. This implies that calves left on their dams are more likely to satisfy their suckling needs, emphasising how important it is when cows and calves are separated, to feed at least twice a day if not more (Veissier *et al.*, 2013). Overall, in terms of health and welfare, there are beneficial implications of feeding calves at least twice if not more per day instead of once, such as providing the calf with the satisfaction of feeling full and supporting the calf's initial growth. Although the main focus of these benefits has previously been on the growth, nutrition and physical health of the calves, an improved mental state shown through certain positive welfare behavioural indicators is also prevalent and is highlighted throughout my thesis.

It is evident from previous literature that there are several positive and negative welfare indicators exhibited by dairy calves. Analysing these behavioural observations along with the effects of different milk allowances, in combination with investigating long-term effects on growth, have rarely been investigated, which has provided a pathway for the novel approach of my thesis. My thesis merges both this behavioural component and the effects of feed provision, enabling any current knowledge around each of these components to be linked to one another as well providing new data, to further develop animal welfare on all farms.

1.6 Thesis aims and outline

In this thesis, I aimed to support positive change in calf rearing practices within the dairy sector by generating new information that will assist farmers to provide their animals with a good life. This was achieved by monitoring positive and negative welfare indicators in the behaviours of young calves and their subsequent liveweights when offered different milk allowances and then managed on pasture until one year of age. My thesis outlines a novel approach relative to previous research conducted within the dairy sector, as it combines behavioural indicators of positive and negative affective welfare with long-term growth metrics.

The experimental trial ran between July 2022 and July 2023. All procedures involving animals in this study were approved by the Animal Ethics Committee at the University of Waikato, under the protocol 1146, and complied with the New Zealand Animal Welfare Act (1999). The trial sequence and key measurements are depicted in Figure 1.3. Briefly, 22 Holstein-Friesian dairy bull calves were used in this trial and were reared inside a roofed shed facility for approximately four weeks from about seven days of age. During this time, calves were assigned to one of two different daily milk allowance treatment groups (either 5 L or 10 L/calf), liveweights were recorded weekly (part of normal farm practise), video behavioural recording occurred for a period of five days, leg accelerometers were used and environmental data collected. Calves were then prepared to leave the indoor rearing facility and go out onto pasture, by separating them into their two allocated milk allowance treatment groups and manually group feeding them on calfeterias over the pen railing. Once the calves were outdoors, a series of further measurements took place, including weekly weighing, video recording of the calves' first two hours on pasture, pre- and post-weaning live behavioural observations and weather data collated from National Institute of Water and Atmospheric Research (NIWA). Once the last calf reached 100 kg liveweight, the calves were transported to a neighbouring farm to graze as one group. Pasture measurements and liveweights were then collected monthly, until the calves were one year of age (Figure 1.3).

In Chapter two, I investigated how low versus high daily milk allowances affects the liveweights and behaviours indicative of both negative and positive affective states of Holstein-Friesian bull calves. Through a series of different measurements (weekly liveweights, leg accelerometers, automatic calf feeder data and 24 h video recordings), I statistically determine whether the milk allowance the calves received had any effect on each of these measurements. A total of 17 behaviours were assessed, including six feed-related behaviours (eating meal, hay, or bentonite; at the milk feeder; drinking water; or ruminating), four lying-related behaviours (lateral and sternal lying, unsure posture, head supported), three grooming-related behaviours (self, other calf, receiving), and others such as standing, exploring, playing or cross-suckling (Napolitano *et al*., 2009; Keeling *et al*., 2021; Papageorgiou and Simitzis 2022). Any links detected between milk allowance, liveweights and behaviours could be used to advise farm managers on the improvement of calf welfare (Fraser & Duncan, 1998; Mellor, 2012).

In Chapter three, I examined the liveweights and behaviours of calves offered different milk allowances, when they were first on pasture, as well as pre- and post-weaning (Jensen *et al*., 1998; Jasper *et al*., 2008; Napolitano *et al*., 2009; Von Keyserlingk *et al*., 2009; Vigors & Lawrence, 2019; Papageorgiou & Simitzis, 2022). The calves stayed on their allocated milk treatments until the last calf reached 75 kg liveweight. They then remained on this initial farm, managed on grazed pasture, until the last calf reached 100 kg liveweight. In this chapter, I described trends of the calves' behaviours during these different time periods. I also conducted a statistical analysis to determine whether the two differing milk allowances (5 or 10 L/calf per day) affected the weekly liveweights of the calves over this period.

In Chapter four, I investigated the liveweights of the calves following their transport to a neighbouring farm where they grazed pasture as one group after reaching the 100 kg liveweight target. In this chapter, I determined the longer-term effects of altering pre-weaning milk allowances on the subsequent liveweights and, therefore, the overall growth performance of these dairy bull beef calves post-weaning (Walton *et al*., 1981; Hill *et al*., 2016; Martin-Gatton College of Agriculture).

Lastly in **Chapter five,** I summarise my findings and discuss the implications of my results with respect to wider literature in the field of animal science with links to animal welfare research.

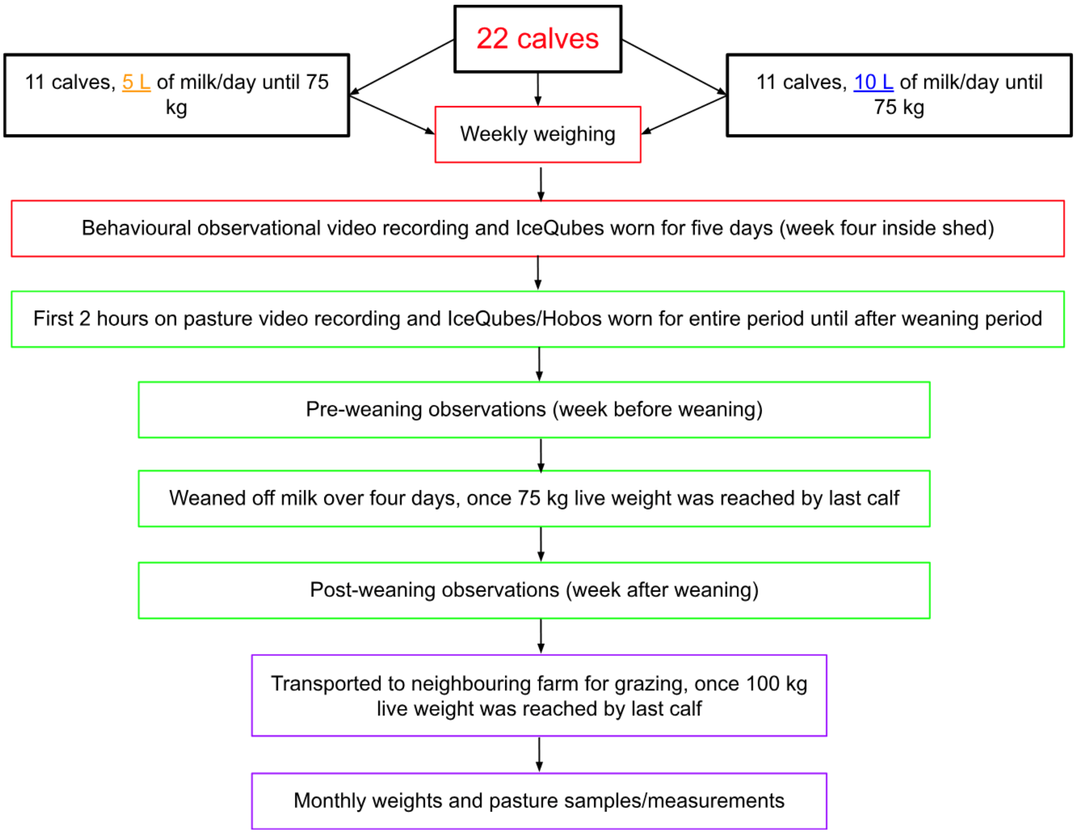

Figure 1.3. Flow diagram of the methods

Chapter 2
The effects of different milk allowances on the behaviour and liveweights of Holstein-Friesian bull calves from two-five weeks of age

2.1 Abstract

Despite the importance of milk for young dairy calves, it is often provided in restricted amounts during rearing after separation from the dam. The aim of this study was to determine the effects of high and low milk allowance on the behaviour and liveweight gain of Holstein-Friesian bull calves. I predicted that calves offered a lower milk allowance will demonstrate behaviours associated with negative welfare, such as increased visits to the milk, hay and meal feeders, cross-suckling and reduced lying, as well as with lower liveweights, compared with calves offered a greater milk allowance. In contrast, I predicted that calves on a higher milk allowance will demonstrate more behaviours associated with positive welfare, such as spending more time self- and allo-grooming, lying down and playing. Twenty-two Holstein-Friesian bull calves were offered either 5 L or 10 L of milk replacer/calf per day via an automatic milk feeder commencing at approximately seven days of age. Calves were weighed weekly, and at four weeks of age their behaviours were recorded, using accelerometers and video observation analysis. Calves offered 5 L of milk/day visited the milk, meal and hay feeders more often, including visiting without a milk reward, indicating signs of hunger ($P=0.028$). They were also less involved in self- and receiving grooming ($P=0.011$ and $P=0.012$) and spent less time lying (55.8 minutes/24 h less, $P=0.024$) than calves on the 10 L/day milk allowance. By comparison, calves offered 10 L of milk/day consumed more milk (+ 1.7 L/day, $P<0.001$) and spent more time lying in lateral ($P<0.001$) and sternal ($P=0.021$) positions, indicating greater comfort. Furthermore, calves offered the lower milk allowance had an average liveweight gain of 9.2 kg compared with 14.9 kg for the calves on 10 L of milk/day, between two to five weeks of age. Overall, calves offered the lower milk volume of 5 L/day showed more signs of hunger, less time lying, were less involved in social interactions and grew slower compared with the calves on 10 L/day. My results suggest that calves should be offered greater milk volumes until weaned, to enhance behaviours associated with positive welfare as well as growth rates.

Key words: Positive and negative welfare indicators, behaviour, milk allowance, growth weight, calves

2.2 Introduction

Calves reared on dairy farms are fed varying milk allowances, which can affect their health and welfare, as well as their growth and subsequent productivity. In 2022, there were over 5.1 million live calves born on New Zealand dairy farms (Figure.NZ, 2023). Typically, calves are removed from the dam within the first 24 h of the calf being born. After this initial cow-calf separation, the calf is taken to a covered rearing facility where it is taught to suckle from a nipple on a manual calfeteria or automatic calf feeder and should be fed at least four litres of gold colostrum within the first six to twelve hours (DairyNZ, 2023b). How a dairy calf is reared in New Zealand depends on the fate of the animal, and whether they are deemed to become a heifer or bull replacement, a bobby calf, or raised for beef. In 2020, approximately 28 % of calves were reared as dairy replacement heifers, 27 % went to the beef industry, 35 % were sold as bobby calves and the remaining 10 % were either born dead, died, euthanised or classified as other (Edwards *et al.*, 2021).

There is not a 'singular' way that dairy farmers in New Zealand rear their calves pre-weaning in terms of feed type, quality or amount offered (DairyNZ, 2023c). Guidelines are, however, provided by the sector and in the Dairy Cattle Code of Welfare (Ministry for Primary Industries, 2019). In the early stages of a calf's life, the rumen, reticulum and omasum are not yet fully developed (Amaral-Phillips *et al.*, 2006). The only functioning component of a newborn calf's stomach is the abomasum, and when the calf suckles, the milk bypasses the rumen through the oesophageal groove into this compartment (Amaral-Phillips *et al.*, 2006). The milk fed to calves initially consists of colostrum for at least the first four days of life, followed by either cow's milk, milk powder replacer or pasteurized waste milk (Lee *et al.*, 2009). Replacement calves and those destined to be reared for beef in New Zealand are typically fed at least 10 % of their body weight each day, ideally in no less than two feeds, for their first five weeks of life, and tend not to be weaned off milk before six weeks of age (DairyNZ, 2023e). Gold colostrum is milk from the first milking and is considered to be of high quality when it has a Brix refractometer reading of >22 %, meaning it has a high IgG content (DairyNZ, 2023b). Cow milk that has not had the fat stripped out of it, is referred to as whole milk and is a higher energy product than milk replacer, due to this higher fat content (Wellert & Hartschuh, 2020). Milk replacer, on the other hand, tends to contain a formula higher in protein and has a more consistent nutrient content. Pasteurized milk is different to both whole milk and milk replacer as it is processed milk, treated with mild heat to reduce milk borne diseases and pathogens (Wellert & Hartschuh, 2020). Although there are benefits to feeding whole milk compared with milk replacer (e.g. the milk is low cost and requires no prior mixing), there are several benefits of using milk replacer (DairyNZ, 2023e). For example, milk replacers can be stored more easily than fresh milk, they contain more known/definite minerals and vitamins, it is not saleable milk coming from the vat, there is less risk of disease transfer from cow to calf via milk pathogens, and milk replacers are very compatible when using automated calf feeders (DairyNZ, 2023e). These beneficial effects are prevalent by the number of farms using milk replacers in a study conducted in Northern Ireland in 2021 (Brown *et al.*, 2021). Those authors investigated calf rearing management practices across 66 farms and reported that 81.8 % of these farmers were feeding their calves milk replacers compared with 18.2 % who were feeding whole milk (Brown *et al.*, 2021).

In addition to different milk types, farmers may offer differing milk volumes, which is the focus of this study. Lower milk allowances tend to consist of four to six litres of milk/calf per day, whereas higher allowances range from eight litres/day to ad-libitum (Jensen & Holm, 2003; Huuskonen & Khalili, 2008; Rosenberger *et al*., 2017). The use of low versus high milk volumes is a topic of discussion, due to the implications these can both have on calves. Previous research has emphasised the importance of feeding higher milk volumes and the positive impacts it has on dairy calf growth rates, both short- and long-term (Rosenberger *et al*., 2017; Burggraaf *et al*., 2020). A clear example of this effect is stated in Burggraaf *et al.* (2020), who reported that calves reared on a higher allowance of eight litres of milk/day remained 31 kg heavier at seven months of age than calves reared on a lower allowance of four litres of milk/day. Furthermore, additional beneficial effects of feeding higher milk allowances on the welfare of calves are evident in several studies, these include fewer vocalisations and visits to the milk feeder, indicating that the animal is obtaining more milk replacer and is therefore full and satisfied. Higher-fed calves also have longer lying times and no increased incidence of disease such as scours due to the higher milk volume (Huuskonen & Khalili, 2008; Rosenberger *et al*., 2017; Jensen & Holm, 2023). Although the physical effects of differing milk volumes on calves is well known and different behaviours associated with affective state (feelings) of calves are beginning to be understood in dairy calves, there are gaps in knowledge around the behaviours associated with positive and negative animal welfare, both short and long-term. I aim to address some of these gaps in my thesis.

According to Fraser (2008), animal welfare consists of overlapping components (biological function, affective state, and natural living) that contribute to an animal's overall wellbeing. When animal welfare practices are not sufficiently met on farms it is generally due to people addressing only one concern, such as the biological aspects (e.g., growth), and ignoring others. This conflict around animal welfare has not arisen due to factual disagreements but primarily due to disagreements around what individuals consider as being the more important factor (Fraser, 2008). A major component of calf welfare is the topic of 'natural living', where for example, the calf is able to express motivated natural behaviours (such as suckling), that are influenced by aspects within its environment (Whalin *et al.*, 2021), which in turn would lead to a more positive affective state.

Positive welfare indicators are extremely important and encouraging to see when exhibited by calves and indicate that the behaviour being performed is due to pleasure (Fraser & Duncan, 1998). When positive affective states are being observed in animals it indicates that multiple domains within animal welfare (nutrition, environment, health, behaviour and mental state) are being met according to the Five Domains framework (Mellor, 2012). Positive welfare behaviour indicators can include play behaviour, allo-grooming, exploration, feeding, ruminating and lying, which may be associated with emotions such as excitement, comfort and satisfaction (Jensen *et al.*, 1998; Napolitano *et al.*, 2009; Papageorgiou & Simitzis, 2022). Negative welfare indicators are the opposite of these and emphasize the lack of pleasure a calf is experiencing by something triggered in its environment (Keeling *et al.*, 2021). Negative behaviours may include physical characteristics, such as ear and tail position, as well as a lack of grooming, increased vocalizations and longer standing times (Doyle & Moran, 2015; Keeling *et al.*, 2021). Understanding the wide range of positive and negative behavioural indicators enables you to see animal welfare from different perspectives, which is the initial step in being able to sustain and encourage positive affective states.

Traditional rearing practices typically focus on the biological functioning (growth and health) of calves. In my thesis, I extend this approach by also investigating the behaviours of the calves associated with affective state and natural living, effectively taking the animals' overall wellbeing into consideration. My thesis examines the effect of different milk allowance on indicators of the animal's wellbeing and, in doing so, I provide new knowledge that helps to address areas where research gaps are currently prominent. This research provides possible future direction and pathways for farmers to rear calves on suitable milk allowances that enables them to demonstrate both positive behavioural welfare and optimal liveweights.

The aim of this study was to determine the effects of high and low milk allowances on the behaviour and growth rate of calves during the first five weeks of their life. The overall hypothesis for this chapter was that different milk allowances (5 L/day and 10 L/day) would cause a difference between the behaviours and liveweights of Holstein-Friesian dairy bull beef calves. Specifically, I predicted that calves fed 5 L/day would have a lower average liveweight and demonstrate more behaviours associated with negative welfare (e.g., cross-suckling, visiting the milk feeder more often, and being more active). Accordingly, I predicted that calves being fed 10 L/day would have higher average liveweights and demonstrate more behaviours associated with positive welfare (e.g., spending more time self- and allo- grooming, lying down and playing).

2.3 Materials and Methods

2.3.1 Animals, Housing and Treatments

The trial was conducted on a Landcorp-Pāmu calf rearing farm in the Taupo region of New Zealand (38.57927° S, 176.16787° E). The experiment was undertaken between July 2022 and July 2023; however, results presented in this chapter are only from the indoor rearing period (July 28th 2022 – August 25th 2022) prior to commencement of weaning onto grazed pasture. All procedures involving animals in this study were approved by the Animal Ethics Committee at the University of Waikato, under the protocol 1146, and complied with the New Zealand Animal Welfare Act (1999). There were welfare concerns raised by the Animal Ethics Committee regarding feeding calves a low milk allowance of 5 L/day; however, as it is common for calves on commercial farms to be fed 10 % of their body weight, I felt this was an important treatment to include. All calves were managed in one group, and provided ad-libitum roughage (hay), meal and water, which was re-filled once or twice a day. Signs of lethargy and illness were monitored in the calves once or twice a day by farm staff and any health concerns addressed. Throughout the project, all calves were managed according to standard farm practice, including receiving vaccinations, drenching and disbudding, or receiving antibiotics, if required. Table 1 provides a detailed description of the trial in this chapter, in particular type and timing of vaccination and drenching of the calves.

There were originally two cohorts of Holstein-Friesian bull calves (n=22/cohort), tested sequentially in the same pen. All calves in each cohort, including both treatments (high and low milk allowance), were managed together as one group. Both cohorts of calves were enrolled in the trial between the ages of approximately seven days old after being collected from three surrounding dairy farms owned by Landcorp-Pāmu in the Wairakei region. Cohort one entered the trial on the 28th of July and Cohort two entered the trial on the 2nd of September. During rearing of Cohort two, there were extensive issues with the automatic feeder resulting in faulty milk powder concentration and, therefore, treatments were not consistently and accurately applied to the animals. Cohort two is, therefore, excluded from the trial and results were not used in the analysis or mentioned further in the methods or results sections. Two calves from Cohort one were also excluded due to sickness (one from each treatment), leaving a total of 20 calves for the trial (refer to the data handling section 2.3.5 for more details).

Calves received colostrum at the three dairy farms supplying animals for this study, prior to arriving at the calf rearing farm. The colostrum given to the animals was tested once a fortnight via a Brix refractometer, which provided an indirect measure of the immunoglobin concentration. Most of the colostrum on these farms was stored in a milk vat and was heated for the first feed given to the calves. Gold colostrum was used daily and was tube fed directly into the abomasum of newborn calves after dam removal and any remaining gold colostrum was given to calves on their second day; therefore, gold colostrum was not stored.

On the 27th of July, before the calves arrived the following day, I set up one indoor pen (4.8m x 9m), placing fresh wood shavings evenly around the surface of the ground (150-200mm deep). A floor area (2400mm x1200mm) and a wooden box (1200mm x 1200mm x1200mm) was set up to hold an automatic calf milk feeder (Model- Forster Technik Lely Calm Automatic Calf Feeder; Lely, Netherlands) (Figure 2.1), which enabled individualised feeding programmes for the calves. The box was put in a corner of the pen and screwed flush with the wall. The milk feeder was placed into the wooden box compartment out of reach of the calves and was fitted and connected to power and

water sources. I then placed two Stallion brand meal bins (length 875mm, width 390mm, depth 200mm) on the front gate of the pen side-by-side, one to be used for meal and one to be used for bentonite (Farmland
s, New Zealand). Bentonite is a clay powder that has excellent absorbent properties due to its surface charge and surface area, therefore binding and absorbing 100% of its dry weight in water (Murray, 2006). The Stallion brand roughage rack (length 260mm, width 550mm, depth 460mm) was placed on a wooden rail on one of the side walls and the Stallion brand water trough, controlled by a bull-cock (length 350mm, depth 200mm, 8 L capacity), was then filled.

Figure 2.1 The Lely automatic calf feeder used in the study. A wooden box kept the feeder secure from the calves.

The calves were provided with meal/Moozlee (Christchurch, New Zealand) (15 % moisture, 18 % protein and 3 % fat), bentonite, bailed hay and water ad-libitum whilst inside the pen and these were refilled and cleaned out daily or earlier when required. Wood shavings inside the pen were also cleaned free of faeces daily.

On the first day the calves arrived in the pen, each calf was injected subcutaneously with 2mls of Salvexin®+B (MSD Animal Health, New Jersey, USA), to protect the animals against diseases caused by *Salmonellab bovis-morbificans, S. hindmarsh, S. typhimurium* and *S. brandenburg.* This was followed by having the hair on their neck covering the jugular shaved with clippers (10cm x 5cm area). Blood was then extracted to fill both a 10 ml red serum vacutainer and 10 ml purple K2 EDTA vacutainer. Blood samples were sent to a commercial laboratory (Hill Laboratories, Hamilton, New Zealand) the same day of extraction, to test for immunoglobulin concentrations using a Goat Anti-Bovine Colostrum Antibody IgG, with a immunoturbidimetric test (Nittobo

America Inc, Murrieta, USA) and a Hitachi Cobas c502 analyser (SVS-Laboratories, 2022). The average IgG concentration for the study calves was 2380.75 mg/dL, with a maximum of 4875.20 mg/dL and minimum of 324.10 mg/dL. Six out of the 22 calves in cohort one had IgG levels below the optimal passive immunity level of 1,000 mg/dL (10g/L) (Lopez *et al.*, 2022).

I then attached a white ear tag with an identification number (one– 22), that had been sprayed with iodine, into the calves' left ear. Each of the calves were weighed using portable scales (custom designed to weigh sheep, goats and calves, made by Leask Engineering, Morrinsville) compatible with a Gallagher 5,000 reader (Gallagher, Hamilton, New Zealand). Photos of the calves' faces, the left-hand side of their body and the right-hand side of their bodies were taken to aid in individual recognition during video analysis of behaviour.

