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Master Thesis in Animal Science

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Effects of Close-UP period duration and feeding level on periparturient performance and health of dairy cows

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Topic description:	An investigation of the effects of feeding acidogenic Close-UP diets for short (14 d) or long (24 d) duration in combination with two feeding levels of Close-UP diets (Control vs. High) on periparturient performance and health parameters of Holstein cows.
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Preface and acknowledgements

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The experimental work was undertaken from June to December 2019 on two commercial dairy farms in Denmark. Great thanks to Hofmansgave and Havredalsgaard for their willingness to let me conduct the experiment and for their assistance with both sampling and registrations.

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Abstract

Nutrition in the dry period is of utmost importance for a dairy cow's performance and health in the subsequent lactation. The objectives of the present study were to investigate the effects of Close-UP feeding duration in combination with two different Close-UP feeding levels on postpartum performance in dairy cows. The study was undertaken on two commercial Danish dairy farms with a 2x2 crossover study design in June to December 2019. The two factors were the duration of Close-UP period (**S**hort; 14 d vs. **L**ong; 24 d) and feeding level, determined by concentrate ratio of Close-UP diet (**C**ontrol; 19 % of DM vs. **H**igh; 38.5 % of DM). Dry and pregnant multiparous Holstein cows (n=207) were randomly assigned to 1 of 4 treatments; **SC** (n=59), **SH** (n=42), **LC** (n=59) or **LH** (n=47). The cows received a Far-OFF diet from dry off (-55 d) to either -24 d (L-group) or -14 d (S-group) relative to their expected parturition. Hereafter, the cows received acidogenic Close-UP diets. After parturition all cows received lactation diets.

Close-UP duration affected colostrum parameters. Higher colostrum yield and a tendency for higher total protein yield at first milk-out was observed for the S-group when compared to the L-group. No effect of feeding level was observed on colostrum parameters. Interactions between duration and feeding level was observed for brix-value, protein percentage and content of alfa-tocopherol in colostrum. Fat- and lactose percentages were similar between the treatment groups. No significant differences in birth weight of calves were obtained.

No significant differences in incidences of milk fever, retained placenta, metritis and ketosis were detected between treatment groups. In addition, no significant differences in severity of udder edema were noted at first milk-out in the four groups.

Close-UP feeding level affected lactation performance. Cows exposed to a high feeding level had an increased energy corrected milk yield and increased protein percentage during the first 50 days in milk when compared to cows fed the C-diet. Moreover, fat to protein ratio in milk tended to be higher in cows fed the H-diet during Close-UP period. No effects of Close-UP duration or interactions between duration and feeding level on lactation performance were detected.

It appears from this experiment that a short Close-UP period with high feeding level is a superior Close-UP strategy for multiparous Holstein cows.

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Abbreviations

AMS = Automatic Milking System

BHBA = Beta-Hydroxybutyrate

BW = Body weight

CP = Crude protein

DCAD = Dietary cation anion difference

DIM = Days in Milk

DM = Dry matter

DMI = Dry Matter Intake

ECM = Energy corrected milk

FP-ratio = Fat to protein ratio

HCl = Hydrochloride acid

Ig = Immunoglobulin

KFL = Kvægbrugets Førsøgslaboratorium

LPL = Lipoprotein lipase

LSM = Least Square Means

NDF = Neutral detergent fiber

NEFA = Non esterified fatty acids

NE_L = Net energy for lactation

OMD = Organic matter digestibility

PTH = parathyroid hormone

SBPM = Sugar beet pulp meal

SCC = Somatic Cell Count

sd = standard deviation

TG = Triglycerides

VLDL = very-low-density lipoproteins

1. Introduction

Rapid physiological, metabolic and nutritional changes occur in the transition period in modern dairy cows (Grummer, 1995). This challenging period in the cow's productive cycle, is associated with a high risk for metabolic disorders that affect subsequent performance and health (Lean et al., 2013; van Saun & Sniffen, 2014). In 2019, 5.6 % of Danish dairy cows died or were euthanized, of which 21 % were culled because of metabolic disorders. In addition, 12 % of the cows that were slaughtered within 60 days in milk, were culled due to metabolic disorders (Raundal & Nielsen, 2020). Several studies suggest that meticulous attention to periparturient diets can improve the overall health and performance of these cows (Douglas et al., 2006; Friggens et al., 2004). This could potentially reduce the number of early cullings.

Feed intake usually decreases as time for parturition approaches. Grummer (1995) and Richards (2011) stated that feed intake is usually decreased 30 to 35 % during the last 3 weeks before parturition, where the major decline in feed intake occurs during the last seven days before parturition (Hayirli et al., 2002). Grummer (1995) also stated that strategies to maximize dry matter intake (DMI) post calving should be initiated prepartum, because a high DMI prepartum is highly correlated to a high DMI postpartum. A high DMI for the high yielding transition cow may have positive effects on subsequent health and production.

Numerous studies have investigated different strategies for dry cow feeding with the aim to maximize especially DMI in the transition cow (Beever, 2006; Mann et al., 2015; Vickers et al., 2013). With one-stage feeding of a dry cow diet with controlled energy density, higher risk of imbalance between nutrient supply and demand may appear. Oversupply of energy in early dry period (Far-OFF) can have negative carryover effects on the peripartum metabolism and feeding during the Close-UP period also affects peripartum metabolism, i.e. energy intake and -balance (Dann et al., 2006).

