MILK FEEDING STRATEGIES TO OPTIMIZE THE TRANSITION TO SOLID FEED IN DAIRY CALVES

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PREFACE AND ACKNOWLEDGEMENT

This master thesis marks the end of my master degree in Agrobiology – Animal Health and Welfare at the University of Aarhus. The thesis corresponds to 45 ECTS and consists of a literature review combined with an experimental part. The experiments were conducted in the facilities at the Cattle Research Centre (DKC) at Aarhus University and were granted by The Danish Cattle Levy Fund. The project investigated the transition from milk to solid feed in dairy bull calves in order to optimize health and welfare of the intensive rosé veal calf production. The experiment and thereby the thesis is conducted interdisciplinary and therefore, calf performance is evaluated in relation to both feed intake and feeding behavior.

The project has given me the opportunity to apply knowledge acquired through the last years of my studies and work experience from practice. The experiments were conducted from September 2016 to April 2017 and lots of unexpected challenges have cost many hours trying to understand why – and how to improve the experiments. Taking a trip down memory lane, the last year has without doubt taught me much more than animal science and I am so grateful for everything that I have learnt. I have many people to thank.

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Annedorte Jensen
ABSTRACT

In rosé veal calf production, the transition from a liquid-based milk replacer diet to a solid feed based diet is a potential risk phase as the calf shifts from depending on energy from milk to solid feed. The objective of this study was to investigate the pre- and post-weaning intake of solid feed and performance if calves were fed by (a) conventional flat-rate (CON; 6.5 L/d from d 1 to 28, and 4 L/d from d 29 to 35 followed by feeding 2 L/d from d 36 to 42 of the study) or step-down (STP; 8 L/d from d 1 to 14, and 5 L/d from d 15-28, followed by feeding 4 L/d from d 29 to 35 and 2 L/d from d 36 to 42 of study) milk feeding methods with the daily allotment available either (b) restricted (RES; maximum portion size: 2.3L per portion) or unrestricted (URES; no maximum portion size) from an automated milk feeder. Two similar experiments were conducted in September to November 2016 (1A) and in February to April 2017 (1B). A total of 64 Holstein bull calves were purchased from seven dairy herds at the age of 11.7 (± 0.7) days and 47.4 (± 0.9) kg body weight. Calves were coded to be 14 d of age in the milk feeder PC at entrance and weaned at 8 weeks of age. In the milk feeding period calves were offered a total of 224 L milk replacer (22% CP, 21% fat). Calves were monitored for two weeks post-weaning. No effects of the milk feeding methods (CON vs. STP) and two milk feeding frequency (RES vs. URES) with respect to overall feed intake and calf performance was detected in this study. The feed conversion efficiency was worse in experiment 1B compared to 1A, possible because calves were suffering from the cold weather and draught in the winter period of experiment 1B. Calves fed RES spent more time occupying the milk feeder in week 4 when calves fed STP and CON were offered 8 and 6.5 L/d, respectively. Calves were challenged by gastrointestinal and respiratory diseases in the first weeks of both experiments, therefore, feed intake and average daily gain was lower than expected. Whether feeding milk replacer through a step-down strategy stimulates the consumption of solid feed, and whether smaller volumes of milk replacer distributed in more meals per day compared with no restriction on milk meal size has a positive effect on calf performance, requires further studies. Furthermore, the long-term effects on veal calf performance must be evaluated.

Key words: dairy calf, step-down, feeding frequency, performance, feeding behavior
ABBREVIATION KEY

AA  Amino acids
ADD  Animal daily dose
ADG  Average daily gain
BHB  Beta-hydroxybutyrate
BW   Body weight
CON  Conventional flat-rate method
CP   Crude protein
DE   Digestible energy
DM   Dry matter
DMI  Dry matter intake
DKC  The Danish Cattle Research Centre
FCE  Feed conversion efficiency
FF   Feeding frequency
GE   Gross energy
G:F  Gain to feed ratio
LCFA Long chain fatty acid
ME   Metabolisable energy
MR   Milk replacer
NE   Net energy
NEFA Non-esterified fatty acid
NRC  Nutrient requirements of dairy cattle
RES  Restricted
STP  Step-down method
T1   Treatment 1: CON vs. STP
T2   Treatment 2: RES vs. URES
URES Unrestricted
VFA  Volatile fatty acid
# TABLE OF CONTENT

## 1 INTRODUCTION

1.1 AIMS OF THE INVESTIGATIONS
1.2 HYPOTHESES
1.3 LIMITATIONS

## 2 DIGESTION AND RUMEN DEVELOPMENT IN THE YOUNG CALF

2.1 RUMEN DEVELOPMENT
2.2 DIGESTION IN THE CALF
2.3 SOLID FEED AND RUMEN DEVELOPMENT

## 3 ENERGY REQUIREMENTS AND GROWTH POTENTIAL

3.1 ENERGY REQUIREMENTS OF CALVES
3.2 GROWTH POTENTIAL OF YOUNG CALVES
3.3 PREDICTION OF AVERAGE DAILY GAIN IN YOUNG CALVES
3.4 DISEASES AFFECTING THE ACTUAL GROWTH RATE

## 4 MILK FEEDING STRATEGIES

4.1 MILK FEEDING FREQUENCY
4.2 STEP-DOWN MILK FEEDING METHOD

## 5 FEEDING BEHAVIORS

5.1 INTRODUCTION TO FEEDING BEHAVIORS
5.2 EXPRESSION OF FEEDING BEHAVIORS
5.3 FEEDING ACTIVITIES
5.4 UNREWARDED VISITS
5.5 RECORDING METHODS

## 6 MATERIALS AND METHODS

6.1 ANIMALS AND HOUSING
6.2 MILK FEEDING PROCEDURE
6.3 EXPERIMENTAL DESIGN AND DIETS
6.4 MEASUREMENTS: INTAKE AND GROWTH
6.5 ANIMAL HEALTH
6.6 Feed Sampling and Analysis 40
6.7 Behavioral Data Collection 40
6.8 Fecal Sampling and Assessment 41
6.9 Blood Samples 42
6.10 Calculations 42
6.11 Statistical Analysis 43

7 Results 45

7.1 Experiment 1A 45
7.2 Experiment 1B 50
7.3 Experiment 1AB 54
7.4 Calf Behavior 58

8 Discussion 62

8.1 Calf Health 62
8.2 Intake of Solid Feed 63
8.3 Calf Performance 65
8.4 Blood Metabolites 68
8.5 Calf Behavior 72
8.6 The Experimental Setup 75

9 Conclusions 76

10 Implications 77

11 References 78

Appendix
1 Introduction

The intensive production of bull calves in Denmark is facing challenges with relation to the use of antibiotics. The threshold limit value set by the Ministry of Environment and Food of Denmark for antibiotics is 1.2 Animal Daily Dose (ADD). A recent register-based study found a large variation in the use of antibiotics (0.05 to 2.20 ADD) between 183 herds that produced calves by purchasing calves directly from dairy herds and raising them to slaughter (Fertner et al., 2016). The study found that the number of calves introduced to the production system was positively associated with the level of antimicrobial use. An increasing size of the production units will therefore most likely increase antibiotic use (Fertner et al., 2016).

The production of dairy breed young bull calves in Denmark is traditionally intensive and separated from the dairy production. Bull calves are purchased from many different dairy farms and mixed, often in relation to weight and/or age, when entering the starter unit. Most calves are transported from the dairy farm and introduced to the intensive production when they are 35 days old on average (Jarltoft, 2017). In young calves, the level of maternal immunity is decreasing and the calf’s ability to produce antibodies is low. Through increased exposure to novel microbes, the calf starts to develop its own antibodies after about 3 weeks of life. At this time the calf is just beginning to have its own antibody responses to environmental microbiota (Hulbert and Moisá, 2016). The calf is, therefore, vulnerable when exposed to stress associated with the introduction to a new environment where factors such as transport, mixing, foreign bacteria, and change in feed ration contribute to a risk period of disease.

Furthermore, the transition from milk to solid feed is a potential risk period where many calves are challenged in relation to their health due to an insufficient nutrient intake. This period potentially results in weight loss and disease. One common practice has been to provide calves with milk twice a day for a total of approximately 10% of the calf’s BW. This milk allowance is somewhat restricted compared to nature, where calves left to nurse their dam or offered milk ad libitum, drink up to 12 L of milk per day (de Passillé and Rushen, 2012). The restricted milk feeding and early weaning methods were developed on encouraging solid feed intake (Eckert et al., 2015). However, there has over the last decade been a movement toward offering more milk in early life to achieve improved health- and welfare, growth rates and feed efficiency (Appleby et al., 2001; Diaz et al., 2001; Jasper and Weary, 2002; Kristensen et al., 2007; Khan et al., 2011b; Rosenberger et al., 2017). The disadvantage of this strategy is a reduced intake of concentrates and, thereby, a slower development of the rumen which affects feed intake and growth negatively post-weaning (Sweeney et al., 2010). So even though a diet based on high milk volumes can result in a rapid and efficient growth, it does little to prepare the pre-ruminant calf for weaning or for utilization of grain and forage based diets (Warner, 1956; Baldwin et al., 2004).

The term ‘weaning strategy’ is widely discussed in the intensive bull calf production industry, and the volume of milk offered in the milk feeding period is distributed very differently among farmers. Most studies investigating the effect of milk ration on solid feed intake, weaning, and performance have evaluated the milk feeding period and excluded the weeks following weaning, which indeed is a period of high risk, because no milk is offered to provide a basic energy supply to the calf. These weeks are of high value and even though the milk ration is reduced gradually over one week, the calves still face challenges due to disease and low daily gain post-weaning. It is believed that a smooth transition from milk to solid feed will re-
duce the use of antibiotics because fewer calves will suffer from lack of energy and, thereby, will be able to cope better with the challenges regarding disease and loss of weight.

To accommodate a smooth transition, different milk feeding strategies have been investigated in the past. The effect of an elevated plan of nutrition has been compared to conventional flat-rate milk feeding and feeding frequency (FF) has been evaluated to observe the effect of milk portion size on the intake of solid feed pre- and post-weaning. The results from previous studies are not unanimous and further studies are therefore required to make a final conclusion.

The objective of this study is to investigate the pre- and post-weaning feeding behavior and performance of Holstein bull calves offered the same amount of milk in the entire milk feeding period either through (a) conventional flat-rate (CON; 6.5 L/d from d 1 to 28, and 4 L/d from d 29 to 35 followed by feeding 2 L/d from d 36 to 42 of the study) or step-down (STP; 8 L/d from d 1 to 14, and 5 L/d from d 15-28, followed by feeding 4 L/d from d 29 to 35 and 2 L/d from d 36 to 42 of study) milk feeding methods with the daily allotment available either (b) restricted (RES; maximum portion size: 2.3L per portion) or unrestricted (URES; no maximum portion size) from an automated milk feeder, when calves are fed the same total amount of calf milk replacer (MR) in the entire milk feeding period.
1.1 Aims of the investigations

The aims of the project are to investigate:

- The effects of STP or CON milk feeding strategies on solid feed intake and average daily gain (ADG) during pre- and post-weaning periods, when both STP and CON are fed the same total amount MR over the entire milk feeding period.

- The effect of milk FF on the early solid feed intake and ADG, when the portion size is unrestricted and calves thereby are allowed to consume the daily ration in one meal (URES) compared to a restricted portion size of maximum 2.3 L (RES).

1.2 Hypotheses

- When introducing the calves to STP, where the daily ration is reduced from 8 L/d in three steps, it is expected to observe a higher total intake of solid feed following the reduction in milk allowances and thereby, a higher ADG compared to calves introduced to CON. This is expected since the transition from milk to solid feed is performed more smoothly allowing the calf to become accustomed to consuming solid feeds.

- Small frequent milk meals (URES) will presumable stimulate the intake of solid feed, because the smaller meals are insufficient to satisfy the calf until it is allowed to enter the automated milk feeder again. Conversely, calves fed URES might have a greater solid feed intake compared to RES, because they experience an extended period between the larger milk feedings.

The project will also investigate the interaction of the two treatments described above. To our knowledge, no studies have investigated this particular interaction in the past. It is believed that the combination of STP and RES will result in the greatest intake of solid feed, which will positively affect the body weight (BW).

1.3 Limitations

Two similar experiments were conducted in September to November 2016 (1A) and February to April 2017 (1B). It was not possible to obtain information regarding birth weight and colostrum feeding of the calves purchased for both experiments. Technical challenges regarding use of the automatic concentrate feeder possibly limited the intake of concentrate in 1A and it was therefore agreed to use feed troughs throughout the repeated (1B). Video recordings in experiment 1A were not used because animal behavior was affected by the technical challenges regarding the automatic concentrate feeder and its interaction with the automated milk feeder. Therefore, behavioral observations were only sampled in experiment 1B. Data obtained from the automatic milk feeder regarding number of visits, occupancy time and portion size was not reliable and is therefore excluded from the thesis.

Time limitations have narrowed the results that are presented in this thesis. Blood was sampled in both experiments but so far only analyzed and presented for the first experiment. Also the behavioral observations were limited to 30 hours distributed over 5 observations days. However, data is recorded for the entire trial period of experiment 1B by using video cameras and these data will be analyzed later.
2 Digestion and rumen development in the young calf

The following sections will give a brief introduction to the anatomy of the pre- and post-weaned calf followed by a description of the digestion of milk and solid feed in relation to rumen development.

2.1 Rumen development

Rumen development has a clear and major impact on the digestive capabilities and thus on the supply of nutrients to the calf (Baldwin et al., 2004). At birth, the compartments of the bovine stomachs are similar to those found in the mature ruminant, but the size and function of each compartment differs significantly (Warner, 1956). In the first weeks of life the calf is monogastric because of an inactive function of the reticulum, rumen and omasum. The abomasum is responsible for the digestion of milk and as the calf begins to consume solid feed, the function of inactive compartments develops (Warner, 1956). The development of the bovine compartments from birth to maturity is illustrated in Figure 1.

Mechanisms controlling the rumen differentiation is not entirely understood and the interrelationship between parameters such as pH, beta-hydroxybutyrate (BHB) and volatile fatty acids (VFA) has not been shown (Baldwin et al., 2004), even though these parameters have been used for analyzing and describing the rumen development in the literature (Coverdale et al., 2004; Lesmeister and Heinrichs, 2004; Suárez et al., 2006a; Khan et al., 2008). The parameters are measured by collecting rumen fluid. Analysis of the rumen after slaughter is likely to be the most accurate method to characterize the rumen development, but this method has the disadvantage that the measures of rumen development cannot be repeated over time (Roth et al., 2009).

![Figure 1](image1.png)

Figure 1 Development of the bovine compartments from birth to maturity (Heinrichs and Jones, 2003).

2.2 Digestion in the calf

Development of the rumen results in a major shift of nutrients being delivered to the peripheral tissues, the intestines and the liver, naturally because the feed is now entering the rumen, where a major part of digestion and metabolism takes place. Variations in the type and form of nutrients offered will affect the nutrients available to support growth, while changing the feed from milk to solid feeds will affect the cellular proliferation of the rumen epithelium remarkably (Baldwin et al., 2004). The offered nutrients also have an impact on the metabolism of the liver. When only offering milk to the preruminant calf, glucose, long-chain
fatty acids and milk-derived amino acids (AA) are primarily intestinally absorbed. When altering the feed toward solid feed the rumen function is developed, and hepatic metabolic activity is increased, because it needs to metabolize VFA, ketones and AA from feed and microbial sources derived from rumen fermentation. These metabolites are absorbed through the rumen papillae- and wall (Baldwin et al., 2004).

2.2.1 Digestion of milk
In the preruminant calf, the esophageal groove directs milk to the abomasum (Ørskov, 1972). Many factors have been reported to affect efficient closure of the esophageal groove in the young calf such as the method of drinking, the composition of the liquid consumed, the temperature of the milk and the age of the calf (Ørskov, 1972; Blowery, 1996). If the calf is fed cows’ milk containing casein, the milk forms a clot in the abomasum caused by the secretion of the enzyme chymosin, which cleaves parts of the molecule (kappa-casein) into a hydrophobic and hydrophilic part. The clot will then be released more slowly from the abomasum to the small intestine compared to MR without casein. In the preruminant calf, the gluconeogenesis is highly regulated and the liver serves as the primary site of ketogenesis (Baldwin et al., 2004). The fact that milk is directed to the abomasum without entering the rumen limits its ability to stimulate rumen development (Warner, 1956). It is well known, that minimal rumen epithelial metabolic activity and VFA absorption is found when the calves are fed milk and no solid feed is offered.

2.2.2 Digestion solid feed
The digestion of concentrate and hay takes place in the rumen as described in the previous section. The concentration of starch and NDF and NDF digestibility, has a major influence on rumen fermentation characteristics and passage rate (Rinne et al., 1997). The dietary content of grass-hay varies among the different species of grasses (e.g. alfalfa and grass-clover) and time of harvesting. With increasing grass maturity the NDF concentration increases, while the NDF digestibility decreases (Rinne et al., 1997). The major compositional difference between grass-hay and concentrate is the content of cellulose. Grass-hay facilitates cellulolytic bacteria and increases acetate production compared to concentrate. The relatively higher content of starch found in concentrate contributes to a production of propionate produced by amylolytic bacteria (Beharka et al., 1998). Both types of feed have a minor content of sugar which contributes to the production of butyrate.

Butyrate is metabolized into BHB in the rumen wall and is known to stimulate the development of the rumen epithelium. Studies have suggested that circulating BHB levels may be an indicator of rumen development and concentrate intake in pre-weaned calves (Khan et al., 2011b). Eckert et al. (2015) found no difference in the concentration of BHB in the blood while treatment groups were consuming the same minimal amounts of concentrate in the first weeks of life. However, the concentration increased significantly when calves were offered less milk and an increase in concentrate intake was seen. Chapman et al. (2017) found a higher concentration of serum BHB in calves fed 3.8 L of MR per day compared to calves fed higher milk volumes, indicating that calves fed a lower milk volume have a higher concentrate intake which improves the rumen development (Quigley et al., 1991; Deelen et al., 2016).
2.3 Solid feed and rumen development

The capacity of the rumen increases remarkably in proportion to the entire gastrointestinal tract around the time of weaning. This is needed to increase the surface area for absorption of VFA, to meet the demands of growth (Baldwin et al., 2004). Weaning occurs only a few months after birth, which is not in agreement with the natural time of weaning, where calves are gradually weaned ending milk intake at the age of ten months (Reinhardt and Reinhardt, 1981). In nature, the calf will gradually transition towards foraging solid feed ensuring that the calf is not weaned from milk until it is able to feed on solid feed only. Solid feed such as calf starter (concentrate) or forage will not pass through the esophageal groove and thus flows from the esophagus and directly to the rumen, where digestion begins. This division of the digestive system in the young ruminant is essential, because the reticulum and rumen are sterile at birth, which means that no bacterial population is established to digest milk in the rumen. When the calf initiates the intake of solid feed, the establishment of ruminal fermentation begins along with a physical and metabolic development, where the physical development includes an increasing volume of the rumen and papillae growth (Warner, 1956; Baldwin et al., 2004). Warner (1956) found that calves fed milk alone showed little changes of the stomach compartments, whereas calves offered solid feed had a noticeable increase in volume and tissue deposition. Also more extensive papillary development of the rumen wall was found. The calves were slaughtered at birth and at the age of 4, 7, 10, 13, and 16 weeks, where changes were evident at 4 weeks and increased steadily over time (Warner, 1956).

2.3.1 Papillae growth

Proliferation and growth of the epithelial cells causes the papillae to increase in length and width, which increases the surface for absorption of the end product of microbial digestion through the rumen wall. Papillae growth is mainly stimulated by the production of butyrate followed by propionate (Baldwin and McLeod, 2000). A continuous presence of VFA maintains rumen papillae growth, size and function (Warner, 1956). Presence and absorption of VFA is therefore indicative of simulated rumen epithelial metabolism, and thought to be the key in initiating rumen epithelial development (Baldwin and McLeod, 2000). Warner (1956) found that calves fed hay and/or grain had a positive effect on the development of the rumen tissue, indicated by the papillary length. Observations made post-mortem indicated that the rumen of newborn calves does not contain of any prominent papillae (Warner, 1956). The same author has described the changes found at the age of four weeks, where the length of the papilla was found to be less than 2 mm in all instances. The papillae growth did not change in the remaining experimental period, where calves were milk-fed until 16 weeks of age (Warner, 1956). By feeding calves with a supplement of grain or hay in the milk feeding period, post-mortem observations found the papillae to be noticeable at four weeks. Kristensen et al. (2007) found similar results, where the weight of the rumen and reticulum increased with a higher intake of concentrate and hay. Likewise, the experiment found a numerical increase in the length of the papillae in the atrium and the ventral ruminal sac in Holstein calves of 5 weeks of age, allowed low milk allowances with increasing intake of concentrate (Kristensen et al., 2007).

The relationship between papillae length and calf weight gain has been consistently found and greater ADG has been associated with longer papillae in four out of eight regions of the rumen (Roth et al., 2009). Calves weaned by a concentrate-method, where calves were fed different amounts of milk depending on the consumption of concentrate, tended to have longer papillae in the left part of the ventral sac (Roth et al., 2009). No other regions of the rumen were affected by the weaning method used in the experiment. The
results were based on calves slaughtered three weeks post-weaning, where the rumen had developed further. Possible differences observed in the rumen in relation to the consumption of solid feed might be larger if the rumen was observed directly after the end of milk provision (Roth et al., 2009).