Following these procedures, I registered the calves with the automatic Lely calf feeder and assigned each calf to a milk allowance (5 L/day or 10 L/day) (Figure 2.2). The treatment groups were balanced by calf liveweights as measured on arrival to the farm. The calves allocated 5 L/day of milk remained on this allowance until weaning; however, for the calves allocated 10 L/day of milk, their allowance was gradually increased by one L/day from 5 L/day until 10 L/day of milk was reached on the fifth day of the trial. There was a gradual increase of milk allowance for the calves on 10 L/day as the calves were only a week old and likely on half this amount at their previous farm. A gradual increase also gave the calves' digestive systems time to adjust and process the milk quantity, to reduce the risk of nutritional scours. Once these calves had reached their 10 L/day allowance, they remained on this amount until weaning at 75 kg liveweight (approximately 12 weeks). I trained the calves how to drink from the feeder by directing them into the feed station and getting them to follow my fingers onto the teat, to then begin suckling and removing milk. Milk was dispensed at approximately 39°C and was automatically pre-mixed at a concentrated rate of 150 g of milk replacer (Ancalf; NZAgbiz Ltd, Hamilton, New Zealand)/L of water. Calves remained on the Ancalf milk replacer (26 % protein, 20 % fat, 43.5 % lactose, 3.5 % moisture and 7 % minerals) until leaving the indoor rearing facility at 5 weeks of age, when they were then transitioned onto Ancalf finisher (22 % protein, 20 % fat, 48.5 % lactose, 3 % moisture and 6.5 % minerals).

Figure 2.2. Automatic Lely calf feeder,
milk drinking station setup.

The Lely feeder registered each calf by its NAIT tag (electronic unique identifier ear tag), which was assigned to one of the milk allowance treatment groups. This milk allowance (5 or 10 L milk/day) was split into three equal allocations (e.g., 5 L/day was divided into three feeds of 1.66 L of milk). The feeder allowed the calves three hours per feed to drink their allocation and if not entirely obtained, this would be added to the next 1.66 L of milk for example. Between the full consumption of each allocation, there was a stand-down period of three hours, meaning if the calf tried to access the milk feeder but had already obtained all its allocated milk allowance for that period, the slider door would not open for the calf to drink (unrewarded visit). If the calf had obtained all its milk allowance for the day, the stand-down period would be longer than three hours and the milk feeder would not allow the calf to drink until the next day began. The automatic Lely calf feeder conducted automatic cleans and was re-filled with milk powder every second day.

Table 1. Timeline of the trial events

Approximate age of calves	Event
1 week	Pen set up, then 22 calves arrived at the farm (Calves vaccinated (two mls of Salvexin), weighed and bloods taken).
1.5 weeks	Security cameras installed around pen.
3 weeks	Calves vaccinated (two mls of Covexin) and disbudded. - The calves were withheld from drinking milk for one morning to undergo a series of veterinarian procedures. The calves were all sedated around 10am, which was followed by being disbudded and vaccinated. The disbudding process involved using hot iron cautery and the vaccination given to the animals was two mls/calf of Covexin 10, which protects the calves against clostridial diseases. Both the disbudding and vaccination procedures were concluded by 14:00 h on the same day. The calves could then proceed to drink from the automatic feeder as normal.
4 weeks	IceQubes and collars attached to calves, calves weighed and painted with ear tag ID. Behavioural video recordings.
5 weeks	Calves split into two separate milk allowance groups and fed manually on calfeteria for two days. Calves left the indoor shed and let out onto pasture.

2.3.2 Behavioural observations

To prepare the calves for behavioural observations, a series of tasks were conducted. Towards the end of the calves' first week in the pen, five security cameras (Univ view 5mp vari focal Turret camera, China) were installed on the wooden shed poles around the pen. At the end of week 3, we attached IceQubes (Icerobotics IceQube; Peacock Technology, Stirling, United Kingdom) to the right hind leg of each calf. A coloured collar was also attached around their neck: Calves one-five (green collars), calves six-10 (yellow collars), calves 11-16 (red collars), calves 17-22 (blue collars). The calves were then weighed and painted (detail water-based tail paint, Fil, Mount Maunganui, New Zealand) with their ear tag number on their left shoulder, right shoulder and lower back, using branding stencil numbers (blue paint was used on black coats and green paint was used on white coats). Each calf also had their tail bone painted, the same colour as the collar around their neck (Figure 2.3). The paint markings were undertaken to assist animal identification when viewing video observation recordings. Continuous, 24-hour video recording commenced that night, for a period of five days; however, only 48.5 hours/calf was used in the analysis due to unforeseen circumstances affecting other hours of data (e.g. farm staff entering the pen resulted in a stand down period of 30 minutes on videos to allow calves to settle again or the feeder not working properly resulted in complete sections of data being un-analysed to minimise any effects on behaviours).

Figure 2.3. A calf showing its coloured collar, matching coloured tail bone to collar colour, its identification number painted on its shoulders and tailbone and a yellow IceQube data logger on its ankle.

To determine whether milk allowances affected the behaviour of approximately five-week-old calves, several behaviours relevant to animal welfare were analysed and compared. Variables that were automatically collected from the Lely calf milk feeder included the frequency of rewarded and unrewarded visits to the feeder, milk consumption and drinking speed (mL/min). Behaviours that were analysed from the IceQube accelerometers included lying behaviour (total time) over five consecutive days. Other behaviours were observed from the video recordings using 5-min instantaneous scan sampling (Altmann, 1974) (Table 2). At 5-minute intervals, each of the 17 behaviours were manually assessed and recorded as being present (1), not present (0) or unsure (.). I carried out all observations and assessed my observer reliability on three separate times (at the start, middle and end) over the period of watching the video recordings. Each time the same five calves were done for a period of two hours each.

Three of these five calves were watched from 08:00-10:00 h and the other two calves were watched from 20:00-22:00 h. The level of reliability was calculated based on the percent agreement, using Microsoft Excel (version 16.75). All behaviours were considered to be in agreement with each other when the reliability was conducted at different times. The intra-observer reliability ranged from 84 % to 100 % between different times the videos were watched, with rumination having the lowest percentage of reliability. I was blind to the milk allowance treatment during video analysis.

The calves were also weighed weekly using Leask engineered scales.

Table 2. Ethogram of the observed behaviours in the calves from the video recordings

Behaviour	Definition
Exploring	Animal is sniffing or licking objects (not other calves).
Ruminating	Animal chews with lateral jaw movement and keeps its head at the same level. No feed is seen in the mouth.
Eating meal	Animal is observed with its head in the meal trough.
Eating bentonite	Animal is observed with its head in the bentonite trough.
Eating hay	Animal is observed with its head over or in the hay rack.
Drinking water	Animal is observed with its head in or above the water trough.
Play running	Animal is observed to be running, galloping and/or kicking its legs into the air.
Lying postures (sternal, lateral, head supported, unsure)	Sternal - Flank is in contact with the ground. Lateral - The weight of the calf is on one side of the body, including the shoulder, barrel and the hip. Head supported - Head is resting on its own body, on another calf or the ground (not including grooming). Unsure- Cannot identify lying position
Upright/Standing	Weight supported by feet/not lying. Includes walking.

Cross-suckling	Animal has its head lowered beneath the stomach of another calf and is bunting, suckling or sniffing the navel or region between the hind legs.
Self-grooming	Animal is licking, sniffing or scratching itself.
Inter-grooming (Other or receiving)	Other calf- Animal is licking or scratching another calf. Receiving- Animal is being licked or scratched by another calf.
At milk feeder	Entire upper body and middle/waist of the calf is inside the feed station.

2.3.3 Environmental conditions

Whilst the calves were in the indoor rearing facility, ambient temperature (°C) and relative humidity (%) were automatically recorded in one-hour intervals via a hand-held Kestrel device (5,000 environmental meter w/link, Kestrel Meters, Boothwyn, USA) that was attached to a shed pole 1 m above calf height.

2.3.4 Feeding and illness challenges during the trial

During this trial, several animals arrived at the farm sick or became sick with nutritional scours that ultimately became mild scours at approximately two weeks of age (one from each treatment group) in two of the weaker calves that originally had lower immunoglobulin levels (<1,000 mg/dL) when arriving at the farm. To reduce the spread of the virus, sick calves were removed by farm staff and placed in a pen that was fully enclosed with black plastic along the side walls and gate front, so that they were isolated from the healthy calves. Whilst the calves were in this isolated pen, they received antibiotic treatment as required, and received 2.5 L of electrolytes (Novolyte) three times per day. The range of antibiotic treatments available on farm included; three mm/dose of Vet-Tet 20 (contains 20 % oxytetracycline fighting against gram positive and gram negative bacteria), three mm/dose of Bivatop (prevents respiratory tract infections and post-parturient infections), three mm/dose of Depocillin (contains procaine penicillin which is susceptible against a range of gram-positive micro-organisms) and one mm/dose of Meloxivet (reduces inflammation, pain and fever).

Additional treatment from veterinarians was also provided when needed.

The automatic calf feeder also encountered water shortages due to water to the feeder being interrupted by other farm processes.

A further problem that was discovered at the end of the trial was downloading data from the cloud. Although the automatic calf feeder was serviced days before the trial began and retrieving information from the cloud on the app during the trial occurred, downloading all of the stored data from the cloud at the end of the trail produced issues, due to the server version and cloud being incompatible.

Figure 2.4 outlines a flow chart of the first phase of procedures that were undertaken inside the rearing facility.

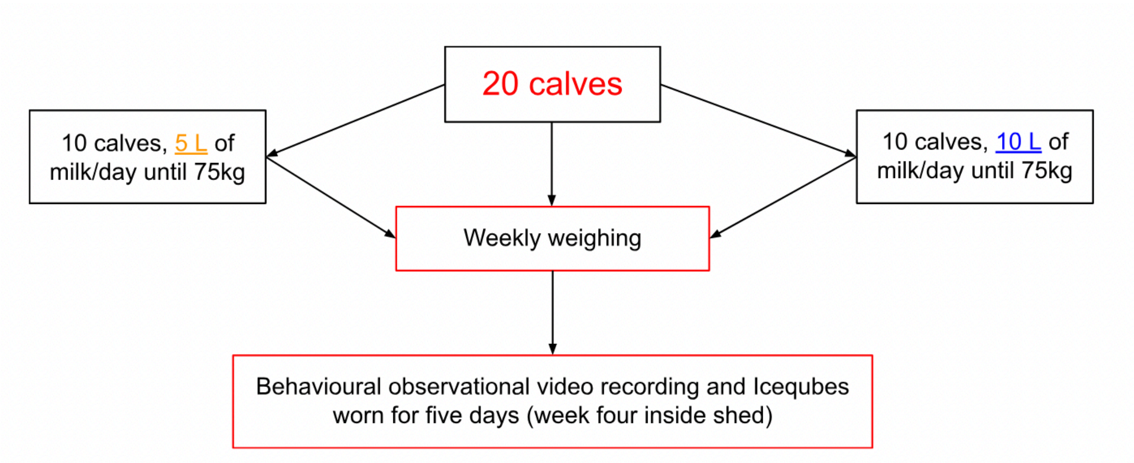

Figure 2.4. Flow diagram of the methods

2.3.5 Data handling

Excluded data

Two calves (one from each treatment) were excluded from all data analyses due to consistent neonatal nutritional scours during the indoor rearing period. A total of 12 days were excluded from the milk feeder data analysis due to issues with the feeder, and data from two calves on one day were excluded due to being in the sick pen without access to the feeder.

Weather data:

Air temperature (°C) and relative humidity (%) were summarised into daily means.

Scan observations

All behaviours were observed in 5-minute scans for 48.5 hours of video recording. For each calf, the percentage of sampling periods where the behaviour was present were calculated for each of the behaviours listed in Table 2, resulting in a single value for each calf, for each behaviour. Behaviours are presented as a percentage of total observations, apart from the lying postures included head supported, which are presented as percentages of total lying observations.

Milk feeder data

Data were collected over the four-week indoor rearing period; however, only two weeks of data were used in the analysis due to issues with the feeder. A daily average was collated for each of the variables on the dates included. This was then converted into one average overall to be used in the ANOVA analysis. The variables recorded were unrewarded (no./day), rewarded (no./day) and total visits (no./day), milk consumption (L/day) and drinking speed (mL/min).

Lying behaviour from data logger

Data from the IceQube devices were recorded in time increments of 15 minutes. The lying time (%/h) per calf was calculated each day (midnight to midnight), which was then converted into an average per calf over the five-day measurement period.

2.3.6 Statistical analyses

Individual calves were considered statistical units. Analyses were conducted in R 4.0.4 (R Core Team, 2021) and mean and standard error of the mean (SEM) values were occasionally calculated using Microsoft Excel (version 16.75). Residual versus fitted plots, Q-Q plots and histograms were initially used to clean the data by checking for constant variance and identifying any potential outliers that could have resulted from faulty technology used for the indoor period of the trial or from abnormalities in the calves like illnesses.

I classified a significant P-value with a difference detected at $P<0.05$.

Liveweights

Liveweight gain data were analysed by conducting analyses of variance, that consisted of a single factor, a fixed effect for treatment and calf as the unit. This involved analysing the weight gain between specified weeks (i.e., weeks one and two, weeks one and three and weeks one and four), which resulted in different levels of significance as expressed in the time series plot. The time series plot is produced over the four-week indoor rearing period, SEM bars were calculated for each of the treatment groups for each of these weeks.

Behaviour using 5-min scan observations

I used a one-way analysis of variance consisting of single factors, treatment as the fixed effect and calves as the experimental unit, to compare the percentage of time spent on each behaviour between the low and high milk feeding allowance groups. A natural log transformation to reduce skewness and help shape normality in the data was applied to the 'at the milk feeder' behaviour. The mean and SED values for this behaviour are therefore presented as log transformations, back transformations are also presented for the averages of this behaviour in brackets for context.

Diurnal patterns were also descriptively presented for eating hay, bentonite and total lying behaviours. The number of times in three hours that the behaviour was observed was summed across all animals for each of the two milk allowance treatment groups. As observations were conducted every five minutes, this resulted in a total of 36 times in 3 hours that the behaviour could have occurred per calf, which is a total of 360 times for the 10 calves in each treatment group. The large gaps in data presented on these time series plots between the 19th and 20th August, were due to only certain sections of data being used (48.5 hr) and not consecutive data due to circumstances that may have affected the behaviours of the calves. Standard deviations (SD) were calculated per calf initially, which were then converted to standard error of the mean (SEM).

Milk feeder data

To compare the behaviour of the calves at the milk feeder between the two treatment groups, I conducted a one-way analysis of variance consisting of single factors, treatment as the fixed effect and calves as the experimental unit, for each of the variables (unrewarded (no./day), rewarded (no./day) and total visits (no./day), milk consumption (L/day) and drinking speed (mL/min)). For the unrewarded, total visits and consumption variables ranked transformed data was used.

Lying behaviour from accelerometers

To compare the lying time between the two milk allowance groups, I conducted a one-way analysis of variance consisting of a single factor, treatment as the fixed effect and calves as the unit. A time series plot was produced showing the average daily lying percentages over a 24-hour period between the two treatment groups. Standard deviations (SD) were calculated per calf initially, which were then converted to standard error of the mean (SEM).

2.4 Results

2.4.1 Environmental conditions

The indoor average daily air temperature was 9.5 °C (SD= 3.10 °C), which ranged from 5.4 °C to 15.1 °C. The average daily relative humidity was 78.4 % (SD=13.9 %). Sunlight could beam into the pen from three sides; however, this was not measured directly.

2.4.2 Behaviour

The behaviour of the calves at approximately five weeks of age is presented in Table 3. Milk allowance had a significant effect on more than half of the behaviours observed. Calves on 10 L of milk/day spent less time eating meal and hay, at the milk feeder and standing, but more time lateral and sternal lying, self-grooming and receiving grooming.

Feeding behaviours

Calves allocated 5 L of milk/day were observed more often eating meal ($F_{1, 18}$= 26.66, $P<0.001$), visiting the milk feeder ($F_{1, 18}$= 31.17, $P<0.001$), and eating hay ($F_{1, 18}$= 5.72, $P=0.028$) compared with calves allocated 10 L/day (Table 3). Calves on 5 L of milk/day were observed more than twice as often visiting the milk feeder and eating hay than the calves offered the greater milk allowance. The 5 L calves were also observed eating meal four times more often than the 10 L calves.

There was no treatment difference in the percentage of observations where calves were ruminating or drinking water (P=0.108 and P=0.862). There was also no significant difference between treatments in the time spent at the bentonite feeder, although this did show a weak trend (P=0.061), with calves offered the greater milk volume observed more often eating bentonite (Table 3).

There was a significant difference in the number of unrewarded visits to the milk feeder between the two milk allowance groups ($F_{1, 18}$=52.780, P<0.001) (Table 4). The calves on 5 L of milk/day visited the milk feeder over 35 more times/day, without being rewarded with milk, compared with the calves on 10 L of milk/day. Furthermore, there was a significant difference in the total number of visits to the milk feeder ($F_{1, 18}$ =38.395, P<0.001), which included rewarded and unrewarded visits. The calves on 10 L of milk/day had a third of the total number of visits to the milk feeder relative to calves on the lower milk allowance (Table 4). The largest average difference on the same day was 107 total visits for the calves on 5 L of milk/day, compared with 10 visits for the calves on 10 L of milk/day. There was no significant difference in the average number of rewarded visits to the milk feeder between milk allowance treatment groups (Table 4).

Based on the consumption variable collected from the milk feeder, on average, neither treatment group drank their full allowance each day (Table 4). The calves offered 5 L of milk/day, however, consumed more of their total allocation, drinking 4.9 L (SED= 0.41) compared with the calves offered 10 L of milk/day who, on average, drank 6.6 L/day (SED= 0.41). The drinking speed variable was also significantly different between treatments ($F_{1, 18}$ =4.619, P=0.045, Table 4), with the calves on 5 L of milk/day, on average drinking 85.1 mL/min faster than the calves on 10 L/day (473.12 vs. 388.01 mL/min, SED=39.60).

Diurnal patterns were also descriptively analysed for eating hay and eating bentonite. Figures 2.5 and 2.6 below are all visual representations of time series plots, where the data points represent the three-hour time periods on each of the days. In particular, the times during the day when the calves on 5 L of milk/day were more interested in eating hay are highlighted in Figure 2.6. These involved a large frequency of observations early afternoon from 12:00-15:00 h on the 18th August and mid-morning from 06:00-09:00 h on the 20th August. Figure 2.6 depicts the frequency of observations in 3 hourly blocks for being observed at the bentonite feeder, which occurred most frequently during the day from 09:00-18:00 h or early in the morning from 03:00-06:00 h, but not very often throughout the night.

Lying behaviours

I measured lying behaviour in two ways, firstly using IceQube sensors for 5 continuous days, and secondly using 5-min scan observations from video recordings over a 48.5-hour period. Based on the IceQube data, on average, the calves offered 10 L milk/day spent more time lying down each day compared with calves on 5 L milk/day (15.48 h/24 h (SEM= 0.331 h) versus 14.55 h/24 h (SEM= 0.18 h), $F_{1, 18}$=6.077, P=0.024) (Figure 2.7). When inspecting diurnal patterns of lying, I found that the calves offered 10 L of milk/day spent, on average, more time lying down during the night from 00:00-05:00 h and from 15:00-00:00 h compared to the 5 L calves (Figure 2.8).

Based on the video observations in Table 3, calves on the 10 L milk allowance were observed lying down more often in both sternal (P=0.021) and lateral (P<0.001) lying positions, with a 5.1 % difference, on average, in the percentage of observations for total lying behaviour between milk allowance treatments ($F_{1, 18}$=11.308, P=0.003). Calves on 5 L of milk/day typically spent less time lying down from 00:00-09:00 h on the 18th and 19th August, and between 09:00 and 12:00 h on the 20th August (Figure 2.9). Calves offered 10 L of milk/day were observed expressing lateral lying behaviours nearly five times more often than the 5 L calves ($F_{1, 18}$=20.776, P<0.0001). The proportion of observations where calves were lying but their position could not be determined was 0.7% and 1.2% for the 5 L and 10 L calves, respectively.

When calves were lying down they were observed, on average, with their heads supported in 25 % of observations; however, there was no difference between treatments (P=0.313, Table 3). The alternative to lying was the percentage of observations standing or being upright. Calves on 5 L of milk/day spent more of their time standing/upright than calves on 10 L of milk/day (P<0.001, Table 3).

Social behaviours, play and exploration

There was a significant difference between the treatment groups for self-grooming behaviour, although this was a small effect of only 1.4 % difference between the groups (P=0.011, Table 3). Receiving grooming was also significantly different between the groups (P=0.012), with the calves offered 10 L of milk observed receiving grooming twice as often as the 5 L group. Calves were observed playing in 0.27 % of all observations, with no statistical difference between groups ($F_{1, 18}$=2.147, P=0.160). However, seven out of the ten calves offered 10 L of milk/day were observed playing at some point, compared with four out of ten calves for the calves offered 5 L of milk/day. There was no significant difference in the percentage of observations for grooming another calf or exploring between the two groups (P=0.121 and P=0.110), although calves offered more milk tended to groom other calves twice as often and explored more.

Cross-suckling

Although cross-suckling frequency ($F_{1, 18}$=0.089, P=0.769) was not significantly different between the two groups, over half (6/10) of the calves on 5 L of milk/day were observed cross-suckling at some point during the five-minute observational scans, compared with a third (3/10) of the calves on 10 L of milk/day.

Table 3. The mean percentage (± SED) of observations for the behaviours of 20 calves, approximately five weeks old, offered different milk allowances (5 or 10 L/day, n=10 calves/treatment). Collated over 48.5 hours. Data for "at milk feeder" were log-transformed prior to statistical analyses, back-transformed means are presented in parentheses.

Behaviour	5 L (%)	10 L (%)	SED (%)	F value	T value	P value
At meal feeder	2.8	0.7	0.40	26.660	5.163	**<0.001**
At milk feeder	-312.3 (4.4)	-384.9 (2.1)	13.01	31.166	5.583	**<0.001**
At hay feeder	0.8	0.4	0.18	5.724	2.393	**0.028**
At bentonite feeder	0.2	0.4	0.11	3.983	1.996	0.061
Ruminating	17.7	13.1	2.74	2.852	1.689	0.108
Drinking water	0.2	0.2	0.09	0.031	0.177	0.862
Total lying	48.2	53.3	1.52	11.308	3.363	**0.003**
Lateral lying (% of total lying)	0.5	2.4	0.40	20.776	4.558	**<0.001**
Sternal lying (% of total lying)	62.3	67.4	2.01	6.447	2.539	**0.021**
Unsure posture	0.7	1.2	0.33	2.356	1.535	0.142
Head supported (% of total lying)	24.0	25.9	1.83	1.079	1.039	0.313
Standing	36.9	29.8	1.89	14.146	3.761	**<0.001**
Self-grooming	1.8	3.2	0.53	7.981	2.825	**0.011**

Receiving grooming	0.5	1.0	0.18	7.862	2.804	**0.012**
Grooming other calf	0.5	1.0	0.29	2.643	1.626	0.121
Exploring	0.5	0.7	0.13	2.830	1.682	0.110
Playing	0.1	0.2	0.051	2.147	1.465	0.160
Cross-suckling	0.5	0.4	0.30	0.0892	0.299	0.769

Table 4. Variables collected via a Lely automatic calf feeder, over two weeks for 20 calves (2-5 weeks old) with different milk allowances (5 or 10 L/day, n=10 calves/treatment), ±SED.