When a two-stage feeding strategy (phase-feeding) is applied, cows are usually switched from Far-OFF to Close-UP feeding around 21 days prior to parturition. The combination of restricted energy intake in the Far-OFF period and the increased energy density of the diets in the Close-UP period would be expected to mitigate the prepartal DMI-depression. Richards (2011) found increased DMI when cows were switched from the Far-OFF diet to the Close-UP diet, which was followed by increased serum insulin levels and decreased serum NEFA-concentrations.

However, these beneficial properties were only maintained during the first week after the switch. This could suggest that shortening the Close-UP period could be beneficial in increasing DMI and energy status immediately prior to calving. However, most literature has focused on extremes in terms of length of Close-UP period and information on Far-OFF feeding is sometimes lacking (Contreras et al., 2004; Corbett, 2002; Mashek & Beede, 2001). Therefore, research is needed to investigate the sensitivity of Close-UP feeding durations in combination with different feeding levels of Close-UP diets to achieve a successful adaptation to lactation.

The objectives of this study are to investigate the effects of feeding an acidogenic Close-UP diet for a short (14 d) or long (24 d) duration on colostrum yield/ -quality, health, milk production and metabolic status of the transition dairy cow. Furthermore, possible effects of Close-UP feeding level (control vs. high) and interactions between Close-UP duration and Close-UP feeding level on postpartum health and performance, will be investigated.

Hypotheses:

The hypotheses to be investigated are:

- A long duration of Close-UP feeding will improve colostrum yield and quality.
- A long duration of Close-UP feeding will negatively affect subsequent milk production.
- High feeding level will improve postpartum milk production.

<u>Research questions:</u>

For investigations of the hypotheses, the following research questions are listed and will be answered/described through the thesis:

- What are the nutrient requirements for dry cows?
- What happens in the mammary gland during involution and what are the biological processes related to adaptation to lactation?
- How are two-stage feeding of dry cows thought to secure a successful adaptation to lactation?

2. Background

The purpose of this literature review is to provide an understanding of the dry cow's requirements for nutrients and the biological processes related to adaptation to lactation, including mammary gland involution, the following colostrogenesis and bone mineral- and lipid mobilization. A thorough understanding of these processes and their derived risks is of paramount importance in the experimental investigation. Furthermore, a description of how two-stage feeding of dry cows are thought to secure a successful adaptation to lactation, will be carried out.

2.1 Nutrient requirements for dry cows

Danish recommendations for feeding of dry cows are based on the cow's requirements for performance in late gestation. Compared to lactation, the cow's requirements are rather low, and the requirements for maintenance, foetal growth and possibly growth of the cow herself are usually easy to meet with the typical feedstuffs available on Danish farms.

The following section will describe the nutritional needs of dry cows with primary focus on energy- and protein requirements. The overall role of nutrition in the dry period is to 1) stop milk secretion (dry off), 2) maintain the cow's needs until requirements related to fetus and onset of lactation appears simultaneously with decreasing ruminal capacity as the fetus develops (3). Knowledge about the requirements of the cows is essential for understanding the complex dilemmas related to dry cow nutrition and evaluating duration of exposure to different nutritional situations.

2.1.1 Energy

According to the Nordic feed evaluation system, Norfor (Volden, 2011), equations to predict energy requirements for maintenance and gestation are based on Dutch equations, where equations to predict energy demand for growth derive from the French system. In Norfor, no growth is assumed in multiparous cow. The energy requirements for maintenance in cows is calculated according to Van Es (1978) and is presented in net energy for maintenance ($NE_{maintenance}$) (MJ NE/d), equation [1] :

$$NE_{main} = 0.29256 \cdot BW^{0.75} \cdot NE_{exercise} \qquad [1]$$

where 0.29256 is a constant that determines maintenance requirement per kg metabolic weight of cows; BW is the bodyweight in kilograms and $NE_{exercise}$ is a factor related to physical activity. It is assumed that cows in loose house systems increase their requirements for $NE_{maintenance}$ by 10 pct. (factor 1.1). The correlation between $NE_{maintenance}$ and BW for a multiparous loose housed cow is shown in Figure 1.

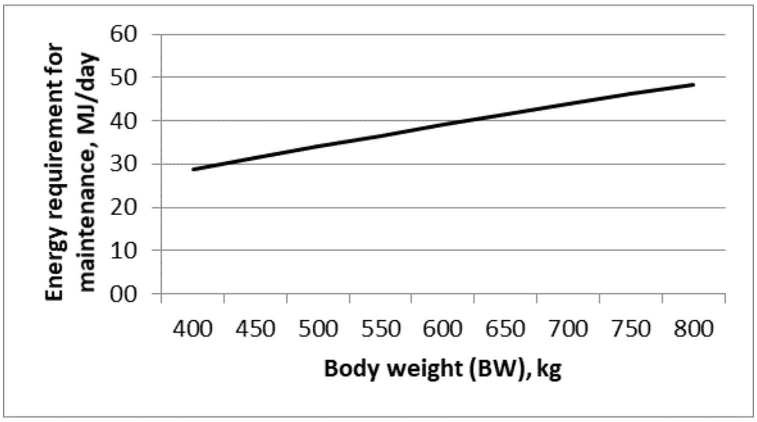

Figure 1 Energy requirements for maintenance as a function of body weight (BW) (400-800 kg)

According to Van Es (1978), the energy requirements for the first 150 days of gestation are negligible (the total gestation length being usually 280-284 days). However, hereafter the energy requirements increase exponentially with each day approaching gestation. The energy requirements in MJ/day for gestation (NE_{gest}) on a specific day of gestation ($gest_{day}$) can be calculated using the equation [2]:

$$NE_{gest} = \frac{BW_{mat}}{600} \cdot e^{0.0144 \cdot gest_{day} - 1.1595} \quad [2]$$

where BW_{mat} is the mature body weight for the breed in kilograms. In Norfor, the mature BW for a cow of Holstein breed is assumed to be 640 kg.

