# Intake-based weaning of dairy calves and the influence of forage type

by

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# Abstract

The objective of this study was to assess the effects of intake-based weaning methods and forage type on behaviour and growth of dairy calves. Holstein calves (n = 108) were randomly assigned to 1 of 3 weaning treatments: milk reduction by age (wean-by-age), individual dry matter intake (DMI) (wean-by-intake), or combination of individual DMI and age (wean-by-combination). Groups of calves were alternately assigned to 1 of 2 forage treatments: grass hay (Hay), or silage-based total mixed ration (TMR) (n = 6 groups per treatment). Until d 30, all calves received 12 L/d of milk. On d 31, milk was reduced by 25% of the individual's average milk intake. For wean-by-age calves (n = 31), milk remained stable until d 62 when milk was reduced until weaning at d 70. For wean-by-intake calves (n = 35), milk was further reduced by 25% once calves consumed 200, 600, and 1150 g DM/d of solid feed. For wean-by-combination calves (n = 35), milk remained stable until calves consumed 200 g DM/d; milk was then reduced until weaning at d 70. If calves failed to reach DMI targets by d 62 (failed-to-wean, n = 10), milk was reduced until weaning at d 70. 27 wean-by-intake calves met all 3 DMI targets (successful-intake) and 33 wean-by-combination calves met the 1 DMI target (successful-combination). Successful-intake and successful-combination calves had greater body weight (BW) at 84 d than wean-by-age calves, followed by failed-to-wean calves (123.0 vs 121.6 vs 117.0 vs 100.1 ± 3.1 kg, respectively). During weaning, successful-intake calves ate more starter than successful-combination, wean-by-age, and failed-to-wean calves (1.18 vs 0.85 vs 0.49 vs 0.14 ± 0.08 kg DM/d, respectively). Hay calves had greater BW at 84 d than TMR calves (124.0 vs 119.0 ± 1.6 kg, respectively). During weaning, Hay calves consumed more starter than TMR calves (0.85 vs 0.65 ± 0.09 kg DM/d, respectively). Intake-based weaning can improve performance of calves that successfully wean, and grass hay can improve starter intake and growth around weaning.

Some calves consume little solid feed before weaning; further research is needed to understand how these calves should be managed.

## Lay Summary

Dairy calves are typically separated from the dam at birth and are provided milk and solid feed by the farmer. In this study I compared feeding behavior and performance of dairy calves weaned based on age, solid feed intake, or a combination of age and solid feed intake, and fed either grass hay or a silage-based total mixed ration (TMR). Calves weaned based on solid feed intake and a combination of age and solid feed intake had greater body weights at 12 wk, consumed less milk, ate more solid feed, and visited the milk feeder more frequently. Calves fed grass hay consumed more solid feed and had greater body weight at 12 wk versus calves fed silage-based TMR.

# Preface

I completed this study with supervision from Dr. Danial Weary from the University of British Columbia. My supervisory committee additionally included Dr. Marina (Nina) von Keyserlingk and Dr. David Kitts from The University of British Columbia. The study presented in this thesis was approved by The University of British Columbia's Animal Care Committee (protocol # A19-0152). Animals were cared for according to the guidelines outlined by the Canadian Council of Animal Care (2009).

A version of Chapter 2 is being prepared for publication, co-authored by Welk, A., H. W. Neave, H.B. Spitzer, M. A. G. von Keyserlingk, and D. M. Weary. A. Welk designed the study in collaboration with the co-authors. A. Welk collected all data, conducted the data analysis, and wrote the manuscript. H. Neave helped with study design, data analysis and interpretation, and writing. H.B. Spitzer helped with data collection and writing. M. A. G. von Keyserlingk helped with study design, interpretation, and writing. D.M. Weary acted in the typical role of supervisor, helping with study design, data analysis, input, and editing of the manuscript drafts. The project received UBC Animal Care Approval (certificate number: A19-0152)

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## List of Abbreviations

ADG = average daily gain

ADF = acid detergent fibre

BW = body weight

CP = crude protein

cm = centimetres

d =day(s)

DM = dry matter

DMI = dry matter intake

g = grams

kg = kilograms(s)

lb = pounds

L = litre

m = metre

min = minutes

ME = metabolizable energy

mo = month(s)

NDF = neutral detergent fibre

SD = standard deviation

SE = standard error

Starter = grain-based solid feed diet for dairy calves

TMR = total mix ration

wk = week(s)

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# Chapter 1: Introduction

The management of dairy calves from birth to weaning and beyond can influence their short- and long-term performance and welfare (Khan et al., 2011a; Gelsinger et al., 2016; Imani et al., 2017). It is common practices to remove calves from the dam soon after birth (Urie et al., 2018; Winder et al., 2018). As a result, it becomes the responsibility of the farmer to care for these young animals. High morbidity and mortality during the calf rearing period remains a challenge for farmers. A 2014 U.S. national wide survey estimated that 5% of dairy calves died and 34% experienced a health disorder before weaning  (Urie et al., 2018). Similarly in Canada, milk-fed calf mortality rate of 6.4% was reported in a 2015 national wide survey (Winder et al., 2018). These figures indicate that there is still much room for improvement in calf rearing methods on dairy farms.

Calf feeding practices can play a major role in promoting calf health, growth, and positive feeding behaviours. Weaning, the transition from a milk-based diet to solid-feed based diet, is a critical time for calves, marking the change from a pseudo-monogastric to a functioning ruminant. Weaning is often described as stressful (Weary et al., 2008). Calves can experience behavioural signs of stress and hunger, weight loss, and increased risk of illness, especially when poor weaning practices are used such as abrupt milk removal at an early age. Adopting a weaning program that encourages solid feed intake before and during weaning is critical in maintaining growth and minimizing stress during this period (Khan et al., 2007a). How well calves cope with weaning will be influenced by many factors, such as milk allowance, weaning method, and the type of solid feed provided (Khan et al., 2011a; 2016).

In this chapter, I will summarize what is known about calf feeding practices and the effects on feeding behaviour and performance of dairy calves. I will first briefly outline the natural feeding behaviour of calves when reared by the dam in semi-natural environments. This will aid as a comparison to current farm practices and help to inform improvements in feeding management practices. As my research focuses on the weaning period, I will cover three main areas that influence a calf's ability to cope with weaning including milk feeding practices, weaning methods, and solid feed type. Finally, I will identify areas for improvements and gaps in literature.

## 1.1 Natural Feeding Behaviour of Calves

In natural systems, calves are reared by the dam. Calves will typically suckle from the dam approximately 8 to 12 times daily during the first few weeks of life, with feeding times lasting 8 to 10 min (see review by Whalin et al., 2021). Milk consumption increases quickly with age with calves having the ability to consume approximately 15 L/d (de Passillé et al., 2008; Borderas et al., 2009). As the calf matures, it becomes less dependent on milk. Calves begin grazing and ruminating at approximately 3 wk and around 5 mo calves spend approximately 5-6 hr grazing (Hutchison et al., 1962). As the calf begins to consume more solid feed, daily nursing declines with nursing frequency averaging 4 times daily at 1 mo and only once daily at 6 mo (Das et al., 2000). Around 8 to 11 mo of age the dam will typically cease nursing her calf (Reinhardt and Reinhardt, 1981). In this context especially, weaning is a gradual process, with the dam reducing her nursing frequency and milk output over the course of several months as the calf becomes nutritionally independent (reviewed by Whalin et al., 2021). The dam encourages this weaning process by rejecting some nursing attempts and showing increased aggression towards her calf (reviewed by Enríquez et al., 2011).

## 1.2 Milk Feeding

### 1.2.1 Conventional Milk Feeding Practices

On dairy farms calves are often fed relatively low milk allowances, approximately 10% of a calf's body weight (BW) (Khan et al., 2011a). For example, in the U.S. over 50% of dairy farmers fed 3.8 – 4.7 L/d of whole milk or milk replacer (USDA, 2016). In Canada, approximately 33% of dairy farmers feed calves ≤ 6 L/d (Winder et al., 2018). Calves are also typically fed 2 milk meals a day (USDA, 2016). These restricted milk-feeding programs were thought to encourage starter intake and thus accelerate weaning, as well as reduce the risk of calf diarrhea and other illness, and reduce the cost associated with feeding and managing milk-fed calves (reviewed by Kertz et al., 1979).

In addition to restricted milk allowances and reduced meal frequency, calves are often fed using an open bucket (Friend and Dellmeier, 1988). Bucket feeding requires calves to learn how to drink from a bucket and does not allow them to perform their natural sucking behaviour. This is thought to result in frustration, a high incidence of non-nutritive oral behaviour, and perhaps also poor health (Margerison et al., 2003). In contrast, feeding calves via a teat can promote natural nursing behaviours such as suckling, head butting, and, in combination with high milk allowances, increased feeding times while reducing non-nutritive oral behaviours (Appleby et al., 2001; Jensen and Budde, 2006). Despite the benefits of nipple feeding have been known for over 50 years (e.g., Wise and Lamaster, 1968; Alexander, 1954), most dairy farmers in the U.S. continue to use open bucket feeding (USDA, 2016). In Canada, 36% of farmers self-reported feeding milk exclusively using open bucket, and a further 11% use a mix of nipple and bucket feeding (Winder et al., 2018).

### 1.2.2 Re-thinking Milk Allowances

During the last two decades several studies have challenged the common practice of feeding restricted quantities of milk, in part due to the high rates of morbidity and mortality discussed above, but also in response to poor growth rates and chronic signs of hunger in pre-weaned calves. Not surprisingly, when calves are offered *ad-libitum* milk, they are able to consume similar amounts of milk compared to calves reared by the dam, reaching up to 12 L/d (Jasper and Weary, 2002). Calves reared by the dam or fed higher milk allowances (often 20% of the calf's BW: DFC-NFACC, 2009) consistently show greater preweaning ADG compared to calves fed more restricted quantities (Jasper and Weary, 2002; Miller-Cushon et al., 2013a; Rosenberger et al., 2017). Calves fed lower milk allowances may attempt to compensate with increased intake of calf starter but only when they are a minimum of 2-3 wk of age (Jasper and Weary, 2002). The inability to consume starter before this time is due to calves having non-functional rumens at this stage of life and are physically unable to utilize energy from solid feed (Drackley, 2008). Therefore, despite consuming twice as much solid feed, calves fed low milk allowances are unable to maintain similar nutrient intake and growth rates compared to calves fed higher quantities of milk before weaning (Jasper and Weary, 2002; Rosenberger et al., 2017). There is also evidence that the increased growth achieved by higher milk intakes during pre-weaning has long-term benefits on milk production and reproduction later in life (Raeth-Knight et al., 2009; Heinrichs and Heinrichs, 2011; Soberon et al., 2012)

In addition to increased growth, calves fed high milk allowances show reduced behavioural signs of chronic hunger and non-nutritive oral behaviours. Newborn calves vocalized less when fed 8 L/d of milk over 6 feedings compared to calves fed 5 L/d of milk over 2 feedings (Thomas et al., 2001). Calves fed *ad libitum* milk had 12-times fewer unrewarded visits at the milk feeder compared to calves fed 4 L/d of milk (De Paula Vieira et al., 2008). Increasing milk allowances also appears to increase play behaviour in calves; Krachun et al. (2010) found that the duration of locomotor play behaviour pre-weaning was greater in calves fed 12 L/d of milk compared to calves fed 6 L/d. When feeding calves using an automated milk feeder, feeding behaviour is also positively impacted by higher milk allowances with calves demonstrating higher meal frequency, longer feeding durations (Appleby et al., 2001; De Paula Vieira et al., 2008; Miller-Cushon et al., 2013a), and more evenly distributed diurnal feeding patterns (Miller-Cushon et al., 2013a). Overall, feeding higher milk allowances (≥ 8 L/d) improves growth and promotes natural feeding behaviours in dairy calves.

## 1.3 Weaning of Dairy Calves

### 1.3.1 The Transition from Milk to Solid Feed

One of the most important changes a dairy calf experiences during the calf rearing period is weaning when they transition from a milk-based diet onto solid feed (typically a combination of calf starter and forage). During the transition from milk to solid feed, the calf undergoes rapid structural, physiological, and microbiological transformations in the rumen and gastrointestinal tract that prepare the calf to become a functional ruminant (Khan et al., 2016). It is important for calves to be consuming adequate amounts of solid feed before weaning occurs as solid feed intake starts ruminal fermentation, triggering the physical and metabolic development of the rumen (Baldwin et al., 2004). Calves with poor solid feed intake before weaning show reduced rumen function, poor growth, and experience prolonged hunger around weaning (reviewed by Weary et al., 2008).

As described above, when the calf is reared by the dam weaning occurs over several months and often not complete until the calf is 8 - 11 mo of age (Reinhardt and Reinhardt, 1981); however, on most farms' calves are weaned 5 - 6 mo earlier and over shorter periods of time, often less than a week. For example, in the U.S., calves are typically weaned are around 9 wk (Urie et al., 2018). Medrano-Galarza et al. (2017) reported that Canadian farms using automated milk feeders had a medium weaning age of 7 wk (25th – 75th percentiles: 6 – 8 wk; n = 79 farms) while farms that fed milk manually had a medium weaning age of 8 wk (25th – 75th percentiles: 6 – 10 wk; n = 523 farms). All farms using automated milk feeders weaned calves gradually with a medium weaning duration of 13 d (25th – 75th percentiles: 9 – 17 d). About 15% of farms that fed milk manually weaned calves abruptly, while the remaining 85% implemented some sort of gradual weaning method with medium weaning duration of 7 d (25th – 75th percentiles: 4 – 14 d). Although reported weaning ages have remained relatively consistent over the past three decades (Waltner-Toews et al., 1986; Spicer et al., 1994; Urie et al., 2018), gradual weaning over longer periods has become more common (Otterby and Linn, 1981; Pettersson et al., 2001), likely owing to increases in milk allowances offered to calves.

### 1.3.2 Gradual Weaning Methods

One risk associated with feeding high milk allowances is reduced calf performance around weaning. Feeding high milk allowances delays solid feed intake compared to calves fed low milk allowances (Appleby et al., 2001; Raeth-Knight et al., 2009). Low solid feed intake before weaning can be problematic, particularly when abrupt weaning (milk reduction in < 7 d) is used, as the calf's rumen is underdeveloped and unprepared for rapid solid feed intake after weaning (Baldwin et al., 2004). As a result, calves fed higher quantities of milk and then weaned abruptly experience reduced weight gain, in some cases weight loss, and increased signs of hunger (van Niekerk et al., 2021; Steele et al., 2017; Sweeny et al., 2010). One method to encourage early solid feed intake in these calves is to gradually wean calves off milk (reducing daily milk volumes incrementally overall several weeks). By reducing milk gradually over several weeks, calves can increase solid feed consumption before complete milk removal, allowing time for the rumen to mature. One of the first published examples of a gradual weaning method is that described by Khan et al. (2007a,b), reducing milk allowance by 50% around 4 wk of age and again around 7 wk to complete weaning at 8 wk of age. Work investigating gradual weaning from high milk allowances has found that this method allows for greater solid feed intake, increased growth, and fewer signs of hunger compared to abrupt weaning (Sweeny et al., 2010; de Passillé et al., 2010). Gradual weaning has been found to reduce both the stress and severity of weight loss compared to abrupt weaning methods. (Khan et al., 2007b; Sweeney et al., 2010; Steele et al., 2017).

Past work has primarily focused on comparing gradual with abrupt weaning programs with only a few studies comparing different gradual weaning methods. For example, studies have compared ‘step-down’ (consisting of 2 or 3 substantial drops in milk ration) and ‘linear’ (consisting of much smaller but more frequent drops in milk) weaning methods but effects on the calf appear to be minor (Welboren et al., 2019; Parsons et al., 2020). Differences in gradual weaning methods are more likely related to duration and age of weaning. For example, calves showed a decrease in digestible energy and growth, as well as high rates of cross-sucking, when gradually weaned off of 12 L/d of milk at 6 weeks of age (de Passillé et al., 2010), but not when weaned at 12 to 13 wk (de Passillé et al., 2011; de Passillé and Rushen, 2016). Additionally, recent research has found that later weaning ages can positively impact rumen development and lead to a more gradual shift in rumen microbial diversity (Eckert et al., 2015; Meale et al., 2017). Overall, recent research suggest that the older high milk-fed calves are weaned, the better they transition onto solid feed (Eckert et al., 2015; de Passillé and Rushen, 2016; Schwarzkopf et al., 2019). However, majority of studies evaluating weaning methods wean calves between 6 to 8 wk (e.g., Eckert et al., 2015; Welboren et al., 2019; Parsons et al., 2020). More research is needed to understand the impact of different weaning methods when calves are weaned at later ages (> 9 wk).