2.3.2 Hay intake
Slight changes are found when observing the rumen development in calves offered mature alfalfa hay in addition to milk compared to calves offered milk only until the age of six weeks (Figure 2). However, it is important to emphasize that the nutritional quality of the hay may have an influence on whether feeding hay in the milk feeding period has a positive effect on the rumen development or not. Feeding an early cut dried grass with a high content of sugar may improve the digestion in the rumen and thereby the development of the papillae tissue. It is well known that physical structure of the feed has a great influence on development, rumen muscularization and volume (Heinrichs, 2005). Large particle size and high fiber content physically increase rumen wall stimulation and, thereby, increases rumen motility and volume (Warner, 1956). Warner (1956) found that calves receiving a supplement of hay had a greater total capacity of the reticulo-rumen and omasum compared to calves only being milk-fed or receiving grain. As described previously, no differences in papillae growth was found among the treatments, therefore, it was concluded that feeding grain without offering hay stimulates rumen development in the milk fed calf. Similarly, Roth et al. (2009) found that consumption of hay had a significant impact on rumen development, the length of the papillae was found to be longer in the left part of the dorsal blind sac, with an increasing intake of hay. No other areas of the rumen were affected by the treatment (Roth et al., 2009). The results described by Warner (1956) were at the time of publication surprising, since it was commonly believed that hay was required for the development of the normal rumen tissue.

Provision of forage for young calves has been discouraged, out of concern that it will displace concentrate intake and thereby influence the rumen development negatively (Hill et al., 2008). However, evidence has been found suggesting that provision of forage does not necessarily reduce concentrate intake (Khan et al., 2011a; Castells et al., 2012). Furthermore studies have found a positive impact on the ruminal environment by reduced acidity of the ruminal fluid (Suárez et al., 2007; Khan et al., 2011a).

Figure 2 Comparison of rumen papillae development at 6 weeks of age in calves fed milk only, milk and mature alfalfa hay or milk and grain (Heinrichs, 2005).
2.3.3 Concentrate intake
Dry matter intake (DMI) is known to have a positive effect on rumen development. Suárez et al. (2006b) described a positive correlation between DMI and empty rumen weight by comparing results from nine experiments with concentrate-fed calves conducted in the period from 1966 to 2006 (Suárez et al., 2006b). The comparison illustrated that DMI stimulates rumen growth, but it is important to emphasize that the correction for differences in dry feed intake is not implemented when comparing the nine results of forestomach growth. Longer papillae has been observed to be positively correlated positively with greater concentrate consumption (Roth et al., 2009).
The physical structure of the concentrate is found to have an important influence on the rumen development, where coarsely or moderately ground concentrate diets have been shown to increase rumen capacity and muscularization (Beharka et al., 1998). Concentrate diets with increased particle size are therefore believed to have a positive effect for overall rumen development (Heinrichs, 2005). The coarsely ground concentrate stimulates papillae growth because of the chemical composition leading to the production of butyrate and propionate and hereby increases the rumen capacity.
3 Energy requirements and growth potential

Nutrition of young calves is of great importance in relation to both health and profitability. Challenges in the pre-weaning period and transition phases can be costly. The postnatal development of the digestive system is highly dependent on nutrition, the amount of milk and solid feed offered has a major influence. A common challenge when raising calves is to ensure that all calves consume the required nutrients in all transition phases, especially post-weaning. Feeding high volumes of milk will positively affect growth rate in the milk feeding period and, therefore, it might be tempting to allow the calves to feed on milk only.

In Denmark, bull calves are fed very differently in the first weeks of life when housed at the dairy farm. The optimal feeding strategy is still broadly discussed, because what is suitable for the dairy farmer might not be beneficial for the farmer raising the bull calves. When milk prices are low, the expenses associated with feeding high levels of milk will be paid back in higher weight gains, since calves heavier than 50 kg purchased from a Danish dairy herd are payed a supplement per kg live weight. Heavy calves fed milk only in the first weeks of life will be extremely challenged when transferred to the intensive bull production unit, since lower amounts of milk replacer with less energy is provided at a time when the calf’s ability to consume solid feed is negligible.

In the following sections energy requirements and the growth potential of calves during the milk feeding period will be presented. Furthermore, the findings on how diseases affect the actual growth rate are described.

3.1 Energy requirements of calves

Energy and thereby energy requirements of calves can be expressed in numerous ways (Figure 3). Gross energy (GE) is the total amount of energy in the feed. The GE can be predicted relatively precisely from the knowledge of the chemical composition of the feed. Not all the energy in the feed is available for the animal, so the portion of GE that is actually digested by the animal is known as digestible energy (DE). The metabolisable energy (ME) is the portion of GE that is useful for metabolism. This value takes the loss of energy through feces, urine and gases into account. Net energy (NE) is the portion of metabolisable energy that is available for maintenance and growth (McDonald, 2011).

![Figure 3] Energy expressed at different levels (McDonald, 2011)
The latest edition of the NRC (Nutrient requirements of dairy cattle) recommendations uses the ME system for calves (NRC, 2001). The daily energy requirement for calves fed on milk only can be predicted in ME by using the equation derived by Toullec (1989) where a maintenance requirement of 100 kcal/kg BW^{0.75} is used (NRC, 2001):

\[ ME \text{ requirement} \left( \frac{M\text{cal}}{d} \right) = 0.1 \, BW^{0.75} + (0.84 \, BW^{0.335}) \,(ADG^{1.2}), \]

where BW and ADG are expressed in kilograms. The first part of the equation expresses the energy required for maintenance, while the second part is a function of both body size and rate of gain, expressing the energy required for ADG. The ME requirements for maintenance under thermoneutral conditions are approximately 7.33 MJ/d for a 45 kg calf (Drackley, 2008). Whole milk contains more ME/kg of solids compared to milk replacers and, therefore, calves need a larger amount or a higher dry matter (DM) content in MR compared to whole milk in order to obtain a similar growth rate. Also the conversion rate from GE to ME is higher for whole milk compared to MR (93 and 90%, respectively). The amounts of milk solids consumed above maintenance can be used for growth (Table 1). ME requirements for maintenance may be underestimated for calves during the first weeks of life because of a high and variable basal metabolic rate observed during this time. Protein requirements are mostly determined by the growth rate and the requirement for maintenance are small (30 g/d for a 45-kg calf) (Drackley, 2008).

Calculating the ME requirements of a calf fed multiple feeds differs from the equation given when calves are fed milk or MR only. NRC (2001) has made several assumptions about the diet that the calf consumes, as well as the efficiency with which ME is used for maintenance and gain. Efficiencies of ME use from solid feeds for maintenance and gain are fixed at 75% and 57%, respectively when using the equation of NRC (2001). The efficiency of use of ME from the total diet is then calculated as the average of the individual efficiencies for milk and solid feed and weighted according to their contribution to the total ME in the diet. The chemical composition of the feeds affects the ME requirement because of the utilization of the energy. Carbohydrate and protein derived from concentrate must be fermented in the rumen, therefore, calves offered a combination of milk and concentrate utilize the GE less efficiently than calves only fed milk. Furthermore, heat production is considered as a loss, which will increase with increased rumen activity and contribute to less energy available for growth (NRC, 2001).

### Table 1 Requirements for metabolisable energy (ME) and apparent digestible protein (ADP) for a 50-kg calf at different rates of ADG under thermoneutral conditions (NRC, 2001).

<table>
<thead>
<tr>
<th>Rate of gain (kg/d)</th>
<th>ME (MJ/d)</th>
<th>ADP (g/d)</th>
<th>Required DM intakea (kg/d)</th>
<th>CP requiredb (% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.87</td>
<td>31</td>
<td>0.40</td>
<td>8.3</td>
</tr>
<tr>
<td>0.20</td>
<td>9.92</td>
<td>78</td>
<td>0.45</td>
<td>18.7</td>
</tr>
<tr>
<td>0.40</td>
<td>12.56</td>
<td>125</td>
<td>0.63</td>
<td>21.4</td>
</tr>
<tr>
<td>0.60</td>
<td>15.49</td>
<td>173</td>
<td>0.78</td>
<td>23.7</td>
</tr>
<tr>
<td>0.80</td>
<td>18.67</td>
<td>220</td>
<td>0.94</td>
<td>25.1</td>
</tr>
<tr>
<td>1.00</td>
<td>21.98</td>
<td>267</td>
<td>1.10</td>
<td>26.1</td>
</tr>
</tbody>
</table>

a Amount of milk replacer DM containing 19.89 MJ ME/kg DM needed to meet ME requirements.
b Amount of CP needed in DM of milk replacer to supply the amount of ADP that matches ME provided, with NRC assumptions and equations. Assumes only milk proteins fed.
3.2 Growth potential of young calves

It is well known that growth differentials are enormous among farms. In practice we see great differences in the volume of MR fed to the calves, therefore, the growth rate is expected to show great variety. Feeding milk or MR ad libitum is known to influence the ADG positively and higher weight gains are found in the pre-weaning period (Appleby et al., 2001; Jasper and Weary, 2002; Borderas et al., 2009; Khan et al., 2011b; Chapman et al., 2016; Rosenberger et al., 2017). The greatest ADG (1.2 kg/d) in the pre-weaning period found in the literature was obtained when calves were fed ad libitum MR until 7 weeks of age with a total DMI of 1.87 kg/d. The MR contributed to 95 % of the DMI (Miller-Cushon et al., 2013). Growth rates of 0.8 to 1.1 kg/d are commonly reported: (Appleby et al., 2001; Jasper and Weary, 2002; Hepola et al., 2008). Calves fed restricted amounts of milk or MR are reported to obtain an ADG in the range of 0.3 to 0.6 kg/d in the milk feeding period (Borderas et al., 2009; Miller-Cushon et al., 2013; Chapman et al., 2016; Rosenberger et al., 2017).

A meta-analysis including data from 20 research trials where 993 bull calves were observed until 8 weeks of age found that intake of concentrate and MR were the most important variables for predicting the growth rate (Bateman et al., 2012). An increasing provision of MR and intake of concentrate improves calf growth, likewise a higher content of fat and CP in the MR has an positive influence on the growth rate (Bateman et al., 2012). On average calf ADG was 615 g across the 20 research trials included in the meta-analysis until d 56, when a variety of different MR was fed with protein contents ranging from 20% to 28% and fat contents ranging from 17% to 22%. Weaning age differed among the 20 trials and the level of MR intake varied from 430 to 1009 g per day in the pre-weaning period (mean = 610 g per day). The meta-analysis presents the minimum and maximum values of ADG (129 and 1161 g/d, respectively), which indicates a major difference among the calves used in the trials (Bateman et al., 2012).

3.3 Prediction of average daily gain in young calves

The development of an accurate calf model predicting growth rates is challenging because several factors such as breed, colostrum, feeding programs, facilities and environmental conditions affect the growth (Souza et al., 2016). Calves require nutrients for growth and maintenance, which greatly depend on the climate and housing of the calves. Furthermore, the immune responses to infectious challenges and stress responses influences the required energy intake (Drackley, 2008). Mathematical models are important tools to estimate nutritional requirements, growth and performance of calves; nonetheless more knowledge is needed to develop models estimating precise growth rates in calves younger than 2 months. The last update of NRC (2001) presents a chapter on the nutrient requirements of the young calf, which has been adopted by many dairy cattle nutritionist. The model presented to predict the ADG in young calves is in good agreement with 16 published research studies presented by NRC (2001), when comparing the actual performance of calves receiving both milk or MR and concentrate with the predicted values. However, the requirements for ruminant calves weighing less than 100 kg do not merge exactly into the requirements for larger calves. When comparing the predicted and the observed ADG from 25 treatments in 19 published studies, half of the predicted values were found to be greater than the observed values, while the other half of the predicted values were smaller than the observed values of ADG (NRC, 2001).

In 2013 the calf model presented by NRC (2001) was evaluated by using 993 individual data from dairy calves in the United States (Hill et al., 2013). Data was obtained from the above-mentioned meta-analysis
of 20 published trials, where calves from 0 to 8 weeks of age were fed different milk replacers that varied in energy and CP content (Bateman et al., 2012). Hill et al. (2013) found a relationship between predicted and observed ADG ($R^2 = 0.42$) and a predicted growth that was limited and well below observed values when rates of ADG were greater than 700 g/d. Souza et al. (2016) evaluated the model by using data from 16 studies involving 51 diets for Brazilian dairy calves under tropical conditions. This paper contradicted Hill et al. (2013) and ADG estimated by the energy available from the diet, was found to be overestimated by 19 g/d when the predicted values calculated in NRC (2001) were compared with the observed values from the previous studies (Souza et al., 2016). The correlation between the predicted and the observed values were low for the energy-allowable gain ($R^2 < 0.25$) indicating a low precision for estimating ADG of dairy calves raised in Brazil (Souza et al., 2016).

Dietary changes, biological values, metabolic coefficients for different nutrient types, effect of colostrum intake on health, environmental temperature and management practices that might stress the calf do all affect the calf on an individual level, therefore, the prediction of the required nutrients to obtain a given ADG is highly complex (Hill et al., 2013).

### 3.4 Diseases affecting the actual growth rate

Since calf health is one of the factors that has an impact on ADG, the occurrence of disease will affect the performance of the calf. Disease is often related to a reduced feed intake, so it is of major importance to treat the calves immediately to avoid weight loss. Animals with subclinical infections also eat less, grow slower and convert feed to product in an inefficient manner. The probability of being treated at least once has been found to increase if less concentrate is consumed by the calf (Roth et al., 2009). Also the proportion of days with fever ($\geq 39.5\, ^{\circ}C$) decreased with increasing concentrate intake (Roth et al., 2009).

To understand why sick animals do not eat, interactions between the immune system and the central nervous system, where motivation to eat is controlled has been investigated. The findings suggest that the reduction in feed intake in sick animals is mediated by inflammatory cytokines which induce the secretion of leptin by transferring a message from the immune system to the endocrine system and the central nervous system (Johnson, 1998). The fact that the feed intake is reduced when the calf is exposed to a clinical or a subclinical disease is very unfortunate, because sick calves have increased energy expenditure associated with the increase in body temperature and production of antibodies. This redirecting of energy resources to support immune responses means energy is not available for productive purposes associated with ADG (Carroll and Forsberg, 2007).

Respiratory disease is one of the major challenges when raising calves in an intensive calf production, therefore, a high antimicrobial usage is found among farmers producing bull calves. 79% of the used antimicrobials in 2014 was registered for respiratory disorders when data from a total of 325 Danish herds were analyzed (Fertner et al., 2016). Respiratory diseases have been shown to have a negative effect on ADG in veal calves and an increasing number of bovine respiratory disease (BRD) have led to an increasingly reduced ADG compared to healthy calves (Pardon et al., 2013). The same author found that diarrhea severely increased the mortality risk and the decreased BW.

The accuracy of the diagnosis is important to gain an effective treating program. Schneider et al. (2009) found lung lesions in 61.9% of the cattle which were observed at slaughter ($n = 1,665$). Lung lesions were found in 60.6% of the cattle that were never treated, which indicates that far more calves might suffer from
respiratory diseases than those being treated. Treatment for BRD was associated with an ADG reduction of 25% in the first 4-6 weeks after introduction to a feedlot compared to calves not being treated. The overall ADG from entrance to slaughter was reduced by 5% indicating that the calves suffered the largest losses in performance during the first period after entering the feedlot (Schneider et al., 2009). Also presence of active bronchial lymph nodes (severe lung lesions) have shown to have a negative effect on ADG (Schneider et al., 2009).

Inadequate nutrition can depress the immune function and thereby increase the risk of getting a disease. The incidence of disease is therefore hypothesized to be lower when calves are fed higher levels of milk in the pre-weaning period. A large proportion of the literature does not present the cases of disease in the experiment. Some studies report that calves remained healthy throughout the experiment, but no further information is available. However, the effect of milk volume and frequency on health has been evaluated in some of studies. Conneely et al. (2014) found that offering milk at a rate of 10 or 15% of birth BW did not have any effect on the probability of a calf experiencing a disease a greater number of times. These finding are in agreement with other experiments feeding different levels of milk or MR (Terré et al., 2007; Bach et al., 2013; Yunta et al., 2015). No effect of feeding frequency was found when health data obtained from dairy calves fed the same amount of milk or MR once or twice per day was evaluated (Kehoe et al., 2007; Gleeson and O’Brien, 2012; Conneely et al., 2014).
4 Milk feeding strategies

It is commonly believed that the capacity of the abomasum may be exceeded causing milk in the rumen, if the calf is fed high volumes of milk. This is unwanted since milk in the rumen can disturb the microbial flora and thereby increase the risk of indigestion and diarrhea. In a new study by Ellingsen et al. (2016) no milk in the rumen was found regardless of intake, when radiographing the calf before, during and immediately after intake of more than 5 L per meal. It is widely reported that high volumes of milk increase the ADG in the pre-weaning period (Appleby et al., 2001; Jasper and Weary, 2002; Borderas et al., 2009; de Passillé et al., 2011; Miller-Cushon et al., 2013; Chapman et al., 2016; Chapman et al., 2017). This strategy has also shown to decrease the intake of solid feed in the pre-weaning and weaning period (Jasper and Weary, 2002; Jensen, 2006; Kristensen et al., 2007; Hill et al., 2009), therefore the optimal weaning strategy to gain high ADG without reducing rumen development is widely discussed.

The following sections will present the effect of milk feeding strategies on the intake of solid feed and ADG. Milk FF and the step-down milk feeding strategy will be presented.

4.1 Milk feeding frequency

Automatic milk feeders allow the calf to consume the daily ration in more meals per day without extra labor costs; therefore, knowing the benefits of milk FF is of high interest among farmers that use automatic milk feeders. Conversely, farmers feeding calves manually might have an interest in feeding the calves once daily to save labor time if the performance and health of the calves remains unchanged.

An analysis of published data on the effects of FF on calves found a positive effect on ADG when increasing the FF from one or two meals per day to four in postweaned calves (Gibson, 1981), but there is a paucity of data describing the effects of increasing milk or MR FF on calf growth and health. More studies have investigated the effect of offering calves the same amount of milk fed in either one or two daily meals, where no increase in ADG in the pre-weaning period is reported (Kehoe et al., 2007; Hulbert et al., 2011; Gleeson and O’Brien, 2012; Conneely et al., 2014). However, Leibholz (1975) suggested that calves fed once daily may pass through a period of nutrient deprivation when the supply of nutrients from the gut to the tissues is insufficient to meet the metabolic requirement. Leibholz (1975) found that this period was between 18 and 24 h after the milk was offered. These findings are in disagreement with Williams et al. (1986) who suggested that calves given MR once daily do not pass through such periods with severe nutrient deprivation. However, substrate utilization and energy balance was found to be affected by the milk FF. Periods of increased oxidation of either fatty acids or CP were found when reducing the meals from four to one daily. By increasing the milk FF (1, 2, 4 or 6 feedings per day), no effect on ADG or efficiency of energy utilization was found (Williams et al., 1986).