Assuming a constant energy balance at 100 pct. during the dry period, there are no energy requirements or -supply related to deposition and mobilization.

The total daily energy requirement for maintenance and gestation, for a multiparous cow of Holstein breed, weighing 640 kg, is illustrated in Figure 2.

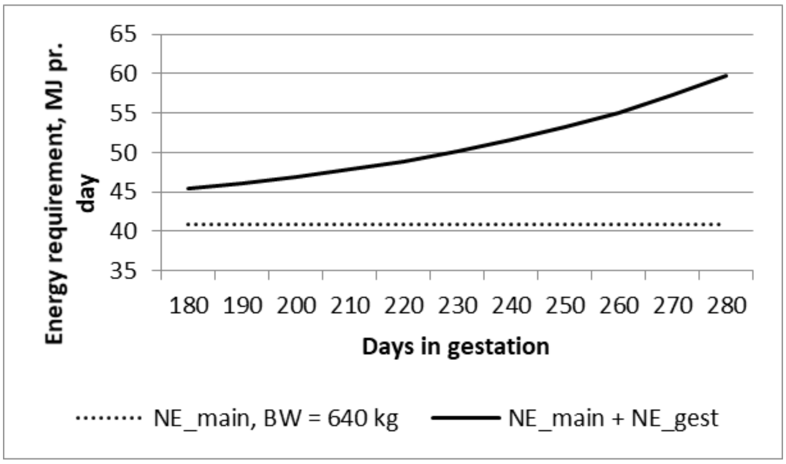

Figure 2 Energy requirement for a multiparous Holstein cow, BW = 640 kg, as a function of days in gestation.

Assuming a dry period of 50 days, the total energy requirement is expected to have increased by app. 10 MJ from day of dry-off to day of parturition. Feeding a TMR to a group of dry cows on different days in gestation, challenges may occur in relation to formulate a ration that meets but does not exceeds the nutrient requirements for the individual dry cow in the group. Different strategies can be implemented practically. One strategy is two-stage feeding, which will be elaborated in section 2.3.

2.1.2 Protein

Besides energy requirements, the dry cow has requirements for protein. Norfor has developed its own equations for protein requirements related to milk production and growth, but according to Nielsen & Volden (2011), protein requirements for maintenance and gestation are based on NRC recommendations. These equations are modified to account for endogenous losses of nitrogen (N) and the requirement for protein is referred to as AAT_N. In the calculation of AAT_N, the amount of N secreted in urine, N being necessary for hair and skin growth as well as endogenous N secretion in faeces is included. Endogenous N secretion in faeces is dependent on DMI, thus the overall AAT_N requirement for maintenance increases as DMI increases. The daily AAT_N requirement for maintenance, $AAT_{N_{main}}$, is calculated according to the equation [3], and is measured in grams per day:

$$AAT_{N_{main}} = \frac{2.75 \cdot BW^{0.5}}{0.67} + \frac{0.2 \cdot BW^{0.6}}{0.67} + \frac{r\_outOM \cdot 0.03 \cdot 0.5 \cdot 3 \cdot 0.4}{0.67} + si\_outOM \cdot 0.025 \cdot 0.5 \qquad [3]$$

where BW is the bodyweight of the cow in kilograms; r_outOM is the amount of organic matter (OM) leaving the rumen and entering the small intestine; si_outOM is the amount of OM leaving the small intestine; 0.67 is a constant utilization coefficient of AAT_N for maintenance; 0.03 reflects the amount of endogenous crude protein (CP), which is 3 % of the OM entering the small intestine from the rumen; 3 is a multiplier to include all endogenous CP produced in the small intestine; 0.5 is AA-N (amino acid-N) proportion of endogenous CP; the factor 0.4 is used to account for reabsorption of 60 % endogenous CP and 0.025 refers to the endogenous CP (2.5 %) entering the large intestine.

Stage of pregnancy determines the requirement of AAT_N for gestation. The formula used in Norfor [4] is modified from National Research Council (NRC, 1985), and the mature BW is used as a scaling factor (BW_mat), reflecting the birth weight of the calf:

$$AAT_{Ngest} = \frac{\frac{BW_{mat}}{600} \cdot 34.375 \cdot e^{8.5357-(13.1201 \cdot e^{-0.00262 \cdot gest_{day}})-0.00262 \cdot gest\_day)}}{0.5} \qquad [4]$$

where AAT_{Ngest} is the daily AAT requirement for gestation in grams per day; gest_day is the actual day of gestation and the coefficient 0.5 refers to the assumed AAT_N utilization for gestation. Figure 3 illustrates estimated requirement for AAT_N as gestation proceeds and that the requirement increases exponentially, with a steep increase in the last trimester of pregnancy.