Another key component to gradual weaning methods is the duration of the milk reduction. There seems to be a general agreement that a milk reduction over a period less than 7 days is considered abrupt (Khan et al., 2007b; Bennetton et al., 2019); however, there is little agreement on the optimum weaning duration. Sweeny et al. (2010) concluded that 10 d was ideal for weaning calves off 12 L/d of whole milk, while Hill et al. (2012) concluded that 14 to 21 d was needed to gradually to wean calves off 1 kg DM/d (approximately 8 L/d) of milk replacer. However, several studies have reported growth slumps during weaning when even a 14-d milk reduction period was implemented (Steele et al 2017; Welboren et al., 2019; Parsons et al., 2020). de Passillé and Rushen (2012) found that weight gain was positively correlated with weaning duration, and that calves with a weaning duration of 23 d compared to 7.5, 10.5, and 14 d had greater growth rates during weaning. Together these results suggest that a weaning duration of 3 wk maybe more appropriate. However, weaning duration will likely be influenced by weaning age and milk allowance. Future research is needed to fully understand the best combination of weaning duration and age, and the effects on growth, feeding behaviour, and weaning distress.

### 1.3.3 Intake-based Weaning

Age is the most common criterion used when deciding to wean calves off milk (USDA, 2016; Medrano-Galarza et al., 2017). However, in semi-natural rearing systems calves wean at variable ages (Reinhardt and Reinhardt, 1981). In intensive rearing systems, large individual variability in when calves begin to consume solid feed is often observed, despite calves being reared under similar conditions. For example, de Passillé and Rushen (2012) found that calves fed 12 L/d of milk differed greatly in when they first consumed 0.2 kg/d or 0.4 kg/d of starter, reporting a range of 22 to 74 d of age. Similarly, Neave et al. (2018) reported a range of 8 to 41 d of age for calves to first consume 40 g of starter (calves fed a range of milk allowances between 6 – 12 L/d). These findings suggest that weaning by age, even with the use of a gradual weaning method, may result in some calves with very low solid feed intakes at the start of weaning.

Weaning based on individual solid feed intake has the advantage of ensuring that each calf consumes minimum levels of solid feed before milk allowance is reduced, thus increasing the likelihood that calves are nutritionally ready for the transition. Dairy farmers have shown some interest in intake-based weaning. In a 2014 USDA survey, 21.5% of U.S. dairy farmers weaned calves based on starter intake (USDA, 2016). In Canada, Medrano-Galarza et al. (2017) found that 50% of farms using manual feeding systems and 16% of farms using automated feeding systems took starter intake into consideration when weaning calves.

Despite interest in intake-based weaning, majority of work investigating weaning methods have used a fixed age as the weaning criterion (i.e., Steele et al 2017; Welboren et al., 2019; Parsons et al., 2020); only a handful of studies have investigated weaning on the basis of individual intake (i.e., Whalin et al., 2022; Bennetton et al., 2019; de Passillé and Rushen, 2012; 2016). The results of these studies suggest that there are benefits to intake-based methods, including higher and more consistent starter intake (Whalin et al., 2022, Benetton et al., 2019; de Passillé and Rushen, 2016), greater ADG during the weaning period (Whalin et al., 2022), with no detriment in postweaning weights for calves that successfully wean by intake versus calves weaned by age (Bennetton et al., 2019; de Passillé and Rushen, 2016). In general, intake-based weaning methods often use 3 to 4 starter intake targets, each resulting in a milk reduction (either based on milk allowances or individual milk consumption) once met (Whalin et al., 2022; Benetton et al., 2019; de Passillé and Rushen, 2012; de Passillé and Rushen, 2016). Starter intake targets of ~ 200 g/d and ~ 1300 g/d have been used to initiate and complete weaning, respectively (Whalin et al., 2022; Benetton et al., 2019; de Passillé and Rushen, 2016). These targets were chosen as they 1) corresponded with the Bovine Alliance on Management and Nutrition (2017) recommendation on ideal starter intakes around weaning (~ 1,300 g/d at weaning) and 2) encourage the most ideal weaning ages and durations (de Passillé and Rushen, 2012). Table 1.1 provides further detail on intake-based weaning methods explored over the past two decades.

Despite the promise of intake-based weaning, there are some weaknesses to methods explored in the studies summarized in Table 1.1. First, milk was abruptly reduced when a step-down method was used (Whalin et al., 2022; Benetton et al., 2019; de Passillé and Rushen, 2012; de Passillé and Rushen, 2016). Additionally, calves could wean themselves within 7 d or less (Benetton et al., 2019; de Passillé and Rushen, 2012; de Passillé and Rushen, 2016). These methods could be considered abrupt and may explain why calves weaned by intake often show a higher frequency of unrewarded visits to the milk feeder compared to calves weaned by age (Benetton et al., 2019; de Passillé and Rushen, 2016). Unrewarded visits are considered a sign of hunger or frustration so this result suggests that calves weaned by intake experience greater hunger during weaning compared to calves weaned by age. Second, weaning at a desirable age is still at the forefront of most studies. Benetton et al. (2019) implemented an initial 25% milk reduction at d 30 of age to encourage starer intake before applying intake targets. Applying an initial milk reduction encourages calves to meet intake targets at an earlier age and progress through weaning at a faster pace. Several studies have also implemented a fixed age at which calves must meet intake targets; if targets are not met by this age calves are still weaned so to ensure that all calves are weaned by a predetermined age (Benetton et al., 2019; de Passillé and Rushen, 2012). Calves that fail to meet intake targets by this age typically gain less weight around weaning relative to calves that met these targets (Benetton et al., 2019; de Passillé and Rushen, 2012). Further research is needed to develop intake-based weaning methods that minimize the negative effects of weaning and to better assess individual calf performance using these methods.

**Table 1.1. Summary of intake-based weaning methods utilized in the literature.**

| Reference | Milk allowance | Weaning method | Failed-Intake | Weaning age |
|---|---|---|---|---|
| **Whalin et al., 2022** | 12 L/d | **Age:** Initial milk reduction by 25% of individual average milk intake at 30 d. Final milk reduction from 42 – 56 d (0.5 ± 0.1 L/d)<br>**Intake:** Initial milk reduction by 25% of individual average milk intake at 30 d. Further 25% milk reductions at each starter intake target (225, 675, 1300 g/d)<br>**Failed-Intake**: Intake calves that failed to meet 225 g/d target by 42 d. Calves experienced another forced 25% milk reduction at 42 d but met the final two targets. | 43% of intake calves (6 out of 14) | **Age:** 56 d<br>**Intake:** 59.6 ± 8.5 d (Range: 46 – 75 d)<br>**Failed:** 73.8 ± 8.0 d (Range: 63 – 84 d) |
| **Bennetton et al. 2019**[1] | 12 L/d | **Age:** Milk reduced to 6 L from 30 – 35 d (1.2 L/d). Final milk reduction from 63 – 70 d (0.86 L/d). | Exp 1: 37.5 % of intake calves (6 out of 16) | **Age:** 70 d<br>**Intake Exp 1**: 52 ± 6.1 d (Range: 40 – 62 d) |

|  |  |  |  |  |
|---|---|---|---|---|
|  |  | **Intake (Exp 1 & Exp 2):** Initial milk reduction by 25% of individual average milk intake at 30 d. Further 25% milk reductions at each starter intake target (225, 675, 1300 g/d)<br>**Failed-Intake:** Intake calves that failed to meet any intake targets by 63 d (Exp 1) or 84 d (Exp2) | Exp 2: 6.5% of calves (3 out of 46) | **Intake Exp 2:** 59.1 ± 9.6 (Range 44 – 84)<br>**Failed Intake:** 70 d (Exp 1) and 91 d (Exp 2) |
| **de Passillé and Rushen, 2016** | 12 L/d | **Early Wean:** Milk gradually reduced from 40 – 48 d (1.5 L/d)<br>**Late Wean:** Milk gradually reduced from 80 – 88 d (1.5 L/d)<br>**Intake:** Milk allowance reduced by 3 L at each starter intake target (200, 600, 1000, 1400 g/d) | N/A | **Early Wean:** 48 d<br>**Late Wean:** 88 d<br>**Intake:** 75.8 ± 10.7 d (range: 58 – 94 d) |
| **de Passillé and Rushen, 2012** | 12 L/d | **Low start, Low end:** Milk allowance reduced by 3 L at each starter intake target (200, 400, 600, 800 g/d)<br>**Low start, High end:** Milk allowance reduced by 3 L at each starter intake target (200, 665, 1135, 1600 g/d)<br>**High start, Low end:** Milk allowance reduced by 3 L at each starter intake target (400, 535, 665, 800 g/d)<br>**High start, High end:** Milk allowance reduced by 3 L at each starter intake target (400, 800, 1200, 1600 g/d)<br>**Failed-Intake:** Calves that failed to meet the any intake target by 74 d | 20% of all calves (11 out of 60) | **Low start, Low end:** 61.1 ± 3.2 d<br>**Low start, High end:** 64.4 ± 2.7 d<br>**High start, Low end:** 58.1 ± 3.2 d<br>**High start, High end:** 69.0 ± 3.1 d<br>**Failed-Intake:** 81 d |
| **Roth et al., 2009**[2] | 6 L/d | **Age:** Milk gradually reduced from 56 – 84 d (0.2 L/d)<br>**Intake:** Weaning started and completed at starter intake targets of 700 and 2000 g/d, respectively. | N/A | **Age:** 84 d<br>**Intake:** 76 d (ranged 45 – 98 d) |

[1] Two experiments were conducted. Exp 1 included wean by age and wean by intake treatments, allowing wean by intake calves until 63 d to meet intake targets. Exp 2 included only wean by intake treatment, allowing calves until 84 d to meet intake targets.

[2] Milk allowance was adjusted daily based on average starter intake over the pervious 4 d and was rounded up to the nearest half or full litre. Milk and starter had a linear relationship.

## 1.4 Solid Feed

### 1.4.1 Rumen Development and Grain-Based Calf Starters

Calves are born with underdeveloped, non-functional rumens, so during the first few weeks of life they rely almost exclusively on milk which is digested in the abomasum (Drackley, 2008). During weaning, calves undergo major physiological changes in their digestive system to shift the location of primary digestion from the abomasum to the rumen (Baldwin et al., 2004). The development of the rumen is initiated by the onset of solid feed and water consumption. As the calf consumes solid feed, the microbial population in the rumen ferments carbohydrates into volatile fatty acids (VFAs) (Dijkstra, 1994). The presence of VFAs stimulates epithelial cell proliferation developing into papillae, figure like projections present on the surface of the rumen responsible for the absorption of digestion end products (Baldwin et al., 2004). The stimulatory effects of VFAs are not equal; butyrate, followed by propionate, provide the greatest stimulation for rumen development while acetate provides little stimulation (Sander et al., 1959). As solid feed intake increases, VFA concentrations increase, and rumen development ensues with total rumen volume increasing and muscle layers thickening (Baldwin et al., 2004). Although rumen fermentation can start at a very young age (~ 2 wks), it takes time for the rumen to develop which is needed for calves to utilize nutrients from solid feed (Baldwin et al., 2004). For instance, it can take papillae 4 wk to grow from 1 mm to 3 mm in length (van Niekerk et al., 2021). Overall, the development of the rumen is a slow process and continues beyond the weaning phase. Thus, weaning methods should be gradual to reflect this process.

Grain-based calf starters were developed to help calves achieve high solid feed intakes at an early age. Calf starters are highly palatable feeds consisting of easily fermentable carbohydrates that maximize VFAs production promoting fast papillae growth and rumen development (Drackley, 2008; Khan et al., 2016). Calf starters are designed to favor the production of butyrate and propionate over the expense of acetate (Khan et al., 2016). Currently, it is recommended to feed calf starters *ad libitum* from the first week of life to 3 mo of age (i.e., after calves are typically weaned) before incorporating forage into the diet (BAMN, 2017). Due to studies demonstrating that forage can decrease calf starter intake, slow digestibility, and increased acetate production (e.g., Hill et al., 2008; 2010), feeding forage preweaning is often discouraged (BAMN, 2017). However, only feeding calf starter can compromise rumen development and health. Providing only starter can over-stimulate the rumen papillae which may cause keratinization or parakeratosis and reduce absorption of VFAs (Khan et al., 2016). Feeding only starter can also reduce ruminal pH leading to ruminal acidosis, which can impair papilla development and increase inflammation (van Niekerk et al., 2021). As such, calves fed high grain-based diets have been found performing non-nutritive oral behaviours such as tongue rolling (Webb et al., 2015), leading to concern for their welfare.

### 1.4.2 Providing Forage to Pre-Weaned Calves

Over the past decade there has been a renewed interest in providing pre-weaned calves with forage (Imani et al., 2017). From a behavioural standpoint, calves consistently select a proportion of hay in their diet and are often observed sorting within mixed rations for long forage fragments (Miller-Cushon et al., 2013b; Costa et al., 2016), suggesting that calves are motivated to consume forage. This is unsurprising given that cattle naturally utilize forages and that calves consume forage when available. The addition of forage in a pre-weaned calf diet has shown to either have no effect or a positive effect on growth and feed intakes (Imani et al., 2017). Several studies have reported increased starter intake and total dry matter intake (DMI) in calves fed starter *and* forage compared to calves only fed starter (i.e., Castells et al., 2012; Horvath and Miller-Cushon, 2019; Khan et al., 2011a). As a result of increased solid feed intake, improvements in ADG have been reported during weaning and post-weaning (Castells et al., 2012; Horvath and Miller-Cushon, 2019). Improved feed intake and growth in calves feed supplemental forage is likely due to improved rumen development and stabilization of the rumen environment. Calves offered supplemental forage have been found to have greater rumen muscle thickness compared to calves only fed starter (Castells et al., 2013; Pazoki et al., 2017). Through increased rumination and salivation, the inclusion of supplemental forage has also been shown to increase rumen pH in both pre-weaned and post-weaned calves (Laarman et al., 2011; Khan et al., 2011b). The presence of forage particles in the rumen stimulates rumen mobility preventing rumen abnormalities such as plaque formation (Suárez et al., 2007; Pazoki et al., 2017). Overall, inclusion of supplemental forage in a pre-weaned calf diet can improve feed intake, growth, and rumen development and prepares the calf for efficient forage digestion after weaning (Khan et al., 2016).

Evidence also suggests that providing supplemental forage to pre-weaned calves can promote positive feeding behaviour and limit non-nutritive oral behaviour. Providing supplemented forage can reduce pen-directed sucking (Horvath and Miller-Cushon, 2017, 2019) and, in individual housed veal calves, tongue rolling (Webb et al., 2015). Supplemental forage has also been shown to increase lying and feeding time during and after weaning (Bagheri et al., 2021; Webb et al., 2015). Interestingly, Horvath and Miller-Cushon (2019) found that calves provided free choice starter and grass hay made fewer unrewarded visits to the milk feeder and spent more time self-grooming during a 10-d weaning period compared to calves only provided starter, suggesting that calves with access to forage during weaning were less hungry and dedicated more time for self maintenance. Overall, these results indicated that forage is important for the development of feeding behaviour and may provided a smoother transition onto solid feed.

Although there is considerable evidence showing the benefits of supplemental forage, discrepancies still arise in the literature (Hill et al., 2019). Inconsistent results are likely due to factors related to the presentation and type of solid feed provided, including physical characteristics of starter and forage, presentation method, and the starter-to-forage ratio. Calves fed ground or pelleted starter compared to texturized starter seem to benefit more from forage supplementation (reviewed by Imani et al., 2017). Although forage particle size has little effect on starter intake and performance (Bagheri et al., 2021; Omidi-Mirzaei et al., 2018), calves have a strong preference for long particle lengths over short particle lengths (Webb et al., 2014a). Additionally, there is evidence that long particle lengths have a positive effect on behaviour; calves fed long forage particle sizes spend more time ruminating and eating forage, and spend less time performing non-nutritive oral behaviours (Omidi-Mirzaei et al., 2018). The starter-to-forage ratio in mixed rations seems to affect feed intake and performance of calves. Hill et al. (2008) found that calves fed higher portions of forage in a mixed ration experienced lower ADG and solid feed intake compared to calves offered lower portions of forage. Presenting starter and forage as mix ration may be problematic as it tailors the diet to the average calf and disregards individual variation in forage consumption seen in pre-weaned calves (Webb et al., 2014b). Offering calves forage and starter separately as a free choice allows individual calves to choose the portion of feed they desire, allowing them to gain the most benefit from each feed source. In a meta-regression analysis, Imani et al. (2017) found that higher levels of forage (> 10% in DM) improved starter intake and ADG with this response being greater in calves offered starter and forage as a free choice rather than as a mixed ration.