Previous investigations on the milk FF are presented in Table 2.
Table 2 Effect of milk feeding frequency on ADG and solid feed intake

<table>
<thead>
<tr>
<th>Reference</th>
<th>No of calves</th>
<th>Milk volumes/d</th>
<th>Treatment</th>
<th>Pre-weaning period</th>
<th>Weaning period</th>
<th>Effect on ADG/BW</th>
<th>Effect on solid feed intake</th>
</tr>
</thead>
</table>
| Williams et al. (1986) | 34 bulls | 40g MR per kg M<sup>0.75</sup> | A) 1 daily feeding  
B) 2 daily feedings  
C) 4 daily feedings  
D) 6 daily feedings | Day 9 – 40 | - | No effect on ADG | No information available |
| Kaufhold et al. (2000) | 14 bulls | Adjusted weekly | A) 2 daily feedings (bucket)  
B) 6 daily feedings (automatic feeder) | Week 11 - 13 | - | No effect on ADG | No solid feed was offered |
| Nussbaum et al. (2002) | 14 bulls | 5 – 11 L | A) Meal size was ranging from 0.5 to 1.5 L per portion. Fed by automatic milk feeder.  
B) Meal size was ranging from 2.5 to 5.5 L per portion. Fed by bucket twice a day. | Day 1 - 28 | - | No effect on ADG | No information available |
| Jensen (2004) | 62 heifers  
130 bulls | 6.4 L | A) 4 daily feedings  
B) 8 daily feedings | Day 12 – 70 | Day 64 - 70 | No effect on ADG | No information available |
| von Keyserlingk et al. (2006) | 28 heifers | Ad libitum | A) Access to milk for 24h/d  
B) Access to milk for 2 daily feedings each of 2 hours | Day 5 – 32 | - | No effect on ADG | No effect of intake of concentrate |
| Kehoe et al. (2007) | 70 heifers  
54 bulls | 10% of BW | A) 2 daily feedings  
B) 2 daily feedings until d 14. From d 15 calves were fed once daily. | Weaned at either 3, 4, 5 or 6 weeks of age. | MR was fed at 5% of birth BW once daily 1 week prior respective weaning age | No effect on ADG | Positive effect of treatment B when calves were weaned at 5 or 6 weeks of age (overall intake from week 1-8) |
| Hulbert et al. (2011) | 44 bulls | 4.45 L | A) 1 daily feeding (day 1 – 45)  
B) 1 daily feeding (day 1 – 24 ) and 2 daily feedings (day 24-45) | Day 1 - 45 | Weaned gradually and completely weaned when they consumed 900 g concentrate for 2 consecutive days | No effect on ADG | No effect on concentrate intake measured pre- or post-weaning |
| Sockett et al. (2011) | 70 heifers | Day 1-7: 815g MR  
Day 8-42: 1135g MR  
Day 43-49: 565g MR | A) 2 daily feedings  
B) 3 daily feedings | Day 1 - 49 | Day 43 - 49 | Positive effect on ADG of treatment B | Day 1-42: No effect Day 43-49: positive effect of treatment B (+26%) |
| Kmickiewycz et al. (2013) | 12 heifers  
12 bulls | 10% of BW (20%CP) | A) 2 daily feedings  
B) 4 daily feedings | Day 1 - 42 | | No effect on ADG | Positive effect of treatment B at weaning |
| Kmickiewycz et al. (2013) | 12 heifers  
12 bulls | 13% of BW (26%CP) | A) 2 daily feedings  
B) 4 daily feedings | Day 1 - 42 | | No effect on ADG | No effect on intake at weaning or post-weaning |
### Table 3 Effect of step-down milk feeding method on ADG and solid feed intake

<table>
<thead>
<tr>
<th>Reference</th>
<th>No of calves</th>
<th>Treatment Milk level per day</th>
<th>Pre-weaning period</th>
<th>Weaning</th>
<th>Effect on ADG/BW</th>
<th>Effect on solid feed intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silper et al. (2014)</td>
<td>54 bulls</td>
<td>A) 4L-60d</td>
<td>Day 1 – 60</td>
<td>Abruptly weaned at d 60. Followed until d 90.</td>
<td>Negative effect of treatment A in d 1-30. Positive effect of treatment C in d 60-90.</td>
<td>No effect pre- or post-weaning</td>
</tr>
<tr>
<td>Daneshvar et al. (2015)</td>
<td>40 bulls</td>
<td>A) 5.5L-56d/2L-59d</td>
<td>Day 3 – 59</td>
<td>Weaned at d 60. Followed to d 74.</td>
<td>No effect on ADG</td>
<td>No effect pre- or post-weaning</td>
</tr>
<tr>
<td>Omidi-Mirzaei et al. (2015)</td>
<td>45 bulls</td>
<td>A) 4L-52d/2L-56d</td>
<td>Day 3 – 56</td>
<td>Weaned at d 56</td>
<td>Greatest effect of treatment C on ADG pre-weaning (d 1-56).</td>
<td>No effect of overall intake (d 1-70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B) 6L-29d/4L-60d</td>
<td></td>
<td></td>
<td>Greatest effect of treatment A on ADG post-weaning (d 56-70)</td>
<td>Positive effect of treatment B post-weaning (d 56-70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C) 6L-60d</td>
<td></td>
<td></td>
<td>Greatest effect of treatment C on the overall ADG (d 1-70)</td>
<td>Positive effect of treatment C pre-weaning.</td>
</tr>
<tr>
<td>Leão et al. (2016)</td>
<td>33 heifers</td>
<td>A) 6L-30d/4L-60d/2L-90d</td>
<td>Day 1 – 90</td>
<td>Weaned at d 90</td>
<td>No effect on ADG</td>
<td>Positive effect of treatment A from d 60-90.</td>
</tr>
<tr>
<td>Daneshvar et al. (2017)</td>
<td>40 bulls</td>
<td>A) 5.5L-56d/2L-59d</td>
<td>Day 3 – 59</td>
<td>Weaned at d 60. Followed to d 74.</td>
<td>No effect on ADG</td>
<td>No effect pre- or post-weaning</td>
</tr>
</tbody>
</table>
4.2 Step-down milk feeding method

Step-down (STP) is a milk feeding method, where the calves are fed high amounts of milk or MR in the first weeks of life followed by elevated plane of nutrition preparing the calves for weaning. Calves consume minimal concentrate during the first weeks of life (Eckert et al., 2015) and an increase of concentrate consumption is not seen until four weeks of age (Warner, 1956). A substantial increase in concentrate intake is not seen until the step-down of milk (Hill et al., 2012; Eckert et al., 2015). The fact that the calves almost fully rely on milk consumption in the first weeks of life is important to accommodate by offering the calf greater amounts of milk, but without reducing or delaying the solid feed consumption before weaning.

STP might be a beneficial milk feeding strategy because the milk is reduced gradually and thereby the calf is stimulated to begin feeding on concentrate before weaning. Studies have documented that the gradual decrease of milk provision for dairy calves enhances the intake of solid feed (Sweeney et al., 2010). Less solid feed consumption by the calves fed high volumes of milk is associated with poor post-weaning performances (Baldwin et al., 2004). The post-weaning performance depends on the weaning strategy, therefore, it is difficult to discuss the effect of the milk feeding strategy post-weaning, without paying attention to the weaning strategy. A decrease in ME intake at step-down and weaning results in lower ADG, which is commonly seen, when calves have been fed high amounts of milk (Jasper and Weary, 2002; Sweeney et al., 2010).

Calves fed milk ad libitum had increased ADG during the first 3 to 4 weeks but this effect disappeared when the control group (fed 4 L of milk per day) increased their concentrate intake (Borderas et al., 2009). Total BW gain from d 1 to d 50 has been found to be higher in calves fed high amounts of milk, because of their initial advantage in gain caused by increased milk consumption. The intake of concentrate was increased post-weaning, but the intake of calves receiving high amounts of milk in the milk feeding period remained below the intake of calves fed low amounts of milk (Borderas et al., 2009). Chapman et al. (2016) found that concentrate intake increased quickly reaching amounts similar to calves fed less MR.

Calves fed high levels of milk are seen to have a BW advantage after weaning, because of the higher intake of energy in the milk feeding period. This advantage was seen to last for four weeks post-weaning when weaned at the age of 12 to 13 weeks compared to one week for calves weaned at the age of 6 to 7 weeks. Like milk FF, there is a paucity of data describing the effects of STP on calf growth and health, but some authors have been investigating step-down milk feeding strategies to accommodate the welfare of the calves (Table 3).
5 Feeding behaviors

Feeding behavior is related to health and welfare, therefore, it is of great importance in relation to the performance of the calf. In the following sections feeding behavior will be described in relation to feeding expression, where time spent engaged in feeding activities and unrewarded visits in the automatic milk feeder will be presented. Additionally a short introduction to recording methods used in behavioral experiments will be presented.

5.1 Introduction to feeding behaviors

Feeding behavior is influenced by both the distribution and type of feed offered, which means that feeding patterns are highly affected by the type of production system. A production system where animals are outside allows the ruminants to spend more time grazing and ruminating compared to ruminants in a barn, where the feed is available only at the feeding-through or bunk. Limited space makes it less likely that ruminants will feed at the same time (Tucker, 2009) because competition for feed will change the feeding behavior towards fewer meals per day of a longer duration (Tripon, 2013).

Feeding behavior of calves is highly dependent on suckling behavior since milk is the most preferred and offered energy source in the first months of life. In nature when the dam nurse the calf, the suckling bout frequency is 4 to 10 times a day lasting 7 to 10 min per bout (de Passillé, 2001). The suckling behavior changes from a high frequency of sucklings (5.6 sucklings per day) of short duration (6 min/suckling) to a lower frequency (3.5 per day) but longer duration (10-11min/suckling) at around three weeks of age (Nicol and Sharafeldin, 1975). Also the intervals between sucklings increase after three weeks of age. The calf will start grazing early in life in the presence of the dam (Miller-Cushon and DeVries, 2015) and time spent grazing will increase rapidly with the age (Nicol and Sharafeldin, 1975). The calf’s grazing time has been found to be affected by the dam’s milk production, where higher milk production reduces the time spent on grazing (Nicol and Sharafeldin, 1975), which is in agreement with present studies finding that offering a greater amount of milk will reduce the intake of solid feed (Borderas et al., 2009; Miller-Cushon et al., 2013; Chapman et al., 2016).

Raising calves in a modern production unit restricts the calves in their feeding behavior as they are fed artificially separated from the dam. The different approaches in milk feeding is found in the method of feeding (bucket vs. teat), frequency, milk volume, weaning age, type of solid feed provided and social grouping. The natural suckling behavior of calves can be accommodated by using automatic milk feeders, allowing the calf to consume the daily milk allowance over several meals compared to bucket feeding. By using computer controlled milk feeding, different feeder setups allow the calf to take fewer and larger meals as it gets older, which is similar to the natural situation (Jensen and Holm, 2003; Jensen, 2009).

It is common practice to provide the calf 10% of the BW, which is less than 50% of the ad libitum intake (Appleby et al., 2001; Khan et al., 2011b), therefore, the restricted milk feeding constrains milk meal patterns (Appleby et al., 2001; De Paula Vieira et al., 2008).
5.2 Expression of feeding behaviors

Little is known about the factors that influence the development of feeding patterns. Feeding patterns might be controlled by satiety factors, therefore, the amount and the energy content of the feed ingested has an influence on the meal distribution. It is known that decisions related to feeding are controlled by complex brain functions, memorizing has great importance for feeding development (Tucker, 2009). Meal initiation may on the other hand depend on cognitive factors, and the development of individual feeding patterns may therefore depend on prior experiences and learning (Miller-Cushon et al., 2013). Learning plays a vital role in diet selection because it enables the animal to select nutritious feeds, avoid toxic feeds (Burritt and Provenza, 1989) and enables the animal to memorize where the preferred food sources can be found (Tucker, 2009). Feed delivery is an external stimulation of feeding behavior. Calves are separated from the cow in an early stage of life in the industrialized animal production and all aspects of the calf’s feeding behavior are therefore influenced by management practices. A variety of different management practices in relation to housing and feeding strategies will influence the expression of the feeding behavior.

What type of feed, how much the calf is offered and when it is fed are factors that will influence the expression of the feeding behavior in calves (Miller-Cushon and DeVries, 2011a). Greter et al. (2010) found a positive effect of an early introduction of a total mixed ration (TMR) to heifer calves, resulting in a more even diurnal feeding pattern, reduction of feed bunk competition and a more solid fecal consistency (Greter et al., 2010). Calves that were not previously fed TMR had a greater peak in feeding activity following the feed delivery, which indicates that the feed delivery method affects the expression of feeding.

Milk feeding level affects the diurnal feeding patterns of calves (Miller-Cushon et al., 2013). The differences in early milk feeding patterns do not seem to effect the development of the longer-term feeding patterns, but they might have a short-term influence. When calves are fed restrictively in the milk feeding period, a greater variability in feeding time and meal size has been observed post-weaning compared to calves fed ad libitum milk replacer in a period of 6 weeks (Miller-Cushon et al., 2013). The restrictive milk feeding might have an influence on the feeding patterns post-weaning if calves are consuming large amounts of concentrate and no forage is offered. Great amounts of e.g. barley-based concentrates will possibly induce an acidic ruminal environment (Kristensen et al., 2007), which can increase the variability in the DMI. The feed delivery method is therefore important for the expression of the feeding behavior, if the feeding management affects the development of the GI tract and the immune function in a negative manner.

The ability of calves to select a nutritionally balanced diet prior to weaning is limited, but the composition of the feeds offered may affect the feeding behavior and patterns of dietary selection (Miller-Cushon and DeVries, 2015). Ruminants develop preferences in relation to feed which is associated with positive post-digestive feedback, such as feed paired with e.g. glucose (Burritt and Provenza, 1992). Dietary selection of concentrate might depend on energy content of the feed, when changes in dietary selection in relation to milk allowances occurs, since calves increase concentrate intake relative to hay intake post-weaning (de Passillé et al., 2011; Rosenberger et al., 2017). Calves preferred long particles of hay compared to chopped hay, when calves were trained to work for roughage rewards in the milk feeding period (Webb et al., 2014). The experiment showed that calves are motivated to work for roughage when fed a high energy diet (milk and concentrate) and calves are thereby able to make choices related to the rumen function and motivation to ruminate (Webb et al., 2014).
5.3 Feeding activities

The time spent engaged in different feeding activities is highly dependent on the amount of milk offered. When fresh milk is delivered, calves peak in feeding activity, also when fed ad libitum milk (Appleby et al., 2001). The diurnal feeding activity of calves provided milk at two feeding levels: 1) ad libitum and 2) restricted at 5 L per day is illustrated in Figure 4. As presented in chapter 4, numerous studies have found an association between the amount of milk offered and consumption of solid feed; a large milk intake reduces the concentrate intake significantly. Feeding large amounts of milk will therefore reduce the time spent engaged in feeding activities in relation to solid feed (Miller-Cushon et al., 2013). Housing also has an influence on feeding behavior, where pair-housing has been shown to have a positive effect on the consumption of solid feeds (Hepola et al., 2006; Jensen et al., 2015).

When offering the same milk allowance in four compared to eight portions, the occupancy of the feeder is lower, indicating that fewer and larger portions may be beneficial for competition and access to the milk feeder (Jensen, 2004). The total frequency of visits to the concentrate feeder has been observed to be higher, when calves are fed low compared to high amounts of milk or MR (Jensen, 2006; Borderas et al., 2009) which is due to a high level of unrewarded occupancy (Jensen, 2006). Borderas et al. (2009) found that calves offered ad libitum milk tripled the occupancy time during weaning compared to the pre-weaning period. These findings are in agreement with Jensen (2006). Although the occupancy time was tripled, it was still significantly lower compared to calves fed high levels of milk. Also the occupancy time of the concentrate feeder is found to be higher before and during the weaning period when calves are offered low amounts of milk (Jensen, 2006).

Figure 4: Diurnal feeding activity of calves provided milk at two feeding levels: ad libitum and restricted at 5 L per day (2.5 L provided at 0800 and 1600). Time spent engaged in non-nutritive sucking is shown for calves provided restricted amounts of milk (Miller-Cushon and DeVries, 2015).
5.4 Unrewarded visits

A high frequency of unrewarded visits to the automatic milk feeder are associated with hunger (Jensen and Holm, 2003; Jensen, 2006; De Paula Vieira et al., 2008). Unrewarded visits are undesirable because the calves are able to block the feeder from calves waiting for a rewarded visit and they may disturb the feeding calves (Jensen and Holm, 2003). More than twice the number and duration of unrewarded visits was found in a study where calves were fed a low milk allowance (3.8 to 4.8L) compared to calves fed a high milk allowance (6.4 to 8L) (Jensen, 2006). de Passillé et al. (2011) found that calves fed 10% of their BW had more unrewarded visits to the feeder compared to calves fed double the amount of MR. Fewer signs of hunger were seen when calves were exposed to a delayed weaning at 12 to 13 week of age compared to an early weaning at 6 to 7 week de Passillé et al. (2011). These findings are in agreement with numerous authors who investigated the feeding behavior of calves fed small or large amounts of milk. Calves fed a low amount of milk made most visits just before the next allowance of milk became available (Jensen and Holm, 2003; De Paula Vieira et al., 2008; Borderas et al., 2009).

Unrewarded visits were not affected by the number of calves per feeder or number of milk portions, when calves were fed a similar amount of MR (Jensen, 2004). Likewise no differences in frequency of unrewarded visits to the feeder was found when calves were offered the same milk allowance in four rather than eight milk portions (Jensen, 2004). Longer meal duration may ensure better fulfilment of the need to suck and thereby reduce the frequency of unrewarded visits; therefore, a reduced milk flow rate could be beneficial. Jensen and Holm (2003) found that a reduced milk flow rate caused an increase in the occupancy of the feeder, because the mean duration of the rewarded visits was increased. Also no reduction of the frequency of unrewarded visits was found, when reducing the milk flow rate. According to this study it is therefore not beneficial to reduce the milk flow rate, since more rewarded occupancy will block the feeder and cause competition for calves waiting for a rewarded visit (Jensen and Holm, 2003).

The frequency of unrewarded visits has been found to decrease over time, suggesting that some learning is involved, because unrewarded visits might reflect that calves are challenged in knowing when they are offered their next meal (Jensen and Holm, 2003). During weaning, the number of unrewarded visits will reflect the milk feeding strategy. Calves fed a high milk allowance will visit the feeder more often, because consumption of solid feed is insufficient to provide the calf with the required energy. Oppositely, calves fed lower milk allowances will rely more on consumption of solid feed, and therefore experience less hunger and fewer unrewarded visits will be observed in the weaning period.

5.5 Recording methods

Recording and analyzing feeding patterns in calves is important, because the feeding behavior is related to the health and welfare of calves (Miller-Cushon and DeVries, 2011b). Time sampling techniques are useful when collecting animal behavior data. The choice of technique is important to minimize the time used observing the behavior and maximize the accuracy of the resulting estimate of feeding time. When deciding what method to use, two levels of decisions must be made. Sampling rules covers the distinction between ad libitum sampling, focal sampling, scan sampling and behavior sampling, while recording rules covers the distinction between continuous recording and time sampling. Time sampling is further divided into instantaneous sampling and one-zero sampling. The sample rules specifies which subject to watch and when. How the behavior is recorded is determined by the recording rules (Martin and Bateson, 2007)
5.5.1 Sampling rules
Ad libitum sampling is a method used when whatever is visible and seems relevant at the time is noted down. If this method is used continuously it is a very time demanding method, because all events need to be observed and analyzed. The method is useful when recording rare but important events. Focal sampling is used when the individual animal is observed, often for several individuals of different elements of behavior. When the whole group of subjects is rapidly scanned at regular intervals, the method used is called scan sampling. Here the behavior of each individual in a group is recorded, where a single scan may take from a few seconds to several min, depending on the number of animals scanned. Finally behavior sampling is used when a specific behavior is recorded, such as fights, where the occurrence of a particular element of behavior is recorded together with the observations of the involved individuals from the group (Martin and Bateson, 2007).

5.5.2 Recording rules
Continuous recording measures the behavior patterns from start to end. This method measures each occurrence providing an exact record of both frequency and duration of the behavior. It is clearly the most time-consuming method since the observations need to be continuous and only one animal can be observed at the time. Some behavior may be recorded using time sampling, which measures behavior at regular intervals. This method is less informative and might not give the observer the exact information about the behavior throughout the period recorded or observed if e.g. the behavior occurs in short episodes. Time sampling can be divided into instantaneous sampling and one-zero sampling (Martin and Bateson, 2007).

5.5.3 Instantaneous and One-Zero sampling
The method of instantaneous sampling is dividing the observation sessions into short sample intervals. Unlike continuous recording, the instantaneous method only records the behavior at the given sample points. Instantaneous recording is most accurate when the recording interval is short relative to the duration of the observed behavior (Martin and Bateson, 2007). From an economically perspective it is of high interest to use instantaneous sampling, but without compromising the quality of the data obtained. Miller-Cushon and DeVries (2011b) have compared calf feeding behavior data obtained from continuous recording with data obtained from instantaneous recordings. Feeding time was reasonably accurately estimated by using instantaneous recording at intervals between 2 and 5 min. When increasing the interval time to 10 min no correlation was found between the continuous- and the instantaneous recording. It is not possible to obtain accurate data to describe meal time and meal frequency when using intervals greater than 30 s and 1 min, respectively, because calves that have more frequent meals are likely to have shorter meals, increasing the possibility of individual meals being entirely missed (Miller-Cushon and DeVries, 2011b).

Like instantaneous sampling, one-zero sampling divides the observations into short sample intervals. At the end of each sampling interval it is noted whether the behavior has been observed or not, irrespective of how long or how often the behavior pattern has occurred in the sampling interval (Martin and Bateson, 2007). This method can be useful when measuring a behavior pattern which is rare or if the animal is only active a few hours a day. Then the method can be used to define the period, where the behavior is observed – and another recording method can then be used to describe the pattern of the behavior.
6 Materials and Methods

This study complies with the Danish ministry of Justice Law No. 382 (June 10, 1987), Act No. 726 (September 9, 1993), concerning experiments with animals and the care of experimental animals. The health of the animals was monitored and sick animals were treated by skilled employees from the Danish Cattle Research Centre (DKC), under veterinarian guidance. The experiment is divided into two sub-experiments each consisting of two blocks. The two experiments (1A and 1B) were conducted from September to November 2016 and from February to April 2017, respectively. The initial objective of this study was to analyze data as one experiment, where animals were blocked in four replicates of 16 male Holstein calves (a total of 64 calves). However, major changes between the two sub-experiments argue that data should be handled as two experiments. The timeline of the experimental procedure is illustrated in Figure 5.

![Timeline of the experimental procedure](image)

**Figure 5** Timeline of the experimental procedure.

6.1 Animals and housing

Animals were blocked in four replicates of 16 calves and a total of 64 male Holstein calves were used in the experiment [Age(d), BW(kg) (mean ± SE): 1A = 12.4 ± 1.2, 47.7 ± 1.4, 1B = 10.9± 0.9, 47.1 ± 1.2]. In experiment 1A, 12 calves were born at DKC, where the experiment took place, while the rest of the calves were purchased from 2 dairy farms located within 30 km from DKC. The history of the calves was not known. This was changed in experiment 1B, where all dairy farms wherefrom calves were purchased were visited before the initiation of the experiment. Thereby calves were chosen according to a physical assessment and not only based on day of birth, as in experiment 1A. Calves in experiment 1B were purchased from four dairy farmers (eight calves from each farm). The visit made it possible to obtain knowledge about the calving area, the feeding level and management in relation to calf health, in contrast to experiment 1A. It was not possible to obtain birth weight for any of the calves. All dairy farmers were requested to offer the calves colostrum within the first 6 hours of life. In the first weeks of life, all calves were housed individually with straw bedding and offered whole milk, MR or a mix of both and concentrate.