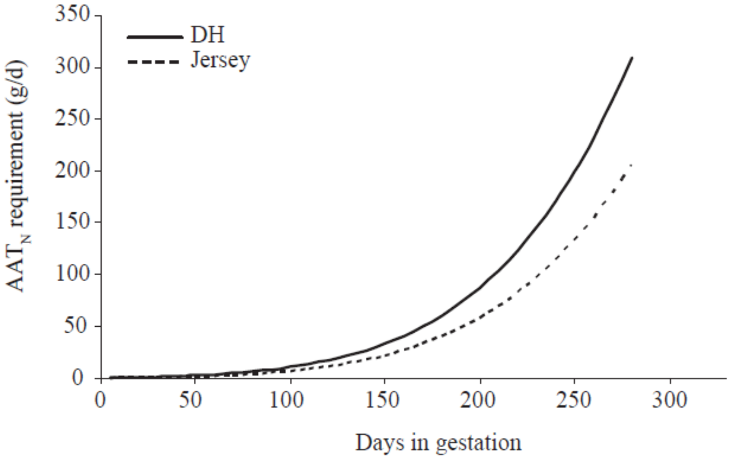

Figure 3 Estimated AAT_N requirement (g/d) for pregnancy depending on days of gestation and mature BW (Jersey: 440 kg and Holstein (DH): 640 kg) (Nielsen & Volden, 2011).

From day of gestation until dry-off, the requirements are easily met by the feed ration assigned for the lactating cows under normal Danish circumstances.

2.2 Biological processes related to a successful adaptation to lactation

With a basic understanding of dry cows' requirements for energy and protein in mind, biological processes related to a successful transition to lactation will be investigated and described in this following section. Around parturition, dairy cows undergo considerable changes in going from pregnancy to lactation and this is when metabolic imbalances most often occur. Onset of lactation combined with decreased appetite due to calving and huge metabolic changes in body reserves, requires optimal nutrition and feeding, in order to aid the cow trough a critical stage of her lactation cycle.

2.2.1 Mammary gland involution

From end of lactation, during the dry period and at onset of lactation, the mammary gland of a dairy cow undergoes changes in cell morphology and cell population. Several studies have showed that dairy cows need a dry period for optimal yield capacity in the subsequent lactation compared to continuous lactation, where milking is continued through to the subsequent lactation (Dias & Allaire, 1982; Kok et al., 2017; Andersen et al., 2005). Cessation of milking at dry off initiates mammary involution. Mammary involution of epithelial cells occurs by apoptosis and according to Smith & Todhunter (1982) the process of involution can be divided into three phases. Soon after milk cessation *active involution* is initiated (1), followed by a *steady state period (2)*, where the mammary gland is non-lactating. Prior to parturition, the gland undergoes *redevelopment (3)* and colostrogenesis begins.

Morphological and structural changes in the mammary gland reflect the different stages of involution. These morphological and structural changes have been studied in cows and described by Holst et al. (1987). The study revealed that already 24 h after milk cessation, large vacuoles appeared in the alveolar epithelial cells, formed from lipid droplets and secretory vesicles. These vacuoles were present until day 21-30 of involution. The epithelial cells remained attached to the basal membrane and only a little loss of alveolar cells was observed. Throughout the period of involution, the epithelial cells were still "active" and were still capable of secretion despite being morphologically different from cells in lactating tissues. Intracellular components related to milk synthesis, such as Golgi-apparatus and the rough endoplasmic reticulum (RER), disappeared in the involuting cells. The intracellular organelles are degraded by two different autophagocytic structures, which are only present about 48-60 h after milk cessation.

Besides morphological and structural changes, composition of the mammary secretions changes during involution. Hurley (1989) investigated how secretion volume increased during the initial few days of involution and found that this was followed by a decrease in volume where secreted components were expected to be reabsorbed. This was followed by a decrease in volume, where secreted components were expected to be reabsorbed. Furthermore, content of lactose, milk fat and milk-specific protein (α-lactalbumin and β-lactoglobulin) also declined during involution. In contrast, total protein concentrations increased in mammary secretions during involution due to increased concentrations of immunoglobulins, lactoferrin and serum albumin in the first few days after milk cessation. Approximately 3 weeks after milk cessation, the involution reached a steady state, where the vacuoles that were formed in the active stage have disappeared. After this, mammary redevelopment began, and the synthesis of colostrum could be initiated. Cessation of milking is the main signal for initiation of involution, however nutrition in the period around and following cessation of milking is an essential management tool to reduce the milk yield abruptly and proper involution is essential for optimal mammary redevelopment.

2.2.2 Colostrogenesis, mammogenesis and onset of lactation

Colostrogenesis is a discrete and finite stage in the mammary redevelopment that comprises the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions (Barrington et al., 2001). An indicator of colostrum quality is the concentrations of immunoglobulins (Ig), especially $IgG_{1.}$ IgG_1 is the predominant colostral Ig essential for passive immunisation of the neonatal calf (Besser & Gay, 1994). During colostrogenesis, up to 500 g of IgG's are transferred into mammary secretions every week, deriving from maternal circulations. The concentrations of IgG_1 and IgG_2 in maternal serum are almost equal, whereas the concentration of IgG_1 in colostrum is 5-10 times higher than IgG_2-concentration (Larson, 1992).

Several studies have investigated the mechanism behind the transfer of IgG_1 into mammary secretions. Larson et al. (1980) found that selective transport of IgG_1 is facilitated by two separate systems. One system is responsible of capturing the IgG_1's from the extracellular fluid, while another system is responsible for internalization and transcytosis of the IgG_1's in order to deliver it to mammary secretions. In addition, Butler (1983) demonstrated that the capturing of IgG_1 in the extracellular fluid is active and is mediated by specific receptors. These receptors are located on the baso-lateral surface of alveolar epithelial cells during colostrogenesis, but are not present during established lactation (Hammer et al., 1969; Kemler et al., 1975).