In addition, milk allowance, weaning method, and housing type could all influence the effects of supplemental forage. Phillips (2004) found that calves housed in groups consumed more forage and spent more time at the forage feeder then individually housed calves. Studies feeding high milk allowances (> 8 L/d) and weaning at later ages (> 8 wks) consistently find that calves are able to consume large amounts forage (15% - 30% of solid feed intake) while maintaining high starter intakes and growth rates (Khan et al., 2011b; Miller-Cushon et al., 2013b; Horvath and Miller-Cushon, 2019). In more recent work, Terler et al. (2022) found that calves fed high quality hay as their only source of solid feed had similar DMI, ADG, and postweaning weights as calves fed 70:30 mix of starter and forage. Within this study, calves were provided *ad libitum* milk for the first weeks of life and weaned at 12 wks of age, demonstrating that high-quality hay can substitute grain-based starter in calves fed high milk rations and weaned at older ages without adverse effects on performance. Currently, the effect of different types of management practices are not well understood, making interpretation of results difficult. Further investigation is needed to disentangle the effects of various management practices on forage consumption and subsequent effects in dairy calves.

### 1.4.3 Forage Type

To date the majority of research investigating supplemental forage for pre-weaned calves have focused on the comparison between calves only fed starter versus calves fed starter and forage (i.e., Khan et al., 2011b; Terré et al., 2013). Only a handful of studies have compared different types of forages, primarily focusing on dry forages such as alfalfa hay, grass hay, ryegrass hay, oat hay, straw, barley straw, etc. (i.e., Suárez et al., 2007; Castells et al., 2012; Omidi-Mirzaei et al., 2018). Overall, studies indicate that higher nutritive forages, such as high-quality grass hay or alfalfa hay, are more willingly consumed by calves compared to lower nutritive forages, such as low-quality hay or straw (Castells et al., 2012; Omidi-Mirzaei et al., 2018). In addition, Webb et al., (2014a) found that calves showed a strong preference for high-quality hay over straw. Although calves consume larger quantities of high nutritive forages, benefits on behaviour and performance are mixed. Castells et al. (2012) found that alfalfa hay reduced starter intake, total DMI, and ADG compared to barley straw in calves fed pelleted starter. In contrast, Omidi-Mirzaei et al. (2018) found that alfalfa hay improved starter intake, total DMI, and feed efficiency with no difference in growth compared to straw in calves fed a texturized starter. Results on high-quality hay seem less mixed. Castells et al. (2012) found that oat hay provided the most benefits to starter intake, total DMI, and growth while Terler et al. (2022) found that calves fed a high-quality grass hay had greater ADG postweaning and showed less sorting for concentrate compared to calves fed a medium-quality grass hay. Although straw has less of an effect on feed intakes and growth compared to other dry forage types, improvements in rumination and reductions in non-nutritive oral behaviour have been found (Castells et al., 2012; Omidi-Mirzaei et al., 2018).

One area of recent interest is incorporating silages, such as corn silage, haylage, or fermented alfalfa, into the pre-weaned calf diet. The fermented nature of silages may enhance digestibility and butyrate access leading to improved rumen development. Silages may also be more practical for dairy farmers to utilize as silages are commonly found on dairy farms and may come at a lower cost compared to dry forages, depending on a farm's geographical location (Kehoe et al., 2021). Most studies investigating silages have focused on corn silage (Overvest et al., 2016; Mirzaei et al., 2017; Kehoe et al., 2019) with some studies investigating alfalfa silage (Khan et al., 2020) and triticale silage (Castells et al., 2012, 2013). Similar to dry forages, research shows that the addition of silage to a calf diet either has no effect or positive effects on growth and performance when compared to calves only fed starter (Castells et al., 2012; Mirzaei et al., 2017). When compared to different types of dry forges, the benefits of silages are less clear. Castells et al. (2012) found that triticale silage increased total DMI and ADG compared to calves fed alfalfa hay but not compared to barley straw, rye-grass hay, or oat hay. Similarly, Mirzaei et al. (2017) reported increased DMI when starter was mixed with corn silage compared to alfalfa hay. It is noted that starter should be fed along side a silage. Studies providing fermented feeds as the only source of solid feed found that calves had lower DMI, reduced weight around weaning, and indications of poor rumen development compared to calves only fed starter (Khan et al., 2020) or calves fed starter and grass hay (Overvest et al., 2016). To date only handful of studies have compared silages to other types of forages (Castells et al., 2012; Overvest et al., 2016; Mirzaei et al., 2017). Further research is needed fully understand the effects of feeding different types of silages on calf feeding behaviour and performance around weaning, and the practical implementation of providing silage to calves on farm.

### 1.5 Aims of the Thesis

Although much progress has been made over the past two decades on pre-weaned dairy calf nutrition and management, weaning continues to be challenging for calves and dairy farmers. Encouraging solid feed intake before, during, and after weaning is critical to weaning success. Weaning method and solid feed type can influence feeding behaviour and performance of calves around weaning. In this chapter, I identified that intake-based weaning is a promising method that allows calves to move through weaning at their own pace. However, methods investigated thus far could be considered abrupt and have led to signs of increased hunger and distress compared to age-based weaning methods. Additionally, providing supplemental forage in the form of a silage may be more practical for dairy farmers and offer similar performance as dry forage, but further work is needed to understand how silage influences feeding behaviour and performance in dairy calves around weaning. Thus, the overall objective of this thesis was to investigate intake-based weaning methods and forage type on dairy calf feeding behaviour and performance around weaning.

# Chapter 2: The effect of intake-based weaning and forage type on feeding behaviour and growth of dairy calves fed by automated feeders

## 2.1 Introduction

Dairy calves are often fed restricted allowances of milk (approximately 4 – 6 L/d) (Medrano-Galarza et al., 2017; USDA, 2016). Providing calves higher allowances of milk (e.g., 10 – 12 L/d) reduces signs of hunger, increases growth (e.g., Jensen and Holm, 2003; Khan et al., 2007b; Miller-Cushon et al., 2013a), and results in increased productivity later in life (Heinrichs and Heinrichs, 2011; Soberon et al., 2012). However, calves fed high allowances of milk typically have low solid feed intake before weaning (Rosenberger et al., 2017), increasing hunger and slowing weight gains when milk is abruptly removed (de Passillé et al., 2011; Dennis et al., 2018).

Gradual weaning can improve starter intake before milk is completely withdrawn. Step-down (e.g., Khan et al., 2007b) and linear (e.g., Sweeney et al., 2010) reductions in milk allowances have been investigated, with the majority of studies have used age as the criterion for when milk allowances are reduced. However, there is considerable variability in when calves begin to consume solid feed (Heinrichs and Heinrichs, 2011; Neave et al., 2018), and some calves may not consume much starter before they reach a certain age (de Passillé and Rushen, 2012). Another approach is to wean calves is when they meet a starter intake target, with the idea that calves are then nutritionally ready to transition to solid feed (Roth et al., 2009; de Passillé and Rushen, 2012, 2016). This method can also allow for the reallocation of milk from calves that wean early to calves that need more time to transition onto solid feed (Benetton et al., 2019).

Weaning by starter intake can result in intermediate weaning ages (de Passillé and Rushen, 2012), reduced milk intake, increased starter intake, and similar postweaning weights compared to calves gradually weaned at a set age (Benetton et al., 2019).

One disadvantage of weaning based on starter intake is that calves show a higher number of unrewarded visits to the milk feeder during the weaning period (10 – 15 times/d versus just 5 times/d for calves weaned by age: de Passillé and Rushen, 2012; Benetton et al., 2019). A high number of unrewarded visits is considered a sign of hunger (De Paula Vieira et al., 2008; Nielsen et al., 2008), specifically a sign of hunger for milk rather than low energy intake (de Passillé and Rushen, 2012). A drawback of previous studies is that when calves reached specific targets for starter intake, milk allowances were reduced abruptly (de Passillé and Rushen, 2012; Benetton et al., 2019). Additionally, if calves responded to reduced milk allowance by immediately increasing their starter intake, this would trigger a further abrupt reduction in milk allowance such that some calves were fully weaned within one week. Previous studies have also reported that approximately 18% of calves fail to meet starter intake targets by a predetermined age (de Passillé and Rushen, 2012; Benetton et al., 2019; Whalin et al., 2022). Thus, further refinements to weaning programs based on individual solid feed intake are needed, perhaps by reducing the milk more gradually once a solid feed intake target is reached.

Forage provision is another important consideration in early calf feeding programs. Recent work has shown that providing forage to calves fed high milk allowances before weaning improves overall solid feed intake, rumen development, and increases rumen pH (Khan et al., 2011a; Laarman et al., 2012). One area of recent interest is incorporating silages, such as a lactating cow TMR, into a pre-weaned calf diet (Overvest et al., 2016; Kehoe et al., 2019; Mirzaei et al., 2017). Silages may provide a palatable forage source that encourages solid feed intake and rumen development (Kehoe et al., 2021). In addition, offering a lactating cow TMR could also act as an alternate source of grain intake, aiding calves that struggle to transition onto calf starters. Studies that offered different types of forage to calves typically focused on calf performance after weaning with little attention to the weaning period. Currently, there is little work exploring behaviour and performance of dairy calves fed dry forages versus silages around weaning.

The use of automated calf feeders and solid feed intake as a criterion for weaning has increased in North America (Medrano-Galarza et al., 2017; USDA, 2016). Despite the increase in use of solid feed intake as a weaning criterion, little is understood about the best practices for weaning calves this way. The first objective of this study was to evaluate feed intake, feeding behavior, and growth of calves weaned by age, individual DMI, and a combination of individual DMI and age. We tested an age-based weaning method as this is still the most common criterion to wean calves (USDA, 2016). We tested an intake-based method similar to Benetton et al. (2019) but added a gradual reduction in milk allowances over a 3-d period after calves met intake targets to eliminate abrupt drops in milk. Finally, we tested a combination of the intake and age involving small incremental reductions in milk over a longer period, but only after meeting an initial DMI target; we expected this method to reduce the large number of unrewarded visits when calves are weaned based on intake alone. Overall, we expected intake-based weaning methods to encourage greater solid feed intake during weaning, resulting in greater solid feed intake after weaning and overall growth. We also anticipated a proportion of calves assigned to the intake-based weaning methods would fail to meet intake targets based on previous work (see de Passillé and Rushen, 2012; Benetton et al., 2019; Whalin et al., 2022).

Our second objective was to explore the effect of providing forage as either a grass hay or a silage based TMR on feeding behavior and growth of calves on different weaning programs. We expected calves fed the silage based TMR would have greater forage consumption, resulting in greater total DMI and improved growth compared to calves fed a grass hay.

## 2.2 Materials and Methods

This experiment was conducted from August 2019 to September 2020 at the UBC Dairy Education and Research Centre in Agassiz, BC, Canada. The animals were cared for according to the guidelines of the Canadian Council of Animal Care (2009), with animal use approved by the UBC Animal Care Committee (protocol # A19-0152).

### 2.2.1 Animals and Housing Management

#### 2.2.1.1 Calf Rearing Period

This experiment used 108 Holstein calves (n = 86 females; n = 22 males) that had an average birth weight of 41.2 ± 5.5 kg (mean ± SD). After separation from the dam (within 6 h after birth), calves were placed in an individual pen bedded with sawdust and bottle-fed 4 L of colostrum (>50 g/L of IgG). If less than 3 L of colostrum was consumed, calves were tube-fed the remaining amount (n = 32). On average calves consumed 3.9 ± 0.3 L (mean ± SD) of colostrum. A blood sample was collected from the jugular vein at 24 h to 48 h after the first feeding of colostrum and the serum was analyzed using a Reichert AR 200 Digital Handheld Refractometer (Reichert Technologies, NY, USA). All calves had serum total protein levels > 5.2 g/dL (as recommended for passive transfer of immunity; Windeyer et al., 2014). While individually housed, calves were bottle fed 8 L/d of whole milk divided into 2 feedings/d. At d 2 of age calves were moved into a sawdust-bedded group pen. Pens were filled as calves were enrolled in the study until 108 calves (12 groups, 9 calves per group) were enrolled. Calves remained in the group pen until the youngest calf of the group completed the calf rearing period (d 84 of age) at which point they were moved to the grower facility (located in a separate barn approximately 30 m from the calf rearing facility).

#### 2.2.1.2 Grower Rearing Period

Eight of the original 12 groups (consisting of *n* = 58 females and *n* = 10 males) were maintained (as a group of 9 animals) and followed until the youngest calf in each group reached d 140 of age. This subset of animal consisted of the last 8 of 12 groups. For the first 4 wk in the grower facility calves were housed in sawdust-bedded group pen (4.7 x 11.8 m) which included 3 automated feeders (RIC; Insentec B.V., Marknesse, FL, Netherlands) and a water trough. For the second 4-wk period calves were housed in a free stall pen (62 $m^2$) consisting of 13 stalls (2.0 x 0.9 m) with deep sand bedding, a feeding alley with 15 self-lock head gates (35 cm, center to center), and a water trough.

### 2.2.2 Pre-weaning and Post-weaning Diets and Forage Treatments

For the first 30 d of age, calves had access to a maximum of 12 L of pasteurized whole milk per day, fed at 40 °C using automated feeders (CF 1000 CS Combi; Delaval Inc., Tumba, SDR, Sweden). Each pen had 1 milk feeder equipped with 1 teat and barriers that restricted access to a single calf. Milk allowances accumulated at a rate of 5% of the daily allowance every hour from midnight to 2000 h, and the milk feeder delivered a minimum amount of 0.5 L and a maximum amount of 9.5 L of milk per visit. Calves were able to split their accumulated milk allowance into as many visits as desired but needed to have the minimum amount of milk available to receive milk. Daily milk intake and the number of unrewarded visits (when calves entered the feeder but were not allowed to drink milk) were automatically recorded by the milk feeder, using RFID ear tags to individually identify calves. After weaning, unrewarded visits to the milk feeder continued to be recorded whenever calves entered the milk feeder and attempted to drink milk. Adjacent to the milk feeder was a starter feeder equipped with a race to restrict access to a single calf. Starter was dispensed in 20 g increments while the calf's head was in the feeder. Daily starter intake was also recorded by the CF 1000 feeder. All calves had *ad libitum* access to a 20% CP texturized calf starter with the main ingredients being soybean meal, flake corn, flake barley, wheat, molasses, and canola meal (Richie Smith Feeds, Inc. Abbotsford, BC, Canada).

Group pens were alternately assigned to 1 of 2 forage treatments (Figure 2.2): chopped grass hay (Hay) or a silage-based lactating cow TMR (TMR) (n = 6 groups per treatment). The hay was a local blend of tall fescue, orchard grass, and ryegrass. The TMR consisted of 32% corn silage, 14% grass silage, 6% alfalfa hay, 2% straw, and 46% concentrate mix consisting primarily of canola, soybean, and corn meal. Hay, TMR, and water were offered *ad libitum* from automated Insentec feeders (RIC; Insentec B.V., Marknesse, FL, Netherlands). For each visit to the feeder, calf number was recorded using RFID tags, and the initial and final time of visit and weight of forage were used to calculate visit duration and forage intake. Barriers were located on each side of the feeders to restrict access to a single calf. Forage was replaced twice daily at 0900 and 1800 h. As forage was fed at the group level, waste and sorting of feed was not recorded. These forage diets were fed throughout the calf rearing period and maintained during the first two weeks after moving to the grower rearing facility. All calves, regardless of forage treatment, were then changed to the same heifer TMR consisting of 26% alfalfa hay, 21% local grass hay, 20% grass silage, 15% straw, 17% heifer grower pellet, and 0.5% mineral mix. The heifer TMR was top dressed with approximately 1.5 kg DM/animal of a 18% CP texturized calf starter (Richie Smith Feeds, Inc. Abbotsford, BC, Canada). This diet was fed until completion of the study at d 140 of age.