Variable	5 L	10 L	SED	F value	T value	P value
Unrewarded visits (no./day)	38.2	3.1	4.84	52.780	7.265	**<0.001**
Total visits (no./day)	49.6	15.2	5.55	38.395	6.196	**<0.001**
Rewarded visits (no./day)	11.3	12.1	1.66	0.211	0.459	0.652
Milk consumption (L/day)	4.9	6.6	0.41	0.17	0.042	**<0.001**
Drinking speed (mL/min)	473.1	388.0	39.60	4.619	2.149	**0.045**

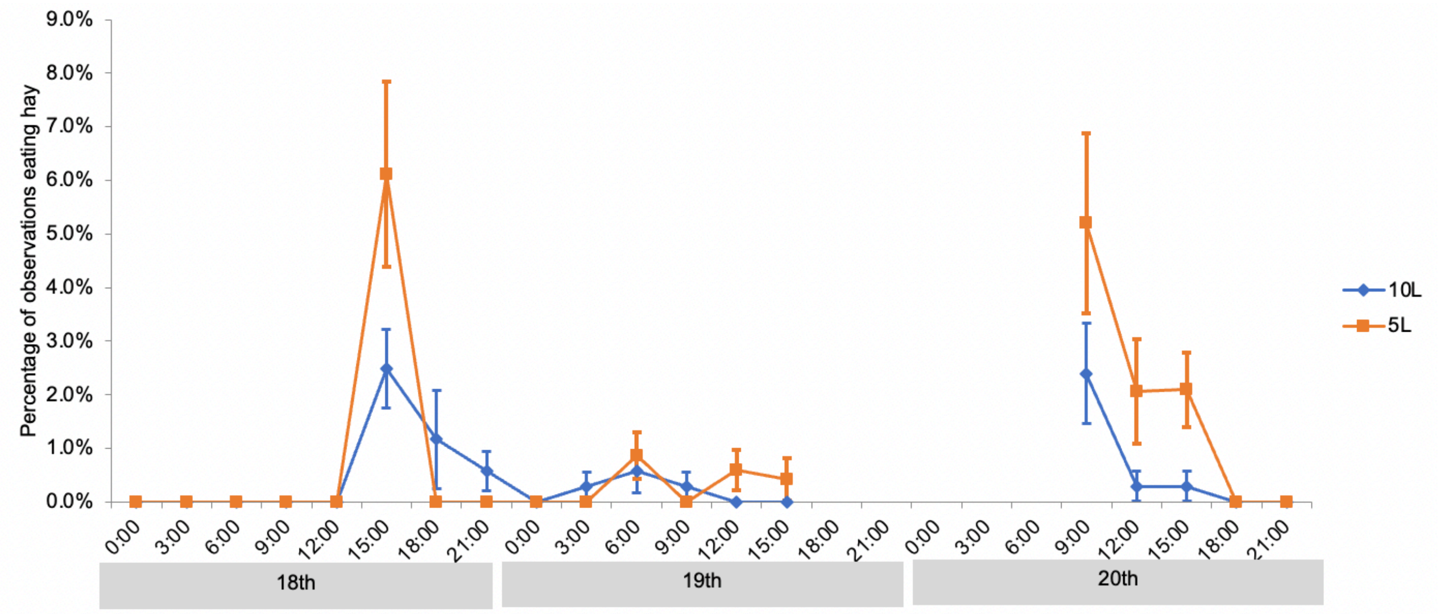

Figure 2.5. The mean percentage of observations in three hourly blocks that 20 calves approximately five weeks old, offered different milk allowances (5 or 10 L/day, n=10 calves/treatment), were observed to be at the hay feeder (±SEM). Collated over 48.5 hours over three days (18^{th} to 20^{th} August).

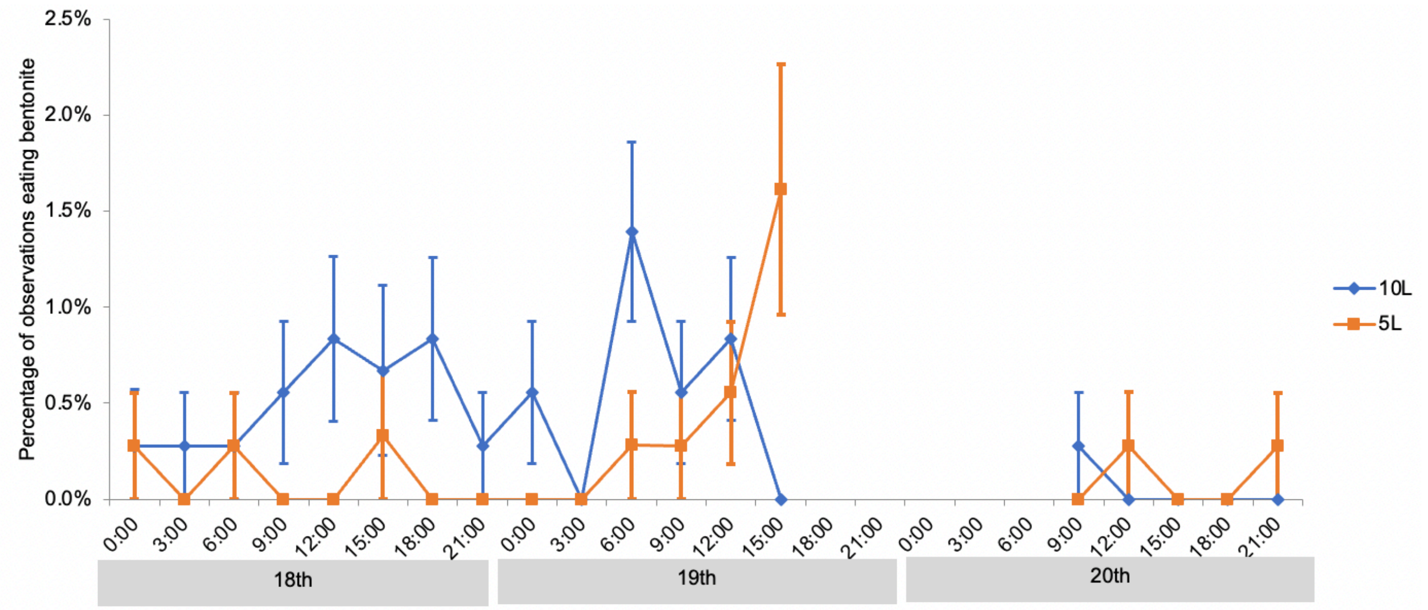

Figure 2.6. The mean percentage of observations in three hourly blocks that 20 calves approximately five weeks old, offered different milk allowances (5 or 10 L/day, n=10 calves/treatment), were observed to be at the bentonite feeder (±SEM). Collated over 48.5 hours over three days (18^{th} to 20^{th} August).

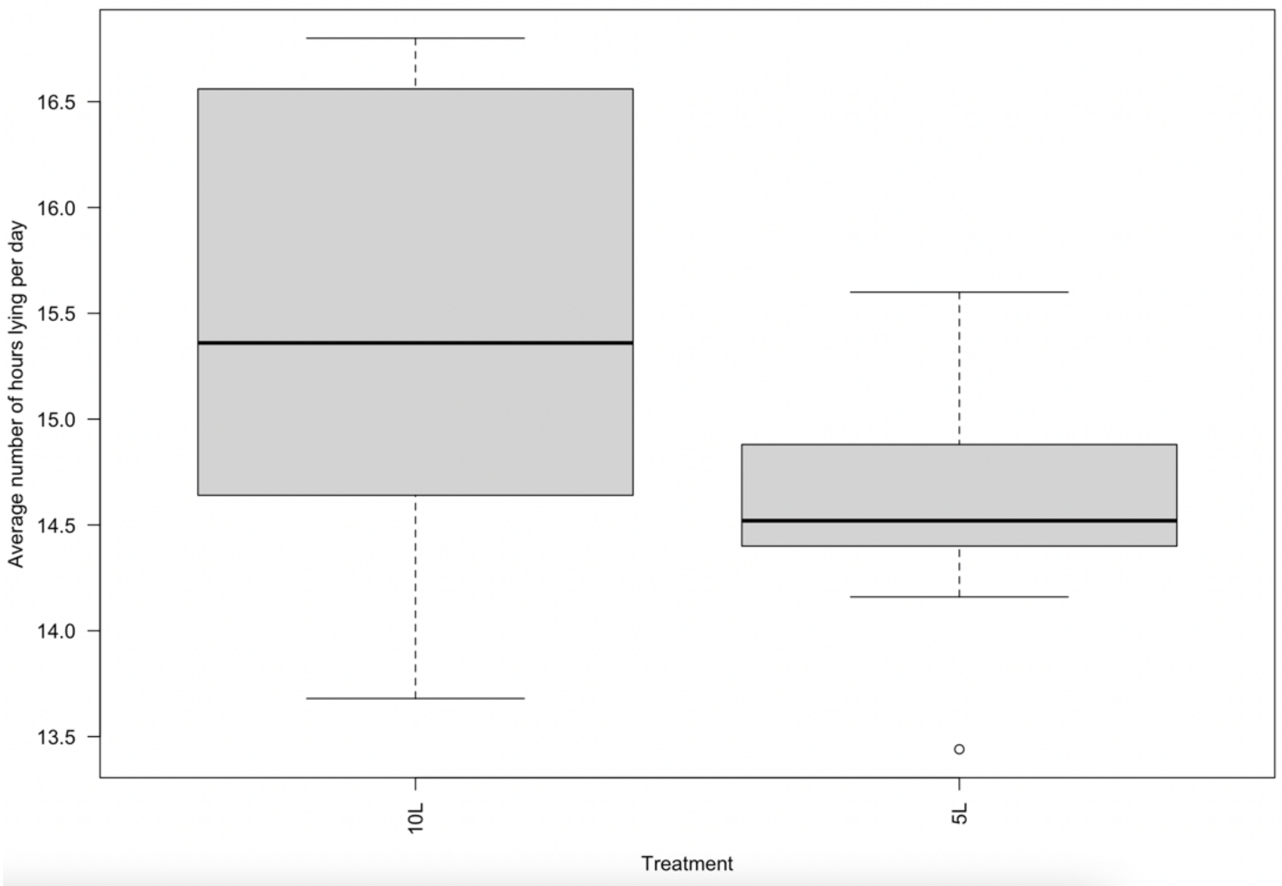

Figure 2.8. The mean number of hours lying/24 hours for 20 calves approximately five weeks old, offered different milk allowances (5 or 10 L/day, n=10 calves/treatment). Data was collated over five days, via IceQube accelerometers. The box plot includes the lower and upper quartile ranges, the inter quartile range, the median and outliers.

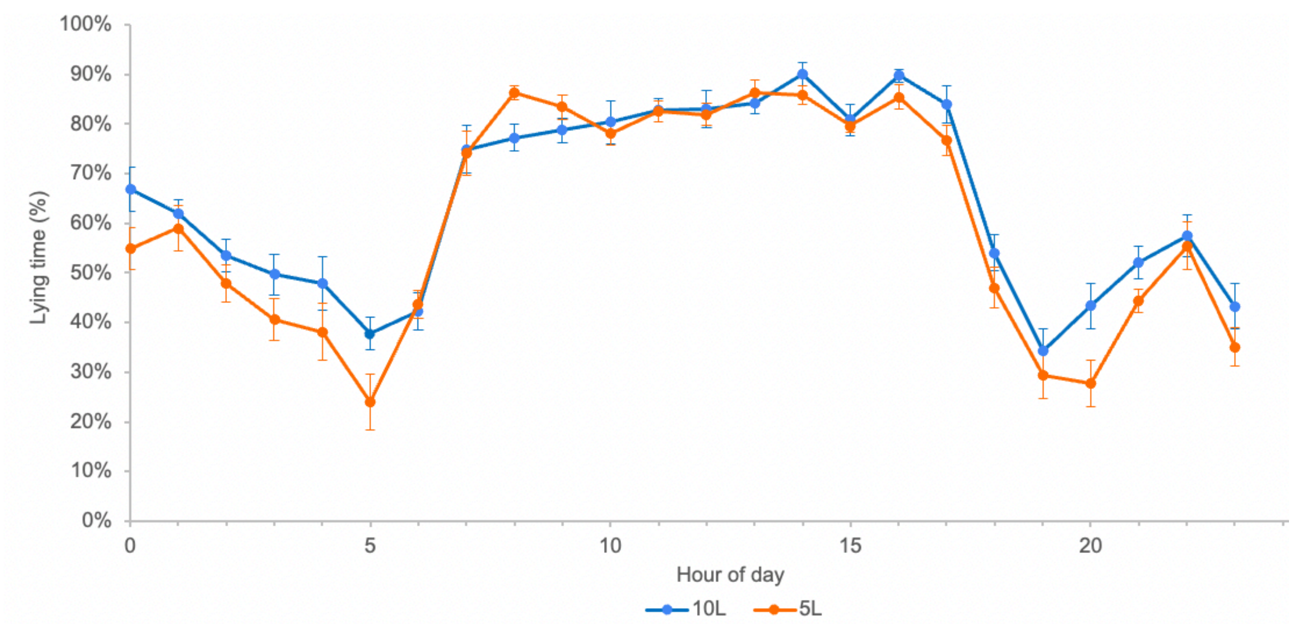

Figure 2.7. Time series plot showing the average diurnal pattern of the percentage of time lying (±SEM) from the IceQube accelerometers, for Holstein-Friesian bull calves offered differing milk allowances (5 or 10 L/day, n=10 calves/treatment). Average for five consecutive days.

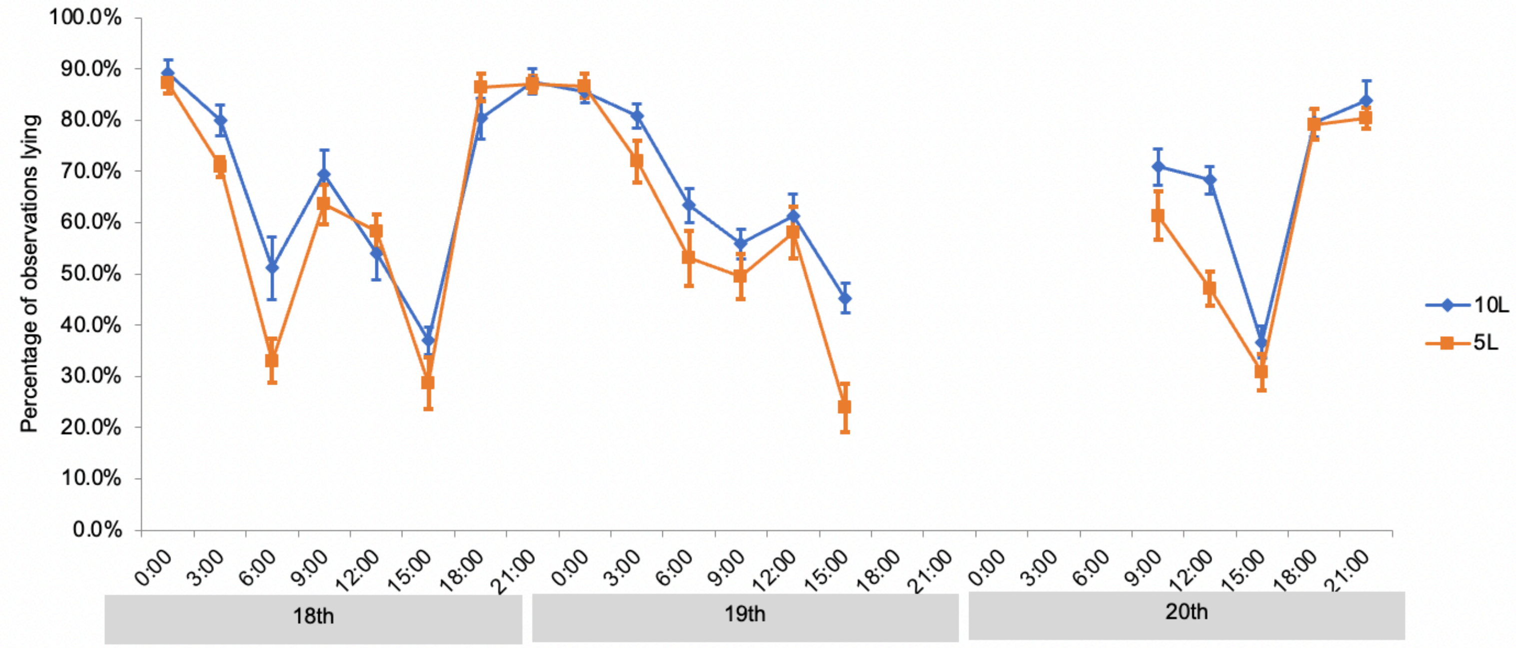

Figure 2.9. The mean percentage of observations in three hourly blocks that 20 calves approximately five weeks old, offered different milk allowances (5 or 10 L/day, n=10 calves/treatment), were observed to be lying (either sternal or lateral), ±SEM. Collated over 48.5 hours over three days (18^{th} to 20^{th} August).

2.4.3 Liveweights

When the calves were first enrolled into the trial, the average liveweight was similar across both treatment groups (39.3 kg, SED=1.47 kg) (Figure 2.10), as treatment allocation was balanced on liveweight. During the four-week experimental period, calves offered a greater milk volume of 10 L of milk/day grew faster between two and five weeks of age than calves on 5 L of milk/day ($P<0.001$, $F_{1, 18}=21.499$, SED=1.23 kg). Over the experimental period, the calves on 5 L of milk/day consistently had lower average liveweights, with the average weight of the two treatment groups when they left the indoor rearing facility on week 4 being 48.5 kg (SEM=1.02 kg) compared with 54.1 kg (SEM=1.02 kg) for the 10 L calves. This was an average liveweight gain at five weeks of age of 9.2 kg (SEM=0.87 kg) for the calves offered 5 L of milk/day compared with 14.9 kg (SEM=0.87 kg) for the calves offered 10 L of milk/day (Figure 2.10).

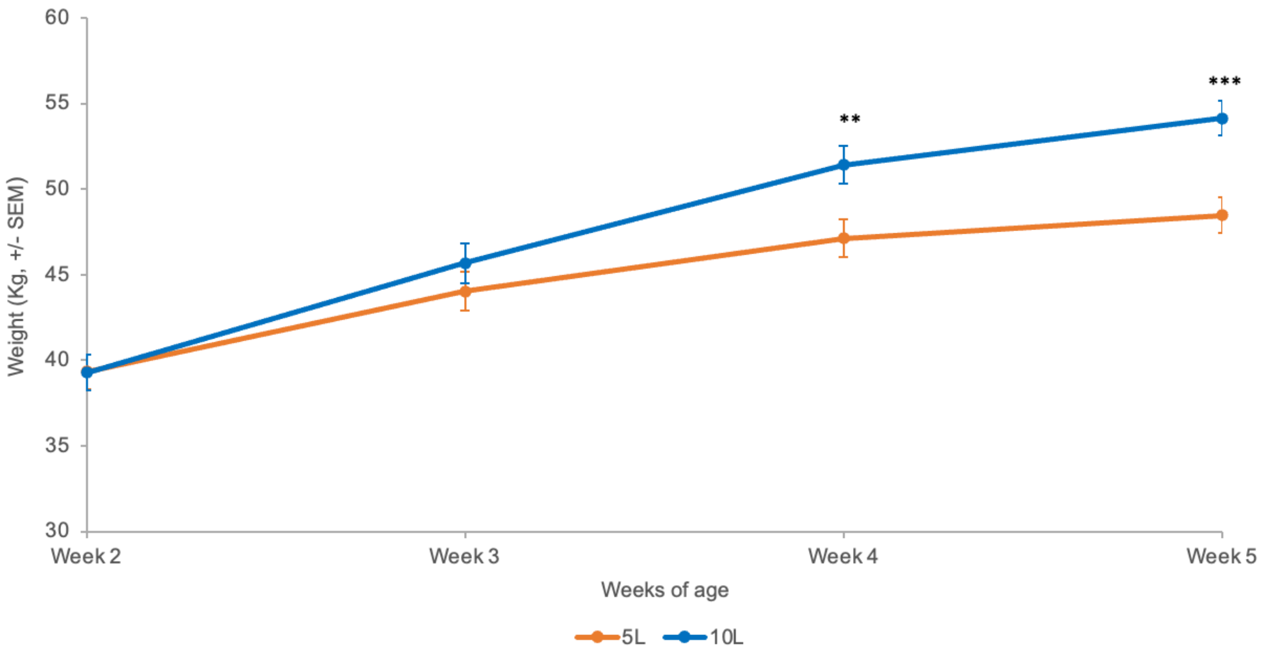

Figure 2.10. Liveweights (±SEM) over a four-week period, for 20 calves aged two to five weeks old whilst inside the indoor rearing facility. Calves were offered different milk allowances (5 or 10 L/day, n=10 calves/treatment). Asterisks (*) showing level of significance (**= P <0.01, ***= P<0.001)

2.5 Discussion

In this study, I observed that offering different milk allowances (5 L/day and 10 L/day) altered several behavioural indicators of welfare and the liveweights of Holstein-Friesian dairy bull beef calves during the first month of age. The calves fed 5 L/day had consistently lower average liveweights and a lower weight gain during this period than calves fed 10 L/day. They spent more time eating supplements (hay and meal), visited the milk feeder without reward more often, had a faster drinking speed, and spent less time lying and more time standing/upright, indicating that they were hungrier and more active. In contrast, the calves fed 10 L/day grew faster and spent more time self- and allo-grooming and lying in both lateral and sternal positions. Therefore, the hypothesis and predictions of my study were confirmed.

2.5.1 Calves offered a lower milk allowance showed feeding behaviours indicative of greater hunger

A key finding from this study was that the feeding behaviours of calves were significantly affected by the differing milk allowances, which included the number of unrewarded and total visits to the milk feeder, consumption of milk, and speed of drinking, as well as the percentage of observations spent at the milk feeder or eating meal, hay, and bentonite. In accordance with previous research (Vieira *et al.*, 2008), my feeding behavioural results provide evidence that calves fed restricted milk allowances show more signs of hunger compared with calves fed ad-libitum milk or a much greater allowance than those on 5 L/day. For example, in a study by Vieira *et al.* (2008), 24 Holstein dairy calves (12 male and 12 female) were fed similar allowances to the calves in my trial, which produced similar results in terms of unrewarded visitations to the automatic milk feeder. In this previous study, calves on the lower milk allowance visited 24 times/day without reward compared with 2.1 times for calves fed ad-libitum, with the calves on 5 L of milk/day in my trial visiting 38 times/day without reward compared with three times per/day for the 10 L calves. Vieira *et al*. (2008) also reported that when a calf on a restricted milk allowance was visiting the milk feeder during a rewarded visit, they would spend twice as much time on the teat, which indicated that the calves were hungrier. When calves show signs of hunger, which is their motivation to eat, their neural homeostatic system is alerting them of low blood glucose concentrations. In comparison, calves on 10 L of milk/day get a sense of satiety due to likely having a greater state of glycaemia, indicating higher levels of glucose in the blood (Vieira *et al.*, 2008). Hunger is not only related to wanting the feeling of being satiated, but also to the hedonic system, which refers to preferred tastes (de Passillé & Rushen, 2006). Indeed, the taste and learned association of the lactose flavour with satiation enhances the calf's drive to drink milk and, in fact, when this lactose flavour was blocked via the use of lactase in a trial using four-18 week old male dairy calves, non-nutritive suckling was reduced (de Passillé & Rushen, 2006). Calves being fed smaller milk volumes, therefore, crave this preferred taste more actively, due to getting less of it.