What regulates colostrogenesis in general and mechanisms that might be affected by nutrition, are essential for evaluating the effects of different Close-UP feeding period durations. It is suggested that synthesis of colostrum is initiated 3-5 weeks prior to parturition (Barrington et al., 2001; Brandon et al., 1971). Occurring simultaneously with colostrum synthesis, the mammary epithelial cells also begin to differentiate in preparation of lactation. Literature suggests that endocrine initiation of colostrogenesis is regulated by estrogen; either alone or in combination with progesterone. In 1971, Smith et al. investigated the role of estrogen and progesterone in the selective transfer of IgG_1 into mammary secretions. The study revealed that estrogen and progesterone administered to cows during the last 4-6 weeks of pregnancy affected the selective transport of IgG_1 to colostrum, and thus the synthesis of colostrum. Convey (1974) found conformingly that serum concentrations of progesterone began to decline 2-3 weeks prepartum, followed by a sharp decline 1-2 days prepartum. This led to the hypothesis that the early decline in progesterone level is the initiating signal for colostrogenesis. This hypothesis was confirmed by Guy et al. (1994) who found incidences where declines in progesterone levels and transfer of IgG 10-30 days before parturition coincided.

Besides the effects of steroid hormones, other hormones might be important in the establishment of colostrogenesis. Hadsell et al. (1992) reported that cows treated with bovine growth hormone (GH) showed an increased IgG_1 concentration in mammary secretions, although no increase was seen at parturition. The mechanism behind this could be the increased mammary blood flow and cellular activity caused by stimulation of insulin-like growth factor-1 (IGF-1) secretion, its selective binding to IGF-BP3 and subsequent binding to specific mammary IGF-binding-protein-3 (IGFBP3) receptors in the mammary gland. Hence, this enhanced blood flow would increase the availability of IgG_1 for transfer to colostrum (Barrington et al., 2001; Rulquin & Vérité, 1993).

It remains unclear whether colostral IgG can be manipulated through the feeding schedule of the dry cow. In theory, increased dietary energy level when altering the diet from Far-OFF to Close-UP might affect the metabolic status of the cow and thereby lead to upregulation of IGF-1 secretion. This is because serum insulin levels are increased due to higher nutrient supply. The mechanism is that increased serum insulin levels facilitate GH-receptor formation in the liver, thereby stimulating hepatic IGF-1 secretion. This could increase IgG_1 transfer, as suggested above. However, the metabolic status of the cow at the time of colostrogenesis might be affected by previous feeding strategy (Dann et al., 2006; Janovick et al., 2011), which might decrease energy uptake when colostrogenesis is initiated. This indicates that the mechanism described could be different.

Cessation of colostrogenesis is also regulated by hormones, and Barrington et al. (2001) suggest that glucocorticoids and prostaglandin F2α play the major role in cessation. Transfer of immunoglobulins to mammary secretions terminates, and lactogenesis initiates. Estrogen levels increase around one month prepartum, whereas increasing levels of cortisol, growth hormone and prolactin are observed approximately one week prepartum. Concentration of serum progesterone decreases sharply around 1-2 days prior to parturition (Akers, 2002; Sjaastad et al., 2010a). Cortisol and prolactin have a synergistic effect on the initiation of lactation, whereas cortisol induces growth of RER and Golgi apparatus of the epithelial cells (recall that these organelles were declining in the involuting epithelial cell, section 2.2.1). This is essential for prolactin to have an optimal effect on binding to receptors in the mammary gland. Prolactin levels are increased 5- to 10-fold around parturition. Prolactin is responsible for the activation of intracellular tyrosine kinases which facilitate the protein-coding in the epithelial cells. In late pregnancy the transcription of the gene for prolactin receptors on the epithelial cells is inhibited by progesterone. When progesterone levels decline, three to four days prior to parturition, the synthesis of prolactin receptors increases, enabling the mammary gland to respond to the greatly increased levels of prolactin from the anterior pituitary (Akers, 2002). Synthesis of the milk-specific protein α-lactalbumin is now initiated in the mammary gland. This protein is essential for the synthesis of lactose; the main driver for milk volume.

To summarize; At dry-off, cessation of milking initiates mammary involution. Involution reaches its final steady stage app. 3 weeks after dry-off and the mammary gland is ready for redevelopment of secretory cells; the basis for colostrogenesis and lactogenesis. Colostrogenesis is regulated and controlled by hormones with estrogen and progesterone being the main drivers in relation to Ig secretion. Lactogenesis is also mediated by hormonal regulation with prolactin being the main driver, and milk-specific proteins can be synthetized. Understanding the principles behind the involution, the colostrogenesis and the onset of lactation, will help to unlock the potential gateways that may be possible to manipulate through nutrition.

2.2.3 Metabolic adaptation

2.2.3.1 Bone mineral metabolism and derived risks

Bone minerals play an important role in the physiological transition of cows from pregnancy to lactation. The demand for minerals depends on both body weight and performance. Performance of dry cows is characterized by the final rapid development of the fetus, uterus and placenta. A few days prior to parturition, large amounts of minerals (especially calcium) is transferred to the colostrum, thus increasing the demand for minerals derived from both feed and bone mobilization. Dairy cows with a high production potential have equivalently greater and more demanding requirements of their regulatory mechanisms in order to avoid suffering from deficiency diseases such as hypocalcaemia. In this section, focus will be on dairy cows' calcium metabolism and derived risks in the transition period, which is essential for a better understanding of the role of nutrition in the dry period.