At arrival at DKC (2 week of age) all calves received injections of 5 mL of vitamin supplement containing vitamin A, D and E (ADEsan Vet). All calves were vaccinated against ringworms, where 2 mL of Bovilis Ringvac Vet was given as a preventive measure. The vaccination was repeated 14 days after arrival. At arrival, the calves were also vaccinated against Bovine Respiratory Syndrome (BRS), where the calves received an injection with 5 mL of Bovilis Bovipast RPS. All calves were reinjected 28 days after arrival. Calves were weighed
both on the day of arrival and the following day and an average BW was calculated to determine the weight at entrance. There were eight calves in each group and calves were housed according to their group in group pens (4.20 x 5.20). The pens were located in a four-sided, roofed but uninsulated stable at DKC. All group pens had sides of tubular metal bars. Each pair of group pens was separated by an open gate, making contact between calves in the two groups possible. The front of each pen had two openings for access to two water bowls, offering the calves water ad libitum. Fresh straw bedding was added every day and a total replacement of the bedding was executed once throughout the 8 weeks experiment.

6.2 Milk feeding procedure

In the milk feeding period all calves were fed milk replacer via an artificial teat in an automated feeder (Urban GmbH & Co. KG, Germany). One Urban CalfMom was used to feed and monitor the four groups of calves. In each group pen there was a milk feeding stall with one teat (one teat for eight calves). The calves were fed 21.6 % CP and 21 % fat calf MR based on whey, plant oil, soya and 13% skim-milk powder (e-lac HP 110, Hamlet Protein, Denmark), mixed at a rate of 140 g/L of water. No citric acid or vitamins were added to the milk solution. The milk feeder was automatically cleaned once a day throughout the milk feeding period at 11am.

The Urban feeding day ran from 9am to 9am and the feeding method was used according to the Urban programming biological feeding, where calves fed RES was allowed MR when a minimum portion of 2 L was earned. At least 5.5 hours had to pass between successive milk portions (0.061L/10 min). If the calf did not consume the milk allowance, the remaining MR was not transferred to the next feeding day.

On the day of arrival all calves were introduced to the feeding stall and guided to the artificial teat, where a portion of approximately two liters of MR was offered. When the calf entered the feed stall it was recorded by the electronic ear tag. The age of the calves varied at entrance (1A: from 4 to 25 d, 1B: from 6 to 17 d) but all calves were coded to be 14 days in the milk feeder PC to allow all calves irrespective of age at entrance to receive MR for 42 d. The computer then automatically recognized the calf and how much MR it was allowed and whether it was permitted to feed at that specific time of the day, when a calf tried to enter the feeder. The calves arrived at DKC on a Wednesday and were offered 6.5 L of MR per day with the restriction of 2.3 L per meal for the first two Urban Feeding Days. Guidance of the calves to the artificial teat was practiced in the two first days and a bar was placed behind the calf to keep the calf in the feeder while MR was permitted to the calf. Friday at 9am the experiment began (start of week 3) and all calves were allocated to their treatment groups and allowed MR for 42 days. The calves were continuously trained by using the bar once a day, if less than four liters of MR per calf were consumed of the allowed daily ration.

6.3 Experimental design and diets

16 calves were allocated to one block consisting of two groups (Figure 7): 1) CON (all calves were offered 6.5 L of MR in week 3 to 6 (d 1 to 28 of the study) and weaned in a period of two weeks offered 4 L/d in week 7 and 2 L/d in week 8) and 2) STP (all calves were offered 8 L/d of MR in weeks 3 to 4 (d 1 to 14 of the study), 5 L/d in weeks 5 to 6 and weaned in a period of two weeks offered 4 L/d in week 7 and 2 L/d in week 8). All calves in the experiment were offered a total of 224 L of MR throughout the milk feeding period (Figure 6).
Materials and Methods

Figure 6 Milk feeding method for calves fed through conventional (CON) and step-down (STP) procedures. Calves were coded to be 14 d of age in the milk feeder PC at entrance to the experimental unit, and all calves were weaned at d 42 (56 d of age).

Figure 7 Experimental design used in experiment 1A conducted in September - November 2016 and experiment 1B conducted in February - April 2017. In each experiment, 32 calves were allocated to two blocks, each consisting of two group-pens. Calves were fed either by conventional (CON) or step-down (STP) milk feeding procedures. In each group calves were allocated to a restricted (RES) or unrestricted (URES) allowance to the daily milk ration.
All calves were offered hay ad libitum (Grønhø Plus, DLG, Denmark, Table 4) and concentrate consisting of 22% crude protein and 5.5% fat (26067 Kalvestart Hamlet, Vestjyllands Andel, Denmark, Table 4). Hay was provided in a shared trough in each group, and measurements of the individual intake were not possible to obtain. In experiment 1A, the concentrate was offered by an automatic concentrate feeder (Urban GmbH & Co. KG, Germany) connected to the same computer as the artificial teat.

Table 4 Chemical composition of feed components

<table>
<thead>
<tr>
<th>Chemical composition¹</th>
<th>Calf Milk Replacer²</th>
<th>SD</th>
<th>Concentrate³</th>
<th>SD</th>
<th>Hay⁴</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>97</td>
<td>0.21</td>
<td>89</td>
<td>0.00</td>
<td>88</td>
<td>0.42</td>
</tr>
<tr>
<td>CP (% of DM)</td>
<td>21.6</td>
<td>0.03</td>
<td>22.0</td>
<td>0.31</td>
<td>14.2</td>
<td>0.03</td>
</tr>
<tr>
<td>CF (% of DM)</td>
<td>21.1</td>
<td>0.12</td>
<td>5.4</td>
<td>0.23</td>
<td>3.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Starch (% of DM)</td>
<td>-</td>
<td>-</td>
<td>3.6</td>
<td>0.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sugar (% of DM)</td>
<td>28.3</td>
<td>0.18</td>
<td>-</td>
<td>-</td>
<td>12.2</td>
<td>0.43</td>
</tr>
<tr>
<td>aNDF (% of DM)</td>
<td>-</td>
<td>-</td>
<td>15.6</td>
<td>0.08</td>
<td>5.3</td>
<td>0.16</td>
</tr>
<tr>
<td>iNDF (g/kg NDF)</td>
<td>-</td>
<td>-</td>
<td>199</td>
<td>0.00</td>
<td>210</td>
<td>0.11</td>
</tr>
<tr>
<td>Ash (% of DM)</td>
<td>6.6</td>
<td>0.01</td>
<td>65.2</td>
<td>0.00</td>
<td>8.65</td>
<td>0.06</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.67</td>
<td>0.02</td>
<td>0.93</td>
<td>0.03</td>
<td>0.36</td>
<td>-</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.63</td>
<td>0.00</td>
<td>0.46</td>
<td>0.01</td>
<td>0.28</td>
<td>-</td>
</tr>
<tr>
<td>Na (%)</td>
<td>0.38</td>
<td>0.00</td>
<td>0.27</td>
<td>0.01</td>
<td>0.19</td>
<td>-</td>
</tr>
<tr>
<td>K (%)</td>
<td>1.30</td>
<td>0.07</td>
<td>0.95</td>
<td>0.01</td>
<td>1.88</td>
<td>-</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.16</td>
<td>0.01</td>
<td>0.21</td>
<td>0.00</td>
<td>0.22</td>
<td>-</td>
</tr>
<tr>
<td>Fe, mg/kg</td>
<td>139</td>
<td>7.07</td>
<td>221</td>
<td>6.36</td>
<td>299</td>
<td>-</td>
</tr>
<tr>
<td>Mn, mg/kg</td>
<td>78</td>
<td>4.31</td>
<td>96</td>
<td>2.83</td>
<td>110</td>
<td>-</td>
</tr>
<tr>
<td>Zn mg/kg</td>
<td>83</td>
<td>1.70</td>
<td>101</td>
<td>5.44</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>Cu, mg/kg</td>
<td>6</td>
<td>0.77</td>
<td>13</td>
<td>0.21</td>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Values obtained from chemical analysis of two feed samples.
² Soya protein based calf milk replacer supplied by Hamlet Protein (Denmark) containing (% in feed) 13% skimmed milk powder, 48.3% whey, 17.6% vegetable oil, 20% HP 110, 0.8% minerals and vitamins and 0.3% Lysine HCl.
³ Calf starter concentrate supplied by Hamlet Protein (Denmark), containing (% in feed) 34.88% barley, 23.17% concentrate from soya protein, 18.04% wheat, 9% grass powder, 5% corn, 3% dried sugar beet.
⁴ Grass hay, Grønhø Plus, supplied by DLG (Denmark). Minerals were analyzed in one feed sample only.

Experiment 1A
In the first two weeks (week 3-4) of the experiment a trough with concentrate was placed in each group pen to lessen the number of new activities introduced. The guidance of the calves using the automatic concentrate feeder began in the second week of the experiment and the trough was removed two weeks after arrival to DKC. The guidance was practiced in the same way as for the milk feeder and all calves were trained until four weeks of age. In the following weeks calves consuming less than 100 g per day were trained once daily.

Experiment 1B
The use of the automatic concentrate feeder caused some challenges in experiment 1A which is why the calves in experiment 1B were offered concentrate in a shared trough throughout the entire experiment. This means that no individual measurements of both hay and concentrate were possible to obtain in exper-
iment 1B. Therefore, it is not possible to evaluate the effect of RES and URES on the individual intake of solid feed, since one group includes both RES and URES calves. The decision to exclude the automatic concentrate feeder was based on doubts about the function of both the milk- and concentrate feeder. Thus, it is assumed that the function of the electronic reader to identify the calves is limited when both feeders are installed closely to each other.

6.4 Measurements: Intake and growth

In experiment 1A, Intake of MR and concentrate consumed from the automatic milk- and concentrate feeder were recorded daily. Numbers of visits and intake per visit was recorded and saved by the computer connected to the Urban CalfMom controlling all automatic feeders in the experiment. In the first two weeks, calves were offered concentrate from a trough where intake was measured three times a week and fresh concentrate was offered and orts replaced. The same procedure was followed when measuring the intake of hay. Orts was removed three times a week and fresh hay was offered whenever they needed a refill to make sure all calves had ad libitum access to hay. All calves were weighed once a week (Thursday). At arrival, weaning and at the end of the experiment, all calves were weighed on two consecutive days (Wednesday and Thursday) to obtain an accurate weekly weight and to account for day-to-day variability.

In experiment 1B, intake of MR was recorded as in experiment 1A. Concentrate and hay was provided from a trough throughout the entire experiment and orts were removed daily and replaced with fresh feed. The intake of feed was calculated by subtracting orts from the amount provided. All calves were weighed once a week (Friday). At arrival, weaning, and in the end of the experiment, all calves were weighed on two consecutive days (Wednesday and Thursday).

6.5 Animal health

All calves were assessed daily for general health and rectal temperature was measured if the calf showed disease symptoms. Calves were given coal orally twice a day for a period of minimum five days if they showed diarrhea symptoms. This strategy was chosen to prevent calves getting watery diarrhea and fever. In severe situations the calves were bottle fed 2 L of electrolyte solution to prevent dehydration and to stabilize the GI-tract. If the calves were exposed to fever and signs of diarrhea were recorded, the calf was given antibiotics.

Experiment 1A

All calves entered DKC in two groups of 16 calves on the 21st of September and 28th of September, respectively, meaning that block 1 was one week ahead of block 2 throughout the experiment. More than 20% of the calves in block 2 were classified as ill the day after arrival so all calves were treated with antibiotics (Draxin Injectable Solution) and Loxicom for pain relief. This treatment contributed to the high number of calves being treated against respiratory disease in the experiment. In total 23 calves were treated against respiratory disease, and nine calves were treated more than once throughout the experiment. All treatments in the experimental period are given in Table 5.

In total, two animals died suffering from an acute intestinal infection (STP-URES) and an acute pneumonia or BRS virus (CON-URES). The first calf died within the first week of the experiment, therefore, a new calf from the same dairy farmer was bought and introduced to the experiment on the same day as the dead calf
was found. The second calf died in week 6, and therefore, no experimental animal was replaced. A Holstein bull calf similar in age and weight to the 31 experimental calves was introduced as a ‘dummy’ to the group to even the group size among the treatments.

**Experiment 1B**

All calves entered DKC on the 1st of February. In total 28 calves were treated against respiratory disease, and 12 calves were treated more than once throughout the experiment. All treatments in the experimental period are given in Table 5. No calves were replaced in the experimental period and data is therefore obtained from 32 calves.

**Table 5** Number of calves treated during the two experiments (1A and 1B). The incidence of gastrointestinal disease was limited and prevented by giving charcoal orally. The number of treatments is given in the parenthesis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con-Res</th>
<th>Con-Ures</th>
<th>Stp-Res</th>
<th>Stp-Ures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>7 (11)</td>
<td>7 (10)</td>
<td>6 (9)</td>
<td>7 (14)</td>
</tr>
<tr>
<td>1B</td>
<td>7 (10)</td>
<td>6 (9)</td>
<td>7 (14)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coal</td>
<td>8 (8)</td>
<td>7 (7)</td>
<td>8 (8)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>-</td>
<td>2 (2)</td>
<td>-</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>-</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>-</td>
<td>-</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>2 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Otitis media</td>
<td>1 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6.6 Feed sampling and analysis

To determine DM and nutrient content of the feed offered in the experiment, samples of hay, concentrate and MR were collected weekly throughout the experiment (for 1A and 1B, respectively) and a chemical analysis was performed to analyze the content of nutrients and minerals (Ca, P, Mg, K, Na, Fe, Mn, Zink, Cu) (Eurofins, Denmark). The chemical components of the feeds are given in Table 4.

6.7 Behavioral data collection

No behavioral data were collected in the experiment 1A. In experiment 1B video-recordings of the behavior of all calves was stored continuously throughout the study. Cameras were connected to a digital video recorder. Four cameras covered each group. One of the cameras was positioned above the group and, thereby, able to record the entire group while the three others were positioned to cover the artificial teat, the concentrate trough and the hay rack. In Experiment 1B the additional behavioral data was obtained by direct observation and this data was used to analyze the foraging behavior (feeding time) during two of the periods where changes in the MR ration occurred (week 4 and week 7). Feeding behavior was also measured using direct observation in week 9 (one week post-weaning). The direct observations were executed the day before (Thursday) and after (Saturday) the change in milk allowance (Friday) (Table 6).
Prior to the direct observations the time of day with the highest activity (7am to 11am and 14pm to 16pm) were determined from continuous video recording for four 24 hour periods. Only these hours were included in the direct observations. To observe the time spent on foraging activities, the four groups were observed instantaneously, where the observation session was divided into short sample intervals of 5-min (Miller-Cushon and DeVries, 2011b). At the beginning of each sample point, a record was made of whether the calf was standing (S), and if the calf was standing it was noted whether it was eating from the trough of concentrate (C), eating from the through of hay (H), or if the calf was occupying the milk feeder (M) (Table 7). At any instant each calf could only be recorded as standing, or not. If standing the calf could be recorded as either ‘concentrate feeder’, ‘hay trough’ or ‘occupying milk feeder’. All calves were marked for individual identification prior to the onset of the behavioral recordings. The data was collected on a hand-held MacBook using the program ‘Excel’ (2010). In the program each sample interval was pre-defined (lines) and each calf was indicated (columns) as presented in the Appendix (Figure 5).

### Table 6 Calf age, milk allowance and weekday of direct observations.

<table>
<thead>
<tr>
<th>Calf age, week*</th>
<th>Weekday</th>
<th>Milk allowance, L/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Thursday</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Saturday</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Thursday</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Saturday</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Thursday</td>
<td>0</td>
</tr>
</tbody>
</table>

* A new week started Friday

---

6.8 Fecal sampling and assessment

Fecal sampling, measurement of rectal temperature, determination of color and consistency of the manure and assessment of the cleanliness of the hind part of the calf were performed on the last day of the experiment (week 10). All calves were handled individually. The rectal temperature was measured followed by fecal sampling from the rectum using plastic gloves to stimulate the calf. The sample was collected in plastic cups identified with the number of the individual calf. The samples were immediately weighed (including the cup) and dried at 60°C for 72 hours in a forced air oven. When removed from the oven, the samples were weighed again. A standard weight of the cup (8.28 g) was subtracted from all measures and the dry matter content of each sample was measured. The consistency and color of all samples were determined right after sampling.
The color of the manure was determined based on a scale ranging from 1 to 6:

- 1: Red/pink (bloody)
- 2: Grey
- 3: Yellow
- 4: Brown
- 5: Green
- 6: Very dark/black

The consistency of manure was determined based on a scale used for manure from cows and ranged from a score of 1 to 5:

- Score 1: Manure is thin and aqueous. It is difficult to see, that it is manure.
- Score 2: Manure is thin and like gruel. Compared to score 1 it looks like manure.
- Score 3: Consistency like a thick porridge.
- Score 4: Manure is thick and prints are seen when fingers are removed.
- Score 5: Solid droppings. Prints are more distinct.

The cleanliness of the hind part of all calves was determined based on a scale ranging from score 1 to 5:

- Score 1: Clean
- Score 2: A little of the hind part is dirty
- Score 3: Some of the hind part is dirty
- Score 4: A big part of the hind part is dirty
- Score 5: Most of the hind part is dirty

6.9 Blood samples

In experiment 1A blood samples were collected on d 1 (Friday, week 3) and d 56 (Thursday, week 10) from 10am to 11am. All calves were handled individually and samples were taken by the vena jugularis. Two samples were collected to be used for serum and plasma analysis, respectively. Blood used for serum analysis was kept at room temperature, while blood collected for plasma analysis was kept on ice when transported from the barn to the laboratories. All samples were labelled and stored in a deep freezer until analyzed (-20°C). Blood was analyzed in the laboratory at Aarhus University, Foulum, where NEFA, glucose, urea and BHB was analyzed by spectrophotometry using the auto analyzer ADVIA 1800. IgG was analyzed with ELISA assays measured by an ELISA reader (Galaxy Polarstar).

6.10 Calculations

All calculations and illustrations were made in Microsoft Excel 2010. The feed conversion efficiency (FCE) and consumption of milk, hay and concentrate was calculated weekly, and the average intake per day was calculated within each week. The ADG was calculated based on one or two weekly weights. The birth weight was not known, therefore, this variable was determined to 43 kg based on the average birth weight of 705 bull calves born at DKC in the period 2011 to 2016. Metabolisable energy (ME) was calculated in the
Materials and Methods

NRC dairy evaluation program (NRC, 2001) with values from the analyses for MR, hay and concentrate. Concentrations of ME for hay, concentrate and MR were estimated to be 8.50, 13.73 and 20.18 MJ/kg, respectively.

Experiment 1A
One dead calf was replaced in week 3 and therefore no ADG was obtained for the new calf from week 2 to week 3. The ADG in week 3 and entrance weight (week 2) were estimated by calculating the ADG from birth (43 kg) to entrance, by using the weight and age at the day of replacement. The consumption of milk, concentrate and hay was estimated using values of the mean intake for the given period measured of calves allocated to same treatments as the replaced calf in the experiment.

6.11 Statistical analysis
Each experiment (1A and 1B) included 2 blocks (B) of 16 calves. In experiment 1A one calf was lost, so only 31 calves were included from this experiment. Calves were blocked according to herd of origin and placing in the barn. Thus, in 3 of the 4 blocks, 8 calves from each of 2 herds founded a block. However, in experiment 1A, 12 calves from one herd and 4 calves from another herd founded block 1. As mean age and BW was slightly different between herds, there was also a difference in mean BW and age at start between blocks 1 and 2 within each experiment.

Most analyses were made separately for the two experiments. Data was analyzed separately for two weeks periods (week 3-4, week 5-6, week 7-8, week 9-10), preweaning period (week 3-8) and overall period (week 3-10). Dry matter intake, BW, ADG, FCE, behavioral observations and blood variables (glucose, NEFA, Urea, BHB and IgG) were analyzed by analysis of variance as a randomized complete block design using the GLM procedure of SAS (SAS Inst. Inc., Cary NC / SAS Institute, 2001). The variables were analyzed using the following model:

\[ Y = \mu + B + T1 + T2 + T1 \times T2 + e, \]

where \( Y \) is the response variable, \( \mu \) is the overall mean, \( B \) is the block effect, \( T \) is the treatment effect, where \( T1 \) is the treatment effect of STP and CON, \( T2 \) is the treatment effect of RES and URES, and \( e \) is the residual error term.

Means were tested using Bartlett’s test for homogeneous variance. The Bartlett’s test only showed variance heterogeneity for the following variables: Occupancy time in the milk feeder obtained in week 4, total standing time in week 4 and 5, and time spent standing inactively in week 5. However, based on statistical experiences it was considered that also these variables were robust enough to be analyzed without any transformations.

Data were tested for normality of the residuals by evaluating the QQ-plots constructed using the UNIVARIATE procedure of SAS. Type 2 SS was used for the F-test. All values were given as LS means ± SEM and significance of effects was declared at \( P < 0.05 \) and tendencies at \( P < 0.10 \).

Weekly weights and ADG for the entire experiments were considered as repeated measures and analyzed as a split-plot design using the autoregressive 1st order covariance structure (AR-1) in the MIXED model.
procedure of SAS. The MIXED model included the fixed effects of block, treatments, and week and the 2- and 3-way interactions between treatments and week.