### 2.2.3 Weaning Treatments

When first moved to the group pen, calves were pseudo-randomly allocated by the researchers to weaning treatments (wean-by-age, wean-by-intake, or wean-by-combination), such that one calf was allocated to each treatment in each consecutive block of 3 calves. The first calf in each block was randomly allocated to treatment using a table of random numbers, and the second calf was randomly allocated in the same way but only within the two remaining treatments available. Each group contained a total of 9 calves (3 blocks, with 3 calves of each treatment). As subsequent blocks were enrolled, we scrutinized birth weight and sex to ensure that these were similar across treatments.

For calves to be eligible to begin weaning at d 31, they had to consume a minimum average milk intake of 6 L/d over the previous 5 d. If calves did not meet this criterion, they remained in the group pen but were excluded from the experiment. This criterion was applied to all weaning treatments. All calves, regardless of treatment, experienced an initial milk reduction at d 31, following previous work employing gradual step-down weaning methods (Benetton et al., 2019). Milk allowance was gradually reduced by 25% of each individual's average milk intake over d 26 to d 30. This reduction took place over 3 d (i.e., milk was reduced by approx.

8%/d) such that on d 33 calves were at 75% of their previous milk intake. After this initial milk reduction, calves began their assigned weaning protocol, described below.

*Wean-by-age:* milk allowance remained stable for the next 29 d. Starting on d 62 milk allowance was gradually reduced over 8 d (again, by approx. 8%/d) such that by d 70 calves were completely weaned.

*Wean-by-intake*: milk allowance was reduced (again by 25% over 3 d) once the calf achieved daily solid feed DMI targets of 200, 600, and 1150 g DM/d (complete weaning). These targets corresponded to the 225, 675, and 1300 g/d of as-fed starter feed intake targets reported by Benetton et al. (2019) (corresponding to 0.5, 1.5, and 3 lb/d targets recommended by the Bovine Alliance on Management and Nutrition, 2017). DM was calculated and corrected on a weekly basis for starter, hay, and TMR using the microwave method described below. Calves needed to consume the target rolling average intake across 3 d, with a daily minimum of no less than 50% of the target. If calves did not meet any target, they were weaned starting at d 62 as described for the wean-by-age treatment. For calves that met the first DMI target but not the second, on d 65 milk allowance was gradually reduced over 6 d (again, by approx. 8%/d) so that on d 70 calves were completely weaned. For calves that met the second DMI target but not the third, on d 68 milk allowance was gradually reduced over 3 d (again, by approx. 8%/d) so that in all cases on d 70 calves were completely weaned.

*Wean-by-combination*: When calves met the first solid feed DMI target of 200 g DM/d (from the wean-by-intake treatment), milk allowance was gradually reduced so that all calves were fully weaned on d 70; the rate of daily milk reduction depended on the age that each calf reached the solid feed intake target. For example, a calf that met the target at d 50 had milk reduced at a rate of 5%/d over 20 d to wean by d 70. Calves that had not reached the DMI target by d 62 were weaned following the wean-by-age treatment.

### 2.2.4 Growth and Health

Within the calf rearing facility, calves were individually weighed and health scored on a weigh scale (Smart1 Scales, Westernscale Inc., Port Coquitlam, BC, Canada) twice per week. Health examinations followed the Wisconsin Dairy Calf Health Scoring chart (University of Wisconsin-Madison, Madison, WI, USA) and were scored for the purpose of controlling for illness (health was not considered an outcome measure). Body measurements were recorded as described by Khan et al. (2007a), including body barrel, heart girth, and wither height.

Visual examinations were performed twice daily (morning and afternoon); any calf showing signs of illness was examined further following the health examination procedure described below and treated accordingly. Milk consumption of each calf was recorded daily. During the pre-weaning phase, calves were assisted to the milk feeder if they failed to visit the feeder that day, or if they consumed < 2 L of milk before 0900 h or < 4 L of milk before 1800 h. Calves showing signs of diarrhea (fecal score = 3) and low milk consumption (< 4 L/d) were treated with an electrolytic solution for 3 d or until fecal score returned to normal; if diarrhea and low milk consumption was accompanied by fever (body temperature ≥ 39.5°C) these calves were also administrated a NSAID (Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, ON, Canada). Calves showing signs of respiratory illness, consisting of nasal discharge (nasal score < 1), pulmonary infection (score = 2), and fever (body temperature ≥ 39.5°C) were treated with an antibiotic (Resflor GOLD®, Intervet Inc. Roseland, NJ, USA). Calves were classified as sick if they had a score 3 or greater for diarrhea, had a score of 4 or greater for pulmonary inflammation, were treated with NSAID or antibiotics, or had a combination of the above at any point during the experiment.

Once moved to the grower rearing facility, calves were individually weighed on a weigh scale (model ZQ375, Avery Weigh-Tronix, Richmond, BC, Canada) twice weekly until calves were 140 d old.

### 2.2.5 Feed Sample Collection and Analysis

Weekly starter, hay, and TMR samples were collected from each group pen before calves had access to the fresh forage. DM was calculated using the microwave method following Oetzel et al., (1993). Briefly, samples (approx. 100 g) were heated in a 700-w microwave oven sequentially for 1.5 min, 1 min, 45 s, 30 s, and 15 s at maximum power. Between each period, the sample was removed from the microwave, cooled for 10 s, and mixed to avoid burning. Immediately following this process, the sample weight was recorded, and the sample was heated again for 15 s. This step was repeated until the difference from the previous sample weight was < 0.1 g for three consecutive intervals. The microwave used was equipped with a rotating plate and digital clock to determine timing. DM percentage was calculated using the difference between the initial and final weight of the sample.

Milk samples were collected once per week from the milk storage tank and analyzed for components (Pacific Milk Analysis Lab, Chilliwack, BC, Canada). Calf starter, hay, and TMR samples were collected weekly, frozen, and pooled into 3-mo periods for nutrient and DM analysis (Cumberland Valley Analytical Services, Waynesboro, PA, USA). All automated feeders were calibrated once per week to verify accurate dispensing of milk, starter portions, and weighing of Hay and TMR. Milk and solid feed nutrient analyses as well as particle length size for forages are reported in Table 2.1.

**Table 2.1. Chemical composition and particle length of feed stuff.** Mean (±SD) chemical composition of milk, calf starter, hay, and TMR, and particle length size of hay and TMR, on a DM basis offered to Holstein dairy calves (n = 108).

| | Milk | Calf Starter | Hay | TMR |
|---|---|---|---|---|
| *Chemical Composition* | | | | |
| DM (%) | 12.2 ± 0.4 | 86.2 ± 0.6 | 85.2 ± 1.1 | 44.6 ± 2.9 |
| CP (%) | 26.8 ± 1.2 | 23.7 ± 1.3 | 14.1 ± 1.0 | 16.4 ± 1.1 |
| Fat (%) | 33.4 ± 1.1 | 5.4 ± 1.1 | 2.5 ± 0.2 | 5.5 ± 0.3 |
| Lactose (%) | 33.2 ± 1.0 | ND[1] | ND | ND |
| NDF (%) | ND | 11.1 ± 0.4 | 56.4 ± 1.3 | 32.1 ± 2.2 |
| ADF (%) | ND | 6.0 ± 0.7 | 32.0 ± 0.8 | 20.4 ± 2.2 |
| Starch (%) | ND | ND | 2.7 ± 0.1 | 23.0 ± 2.0 |
| Ash (%) | ND | 7.6 ± 0.6 | 7.6 ± 1.3 | 8.0 ± 0.2 |
| ME[2] (Mcal/kg) | 5.1 ± 0.05 | 3.2 ± 0.08 | 2.4 ± 0.06 | 2.8 ± 0.08 |
| *Particle length[3]* | | | | |
| Long (%) | ND | ND | 58.4 ± 9.5 | 23.8 ± 7.6 |
| Medium (%) | ND | ND | 20.3 ± 4.4 | 35.6 ± 5.8 |
| Short (%) | ND | ND | 17.4 ± 4.4 | 27.8 ± 1.9 |
| Fine (%) | ND | ND | 3.9 ± 2.4 | 12.8 ± 2.2 |

[1] ND = not determined

[2] ME = TDN x 0.04409 x 0.82; calculated according to NRC (2001) equations.

[3] Particle separated, using a Penn State Particle Separator, into 4 fractions: long (> 19 mm), medium, (< 19, > 8 mm), short (< 8, > 1.18 mm), and fine (< 1.18 mm).

### 2.2.6 Statistical Analysis

All statistical analyses were performed in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA), with calf as the experimental unit for weaning treatments and group as the experimental unit for forage treatments. Our a priori power analysis was based upon the weaning treatment, using calf as the experimental unit, and unrewarded visits as the primary outcome measure. Using estimates of effect size and variance from Benetton et al. (2019), and power set at 0.8 and alpha at 0.05, we calculated a required sample of 36 calves for each of our 3 weaning treatments.

Data were scrutinized using probability distribution plots. The variables starter DMI, forage DMI, and unrewarded visits during the pre-weaning period were square-root transformed to normalize residuals. Seven calves were removed from the analysis: 2 calves died (1 in the wean-by-age receiving TMR treatment combination, and 1 in the wean-by-intake receiving TMR treatment combination); 3 calves had a BW at 84 d more than 3 SD lower than the mean (2 in the wean-by-age receiving Hay treatment combination, and 1 in the wean-by-age receiving TMR treatment combination), and 2 calves failed to meet the criterion of average milk intake > 6 L/d at the time of initial stepdown on d 31 (1 in the wean-by-combination receiving TMR treatment combination, and 1 in the wean-by-age receiving TMR treatment combination).

Some calves in the wean-by-intake and wean-by-combination treatments did not meet intake targets by d 62 and were thus classified as 'failed-to-wean'. We predicted that the effect of treatment would vary depending on whether calves successfully met these DMI targets, so we considered calves that failed-to-wean as a separate weaning outcome (failed-to-wean; n = 10). These calves were compared with those that were successfully weaned in the wean-by-intake treatment (successful-intake; met all 3 intake targets by d 62; n = 27), calves that were successfully weaned in the wean-by-combination treatment (successful-combination; met the intake target by d 62; n = 33), and all calves in the wean-by-age treatment (n = 31). Blinding was not possible as researchers needed to know assigned treatments to apply weaning methods and provide correct forage type.

The effects of weaning treatment, forage treatment, and the weaning x forage interaction were tested using a mixed model. Data for feed intake, feeding behavior, and growth were summarized into 5 periods: preweaning (d 2 to 30), weaning (d 31 to 69), postweaning (d 70 to 84), total calf-rearing (d 2 to 84), and heifer-rearing (d 85 to d 140). The following response variables were tested separately for each period: feed intake (milk intake, starter DMI, forage DMI, total DMI), feeding behavior (unrewarded visits to the milk feeder), body measurements (rate of change and final measures of body barrel, heart girth, and withers height), ADG, gain-to-feed ratio, and final weight at d 84. We also analyzed the effect of weaning treatment, forage treatment, and the weaning x forage interaction on ADG and final weight at d 140 in the grower rearing period. All models included the fixed effects of sex, birthweight, order that calves were introduced into the group pen, and sickness (dichotomous variable, sick or not sick during the experiment). Group was specified as the subject and calf was specified as a repeated measure within each group. Compound symmetry was selected as the covariance structure based on AIC, and the Kenward-Roger method was used for computing the denominator degrees of freedom.

Results are reported as least-squares means and SE for each period. Significance was declared at $P \leq 0.05$ and tendencies at $\leq 0.1$. No weaning x forage was found in any model so results are presented for these treatments separately and the interaction was removed from the model. For descriptive purposes, we graphed weekly milk, starter and forage intakes, BW, ADG, and unrewarded visits at the milk feeder for each weaning treatments (i.e., wean-by-age, successful-intake, successful-combination, and failed-to-wean), and weekly starter and forage intakes, and BW for each forage treatment (i.e., Hay and TMR).

## 2.3 Results

### 2.3.1 Weaning Treatments

#### 2.3.1.1 Descriptive Results: Age and Duration of Weaning

Ten calves (14.3%) failed to meet all the intake targets in the wean-by-intake and wean-by-combination treatments. Within the wean-by-intake treatment, 8 calves (22.9%) failed to complete weaning; 3 calves did not meet any target while the remaining 5 only met the first target of 200 g DM/d. Within the wean-by-combination treatment, 2 calves (5.7%) failed to meet the initial target of 200 g DM/d and thus failed to complete weaning. The mean (± SD) weaning age for the successful-intake calves was 56.3 ± 5.8 d (range: 48 – 70 d) and the mean (± SD) duration of weaning (from time to meet first target of 200 g DM/d to complete weaning) was 16.3 ± 4.0 d (range: 12 – 26 d). For successful-combination calves, the duration of weaning was nearly double that of successful-intake calves (28.3 ± 6.1 d, range: 10 – 34 d). Milk allowance after the initial step-down on d 31 was similar across all weaning treatments (wean-by-age: 7.4 ± 1.0 L/d; successful-intake: 7.1 ± 1.0 L/d; successful-combination; 7.3 ± 0.9 L/d; failed-to-wean: 7.1 ± 0.8 L/d).

#### 2.3.1.2 Milk and Feed Intake

Before weaning, milk intakes were similar across weaning treatments (Figure 2.1A). Once weaning began, milk intakes followed the pattern expected based on the weaning protocol: wean-by-age calves consumed 3.2 L/d more than successful-intake calves ($t_{1,83.1} = 16.0$, $P < 0.001$) and 1.7 L/d more than successful-combination calves ($t_{1,81.1} = 9.1$, $P < 0.001$) (Table 2.2). Failed-to-wean calves tended to consume on average 0.5 L/d less milk than wean-by-age calves ($t_{1,89.3} = 1.9$, $P = 0.06$).

Starter intake increased with age, especially starting after the initial milk reduction at day 31 for wean-by-age, successful-intake, and successful-combination calves (Figure 2.1B). Successful-intake calves consumed the most starter during weaning, averaging 0.33 kg DM/d more than successful-combination calves ($t_{1,82.3} = 3.7$, $P < 0.001$), and 0.69 kg DM/d more than wean-by-age calves ($t_{1,83.4} = 9.4$, $P < 0.001$). After weaning, successful-combination calves were able to catch up to successful-intake calves such that starter intakes were similar; however, wean-by-age calves continued to consume less starter than successful-intake and successful-combination calves ($t_{1,8.26} > 3.3$, $P < 0.001$). Over the total calf rearing period, wean-by-age calves had the lowest total DMI (vs. successful-intake: $t_{1,83.9} = 5.1$, $P < 0.001$; vs. successful-combination: $t_{1,82.8} = 3.7$, $P < 0.001$).

Failed-to-wean calves consumed little starter until week 9. Before weaning, failed-to-wean calves consumed approximately 4 times less starter than calves on all other weaning treatments ($t_{1,84.9} > 2.9$, $P < 0.004$). Failed-to-wean calves continued to consume less starter during weaning ($t1,89.3 > 4.3$, $P < 0.001$) and after weaning ($t_{1,87.2} > 2.7$, $P < 0.009$), and had the lowest total DMI over the calf rearing period ($t_{1,88.2} > 3.0$, $P < 0.004$).

Forage intake followed a similar pattern to starter intakes, increasing around week 5 for successful-intake, successful-combination, and wean-by-age calves, but only at week 9 for failed-to-wean calves (Figure 2.1C). During weaning, successful-intake and successful-combination calves consumed (or tended to consume) more forage than wean-by-age calves ($t_{1,82.5} > 1.7$, $P < 0.09$), while failed-to-wean calves consumed (or tended to consume) very little forage compared to the other weaning treatments ($t_{1,86.9} >1.8$, $P < 0.08$). Before and after weaning, there were no differences in forage intake among weaning treatments.

**Figure 2.1. Milk, starter, and forage intake of calves in the wean-by-age, successful-intake, successful-combination, and failed-to-wean weaning treatments from 1 to 12 wk.**
Arithmetic means (±SE) for descriptive purposes of A) milk intake, B) starter intake, and C) forage intake for Holstein calves aged 2 to 84 d. Values are shown separately for calves in the wean-by-age (n = 31), successful-intake (n = 27), successful-combination (n = 33), and failed-to-wean (n = 10) weaning treatments.