The skeleton of the cow contains 99 % of the calcium in the body and the amount deposited into bones decreases with increasing age. When ruminants reach one year of age, the amount of calcium deposited in the bones reaches a maximum, and the rate of deposition declines hereafter, levelling out around 9 years of age. In plasma, around 50 % of the Ca is found in its ionized form (Ca^{2+}). The remaining part (40 %) is found bound to proteins, mainly albumin and 10 % is bound to complexes such as bicarbonate and citrate (Horst, 1986).

Calcium demand is mainly driven by mammary secretions. Before parturition the daily requirement for calcium is below 20 g, which includes the amount of calcium excreted in the faeces and urine as well as calcium for foetal development and growth (DeGaris & Lean, 2008; Friggens et al., 2004). A study by Goff et al. (2002) showed that the demand for calcium is solely a result of the calcium drain of lactation, and not due to the act of parturition. This was illustrated when plasma calcium concentrations were compared between mastectomized cows and intact cows around the periparturient period (Figure 4). Therefore, the strong demand for calcium appears when colostrum synthesis is initiated a few days prior to parturition.

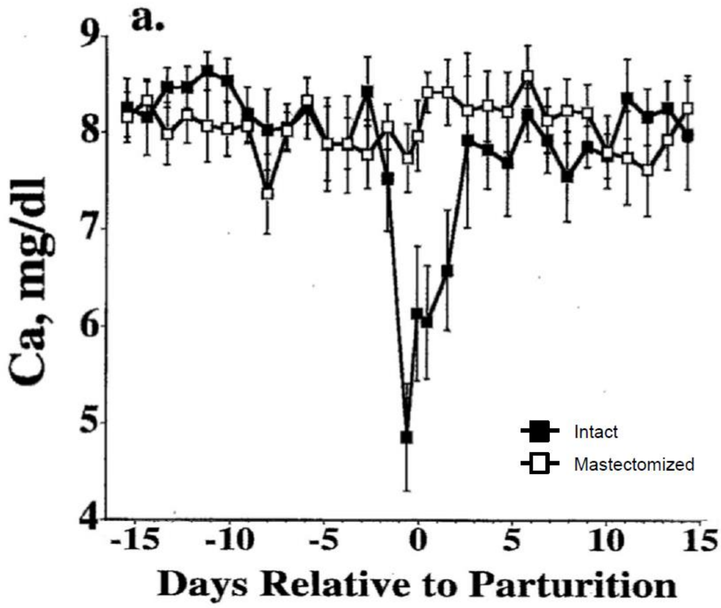

Figure 4 Plasma calcium concentrations in intact and mastectomized cows during the periparturient period (Goff et al., 2002)

Circulating plasma calcium reserves are limited in the cow, which is why the demand must be compensated through increased intestinal and ruminal absorption and via bone mobilization. The blood plasma concentration of calcium is regulated to maintain an almost constant level of around 2.0-2.5 mmol/L, except for the time around parturition, where there is a sudden drop in plasma calcium concentrations due to excretion of calcium in colostrum (Hernández-Castellano et al., 2017). Cows are able to handle a drop in plasma calcium concentrations, but clinical signs of hypocalcaemia are observed when plasma concentrations of calcium decline to below 1.4 mmol/L. Subclinical hypocalcaemia appears when plasma calcium levels are 2.0-1.4 mmol/L (DeGaris & Lean, 2008). In all cases, hypocalcaemia is caused by an imbalance between outflux in milk and influx to the extracellular matrix of ionized calcium (Ca^{2+}). Severe cases of clinical hypocalcaemia are associated with muscle lameness (stasis) and may cause death (DeGaris & Lean, 2008). The disease can be cured by acute intravenous administration of calcium.

The level of calcium in the plasma is regulated through a complex regulatory mechanism of the transition cow. The homoeostasis includes the parathyroid hormone, PTH, the active form of vitamin D (1,25-dihydroxivitamin D/calcitriol) and calcitonin. These three hormones are responsible of the maintenance of the plasma calcium concentration aiming for 2-2.5 mmol/L. The PTH hormone is the overall driver for the increase of [Ca^{2+}] in the extracellular fluid when the concentration is low, thereby preventing hypocalcaemia. PTH is a peptide hormone, with a relatively short half-life in plasma (10 min), resulting in its rapid degradation (Sjaastad et al., 2010b). Therefore, secretion of PTH from the parathyroid glands needs to be regulated continuously. A review by Horst et al. (1997) revealed that release of PTH is regulated through negative feedback mechanism. Low [Ca^{2+}] is a stimulus to enhanced PTH secretion. Increased PTH secretion results in a rise in [Ca^{2+}], thereby PTH level declines again due to increased levels of Ca^{2+} in the extracellular fluid deriving from mainly bone resorption. However Ramberg et al. (1984) described that animals in early lactation depend mostly on intestinal absorption, especially when fed high Ca levels. PTH is furthermore responsible for the renal formation of the active form of vitamin D, which is the second hormone affecting the calcium homeostasis. The overall role of calcitriol/vitamin D is to enhance the absorption of Ca^{2+} from the small intestine to the blood. The hormone binds to intracellular receptors in the epithelial cells of the intestines responsible for transporting Ca^{2+}, hence increasing [Ca^{2+}]. PTH is thereby indirectly affecting intestinal absorption of calcium through calcitriol because PTH stimulates the activity of 1-α-hydroxylase in the kidneys, which are responsible for the hydroxylation of the metabolites of vitamin D into the active form (calcitriol) (Horst, 1986).