Calf was designated as a random effect and included as calf(T1*T2) for testing the effects of the main-plot factors (T1, T2 and T1*T2). The Kenward-Roger method was used for calculation of the degrees of freedom. The variables were analyzed using the following model:

\[ Y = \mu + B + T1 + T2 + T1 \times T2 + \text{calf}(T1 \times T2) + \text{week} + T1 \times \text{week} + T2 \times \text{week} + T1 \times T2 \times \text{week} + e \]

Additionally, weight and ADG data from experiment 1A and 1B were analyzed as one experiment, referred to as experiment 1AB. Data was analyzed separately for two weeks periods (week 3-4, week 5-6, week 7-8, week 9-10), preweaning period (week 3-8) and overall period (week 3-10). With one observation per calf (n=63), data were analyzed using the GLM procedure of SAS. The model included the combined effect of the two experiments and the two blocks within each experiment. The variables were analyzed using the following model:

\[ Y = \mu + \text{EXP} + B(\text{EXP}) + T1 + T2 \times T1 + e, \]

Where EXP is the experiment and B(EXP) is the block within each experiment. Also, weekly weights and ADG were analyzed using the MIXED model procedure of SAS:

\[ Y = \mu + \text{EXP} + B(\text{EXP}) + T1 + T2 + T1 \times T2 + \text{calf}(T1 \times T2) + \text{week} + T1 \times \text{week} + T2 \times \text{week} + T1 \times T2 \times \text{week} + e, \]

where all factors are designated as described above.
7 Results

The results obtained from the two experiments 1A and 1B are presented separately. Feed intake, performance and fecal score were recorded and analyzed in both experiments. Blood metabolites and behavioral observations are only analyzed for experiment 1A and 1B, respectively. ME intake is calculated by using the computer model from NRC (2001), which calculates the values for varied proportions of DMI from MR and solid feed. Treatment 1 (CON and STP) and treatment 2 (RES and URES) will be referred to as T1 and T2, respectively. Additionally, weight and ADG data from experiment 1A and 1B were analyzed as one experiment, referred to as experiment 1AB.

7.1 Experiment 1A

7.1.1 Blood metabolites

Data of blood metabolites are listed in Table 8. No difference in blood glucose was observed between treatment groups on d 1 of study. A significant effect (P=0.047) of T2 was found at the end of the trial at d 56 where calves fed URES had a higher concentration of glucose in blood plasma. No difference in concentration of blood NEFA, Urea and BHB were observed among treatment groups. No knowledge about colostrum feeding; quality, quantity and time of feeding after calving was obtained from the dairy herds. Minimum and maximum values of IgG were found to be 0.29 and 28.37 mg/ml for calves being 8 and 13 d of age at the day of blood sampling, respectively. The IgG concentration was found to be affected by T2 at the end of the trial, calves fed RES had a greater concentration of IgG compared to calves fed URES (13.5 and 11.0 mg/ml, respectively).

Table 8 Blood concentrations of metabolites in Holstein bull calves fed milk replacer either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1A.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CON</th>
<th>STP</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>RES</td>
<td>URES</td>
<td>RES</td>
</tr>
<tr>
<td>Day 1</td>
<td>6.18</td>
<td>6.49</td>
<td>5.83</td>
</tr>
<tr>
<td>Day 56</td>
<td>5.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEFA, µekv/L</td>
<td>Day 1</td>
<td>122</td>
<td>226</td>
</tr>
<tr>
<td>Day 56</td>
<td>71.0</td>
<td>56.8</td>
<td>87.3</td>
</tr>
<tr>
<td>Urea, mM</td>
<td>Day 1</td>
<td>2.36</td>
<td>2.23</td>
</tr>
<tr>
<td>Day 56</td>
<td>3.86</td>
<td>4.18</td>
<td>3.44</td>
</tr>
<tr>
<td>BHB, mM</td>
<td>Day 1</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>Day 56</td>
<td>0.45</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td>IgG mg/ml</td>
<td>Day 1</td>
<td>11.1</td>
<td>11.4</td>
</tr>
<tr>
<td>Day 56</td>
<td>12.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
7.1.2 Fecal score

No differences in DM, consistency, cleanliness and rectal temperature were found between treatment groups in week 10 (Table 9). The score of fecal color was significant greater (P=0.02) for calves fed STP, indicating a more green/darker fecal color, compared to calves fed CON.

Table 9 Fecal score and rectal temperature in Holstein bull calves fed milk replacer either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1A.

<table>
<thead>
<tr>
<th></th>
<th>CON RES</th>
<th>CON URES</th>
<th>STP RES</th>
<th>STP URES</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>17.53</td>
<td>17.36</td>
<td>16.5</td>
<td>15.76</td>
<td>1.47</td>
<td>NS</td>
</tr>
<tr>
<td>Color*</td>
<td>4.00a</td>
<td>4.00a</td>
<td>4.63b</td>
<td>4.27b</td>
<td>0.20</td>
<td>0.02 NS</td>
</tr>
<tr>
<td>Consistency**</td>
<td>2.88</td>
<td>2.75</td>
<td>2.88</td>
<td>2.69</td>
<td>0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Cleanliness**</td>
<td>1.88</td>
<td>1.63</td>
<td>2.06</td>
<td>1.90</td>
<td>0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.94</td>
<td>38.89</td>
<td>38.75</td>
<td>38.75</td>
<td>0.15</td>
<td>NS</td>
</tr>
</tbody>
</table>

Calves were 10 weeks of age. The method of fecal sampling and scoring is described in section 6.8.

*The scale was ranging from 1 to 6  **The scale was ranging from 1 to 5
a,b Means within a row with different superscripts are significantly different (P < 0.05)

7.1.3 Feed intake and performance

Feed intake, weight and ADG of calves during the pre-weaning, weaning and post-weaning period are listed in Table 10. Milk data reflects the treatments and a significant effect of T1 in week 3-4 and week 5-6 is therefore a result of the experimental setup. In week 5-6 significant effects were also found of T2 and T1xT2. The MR intake in week 5-6 was lower for calves fed CON-URES (5.73 L/d) compared to calves fed CON-RES (6.25 L/d).

No differences in concentrate intake were found in the first two weeks of the experiment, but T1 was observed to have a positive effect on the intake of concentrate in week 5-6 (P=0.005), when calves were fed STP compared to CON. No other periods showed any differences of concentrate intake, when comparing the observations between the treatment groups. No statistical analyses was performed on the intake of hay, but a numerical higher intake of hay was generally found on group level, when calves were fed STP compared to CON. The intake of concentrate was found to be affected by block (P=0.011), where calves in block 2 only consumed 73 % of the total amount consumed by calves in block 1. No differences between treatment groups were found in concentrate intake. The relationship between intake of concentrate in the pre- and post-weaning period is illustrated in Figure 8 ($R^2 =0.42$).

No differences were found, when the DMI, ME and FCE were evaluated among the treatment groups. In the pre-weaning period calves fed CON-RES tended (P=0.06) to use less MJ per kilo of gained BW compared to the other treatment groups, when FCE was not including the intake of hay.

No differences in BW were found when comparing the four treatment groups. As expected, week had a significant effect (P<0.001) and all weeks differed (Figure 9). The ADG in week 5-6 was significant affected by T1 and T2, and calves fed CON-RES were found to have a higher ADG compared to the three other treatment groups. T1 x week was significant for weekly ADG (Figure 10) and ADG (Figure 11) (P =0.046 and P=0.031, respectively). No correlation between observed and predicted ADG was found, when the predicted ADG was calculated by using the computer version of NRC (2001) (Figure 12).
**Table 10** Dry matter intake and performance during pre-weaning, weaning and post-weaning stages in Holstein bull calves fed milk replacer either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1A.¹

<table>
<thead>
<tr>
<th>Week 3-4</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (L/d)</td>
<td>5.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concentrate (kg/d)</td>
<td>0.03</td>
<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Hay (kg/d)</td>
<td>0.03</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>52.9</td>
<td>51.6</td>
<td>53.0</td>
<td>53.0</td>
<td>2.40</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.41</td>
<td>0.40</td>
<td>0.48</td>
<td>0.45</td>
<td>0.08</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 5-6</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (L/d)</td>
<td>6.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Concentrate (kg/d)</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.005</td>
</tr>
<tr>
<td>Hay (kg/d)</td>
<td>0.09</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>64.6</td>
<td>60.8</td>
<td>62.1</td>
<td>60.9</td>
<td>2.59</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06</td>
<td>0.046</td>
</tr>
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<table>
<thead>
<tr>
<th>Week 7-8</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Buck (kg/d)</td>
<td>2.92</td>
<td>2.94</td>
<td>2.94</td>
<td>2.96</td>
<td>0.02</td>
<td>NS</td>
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<tr>
<td>Concentrate (kg/d)</td>
<td>0.86</td>
<td>0.97</td>
<td>0.97</td>
<td>1.01</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Hay (kg/d)</td>
<td>0.20</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>77.2</td>
<td>72.5</td>
<td>75.2</td>
<td>72.7</td>
<td>3.47</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.91</td>
<td>0.90</td>
<td>0.90</td>
<td>0.85</td>
<td>0.09</td>
<td>NS</td>
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<table>
<thead>
<tr>
<th>Week 9-10</th>
<th>Post-weaning</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buck (kg/d)</td>
<td>1.97</td>
<td>1.84</td>
<td>2.11</td>
<td>1.94</td>
<td>0.20</td>
<td>NS</td>
<td>NS</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 3-8</th>
<th>Pre-weaning</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buck (kg/d)</td>
<td>4.99</td>
<td>4.69</td>
<td>4.82</td>
<td>4.82</td>
<td>0.09</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Concentrate (kg/d)</td>
<td>0.34</td>
<td>0.40</td>
<td>0.43</td>
<td>0.45</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Hay (kg/d)</td>
<td>0.11</td>
<td>0.13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>64.9</td>
<td>61.6</td>
<td>63.4</td>
<td>62.2</td>
<td>2.77</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.74</td>
<td>0.65</td>
<td>0.70</td>
<td>0.63</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>1.07</td>
<td>1.09</td>
<td>1.15</td>
<td>1.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ME* (MJ/d)</td>
<td>18.55</td>
<td>18.71</td>
<td>19.55</td>
<td>19.76</td>
<td>0.92</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ME** (MJ/d)</td>
<td>19.64</td>
<td>19.68</td>
<td>20.64</td>
<td>20.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FCE* (MJ/kg)</td>
<td>25.41</td>
<td>29.35</td>
<td>29.39</td>
<td>31.74</td>
<td>1.72</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FCE** (MJ/kg)</td>
<td>23.53</td>
<td>21.27</td>
<td>22.69</td>
<td>21.65</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Week 3-10</th>
<th>Trial period</th>
<th>CON (RES)</th>
<th>CON (URES)</th>
<th>STP (RES)</th>
<th>STP (URES)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buck (kg/d)</td>
<td>0.75</td>
<td>0.76</td>
<td>0.85</td>
<td>0.82</td>
<td>0.08</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Concentrate (kg/d)</td>
<td>0.14</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>72.1</td>
<td>68.2</td>
<td>70.8</td>
<td>69.0</td>
<td>3.15</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.87</td>
<td>0.80</td>
<td>0.87</td>
<td>0.81</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ Milk and concentrate data were averaged weekly (kg/d) for each calf. Hay was only measured on group level (n=8 calves). ADG is illustrating the total body weight gain from day 1 until the respective period.

* The calculation is based on the daily intake of milk and concentrate. Data was obtained from the automated milk feeder and automatic concentrate feeder.

** The calculation was based on the intake of milk, concentrate and hay, where the intake of hay was measured on group level (n=8 calves). The daily intake is thereby an average estimate calculated on the gross intake.

<sup>a</sup> Means within a row with different superscripts are significantly different (P < 0.05)
**Results**

**Figure 8** Relationship between intake of concentrate in the pre- and post-weaning periods. Data was obtained for 31 calves consuming concentrate from an automatic concentrate feeder in experiment 1A.

![Intake of concentrate](image1.png)

**Figure 9** Body weight (BW) of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration in experiment 1A. All weeks are significantly different (P<0.001).

![Body weight](image2.png)
Results

Figure 10 Weekly ADG (kg/d) of Holstein bull calves fed either by conventional (CON) or step-down (STP) in experiment 1A. Weeks with different superscripts are significantly different (P<0.05). * CON and STP differs (P<0.05) within week.

Figure 11 ADG of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures in experiment 1A. Weeks with different superscripts are significantly different (P<0.05).
Figure 12 Relationship between ADG (kg/d) observed in the milk feeding period (week 3-8) determined by weekly BW measurements and the predicted ADG (kg/d) by NRC (2001) estimated when knowing the individual feed intake of Holstein bull calves in experiment 1A.

7.2 Experiment 1B

7.2.1 Fecal score

Results are listed in Table 11. No differences in color, consistency, cleanliness and rectal temperature were found between treatment groups in week 10. T1 x T2 tended (P=0.07) to have an effect on fecal DM (%).

Table 11 Fecal score and rectal temperature in week 10 measured in Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1B.1

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>STP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RES</td>
<td>URES</td>
<td>RES</td>
<td>URES</td>
</tr>
<tr>
<td>DM (%)</td>
<td>17.51</td>
<td>14.77</td>
<td>16.32</td>
<td>17.77</td>
</tr>
<tr>
<td>Color*</td>
<td>4.00</td>
<td>4.00</td>
<td>4.13</td>
<td>4.00</td>
</tr>
<tr>
<td>Consistency**</td>
<td>3.06</td>
<td>3.11</td>
<td>3.00</td>
<td>2.69</td>
</tr>
<tr>
<td>Cleanliness**</td>
<td>2.00</td>
<td>2.13</td>
<td>1.88</td>
<td>1.63</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.64</td>
<td>38.98</td>
<td>38.81</td>
<td>38.98</td>
</tr>
</tbody>
</table>

1 Calves were 10 weeks of age. The method of fecal sampling and scoring is described in section 6.8

*The scale was ranging from 1 to 6 **The scale was ranging from 1 to 5
7.2.2 Feed intake and performance

Feed intake, BW and ADG of calves during the pre-weaning, weaning and post-weaning period are listed in Table 12. As in experiment 1A, milk data reflects the treatment and a significant effect of T1 in week 3-4 and week 5-6 is therefore a result of the experimental setup. In week 5-6 significant effects were also found of T2 and of the interaction between treatment 1 and 2. The MR intake in week 5-6 was lower for calves fed CON-URES (6.4 L/d) compared to calves fed CON-RES (6.5 L/d).

No statistical analysis was performed on the intake of hay and concentrate. However, a numerical higher intake of concentrate was generally found throughout the experiment, when calves were fed STP compared to CON. The DMI, ME and FCE were calculated on group level and no statistical analyses were conducted.

No differences in BW were found when comparing the four treatment groups, but all weights were different between weeks (P<0.001). ADG measured from week 3-10 showed a tendency (P=0.08) of T1 x T2 where CON-RES was observed to be lower compared to the other treatment groups. Illustrations of BW, weekly ADG and total ADG are shown in Figure 13, Figure 14 and Figure 15. T1 x week was found to have a significant effect on ADG and weekly ADG (P<0.001 and P=0.019, respectively). T1 x T2 x week had a significant effect on ADG (P = 0.002) and weekly ADG was affected by T1 x T2 (P=0.023).

![Figure 13 Body weight (BW) of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration in experiment 1B. All weeks are significantly different (P<0.05).]
Table 12 Dry matter intake and performance during pre-weaning, weaning and post-weaning stages in Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1B. 

<table>
<thead>
<tr>
<th></th>
<th>CON RES</th>
<th>CON URES</th>
<th>STP RES</th>
<th>STP URES</th>
<th>SEM</th>
<th>T1</th>
<th>T2</th>
<th>T1 x T2</th>
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<tbody>
<tr>
<td>ADG (g/d)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>6.07</td>
<td>6.2</td>
<td>7.01</td>
<td>6.89</td>
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<td>NS</td>
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<tr>
<td>BW (kg)</td>
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<td>52.9</td>
<td>53.9</td>
<td>51.8</td>
<td>2.04</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ME* (MJ/kg)</td>
<td>0.42</td>
<td>0.48</td>
<td>0.45</td>
<td>0.46</td>
<td>0.07</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FCE* (MJ/kg)</td>
<td>0.95</td>
<td>1.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Week 3-4 Milk (L/d)</td>
<td>6.5</td>
<td>6.4</td>
<td>4.98</td>
<td>4.99</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td>0.04</td>
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<td>Concentrate (kg/d)</td>
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<tr>
<td>Hay (kg/d)</td>
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<td>BW (kg)</td>
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<td>60.9</td>
<td>2.68</td>
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<td>NS</td>
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<td>ADG (g/d)</td>
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<td>0.77</td>
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<tr>
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<td>1.26</td>
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</tr>
<tr>
<td>ME* (MJ/d)</td>
<td>23.82</td>
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<td>30.23</td>
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<tr>
<td>Week 7-8 Milk (L/d)</td>
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<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>0.00</td>
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<td>NS</td>
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<td>Hay (kg/d)</td>
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<td>0.98</td>
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<td>23.36</td>
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<tr>
<td>Week 9-10 Post-weaning Concentrate (kg/d)</td>
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<td>Hay (kg/d)</td>
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<td>0.23</td>
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<tr>
<td>BW (kg)</td>
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<td>91.8</td>
<td>3.95</td>
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<td>ADG (g/d)</td>
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<td>1.46</td>
<td>1.46</td>
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<td>NS</td>
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<td>DMI* (kg/d)</td>
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<td>2.73</td>
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<tr>
<td>ME* (MJ/d)</td>
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<td>FCE* (MJ/kg)</td>
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<tr>
<td>Week 3-8 Pre-weaning Milk (L/d)</td>
<td>5.21</td>
<td>5.23</td>
<td>5.04</td>
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<td>0.07</td>
<td>0.01</td>
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<tr>
<td>Concentrate (kg/d)</td>
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<tr>
<td>Hay (kg/d)</td>
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<td>0.10</td>
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<tr>
<td>BW (kg)</td>
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<td>61.2</td>
<td>62.1</td>
<td>59.5</td>
<td>2.48</td>
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<tr>
<td>ADG (g/d)</td>
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<td>0.74</td>
<td>0.75</td>
<td>0.71</td>
<td>0.06</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>DMI* (kg/d)</td>
<td>1.24</td>
<td>1.36</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>ME* (MJ/d)</td>
<td>22.48</td>
<td>24.16</td>
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<tr>
<td>FCE* (MJ/kg)</td>
<td>34.25</td>
<td>34.88</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Week 3-10 Trial period Concentrate (kg/d)</td>
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<td>1.21</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>Hay (kg/d)</td>
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<td>0.13</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>BW (kg)</td>
<td>64.5</td>
<td>68.8</td>
<td>69.8</td>
<td>66.7</td>
<td>2.77</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>0.79</td>
<td>0.93</td>
<td>0.94</td>
<td>0.88</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>0.08</td>
</tr>
<tr>
<td>DMI* (kg/d)</td>
<td>1.77</td>
<td>1.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ME* (MJ/d)</td>
<td>30.06</td>
<td>31.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>FCE* (MJ/kg)</td>
<td>35.84</td>
<td>35.84</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

* Milk data was averaged by week for each calf. Hay and concentrate was only measured on group level (n=8 calves). Body weight was obtained once or twice (week 8 and 10) and ADG is calculated on a weekly basis.
* DMI, ME and FCE were calculated based on the intake of milk, hay and concentrate, where the intake of hay and concentrate was measured on group level (n=8 calves). The daily intake is thereby an average estimate calculated on the gross intake.
* Means within a row with different superscripts are significantly different (P < 0.05)
Figure 14 Weekly ADG (kg/d) of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration in experiment 1B. Weeks with different superscripts are significantly different (P<0.05). * CON and STP differs (P<0.05) within week.

Figure 15 Total ADG (kg/d) of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration in experiment 1B. Weeks with different superscripts are significantly different (P<0.05).
7.3 Experiment 1AB

Relations between age and weight at entrance to DKC and ADG obtained in the trial period (Week 3 – 10) are illustrated in Figure 16 and Figure 17. Body weight and ADG obtained in experiment 1A and 1B were analyzed as one experiment (1AB) to evaluate the effects of treatments when analyzing data obtained from 63 calves. Furthermore, the analysis was conducted to investigate possible differences between blocks within each experiment and the two experiments. Feed intake and FCE were not analyzed in experiment 1AB because no recordings of feed intake of the individual calf were obtained in experiment 1B.

7.3.1 Feed intake and performance

The intake of concentrate tended to differ between the two experiments (P = 0.08), where calves in experiment 1B ate more concentrate compared to calves in experiment 1A in all weeks (Figure 18). In the post-weaning period, the calves consumed significantly more concentrate in experiment 1B compared to 1A (2.78 vs. 1.97 kg/d).