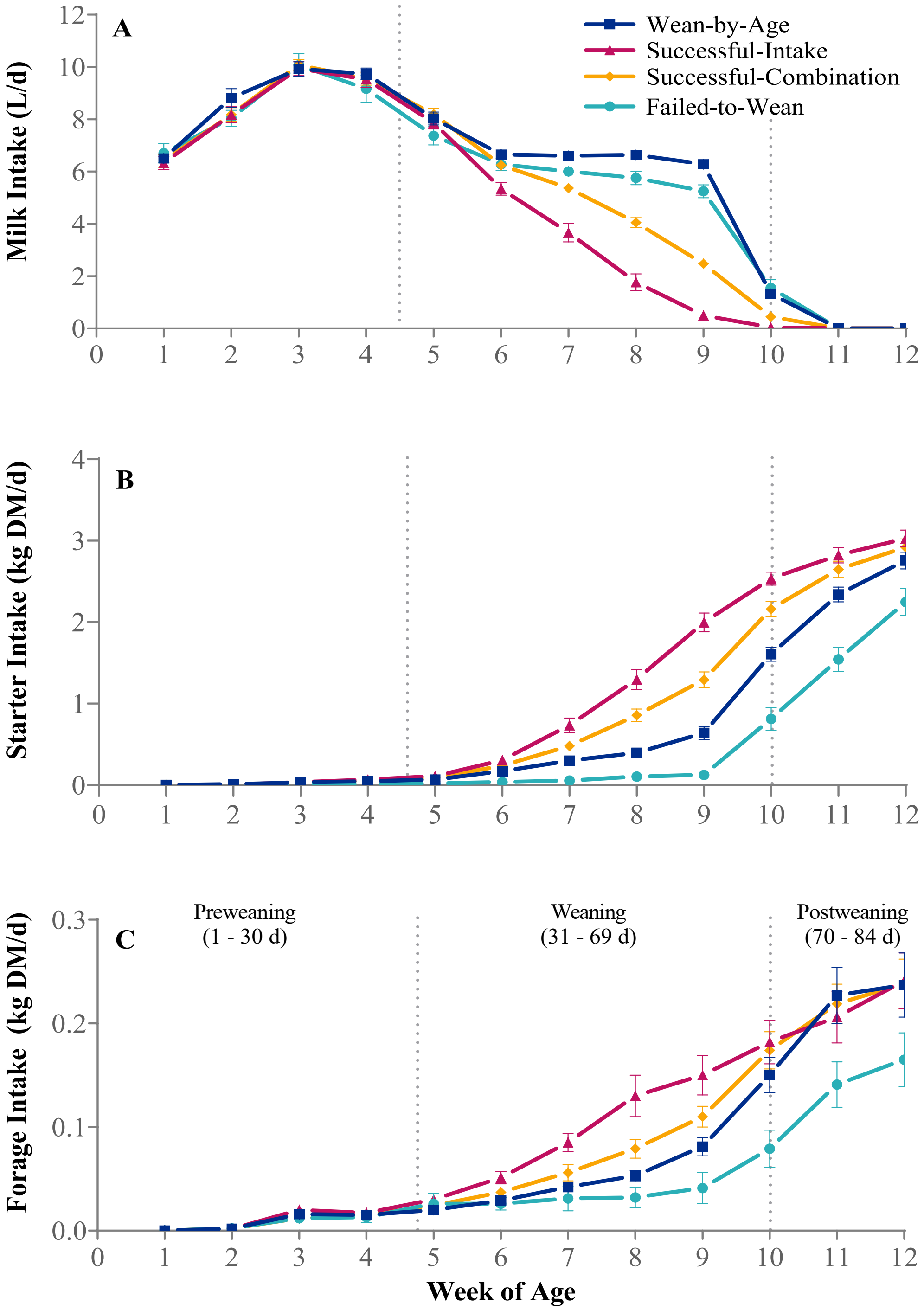


**Table 2.2. Feed intake and behaviour of calves in the wean-by-age, successful-intake, successful-combination, and failed-to-wean weaning treatments.** Least-square means (±SE) of daily milk intake, starter DMI, forage DMI, total DMI, and number of unrewarded visits to the milk feeder of Holstein calves in the wean-by-age (n = 31), successful-intake (n = 27), successful-combination (n = 33), and failed-to-wean (n = 10) weaning treatments. Results are shown separately for each period.

| Response variable | Wean by-Age | Successful-Intake | Successful-Combination | Failed-to-wean | SE | *P*-value |
|---|---|---|---|---|---|---|
| *Pre-weaning (d 2 to 30)* | | | | | | |
| Milk Intake (L/d) | 8.6 | 8.4 | 8.7 | 8.7 | 0.29 | 0.64 |
| Starter DMI (kg/d)$^1$ | $0.024^{a}$ | $0.029^{a}$ | $0.022^{a}$ | $0.005^{b}$ | 0.004 | 0.004 |
| Forage DMI (kg/d)$^1$ | 0.006 | 0.007 | 0.006 | 0.005 | 0.001 | 0.74 |
| Unrewarded visits (no./d)$^1$ | 1.7 | 1.3 | 1.4 | 1.0 | 0.40 | 0.71 |
| *Weaning (d 31 to 69)* | | | | | | |
| Milk Intake (L/d) | $5.9^{a, x}$ | $2.8^{b}$ | $4.2^{c}$ | $5.4^{a, y}$ | 0.26 | <0.001 |
| Starter DMI (kg/d)$^1$ | $0.49^{a}$ | $1.18^{b}$ | $0.85^{c}$ | $0.14^{d}$ | 0.08 | <0.001 |
| Forage DMI (kg/d)$^1$ | $0.08^{a, x}$ | $0.12^{b}$ | $0.10^{ab, y}$ | $0.05^{ac, y}$ | 0.01 | <0.001 |
| Unrewarded visits (no./d)$^1$ | $7.1^{a}$ | $10.6^{b}$ | $10.8^{b}$ | $7.0^{a}$ | 0.90 | <0.001 |
| *Post-weaning (d 70 to 84)* | | | | | | |
| Starter DMI (kg/d)$^1$ | $2.47^{a}$ | $2.89^{b}$ | $2.74^{b}$ | $1.88^{c}$ | 0.10 | <0.001 |
| Forage DMI (kg/d)$^1$ | 0.23 | 0.22 | 0.23 | 0.15 | 0.03 | 0.38 |
| Unrewarded visits (no./d)$^1$ | $4.6^{a}$ | $1.8^{b}$ | $3.7^{a, x}$ | $5.1^{a, y}$ | 1.10 | <0.001 |
| *Total calf-rearing (d 2 to 84)* | | | | | | |
| Total DMI (kg) | $114.6^{a}$ | $135.1^{b}$ | $128.7^{b}$ | $97.4^{c}$ | 5.41 | <0.001 |
| Unrewarded visits (no.) | $372.0^{a}$ | $457.9^{bc}$ | $498.9^{b}$ | $359.6^{ac}$ | 53.0 | 0.005 |

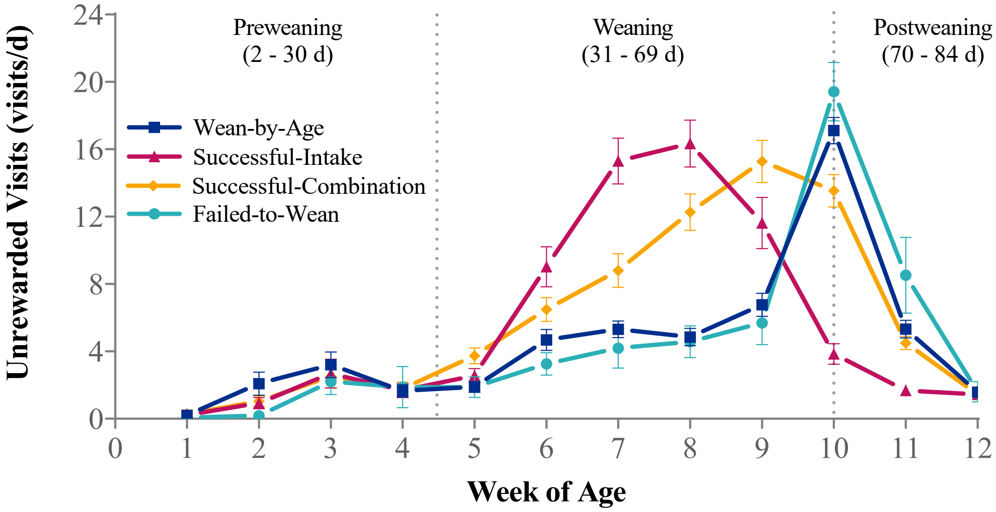
$^{a,b,c,d;\ x,y}$ Means with different superscripts within a row indicate a significant difference ($^{a,b,c,}$ $P \leq 0.05$) or a tendency ($^{x,y}$ $0.05 < P \leq 0.1$) between wean-by-age, successful-intake, and successful-combination calves.

$^1$ Analysis was performed on square-root-transformed data; raw means and SE are reported.

### 2.3.1.3 Unrewarded Visits

Unrewarded visits to the milk feeder increased around week 5 for successful-intake and successful-combination calves, resulting in a higher number of unrewarded visits during weaning compared to wean-by-age and failed-to-wean calves ($t_{1,89.8} > 3.3$, $P < 0.002$). Wean-by-age and failed-to-wean calves showed relatively few unrewarded visits until week 9 but visits increased substantially at week 10 as milk allowance decreased (Figure 2). For all weaning treatments, unrewarded visits decreased after weaning, but successful-intake calves showed the fewest visits ($t_{1,86.6} > 5.2$, $P < 0.001$). Over the calf-rearing period, successful-intake and successful-combination calves a higher total number of unrewarded visits compared to wean-by-age calves ($t_{1,84.9} > 2.1$, $P < 0.04$). Failed-to-wean calves had a similar number of total unrewarded visits to wean-by-age and successful-intake calves, but a lower number of total unrewarded visits compared to successful-combination calves ($t_{1,90.0} = 2.4$, $P < 0.02$).

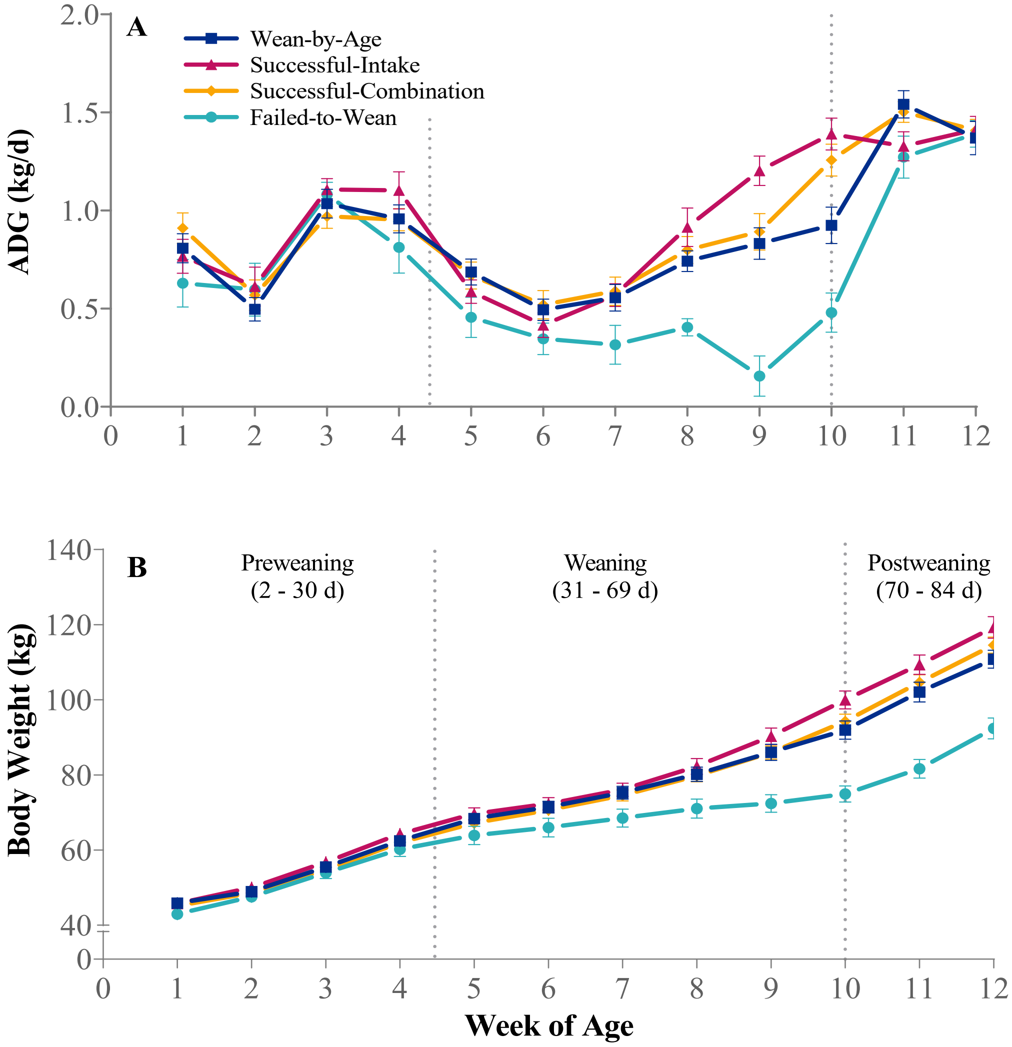
**Figure 2.2. Unrewarded visits at the milk feeder of calves in the wean-by-age, successful-intake, successful-combination, and failed-to-wean weaning treatments from 1 to 12 wk.** Arithmetic means (±SE) for descriptive purposes unrewarded visits at the milk feeder for Holstein calves aged 2 to 84 d. Values are shown separately for calves in the wean-by-age (n = 31), successful-intake (n = 27), successful-combination (n = 33), and failed-to-wean (n = 10) weaning treatments.



### 2.3.1.4 Growth

There were few differences in BW between weaning treatments until week 7, after which failed-to-wean calves showed lower BW for the remainder of the calf rearing period (Figure 2.3). Failed-to-wean calves had the lowest ADG during weaning ($t_{1,88.9} > 3.7$, $P < 0.001$), such that their final BW at 84 d of age was approximately 20 kg less than calves in other weaning treatments ($t_{1,91.2} > 6.5$, $P < 0.001$). Successful-intake and successful-combination calves had similar ADG during weaning and had a similar final BW at d 84 (Table 2.3). However, wean-by-age calves had reduced ADG during weaning compared to successful-intake ($t_{1,83.6} = 2.6$, $P = 0.01$) and successful-combination calves ($t_{1,82.1} = 2.3$, $P = 0.03$), such that their final BW was approximately 6 kg lower at d 84 of age ($t_{1,81.9} > 2.0$, $P < 0.04$).

**Figure 2.3. ADG and BW of calves in the wean-by-age, successful-intake, successful-combination, and failed-to-wean weaning treatments.** Arithmetic means (±SE) for descriptive purposes of A) ADG and B) BW for Holstein calves aged 2 to 84 d. Values are shown separately for calves in the wean-by-age (n = 31), successful-intake (n = 27), successful-combination (n = 33), and failed-to-wean (n = 10) treatments.



Failed-to-wean calves tended to show a lower gain-to-feed ratio before weaning compared to calves in the other weaning treatments ($t_{1,87.8}$ >1.8, $P < 0.07$), but showed the highest gain-to-feed ratio after weaning ($t_{1,83.9} > 4.0$, $P < 0.001$). Wean-by-age calves also had a higher gain-to-feed ratio after weaning compared to successful-intake ($t_{1,81.7} = 5.1$, $P < 0.001$) and successful-combination calves ($t_{1,71.4} = 3.6$, $P < 0.001$). However, over the total calf rearing period, no differences were found between weaning treatments.