Restricted milk feeding on farms is common practice; however, research has emerged that this type of feeding not only has negative health and performance implications but also detrimental cognitive effects (Lecorps *et al.*, 2023). One study used 15 bottles placed around an arena (four full of milk) to test the working memory, general memory and reference memory of calves (Lecorps *et al.*, 2023). Two experiments were conducted for a period of 18 days, both which reduced milk by 50 % on day 12. The first experiment did not change the location of the full milk bottles; however, the second experiment did. Restricted milk allowance already results in cumulative hunger that distresses calves; therefore, when the location of the bottles was also changed in experiment two, the calves' ability to focus on the task and activate their working memory was severely impacted. Hunger and cognitive performance are best interpreted as a U-shaped relationship, with increased signs of hunger (e.g. more visitations to the milk feeder or enhanced vocalizations) being located on the extreme end of the U. This extreme end leads to decreased self-control and poorer decision making; therefore, making the understanding of this link between the emotions of calves and their cognitive ability extremely important (Lecorps *et al.*, 2023). Vocalizations were not recorded in my study, as the calves in my trial were managed in a large facility with hundreds of other calves nearby, making it difficult to distinguish the individual vocalizations of the trial animals. Hunger, however, is not always a negative behavioural indicator as it depends on what the animal is being driven by in terms of what milk allocation they are being fed (Lecorps *et al.*, 2023). Calves fed restricted milk allowances tend to be driven by negative emotions in which animals want to relieve their hunger, whereas motivational hunger is what drives calves offered a more generous milk allowance, where they seek a positive experience associated with consuming food (Lecorps *et al.*, 2023). In relation to my study, calves offered 5 L of milk/day were most likely driven by hunger, whereas the calves offered 10 L of milk/day could have been driven by the pleasure of being able to suckle more often with reward, receiving more frequently a fully satisfied pleasurable feeling.

In addition to milk intake, feeding also includes meal, hay, other supplements and water consumption. To trigger the development of the rumen, a series of components need to be initiated, such as solid feed consumption, fermentation processes, absorption mechanisms and the establishment of the anaerobic ruminal microbial ecosystem (Khan *et al.*, 2011). Each of these components, along with changes in the hepatic and intestinal tract, enable calves to grow and develop, transitioning from solely relying on the nutrients from milk to also extracting the nutrients out of solid feed (Baldwin *et al.*, 2004). Feeding restricted amounts of milk significantly enhances solid feed intake because the animals are hungrier (Baldwin *et al.*, 2004). I found support for this in my study, where calves on 5 L of milk/day spent four times as often eating meal and two times as often eating hay than calves on 10 L of milk/day. Although it has been reported that calves offered lower milk allowances and obtain greater amounts of solid feed have heavier forestomaches (as a percentage of body weight), the ruminal environment of calves on both restricted and generous milk allowances all constitute the same characteristics, including a low pH and a high concentration of volatile fatty acids

(Kristensen *et al.*, 2007). Additionally, the length of the papillae in the ruminal sac was also not affected by differing milk allowances, indicating that although calves on restricted milk allowances may have a rumen that initially develops quicker, the calves on greater milk allowances still have the capacity to fulfil the same sort of development, as well as have a better start to life (Roth *et al.*, 2009). Ultimately, calves offered lower milk allowances rely on the consumption of solid feed to obtain their nutrients earlier on in life and, therefore, seek out nutrition from other sources due to being restricted on milk intake (Jensen, 2017).

Unexpectedly, I found that calves fed 10 L of milk/day tended to eat more bentonite than the calves on 5 L of milk/day, but the reasons for this are unclear. Bentonite is an absorbent aluminium phyllosilicate clay, which acts as a binding agent in the stomach (Moosavi, 2017). It is a detoxifying agent that absorbs toxins and increases gut flora activity (Moosavi, 2017). Cieszynski (1980) states that bentonite has the capacity to absorb five times its weight in water, which could indicate why the calves in my trial who were offered a greater milk allowance were seen eating this product twice as often as those on a lower milk allowance. Calves on 10 L of milk/day drank more liquid milk feed; therefore, I speculate that they were potentially more in need of a binding agent. However, more research is encouraged to further explore this possibility.

I found no significant differences in water drinking or ruminating behaviours between the two treatment groups within this trial. It is possible that the scan interval I used was not short enough to detect treatment differences in drinking behaviour. It has been suggested previously that a 30-second instantaneous sampling method is best to use when monitoring drinking behaviours, due to this type of behaviour being of very short duration and, therefore, less likely to be observed using a five-minute scan interval (Chen *et al.*, 2016). Nevertheless, the proportion of calves eating more hay has been shown to be associated with drinking more water (Lowe *et al.*, 2022); therefore, I expected to detect a greater percentage of water drinking observations for the 5 L treatment group as they spent more time at the hay feeder. Although statistically not significant, calves on 5 L of milk/day also had a slightly greater (1.3 times) percentage of rumination observations than the 10 L of milk/day group, which does align with the prediction from Swanson and Harris Jr (1958) that rumination has also been found to be positively correlated with dry feed consumption. I encourage further research in this area, to determine whether milk volume affects the behaviours of drinking water, ruminating and bentonite ingestion and how these relate to other behaviours demonstrated by calves.

2.5.2 Calves offered a greater milk volume showed increased lying behaviour, portraying comfort and satiety

I also investigated the effect of milk allowance on the lying behaviours of the calves, which tend to be positive behavioural indicators relating to comfort. Based on the IceQube data, calves offered the greater milk volume spent, on average, 56 minutes longer lying each day than the calves on the lower milk volume. Borderas *et al.* (2009) reported similar results, with calves aged four to five weeks of age fed higher milk allowances (12 L of milk/day) spending more time lying (17.3 versus 16.9 h/d) compared with calves offered lower milk volumes (4 L of milk/day). This is also indirectly supported by Vieira *et al.* (2008), who observed that calves on restricted allowances spent 1 h longer standing each day. Moreover, in my study, the treatment differences in daily lying time, derived from accelerometer sensor data, were consistent with those determined from video recordings, whereby calves on the 10 L/day allowance had a greater percentage of observations of total lying behaviour than those on the 5 L/day allowance.

Lying behaviour is an important welfare indicator in cattle (Haley *et al.*, 2000). When lying is observed in cattle, it is often a positive behavioural indicator and can be used to reflect the welfare of an animal, because it leads to positive affective states (Papageorgiou & Simitzis, 2022). The process of lying down in cattle begins by the animal sniffing the surface of the ground, followed by the front knees bending, the neck stretching out, and the hind legs and hips coming to the ground (Tucker *et al.*, 2021). When animals do not spend enough of their time lying, the hormonal responses associated with the hypothalamo-pituitary-adrenal axis may become affected, which may lead to stress (Tucker *et al.*, 2021). The lying position and duration demonstrated by a calf not only depends on their relaxed state, but also on the amount of space the calf has surrounding it (Færevik *et al.*, 2008) and the type of surface the animals are lying on (Sutherland *et al.*, 2013). When calves are in a lateral lying position with their legs stretched out, they are demonstrating a state of complete relaxed recumbence (Færevik *et al.*, 2008). Importantly, in my study, the calves on 10 L of milk/day spent significantly more time lateral lying as well as sternal lying than the 5 L calves. This increased lateral lying could be due to the calves offered more milk being more thermally comfortable during this observation period in cooler temperatures 9.5 °C (SD= 3.10 °C), (range: 5.4°C – 15.1 °C). Sternal resting positions (legs tucked underneath the body) are often conducted to minimize heat loss (conserve energy) (Tucker *et al.*, 2007), therefore potentially the calves on less milk were more thermally challenged. It is evident that calves fed a greater milk allowance would more often find themselves in a state of relaxed recumbence, which can be interpreted as being comfortable, calm and with a feeling of satiety. Therefore, my results indicate that calves fed a higher milk allowance are likely in a more positive affective state than calves fed less milk.

2.5.3 Calves offered a greater milk allowance expressed social behaviours associated with positive welfare

Another important component of calf welfare is their expression of social behaviours, which refers to grooming, playing and exploring. These behaviours are associated with positive affective states in cattle (Jensen *et al.*, 1998; Horvath & Miller-Cushon, 2019; Papageorgiou & Simitzis, 2022). Both self-grooming and allo-grooming provide benefits, including building social relationships, hygienic maintenance, and reducing the heart rate of the calf receiving the grooming (Horvath & Miller-Cushon, 2019). Both types of grooming are influenced by the surrounding environment, such as the type of bedding the animals are lying on or internal issues, such as illnesses spreading around the animals. The environment ultimately contributes to whether the behaviour is likely to occur and, therefore, this behaviour is a good indication of the animal's welfare. In a previous study (Horvath & Miller-Cushon, 2019), allo-grooming was less frequently expressed in younger calves compared with older calves, which is consistent with the relatively low percentage of observations for these behaviours in my study of two to five week old calves. However, it is enlightening in this present study that the behaviours of self-grooming and receiving grooming were significantly greater in calves fed 10 L/day, which indicates that the milk allowance given to calves is an important contributing factor to their expression of social and grooming behaviours that indicate positive affective state.

Play behaviour can be observed in two forms. The initial aspect is locomotor and the latter is social play (Jensen *et al.*, 1998). Locomotor play is observed in young calves, including bucking, kicking and galloping versus non-reproductive mounting or fighting, which is seen in older stock (Jensen *et al.*, 1998). Somers (2013), observed the play behaviours of calves kept outdoors on large areas of pasture for 5.5 h/day over 30 days and nearly 60 % of calves (two out of the average 3.79 group size) were observed playing, with running observed most often (458 times), followed by head butting (100 times) and bucking (81 times). Although play behaviour in my study was not significantly different between the two treatment groups, the behaviour was still exhibited by some of the calves. The lack of behavioural differences between treatments could have been due to the relatively low percentage of observations involving play behaviours because the calves were managed indoors in a more confined space rather than outside on pasture. An open area providing sufficient space has been previously outlined as an essential factor in the expression of play (Jensen *et al.*, 1998). Additionally, Jensen *et al.* (1998) conducted continuous recording of play behaviours and analysed four different types of play (locomotor, social, object and straw play). Hence, a possible limitation in my study could have been that I only analysed play running behaviours rather than bucks, kicks or the other versions of play, and that 5-minute observational scans may have been too infrequent to observe shorter occurrences of play and not suitable to detect any differences in frequencies of play events, for example, kicks and bucks.

Animals require exploring behaviour to acquire information from their surrounding environment. By exploring novel objects, they feel more in control (Papageorgiou & Simitzis, 2022). Ruminants tend to explore because it is a motivational need to forage for food and, therefore, the drive behind this behaviour is that it is self-rewarding and reinforcing (Papageorgiou & Simitzis, 2022). Exploring has been shown to occur more frequently in complex enriched environments; for example, bulls spent more time in forest areas of their paddock, due to it being more stimulating to conduct exploratory behaviours compared with confined unstimulating areas (Tuomisto *et al.*, 2008). This may indicate why there was a lack of exploring behaviours in my trial, where the calves were observed in a small, basic and uncomplex indoor rearing facility.

Although positive behavioural indicators are becoming increasingly important when observing and assessing the welfare of calves, negative behavioural indicators are still important to fully understand the welfare status of animals. Cross-suckling is one of these unwanted behaviours that calves often exhibit in relation to hunger and the need to suckle (Margerison *et al.*, 2003). This behaviour involves searching vertically up the leg of another animal, and then attempting to find a teat at approximate udder height, which is then followed by bunting or sucking on areas within this region. In calves who are artificially fed by a milk feeder, cross-suckling tends to be the most frequent one minute after the milk feeder is removed, indicating the calves would preferably have kept drinking and/or suckling. The duration of this cross-suckling remains very frequent for at least 10 minutes after the feeding has finished (Margerison *et al.*, 2003; Jensen, 2003). Non-nutritive suckling is a negative behavioural indicator because it emphasises frustration in the calf and a lack of satisfaction (de Passillé, 2001). Previous literature emphasises two ways of reducing this behaviour, which involves either feeding volumes of milk more frequently or feeding larger volumes of milk/day (Rushen & de Passillé, 1995). Both alternative feeding regimes better mimic nature relative to rearing systems with restrictive once-a-day feeding. In my study, there was no statistical difference in cross-suckling behaviour between milk allowance treatment groups, despite evidence from feeding behaviours that the calves fed a low milk allowance were hungrier, although both groups were allocated milk three times a day. It would be interesting to investigate cross-suckling behaviour over a longer period of time using more frequent or continuous observations rather than 5-minute scans.

2.5.4 Different milk allowances offered to young calves affect their liveweight

Feeding calves restricted milk volumes also affected their liveweights. The calves fed a high milk allowance of 10 L/day gained almost 6 kg more liveweight between two to five weeks of age, resulting in a greater liveweight at the end of this indoor rearing period than those fed a low milk allowance of 5 L/day. As outlined in Vieira *et al.* (2008), feeding smaller amounts of milk to young calves restricts their growth and development, due to not accounting for their dietary energy and protein requirements. Young calves produce an enzyme in large volumes called pregastric esterase as well as pancreatic lipase (Gooden, 1973). These enzymes work together in an acidic environment (provided via hydrochloric acid secretion into the abomasum) to break down milk into free fatty acids and glycerol (Moran, 2012). These fatty acids and glycerol are absorbed and used as energy for the calf, while proteins get broken down more slowly into amino acids and peptides that are absorbed as a source of protein later on (Moran, 2012). Calves on greater milk allowances, therefore, are provided with a greater source of energy and proteins to enhance greater liveweight gains. My results are consistent with this principle and align with several previous studies such a Rosenberger *et al.* (2017) who studied 56 Holstein-Friesian calves on different milk allowances and reported a 0.77 kg/d liveweight gain for calves on 6 L/day relative to a 0.90 kg/d weight gain for calves on 12 L/day. Furthermore, in that study, calves fed smaller milk volumes tried to compensate for insufficient milk by increasing their solid feed intake and ate significantly more hay, which is consistent with my observations in the present study, that calves on 5 L of milk spent significantly more time at the hay and meal feeders than those on 10 L of milk. These are indicative signs of persistent hunger and the weight gain differences support these behavioural observations. It is therefore evident that both behavioural and physical components should be looked at cohesively when observing calves, as these aspects can effectively support one another to obtain a holistic picture of animal wellbeing. Rosenberger *et al.* (2017) outlined that weight gain advantages in the early ages of a calf can continue to persist right up to weaning. Consequently, I investigate this further in Chapter three and four of my thesis, including the post-weaning period to quantify the longer-term effects of differing milk allowances on both behaviours and liveweights as the calf matures.

2.5.5 Study limitations

There were several limitations throughout the trial that could potentially have affected the behavioural measurements and liveweights of the calves. These included calves being treated or excluded from the trial due to being sick with neonatal nutritional scours, and the re-occurring issues with the milk feeder data (water shortages and cloud data retrieval). Both issues resulted in more than half of the data being excluded. Water shortages to the automatic calf feeder resulted in short periods of time where the calves could not drink milk. Sick calves and issues with the feeder could have had an effect on why the calves offered 10 L of milk/day did not, on average, consume the total amount of milk, compared with Lowe *et al.* (2022) whose calves consumed nearly all of their 10 L/day allocation. Calves offered 5 L of milk/day consumed, on average, 4.9 L/day, which was almost all of their allocation and equivalent to approximately 12.5 % of their liveweight at enrolment of the trial (approximately seven days old), whereas calves offered 10 L of milk/day consumed 6.6 L/day, equivalent to approximately 16.8 % of their liveweight at enrolment. Nevertheless, the relatively small number of unrewarded visits to the feeder each day in the 10 L/day calves compared with those on the 5 L/day (three verse 38 visits), indicates that they were significantly less hungry and had obtained most of their desired milk intake. I would encourage further experimental work to investigate the effect of milk allowance on calves, including examining the effect of bentonite and what urges calves to obtain this supplement, potential meat quality differences between small and large milk volumes, and the financial cost and benefit of feeding calves greater milk volumes to determine whether one would ultimately save in solid feed expenses. Addressing these areas of further research will enable to define optimal calf rearing regimes for surplus, non-replacement dairy calves, in terms of animal welfare and liveweights.

2.6 Conclusion

This study confirms that different milk allowances (5 L/day and 10 L/day) alter the behaviour and growth rates of Holstein-Friesian bull calves. Calves fed the lower milk volume expressed more frequently behaviours associated with negative welfare including visiting the milk feeder more often, being observed at the solid feed stations more frequently and spending more time standing/upright. By comparison, calves fed the higher milk volume had a greater expression of behaviours associated with positive welfare, such as self- and allo-grooming, lying in both lateral and sternal positions, and a greater daily lying time, indicating improved calf comfort. The liveweight prediction was also confirmed in this study, with the calves fed the higher milk allowance exhibiting faster growth, which resulted in a greater liveweight at five weeks of age when they left the indoor rearing facility. Collectively, these results indicate opportunities for enhancement of both liveweight gain and positive affective welfare in dairy calves on farms, by selecting milk allowances that more closely reflect the nutritional requirements and behavioural needs of calves.

Chapter 3
The behaviour and liveweight of Holstein-Friesian bull calves fed different milk allowances on their first day on pasture and around weaning

3.1 Abstract

Feeding calves generous volumes of milk provides them with additive physical and behavioural benefits. The aim of this study was to describe the behaviour and growth rate of calves when first released on pasture, after an indoor rearing period on either a high (10 L) or low (5 L) milk allowance (n=11 calves/treatment), and around weaning. Twenty Holstein-Friesian bull calves were transferred onto pasture with supplements (meal and hay) at five weeks of age and managed in two separate groups, depending on milk allowance. The calves were managed in these groups until they were weaned off calf milk replacer at 75 kg (approximately 12 weeks of age). The calves were weighed weekly, and their behaviour recorded for two hours when they were first let out onto pasture. Lying behaviour was recorded for 72 hours using accelerometers. The behaviour around weaning was also recorded for 2.5 hours prior to weaning and 2.5 hours post-weaning. In addition, lying behaviour was recorded for 10 days around weaning using accelerometers. The behaviour is presented descriptively due to a lack of treatment replication (only 1 group per treatment). When calves were first released onto pasture the main behaviour for the group offered 5 L of milk/day was standing, whereas 10 L calves were observed lying down and ruminating more. Around the weaning period for the calves offered 5 L of milk/day grazing and ruminating increased from the pre-weaning to post-weaning period, whilst lying decreased. For the calves offered 10 L of milk/day all feeding behaviours increased from the pre-weaning to post-weaning periods (except for milk), whilst lying and grooming behaviours decreased. The liveweights from five to 11.5 weeks of age were significantly different between treatment groups, however over the weaning period from 12 to 13 weeks of age this liveweight difference was smaller and by 14 weeks of age, the difference was only 5.6 kg (not significant). When calves were first exposed to pasture, the group offered the higher milk volume demonstrated positive welfare behavioural indicators for example more lying, self-grooming and ruminating, whereas the calves on 5 L of milk/day were spending most of their time standing (in agreement with the results from Chapter two). Prior to weaning calves offered 5 L of milk/day were more engaged in feeding activities, likely seeking out other sources of nutrition compared to calves offered 10 L

of milk/day who conducted more comfort related behaviours such as lying and grooming. Calves should be offered higher milk allowances to encourage behaviours associated with positive affective states such as comfort and exploratory behaviours (social, grooming, exploring, playing, ruminating and lying).

Key words: Positive and negative welfare indicators, behaviour, milk allowance, growth rate, pasture, weaning.

3.2 Introduction

The ability of an animal to express its 'normal' or 'natural' behaviours is a key aspect of positive animal welfare. In order for a calf to express its natural behaviours when exposed to significant life changes, the calf needs to be supported sufficiently in all areas of animal welfare (nutrition, the environment, health, behaviour, and mental state of the animal) so that it can adjust and develop adequately (Mellor, 2012). For a calf that has been separated from birth from its dam, important life changes that occur from about five weeks of age until weaning include the internal development of the rumen, being let out onto pasture since being born and being weaned off milk to rely on a solid feed diet.

There is not a singular way that dairy farmers in New Zealand rear their calves pre-weaning in terms of feed type, quality or amount offered (DairyNZ, 2023c). This ultimately results in significant variation in calf rearing management practices, leading to differences between farms in the physical and behavioural aspects of these animals. One very important source of variation in calf rearing is the milk allowance offered, with the volume given often being determined by a percentage of the animal's body weight at birth (Abbas *et al.*, 2017). Farmers typically feed calves 10% of their body weight; however, this is a relatively low volume of milk/day. Greater liveweight differences and average daily weight gains are prevalent in calves offered greater milk volumes (Abbas *et al.*, 2017). In addition, there is increasing evidence of behavioural differences in response to altered milk allowances, with positive welfare behavioural indicators, such as play, allo-grooming, exploration, ruminating and lying, generally more prominently expressed by calves offered greater milk volumes (Jensen *et al.*, 1998; Napolitano *et al.*, 2009; Papageorgiou & Simitzis, 2022). In my previous chapter (Chapter two), I found a significant effect of milk allowance on both the liveweights and behaviour of dairy bull calves during two to five weeks of age. Calves offered 10 L of milk/day conducted more self- and allo-grooming, were more frequently observed in lateral and sternal lying positions and spent longer lying each day (55.8 minutes more), indicating greater levels of comfort and relaxation than calves offered 5 L of milk/day. The 5 L/day calves conducted 35 times more unrewarded visits to the milk feeder each day and spent more time at the meal and hay feeders, indicating signs of hunger by seeking out more nutrition. Furthermore, calves offered the lower milk allowance had an average liveweight gain of 9.2 kg relative to 14.9 kg for the calves on 10 L of milk/day, during the four-week period outlined in Chapter two.

Another major event a young calf experiences is receiving access to pasture for the first time since birth, which enables animals to express more of their natural behaviours such as exploring and grazing (von Keyserlingk *et al.*, 2009). While suckling is an instinctive behaviour for a calf, they need to learn grazing behaviour and do so by exploring (Charlton & Rutter, 2017). Exploration occurs in an environment where an animal needs to obtain information about its surroundings in order to feel in control of it (Papageorgiou & Simitzis, 2022). Exploring comes in different forms, such as being inquisitive or inspective (Keeling *et al.*, 2021). Inquisitive behaviour refers to the calf looking for novel objects, whereas inspective behaviour involves a specific situation where the animal is confronted with an unusual stimulus (Keeling *et al.*, 2021). Additionally, play behaviour can also be demonstrated by calves in new environments, for example when they are re-exposed to pasture (Mintline *et al.*, 2013). Play behaviour is considered to be a positive welfare indicator because it is associated with an endorphin release, is a self-rewarding pleasurable behaviour and is enhanced in favourable conditions and environments (Mintline *et al.*, 2013). In Chapter two, I determined that calves offered the greater milk volume demonstrated more behaviours associated with positive affective state. I hypothesise that this trend will continue when they are first re-exposed to pasture at five weeks of age.