Weight and ADG data are listed in Table 13. No significant effects of T1, T2 or T1 x T2 were seen, when data was analyzed as one experiment. The effects of experiment and block (block 1 and block 2) in each of the two experiments on weight (kg) and ADG (kg/d) of calves during pre-weaning, weaning and post-weaning stages are listed in Table 14. Weights were significantly different between blocks in all periods, where block 1 in experiment 1B was significantly lower than block 2 in all periods. ADG was significantly different between blocks in week 5-6, 7-8, 3-8 and 3-10, where ADG obtained in block 1 was significantly lower than block 2 in experiment 1B. Differences were seen in ADG between the two experiments in week 7-8 (P=0.031). Calves in experiment 1B performed better than calves in experiment 1A (1.006 vs. 0.887 kg/d, respectively). Weekly ADG (Figure 19) was affected by T1 x week (P=0.019).

\[ R^2 = 0.1658 \]
\[ R^2 = 0.3063 \]
Table 13 Mean body weight (kg) and ADG (kg/d) during pre-weaning, weaning and post-weaning stages in Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2) in experiment 1AB.¹

<table>
<thead>
<tr>
<th></th>
<th>CON RES</th>
<th>CON URES</th>
<th>STP RES</th>
<th>STP URES</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
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<td><strong>Week 3-4</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BW (kg)</td>
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<td>52.3</td>
<td>53.4</td>
<td>52.4</td>
<td>1.54</td>
<td>NS</td>
</tr>
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<td>ADG (g/d)</td>
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<td>0.46</td>
<td>0.46</td>
<td>0.06</td>
<td>NS</td>
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<td><strong>Week 5-6</strong></td>
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<td></td>
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</tr>
<tr>
<td>BW (kg)</td>
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<td>61.7</td>
<td>62.7</td>
<td>60.9</td>
<td>1.86</td>
<td>NS</td>
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<tr>
<td>ADG (g/d)</td>
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<td>0.80</td>
<td>0.70</td>
<td>0.06</td>
<td>NS</td>
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<tr>
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<tr>
<td>BW (kg)</td>
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<td>73.8</td>
<td>75.6</td>
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<td>ADG (g/d)</td>
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<td>0.96</td>
<td>0.92</td>
<td>0.06</td>
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<td>95.0</td>
<td>90.7</td>
<td>2.95</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>1.00</td>
<td>1.05</td>
<td>1.13</td>
<td>1.10</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Week 3-8</strong></td>
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</tr>
<tr>
<td>BW (kg)</td>
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<td>61.4</td>
<td>62.7</td>
<td>60.9</td>
<td>1.84</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>0.68</td>
<td>0.69</td>
<td>0.72</td>
<td>0.67</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Week 3-10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td>68.3</td>
<td>68.5</td>
<td>70.3</td>
<td>67.9</td>
<td>2.08</td>
<td>NS</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>0.83</td>
<td>0.86</td>
<td>0.90</td>
<td>0.85</td>
<td>0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹ Body weight (BW) was obtained once or twice (week 8 and 10) and ADG was calculated weekly.
**Results**

Table 14 Effects of experiment and block within experiment (Block (EXP)) in two experiments (1A and 1B) on BW (kg) and ADG (kg/d) of calves during pre-weaning, weaning and post-weaning stages in Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration in experiment 1AB.¹

<table>
<thead>
<tr>
<th>Week 3-4</th>
<th>Experiment 1A</th>
<th>Experiment 1B</th>
<th>SEM</th>
<th>Block (EXP)</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Block1</td>
<td>Block2</td>
<td>Block1</td>
<td>Block2</td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td>53.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49</td>
</tr>
<tr>
<td>ADG (g/d)</td>
<td>0.38</td>
<td>0.50</td>
<td>0.46</td>
<td>0.45</td>
<td>0.05</td>
</tr>
</tbody>
</table>

| Week 5-6 |          |               |     |             |             |
| BW (kg)  | 62.1<sup>a</sup> | 62.0<sup>b</sup> | 57.3<sup>a</sup> | 65.7<sup>b</sup> | 1.80 | 0.007 | NS |
| ADG (g/d) | 0.72<sup>a</sup> | 0.77<sup>b</sup> | 0.63<sup>a</sup> | 0.88<sup>b</sup> | 0.05 | 0.008 | NS |

| Week 7-8 |          |               |     |             |             |
| BW (kg)  | 75.0<sup>a</sup> | 73.8<sup>c</sup> | 68.2<sup>b</sup> | 78.7<sup>a</sup> | 2.33 | 0.009 | NS |
| ADG (g/d) | 0.93<sup>a</sup> | 0.84<sup>a</sup> | 0.91<sup>a</sup> | 1.10<sup>b</sup> | 0.05 | 0.025 | 0.031 |

| Week 9-10 Post-weaning |          |               |     |             |             |
| BW (kg)  | 92.9<sup>a</sup> | 89.3<sup>a</sup> | 86.5<sup>a</sup> | 99.3<sup>b</sup> | 2.85 | 0.007 | NS |
| ADG (g/d) | 1.34 | 1.21 | 1.29 | 1.42 | 0.06 | NS | NS |

| Week 3-8 Preweaning |          |               |     |             |             |
| BW (kg)  | 63.6<sup>a</sup> | 62.5<sup>b</sup> | 56.3<sup>b</sup> | 64.1<sup>a</sup> | 1.77 | 0.012 | NS |
| ADG (g/d) | 0.66<sup>a</sup> | 0.69<sup>b</sup> | 0.64<sup>a</sup> | 0.77<sup>b</sup> | 0.04 | 0.039 | NS |

| Week 3-10 Trial period |          |               |     |             |             |
| BW (kg)  | 70.9<sup>a</sup> | 69.2<sup>a</sup> | 63.0<sup>b</sup> | 71.9<sup>a</sup> | 2.00 | 0.010 | NS |
| ADG (g/d) | 0.84<sup>a</sup> | 0.83<sup>a</sup> | 0.82<sup>a</sup> | 0.95<sup>b</sup> | 0.05 | 0.044 | NS |

¹ Body weight was obtained once or twice (week 8 and 10) and ADG was calculated weekly.

<sup>a,b</sup> Means within a row with different superscripts are significantly different (P < 0.05)
Figure 18 Comparison of intake of concentrate (kg/calf/d) during week 3-10 between two similar experiments (1A and 1B) where 1A was conducted in September-October and calves were fed concentrate in an automatic feeder. Experiment 1B was conducted in February-April and calves were fed concentrate in a trough.

Figure 19 Weekly ADG (kg/d) of Holstein bull calves fed either by conventional (CON) or step-down (STP) procedures. Data is obtained in two similar experiments (1A and 1B) and analyzed as one experiment. Weeks with different superscripts are significantly different (P<0.05).* CON and STP differs (P<0.05) within week.
7.4 Calf Behavior

Direct observations were obtained in experiment 1B to evaluate the feeding behavior of the calves in relation to occupancy time in the milk feeder and time spent eating hay and concentrate, when allocated to different milk feeding strategies. The objective was to investigate the behavior in the pre-and post-step periods for calves fed STP in week 5 and 7. Furthermore, calves were observed in week 9 (Materials and Method: Table 6).

7.4.1 Feeding behavior

Results are listed in Table 15. Calves fed URES spent more time eating concentrate when fed CON compared to calves fed STP (2.1 vs. 0.7 min/h, respectively) in week 5, where the milk allowance was reduced from 8 to 5L/d for calves fed STP. Post-weaning (week 9), there was a tendency (P=0.08) that calves fed RES spent more time eating concentrate compared to URES. The time spent on eating concentrate was significant different between weeks (P<0.001) (Figure 20).

An effect of T2 on the intake of hay was found in week 4 and 8, where calves fed RES spent more time eating hay in week 4 compared to calves fed URES. Conversely, T2 was found to increase the time spent on eating hay for calves fed URES compared to RES in week 8, where calves were offered 2 L MR per day. A significant effect of week (P=0.024) and T2 x week (P=0.042) was found when analyzing feeding behavior in relation to intake of hay (Figure 21).

In week 4, calves fed CON-RES spent significantly more time in the automatic milk feeder compared to the other treatment groups. However, no other significant effects were found on occupancy time in the milk feeder between the treatment groups in the following weeks, but a significant effect (P<0.001) of week was found (Figure 22).

7.4.2 Standing behavior

Calves fed RES spent significantly more time standing inactively compared to calves fed URES in week 4 and 5 (16.3 vs. 11.6 min/h and 17.9 vs. 14.5 min/h, respectively). Also, the total standing time was affected by T2 in week 4 and 5, where calves spent more time standing when fed RES compared to URES (25.2 vs.16.8 min/h and 25.9 vs. 19.9 min/h, respectively). No significant effect of week was found, but T2 x week effect was significant (P=0.0231) (Figure 23).
Table 15 Feeding behavior in Holstein bull calves fed milk replacer either by conventional (CON) or step-down (STP) procedures (T1) with restricted (RES) or unrestricted (URES) allowance to the daily milk ration (T2).1

<table>
<thead>
<tr>
<th>Week</th>
<th>Concentrate, min/h</th>
<th>Hay, min/h</th>
<th>Occupying the milk feeder, min/h</th>
<th>Standing inactively, min/h</th>
<th>Standing total, min/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON RES URES SEM T1</td>
<td>STP RES URES SEM T1</td>
<td>SEM T1</td>
<td>SEM T1 x T2</td>
<td>SEM T1</td>
</tr>
<tr>
<td>4</td>
<td>1.7 0.8 1.7 2.0 0.4 NS NS NS</td>
<td>1.4ab 2.1a 2.0ab 0.7b 0.4 NS NS 0.033 NS</td>
<td>2.5a 1.4b 3.1a 2.0b 0.5 NS 0.047 NS</td>
<td>5.9a 1.5b 3.0b 2.5b 0.6 NS 0.001 0.003</td>
<td>16.2a 10.9b 16.4a 12.4b 1.8 NS 0.013 NS</td>
</tr>
<tr>
<td>5</td>
<td>5.8 3.0 5.8 4.3 0.6 NS NS NS</td>
<td>5.8 3.0 5.8 4.3 0.6 NS NS 0.003 NS</td>
<td>3.8 2.2 1.9 1.4 0.7 0.06 NS NS</td>
<td>19.4a 14.0b 16.4a 15.0b 1.2 NS 0.009 NS</td>
<td>19.4a 14.0b 16.4a 15.0b 1.2 NS 0.009 NS</td>
</tr>
<tr>
<td>7</td>
<td>2.7 3.0 2.8 3.2 0.6 NS NS NS</td>
<td>2.7 3.0 2.8 3.2 0.6 NS NS 0.033 NS</td>
<td>3.1 2.8 3.4 4.2 0.7 NS NS NS</td>
<td>4.6 4.5 3.5 4.6 0.7 NS NS NS</td>
<td>22.3 19.9 21.0 22.0 1.6 NS NS NS</td>
</tr>
<tr>
<td>8</td>
<td>4.6 4.5 3.5 4.6 0.7 NS NS NS</td>
<td>4.6 4.5 3.5 4.6 0.7 NS NS 0.003 NS</td>
<td>2.4a 3.9b 2.9a 4.7b 0.6 NS 0.011 NS</td>
<td>4.7 3.0 4.3 3.8 0.6 NS 0.08 NS</td>
<td>4.7 3.0 4.3 3.8 0.6 NS 0.08 NS</td>
</tr>
<tr>
<td>9</td>
<td>4.7 3.0 4.3 3.8 0.6 NS NS NS</td>
<td>4.7 3.0 4.3 3.8 0.6 NS NS 0.003 NS</td>
<td>2.5 2.6 2.7 2.9 0.6 NS NS NS</td>
<td>0.1 0.3 0.5 0.1 0.2 NS NS 0.08</td>
<td>15.4 15.2 14.5 15.0 1.5 NS NS NS</td>
</tr>
</tbody>
</table>

1 All data was obtained by direct observations by using the method of instantaneous sampling with 5 min intervals. The observations were obtained once per week from 7am to 11am and 14pm to 16pm. All values are given in min/h, calculated as an average of the six hours per day.

ab Means within a row with different superscripts are significantly different (P < 0.05)
Figure 20 Mean feeding time of concentrate (min/h). Values are shown separately for calves fed milk replacer either by conventional (CON) or step-down (STEP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration. Weeks with different superscripts are significantly different (P<0.05).* RES and UURES differs (P<0.05) within week.

Figure 21 Mean feeding time of hay (min/h). Values are shown separately for calves fed milk replacer either restricted (RES) or unrestricted (URES) allowance to the daily milk ration. Weeks with different superscripts are significantly different (P<0.05).* RES and URES differs (P<0.05) within week.
Figure 22  Mean occupancy time (min/h) in the automated milk feeder. Values are shown separately for calves fed milk replacer either restricted (RES) or unrestricted (URES) allowance to the daily milk ration. Weeks with different superscripts are significantly different (P<0.05). * RES and UURES differs (P<0.05) within week.

Figure 23  Mean time spent on standing (min/h). Values are shown separately for calves fed milk replacer either by conventional (CON) or stepdown (STEP) procedures with restricted (RES) or unrestricted (URES) allowance to the daily milk ration. No significant effect of week was found. * RES and URES differs (P<0.05) within week.
8 Discussion

8.1 Calf health

8.1.1 Disease

In both experiments calves were challenged by respiratory and gastrointestinal diseases in the first weeks of the experiment. The calves were moved from the dairy farms at an average age of 12 days, which is a period of high risk, especially if calves are introduced to several changes. Calves were all housed individually at the dairy farms and the introduction to the experiment therefore caused several stress factors; transport, mixing and change of feed might have contributed to the incidence of disease. Furthermore, age at entrance might also have an effect of the observed health challenges as older calves at arrival has less risk to develop diarrhea (Pardon et al., 2013).

In experiment 1A one calf died because of an acute intestinal infection. This was ascertained by the veterinarian when doing an autopsy, where enlarged mesenteric lymph nodes were found in the membrane of the small intestine. This could indicate that the gastrointestinal challenges were caused by pathogens, which affect calves with reduced resistance the most. However, all calves consumed their daily allowance of MR and therefore the incidence of diarrhea did not affect the MR intake. The incidences of diarrhea possibly caused a reduced intake of solid feed and reduced the feed efficiency because the intestine's ability to absorb important nutrients might have been compromised. When calves are challenged by a gastrointestinal disease, they will more likely also be exposed to respiratory diseases (Svensson et al., 2006; Hultgren et al., 2008; Pardon et al., 2013) possible because of their reduced resistance and lower nutritional status. Therefore the high number of calves exposed to a respiratory disease might be related to the occurrence of diarrhea. However, the association between diarrhea and respiratory disease may be linked to common predisposing management factors, such as inadequate colostrum management (Svensson et al., 2006).

Calves in experiment 1B were born in January 2017 which was a wet and cold month. All four farmers wherefrom calves were purchased were visited the day before the arrival to DKC, and only healthy calves were accepted for use in the experiment. But when the calves arrived at DKC, some calves already showed signs of gastrointestinal challenges. In week 3 and 4 calves were exposed to wet and windy weather and the stables were not optimal for wind protection. Draught caused calves to spend more energy to maintain the body temperature. Calves housed in herds where draught was detected in the pen, were found to have a heightened risk of having increased respiratory sounds at lung auscultation compared to calves in herds where no draught was detected (Lundborg et al., 2005). However, no differences of treatments regarding respiratory and gastrointestinal diseases were found between group-pens. Presence of draught was not determined in the present study and it is therefore not possible to discuss the effect of draught between the four group-pens.

As described, several factors could have contributed to calves suffering from diarrhea in the experiments. An interesting perspective in this discussion is whether the MR could have induced any of the gastrointestinal challenges observed. The importance of abomasal coagulation of the casein in milk or MR has been debated for many years. This discussion will not be included in this thesis, but it is important to emphasize
that using MR with 13% skim milk, as used in the present experiment, might cause changes in the digestion and absorption of the MR. A MR consisting of 13% skim milk will not clot in the abomasum. This might result in more rapid-flow of the undigested protein into the duodenum and thereby overwhelming the digestive capacity and causing a more fluid consistency (Roy, 1970).

Health observation and treating of sick calves was done by several of the persons involved in the experiment and therefore the identification of sick calves was unfortunately somewhat different depending on former experience. Treatment of diarrhea was not distinguished between ‘preventing’ and ‘treating’, but calves were fed electrolytes if signs of dehydration were seen. An improved registration of clinical signs and decision for treatments of the calf would make data more useful as it would be interesting to analyze the effect of IgG at entrance on the number of incidences of diarrhea and the effect of diarrhea on ADG in the first weeks of the calf’s life.

8.1.2 Fecal scores

No differences in DM content, consistency, cleanliness and rectal temperature were found between treatment groups, when feces were sampled and dried in week 10. In experiment 1A the color of the feces was darker for calves fed STP compared to CON. This can be explained by the observation that STP calves in general ate more grass hay in week 9-10 compared with CON. However, this observation was only numerical, since no statistical analysis was made. No differences in feces color were found in experiment 1B.

Feces were only sampled once during the experiment. To be able to evaluate the effects of treatment on the fecal scores, more samples are needed. Khan et al. (2007) and Omidi-Mirzaei et al. (2015) obtained information regarding fluidity, consistency and odor on a daily basis in their study. This was possible because calves were housed individually. Calves in the present study were housed in groups and, therefore, it would have been extremely time consuming to collect feces from all calves on a daily basis. It would have been beneficial to gain more knowledge about the fluidity of feces, when calves were allocated to different milk feeding strategies. However, no differences were observed for fecal score among the treatments during the pre-weaning, post-weaning, and overall periods in the two studies conducted by Khan et al. (2007) and Omidi-Mirzaei et al. (2015).

8.2 Intake of solid feed

8.2.1. Feed intake

Low intakes of solid feed were observed until calves were approximately 5 weeks of age (< 100g/d), which is in agreement with similar studies (Leão et al., 2016; Overvest et al., 2016). Intake of concentrate was individually measured in experiment 1A from week 5 to week 10. No overall treatment effect was found in the pre- or post-weaning period, but calves fed STP consumed more concentrate in week 5-6 than CON calves. This effect was also seen in experiment 1B, but since no individual measures were obtained, no statistical analysis was made. It was expected that calves fed STP would have a greater intake of concentrate when the milk allowance was reduced from 8 to 5L/d. The hypothesis was that calves fed STP would rely more on the consumption of solid feed to compensate for the lack of energy from the reduced milk allowance. Khan et al. (2007) found a similar effect, when the milk allowance was reduced from 20 to 10% of BW
at d 26 of age. Calves in the post-step period consumed significantly more concentrate and hay, compared to calves fed 10% of BW for the entire pre-weaning period. The positive effect of DMI continued in the post-weaning phase (51 to 90 d of age) which was similar to that reported by Omidi-Mirzaei et al. (2015). Leão et al. (2016) found no significant overall effect of three different step-down milk feeding strategies on the intake of concentrate, but a numerical difference was found, where calves fed 6L-30d/4L-60d/2L-90d had a greater intake of concentrate in the total trial period compared to calves fed 8L-30d/6L-60d/3L-90d (0.98 vs. 0.75 kg/d). In the last period (d 60 to 90) the intake of concentrate between the two treatments were significantly different, and calves fed 2 L/d had a greater intake (Leão et al., 2016). Silper et al. (2014) found no differences in concentrate intake in the post- or post-weaning period when calves were fed three different milk feeding strategies until 60 d of age (4L-60d; 6L-29d/4L-60d; 6L-60d).

Increased milk intake in the pre-weaning period combined with a step-down strategy will increase the digestive tract capacity and may result in a greater demand for nutrients and thereby an increased consumption of solid feed during the post-step phase (Khan et al., 2007). Higher consumption rate of solid feed pre-weaning by STP calves will initiate ruminal activity earlier than calves fed CON. When great amounts of milk or MR is offered in the pre-weaning period, it is well known that the consumption of solid feed is lower compared to calves fed lower amounts of milk or MR. However, when using enhanced milk feeding programs in the first four weeks of the calf’s life, no major difference in solid feed intake is found, because the intake of concentrate in generally is low (Khan et al., 2007). Therefore, high milk allowances in the first four weeks of the pre-weaning period will most likely not depress the solid feed intake post-weaning. Furthermore, young calves are unable to compensate for low milk intake by increasing their concentrate intake during the first weeks of life (Khan et al., 2011b), and therefore higher milk allowances are of great advantage if practiced in this early period of life.

The difference in MR allowances for calves fed CON and STP was 1.5 L/d in week 3-4 and total amount of MR was similar among treatment groups. The method used was similar to the experiments conducted by Daneshvar et al. (2015) and Daneshvar et al. (2017) where no differences in DMI during the pre- and post-weaning period were reported. The modest difference in milk allowance between CON and STP in the present experiment could explain the lack of effect on the concentrate intake post-weaning. Furthermore, the total amount of MR were lower in the present experiment, Daneshvar et al. (2015) and Daneshvar et al. (2017) when compared to Khan et al. (2007) and Omidi-Mirzaei et al. (2015) (224, 311, 311, 375 and 424 L, respectively). However, calves were 2 weeks of age when introduced to the present study, and therefore the total amount of MR consumed was lower compared to similar studies using calves from 1 d of age.

No effect of FF on the intake of concentrate was found in the present experiment. This result is in agreement with other studies investigating the effect of FF (von Keyserlingk et al., 2006; Hulbert et al., 2011). However, the methods used in those experiments differ significantly from T2 in the present experiment. Sockett et al. (2011) found a numerical difference in concentrate intake when heifer calves were fed the same amount of MR until d 49 of age, distributed in either two or three meals per day. Calves fed three times per day had a greater intake of concentrate in the pre-weaning period (d 1-42) compared to calves fed twice a day (3.3 vs. 3.9 kg). This effect was significant in the weaning period (d 43-49). However, no data from the post-weaning period was presented and therefore it is not known whether the positive effect of three times milk feeding per day was extended into the post-weaning period.
8.2.2 Intake of concentrate pre- and post-weaning
The relationship of the intake of concentrate in the pre- and post-weaning period is illustrated in Figure 11. The correlation coefficient ($R^2 = 0.42$) indicates that 42% of the variation in one variable is related to the variation in the other. The intake of concentrate in the pre-weaning period may therefore be relatively important to the level of consumed concentrate in the following weeks post-weaning. High intake of concentrate before weaning is found to help ensure intake and sustain a desirable growth rate post-weaning (Kertz et al., 1979).