The third hormone involved in the homeostasis is calcitonin, a peptide hormone produced in the thyroid gland. The concentration of calcitonin is positively correlated to the concentration of Ca^{2+} in blood. Calcitonin is thereby responsible for the mineralisation of bone minerals, and the main target cells for calcitonin are the osteoclasts, which are essential in mineral mineralisation. Calcitonin reduces to rate of bone resorption due to a lower activity of active osteoclasts on the surfaces of the bones and thereby acts as the down-regulator of elevated Ca^{2+} levels above normal (Hernández-Castellano et al., 2019).

With a basic understanding of the regulatory mechanism of calcium homeostasis in mind, it is possible to evaluate the derived risks of implications of the homeostasis, such as when the blood plasma concentrations are below 2.0 mmol/L. At the onset of lactation, where demand for calcium is high and the feed intake is reduced due to parturition (Douglas et al., 2006; Grummer, 1995), the abovementioned stimuli generally result in increased resorption of calcium from bones and intestinal absorption. However, there are cows that either cannot fulfil their mammary demand for calcium or suffer from an acute inability to resorb calcium from their bones (Horst, 1986). The latter was investigated by Rowland et al. (1972) who observed impairment of osteoclast function in cows that suffered from clinical hypocalcaemia.

Hypocalcaemia is a well-known production disease in dairy herds and have been a research topic for the past 50 years. A Danish report on the number of cows treated for periparturient hypocalcaemia in 2013-2015 showed that dairy cows were treated for hypocalcaemia in 3.6 % of the total calvings (3.3 % and 7.4 % for Holstein and Jersey, respectively) (Strudsholm et al., 2016), thus indicating the prevalence of the acute state of the disease. This aligns with the incidence found by others (DeGaris & Lean, 2008; Goff, 2008) In addition, Reinhardt et al., (2011) reported incidences of subclinical hypocalcaemia in 25%, 41%, 49%, 51%, 54% and 42% of 1st–6th lactation US-dairy cows, respectively. Despite that the severity of the disease in clinical cases is greater than in the subclinical cases, the importance of the subclinical cases should not be underestimated. Rodríguez et al. (2017) investigated the associations between subclinical hypocalcaemia and postparturient diseases affecting production and longevity. The authors reported that cows suffering from subclinical hypocalcaemia had 3.7-, 5.5-, 3.4- and 4.3-times greater risk for experiencing displaced abomasum, ketosis, retained placenta and metritis, respectively. Furthermore, cows prone to subclinical hypocalcaemia showed their first estrus later than normocalcemic cows. Despite the fact that the study perceives that subclinical hypocalcaemia occurs when serum [Ca] is below 2.4 mmol/L, which could lead to overestimation of the odds ratios listed, other studies confirm that subclinical hypocalcaemia affects health and production post-partum (Goff, 2008). Therefore, preventive strategies should be implemented in dairy operations as cases of hypocalcaemia is just the tip of an iceberg.

To summarize, an enormous demand for bone minerals in mammary output drives the cow's bone mineral mobilization, where the main homeostatic hormones include PTH, vitamin D and calcitonin. When the mobilization capacity cannot fulfil the requirement and the serum concentration of calcium drops below 2.0 mmol/L, the cows are of greater risk of suffering from the deficiency disease milk fever, either clinical or subclinical. Any stage of milk fever can be associated to several other production diseases and may affect production and longevity, which is why any incidence of milk fever should be avoided in the herd. Feeding related strategies can be implemented in order to alter the regulatory mechanisms and prime the cow for greater calcium mobilization at onset of lactation.

2.2.3.2 Lipid mobilization capacity and derived risks

The coordination and regulation of lipid metabolism in the liver, adipose tissue and mammary gland of a dairy cow are key components of the adaptation to lactation. At a time where energy demand for milk production is at its highest and DMI and thus nutrient supply lags far behind, the cow needs energy from its body reserves; mainly deriving from adipose tissue. Extreme mobilization of fat is associated with an increased risk for fatty liver syndrome (Roberts et al., 1981), ketosis and derived risks (Drackley, 1999). Feeding in the dry period affects the cow's capacity for lipid mobilization (Dann et al., 2006) and thus the risk for developing related diseases that affect production and health. To illustrate the importance of the timing of the nutritional signalling in this critical period, the following section will focus on the mechanisms behind the lipid mobilization occurring in the periparturient cow and the derived risks when the mobilization capacity is exceeded.

Plasma pools of metabolites vary according to state of lactation. A review by Adewuyi et al. (2005) revealed that the serum concentration of non-esterified fatty acids (NEFA) began to rise a few days prior to parturition. More detailed, Grummer (1993) illustrated that NEFA concentrations increased twofold from 17 to 2 days prior to parturition and increased twofold again to peak at parturition. This is of interest because serum NEFA concentrations indirectly reflect the hepatic fatty acid uptake and thereby provides an insight into the fatty liver development. In addition, a study by Reynolds et al. (2003) showed that during the periparturient period the blood flow to the liver also increased significantly from 1147 L/h at nine days prepartum to 2187 L/h eleven days postpartum, which is an important contributing factor for increased hepatic NEFA uptake.