Weaning is a further event that occurs in a calf's life within the first few months of age and involves the removal of milk from the animal's diet. Weaning can occur abruptly or gradually, depending on the preference of each farmer (Jasper *et al.*, 2008). Generally, however, it is recommended that calves commence weaning at no earlier than six weeks of age, and that a step-wise process is used over a period of one-two weeks (DairyNZ, 2023d). Weaning via a step-wise process is gradual and slow, allowing the animal to transition from a predominantly milk diet to completely solid diet. Weaning can be a stressful and unfavourable time for calves as they lose their main source of nutrients and are required to adapt quickly to maintain sufficient growth and a positive welfare status (DairyNZ, 2023d). Gradual weaning has a number of positive outcomes, such as increased overall energy due to slower adaptation of the gastrointestinal intestinal tract, and results in greater growth rates or liveweights post-weaning (Steele *et al.*, 2017) Scoley *et al.* (2019). Furthermore, gradually weaned calves have reduced vocalizations post-weaning (Bittar *et al.*, 2020). The calves in my trial were gradually weaned off their milk diet, which meant their digestive systems could transition onto a pasture-based diet more adequately. This was particularly important for the calves offered the higher milk allowance. This gradual weaning process was also implemented to support their welfare, instead of abruptly removing their milk source, which would be expected to create undesired stress.

Behaviours prior to weaning, during and after weaning are likely to differ, due to the calf adapting to a new way of living and potentially not feeling a state of satiety and comfort until they have adapted to a solely solid diet (Jasper *et al.*, 2008). When the calves' environment is stable and the animals are comfortable (particularly prior to a stressful event such as weaning), they have greater expression of behavioural indicators of positive welfare such as lying, ruminating and grooming (Vigors & Lawrence, 2019). After calves are weaned their behaviours tend to vary; for example, eating time often increases and lying time tends to decrease (Lynch *et al.*, 2019). Calves also tend to exhibit increased activity in the form of paced walking post-weaning (Enríquez *et al.*, 2010). Additionally, cross-suckling (on other calves or objects) tends to become more prominent, as well as a lack of play post-weaning (Jasper *et al.*, 2008). Reduced play occurs as the animal's emotions become affected by the gradual absence of the milk feeding process. It is not uncommon for calves to become attached to an inanimate object or some form of the milk delivery process; therefore, when this is removed, the animals overall emotional status can be impacted (Jasper *et al.*, 2008). Additionally, liveweight differences for calves offered different milk allowances prior to weaning tend to be prominent and these can persist beyond weaning (Rosenberger *et al.*, 2017).

Feeding calves different milk allowances affects both their liveweights and behaviours. Observing calves when first exposed to new conditions, such as their first hours outside on pasture and around the weaning period, enables our understanding of their behavioural responses and potential affective states. Investigating the effect of milk allowance on these behaviours, provides insights that may help to identify opportunities to improve calf welfare. Additionally, it opens future pathways to potentially rear non-replacement calves for longer on suitable milk allowances, enabling them to demonstrate positive behavioural indicators and sufficient liveweights. This would allow them to be more suitable for beef production, rather than being slaughtered early in life due to being considered unprofitable animals (Van Dyke *et al.*, 2021). Rearing these animals for longer on dairy farms could grow our meat sector and help reduce negative stigma around the early slaughter of bobby calves.

The aim of this study was to describe the liveweights and behaviours of calves fed differing milk allowances when they are first re-exposed to pasture and around the weaning period. The overall hypothesis for this chapter was that the differing milk allowances (5 L and 10 L) would cause differences in the activity, behaviours and liveweights of Holstein-Friesian bull calves whilst outdoors on pasture for the first time and around the weaning period. Specifically, I predicted that calves fed 5 L of milk/day would have a lower average liveweight and demonstrate behaviours associated with negative welfare, such as spending less time exploring and lying down, but more time being active compared with calves fed 10 L/day, who would demonstrate behaviours associated with positive welfare, such as self- and allo-grooming and more time lying.

3.3 Materials and Methods

All procedures involving animals in this study were approved by the Animal Ethics Committee at the University of Waikato, under the protocol 1146, and complied with the New Zealand Animal Welfare Act (1999).

3.3.1 Previous management of calves when reared indoors (Chapter 2)

Prior to the start of the current study, 22 Holstein-Friesian bull calves were reared inside a shed facility for approximately four weeks as one group, and individually offered either a 5 or 10 L daily milk allowance using an automatic milk feeder with free access to meal and hay. Treatments were balanced on liveweight at arrival to the farm at five to seven days of age (n=11 calves/treatment). Refer to Chapter two for a detailed description of the methods, leading up to this chapter. Two calves (one from each treatment) were excluded from all analyses due to persistent illness, i.e. n=10 calves/treatment.

3.3.2 Liveweights

The calves were weighed weekly using Leask engineered scales. Once the calves outgrew these custom-designed scales, they were weighed on static scales (Gallagher TW5 and Gallagher wireless load bars).

3.3.3 Calves outdoors on pasture

It was not possible to feed the calves individually when they were on pasture and, therefore, they were manually group fed their daily milk allowances and managed in two separate groups (one group per treatment), separated by electric tape fences. The groups were managed in the same paddock (0.9 ha) over the spring months (September, October and November), where the pasture consisted mainly of perennial ryegrass (Mason Jones, personal communication). Both groups had access to tree shade and were fed milk using two separate 12-teat Stallion manual calfeterias. The calves were offered 5 or 10 L/calf per day of Ancalf finisher milk replacer split across two feeds per day at 07:00 h and 16:30 h (Ancalf; NZAgbiz Ltd, Hamilton, New Zealand). Both groups of calves were offered ad-libitum meal (Jswap-Corn, Gluten and DDG pellet blend), consisting of 50 % DDG, 47 % Corn Gluten Pellets, 3 % Mineral Boost Bovatec, 20.87 % Protein, 12.39 ME/kg, 85.89 %DM, 10.26 % Starch and 27.16 % NDF (Jswap, Tauranga). Meal was provided in a meal trough (200L performance product sledge frame trough) placed on the ground in the paddock, and a bale of hay (length 1950mm, width 1000mm and depth 900mm) was placed in the centre of the paddock, with free access to water in a concrete water trough (external measurement 1650mm diameter, internal measurement 1500mm diameter and depth 330mm). The two groups shared one water trough, separated by a wire tape. Vaccination procedures were conducted as per standard farm practice and occurred while calves were drinking from the manual milk feeder or whilst they were lined up in the yards. All drenching procedures were conducted in the yards. Vaccinations and drenches received by the trial animals are described in Table 6.

3.3.4 Behaviour during the first 2 hours on pasture

The behaviour of the two groups of calves when first transferred onto pasture was recorded for two hours (between 12:15 h and 14:15 h) using hand-held video cameras (90X Panasonic i.zoom hybrid O.I.S camera; Panasonic, Auckland, New Zealand) attached to a tripod. The calves were recorded in numerical order according to their ear tags, and each calf was recorded for one minute and five seconds (to get the full minute of recording) before recording the next calf. During the first hour, each calf was recorded four times to obtain a total time of four minutes and 20 seconds per calf. One operator recorded the 10 L group and another operator recorded the 5 L group. For the second hour, each calf was recorded three times to obtain a total time of three minutes and 15 seconds per calf. Each recording was truncated to one minute, which resulted in a total of seven minutes of video recording per calf. The behaviour of calves was scored using 1-0 sampling (Altmann, 1974). The entire minute (for each of the seven minutes/calf) was viewed and the observer recorded whether any of the behaviours listed in Table 5 were present (1), not present (0) or unsure (.). I carried out all observations and assessed my observer reliability once after watching half of the videos. For my reliability videos, I watched one minute for five randomly selected calves from each treatment group. The level of reliability was calculated based on the percent agreement, using Microsoft Excel (version 16.75). All behaviour observations were considered to be in agreement with each other. The intra-observer reliability ranged from 90 % to 100 % between different times the videos were watched, with the lowest percentages of reliability detected for head-supported lying and self-grooming behaviours.

IceQube accelerometers (Icerobotics IceQube, Peacocks Technology, United Kingdom) were attached to the right hind leg of each calf at four weeks of age. These data loggers were used to record lying behaviour (total time) for the first 72 hours on pasture. The IceQube data loggers were removed and replaced with HOBO accelerometer data loggers at eight weeks of age to the right hind leg (HOBO Pendant G- Part number UA-004-64, Onset Computer Corporation, United States) (Figure 3.1), to reduce some rubbing that was observed on the animals' legs. For a detailed description of the trial events leading up to and including this chapter, refer to Table 6.

Figure 3.1. An IceQube data logger (left) and a HOBO data logger (right). Both devices are accelerometers that track lying behaviour (IceQubes also track step count).

3.3.5 Behaviour pre-weaning

Weaning occurred when the lightest calf in the groups had reached 75 kg liveweight, which occurred at approximately 12 weeks of age. Two days prior to weaning, both groups of calves were painted with individual identification marks/symbol using paint (detail water-based tail paint; Fil, Mount Maunganui, New Zealand). Live 10-min instantaneous scan sampling was conducted on the following day to observe calves for two hours and 30 minutes (10:30 h - 13:00 h, three and a half hours after milk was offered) (Altmann, 1974). Two people observed the two groups (one person per group) in numerical order (using ear tag numbering) recording behaviours observed for each calf in their group (Table 5). Two and a half hours of data collection occurred, followed by 20 minutes of inter-observer reliability recordings. The inter-observer reliability was conducted four times via live 5-minute instantaneous scan sampling, whereby the two individuals observed the same calves at the same time and recorded the calves' behaviour (Table 5). The two treatment groups were observed for a total of 10 minutes each. The level of reliability was calculated based on the percent agreement, using Microsoft Excel (version 16.75). All behaviour observations were in agreement with each other based upon the inter-observer reliabilities, which ranged from 95% to 100 %, with the lowest percentages of reliability detected for sternal lying and rumination behaviours.

Lying behaviour was recorded using HOBO accelerometers. The HOBO accelerometer devices have previously been validated for use on calves (Bonk *et al.*, 2013). The HOBOs were checked weekly for any signs of rubbing/discomfort.

3.3.6 Weaning process

Weaning occurred once all calves had reached 75 kg liveweight. The daily milk allowance was gradually reduced over five days using the following protocol, with ad-libitum hay and meal.

Day 1: Calves on the 10 L/day treatment were reduced to a daily allowance of 5 L of milk/calf, which was group fed using a calfeteria as described in section 3.3.2. Calves on the 5 L/ day treatment were reduced to 2.5 L of milk/calf per day.

Day 2: Calves in the 10 L treatment group remained on the reduced allowance of 5 L of milk/calf per day, and those in the 5 L group remained on 2.5 L of milk/calf per day.

Day 3: The milk allowance of calves in the 10 L treatment group was further reduced to 2.5 L of milk/calf per day, whereas those in the 5 L group remained on 2.5 L of milk/calf per day.

Day 4: Calves in the 10 L and 5 L treatment groups remained on the reduced allowance of 2.5 L of milk/calf per day .

Day 5: Calves in both treatment groups did not receive any milk, completing the weaning process.

3.3.7 Behaviour post-weaning

On day five (first day weaned off milk), the calves were weighed and then re-painted with their individual identification symbol. Post-weaning observations occurred the following day (using live 10-min instantaneous scan sampling following the same procedure as described for pre-weaning observations, but observations were conducted from 9:50-12:50 h). Two and a half hours of data collection occurred, followed by 30 minutes of inter-observer reliability recordings. The inter-observer reliability was conducted three times via live 10-minute instantaneous scan sampling, where the two individuals observed the same calves at the same time and recorded the calves' behaviour (Table 5). The level of reliability was calculated based on the percent agreement, using Microsoft Excel (version 16.75). All behaviour observations were in agreement with each other based upon inter-observer reliabilities of 100 %.

Figure 3.2 outlines a flow chart of the second phase of procedures that were undertaken outdoors on pasture.

Table 5. Ethogram of calf behaviours recorded outdoors.

Exploring	Animal is sniffing or licking objects (not including other calves).
Ruminating	Animal chews with lateral jaw movement and keeps its head at the same level. No feed is seen in the mouth.
Eating meal	Animal is observed with its head in the meal trough.
Eating hay	Animal is observed to have its head over and in the hay rack.
Nibbling	Animal comes into contact with grass, sniffs or explores the grass/roughage by moving its mouth over it and performs small quick bites.
Grazing	Animal grabs and ingest the forage. Mouth is to be observed chewing. The calf may be stationary or moving forward.
Drinking water	Animal is observed to have its head in or above the water trough.
Play running	Animal is observed to be running, galloping and/or kicking its legs into the air.
Lying postures (sternal, lateral, head supported)	Sternal - Flank is in contact with the ground. Lateral - The weight of the calf is on one side of the body, including the shoulder, barrel and the hip. Head supported - Head is resting on its own body, on the ground, on another calf or the ground (not including grooming).
Upright/Standing	Weight supported by feet/not lying. Includes walking.
Cross-suckling	Animal has its head lowered beneath the stomach of another calf and is bunting, sucking or sniffing the navel or region between the hind legs.
Self-grooming	Animal is licking, sniffing or scratching itself.
Inter-grooming	Animal is licking another calf.
At milk feeder	The calf is observed to have its mouth around one of the artificial teats at the milk feeder.
Shade	Visible shade- A shadow present on the ground. Shade use- Any part of the body is observed to be in the shade.

Table 6. Timeline of the trial events

Approximate age of calves	Event
5 weeks	From this age, calves were reared outdoors on milk, pasture, meal and hay supplements.
8 weeks	IceQubes were removed on all calves and replaced with HOBOs
11.5 weeks	Calves painted with identification marks for pre-weaning observations.
11.5 weeks	Pre-weaning observations.
12 weeks	Calves given second vaccinations (2mls of Covexin and 2mls of Salvexin). Calves weaned off milk.
12.5 weeks	Calves painted with identification marks for post-weaning observations.
13 weeks	Post-weaning observations.
14 weeks	Calves drenched (6mls of Concur drench).
21 weeks	Calves drenched (6mls of Concur drench and 12mls of Zolvex drench). Calves transported via truck to neighbouring farm to graze pasture.

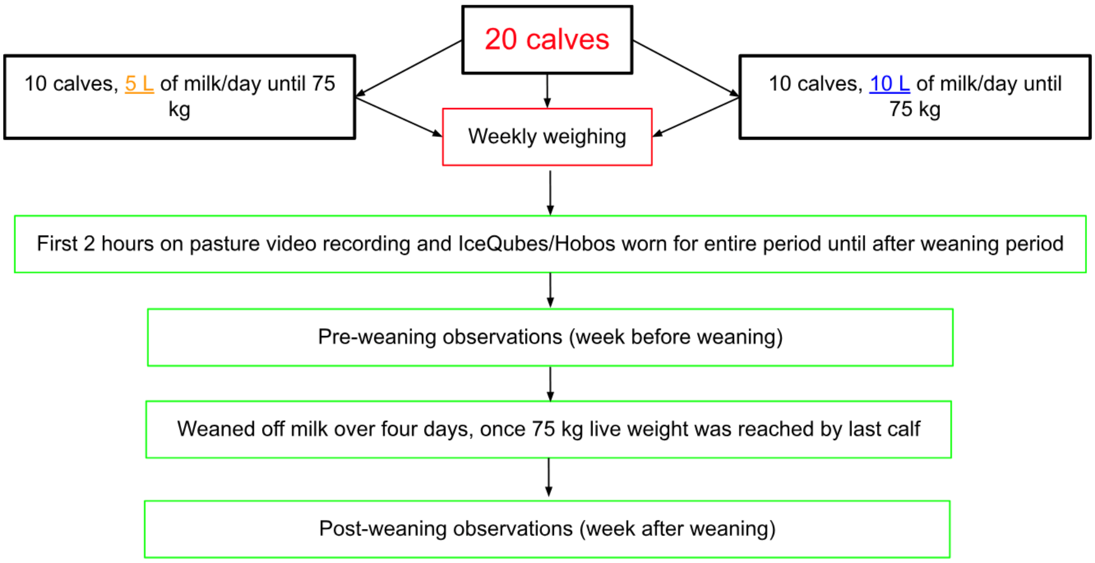

Figure 3.2. Flow diagram of the methods whilst the 20 calves were outdoors on pasture until weaned off milk.

3.3.8 Environmental conditions

Weather data (air temperature °C and precipitation mm) were retrieved from the nearest National Institute of Water and Atmospheric Research weather station (Station 41429), which was located 13.4 km away from the farm. Data were recorded in ten-minutes intervals.

3.3.9 Data handling

Excluded data

One HOBO accelerometer attached to an animal from the 5 L treatment group produced faulty data; therefore, accelerometer data from this animal were excluded from the analysis.

Weather data

Air temperature (°C) and precipitation (mm) were summarised into daily means using Microsoft Excel (version 16.75).

Behaviour observations

During each of the behavioural observation periods, the percentage of sampling periods where the behaviour was present was calculated for each calf for each behaviour. The behaviours observed for the first two hours on pasture were grazing grass, nibbling, drinking water and milk, eating meal/bentonite and hay, ruminating, lateral and sternal lying (and any unsure lying postures), head supported whilst lying, standing/upright, self-grooming, receiving grooming, grooming another calf, exploring, playing and cross-suckling (Table 5). The behaviours were the same as for first exposure to pasture except meal/hay instead of meal/bentonite was observed and, additionally, visible shade and in shade variables were recorded (Table 5). All behaviours are presented as a percentage of observations (Tables 7 and 8), the lying postures and head supported behaviours are presented as percentages of total lying observations.

Lying behaviour from data loggers

Data from the IceQube devices were recorded in time increments of 15 minutes. The lying time (%/h) was calculated for each calf and averaged for the first 72 hours on pasture. Data from the HOBO devices were summarised in time increments of one hour. Each of the weaning phases (pre-weaning, during-weaning and post-weaning) are described separately, where for each phase the total lying time (%/h) was summarised per calf.

3.3.10 Analyses

For this chapter, there was only one experimental unit per treatment as the calves were group fed their daily milk allowance rather than individually fed. Hence, inferential statistics on the effect of milk allowance were not performed on behavioural data due to the lack of treatment replication, and all data are presented descriptively except for liveweight data.

Weather

Mean values and standard deviation (SD) for the weather data during the three observation days (first day on pasture, pre- and post-weaning) were generated using Microsoft Excel (version 16.75).

Liveweights

Liveweight gain data were analysed by conducting analyses of variance, which consisted of a single factor, a fixed effect for treatment, and calf as the experimental unit. This involved analysing the weight gain between specified weeks, which resulted in different levels of significance as expressed in the time series plot. The time series plot is produced over a period of ten weeks, SEM were calculated for each of the treatment groups for each of these weeks.

Behaviour

The percentage of sampling periods where the behaviour was present was calculated for each calf and then averaged per treatment group for each of the sample periods (initial two hours on pasture, pre-weaning and post-weaning observations). Means and SEM are presented.

IceQubes and HOBOs

To convert the lying percentage averages for the treatment groups produced into minutes, the values were divided by 100, multiplied by 60 and then by 24.
For the IceQube data (first two hours on pasture) a time-series plot was produced showing the average daily lying percentages over a 24-hour period of the two treatment groups. Standard deviations were calculated per treatment initially, which were then converted to SEM.

For the HOBO data (weaning period) a bar graph was produced. SEM were calculated for each of the periods, with pre-weaning spanning over three days, during-weaning spanning over four days and post-weaning spanning over three days.

For each period, an average was collated per hour/day for each of the calves, which was then calculated into one average/calf over the specified period, this was finally summarised into treatment groups, to be presented in the plots with SEM.

3.4 Results

3.4.1 Environmental conditions

The average air temperature on the first day at pasture was 9.5 °C (range: 6.8 – 13 °C) and the total rainfall was 2.0 mm. The average air temperature on the day of pre-weaning observations was 12.7 °C (range: 9.3 –16.4 °C), and on the day of post-weaning observations the average air temperature was 11.4 °C (range: 6.9 – 16.2 °C). There was no rainfall during the pre- and post-weaning observations.

3.4.2 Behaviour at first exposure to pasture

The percentages of observations for various behaviours during the calves' first two hours on pasture are outlined in Table 7. There were no observations of calves eating hay, meal or bentonite, drinking water or milk, grazing, lateral lying, receiving grooming, play; therefore, these behaviours are not presented in Table 7.

Calves offered 5 L of milk/day

During the two-hour observational period, calves offered the lower milk volume of 5 L/day spent most of the observations standing/upright (92.9 % of observations). They were ruminating in 27.8 % of the observations and undertaking exploratory behaviour in 12.9 % of observations. They were also observed nibbling in a small proportion of observations (3.1 %). Grooming behaviours were not frequently observed, with grooming another calf observed in 2.5 % of observations and self-grooming in 1.7 % of observations. Cross-suckling was only observed in this 5 L group of calves (1.7 % of observations). Lying time (first 72 hours on pasture) recorded via IceQube data loggers was, on average, 14.2 h/24 h (SEM=0.146 h) for this group.

Calves offered 10 L of milk/day

In contrast, calves offered the greater milk volume of 10 L/day spent most of the observations lying down and ruminating during the two-hour observational period (Table 7); the calves were lying in 76.7 % of the observations and ruminating in 70.7 % of the observations. When the calves were lying, their heads were supported in 5.8 % of observations. They were also seen exploring their environment in 15 % of observations and nibbling in 9.2 % of observations. Self-grooming was also observed more frequently in this group of calves compared with the 5 L group (3.7 % vs. 1.7 %), whereas grooming another calf was observed at a similar frequency (2.5 %) to those of 5 L calves (Table 7). Cross-suckling was not observed in this group of calves. Lying times for this group, recorded using IceQube, averaged 15.0 h/24 h (SEM=0.137 h) during the first 72 hours on pasture.

Figure 3.3 outlines the average diurnal lying patterns of the calves during their first time back out on pasture. The 24-hour period is an average of three consecutive 24-hour periods (a total of 72 hours), with calves on 10 L of milk/day spending more time lying between 08:00 h - 09:00 h, 11:00 h - 14:00 h and 19:00 h - 24:00 h than calves on 5 L of milk/day.

Table 7. Behaviour (% of observations, ±SEM) during the first two hours on pasture of 20 Holstein-Friesian bull calves offered different milk allowances (5 or 10 L/day, n=10 calves/treatment).