8.2.3 Effect of experiment
Calves in experiment 1B tended to eat more concentrate throughout the study and especially in the post-weaning period were the intake of concentrate was significantly greater than in experiment 1A (2.78 vs 1.97 kg/d). This difference can be explained by several factors, mostly influenced by the experimental setup, season and management. Calves in experiment 1A were introduced to an automatic concentrate feeder, which may have caused some challenges to the calves regarding feed intake. The calves were trained to use the feeder, but because of technical issues, the calves might have been unrewarded several times. Calves in experiment 1B were fed in a trough throughout the experiment and were thereby not challenged by technical issues. Furthermore, calves in experiment 1B were able to eat concentrate at the same time as other calves in the pen, compared to calves using the automatic feeder. This might have stimulated the intake of concentrate because of social facilitation (De Paula Vieira et al., 2010; Jensen et al., 2015). Finally, all calves in experiment 1B were purchased from dairy farmers, where all calves were offered concentrate from d 1. This was not entirely known for calves purchased in experiment 1A. The fact that the calves were introduced to concentrate from a very young age may have had a positive effect on the concentrate intake later in the milk feeding period (Miller-Cushon and DeVries, 2011a). The intake of concentrate was already greater in week 3-4 for calves in experiment 1B compared with 1A indicating that they were more likely to be familiar with concentrate as a feed source.

8.3 Calf performance
8.3.1 Average daily gain
Overall ADG was similar in both experiments (1A = 0.68 kg/d, 1B = 0.71 kg/d) when calculated from d 14 to 56. These results are in agreement with Jensen (2004), where calves gained 0.701 kg/d in the milk feeding period (d 13-70). However, more of the previous studies evaluated the milk feeding period from calving or d 3 of life until weaning. To be able to compare the ADG obtained in the present study with previous studies, the ADG from calving to initiation of the experiment was calculated (0.39 and 0.33 kg/d for experiment 1A and 1B, respectively). This information was used to calculate the ADG in the milk feeding period from d 1 – 56 (0.62 and 0.63 for experiment 1A and 1B, respectively). These findings are in perfect agreement with Daneshvar et al. (2015) when calves were allocated to either CON or STP and had access to forage. Other studies report lower levels of ADG in the pre-weaning period in the range of 0.4 to 0.79 kg/d depending on the amount of MR offered (Kehoe et al., 2007; Khan et al., 2007; Omidi-Mirzaei et al., 2015). Calves fed the same amounts of MR (1.08kg/calf/d) distributed in two or three meals per day in the pre-weaning period gained 0.60 and 0.71 kg/d, respectively (Sockett et al., 2011).
ADG did not differ between treatment groups in the pre-weaning, weaning and post-weaning period in either of the two experiments in the present study. Weekly ADG was higher for calves fed STP compared to CON in week 5 in experiment 1A. This effect was only numerically found in experiment 1B. Reduced ADG for calves fed STP in week 5 was expected, because MR allowance was reduced from 8 to 5 L/d. In general, studies investigating the effect of step-down methods, report the pre- and postweaning performance, but no weekly data around step-down is found in the literature. Therefore, it is only possible to discuss the overall effect of ADG in the pre- and postweaning period. However, it is commonly known that calves fed high levels of milk or MR will have a lower ADG in the weeks following milk reduction (Jasper and Weary, 2002; Sweeney et al., 2010; Eckert et al., 2015)

In experiment 1A, ADG was found to differ between treatment groups in week 5-6, where calves fed CON-RES had a greater ADG compared to the other treatment groups. No effects of treatments were found on the intake of solid feed in week 5-6 but the MR consumption was greater for calves fed CON-RES compared to CON-URES. This was not expected since calves were offered the same daily amount of MR. Calves fed CON-URES and STP-RES tended to perform better than the other treatment groups in the trial period (week 3-10) in experiment 1B. However, this tendency was possible derived by the significant effect found between treatments in week 9-10. There is no logical explanation as why calves perform better when fed CON-URES and STP-RES compared to CON-RES and STP-URES in the postweaning period. When ADG was analyzed as one experiment (experiment 1AB) no interaction of the two treatments were found.

No previous studies have investigated the effect of FF where calves had free access to the daily milk ration and thereby had the opportunity to control the portion size themselves. Unfortunately, lack of data from the automatic milk feeder makes it impossible to discuss the FF of calves fed URES, since the number of rewarded visits in the present study is not known. However, Sockett et al. (2011) reported a significant greater BW gain in the pre-weaning period (d 1-42), when calves were fed MR three times versus twice a day (29.9 vs. 25.1 kg). Furthermore, Nussbaum et al. (2002) reported a numerical greater ADG for calves fed RES (meal size was ranging from 0.5 to 1.5 L per portion) compared to calves fed twice a day from d 1-28 (0.61 and 0.71 kg/d, respectively). In both studies, calves were fed greater amounts of MR and milk compared to the present and other studies who did not find any effect on ADG when increasing the FF (Williams et al., 1986; Jensen, 2004; Kmicikewycz et al., 2013). This could indicate that the effect of increasing FF is valuable when calves are fed higher levels of milk or MR in the pre-weaning period.

Previous studies have seen an effect of step-down milk feeding strategies when calves in the pre-weaning period consumed a greater total amount of MR compared to calves fed conventionally (Khan et al., 2007; Omidi-Mirzaei et al., 2015). However, use of different amounts of milk or MR when evaluating feeding strategies makes it difficult to discuss the true effect of step-down. Daneshvar et al. (2015) and Daneshvar et al. (2017) did not find any differences in ADG pre-weaning when calves were fed step-down or conventional methods similar to STP and CON in the present experiment. For calves to experience a smooth transition from milk to solid feed at weaning, it is crucial that calves do not loose BW at this time. The objective of the present study was to determine the optimal feeding strategy to reduce possible negative effects of weaning. Calves remained healthy in the transition period and no calves were exposed to weight loss. No differences in ADG were found between the four blocks and two experiments.
In the post-weaning period the calves gained 1.28 and 1.37 kg/d in experiment 1A and 1B, respectively. Other studies report values within the range of 0.72 to 1.31 kg/d when calves were followed 14 d post-weaning (Kehoe et al., 2007; Daneshvar et al., 2015; Omidi-Mirzaei et al., 2015; Daneshvar et al., 2017). Calves with access to hay in the pre- and post-weaning period had the greatest ADG of 1.31 kg/d (Daneshvar et al., 2015). It has been widely recognized that feeding forage can increase post-weaning total DMI possible because of increased gut fill (Coverdale et al., 2004; Khan et al., 2011a; Castells et al., 2012). Two meta-analyses were recently conducted to evaluate the effects of forage provision to dairy calves on growth performance and rumen fermentation (Suarez-Mena et al., 2016; Imani et al., 2017). Both analyses report that improving the ADG was greater for calves fed a high level of forage, where the overall ADG response was lower for calves offered forages with textured concentrates compared to calves offered ground starter feed. However, the potential of forages to affect the concentrate intake and thereby performance depends on more factors such as source, level and method of forage feeding, and the physical form of the concentrate provided (Imani et al., 2017). Furthermore, Suarez-Mena et al. (2016) found that effects of feeding forage was greatly depended on calf age during the experiment.

### 8.3.2 Predicted vs. observed ADG

The predicted ADG was determined by using the computer version of NRC (2001) for calves in experiment 1A where MR and intake of concentrate were recorded individually for all the calves. As illustrated in Figure 12 no relationship was found between the predicted and observed ADG in the milk feeding period (week 3–8). Dietary changes, the biological values, metabolic coefficients for different nutrient types, effect of colostrum intake on health and environmental temperature and management practices that might stress the calf do all affect the calf on an individual level. Therefore the prediction of the required nutrients to obtain a given ADG is highly complex (Hill et al., 2013). Efficiencies of ME use from dry feeds for maintenance and gain is fixed at 75 and 57%, respectively, when using the model by NRC (2001). It was not expected to find a great relationship between the predicted and observed ADG, especially because of the small number of observations (n = 31), and therefore great variation between calves will be expressed. Calves were purchased from different farms and calves where therefore exposed to different management factors in their first weeks of life. This may have affected the ability to cope with the challenges during the experiment. Hill et al. (2013) found a predicted growth that was limited and well below observed values when rates of gain were greater than 700 g/d. With 993 observations a reasonable relationship between the predicted and observed ADG was found ($R^2 = 0.42$).

It is generally found that the calculations of NRC (2001) are reasonably accurate when predicting the ADG for the first four weeks of the calf’s life (Mike Van Amburgh, personal communication, May 17, 2017). However, the accuracy of the prediction decreases, as the calf starts eating concentrates in greater amounts.

### 8.3.3 Feed conversion efficiency

Calves fed CON-RES in experiment 1A tended to have a lower FCE compared to the three other treatment groups in the milk feeding period (week 3–8). Sockett et al. (2011) reported similarly a greater gain to feed ratio (G:F) when calves were fed the same amount of MR three times versus twice daily (0.61 vs. 0.52). However, this effect was not found in other studies investigating the effect of FF (Kehoe et al., 2007; Hubert et al., 2011; Kmicikewycz et al., 2013).
The FCE was numerical higher in experiment 1B compared to 1A for week 3-8 and post-weaning (week 9-10). This can be explained by the lower temperatures and draught that calves were exposed to in experiment 1B. In the first four weeks of the experiment, the diurnal mean temperature was 8.1 and 1.8 °C in experiment 1A and 1B, respectively. The thermoneutral zone in very young calves ranges from 15 to 25°C and shifts depending on factors such as age, feed intake and length and thickness of the hair coat (NRC, 2001). According to NRC (2001) the maintenance energy requirement is increased by 13 and 40% when calves older than three weeks are housed where the temperature is 5 and -5 °C, respectively. No individual feed intakes were obtained in experiment 1B and FCE was therefore calculated at group level. Furthermore, the individual intake of concentrate in experiment 1A must be evaluated with cautiousness, because calves were able to ‘steal’ portions of concentrate from other calves, if they left the automatic feeder without eating their rewarded portion. The automatic feeder did not include a scale system with the possibility to weigh the orts.

In the milk feeding period, G:F is in agreement with values reported by other authors investigating the effect of milk feeding strategies (1A = 0.6, 1B = 0.55) (Hulbert et al., 2011; Sockett et al., 2011; Kmickewycz et al., 2013; Daneshvar et al., 2015; Omidi-Mirzaei et al., 2015; Leão et al., 2016; Daneshvar et al., 2017). Lower values were reported by Khan et al. (2007), where calves fed STP had a significantly greater G:F compared to calves fed CON (0.46 vs. 0.40). Calves were fed whole milk, the study was conducted in April in South Korea and the incidence of diarrhea was low. No other signs of disease was noticed and the low G:F is therefore somehow surprising.

The post-weaning FCE did not differ between treatments in the present study, but seemed to decrease for all treatment groups compared with the milk feeding period. This means that less energy was required to gain weight post-weaning. This was contrary to previous findings, where post-weaning G:F was decreased, illustrating that more energy was required to gain weight (Khan et al., 2007; Hulbert et al., 2011; Daneshvar et al., 2015; Omidi-Mirzaei et al., 2015; Daneshvar et al., 2017). The post-weaning period reported by Khan et al. (2007) was 30 d, but all other studies mentioned above had a post-weaning period of 14 d as in the present study. Daneshvar et al. (2015) found that post-weaning G:F was greater for calves fed CON when forage was provided compared to calves fed CON without forage provision and STP with or without forage provision. Calves fed STP without forage provision had a significantly lower G:F compared to other treatment groups post-weaning (Daneshvar et al., 2015). None of the studies that found a lower G:F were providing hay in the pre- or post-weaning periods. This could indicate that provision of hay in the present experiment had a positive influence of the feed utilization in the post-weaning period (Coverdale et al., 2004).

8.4 Blood metabolites

8.4.1 Blood glucose

No differences in blood glucose were found between treatment groups, when blood was sampled and analyzed at d 1 of the experiment (14 d of age). The level of blood glucose was found to range from 5.83 to 6.49 mM, which is in agreement with other studies investigating milk feeding strategies (Kehoe et al., 2007; Khan et al., 2007; Daneshvar et al., 2015; Omidi-Mirzaei et al., 2015; Yunta et al., 2015; Leão et al., 2016;
Daneshvar et al., 2017). Blood samples were collected from 10am to 12am, where calves possibly had been rewarded in the automated milk feeder since 9am. When comparing the results from the present experiment with other studies it is important to notice the time of sampling and especially whether blood was sampled before or after the morning feeding. Silper et al. (2014) found that average glucose concentration for all sampling ages up to 60 d old was lower than values found in the literature which most likely was related to the long interval between the evening MR feeding and the blood sampling next morning (16 h). In the pre-weaning period where concentrate intake is negligible, low values of blood glucose might indicate that calves are exposed to hypoglycemia, if blood is sampled before the morning milk feeding (Silper et al., 2014). When feeding high levels of milk or MR, concentrations of blood glucose will likely increase, mainly due to a greater consumption of lactose (Yunta et al., 2015).

Two weeks post-weaning the level of blood glucose was effected in T2 calves, where higher concentrations were found in calves fed URES compared to RES. It has not been possible to find any results of blood glucose in studies investigating FF, when calves were fed milk or MR distributed in more than two daily portions. Kehoe et al. (2007) did not find any differences in the level of blood glucose when calves were fed MR once compared to twice daily. Blood was sampled from 10am to 12am and the increased level of blood glucose found in calves fed URES may indicate that those calves consumed concentrate in the time period similar to when their daily MR ration was offered from the automatic milk feeder in the preweaning period.

No numerical differences were observed between d 1 and d 56. This is in agreement with Quigley et al. (1991), but differs from results presented by Silper et al. (2014) who found that post-weaning glucose was higher than during the pre-weaning period, when calves were abruptly weaned at d. 60 and blood was sampled at d 75 and d 90.

However, the concentration was reasonably low in the pre-weaning period which might have affected the significant increase from pre- to post-weaning in blood glucose concentration. Thermal stress increases the energy requirements for maintenance resulting in a faster utilization of the circulation glucose (Drackley, 2008) and it is therefore argued that low concentrations of glucose in the pre-weaning period might be related to thermoregulatory ability (Silper et al., 2014).

It was expected that when concentrate intake increased, rumen fermentation would be more established and lower blood glucose would therefore be observed, because of hepatic gluconeogenesis with propionate as the main substrate (Leão et al., 2016). However, no differences in concentrate intake in the post-weaning period were found in the present study, and thus no difference in blood glucose could be expected between treatments. Khan et al. (2007) found a lower concentration of blood glucose in calves fed STP on d 40 and argued that the observed differences might be a result of calves consuming more solid feed and therefore possibly relied more on end products of fermentation to derive energy needs. Calves were not weaned at d 40 and both treatment groups consumed 10% MR of BW. However, blood was sampled before the morning feeding, where lactose consumed from the preceding milk feeding was already metabolized.

To evaluate the effect of the treatments in the milk feeding period in the present study, more blood samples were needed. It would have been of great value to obtain information of blood metabolites when the milk allowance was reduced from 8 to 5L/d for calves fed STP. This blood sampling was therefore part of experiment 1B (not analyzed yet). However, no differences were found in the concentration of blood glu-
cose by Daneshvar et al. (2015) at d 35 (before step-down) and d 74 (end of trial), when calves were fed step-wise compared to conventional milk feeding procedures.

8.4.2 Blood urea

No differences in concentration of blood urea were found among treatment groups, when blood was sampled and analyzed at d 1 of the experiment (14 d of age) and d 56 (10 weeks of age). This is in good agreement with the similar feed intake in the four treatment groups. The concentration was found to increase numerically between d 1 and d 56 which is a result of greater intake of CP (Daneshvar et al., 2017). The results and ranges of values are in agreement with other studies investigating different milk feeding strategies. Daneshvar et al. (2015) and Daneshvar et al. (2017) found no differences in blood urea, when concentrations were analyzed at d 35 (before step-down) and d 74 (end of trial), when calves were fed step-wise compared to conventional milk feeding procedures. Both studies reported similar results as found in the present experiment, where the concentration of blood urea was increasing post-weaning (Daneshvar et al., 2015; Daneshvar et al., 2017). Kehoe et al. (2007) found no differences in concentration of blood urea when calves were fed once compared to twice daily.

Khan et al. (2007) found greater concentrations of blood urea at d 50 (weaning) and d 60 in calves fed through the STP procedure compared with calves fed through CON. The explanation for this could be that DMI was significantly higher for calves fed STP leading to higher intakes of CP and rates of protein degradation.

8.4.3 Blood BHB

No differences in concentration of blood BHB were found among treatment groups, when blood was sampled and analyzed at d 1 of the experiment (14 d of age) and d 56 (10 weeks of age). The concentration of blood BHB was found to increase numerically between d 1 and d 56 which is a result of greater intake of concentrate (Eckert et al., 2015).

Omidi-Mirzaei et al. (2015) found no differences in BHB between STP and CON in the pre- and post-weaning period. However, their study found that BHB was lower when calves were fed step-up/step-down milk feeding compared to STP in the pre-weaning period (0.08 vs. 0.18 mM). Values reported by Omidi-Mirzaei et al. (2015) were lower compared to values obtained in the present study (0.13 vs. 0.23 mM, respectively), where the concentration found by Omidi-Mirzaei et al. (2015) was based on three samples obtained at d 21, 35 and 49 of age in the pre-weaning period. No numerical differences were found post-weaning, when calves were 70 days of age.

Khan et al. (2011b) have suggested that circulating BHB levels may be an indicator of rumen development and concentrate intake in pre-weaned calves. Chapman et al. (2017) found a higher concentration of serum BHB in calves fed 3.8 L/d of MR compared to calves fed higher milk volumes, indicating that calves fed a lower milk volume have a higher starter intake improving the rumen development (Quigley et al., 1991; Deelen et al., 2016). However, in the present study no relationship between intake of concentrate and circulating BHB was found ($R^2=0.01$) as illustrated in Figure 24. More samples were needed to evaluate the effects of T1 and T2 in the present experiment. Also, more samples were needed to adjust for the day-to-day variability in feed intake and diurnal feeding patterning in relation to the time of sampling.
Discussion

8.4.4 Plasma NEFA

No differences in concentration of NEFA were found between treatment groups, when blood was sampled and analyzed at d 1 of the experiment (14 d of age) and d 56 (10 weeks of age). The concentration was found to decline numerically between d 1 and d 56, which is in agreement with other studies (Hammon et al., 2002; Quigley et al., 2006; Kmicikewycz et al., 2013). Values ranged from 0.12 to 0.23 mM found when calves were 14 d of age, which is within range of values reported by other studies (Hammon et al., 2002; Kmicikewycz et al., 2013).

Increased concentrations of NEFA may indicate adipose mobilization in support of energy demands. Hammon et al. (2002) found that NEFA concentration were lower when calves were fed ad libitum milk feeding compared to restricted milk feeding, arguing that inhibition of fat mobilization was greater with higher feeding intensity.

8.4.5 IgG

No differences in IgG concentration were found between treatment groups, when blood was sampled and analyzed at d 1 of the experiment (14 d of age). However, there was a difference between calves fed RES and URES at d 56 (13.93 and 11.00 mg/ml, respectively).

Values found in the present experiment is in the range of those reported by other studies investigating milk feeding strategies in young dairy calves (Mee et al., 1996; Sockett et al., 2011). Serum IgG concentration between 24 to 36 h and 3 week of age was 18.2 and 8.6 mg/ml, respectively, when calves were fed 2 L of colostrum immediately after calving (Mee et al., 1996), indicating that the concentration of IgG declines rapidly within the first weeks of life. No effects of time of sampling were analyzed in the present study.

Figure 24 Relationship between concentrate intake in week 10 (kg/d) and circulating BHB levels (mM) at d 56.
8.5 Calf Behavior

8.5.1 Standing time

Standing time was found to be greater for calves fed RES compared to calves fed URES in week 4 and week 5. No differences were found when calves were fed same amounts of MR in week 7 and week 8. Neither was any effect of treatments found post-weaning. Calf activity is directly affected by feeding times (Kienitz et al., 2017) and since calves fed RES were offered their MR allowance in more meals per day compared to URES, this effect may reflect more activity due to visiting the milk feeder more often. Also calves fed RES may have had difficulties in predicting when milk was accessible to them and this may explain more activity in the first weeks.

There is a paucity of data in the literature describing the lying pattern of young calves fed MR with different feeding frequencies. Feeding calves 8 MR portions per day lead to an increased duration time of all visits to the milk feeder and number of rewarded visits compared to calves offered the same milk allowance in 4 portions (Jensen, 2006; Rasmussen et al., 2006). However, Rasmussen et al. (2006) did not find any difference in total standing time when calves were fed four and eight portions a day.

In average, calves stood for 22 min/h. This finding is in agreement with Castells et al. (2012), who reported values in the range of 19 to 24 min/h depending on the forage source provided when calves were observed once a week for 2 weeks before and 2 weeks after weaning. Eckert et al. (2015) observed calves from -1.5 to -0.5 h before the afternoon milk delivery three days a week and reported a greater standing time compared to the findings in the present study (26 min/h when calves were weaned at 8 weeks).