Daily growth in structural body measurements did not differ between treatments before weaning. During weaning, failed-to-wean calves had decreased body barrel, heart girth, and wither height growth compared to calves in other weaning treatments ($t_{1,91.6} > 2.2$, $P < 0.03$). Wean-by-age calves had reduced body barrel and heart girth growth compared to successful-intake and successful-combination calves ($t_{1,82.8}> 2.3$, $P < 0.02$). Calves generally had similar structural growth measures after weaning, except wean-by-age and failed-to-wean calves had greater body barrel growth compared to successful-intake and successful-combination calves ($t_{1,81.9} > 2.4$, $P < 0.02$). At the end of the calf rearing period, successful-intake and successful-combination calves had similar final structural body measures, wean-by-age calves had reduced (or tended to have reduced) final body barrel and heart girth compared to successful-intake and successful-combination ($t_{1,82.3} > 2.0$, $P < 0.06$) calves. Failed-to-wean calves had the lowest final body measures compared to calves in other weaning treatments ($t_{1,87.8} > 2.9$, $P < 0.005$).

During the grower rearing period, successful-combination calves had the highest (or tended to have highest) final weight at d 140 compared to calves in other weaning treatments ($t_{1,52.4} > 1.8$, $P < 0.08$). Successful-combination and failed-to-wean calves tended to have higher ADG compared to wean-by-age and successful-intake calves ($t_{1,56.1} > 1.8$, $P < 0.07$).

**Table 2.3. Growth measurements of calves in the wean-by-age, successful-intake, successful-combination, and failed-to-wean weaning treatments.** Least-square means (±SE) of growth measurements of Holstein calves in the wean-by-age (n = 31), successful-intake (n = 27), successful-combination (n = 33), and failed-to-wean (n = 10) weaning treatments. Results are shown separately for each period.

| Response variable | Wean-by-Age[1] | Successful-Intake[1] | Successful-Combination[1] | Failed-to-Wean[1] | SE | *P*-value |
|---|---|---|---|---|---|---|
| *Pre-weaning (d 2 to 30)* | | | | | | |
| Heart girth (HG, cm/d) | 0.43 | 0.48 | 0.44 | 0.44 | 0.04 | 0.46 |
| Body barrel (BB, cm/d) | 0.55 | 0.63 | 0.58 | 0.62 | 0.07 | 0.37 |
| Wither height (WH cm/d) | 0.27 | 0.29 | 0.25 | 0.27 | 0.03 | 0.24 |
| ADG | 0.83 | 0.88 | 0.85 | 0.74 | 0.05 | 0.12 |
| Gain: feed (kg/kg of DM) | $0.83^{ab, x}$ | $0.89^{a}$ | $0.83^{ab, x}$ | $0.71^{b, y}$ | 0.06 | 0.05 |
| *Weaning (d 31 to 69)* | | | | | | |
| HG (cm/d) | $0.26^{a}$ | $0.30^{b}$ | $0.31^{b}$ | $0.19^{c}$ | 0.03 | <0.001 |
| BB (cm/d) | $0.46^{a}$ | $0.54^{b}$ | $0.54^{b}$ | $0.20^{c}$ | 0.05 | <0.001 |
| WH (cm/d) | $0.21^{a}$ | $0.20^{a}$ | $0.21^{a}$ | $0.16^{b}$ | 0.02 | 0.02 |
| ADG | $0.71^{a}$ | $0.85^{b}$ | $0.82^{b}$ | $0.44^{c}$ | 0.07 | <0.001 |
| Gain: feed (kg/kg of DM) | 0.55 | 0.50 | 0.54 | 0.48 | 0.04 | 0.11 |
| *Post-weaning (d 70 to 84)* | | | | | | |
| HG (cm/d) | 0.49 | 0.43 | 0.45 | 0.42 | 0.05 | 0.51 |
| BB (cm/d) | $0.83^{a}$ | $0.55^{b}$ | $0.61^{b}$ | $0.95^{a}$ | 0.12 | 0.003 |
| WH (cm/d) | $0.19^{a}$ | $0.27^{b}$ | $0.25^{b}$ | $0.22^{ab}$ | 0.04 | 0.04 |
| ADG | 1.52 | 1.41 | 1.49 | 1.41 | 0.08 | 0.21 |
| Gain: feed (kg/kg of DM) | $0.60^{a}$ | $0.48^{b}$ | $0.52^{b}$ | $0.74^{c}$ | 0.04 | <0.001 |
| *Total calf-rearing (d 2 to 84)* | | | | | | |
| Final HG | $111.0^{a, x}$ | $112.6^{b}$ | $112.9^{ab, y}$ | $106.2^{c}$ | 1.15 | <0.001 |
| Final BB | $129.5^{a, x}$ | $132.6^{b}$ | $131.9^{ab, y}$ | $124.2^{c}$ | 1.73 | <0.001 |
| Final WH | $97.0^{a}$ | $98.4^{b}$ | $97.7^{ab}$ | $94.3^{c}$ | 0.82 | <0.001 |
| Birth weight | 42.8 | 42.8 | 42.1 | 41.6 | 1.74 | 0.88 |
| Final weight d 84 | $117.0^{a}$ | $123.0^{b}$ | $121.6^{b}$ | $100.1^{c}$ | 3.11 | <0.001 |
| ADG | $0.89^{a}$ | $0.95^{b}$ | $0.95^{b}$ | $0.71^{c}$ | 0.04 | <0.001 |
| Gain: Feed (kg/kg of DM) | 0.66 | 0.64 | 0.64 | 0.60 | 0.03 | 0.17 |
| *Heifer-rearing (d 84 to 140)* | | | | | | |
| Initial weight | $116.4^{a}$ | $117.8^{a}$ | $120.5^{a}$ | $98.2^{b}$ | 3.73 | <0.001 |
| Final weight d 140 | $166.2^{a, x}$ | $168.1^{ab, x}$ | $176.1^{b, y}$ | $155.2^{a, y}$ | 5.49 | 0.007 |
| ADG | $0.86^{a, x}$ | $0.86^{a, x}$ | $0.96^{b}$ | $0.99^{ab, y}$ | 0.06 | 0.05 |

$^{a,b,c,d;\ x,y}$ Means with different superscripts within a row indicate a significant difference ($^{a,b,c,}$ $P \le 0.05$) or a tendency ($^{x,y}$ $0.05 < P \le 0.1$) between wean-by-age, successful-intake, and successful-combination calves.

#### 2.3.1.5 Post-hoc Classification of Wean-by-Age Calves

The analysis presented above follows our original intention of the wean-by-age treatment group, which was to reflect standard farm practice of weaning all calves based on age regardless of solid feed intake. However, the lower average BW of calves in wean-by-age treatment group could have been driven by 3 calves that were consuming little solid feed before the final milk reduction at d 62. Indeed, if these calves had been in the wean-by-intake or wean-by-combination treatments, they would have been classified as failed-to-wean calves and analyzed separately. We therefore tested whether the results for the wean-by-age calves were driven by the 3 calves that consumed less than 200 g DM/d before 62 d of age (the first intake target for wean-by-intake and wean-by-combination treatments). Calves that were consuming sufficient solid feed before weaning on the wean-by-age treatment (successful-age; n = 28) were compared to successful-intake, successful-combination, and failed-to-wean calves (n = 13 with the 3 calves from wean-by-age treatment). This new analysis was performed as described in the statistical analysis section.

Some of the differences in growth between the wean-by-age treatment and other weaning treatments were due to these 3 calves that failed to consume solid feed early. When these 'failed' calves were removed from the wean-by-age treatment, there was no longer a substantial weight difference; successful-age calves only tended to weigh about 4 kg less (previously 6 kg less) than successful-intake calves ($t_{1,83.5} = 1.9$, $P = 0.06$). Similarly, ADG during weaning only tended to be different between successful-age and successful-intake calves ($t_{1,83.3} = 2.03$, $P = 0.06$), with differences no longer seen postweaning or over the calf rearing period. No difference in final BW was found between successful-age and successful-combination calves as previously seen. Along the same line, there were no longer differences in structural body growth measurements between successful-age, successful-intake, and successful-combination calves during the calf rearing period.

Feed intake and unrewarded visits at the milk feeder did not change when ‘failed’ calves were removed from the wean-by-age treatment. Compared to successful-intake and successful-combination calves, successful-age calves had higher milk intakes ($t_{1,79.3} > 8.9$, $P < 0.001$) and lower starter intakes ($t_{1,82.7} > 9.0$, $P < 0.001$) during weaning. Postweaning, successful-age calves had lower starter intakes ($t_{1,82.8} > 5.4$, $P < 0.01$), resulting in an overall lower total DMI ($t_{1,83.7} > 3.1$, $P < 0.002$) across the calf-rearing period compared to successful-intake and successful-combination calves. Unrewarded visits were again lower for successful-age calves during weaning ($t_{1,83.8} > 4.1$, $P < 0.001$) and across the calf rearing period ($t_{1,83.8} > 2.1$, $P < 0.04$) compared to successful-intake and successful-combination calves. Removing ‘failed’ calves from the wean-by-age treatment had no effect on forage treatments, so these results are not presented.

### 2.3.2 Forage Treatments

#### 2.3.2.1 Descriptive Results: Age and Duration of Weaning

Weaning age and duration were similar in groups assigned to Hay vs TMR treatments, with no evidence of an interaction with weaning method. An equal number of calves failed to meet intake targets within the wean-by-intake (n = 4 Hay; n = 4 TMR) and wean-by-combination (n = 1 Hay; n = 1 TMR) treatments. Within the successful-intake treatments, Hay and TMR calves had similar weaning ages (56.4 ± 5.6, range: 49 – 63; vs. 56.2 ± 6.1, range: 48 – 70, respectively) and weaning durations (17.0 ± 4.3, range 12 – 26; vs. 16.6 ± 3.7, range: 12 – 22, respectively). Within the successful-combination treatments, Hay calves had a longer weaning duration with less individual variability compared to TMR calves (30.9 ± 3.3, range: 24 – 34; vs 25.5 ± 7.1, range: 10 – 34, respectively). Milk allowances after the initial step-down on d 31 were also similar between forage treatments (Hay: 7.5 ± 0.8; TMR: 7.0 ± 1.0). Weekly starter intake, forage intake and BW for forage treatments are illustrated in Figure 4.

#### 2.3.2.2 Feed Intake, Unrewarded Visits, and Growth

Starter and forage intakes increased with age, especially starting at week 5 for both Hay and TMR calves. Before weaning Hay calves tended to consume more forage than TMR calves ($F_{1,9.5} = 4.1$, $P = 0.07$) (Table 2.4). During weaning, Hay and TMR calves consumed similar amounts of forage, but Hay calves consumed more starter ($F_{1,8.5} = 8.8$, $P = 0.02$). After weaning, Hay calves continued to consume more starter ($F_{1,10.0} = 9.4$, $P = 0.01$) while TMR calves began to consume more forage ($F_{1,10.0} = 40.8$, $P < 0.001$). High starter intake resulted in Hay calves consuming 13 kg more total DM than TMR calves over the total calf-rearing period ($F_{1,9.37} = 9.4$, $P = 0.01$).

There was no effect of forage treatment on unrewarded visits before or during weaning, but TMR calves showed more unrewarded visits after weaning compared to Hay calves ($F_{1,8.7} = 12.0$, $P = 0.007$). This difference in unrewarded visits was not evident over the total calf-rearing period.

**Figure 2.4. Starter intake, forage intake, and BW of calves receiving Hay or TMR from 1 to 12 wk.** Arithmetic means (±SE) for descriptive purposes of A) starter intake, B) forage intake, and C) body weight for Holstein calves aged 2 to 84 d. Values are shown separately for groups of calves receiving Hay (n = 6 groups) or TMR (n = 6 groups).

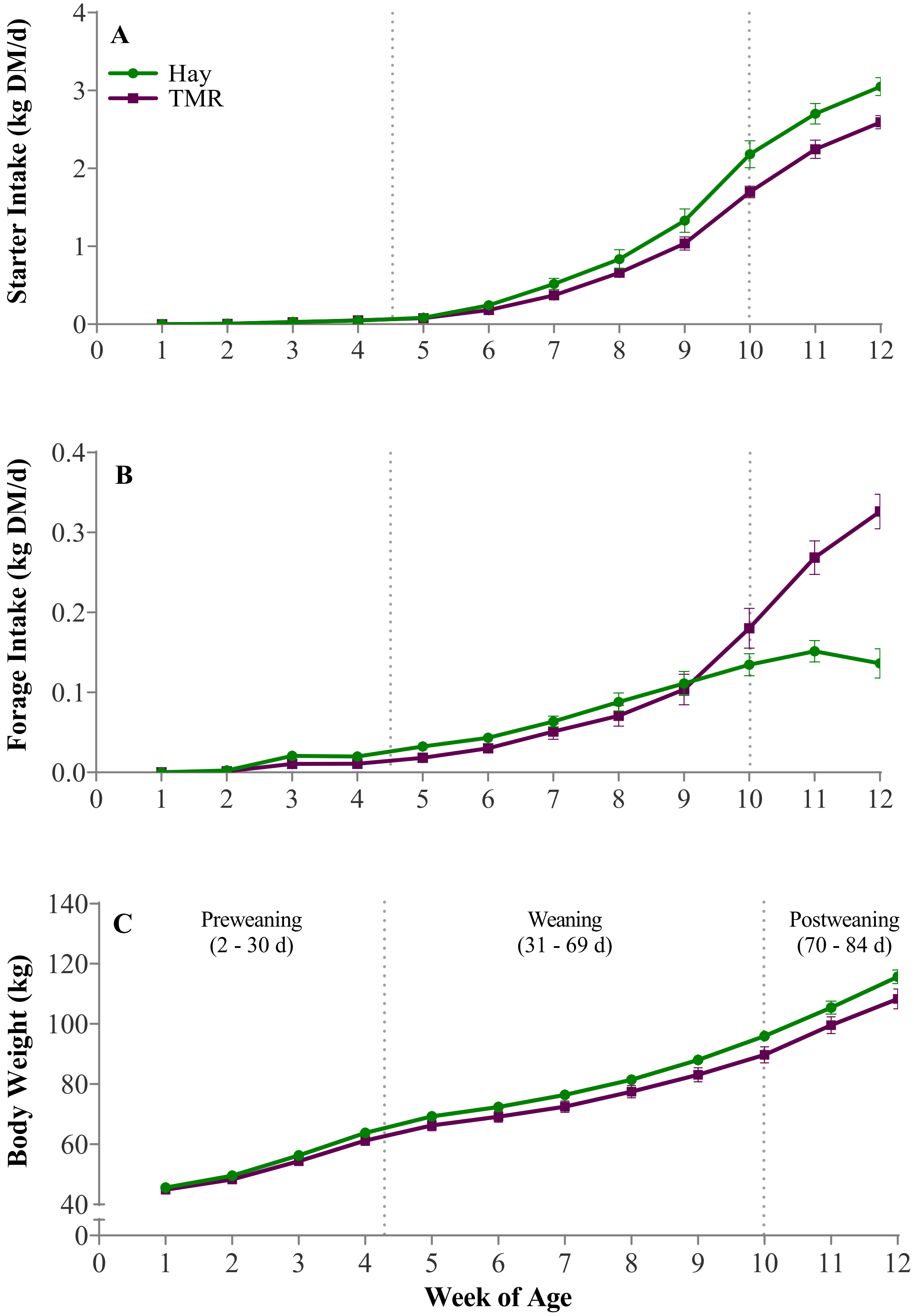


**Table 2.4. Feed intake and behaviour of calves receiving Hay or TMR.** Least-square means (±SE) of daily milk intake, starter DMI, forage DMI, total DMI, and number of unrewarded visits to the milk feeder of groups of Holstein calves receiving Hay (n = 6 groups) and TMR (n = 6 groups). Results are shown separately for each period.

| Response variable | Hay | TMR | SE | *P*-value |
|---|---|---|---|---|
| *Pre-weaning (d 2 to 30)* | | | | |
| Milk Intake (L/d) | 8.8 | 8.4 | 0.21 | 0.22 |
| Starter DMI (kg/d)[1] | 0.022 | 0.022 | 0.004 | 0.86 |
| Forage DMI (kg/d)[1] | 0.008 | 0.005 | 0.001 | 0.07 |
| Unrewarded visits (no./d)[1] | 1.5 | 1.3 | 0.28 | 0.54 |
| *Weaning (d 31 to 69)* | | | | |
| Milk Intake (L/d) | 4.6 | 4.5 | 0.15 | 0.64 |
| Starter DMI (kg/d)[1] | 0.85 | 0.65 | 0.09 | 0.02 |
| Forage DMI (kg/d)[1] | 0.09 | 0.09 | 0.01 | 0.99 |
| Unrewarded visits (no./d)[1] | 9.4 | 8.9 | 0.50 | 0.64 |
| *Post-weaning (d 70 to 84)* | | | | |
| Starter DMI (kg/d)[1] | 2.81 | 2.39 | 0.12 | 0.01 |
| Forage DMI (kg/d)[1] | 0.14 | 0.30 | 0.02 | <0.001 |
| Unrewarded visits (no./d)[1] | 3.3 | 4.0 | 0.32 | 0.007 |
| *Total calf-rearing (d 2 to 84)* | | | | |
| Total DMI (kg) | 125.4 | 112.5 | 3.43 | 0.01 |
| Unrewarded visits (no.) | 426.1 | 418.0 | 28.8 | 0.81 |

[1] Analysis was performed on square-root-transformed data; raw means and SE are reported.