Behaviour	**5 L (%)**	**SEM 5 L (%)**	**10 L (%)**	**SEM 10 L (%)**
Nibbling	3.1	2.07	9.2	5.34
Ruminating	27.8	7.82	70.7	13.75
Head rest (% of total lying)	2.9	1.90	5.8	3.94
Total lying	7.1	5.73	76.7	8.46
Unsure posture (% of total lying)	0	0.00	1.4	1.43
Standing	92.9	5.73	27.1	5.65
Grooming other	2.5	2.50	2.5	2.50
Self-grooming	1.7	1.67	3.7	3.33
Exploring	12.9	6.55	15.0	5.64
Cross-suckling	1.7	1.67	0	0.00

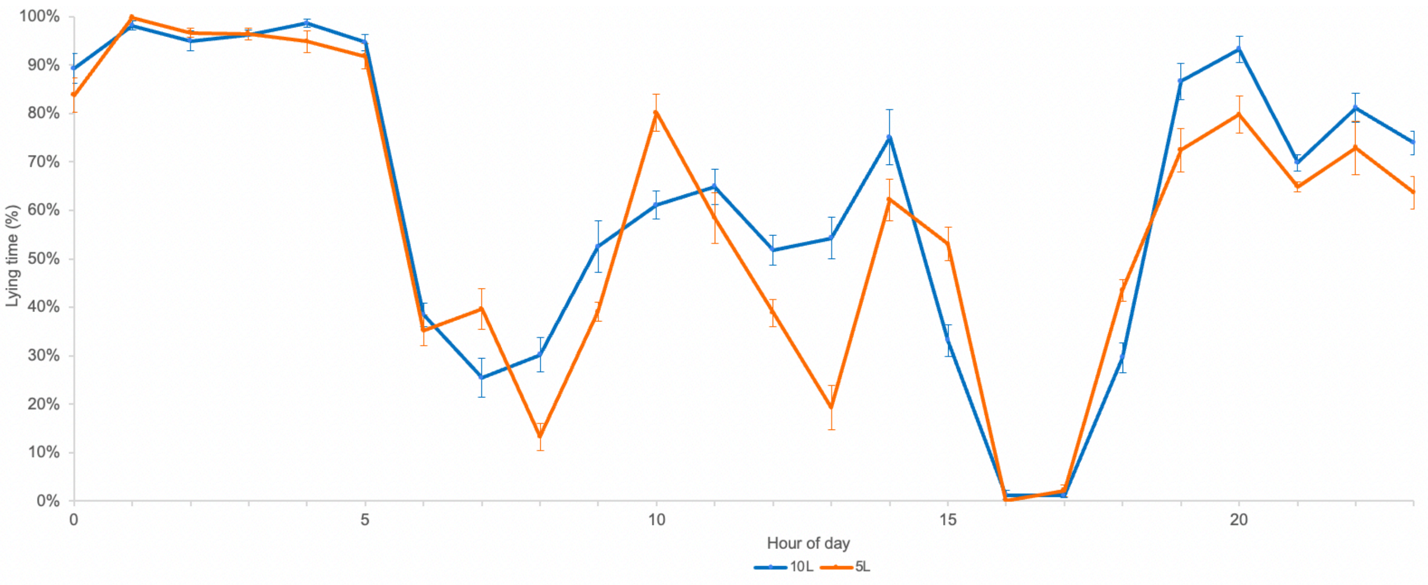

Figure 3.3. The diurnal lying patterns of 20 five week old Holstein-Friesian bull calves offered different milk allowances (5 or 10 L/day, n=10 calves/treatment), when first exposed to pasture conditions after an indoor rearing period. The average daily lying percentages per hour (±SEM) collated from IceQube accelerometers, for the calves on the different milk allowances. Averaged over 72 hours (three consecutive 24-hour time periods).

3.4.3 Behaviours- Pre- and post-weaning

Calves offered 5 L of milk/day

The percentage of observations for various feeding behaviours varied between pre- and post-weaning periods for the calves offered 5 L of milk/day (Table 8). Grazing behaviour increased from 13.3 % of observations pre-weaning to 38.7 % post-weaning (Table 8). Nibbling decreased following weaning, going from 3.8 % pre-weaning to zero occurrences post-weaning. The same occurred for drinking water, which was 2.5 % of observations pre-weaning and zero percent of observations post-weaning. Eating meal/hay also declined by 5.8 % post-weaning. Ruminating increased by 3.1 % and observations at the milk feeder were not present at either sampling period for this group of calves (Table 8).

Lying behaviours also varied between pre- and post-weaning periods for the 5 L calves, but not to the same extent as feeding behaviours. Total lying percentage decreased by 9.6 % post-weaning, and sternal lying as percentage of total lying decreased by 9 %. Data recorded via the HOBO accelerometers show the average daily lying time decreased from 15.17 h (SEM= 0.192 h) pre-weaning to 13.85 h (SEM= 0.228 h) during-weaning, which was a difference of one hour and 19.2 minutes (Figure 3.4). From the during-weaning to post-weaning period, the average daily lying time slightly increased to 14.06 h (SEM= 0.288 h), which was an increase of 12.96 minutes (Figure 3.4). Self-grooming did not change during the weaning period, and allo-grooming, exploring, cross-suckling and shade use were not observed.

Calves offered 10 L of milk/day

Both feeding and lying behaviours varied between pre- and post-weaning sampling periods for calves offered 10 L of milk/day (Table 8).

Grazing behaviour increased from 2.6 % of observations pre-weaning to 35.3 % post-weaning, which is a large difference of 32.7 %. In addition, ruminating increased from 11.7 % to 14.7 % (Table 8).

All lying behaviours decreased from the pre-weaning sampling period to the post-weaning sampling period (Table 8). The percentage of observations for total lying more than halved during the post-weaning sampling period compared with pre-weaning period. As a percentage of total lying observations, sternal lying decreased from 60.6 % to 28.7 %, whereas lateral lying was reduced from 12.6 % to 2.7 %, indicating that these behaviours were conducted two to three times less often during the post-weaning period. Calves also had their heads supported in fewer lying observations post-weaning (2.7 % of total lying) compared with pre-weaning (12.6 % of total lying). Data recorded via the HOBO accelerometers indicated that the average daily lying time decreased from 15.48 h (SEM= 0.182 h) pre-weaning to 13.49 h (SEM= 0.216 h) during-weaning, which was an approximately two-hour reduction (Figure 3.4). From the during-weaning to post-weaning period, however, there was a small 14.4-minute increase in average daily lying time to 13.73 h (SEM= 0.274 h), which was 1 hour 45 minutes less than the average daily lying time in the pre-weaning sampling period (Figure 3.4).

Social behaviours also decreased post-weaning in the 10 L calves. Grooming another calf decreased 7-fold (from 3.3 % to 0.47 % of observations) and self-grooming decreased 1.7-fold (from 15.0 % to 8.7 % of observations), whereas receiving grooming decreased from 1.3 % of observations pre-weaning to zero occurrences post-weaning (Table 8). Exploratory behaviour was similar pre-weaning and post-weaning. Cross-suckling and shade use were only observed post-weaning for this group, increasing to 0.67 % and 5.3 % of observations, respectively (Table 8).

The behavioural results presented in table 8 are more similar post-weaning than pre-weaning.

Table 8. Behaviour (% of observations, ±SEM) pre- and post-weaning of 20 Holstein-Friesian bull calves (> 75 kg) offered different milk allowances (5 or 10 L/day, n=10 calves/treatment) whilst managed on pasture. Calves were observed for 2.5 h pre- and post-weaning.

	5 L (%)		**SEM (%)**		**10 L (%)**		**SEM (%)**	
Behaviour	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
Grazing grass	13.3	38.7	3.80	7.22	2.6	35.3	2.03	4.87
Nibbling	3.8	0	1.67	0	1.3	2	0.86	1.02
Drinking water	2.5	0	1.38	0	1.9	2	0.95	1.02
Eating meal/hay	15.8	10	3.45	2.05	8.9	9.3	1.67	2.47
Ruminating	16.9	20.0	4.38	3.72	11.7	14.7	3.65	2.95
At milk feeder[1]	0	-	0	-	0.6	-	0.63	-
Head supported (% of total lying)	4.5	4.7	1.64	2.00	12.6	2.7	3.49	1.09
Sternal lying (% of total lying)	51.0	42.0	4.19	5.07	60.6	28.7	4.85	3.86
Lateral lying (% of total lying)	1.9	1.3	1.33	0.89	6.9	2	2.54	1.02
Total lying	52.9	43.3	4.45	5.47	67.5	30.7	4.73	4.24
Standing	47.1	56.7	4.45	5.47	32.5	69.3	4.73	4.24
Grooming other	0	0	0	0	3.8	0.47	1.38	0.67
Self-grooming	8.3	8.0	1.93	2.18	15.0	8.7	2.98	2.99
Receive-grooming	0	0	0	0	1.3	0	0.83	0
Exploring	0	0	0	0	1.3	1.3	0.86	0.89
Cross-suckling	0	0	0	0	0	0.67	0	0.67
Shade use	0	0	0	0	0	5.3	0	1.66

[1] No milk was available post-weaning

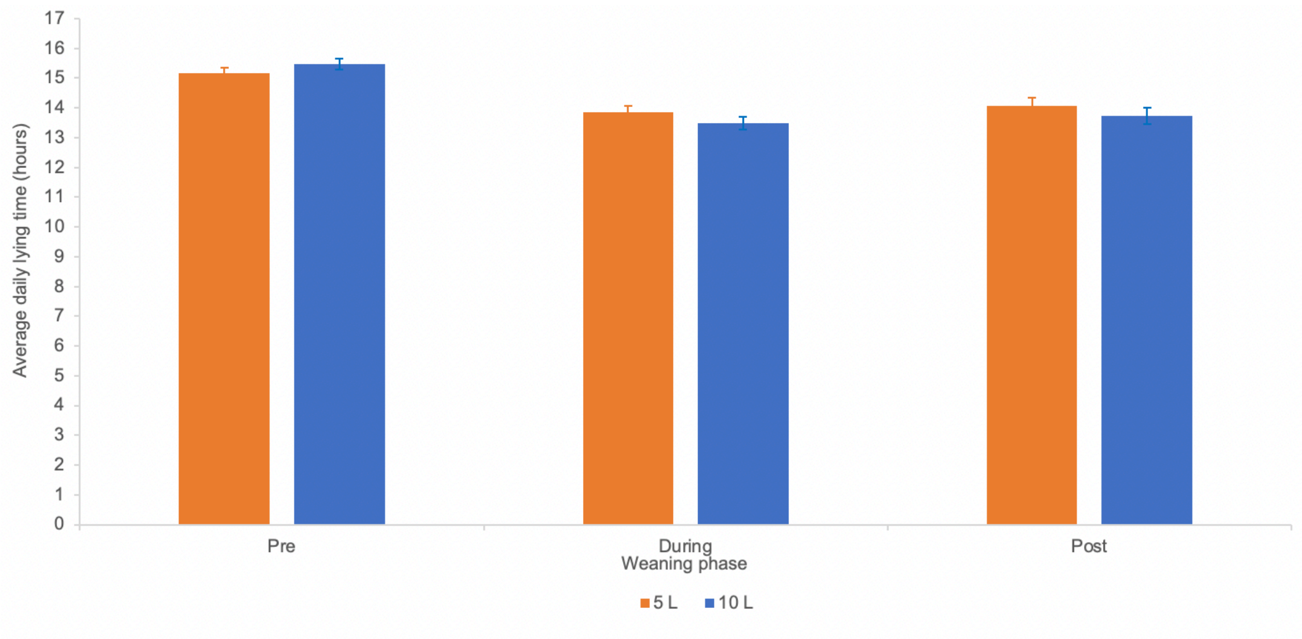

Figure 3.4. Daily lying percentage averages (±SEM), derived from HOBO accelerometers, for 20 Holstein-Friesian bull calves for the pre-weaning, during weaning and post-weaning periods. Calves were offered different milk allowances (5 or 10 L/day, n=10 calves/treatment) until weaning when all animals had reached 75 kg liveweight, at approximately 12 weeks of age.

3.4.4 Liveweights

Calves offered 10 L of milk/day were consistently heavier than the calves offered 5 L of milk/day during the outdoor rearing period until weaning (Figure 3.5). The greatest difference between the milk allowance treatment groups was at six weeks of age, with a difference between the two treatment groups of 7.9 kg liveweight (SED=1.63 kg, $F_{1, 18}$=23.44, P<0.001). For the following two weeks, the liveweight difference was 7.35 kg. From week nine to 14, the liveweight difference between the treatment groups began to get slightly smaller. By week 14 (after weaning at week 12), the difference between the 5 L and 10 L groups was 5.55 kg liveweight (P=0.076, $F_{1, 18}$=3.54, SED=2.95 kg).

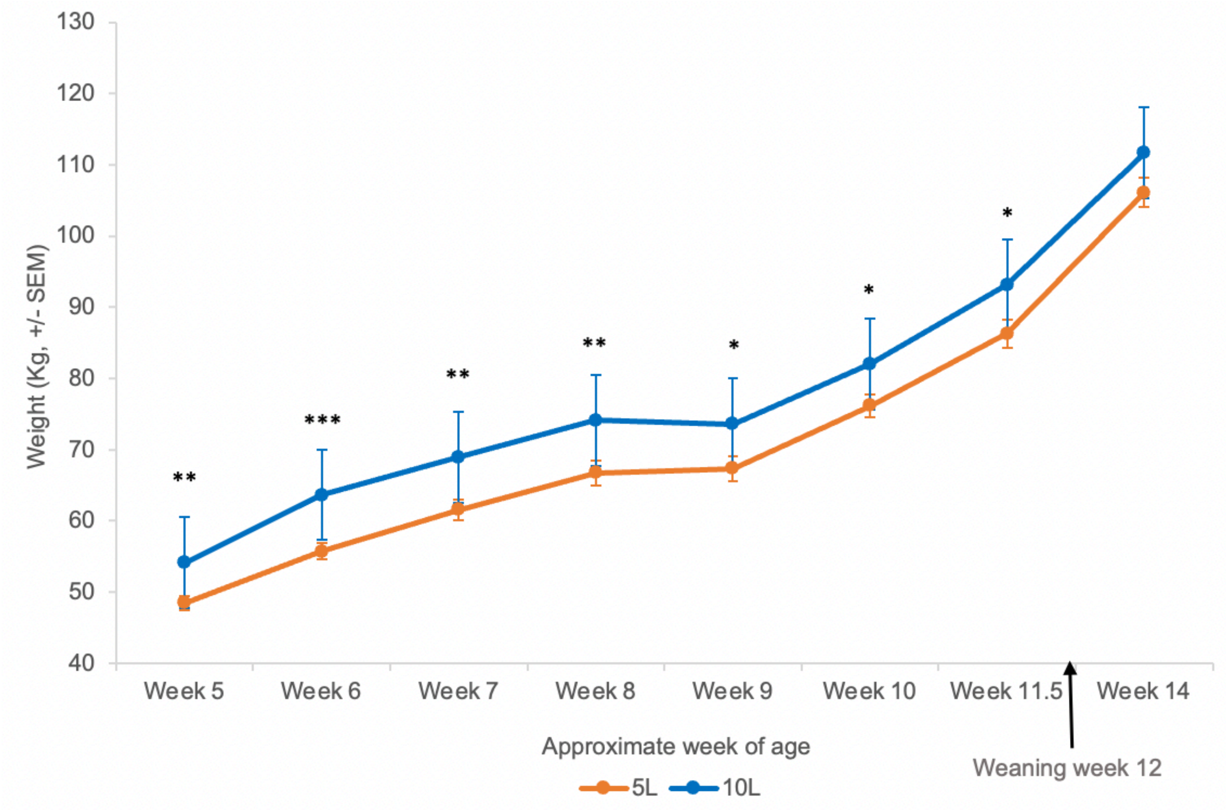

Figure 3.5 The average liveweights (±SEM) from approximately 5 weeks of age (when the calves had been let outdoors on pasture for the first time) until 14 weeks of age (once all calves had been weaned off milk) for 20 Holstein-Friesian bull calves offered different milk allowances (5 or 10 L/day, n=10 calves/treatment). Asterisks (*) showing level of significance (*= P<0.05, **= P <0.01, ***= P<0.001).

3.5 Discussion

Milk allowance and important life changes that occur to calves, such as being re-exposed to pasture after birth and when being weaned affect the liveweights and behaviours of Holstein-Friesian bull calves.

The calves fed 5 L/day had a lower average liveweight and, therefore, a lower weight gain from five weeks of age until 12 weeks of age, when they were gradually weaned off milk. For the calves' initial period on pasture, the dominant behaviour for the group offered 5 L of milk/day was standing/upright, whereas the calves offered 10 L of milk/day spent most of their time lying down. The calves offered 10 L of milk/day were also observed conducting more ruminating, exploring and grooming behaviours, which are positive behavioural indicators of welfare, compared with the calves offered 5 L of milk/day. Cross-suckling was only observed in the calves fed the lower milk allowance.

During the pre-weaning period, the calves offered 5 L of milk/day were observed more often engaging in feeding behaviours (grazing grass and eating meal/hay) and had reduced lying times relative to the calves offered 10 L of milk/day, who were more engaged in lying and social behaviours (grooming and exploring). Post-weaning, the behaviours between the two treatment groups were similar, with feeding and activity increasing as the calves adapted to seeking nutrition primarily through grazed pasture.

When calves are let outside for the first time since birth, they undergo a series of changes that can affect their behaviours. Furthermore, offering different milk volumes can affect these behavioural differences. When first let out onto pasture, the calves that were offered 5 L of milk/day were observed standing/upright in 65.8 % of observations during the 2 h observation period, compared with 27.1 % for the 10 L calves. Calves offered 10 L of milk/day spent over three quarters of an hour longer lying per 24 h during the first 72 hours at pasture. A potential reason for these behavioural differences could have been the weather conditions the calves were exposed to outdoors. Outside, the full effect of low temperatures and wind gusts are felt by the animal, which effectively results in the calf needing more energy to function, as they try to conserve body heat at a greater intensity (Graunke *et al.*, 2011). In order to try conserve body heat, calves will limit the amount of surface area exposed to the rain and wind (Tucker *et al.*, 2007). The first day the calves were on pasture, the average temperature was 9.5 °C and the total rainfall was 2.0 mm, this cool temperature along with rainfall showers and the explanation provided by Tucker *et al.* (2007) (limiting surface exposed to rain and wind), could be a potential reason for why the 5 L calves offered less milk in my study were observed huddling tightly together over this 2 h period and conducted more standing in general. The calves offered 10 L spent more time lying, indicating comfort and relaxation. Longer lying times were also previously observed for the 10 L calves from two to five weeks of age when the calves were reared inside the indoor rearing facility (Chapter Two).

The results of this chapter also indicated that the calves offered more milk were less hungry outdoors compared with the calves offered 5 L of milk/day who may have been lying less because they were hungry or cold. The calves on the greater milk volume potentially could have more energy stored, ultimately conducting more internal diet-ingested thermogenesis processes, preserving their heat individually (Silva & Bittar, 2019). Furthermore, the percentage of observations for lying when their head was supported was 2.9 % higher for calves offered 10 L of milk/day compared with 5 L of milk/day. Hänninen (2007), outlines that animals in a state of Rapid Eye Movement (REM) sleep are harder to wake up than animals in a state of Non-Rapid Eye Movement and that animals who have their head supported while resting, more often go into these REM states (61 % compared to 55 %). This, therefore, suggests that the calves offered more milk in this study were in a deeper state of rest and comfort.

It has also been shown, that animals will conduct more rumination behaviours whilst lying down compared to when standing, which could suggest why the 10 L calves were observed doing this for a large number of observations, suggesting again that they were less hungry (Tucker *et al.*, 2021). Additionally to this, solid feed intake has been found to be correlated with rumination, and for the calves offered 10 L of milk/day nibbling was present in 9.2 % of the observations in this initial period, which could explain why these calves also conducted more ruminating in comparison to calves offered less milk (Swanson & Harris Jr, 1958). A further finding from this initial period on pasture was that calves offered the lower milk volume were the only ones observed conducting cross-suckling, which was in line with earlier findings from Jung and Lidfors (2001) that calves offered more milk conducted significantly less cross-suckling than those offered smaller milk volumes. Calves offered 10 L of milk/day in contrast were observed self-grooming 2 % more of the time, indicating that they found a sense of pleasure and satisfaction in conducting hygiene maintenance compared to calves offered less milk who conducted more hunger related behaviours evidently prioritising different things (Horvath & Miller-Cushon, 2019).

From five weeks of age until weaned of the milk the rumen inside of a calf undergoes significant developments to best withdraw nutrients from its solid feed. When a calf ingests solid food the volatile fatty acids produced via fermentation processes activate the development of ruminal papillae, contributing to the muscle development and enlargement of the rumen (Sander *et al.*, 1959). The most common solid food fed to calves is meal, hay and pasture, however it is meal that is the main dietary component contributing most rapidly to rumen development (DairyNZ, 2023c). Fibres such as hay and pasture on the other hand help to develop muscle layers within the rumen, and prevent the papillae from becoming clumped together (DairyNZ, 2023c). Hay provided to calves should ideally have an energy density of 12-13 MJ ME kg and a protein content of at least 20 % to sufficiently aid in developing the rumen wall, as spring pasture alone has too low fibre, is high in water content and therefore offers suboptimal energy density for a young calf (Geenty & Morris, 2017). The calves in this study were offered meal and hay until they were weaned off milk, which was important for the rumen developmental process. Providing calves with premium food quality gives them a better chance to thrive in their new environment and by continuing to provide the same sort of meal and hay that was provided to them indoors, it meant the calves did not have to process more change when going outside.

Weaning is the process when animals loose access to milk (Budzynska & Weary, 2008). Under natural conditions in the wild, this process occurs over a long period of time, however often for domesticated animals on farms this occurs much more rapidly (Budzynska & Weary, 2008). Gradual weaning is a process that occurs over an extended period and can either involve the slow removal of milk or the substitution of milk for warm water (Bach *et al.*, 2013). Gradual weaning by substituting the milk for water ultimately still provides the calf with the satisfaction of being full and additionally allows the calf to continue preforming suckling behaviours, providing them with a sense of satiety for longer (Budzynska & Weary, 2008). Abrupt weaning on the other hand, results in the calf receiving no intervention, so no milk or water is provided to the calf and teat suckling is abruptly stopped after a single feed without prior preparation (Bach *et al.*, 2013). Common practise on the farm in Taupo where this research was conducted was to substitute milk for water. However, for this trial milk was gradually removed and was not substituted by water, as we were concerned this may have affected the 10 L calves negatively. Loss of milk and routine for calves may result in unwanted distress and is therefore a welfare concern on farms if not approached appropriately (Budzynska & Weary, 2008). Calves offered greater milk allowances tend to be physically affected more during the weaning period in terms of poorer liveweight gains, however calves offered less milk volume tend to have greater behavioural implications such as increased vocalizations, increased activity, unrewarded visits to the milk feeder and increased intake of solid feed, demonstrating persistent signs of hunger (Budzynska & Weary, 2008; Rosenberger *et al.*, 2017).

Prior to weaning, calves fed greater milk volumes have been found to have larger liveweights and grow faster, compared to calves offered lower milk volumes (Bach *et al.*, 2013). However, during the weaning period these liveweight differences have been shown to decrease between calves offered differing milk allowances and can result in the calves on the lower milk volumes having a greater weight gain. For example, a study on eighty Holstein calves found a 977 g/d weight gain for calves fed 6 L of milk/day compared to 857 g/d weight gain for calves fed 8 L of milk/day (Bach *et al.*, 2013). The results of the current study are in agreement with those findings, with the liveweight difference becoming smaller around the weaning period, compared to pre-weaning. The liveweight difference between the two treatment groups was not significant after weaning, with a difference of only 5.55 kg. A reduction in liveweight differences between calves from different milk allowance treatment groups is often due to calves offered the greater milk volume having their main consumption of nutrients diminished, resulting in these calves quickly needing to find solid feed that is just as palatable and interesting.