The effect of week in the present study was not significant when observing the total time standing, indicating that no differences in activity was found pre- and post-weaning. Similar results were reported by Overvest et al. (2016) where no differences in lying time were found pre-and post-weaning when calves were fed different feed types in the first 12 weeks of life. These findings are contradictory to results reported by Eckert et al. (2015) who found that calves weaned at 8 weeks of age lied down for 43 min/h two weeks pre-weaning and 31 min/h two weeks post-weaning, indicating more activity with transition to solid feed. Differences between these results and the ones obtained in the present study can be found in the variation in the milk feeding management and method of observing the calf behavior. Eckert et al. (2015) offered each calf up to 12 L/d of MR once per d until 38 d of age, and calves were gradually weaned by reducing MR amounts with 1 L/d until d 50. The high volumes of MR fed in the pre-weaning period may have contributed to calves being more inactive as minor time was spent on consuming solid feed. Thereby, differences in time spent standing will be remarkably greater pre- and post-weaning compared to calves fed lower amounts of MR where time spent on other feeding activities will increase the time of standing in the pre-weaning period. However, Jensen and Holm (2003) found no effect of milk allowance and milk flow rate on the duration of lying, when calves were fed high or low amounts of MR. Neither did von Keyserlingk et al. (2006) find any effects of periodic milk availability on the daily pattern of lying, when behavior was measured by using scan sampling for two consecutive days at the age of 32 d. No difference was found in standing or lying time, when calves were fed milk once or twice daily (Kienitz et al., 2017).
8.5.2 Feeding time

In week 4, calves fed CON-RES spent significantly more time in the automatic milk feeder compared to the other treatment groups. The observations did not distinguish between rewarded and unrewarded visits, but the results indicate that calves fed a lower amount of MR with restricted portion size spent more time occupying the feeder. Calves fed RES might occupy the feeder for longer duration time after the milk allowance was reached in hope of receiving more MR. Jensen (2006) argued that calves might have difficulties in predicting when milk is accessible to them in the first weeks of usage of the automated feeder and therefore more visits are in general observed for calves fed restrictive. Also, a higher frequency of unrewarded visits to the automatic milk feeder are in previous studies found in calves on a low milk allowance and is associated with hunger (Jensen and Holm, 2003; Jensen, 2006; De Paula Vieira et al., 2008; Jensen, 2009). Calves fed CON are therefore expected to visit the feeder more frequently than calves fed STP in week 4 and week 5. The frequency of unrewarded visits has been found to decrease over time (Jensen, 2009), suggesting that some learning is involved, because unrewarded visits might reflect that calves are challenged in knowing when they are offered their next meal (Jensen and Holm, 2003). The decreasing number of unrewarded visits over time might also reflect that the calves are less exposed to hunger, because the consumption of solid feed has increased.

Data has unfortunately been difficult to withdraw from the milk feeder computer and has not been possible to include in the present discussion. Information about rewarded and unrewarded visits is therefore not known. It is interesting to know how many visits URES calves spent to consume their daily allowance when offered free access. It is expected that calves fed URES consume larger portions compared to RES. Senn et al. (2000) found that large meals were associated with longer post-meal intervals. This finding supports the hypothesis that calves fed greater amounts of milk or MR may tend to pattern their intake in fewer and larger meals, when given the opportunity (Jensen, 2009). It is furthermore expected, that calves consume most of their allowance immediately after the new feeding day begins (Appleby et al., 2001; von Keyserlingk et al., 2006; Miller-Cushon and DeVries, 2015). However, high-fed calves (12 L/d) distribute their visits throughout the day, whereas low-fed (4L/d) calves show an increase in the number of visits around the time when the milk become available to them at the feeder (Borderas et al., 2009).

As expected, time spent eating concentrate and hay increased over time for all treatment groups, which is attributable to the increased consumption as calves grew and transitioned to solid feed. This is in agreement with other studies (Jensen, 2006; Eckert et al., 2015; Overvest et al., 2016).

In week 5, milk allowance was reduced from 8 to 5 L/d for calves fed STP. Calves fed CON were offered 6.5 L/d as in week 4. This reduction was expected to have a positive effect on the intake of solid feed, because calves would compensate for lack of energy by eating more concentrate. The intake of concentrate (kg/calf/d) was not statistically analyzed because data was obtained on group level (n=4), but a numerical difference between CON and STP was found in week 5 (0.285 vs. 0.389 kg/calf/d, respectively). However, the results obtained by direct observations showed that calves fed CON spent more time eating concentrate compared to calves fed STP. Furthermore, the concentrate intake was significantly different between CON-URES and STP-URES in week 5 (2.1 vs. 0.7 min/h, respectively). No information of the intake of solid feed was available regarding RES and URES and it is therefore not possible to link the time spent eating solid feed with the actual feed intake. Observations were made from 22-31 h after the step-down in MR allowance and therefore calves fed STP may not yet have responded to the lower energy intake at the time of observation.
An effect of RES/URES on the intake of hay was found in week 4 and 8, where calves fed RES consumed more hay in week 4 compared to calves fed URES. This could indicate that several small milk portions do not satiate the calf as much as fewer and larger portions do. When calves seek the milk feeder without any reward, they will more likely spend time on other feeding activities. The mean time spent in feeding activities when fed RES and URES are illustrated in the Appendix, Figure 6-7. The pattern indicates that calves fed RES spent most time occupying the milk feeder and eating solid feed in the period from 8am to 9am (the last hour of the feeding day). In this period orts were removed and fresh concentrate and hay was provided. The increased frequency of visits to the milk feeder might indicate that calves fed RES were hungry (Jensen and Holm, 2003). Calves fed URES were more likely to visit the feeder in the afternoon (Appendix, Figure 7). It has not been possible to find research investigating the effect of FF on the feeding behavior in relation to solid feed intake. However, results from the present study indicate that calves fed RES are likely to seek other feeding activities when unrewarded in the milk feeder (Appendix, Figure 6-7).

Conversely from week 4 to week 8, T2 was found to increase the intake of hay for calves fed URES compared to RES, where all calves were offered 2 L MR per day and all calves were allowed to consume their daily MR allowance in one portion. No difference was found in time spent consuming concentrate between the treatment groups in week 8. The mean time spent on feeding activities for calves fed RES and URES in week 8 is illustrated in Appendix, Figure 8-9. The pattern over the six hours of observations looked similar between the treatment groups, but calves fed RES seemed to spend more time occupying the milk feeder in the first hour of the feeding day (9-10am). Both treatment groups consumed the most hay from 8-9am where orts were replaced by fresh feed and activity in the barn stimulated the calves to stand up. However, calves fed URES spent significantly more time consuming hay compared to RES in week 8. Previous research investigating the effect of FF did not provide hay, and it is therefore difficult to discuss the effect of FF on the intake of concentrate and hay separately. Sockett et al. (2011) found a positive effect of the intake of concentrate (+26%) in the weaning period when calves were fed MR in three daily feedings compared to two daily feedings. Thereby this study found a positive effect of more meals per day, which is contradicting with results from the present study. However, the number of daily feedings for all calves is not yet known and it is only hypothesized that calves fed URES would be likely to consume larger meals and thereby less portions per day compared to calves fed RES.

8.5.3 Recording method

To minimize the time required for observation, the method of instantaneous sampling was used in experiment 1B. By observing the calves by using direct observation it was possible to see whether the calf was actually eating and if any irregularities occurred during the observation period, this would be observed. The time periods of observation were determined from continuous video recording and from a practical point of view.

Instantaneous sampling with 5 min intervals has been demonstrated to be reasonably accurately ($R^2 = 0.79$) to assess the daily feeding behavior (Miller-Cushon and DeVries, 2011b). It was considered to further reduce the interval to obtain an even more accurate estimate of the feeding behavior, but it was anticipated that the observations would be too demanding when only one observer should record all events for 32 calves.

The behavior could also have been monitored by direct observations from 8am to 10am, where calves were observed for 1 h immediately following the new feeding day with access to milk and one hour additional
thereafter. This method would obtain data from more consecutive days with observations in the same time period of the day without increasing time spent on direct observations. This method was used in other studies investigating feeding behavior in relation to solid feed intake (Castells et al., 2012; Hosseini et al., 2015). More observation days would possibly also have improved the reliability of data since it is known that solid feed intake can vary from day to day depending on the milk feeding level (Miller-Cushon et al., 2013). Increased daily variation in DMI might be related to health and weather conditions. All calves in the present study were checked from the outside of the group-pen in the mornings before data was collected and no calves were identified as sick on these days. However, some calves might have had subclinical signs which might have affected their activity and thereby the feeding behavior. Furthermore, the observation days in week 4 and week 5 were very cold and windy, where calves due to draught in the barn, were mainly lying in groups trying to cope with the cold.

8.6 The experimental setup

The effect of block within experiment was significant, when data of BW and total ADG was analyzed in experiment 1AB. This suggests that the blocking was effective, as there was a difference between the two blocks in each experiment. In experiment 1A block 1 was generally performing better than block 2 and in experiment 1B block 1 was generally performing worse than block 2. When calves at the start of each experiment were allocated to the group-pens, calves from two herds were randomly allocated to each block according to the experimental setup for experiment 1A and 1B, respectively (Figure 7). The average weight of each experimental block was random and was an effect of two herds having older and heavier calves than the two other herds in each experiment. Factors such as herd of origin, age, BW, group-pen, draught and diurnal temperature will affect the results of the experiments. However, age and BW between treatment groups within the block were similar and calves were blocked according to herd of origin. However, if continuously observations had been recorded, the presence of draught could have been determined and any negative effect of this been circumvented before it affected the calves of the different pens in a random or systematic way. Furthermore, information on placing of the calf pen within the building could have been included in the statistical models to adjust for possible variations within block. The block effect illustrates the importance of homogeneous treatment groups within blocks.
9 Conclusions

In summary, no significant effects of the milk feeding methods (STP and CON) and milk feeding frequency (RES and URES) with respect to overall feed intake and calf performance were detected in this study. No interactions between the two milk feeding strategies were found when evaluated in the pre- and post-weaning period. Overall, ADG did not differ between the two experiments, but block within experiment was found to have a significant effect on ADG in the pre- and post-weaning periods. As hypothesized, calves fed STP consumed more concentrate in week 5-6 compared to calves fed CON. Calves fed URES had a higher concentration of blood glucose two weeks post-weaning compared to calves fed RES. Intake of concentrate was lower in experiment 1A compared to 1B. Challenges regarding use of the automatic concentrate feeder in experiment 1A may have limited the intake of concentrate. In experiment 1B, FCE was numerically worse compared to 1A which could indicate that the calves were suffering from the cold weather and draught in the winter period. No overall differences were found in relation to fecal scores in week 10.

Calves fed RES spent more time occupying the milk feeder, when calves fed STP and CON were offered 8 and 6.5 L of MR per d, respectively. Furthermore, calves fed RES were more likely to spend more time standing compared to calves fed URES in week 4 and week 5. Calves were challenged by gastrointestinal and respiratory diseases in the first weeks of both experiment 1A and 1B, therefore, feed intake and ADG were somewhat lower than expected. Therefore, the effects of the milk feeding strategies investigated in this experiment may not have been expressed as well, as if calves had remained healthy throughout the entire trial period. Finally, it was concluded that calves in the present study performed better post-weaning compared to similar studies, which may be explained by the provision of hay in the pre- and post-weaning period.

Whether feeding MR through a step-down strategy stimulates the consumption of solid feed, and whether smaller volumes of MR distributed in more meals per day compared with no restriction on milk meal sizes have a positive effect on calf performance requires further studies.
10 Implications

Results obtained in the present study reported no differences in calf performance when evaluated from two weeks of age until two weeks post-weaning. It would be interesting to investigate whether the effect of step-down milk feeding strategies would be more pronounced if calves were fed 8 L of MR for the first four weeks of life. Furthermore, it would be of great interest to investigate the effect of both treatments if calves were not purchased from different dairies. This would possibly improve the health status of the calves in the pre-weaning period. However, the present study reflects the ‘real life’ challenges in the intensive calf production.

No overall effects were found when calves were fed restrictive (max 2.3 L/portion) compared to calves fed unrestricted in the present study. However, the effect of feeding milk or MR three times a day, compared to twice daily, is still believed to have a great effect of feed efficiency and ADG (Sockett et al., 2011). Feeding a higher level of nutrition in the milk feeding period has become a popular choice for heifer calves. Maybe the point is not about how much to feed them, but how the nutrition that the calf receives can impact the ADG in the post-weaning period. It is believed that bull calves consuming higher amounts of solid feed in the pre-weaning period will have advantages in the fattening period, because of greater rumen capacity and fermentation.

Studies have shown a great capacity of the abomasum and no increase in occurrence of diarrhea when calves are fed a larger amount of milk or MR - but is this the right argument for feeding high levels of milk or MR in one or two daily feedings? Smaller volumes fed more frequently may result in less digestive disturbances and keep the calf in a positive energy balance at all times of day, all year around. For practical use, further studies are required to evaluate the effect of two versus three daily feedings, when feeding great amounts of milk or MR. Previous studies investigating feeding frequency tend to use automatic milk feeder and these findings might therefore differ from the effect found when feeding milk or MR manually, which is common practice in dairy and veal calf herds.

When evaluating the results obtained from the present study, it may be beneficial to allocate calves to URES when using automatic milk feeders in the intensive bull calf production. This will limit the occupancy of the milk feeders and thereby reduce the competition of calves and allow more calves per feeder. However, recordings of time spent occupying the milk feeder and number of rewarded/ unrewarded visits need to be better evaluated in order to be able to conclude on the exact value of URES compared to RES.

To investigate whether any of the treatments have prolonged effects on carcass weight and classification, results obtained at slaughter will be evaluated. Calves will be slaughtered at 10 months of age.
References

11 References


References


Abstract

Title: Performance of calves fed a fixed amount of milk replacer with or without restrictions on meal size

The abstract has been accepted by the Scientific Committee to be presented at The Annual Meeting of the European Federation of Animal Science (EAAP) in Tallinn, Estonia (August 2017).
Figure 1 Milk replacer, e-lac HP 110
Figure 2 Feed analysis from Eurofins: Grass hay
## 26067 Kalvestart Hamlet

### Tilskudsblanding til kalve
Må kun anvendes med indtil 500 g pr. kg af det daglige foder

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<th>ANALYTISKE BESTANDDELE</th>
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<td>104 FE pr. 100 kg.</td>
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### TILÆTningsstoffer
Emergensorrengøring:

| 10,00 I.E./g | A-vitamin (3a672a) |
| 1,00 I.E./g  | D-vitamin (6071)   |
| 76,03 mg/kg  | E-vit. all-rac-alpha-tocopherylacetat (3a700) |
| 30,11 mg/kg  | E-vit. RRR-alpha-tocopherylacetat (3a700) |
| 5,00 mg/kg   | B1-vitamin         |
| 80,00 mg/kg  | Jern(III)sulfat monohydrat (3B3E1) |
| 1,77 mg/kg   | Calciumiodat anhydrat (3B2E) |
| 25,00 mg/kg  | Kobber(II)sulfat pentahydrat (3B5E4) |
| 187,50 mg/kg | Mangansulfat monohydrat (3B5E5) |
| 0,33 mg/kg   | Koboltcarbonat monohydrat (3B3E3) |
| 171,43 mg/kg | Zinksulfat monohydrat (3B5E6) |
| 0,55 mg/kg   | Natrumselen (3B8.11) |
| 0,02 mg/kg   | Organisk selen (3B8.11) |
|              | Antioxidant |

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Vestyjllands Andel, Vesterkær 16, 6950 Ringkøbing
Fabrik Bonis, Godkendelser- og registreringsnummer: 206-G756800
Batchnr.

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Figure 3 Concentrate, 26067 Kalvestart Hamlet
Calf Requirements

Major Inputs Used to Compute Young Calf Requirements

- Calf Body Weight: 60 (kg)
- Temperature: 20.0 deg. C
- Diet ME: 4.02 (Mcal/kg)
- Diet NEm: 3.28 (Mcal/kg)
- Diet NEg: 2.57 (Mcal/kg)

Calculation of Young Calf Requirements

Allowable Gain

- Energy Allowable ADG: 0.42 (kg/day)
- ADP Allowable Gain: 0.42 (kg/day)

Maintenance Requirement Calculations

- Total Milk Dry Matter Intake: 0.45 (kg/day)
- Total Starter Dry Matter Intake: 0.45 (kg/day)
- Net Energy Basal Maintenance Requirement: 0.086 (Mcal/day/BW^0.75)
- Temperature Multiplier: 1.00
- Net Energy for Maintenance: 1.85 (Mcal/day)
- Dry Matter Intake Required for Maintenance: 0.57 (kg/day)
- Efficiency of use of ME for NEm: 0.82
- Metabolizable Energy Required for Maintenance: 2.27 (Mcal/day)
- Apparently Digested Protein Required for Maintenance: 41 (g/day)
- Crude Protein Required for Maintenance: 48 (g/day)

Growth Requirement Calculations

- Intake Available for Growth: 0.34 (kg/day)
- Net Energy Available for Growth: 0.88 (Mcal/day)
- Efficiency of use of ME for NEg: 0.64
- Metabolizable Energy Available for Growth: 1.37 (Mcal/day)
- Apparently Digested Protein Required for Growth: 105 (g/day)
- Crude Protein Required for Growth: 125 (g/day)

Protein & Fat Values

- Total ADP Required: 146 (g/day)
- Total CP Required: 173 (g/day)
- ADP Balance: 0 (g/day)
- CP Balance: 0 (g/day)
- Dietary Fat: 11.5%

Figure 4 Calculation of young calf requirements (NRC, 2001).
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**Figure 5** Registration sheet used for direct observations in the stable. S = The calf is standing inactively, C = The calf is standing with the head placed in the through providing concentrate and/or observed chewing with the head raised from the through, H = The calf is standing with the head placed in the through providing hay and/or observed chewing with the head raised from the trough, M = The calf is standing in the milk feeder sucking on the teat or inactivity.
**Figure 6** Mean time spent in the automated milk feeder (MR), at the hay rack (Hay) and at the concentrate trough (Concentrate) measured in min/h. Values are shown separately for calves fed restrictive (RES). Data was obtained by direct observations using instantaneous sampling with 5 min recording interval in week 4.

**Figure 7** Mean time spent in the automated milk feeder (MR), at the hay rack (Hay) and at the concentrate trough (Concentrate) measured in min/h. Values are shown separately for calves fed unrestricted (URES). Data was obtained by direct observations using instantaneous sampling with 5 min recording interval in week 4.
**Figure 8** Mean time spent in the automated milk feeder (MR), at the hay rack (Hay) and at the concentrate trough (Concentrate) measured in min/h. Values are shown separately for calves fed unrestrictive (URES). Data was obtained by direct observations using instantaneous sampling with 5 min recording interval in week 8.

**Figure 9** Mean time spent in the automated milk feeder (MR), at the hay rack (Hay) and at the concentrate trough (Concentrate) measured in min/h. Values are shown separately for calves fed unrestrictive (URES). Data was obtained by direct observations using instantaneous sampling with 5 min recording interval in week 8.
Performance of calves fed a fixed amount of milk replacer with or without restrictions on meal size

Jensen, A., Juhl, C., Jensen, M.B., Vestergaard, M.

In rosé veal calf production, the transition period from a liquid-based milk replacer diet to a solid feed-based diet poses a potential risk of lag in growth. Calves are typically purchased at 2-4 weeks of age and fed limited (5-6 L/d) amounts of milk replacer (MR). In this type of production, it is important to obtain a high growth rate after weaning and to avoid diseases to be able to market these calves before 10 months of age. The hypothesis was that a reduction in milk allowance and meal size would encourage calves to eat more concentrate during and after gradual weaning. The hypothesis was to investigate the pre- and post-weaning intake of solid feed and performance through either a conventional flat-rate (CON) or a step-down (STEP) milk feeding protocol combined with either a restricted (RES) or an unrestricted (URES) meal size offered from the automated milk feeder. A total of 32 calves (in blocks of 16) were purchased from three dairy herds at the age of 12.4 (± 1.2) days and 47.7 (± 1.4) kg LW. Each block comprised 2 pens, allocated to either CON or STEP. Within each pen of 8 calves, 4 calves were allocated to RES (min. 4 meals/d) and 4 to URES (min. 1 meal/d). Until weaning at 8 weeks of age, all calves were offered a total of 224 L MR (21% CP, 20% fat). Calves had free access to a pelleted concentrate (19% CP) and to artificially-dried, chopped, grass-hay (14% CP, 13% sugar). MR and concentrate intake were recorded individually via automated feeders. Starter intake did not differ between treatments in the pre- or post-weaning period, increasing from 1.33 in week 8 to 2.11 kg/d in week 10. Hay intake was 0.11 and 0.13 kg/d in calves fed CON and STEP, respectively, in week 3-8 and 0.14 and 0.17 kg/d in week 3-10. ADG was numerically higher in calves fed RES (0.73 kg/d) compared to URES (0.65 kg/d) (P<0.12) in week 3-8. ADG was numerically higher in calves fed STEP (1.34 kg/d) compared to CON (1.22 kg/d) in week 9-10 (P=0.20). Whether feeding MR through a step-down strategy stimulates the consumption of solid feed, and whether smaller volumes of MR distributed in more meals per day compared with no restriction on milk meal sizes have a positive effect on calf performance require further studies.