There were few differences in BW between forage treatments until week 7, after which TMR calves showed lower BW for the remainder of the calf-rearing period (Figure 4). Hay calves weighed about 6 kg more at d 84 compared to TMR calves ($F_{1,8.54} = 7.4$, $P = 0.02$). During weaning period Hay calves tended to have higher ADG than TMR calves ($F_{1,9.17} = 4.2$, $P = 0.07$). No differences in feed efficiency or structural growth were found between forage treatments (Table 2.5). During the grower rearing period, TMR calves gained 0.11 kg/d more than Hay calves ($F_{1,6.4} = 9.4$, $P = 0.02$) and were able to compensate for their previously reduced growth such that at d 140 there was no difference in BW between forage treatments.

**Table 2.5. Growth measurements of calves receiving Hay or TMR.** Least-square means (±SE) of growth measurements of groups of Holstein calves receiving Hay (n = 6 groups) and TMR (n = 6 groups). Results are shown separately for each period.

| Response variable | Hay | TMR | SE | *P*-value |
|---|---|---|---|---|
| *Pre-weaning (d 2 to 30)* | | | | |
| Heart girth (HG, cm/d) | 0.44 | 0.45 | 0.03 | 0.95 |
| Body barrel (BB, cm/d) | 0.58 | 0.61 | 0.04 | 0.60 |
| Wither height (WH, cm/d) | 0.27 | 0.26 | 0.02 | 0.66 |
| ADG | 0.86 | 0.79 | 0.04 | 0.25 |
| Gain: feed (kg/kg of DM) | 0.83 | 0.79 | 0.04 | 0.49 |
| *Weaning (d 31 to 69)* | | | | |
| HG (cm/d) | 0.28 | 0.25 | 0.02 | 0.17 |
| BB (cm/d) | 0.44 | 0.43 | 0.03 | 0.67 |
| WH (cm/d) | 0.20 | 0.19 | 0.01 | 0.24 |
| ADG | 0.75 | 0.65 | 0.04 | 0.07 |
| Gain: feed (kg/kg of DM) | 0.52 | 0.52 | 0.02 | 0.99 |
| *Post-weaning (d 70 to 84)* | | | | |
| HG (cm/d) | 0.43 | 0.46 | 0.03 | 0.55 |
| BB (cm/d) | 0.68 | 0.79 | 0.08 | 0.31 |
| WH (cm/d) | 0.22 | 0.25 | 0.02 | 0.28 |
| ADG | 1.45 | 1.46 | 0.05 | 0.91 |
| Gain: feed (kg/kg of DM) | 0.56 | 0.61 | 0.03 | 0.24 |
| *Total Calf-Rearing (d 2 to 84)* | | | | |
| Final HG | 110.9 | 110.4 | 0.78 | 0.60 |
| Final BB | 129.6 | 129.6 | 1.09 | 0.97 |
| Final WH | 96.6 | 97.1 | 0.49 | 0.42 |
| Birth weight | 42.8 | 41.9 | 0.91 | 0.39 |
| Final weight d 84 | 118.0 | 112.9 | 1.66 | 0.02 |
| ADG | 0.91 | 0.84 | 0.02 | 0.07 |
| Gain: Feed (kg/kg of DM) | 0.64 | 0.64 | 0.02 | 0.99 |
| *Heifer-rearing (d 84 to 140)* | | | | |
| Initial weight | 116.3 | 110.1 | 1.50 | 0.03 |
| Final weight | 167.1 | 165.7 | 2.68 | 0.60 |
| ADG | 0.87 | 0.97 | 0.03 | 0.02 |

## 2.4 Discussion

### 2.4.1 Weaning Treatments

To smoothly transition onto solid feed, calves need to be consuming solid feed prior to milk removal. Weaning strategy is especially important to consider when feeding larger volumes of milk (Khan et al., 2011a). Using solid feed intake as a criterion ensures that weaning is only started when calves are consuming solid feed (Roth et al., 2009; de Passillé and Rushen, 2012), and thus allows calves to go through weaning at their own pace (Benetton et al., 2019). Our study compared two intake-based weaning methods, one using 3 intake targets and a step-down reduction method (wean-by-intake) and the other using 1 intake target and a linear reduction method (wean-by-combination). Compared to previous studies examining intake-based weaning methods (de Passillé and Rushen, 2012, 2016; Benetton et al., 2019), our treatments were designed to be more gradual and thus reduce unrewarded visits to the milk feeder and minimize growth checks around weaning.

The two intake-based weaning methods (successful-intake and successful-combination) resulted in similar BW and structural body measures at 84 d while weaned-by-age calves had reduced BW, body barrel, heart girth, and wither height at 84 d. This lower BW seen in wean-by-age calves was likely driven by low starter intake during the weaning period (0.7 kg DM/d less starter than successful-intake and 0.4 kg DM/d less than successful-combination calves). At week 9, before the final milk reduction, wean-by-age calves were only consuming approximately 0.64 kg DM/d (~ 0.72 kg/d). Although this aligns with recommended starter intake for weaning calves (~ 0.68 kg/d of starter: USDA, 2016), this is far less than 2 kg DM/d and 1.3 kg DM/d of starter intake consumed by successful-intake and successful-combination at week 9, respectively. When calves with low solid feed intake were removed from the wean-by-age treatment in the post-hoc analysis, differences in growth were no longer present between successful-age calves and successful-intake and successful-combination calves. Reduced starter intake before weaning delays rumen development and reduces rumen capacity, leaving the calf unprepared for solid feed consumption, digestion, and growth after weaning. Reduced rumen capacity and development is also likely why wean-by-age calves were unable to consume similar amounts of solid feed after weaning compared to successful-intake and successful-combination calves. Wean-by-age calves would have likely benefited from a longer weaning duration than the 1-wk milk reduction implemented at 62 d. Weaning calves from high milk rations over a short period is known to result in growth checks around weaning (Sweeney et al., 2010). Overall, intake-based weaning method used within our study encouraged greater consumption of solid feed during weaning, for calves that successfully weaned, compared to the age-based weaning method, better preparing calves for digestion and growth after weaning.

Not all calves were able to meet the final weaning target of 1150 g DM/d of solid feed for wean-by-intake treatment and 200 g DM/d for wean-by-combination treatment; due to the nature of our study these failed-to-wean calves followed a nearly identical weaning plan to those in the weaned-by-age treatment and thus had similar milk intakes. However, these calves consumed less starter and had an overall lower total DMI over the calf rearing period. As a result, failed-to-wean calves had reduced hip height, heart girth, and body barrel at the end of calf rearing period. They also weighed about 20 kg less than their successful counterparts and were not able make up the weight difference in the grower period (weighing about 15 kg less at 140 d). Similar results were found by de Passillé and Rushen (2012) where 11 out of 60 calves failed to meet either the 200 g/d or 400 g/d starter intake targets before d 74 of age. Benetton et al. (2019) found that 6 out of 16 calves failed to reach the final starter intake target of 1600 g/d by 62 d of age; when given until 84 d to wean, 3 out of 46 calves still failed to reach the final target. Both studies reported reductions in final BW compared to their successful counterparts. de Passillé and Rushen (2012) suggested that these failed-to-wean calves may be smaller and that intake targets should be adjusted according to calf birth weight (Greenwood et al., 1997). However, in our study (like Benetton et al., 2019) we found that birth weight was similar in calves on the different weaning treatments, suggesting that a low initial weight was not the cause of calves failing to meet intake targets. One interesting finding from our study was that failed-to-wean calves tended to have a lower preweaning gain-to-feed ratio; this became significant when ‘failed’ calves from the wean-by-age treatment were included. This finding suggests that failed-to-wean calves may be less efficient in converting feed nutrients into body mass and may explain the finding of de Passillé and Rushen (2012) where failed calves already weighed less at 20 d of age.

The use of automated starter and forage feeders that restrict use to a single calf may have influenced the number of failed-to-wean calves seen in this study, as calves fed this way must find the feeder and feed on their own. Under naturalistic conditions, dairy cattle graze together and calves rely on older animals to learn where and what feed to consume (reviewed by Whalin et al., 2021). Calves that consume very little solid feed before weaning may be less exploratory, and struggle to find and learn how to use the automated feeders, as reported by Neave et al. (2018, 2019). In addition, because the feeders we used restricted entry to one calf at a time, calves may have experienced competitive interactions at the feeder; low-intake calves may be less dominant and thus struggle to access the feeders or may be more often displaced. Additionally, high milk allowances can increase the risk that some calves consume little solid feed before weaning (Khan et al., 2016), but low solid feed intakes can be an issue even when calves are fed less milk. For example, Roth et al. (2009) reported weaning ages between 45 – 98 d in calves fed 6 L/d of milk and weaned based on starter intake (using an initial target of 0.7 kg/d and a final target 2 kg/d). Neave et al. (2018) also found large variability in age (6 – 29 d) to first consume 40 g/d of starter and variation in starter DMI over 7 – 98 d (0.33 – 1.24 kg/d) in calves fed 6 L/d of milk. An advantage of weaning methods based on intake is that these programs help identify calves that consume little solid feed before weaning; these calves might not be identified on farms that wean by age. One implication of our results is that monitoring for low intake would be beneficial regardless of weaning method, but new research will be required to determine how best to manage these low-intake calves.

Milk intakes were variable before weaning, and averaged between 8 to 9 L/d despite offering 12 L/d; variability in milk intake has been previously reported in calves fed high or *ad libitum* amounts of milk (e.g., Dennis et al., 2018). Differences between amount of milk allocated and amount consumed has been reported in calves fed by automated feeders (Nielsen et al., 2008; Sweeney et al., 2010; Benetton et al., 2019), and even calves fed restricted milk allowances (6 L/d) do not always consume their full allotment (Rosenberger et al., 2017). Differences between milk allowance versus milk consumed may relate to how automated feeders allocate milk throughout the day and how individual calves interact with the feeder. For calves to receive milk from the automated feeders, the minimum milk allocation must have accrued. We set the minimum milk allocation to 0.5 L so that calves were able to ingest small portions of milk over several meals, but his type of meal pattern can increase milk feeder occupancy and competition (Senn et al., 2000; Jensen, 2004; Jensen, 2009), potentially reducing intake. Also, the final milk allowance was allocated at 2000 h, perhaps preventing some calves from receiving milk at night.

Contrary to our expectations, both intake-based weaning methods (successful-intake and successful-combination calves) resulted in high numbers of unrewarded visits, despite introducing a gradual removal of milk after each intake target. Benetton et al. (2019) and de Passillé and Rushen (2016) also found high number of unrewarded visits in calves weaned based on intake. Unrewarded visits to the milk feeder are considered a sign of hunger, with restricted-fed calves having more frequent unrewarded visits than those provided higher milk allowances (Jensen, 2006; De Paula Vieira et al., 2008), which would imply that calves weaned based on a solid feed intake experienced more hunger during weaning compared to weaned-by-age and failed-to-wean calves. Indeed, hunger could have driven calves to reach the intake targets quickly, resulting in further reduction in milk. However, this result warrants further investigation as it appears contradictory that the wean-by-intake and wean-by-combination calves, who have higher ADG and feed consumption, are more hungry than wean-by-age and failed-to-wean calves with poorer growth and intake. It is possible that factors other than hunger, such as frustration, may have influenced the number of unrewarded visits at the milk feeder. Frustration occurs when an individual is kept from attaining a goal at the time they expect to receive it (Berkowitz, 1989). Previous work has suggested that animals experience frustration when an expected reward is not delivered (Amsel, 1958; Carlstead, 1986). During weaning, automated milk feeders reduce the amount of milk and the times at which milk is available, potentially creating a frustrating situation for the calf. Given that calves have consistent feeding times (Jensen, 2004), the inconsistency and uncertainty of when and how much milk calves will have available may lead to further attempts to access milk. This could also explain the low frequency of unrewarded visits seen in wean-by-age and failed-to-wean calves at 5 to 9 wk when milk allowance remained consistent, and the high frequency of unrewarded visits at 10 wk when milk allowance was reduced for these calves. We encourage future work to develop and test predictions distinguishing feelings of hunger and frustration around weaning.

### 2.4.2 Forage Treatments

Over the past two decades there has been a renewed interest in providing milk-fed calves access to forage (Coverdale et al., 2004; Castells et al., 2012, 2013; Pazoki et al., 2017). However, only a few studies have compared different types of forages (Castells et al., 2012; Webb et al., 2014), with little agreement on the best type of forage to feed calves at this stage of life. Since calves typically transition onto a fermented ration later in life, our second objective was to compare feed intake, behavior, and growth of calves fed either a grass hay or a lactating cow TMR and how these forage types influence the weaning transition.

During weaning, Hay calves had greater total DMI and ADG compared to TMR calves, resulting in a greater final BW at 84 d. This higher total DMI was driven by Hay calves consuming more starter during weaning compared to TMR calves. TMR calves were likely unable to achieve the same total DMI as Hay calves due to the high moisture of the TMR. Overvest et al. (2016) also found that calves fed a silage based TMR, similar to the one fed in our study, had reduced DMI and final BW compared to calves fed starter and hay separately. Interestingly, they found similar feed intake across all treatments on an as-fed basis and similar gain-to-feed ratios after weaning, suggesting that the digestibility of the TMR was not the cause of reduced growth but rather that the calves fed TMR were unable to achieve equivalent DMI. Khan et al. (2020) found that calves fed a forage-based starter (fermented alfalfa), with a similar moisture content (45% DM) to our TMR, had lower solid feed DMI during the milk-feeding period and reduced growth during and after weaning. The high moisture of the TMR also helps explain why TMR calves during weaning had reduced starter DMI but similar forage DMI and milk intakes compared to Hay calves. Young calves have a smaller rumen capacity to accommodate forage bulk before and during weaning, so consumption of forage with high moisture may increase gut fill to the extent that DMI and nutrient supply are reduced (Khan et al., 2016). The reduced starter intake during weaning likely left TMR calves hungry, especially around complete milk removal. This may explain why TMR calves trended to have greater unrewarded visits at the milk feeder after weaning compared to Hay calves. Overall, the increase total DMI and ADG during weaning combined with the reduced number of unrewarded visits after weaning suggest that Hay calves had smoother transition on solid feed compared to TMR.

After weaning TMR calves consumed less starter compared to Hay calves but had similar ADG, likely due to their higher forage intake after weaning. The TMR had a greater level of energy and protein than the hay due to the inclusion of concentrate primarily comprised of soybean meal and corn meal, potentially making the TMR more palatable. Miller-Cushon et al. (2014) demonstrated that calves exhibit clear preference for certain high-energy and high-protein feed types, including soybean meal and corn meal. Since the TMR had both forage and concentrate, TMR calves were likely getting a portion of their grain source from the TMR, explaining the lower starter intake of TMR calves during and after weaning. This effect would be more pronounced after weaning, when milk was no longer available, and calves were required to select their diet from starter and forage. In addition, forage particle length and bulk can affect feed intake; shorter particle lengths and higher density feeds result in greater digestibility, higher intakes, and reduced rumen fill (Coon et al., 2018; Omidi-Mirzaei et al., 2018). The shorter particle length in the TMR compared to the hay may have promoted higher intake of the TMR. The fermentation of the TMR likely improved digestibility as microbes had already begun breaking down the TMR making nutrients more accessible to the calf. Another explanation for the increase in forage intake in TMR calves could be changes in the rumen. The fermented feeds in the TMR may have created a more favorable environment for butyrate production (Esdale et al., 1968), allowing for more rapid rumen development and a greater capacity for forage intake. Overall, it is unclear why TMR calves had greater intake of forage after weaning. An improved understanding of how fermented forages affect rumen development as well as preferences for different forage types may shed more light on this finding.