Prior to weaning, the calves in the current trial that were offered 5 L of milk/day were more frequently observed at the meal/hay feed stations than the 10 L calves (15.8 % vs. 8.9 %). This was similar to the results presented in the previous chapter when the calves were reared indoors and indicates that the 5 L calves were searching for nutrition elsewhere when milk was not readily accessible. Hunger has been shown to have long term effects on the working memory of the animal affecting their ability to concentrate (Lecorps *et al.*, 2023). These long term concentration implications indicate that cognitive performance is driven by how full or hungry the calf feels (Lecorps *et al.*, 2023). Although hunger was likely prevalent amongst the calves offered 5 L of milk/day and during the indoor period (Chapter two), feeding preference when on pasture appears to also have an influence on the results, and supports again why the calves offered 10 L of milk were not grazing as often, being more dependent on milk. Offering calves different food sources such as meal, hay and pasture, gives the animals more choice over their preferred food, enabling them to express their natural behaviours (Whalin *et al.*, 2021). Calves offered 5 L of milk/day were observed grazing in a larger proportion of observations compared to the 10 L calves (13.3 % vs. 2.6 %) prior to weaning. As mentioned in Whalin *et al.* (2021), grass is a valuable resource for calves, which is a possible explanation for why the calves in this study who were offered less milk were observed grazing this unlimited food source more often prior to weaning, whereas the 10 L calves more favoured the milk source. Due to grazing being a movement induced activity and the calves having access to lots of space, it was not surprising the 5 L calves spent less time lying down. Whilst the 5 L calves were grazing more, the 10 L calves were grooming more. Grooming other calves is a positive behavioural indicator and was only observed in the group offered 10 L of milk/day pre-weaning, again similar to results presented in Chapter two. According to Whalin *et al.* (2021) animals who conduct grooming behaviours tend to be more content and choose to perform licking/grooming behaviours with preferred partners, rather than choosing to consume food, which is what the calves offered 5 L of milk/day did in the current study, by spending more of their time foraging.

Behaviours differed between the pre-weaning period and the post-weaning period. Post-weaning all calves were observed to be at the meal/hay feeder for a similar number of occurrences, which was consistent with that of Rosenberger *et al.* (2017) who found post-weaning calf starter and hay intake to be similar across milk allowance treatment groups. Increased restlessness and reduced resting behaviours also commonly increase in mammals after weaning which can be interpreted as negative welfare indicators. Previous work aligns with the current findings, as standing/walking activity observed in the animals increased more post-weaning (Budzynska & Weary, 2008). Neave *et al.* (2018) suggested that calves who conduct more exploratory behaviours also have greater solid feed intakes. This was not supported by the current results, as the calves offered 10 L of milk/day were observed exploring more and the calves on 5 L of milk/day were observed at the meal/hay feed stations and grazing grass more. Nibbling can also be considered as a way of exploring due to being an intrinsic need for exploratory behaviours and was also greater for calves offered more milk (Leruste *et al.*, 2014). A potential explanation for this discussion point in Neave *et al.* (2018), could be due to the fact that exploring is a motivational need in order to forage for food, so theoretically calves who forage subconsciously explore more (Papageorgiou & Simitzis, 2022). Another further important finding was that post-weaning cross-suckling only was observed in calves who were offered the greater milk volume. This can be explained by the motivation behind cross-suckling, which involves the need and want to ingest milk to obtain the taste of the lactose (de Passillé, 2001). Shade usage was another behaviour post-weaning that was only observed in calves offered 10 L of milk/day. The average temperature during the post-weaning observations was 11.4 °C and there was no rainfall. The trees in this paddock were located where the calves had been fed milk pre-weaning, and it is possible that the calves were occupying the shade in this area, due to the previous location of the milk feeder.

A key limitation of this study is that the calves were managed in two groups; only one per treatment. This was because it was not possible to feed the calves individually when on pasture on the farm used for this experiment, so animals had to be fed at a group level. The two treatment groups (5 L of milk/day and 10 L of milk/day) were physically separated by a fence during this outdoor period to adequately provide the different milk allowances to the groups. This meant the calves within a group influenced each other's behaviour and because there was only one group/treatment for this chapter, interpretation of the behavioural results needs to be undertaken with caution due to the lack of appropriate replication of treatment groups. This study could, therefore, be repeated using an outdoor automatic Lely calf feeder to ensure each calf is offered their allocated individual treatment, which would enable an analysis with individual calf as the experimental unit. It would also enable individual weaning programs, which is likely beneficial to their welfare. Alternatively, a larger experiment involving multiple groups of calves per milk allowance treatment would be required, with group as the experiment unit.

3.6 Conclusion

Consistent with literature and confirming this chapter's hypotheses, this research indicates that Holstein-Friesian bull calves offered different milk allowances (5 L and 10 L) demonstrate differences in behavioural indicators of welfare when first exposed to pasture and around the weaning period, as well as altered liveweight gains. When calves were first exposed to pasture, the group offered the higher daily milk allowance had greater expression of positive welfare behavioural indicators, including spending more time lying, self-grooming and ruminating, whereas the calves on lower allowance spent most of their time standing/upright (in agreement with the results from Chapter two). Prior to weaning, calves offered 5 L of milk/day were more engaged in feeding activities, likely seeking out other sources of nutrition, compared with calves offered 10 L of milk/day which conducted more comfort-related behaviours such as lying and grooming. Post-weaning, the behaviours of the calves offered 10 L of milk/day became similar to those offered 5 L of milk/day, engaging in more feeding and activity-related behaviours, most likely due to their main food source being removed. Offering a greater milk allowance to calves can have positive implications on the animals' affective state and, therefore, overall welfare.

Chapter 4
Growth rates from five to 12 months of age of Holstein-Friesian bull calves who were fed high and low milk allowances pre-weaning

4.1 Abstract

The aim of this study was to determine whether the independent variable of different milk allowance (low: 5 L, high: 10 L) pre-weaning causes a long-term difference in liveweights of Holstein-Friesian bull calves until one year of age. Twenty Holstein-Friesian bull calves were reared on different milk allowances (low versus high) until 12 weeks of age when they were weaned. The calves were then reared on pasture as one group until one year of age, via a rotational grazing system. The calves were weighed monthly from five months until one year of age, with additional pasture measurements and samples also being taken. The liveweights of the Holstein-Friesian bull calves were not significantly different between the treatments at any point during this time period. Pasture measurements including average coverage and composition altered over time as the seasons changed. The average pasture cover increased from 2155 kgDM ha in January to 2348 kgDM ha in June and more than half of the composition values decreased from summer to winter, except for the ash, crude fat, lignin, neutral detergent fibre, crude protein and nitrogen components which increased.

Based on the results from this chapter, the milk allowance offered to the calves pre-weaning did not have a long-term effect on liveweights.

Key words: Pasture, rotational grazing, liveweight, milk allowance

4.2 Introduction

Liveweights are a physical indication of an animals performance including their health status relating to how well a calf can digest and absorb nutrients from the food, thrive when exposed to a range of weather conditions and fight illnesses, and still be able to put on weight (Donovan *et al.*, 1998). There is conflicting literature around liveweight differences post-weaning after being offered different milk volumes. According to Hill *et al.* (2016), calves offered more milk can have impaired rumens beyond the weaning period, due to choosing to obtain less solid feed at a younger age, which in time can affect and slow the liveweights of these calves. Groenendijk *et al.* (2018) however, discussed the importance of B-hydroxybutyrate as a good indication of how developed the rumen is and found that there was no significant difference between the rumens of the calves offered different milk allowances and the level of this chemical post-weaning, emphasising that rumen development post-weaning is similar. Additional to research around rumen development, literature on liveweights of calves indicates that providing larger milk volumes can have positive implications on calf liveweights persisting well beyond the weaning period, when provided with other adjacent contributing factors such as conducting a gradual weaning process (to allow for a slow stable transition) and the animals being able to exhibit their natural behaviours, which effectively contributes to a calf's overall wellbeing and liveweight (Mellor, 2012; Rosenberger *et al*., 2017). Although this is the case when other contributing factors are sufficiently met, other literature report that liveweight gains can decrease overtime, particularly around the weaning period for calves offered greater milk volumes compared to smaller volumes (Khouri & Pickering 1968; Jasper & Weary 2002).

Pasture type, quality and availability are important underlying components contributing to animal consumption and therefore the liveweight of a calf. The pasture calves are reared on in New Zealand after the weaning period differs depending on the type of terrain, soil and climate the farm is located in, however two popular grass species around the country persisting in most environments are perennial ryegrass (Lolium perenne, L.) and brown top (Agrostis capillaris, L.) (Guevara-Escobar *et al*., 2007). To avoid limiting animal performance, high quality pasture composition is critical, which therefore highlights the importance of pasture and grazing management. One common difference observed on farms however in relation to this pasture management is whether to conduct rotational or continuous grazing (Martin-Gatton College of Agriculture).

Rotational grazing involves a large area of pasture being divided into smaller areas, enabling easy movement of stock between paddocks and promoting pasture regrowth and productivity, however this method does require more labour than continuous grazing (Martin-Gatton College of Agriculture). Continuous grazing on the other hand, has stock remaining in large paddocks for a longer period of time resulting in less labour, but reduced herbage regrowth and only certain herbages can really withstand this type of grazing pressure (Martin-Gatton College of Agriculture). Although both methods can be effective in their own way, rotational grazing appears to have extremely beneficial effects, by almost doubling the liveweights of animals compared with those reared under a continuous grazing system (Walton *et al.*, 1981).

In my previous chapters, calves fed 10 L of milk/day grew faster until weaning compared to calves fed 5 L/day. Along with liveweight differences, the behaviours exhibited by the calves also varied between the two treatment groups during new experiences the calves were exposed to (first two hours on pasture and over the weaning period). Calves offered a higher milk allowance demonstrated behaviours associated with positive welfare such as increased lying, ruminating, grooming and exploring which is consistent with findings of others (Swanson & Harris Jr, 1958; Hänninen, 2007; Silva & Bittar, 2019; Tucker et al., 2021). This was compared to calves offered a lower milk allowance which demonstrated more behaviours associated with negative welfare such as cross suckling, reduced lying/increased activity and more visits to feed stations which is also in accordance with findings of others (Budzynska & Weary, 2008; Rosenberger *et al*., 2017).
We therefore now have a better understanding of the effects different milk allowances have on the liveweights and behaviours leading up to and including weaning in Holstein-Friesian bull calves, however the gap in knowledge addressed in this chapter is the liveweight implications post-weaning. Monitoring the liveweights of calves after being weaned of milk until one year of age, highlights the potential effects that differing milk allowances pre-weaning can have on the future performance of a calf. Monitoring liveweights of Holstein-Friesian bull calves while managed on a rotational pasture grazing system, gives an indication of how non-replacement calves in future could potentially be reared. Rearing these calves for longer on dairy farms could grow our meat industry and reduce the stigma around the early slaughter of bobby calves.

The aim for this chapter was to determine if there is a long-term effect of different milk allowances (5 L and 10 L) fed prior to weaning, on the long-term growth weights until one year of age. The overall hypothesis for this chapter was that the independent variable of differing milk allowances (5 L and 10 L) will cause a difference between the liveweights of Holstein-Friesian bulls reared for beef. I predicted that calves being fed 5 L of milk/day will have a lower average liveweight at one year of age compared to calves being fed 10 L of milk/day pre-weaning.

4.3 Materials and Methods

4.3.1 Previous rearing

The experiment was undertaken between July 2022 and July 2023. All procedures involving animals in this study were approved by the Animal Ethics Committee at the University of Waikato (protocol no. 1146) under the New Zealand Animal Welfare Act (1999). The 22 enrolled calves in the trial were reared as one group inside a shed facility for approximately four weeks. Each calf was allocated either a high (10 L) or low (5 L) milk allowance per day via an automated calf feeder. Meal and hay were provided ad-libitum. The calves were moved outdoors onto pasture at five weeks of age, where a series of further procedures took place including video recording of the calves during the first two hours on pasture, and two hour and 30 minute live behavioural observations pre- and post-weaning (weaning was conducted gradually over four days). Refer to chapters one, two and three for detailed descriptions of all the methods leading up to this chapter. Two calves (one from each treatment) were excluded from all analyses due to persistent illness.

Once the last calf reached 100 kg liveweight, the calves were transported to a neighbouring farm to graze. For a detailed description of trial events leading up to and including this chapter, particularly type and timing of vaccination and drenching of the calves, see Table 9.

4.3.2 Liveweights

Once the calves had been transported from their current farm to the neighbouring farm (38.90434° S, 176.38787° E) to graze, the calves were weighed monthly using farm scales (Tru-Test Datamars cattle crate heavy duty weigh bar scales, ID5000, New Zealand).

4.3.3 Calf management

Forty-six calves including my 20 focal calves were run together as one mob to graze from 21 weeks of age to 32.5 weeks of age. At 32.5 weeks of age an additional 54 calves joined my mob of calves to result in a total of 100 calves.

Drinking water was provided in standard 500 L concrete farm troughs. Pasture break allocations were changed every two and a half days and ranged from approximately 1 ha to 1.4 ha/100 calves. Silage supplements were fed to the calves daily, at 43.5 weeks of age each calf was offered 4.55 kg/day and from 47.5 weeks of age each calf was offered 5 kg/day. Silage was fed to calves between 09:00 h - 09:30 h, in a narrow strip along the fence line using a four-tonne feed out wagon. Shelter was generally not provided, however a few paddocks offered tree shelter.

The calves in this trial were managed as per normal farm practise, which included vaccinations, drenching and veterinary procedures, refer to Table 9.

4.3.4 Pasture sampling

Pasture measurements were undertaken and samples for pasture analyses obtained monthly. Pre-grazing pasture measurements were taken initially by walking in an M shape from one end of the paddock to the other end of the paddock using a rising plate meter (Tru-Test Electronic Plate Meter EC09, Farm Source, Hamilton, New Zealand). Every three steps I placed the plate meter flat on the pasture. Approximately 93 readings were obtained per paddock. Once I reached the end of the paddock, I recorded the number of plate measurements and average height specified on the device. I then walked back to the other side of the paddock, again in an M shape but this time obtaining pasture snip samples using electric cutters (Lanati cord free shearing and clipping hand piece, Rurtec, Hamilton, New Zealand), every 10-15 steps depending on how big the paddock was. The grass was cut just above soil height in front of my foot on the ground. I then picked up the cut grass and added it to a large (60cmx45cm) clear plastic sample bag. The bigger the paddock the more steps were taken before taking the pasture sample, a minimum of 30 samples were collected from each paddock. Two and a half days later I would take post-grazing pasture measurements which were taken in the same way as the pre-grazing pasture measurements (except for the post-grazing pasture measurements no pasture snip samples were taken, only plate meter measurements were conducted). These data and samples were collected monthly prior to calves going into a new paddock and after the calves left the paddock to estimate pasture intake/calf/day. The nutritional composition of the pasture samples was processed by Hill Laboratories using near-infrared spectroscopy analysis. The pasture on this farm consisted approximately of 70 % ryegrass, 25 % clover, 3 % dandelion and 2 % plantain (Lee Church, personal communication).

4.3.5 Recording of environmental conditions

Weather data for the grazing period while the calves were on the neighbouring farm (second farm) was retrieved from the nearest NIWA weather station located 36.6km away from the farm (Station 41429). Data was collected daily, and summarized into monthly averages.

4.3.6 Data handling

Missing data

One calf was removed from the trial at approximately 10 months of age due to bullying (non-reproductive mounting) within the herd causing consistent injury to the calf. To handle missing liveweight values for this calf a REML splines analysis was conducted when looking at liveweights over time.

Pasture and supplement data

Pasture samples were delivered to Hill laboratories, where the components within the pasture were analysed using NIR analyses.

Pasture intake

To calculate the pasture consumed/calf/day, firstly the average pasture cover was calculated via a plate meter using the standard recommended formula for Winter Spring Early summer ryegrass dominant Dairy Pasture (140xheight+500). The height variable is measured by the plate meter device, in units of 0.5cms. Once the average pasture cover was collected via the plate meter, the kg of dry matter/hectare/day/calf was calculated. This was done by firstly subtracting the average post-pasture cover from the pre-pasture cover. Secondly this value was multiplied by the paddock hectare size (provided by the farm manager) the calves had access to graze in. This was then divided by the number of calves in the paddock and then finally divided by the number of days the calves spent in that paddock grazing.

Figure 4.1 outlines a flow chart of the third phase of procedures that were undertaken outdoors on pasture until the calves reached approximately one year of age.

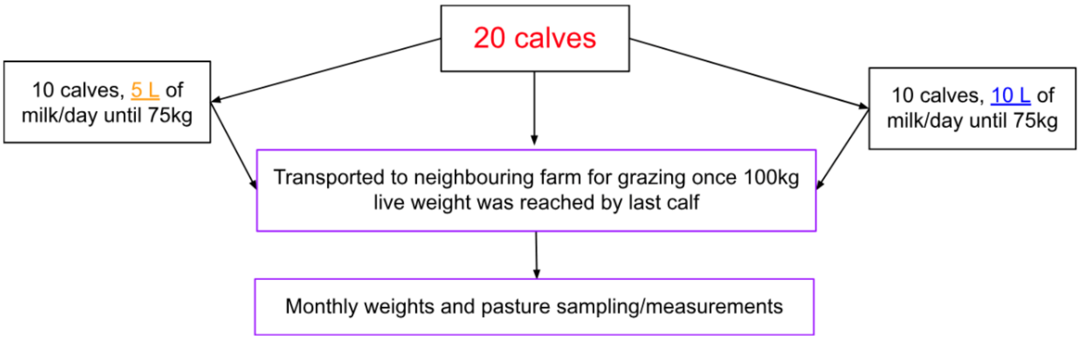

Figure 4.1. Flow diagram of summary methods

Table 9. Timeline of the trial events

Approximate age of calves	**Event**
21 weeks	From this age calves continue to be reared on pasture.
25.5 weeks	Calves given 9mls of matrix C drench and 2mls of B12.
30 weeks	Calves given 2mls of B12.
32.5 weeks	An additional 54 calves joined my calves to graze. 100 calves total in mob.
35 weeks	Calves given 4mls B12 selenium.
35.5 weeks	Calf #7, blister on rump was lanced and given 8mls of penicillin.
38 weeks	Calves given 17mls of matrix C and 4mls of B12.
39 weeks	Calf #7, blister on rump lanced for a second time and was removed from trial due to bullying.
43.5 weeks	All calves now getting supplement of silage (4.55 kg/calf/day).
47 weeks	Faecal egg count done on the calves, conducted fort-nightly from now.
47.5 weeks	Calf #44, very sick lost 20 kg since last weigh a month ago. Given 40mls of bivatop twice. All calves getting 5 kg of silage/calf/day.
52.5 weeks	Last monthly weighing of the calves. Animals left under farm management.

4.3.7 Statistical analyses

Individual calves were considered statistical units. Simple ANOVA analyses were conducted looking at weight gain over the whole time period using R 4.0.4 (R Core Team, 2021). The Weight gain ANOVA analyses consisted of a single factor and a fixed effect of treatment. A time series plot was produced over a period of 32 weeks, SEM bars were calculated for each of the treatment groups for each of these weeks presented in the plot. A liveweight REML splines analysis using GenStat (version 23;

VSN International Ltd.) was also conducted. The REML spline consisted of a fixed effect for treatment and random terms for calf and day spline (overall, by treatment and by calf). A time series liveweight plot was produced over the entire 52-week period that presented predicted means and 95 % confidence intervals.

All other values provided including weather data, pasture data and calculated pasture intake are descriptively presented using Microsoft Excel (version 16.75). Monthly means and standard deviation (SD) are presented.

We classify a significant P-value of <0.05.

4.4 Results

4.4.1 Environmental conditions

The average air temperature whilst the calves were being reared outside on pasture on the neighbouring (second farm) from the middle of December until the end of July was 14.6 °C (SD=3.48 °C), which ranged from a minimum of 5.5 °C to a maximum of 23.7 °C. The total rainfall over this period that the calves were exposed too was 983.8 mm. For a detailed description of the temperatures and total rainfall refer to Table 10.

Table 10. The daily minimum, maximum and average temperature (±SD) (summarised into monthly values) and total precipitation values/month (±SD), that the 20 Holstein-Friesian bull calves were exposed to whilst managed on pasture between December 2022 and July 2023.

Month	Min-temp (°C)	Max-temp (°C)	Av-temp (°C)	SD temp (°C)	Total-rainfall (mm)
December	12.9	21.7	17.6	2.28	131.4
January	14.8	22.3	18.5	1.61	219.4
February	14.5	23.7	18.5	2.11	116.2
March	10.9	19.5	15.8	2.57	78.6
April	8.8	18.4	14.6	2.43	70.0
May	5.5	18.6	12.6	3.41	238.6
June	6.2	14.5	9.8	2.12	52.4
July	5.55	12.6	9.21	2.18	77.2

4.4.2 Liveweights

Overall, there were no significant liveweight differences between the two treatment groups (P=0.158, $F_{1, 17}$=2.18, SED= 7.48 kg) (Figure 4.2). The greatest difference between the calves offered 5 L of milk/day and 10 L of milk/day during this time period in Figure 4.2 was around 20 weeks, with the average difference being 8.5 kg (SED= 5.75 kg). Also evident in this figure is that the largest increase in liveweight for all calves occurred between 30 and 35 weeks of age (approximately seven to nine months of age). The average weight gain of the calves from 30-35 weeks of age for the 5 L and 10 L calves was 33.35 kg and 30.1 kg (SED= 2.81 kg).

Figure 4.3 shows a time series plot of the modelled weight over time, produced via a REML splines analysis. It is evident in this figure that the pattern is not the same between the two treatment groups, which was supported by the REML spline analysis for the shape of the curve which resulted in a significant result (P=0.019). The linear REML spline analysis however, did not produce a significant result due to the calves starting and finishing the trial at similar weights (P=0.587 $F_{1, 18}$= 0.31).

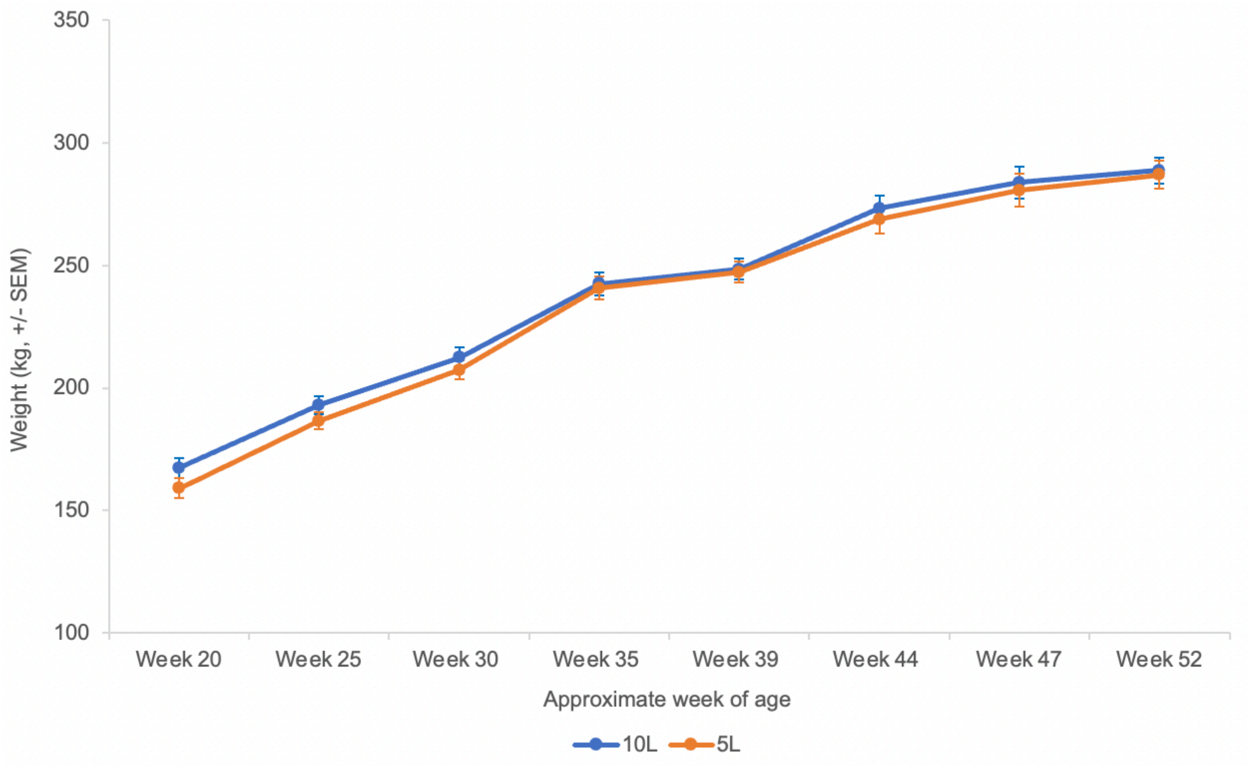

Figure 4.2. The average liveweights (±SEM) over time from approximately 20.5 weeks of age until approximately 52.5 weeks of age for 20 Holstein-Friesian bull calves managed on pasture. The calves had been offered different milk allowances pre-weaning (5 or 10 L/day, n=10 calves/treatment).

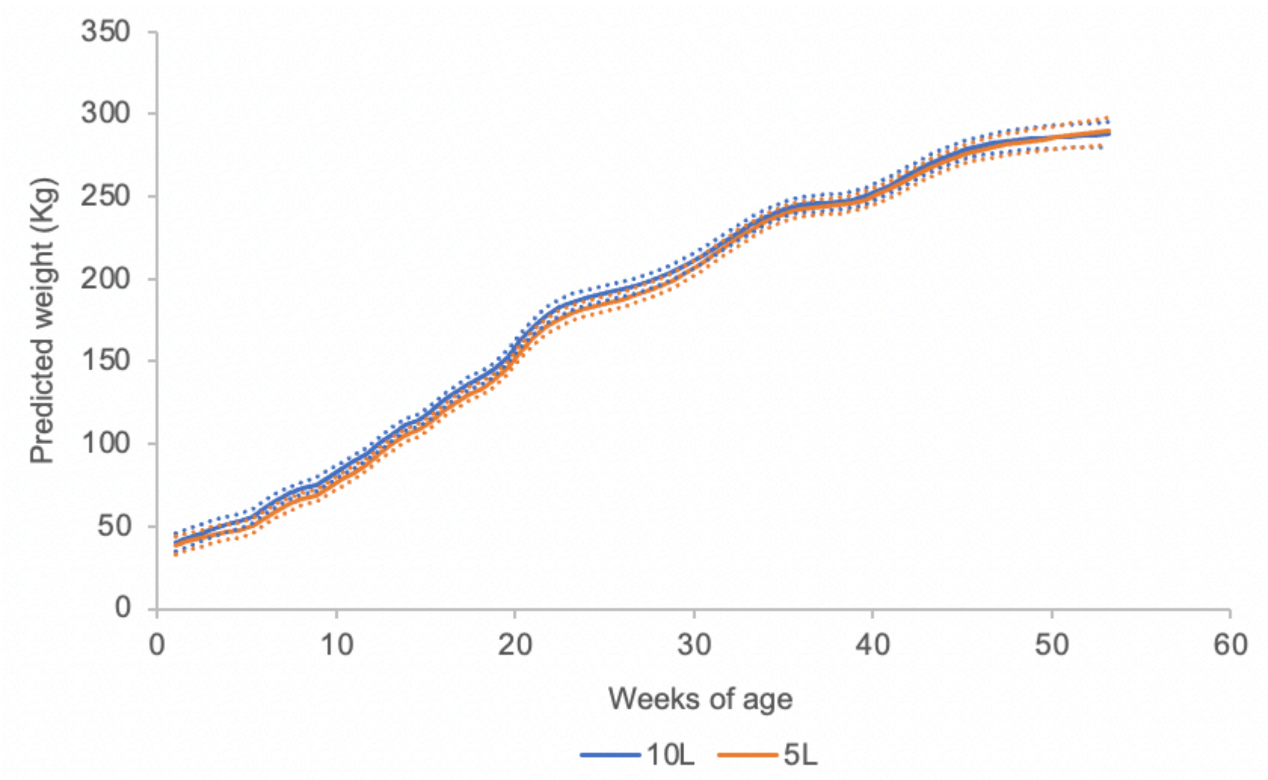

Figure 4.3. Modelled weight over time from one week of age until one year of age for 20 Holstein-Friesian bull calves managed on pasture and offered differing milk allowances (5 or 10 L/day, n=10 calves/treatment) pre-weaning. The figure demonstrates 95 % confidence intervals (dotted lines).

4.4.3 Pasture and supplement composition

Table 11 outlines the pasture composition consumed by the 20 Holstein-Friesian bulls whilst grazing. It is evident in this table that over time the pasture composition changed, in particular the crude protein dry matter percentage increasing by 4.9 %, the soluble sugar dry matter percentage increasing by 12.1 % (more than double then when the calves were 25.5 weeks of age) and the Non-Structural Carbohydrate dry matter percentage increasing by 6.3 % over this time period.

The meal composition at 22 weeks of age can be found in Table 12 along with the silage composition. The silage supplement analyses produced two results higher and three results lower than the normal ranges provided by Hill Laboratories. Dry matter was considered to be 9.2 % higher than the medium interval range and the neutral detergent fibre was 2.7 % higher. Nitrogen was considered to be lower than the medium interval range (0.3 % lower), crude protein was 1.4 % lower and digestibility of organic matter was 1.7 % lower.

4.4.4 Pasture and supplement intake

The approximate calculated amount of pasture consumed over 22.5 weeks also greatly varied. At 30 weeks of age the calves were consuming approximately 7.0 kg/DM/hectare/day compared to 0.95 kg/DM/hectare/day at 52.5 weeks of age, which is a 6.06 kg/DM/hectare/day difference (Table 13). It is important to note that from 43.5 weeks of age, the calves were offered 4.55 kg of silage/day and from 52.5 weeks of age the calves were offered 5.0 kg of silage/calf/day, however this is still a consumed feed reduction of 1.06 kg/DM/hectare/day at approximately one year of age. At 52.5 weeks of age during the winter months the pasture cover was limiting being only 1690 kgDM/ha compared to during the summer months at 30 weeks of age, being 2852 kgDM/ha which is a difference of 1162 kgDM/ha.

Table 11. The pasture composition (±SD) consumed by 20 Holstein-Friesian bull calves whilst reared from approximately 25.5 weeks of age until one year of age (52.5 weeks). DM in this table refers to dry matter.

Pasture Component	25.5 weeks January	30 weeks February	35 weeks March	39 weeks April	44 weeks May	47.5 weeks June	52.5 weeks July	SD
Nitrogen (%)	2.3	2.6	3.3	3	3.2	2.8	3	0.35
Nitrogen (% DM)	2.4	2.7	3.6	3.1	3.3	2.9	3.1	0.39
Dry Matter (%)	20.6	15.1	16.4	14.5	13.6	14	19.4	2.75
Crude Protein (% DM)	14.8	17	22.5	19.7	20.7	18.4	19.7	2.52
Acid Detergent Fibre (%DM)	25	26.9	24.4	25.4	25.6	25.3	23.1	1.16
Neutral Detergent Fibre (%DM)	44	49.5	42.4	45.5	49.1	45.2	44.5	2.63
Lignin (%DM)	6.5	7.3	9.3	8.9	9	8	9.1	1.07
Ash (%DM)	9.4	9.7	11.1	11.7	11.6	10.8	10.1	0.91
Organic Matter (%DM)	90.6	90.3	88.9	88.3	88.4	89.2	89.9	0.91
Soluble Sugars (%DM)	18.5	7.6	7.4	4.7	4.6	15.3	6.4	5.46
Starch (%DM)	1.9	0.8	1	0.6	0.5	0.6	<0.5	0.52
Crude Fat (%DM)	3.2	3.1	3.2	3.1	3	2.7	3.5	0.24
Digestibility of Organic Matter in (% DM)	70.9	62.6	63.3	61.4	60.2	62.3	69.3	4.12
Metabolizable Energy (MJ/kgDM)	11.3	10	10.1	9.8	9.6	10	11.1	0.66
Non-Structural Carbohydrate (% DM)	28.6	20.6	20.8	20.1	15.6	22.9	22.3	3.90
Organic matter digestibility in-vivo (% DM)	78.3	69.3	71.2	69.5	68.1	69.9	77	4.05

Table 12. Supplement composition values (meal and silage) consumed by 20 Holstein-Friesian bull calves whilst reared from approximately 22 weeks of age until a year. DM in this table refers to dry matter.

Component	**22 weeks- Meal December**	**52.5 weeks- Silage July**
Nitrogen (%)	3.1	1.9
Nitrogen (% DM)	3.3	2
Dry Matter (%)	89.9	39.2
Crude Protein (% DM)	20.4	12.6
Acid Detergent Fibre (%DM)	9.6	29.8
Neutral Detergent Fibre (%DM)	29.1	47.7
Lignin (%DM)	3.1	7.6
Ash (%DM)	8	10.8
Organic Matter (%DM)	92	89.2
Soluble Sugars (%DM)	8.7	9.6
Starch (%DM)	9.7	0.9
Crude Fat (%DM)	5.1	3.3
Digestibility of Organic Matter (% DM)	77.2	63.3
Metabolizable Energy (MJ/kgDM)	12.1	10.1
Non-structural carbohydrate (%DM)	37.3	25.6
OMD in-vivi (%DM)	83.9	70.3
pH	-	4.1
Ammonium-N (% DM)	-	0.12
Ammonium-N/Total-N Ratio (%)	-	6.5
Latic Acid (% DM)	-	6.8

Table 13. The approximate amount of pasture consumed/calf/day (±SD), for 20 Holstein-Friesian bull calves whilst reared from approximately 30 weeks of age until a year.

Variable	**30 weeks February**	**35 weeks March**	**39 weeks April**	**44 weeks May**	**47 weeks June**	**52.5 weeks July**	**SD**
Pasture consumed/calf/day (kg/DM/hectare/day)	7.01	7.84	5.55	2.31	8.46	0.95	3.07
Silage (kg/day)	-	-	-	4.55	5.0	5.0	0.21

4.5 Discussion

This study aimed to assess the effects of pre-weaning milk allowance on the longer-term liveweights of Holstein-Friesian bull calves from approximately five months until one year of age. With respect to the hypothesis and prediction for this chapter, both were rejected. The hypothesis for my final chapter was that there would be a difference in liveweights over this time period and my prediction was that calves offered 10 L of milk/day at a young age, would having heavier liveweights persisting beyond weaning until one year of age, however this was not proven. Initially at 20 weeks there was a liveweight numerical difference of 8.5 kg between the two treatment groups, which was actually the greatest liveweight difference throughout the time period of this chapter (five-12 months of age), however this was not a significant difference.

There is varying literature around whether or not different milk allowances offered to calves will have long-term liveweight implications. A study conducted by Burggraaf *et al.* (2020), found a significant liveweight difference at seven months of age of 31 kg's, which was not replicated by the results in my chapter. The findings from my study were however supported by the work of others within this area, ultimately not linking milk allowances with long term liveweights. The results from my study reflect the trend portrayed in Khouri and Pickering (1968) who also found that overtime liveweight gains decreased for calves offered ad-libitum milk compared to a smaller volume. By approximately one year of age the calves in my study that were offered the greater milk volume of 10 L of milk/day had an average liveweight of 287 kg compared to the calves offered 5 L of milk/day that had an average liveweight of 289 kg, which shows that there was no difference in their liveweights. This finding was further supported by Jasper and Weary (2002), who found that over the weaning and post-weaning periods there were no weight gain differences between their two treatment groups either fed conventionally (10 % of body weight) or ad-libitum. They also mention that solid feed intake after weaning was not affected by the treatment groups, which was sustained by the results in the current study, with both treatment groups being observed at the meal/hay feeders a similar amount post-weaning (Chapter three) (Jasper & Weary, 2002). Although actual solids intake was not measured in my thesis.

There is research by others suggesting different reasons as to why calves offered greater milk volumes produce not as fast growth rates when they get older, resulting in lower liveweights. Hill *et al.* (2016) suggests calves offered more milk effectively have impaired rumens, however Groenendijk *et al.* (2018) found that rumen development was similar between treatment groups based on the B-hydroxybutyrate chemical. Schäff *et al.* (2018) appear to align with the results from Hill *et al.* (2016), outlining that calves fed restricted milk volumes have a greater density of rumen papillae helping to digest solid feed, compared to calves offered more milk. There is evidently contradicting research around this idea of rumen development and therefore more research is required to better understand the mechanisms of rumen development and sustained effects on growth rates.

Additional factors contributing to an animal's liveweight once weaned off milk and reared until one year of age are grazing management and pasture composition. Rotational grazing (which was utilized in the current trial) tends to provide more beneficial implications when compared to conventional beef grazing management (Martin-Gatton College of Agriculture). Rotational grazing generally provides a number of positive outcomes such as allowing easy movement of stock, promotes rapid pasture regrowth and enhances liveweight production (Walton *et al.*, 1981). The additive benefits of rotational grazing therefore suggest that the calves in my trial were given a suffice chance to grow and develop over this time period.

The nutritive pasture composition varied over time with changing seasons, which ultimately can have further implications on the animal consuming it. Carbohydrates such as sugar, starch, pectin, hemicellulose, cellulose and lignin make up approximately 75 % of plant dry matter, which therefore supports why in my trial most of these particularly starch and soluble sugars decreased from summer to winter, as the rain in the winter months increased and light decreased thus reducing the dry matter (DairyNZ, 2023f). Also aligning with most of the pasture composition values produced throughout the duration of my trial were those outlined in Machado *et al.* (2005). Those authors found that from summer through to winter, the average percentage of non-structural carbohydrates decreased, in comparison to ash, organic matter digestibility, acid detergent fibres, neutral detergent fibres, lipids, proteins and metabolizable energy which all increased. The results from my study presented in Table 11, align with the majority of these results, apart from organic matter digestibility and metabolizable energy which decreased instead. Over this time period the herbage average mass outlined in Machado *et al.* (2005) also increased, which additionally supported the pasture cover findings from my trial increasing from 2155 kgDM ha in January to 2348 kgDM ha at the beginning of winter in June.

Liveweights and pasture changes over time are important factors to monitor, in order to ensure the animals are healthy and thriving. I weighed the bulls monthly and also took pasture samples on a monthly basis. It would have been interesting to monitor pasture changes more closely to relate these more directly to the liveweights.

4.6 Conclusion

Calves fed different milk allowances from approximately seven days of age until weaning at 12 weeks of age overall had similar growth rates post-weaning until 1 year of age, although calves fed 10L pre-weaning had numerically higher growth rates at an early age post-weaning. Although this research does not confirm the idea that milk allowance influences liveweights long-term, the effects different milk allowance had on calves prior to weaning has prominent effects on the liveweights and behaviours during that period in their life (Chapter two and Chapter three) and ultimately their welfare.

Chapter 5
Discussion

5.1 Summary

This thesis aimed to support positive change in calf rearing practises within the dairy industry by generating information on how to rear calves so that they have a good life. This was done by monitoring the behaviours of young calves and subsequent liveweights when offered different milk allowances and managed on pasture until 12 months of age. Here I will discuss the plausibility and implications of low versus high milk allowance as a possible explanation for the different behaviours and liveweight gains seen over one year, as well discussing limitations of this study and where I believe future research should be focussed.

5.1.1 Behaviours

From one until five weeks of age (Chapter two), twenty Holstein-Friesian bull calves were reared in an indoor rearing facility on a Landcorp-Pāmu calf rearing farm in the Taupo region of New Zealand (38.57927° S, 176.16787° E) and were offered one of two milk allowances (5 L of milk/day or 10 L of milk/day). From five to 12 weeks of age (Chapter three) the calves grazed pasture outdoors and were then weaned off milk at 12 weeks. Over this three-month period, behaviours of the calves associated with positive or negative welfare were monitored at four different time periods (four weeks of age, initial two hours on pasture, pre-weaning and post-weaning). When behaviours associated with positive welfare are demonstrated by calves it is an encouraging sign that a number of the five domains within animal welfare (nutrition, environment, health, behaviour and mental state) are being met ((Mellor, 2012). The fifth component (mental or affective state) refers to the overall experience of the animal which includes emotions such as anxiety, distress, frustration, boredom, isolation or excitement, relaxation and calmness, as well as physical feelings of hunger, thirst, weakness, breathlessness or strength, energetic and fullness (Mellor, 2012). The behaviours demonstrated by calves are therefore indicative on the affective state the animal is experiencing, positive behavioural indicators are essentially also an expression of a positive affective state.

I observed significant behavioural differences in the calves at four weeks of age (Chapter 2), along with encouraging descriptive results for the further three behavioural observational periods whilst the calves were outside on pasture (Chapter three). The majority of the behavioural results I presented throughout my thesis suggested that calves offered the greater milk volume demonstrated more behaviours associated with positive welfare such as grooming and increased lying which effectively also means the calves may be experiencing a positive affective state such as excitement, relaxation, calmness and fullness (Jensen *et al*., 1998; Napolitano *et al*., 2009; Mellor, 2012; Keeling *et al*., 2021; Papageorgiou & Simitzis, 2022). This was compared with the calves that were fed a restricted milk allowance and more frequently demonstrated behaviours associated with negative welfare such as increased activity and visiting the milk, meal and hay feeders more often, and potentially expressing a negative affective state such as frustration, anxiety and distress (Jensen *et al*., 1998; Napolitano *et al*., 2009; Mellor, 2012; Keeling *et al*., 2021; Papageorgiou & Simitzis, 2022). Although it is common practise for farmers to feed their calves 10 % of the animals' body weight at birth (conventional), restricted milk volumes can have detrimental cognitive implications as well as reducing the opportunity for the animal to express positively motivated natural behaviours (Whalin *et al*., 2021; Lecorps *et al*., 2023). My results evidently highlight the importance of feeding greater milk volumes to calves, to result in the performance of more desirable behaviours.

5.1.2 Liveweights

The liveweights of the calves in my trial were recorded weekly (Chapter two and three) until the last calf reached 100 kg at approximately 14 weeks of age. The calves were then transported to a neighbouring farm to graze (38.90434° S, 176.38787° E), where liveweights were recorded monthly (Chapter four). From one to five weeks of age (Chapter two), statistical differences were observed between the liveweights of the calves offered differing milk allowances, which persisted until weaning (Chapter three). Literature widely supports the findings of my work emphasising that restricted milk volumes reduce liveweights prior to weaning (Vieira *et al*., 2008; Bach *et al*., 2013; Rosenberger *et al*., 2017). Post-weaning I found the liveweight gap between the two treatment groups became minimal, which was again supported by wider literature including that of Jasper and Weary (2002). However, looking at other aspects of welfare, (I.e., cognitive ability) or health (I.e., immune function), could be valuable in determining whether these component are influences by early life nutrition.

5.1.3 Limitations and future directions

I believe that the main limitation of the research in my thesis, is the fact that I am human and not a calf. As humans we perceive the world differently to calves, essentially because we have different life purposes, have different senses, and are ultimately composed completely different. Although this is the case, we use research principles, to objectively collect behavioural data, to then compare and interpret the results of different studies, enabling us to best confirm the behaviours being demonstrated by the calves. To me, not being a calf is a limitation of my study, however it is also the underlying principle of my research, because being able to interpret their behaviours and understand these animals, helps us to provide them with a good life. My thesis provides information that can be replicated, to ultimately contribute to a wide range of robust data across a series of conditions.

A second limitation was the removal of cohort 2 from my analysis. The entire methodology was carried out for this second cohort, but due to consistent issues with the automatic milk feeder resulting in faulty milk powder concentrations, treatments (5 and 10 L of milk/day) were not consistently and accurately applied to the animals. This unfortunately reduced the statistical power throughout my thesis.

A key limitation of my project was the reduced ability to conduct a statistical analysis on the behaviours observed in Chapter three. The main aim of my thesis was to offer two groups of calves' different milk allowances to observe the effect on the calves' liveweights and behaviours. Due to only one person located on the Taupo farm feeding my calves, it was not time efficient or possible to have several smaller groups managed outdoors (fewer calves/group, but more groups to add replication to my study), when the automatic calf feeder could not be used outside. Therefore, only two groups were managed outside and were kept separate to sufficiently feed the two varying milk allowances. Producing statistical results in Chapter three would have helped to further strengthen and link milk allowance to the behaviours of my calves.

Based on the important findings produced in this trial in regard to the beneficial outcomes of providing more milk to calves, there is room for further research in this area. In future I would like to address the financial costs for farmers rearing calves (who aren't usually kept on farm) until one year of age, look into potential land available to produce more calf rearing farms and finally make farmers aware of the behaviours their calves are exhibiting, so they become more knowledgeable themselves and can monitor signs of positive and negative affective states and therefore welfare in their own animals.

My research has given insight into the beneficial implications of feeding 10 L of milk/day and the negative affective state calves exhibit when fed less milk (5 L of milk/day). Therefore, if I addressed the costs of feeding greater milk volumes to calves and if these costs were justifiable for farmers and land availability allowed for it, in future all calves including bobby calves could be reared on greater milk volumes for longer. This in turn could grow our meat industry and reduce the stigma around the early slaughter of bobby calves. The proposed calf rearing development, provides a new potential pathway for all calves, ultimately giving them a good life.

5.1.4 Concluding remarks

The primary finding from this research project was that offering greater milk allowances (10 vs. 5 L of milk/day) to Holstein-Friesian bull calves resulted in more behaviours associated with positive welfare and fewer behaviours associated with negative welfare, as well as greater liveweights until weaning. Positive behavioural indicators that were more often demonstrated by the calves offered 10 L of milk included spending more time lying and engaging in social behaviours such as grooming, playing and exploring, relative to calves offered 5 L of milk/day who demonstrated more negative behavioural indicators such as engaging in more standing, feed related behaviours (visiting the milk, hay and meal feeders more often) and cross-suckling. Although there was no significant difference between the liveweights of the calves from 14 weeks to one year of age, the effect of milk allowance on behaviours and liveweights at a young age are critically important to support the development, functioning and affective state of the animal contributing to the calves' overall well-being.

As my Masters has come to an end, I have pondered on the idea of what I aspire to do next. Almost two years ago, at the beginning of my post-graduate degree, I wanted to conduct research that would improve the life of animals and today that is still my goal. I have a huge passion for calves and based upon the results of my study, particularly in terms of animal welfare and the prevalent affective states and behavioural indicators that calves demonstrate, I believe that there are opportunities to develop calf rearing practices that promote behaviours indicative of positive animal welfare as well as optimise animal growth and productivity. Therefore, continuing to support positive change for animals within the dairy sector is my future ambition.

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