During the grower rearing period, TMR calves had higher ADG resulting in similar final BW compared to Hay calves at 140 d. These results suggest that the TMR calves were able to compensate for their reduced BW during the milk-feeding period. Consuming greater amounts of forage post-weaning and having more experience with a mixed ration during the calf rearing period, may have allowed TMR calves to transition onto the heifer TMR more easily. There is some evidence suggesting that early experience with feed types can influence longer-term feeding behaviors in ruminants (Miller-Cushon and DeVries, 2015). For example, Simitzis et al. (2008) reported that lambs exposed to oregano-supplemented feed in the first 2 mo of life continued to show preference for this flavor after 9 mo of age. In dairy calves, Miller-Cushon and DeVries (2011) found that calves exposed to either concentrate or hay during weaning selectively consumed the familiar feed when switched to a mixed ration. These results may be due to food neophobia (i.e. a reluctance to consume unfamiliar foods; (Provenza and Balph, 1987) when exposed to the new diet in the grower rearing period. Food neophobia is well documented in dairy cattle, including dairy calves (Costa et al., 2014), creating a challenge when transitioning to new diets (Launchbaugh et al., 1997). Exposure to a variety of feed types early in life is known to reduce food neophobia in sheep (Catanese et al., 2012; Villalba et al., 2012).

Given that growing dairy calves are often transitioned onto fermented feeds, there is merit in investigating if feeding fermented forages early in life promotes feeding behavior and rumen development later in life.

## 2.5 Conclusion

Calves weaned using an intake criterion showed greater solid feed intake, postweaning weights, and structural growth compared to calves weaned at a fixed age. These results illustrate that weaning based on intake is a promising strategy to manage weaning at the individual level and ensure calves are consuming solid feed before weaning; however unrewarded visits to the milk feeder were higher in calves weaned based on intake, potentially indicating hunger or frustration during weaning. Some calves (regardless of treatment) consume little solid feed before weaning; weaning methods based upon intake can help identify these animals, but how they should be managed is not clear. Forage type can influence the transition onto solid feed. Offering grass hay to milk fed calves can improve solid feed intake and ADG during weaning resulting in greater BW and fewer signs of hunger postweaning compared to calves fed a lactating cow TMR.

# Chapter 3: General Discussion

## 3.1 Thesis Findings

The overall objective of my research was to investigate different intake-based weaning methods and forage type on feeding behaviour, feed intakes, and growth in dairy calves around weaning. In Chapter 1, I reviewed the literature on dairy calf feeding management focusing on three main areas that influence weaning success: milk feeding practices, weaning methods, and solid feed type. Over the past two decades much research has focused on the short- and long-term benefits of feeding high allowances of milk to dairy calves (Soberon et al., 2012; Rosenberger et al., 2017). One criticism of high milk allowances is calves are often consuming little solid feed before weaning, resulting in reduced growth and chronic signs of hunger around weaning when conventional weaning methods are used such as abrupt milk removal at a young age (de Passillé et al., 2011; Sweeney et al., 2010). Intake-based weaning methods have shown promise in promoting feed intake and weight gain during weaning (de Passillé and Rushen, 2016; Benetton et al., 2019; Whalin et al., 2022); however, methods investigated thus far have resulted in signs of hunger and a high number of calves unable to complete the set targets for solid feed intake (Benetton et al., 2019; de Passillé and Rushen, 2016). In addition to milk allowance and weaning method, access to forage plays a major role on feed intake, growth, and development of calves before, during, and after weaning (Khan et al., 2016; Imani et al., 2017). Despite the numerous benefits of feeding forages to milk-fed calves, several studies have attempted to define the most appropriate type and presentation of forage with little agreement about the most optimal feeding strategies.

In Chapter 2, I explored different intake-based weaning methods and forage type and the relationship between feeding behaviour and growth in dairy calves fed by automated feeders.

Two intake-based weaning methods were compared to an age-based weaning method. The intake-based weaning methods were designed to be more gradual compared to past methods assessed in the literature (Benetton et al., 2019; Whalin et al., 2022). Previous work has demonstrated that weaning duration is positively correlated with weight gain and total digestible energy, suggesting that longer weaning durations result in a smoother transition onto solid feed (de Passillé and Rushen, 2012). The wean-by-intake method included 3 intake targets with a 3-d gradual milk reduction at each step-down, requiring calves to have a minimum 2 week weaning duration. Within this treatment, calves could wean at variable ages. The wean-by-combination method included 1 intake target, but calves were required to wean at 70 d; thus, the duration of milk reduction differed between calves. Overall, I found that calves who successfully reached intake targets on both wean-by-intake and wean-by-combination treatments consumed more solid feed during and after weaning resulting in greater BW at 84 d compared to calves weaned by age. However, successful-intake and successful-combination calves had higher unrewarded visits at the milk feeder, suggesting they were hungrier or frustrated during weaning compared to calves weaned by age. I also found that 10% of calves assigned to the intake-based weaning methods failed to reach intake targets. These failed-to-wean calves consumed little solid feed before 62 d and weighed on average 20 kg less at 84 d then their successful counterparts and calves weaned by age.

I also compared two forage types, silage-based TMR versus grass hay, on feeding behaviour and growth around weaning. During weaning I found that calves fed TMR had reduced starter intake and tended to have lower ADG resulting in reduced BW at 84 d compared to calves fed hay. Additionally, calves fed TMR tended to show a greater number of unrewarded visits at the milk feeder post-weaning, suggesting that these calves were hungrier than calves fed hay after weaning. Overall, these results favour the use of hay rather than silage during weaning. After weaning, however, calves fed TMR were able to achieve similar solid feed intakes and ADG compared to calves fed hay, and during the grower rearing period calves fed TMR had greater ADG than calves fed hay resulting in a similar final BW at 5 mo, indicating no long-term effects of the forage treatment.

## 3.2 Limitations

The conclusions from this thesis are limited by the facility design and management of our facility. In Chapter 2, automatic milk, starter, forage, and water feeders were used to collect feed intake and behaviour data of calves. As a result of the design of these systems, only one calf could feed at a given time. The design of these feeders does not align with the natural feeding behaviour of cattle, where dairy cattle graze together and social facilitation plays a major role in the development of calf feeding behaviours (reviewed by Whalin et al., 2021). By limiting opportunities for social feeding, feed intake and behaviour at the automated feeders may have been affected, including the age when calves first began to consume solid feed. Some calves may have benefited from social feeding and facilitation, such as the less exploratory calves (Neave et al., 2018; Whalin et al., 2022).

Many dairy farms still use manual milk feeding methods (Medrano-Galarza et al., 2017; USDA, 2016), making implementation of the treatments applied in the current study difficult. However, one strength of the current study is that the weaning treatments were tested within group, and multiple groups were assessed providing some basis to generalize our conclusions. Overall, some aspects of the facility design, including the size and layout of the pens used as well as the aspects of the automated milk and starter feeders, likely affected study results.

One weakness of the experimental design described in Chapter 2 is that the effect of forage treatment was tested at the group level, providing a less powerful test of this treatment. The power analysis of this experiment was based upon weaning treatments, using calf as the experimental unit and unrewarded visits at the primary outcome measure. Forage treatment was a secondary objective of this study and, due to facility constraints, forage treatments had to be tested at the group level. Although we found treatment difference, notably in feeding intakes around weaning, the lack of significant results in other measures may have been due to low power. Future work should use more groups or apply more sensitive within group testing methods when investigating the effects of forage type.

Another limitation of this study was using unrewarded visits to the milk feeder as the only measure to assess how calves were coping with weaning, following the methods of earlier studies (Benetton et al., 2019; de Passillé and Rushen, 2016; Sweeny et al., 2010). In Chapter 2, I found that successful-intake and successful-combination calves had higher number of unrewarded visits compared to wean-by-age and failed-to-wean calves during weaning, despite having superior growth and feed intakes. I argued that factors other than hunger, such as frustration, may have influenced the number of unrewarded visits at the milk feeder; more work is required to better understand the affective response during weaning, and what inferences can be based on changes in non-nutritive visits to the feeder. Other behavioural measure such as lying time (Horvath and Miller-Cushon, 2019), play behaviour (Krachun et al., 2010), or non-nutritive sucking (de Passillé et al., 2010) could help better understand the effect of intake-based weaning on calf behaviours. Recently, saliva and hair cortisol has been used to assess short- and longer-term stress in calves (González et al., 2010; Welboren et al., 2019). These cortisol measures could provide another way to evaluate stress around weaning. Additionally, using a cognitive test could help better understand the effect of weaning methods on the emotional state of calves (Neave et al., 2013; Grimberg-Henrici et al., 2016). Future work assessing weaning methods should include multiple measure of weaning distress to distinguish between feelings such as hunger and frustration.

## 3.3 Future Research

A major finding of Chapter 2 was that intake-based weaning can improve solid feed intake and weight gain during and after weaning. Although results from Chapter 2 are promising, the practical implication of intake-based weaning on commercial farms may be a challenge. Due to software limitations, time of each milk reduction and individual milk allowances had to be manually calculated and implemented for the intake-based weaning treatments. This procedure was time consuming for the wean-by-intake treatment as three intake targets were required. Currently, there is little information on how weaning methods are implemented on farm let alone intake-based weaning methods. Russell et al. (2022) found a wide range of weaning methods were used on farms in Western Canada ranging from abrupt weaning to a 2-mo gradual reduction in milk. In Chapter 1, it was noted that there is an increasing interest in using intake-based weaning. A surprising result from Medrano-Galarza et al. (2017) was that 50% of farms feeding milk manually took starter intake into account when weaning calves while only 16% of farms using automated milk feeders used an intake criterion. Future research should investigate weaning methods at the farm level and how they impact weaning success. Further, understanding dairy farmers views using similar methods to that reported by Russell et al., (2022) but focused specifically on weaning and the challenges and barriers associated with intake-based weaning would help inform future research on weaning methods.

An obvious follow up to Chapter 2 is to investigate why failed-to-wean calves struggle to transition onto solid feed and how to manage weaning for these individuals. In Chapter 2, 10% of calves assigned to an intake-based weaning method failed to consume sufficient solid feed before 9 wk. To the best of our knowledge, failed-to-wean calves were otherwise similar to their successful counterparts and wean-by-age calves. For example, failed-to-wean calves all had serum total protein levels > 5.2 g/dL indicating sufficient passive transfer, and were similar in birth weight to calves in the other groups. These are also not unique to Holsteins as Whalin et al. (2022) found that 6 out of 16 Norwegian Reds calves failed to meet intake targets by 56 d. A novel finding from Chapter 2 was that failed-to-wean calves had a lower pre-weaning gain-to-feed ratio, indicating that they are not converting energy as efficiently compared to calves in the other weaning treatments. This result suggests that there may be differences in rumen physiology and morphology; however, no study to date has measured rumen development in failed-to-wean calves. It is likely that fewer calves would fail to wean if weaning age was delayed beyond 10 wk as used in this study. In semi-natural rearing systems, calves wean between 8 to 10 mo (Reinhardt and Reinhardt, 1981). Failed-to-wean calves may need a longer period of time on milk and to become familiar with solid feed. Previous work has found that allowing calves to wean later (i.e., 84 d) can reduced the number of calves that failed to reach intake targets (Benetton et al., 2019). Future work on intake-based weaning should allow longer periods for calves to meet intake targets. Another factor impacting failed-to-wean calves may be social competition in the group pen. Competition around individual automated feeders can impact meal patterns and feed intakes (Jensen, 2006; von Keyserlingk et al., 2004). Failed-to-wean calve may have been less dominate and struggled to access resources around weaning. Currently there is little work on social competition in dairy calves. Further research should investigate how competition for resources around weaning impacts individual calves. Finally, the long-term performance and productivity of failed-to-wean calves in unclear. Although this study, along with previous works (see Benetton et al., 2019), indicate that failed-to-wean calves are unable to compensate for reduce growth by 5 mo, the future reproductive and lactation performance of these animals is unknown. Understanding why some calves struggle to transition onto solid feed and how we can improve their weaning experience is needed.

Although calves fed a silage-based TMR had reduced performance during weaning and greater signs of hunger after weaning, high TMR intake post-weaning and greater ADG during the grower rearing period suggest that introduction of silage during the milk feeding period may have longer-term benefits. These post-weaning results warrant further investigation into providing silages to milk-fed calves. An obvious follow up experiment would be to provide calves with a silage that has a lower moisture content. The high moisture content in the TMR (45% DM) likely contributed to the reduced DMI and growth during weaning (Overvest et al., 2016; Khan et al., 2020). Providing a silage with lower moisture may help increase DMI and reduce gut fill, improving weight gain during weaning. Another potential follow-up experiment would be to provide calves with both hay and TMR along with calf starter. This would give calves more agency over their diet and provide calves with exposure to silage, potentially reducing food aversion later in life. Past work has criticized feeding management practices of calves as it is typically tailored to the average calf and neglects the individual variation in consumption of different solid feed types (Atwood et al., 2001). There is some evidence demonstrating that calves provided a variety of feed types can select their own diets, meeting their individual needs while maximizing energy intake and gain-to-feed ratio (Atwood et al., 2001; Webb et al., 2014b). Finally, my results, along with other works (Kehoe et al., 2019;

Mirzaei et al., 2017), suggest that milk-fed calves are willing to consume silages; however, little in know about what types of forages calves prefer. Webb et al. (2014a) was one of the first studies to explore calves' preferences for forage types using double demand conditioning, finding that calves prefer high quality hay over straw and long particle sizes over short. Future research on using such methodologies to better understand calf preference for silages and other feed types is warranted. Given the economic potential (Kehoe et al., 2021) and performance benefits of silages, further research should investigate ways to incorporate silage into a pre-weaned calf diet that improves solid feed intake and growth around weaning. However, it is acknowledged that silages must be managed differently compared to dry forages. Within in this study we provided fresh forage twice a day to ensure that the lactating cow TMR would not spoil. This may be a barrier for some dairy farmers so future work should also assess the practicality of implementing silages on farm.

Finally, our study is one of the few to follow calves beyond the first few weeks after weaning (e.g., Benetton et al., 2019; Dennis et al., 2018). Currently, we have little understating of how different weaning programs and forage types impact future performance. Although we only used a subset of animals and were unable to collect feed intake, this data resulted in two important findings: 1) failed calves were unable to compensate for their reduced growth during the heifer-rearing period, and 2) TMR calves had greater ADG during the heifer-rearing period resulting in similar final BW at 140 d of age. These findings support other work showing evidence that calf performance and management practices used during the milk feeding and weaning period can affect future productivity and performance later in life (Heinrichs and Heinrichs, 2011; Soberon et al., 2012). However, it should be noted that there was a greater variability in final weights reported at 140 d than the weights reported at 84 d. Bazeley et al.

(2016) has also reported large variability in growth rates on UK farms in heifers 60 d to 420 d of age. These authors also found that growth rates between 30 d to 180 d was a strong predictor if calves would meet a pre-breeding target wight 374 kg by 420 d (14 mo of age). Although health, performance, and management practices used during the milk feeding and weaning period explain a portion of variability in growth rates, other factors likely influence this variability such as health, housing management, and nutrition management during the grower rearing period. Currently there is little research on heifer behavior, health, and growth from 3 mo of age to first breeding. I encourage future work on calf management practices to assess long-term implications. More research is also needed to understand how management practices used during the heifer grower rearing period (i.e., post-weaning to first breeding) impacts the welfare of these animals.

## 3.4 General Conclusion

My thesis contributes to the growing body of literature on intake-based weaning methods and on providing forage to milk-fed dairy calves. This work described in this thesis shows that an inclusion of an intake criterion in a weaning method can help calves have smoother transition from milk to solid feed by increasing total solid feed intake and growth during and after weaning. However, not all calves are able to meet the intake criteria, warranting further investigation into this type of weaning method. This research provides some of the first evidence showing that forage type can influence performance of dairy calves during weaning. Providing a silage-based TMR reduced solid feed intake and growth during weaning and increased signs of hunger after weaning. However, calves fed a silage-based TMR had increased weight gain during the grower rearing period, indicating that calves may benefit from early life exposer to silage. Future work on weaning methods and forage provisions in dairy calves should assess the longer-term implications.

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