According to Grummer (1993) fatty liver occurs when: *"the rate of hepatic acid esterification exceeds the rate of TG (triglyceride) disappearance (...)"* in the liver. In other words, fatty liver is a result of extreme accumulation of TG's in the liver. TG accumulation is a consequence of an increased lipolysis of adipose tissue striving to compensate for the cow's negative energy balance. Energy deficient cows in early lactation have decreased levels of insulin. When insulin levels are low, hepatic IGF-1 secretion is also low. Due to inhibited negative feedback on the secretion of GH from the pituitary gland, the GH-concentration increases (Florini et al., 1996). Thereby, GH is one of the stimulators for lipolytic activity in adipose tissue (by an increased effect of the catecholamines), thus exporting the fatty acid as NEFA (and the remaining glycerol) to the blood. In addition to the hydrolysis of TG from adipose tissue, hydrolysis of the lipoproteins' TG in the capillary wall also occurs, where lipoprotein lipase (LPL) is the responsible enzyme for the hydrolysis (Madsen & Nielsen, 2003). NEFA is transported for uptake in the liver, where it is 1) used in the synthesis of lipoproteins or 2) oxidized to acetyl-

CoA. Acetyl-CoA is either a substrate for the glucogenesis or a substrate for the ketogenesis to form ketones. The quantitative export of lipoproteins as very-low-density lipoproteins (VLDL) from the liver is very low in ruminants (Drackley, 1999). Therefore, to avoid NEFA concentrations to reach toxic levels in the blood, the liver needs to store the lipoproteins as TG (hepatic acid esterification) or oxidize acetyl-CoA in the citric acid cycle (TCA) (glucogenesis) or form ketones (acetate). However, an infiltration of TG occurs in energy deficient cows, because of a reduced capacity for oxidation in the TCA-cycle. The TCA-cycle needs sufficient amounts of glucogenic substrates; propionate or amino acids in ruminants (White, 2015) . This is the limiting factor when cows are in extreme need of energy, therefore the only pathway to dispose NEFA from the blood, when sufficient amounts of glucogenic substrates are absent, is to store the NEFA as TG in the liver. According to White (2015) liver lipid accumulation is therefore a combination of several hepatic pathways: 1) increased lipolysis and fatty acids delivered to the liver, 2) increased fatty acid synthesis in the liver, 3) decreased hepatic oxidation of fatty acids and 4) impaired export of TG as VLDL. An accumulation of TG-droplets in the liver inhibits the function of the hepatocytes (Johannsen et al., 1993).

Fatty liver per se is not dangerous for the cow but rather dependent on its severity while still being associated with decreased metabolic function of the liver (Drackley, 1999; Grummer, 1993), which, especially in combination with other transition challenges, affects production and health. Normal liver-TG accumulation is by Bobe et al. (2004) perceived to occur when the percentage of TG in wet liver tissue is below 1 %. 1-5 % liver-TG is categorized as mild fatty liver, 5-10 % is moderate fatty liver and >10 % is severe fatty liver (Bobe et al., 2004). These authors also reported an overview of the incidence of the moderate and severe cases of fatty liver, based on nine different studies representing intensive dairy breeds in Europe (7), US (1) and Asia (1). The incidence of moderate cases between the studies varied from 20 % (US) to 65 % (France) and the incidence of severe cases varied from 5 % (UK, France) to 24 % (US).

Ketosis (ketonemia) is strongly associated to TG-accumulation in the liver. Biopsies from livers of healthy, mildly-ketotic and severely-ketotic cows were examined for fat content in a Finnish study by Gröhn et al. (1983). Fat percentages in livers were 5.4, 8.4 and 10.4 % in healthy, mildly-ketotic and severely-ketotic cows, respectively. Independent of how the distinguishing between the severity of ketosis in these specific cows were done, the results indicate an association between the two diseases. Ketosis occurs when ketones accumulate in the blood and the rate of ketone synthesis in the liver exceeds the rate of oxidation. Ketone formation is a result of hepatic ketogenesis and is a process which is enhanced when glucogenic substrates are insufficient, and acetoacetate is transported to the blood for utilization by other tissues (Grummer, 1993). Simultaneously the concentration of another ketone, β-hydroxybutyrate (BHBA), in the blood, milk and urine increase, thus [BHBA] can be used as a risk indicator of ketosis (Nielsen et al., 2005). Ketones are utilized by the different tissues, however, ketones are moderately strong acids that can cause metabolic acidosis, when they accumulate in the circulating blood. Dairy cows prone to ketosis may have decreased milk yield with a higher fat percentage, as ketones are a precursor for lipogenesis in the mammary gland (Bergman, 1971). Ketosis negatively affects DMI (Grummer, 1993), which is very inappropriate for the present stressed metabolism of the transition cow.

Common for most metabolic challenges occurring around parturition is that they are a consequence of a greater requirement of nutrients combined with an insufficient capacity for nutrient uptake and supply. In this case; Increased fatty acid mobilization is a result of a huge demand for energy. When glucogenic substrates are absent, TG's accumulates in the liver and hepatic ketone synthesis exceeds the rate of oxidation. Often this stage is associated to DMI depression, which worsens the metabolic stress and intensifies the struggle of adaptation to lactation.

2.3 Two-stage feeding of dry cows – a prepartum feeding strategy

One main argument for phase-feeding of dry cows is to reduce the risk of imbalance in nutrient supply and demand, which might be present when offering a single-stage diet throughout the entire dry period (Figure 2). Imbalance in nutrient supply and demand might be associated with periparturient metabolic problems affecting production and health. Several studies agree that an oversupply of energy in early dry period is associated to long term inexpedient effects on metabolism, production and health (Beever, 2006; Dann et al., 2006; Douglas et al., 2006; Fronk et al., 1980), however low energy feeding throughout the dry period does not comply with the increased nutrient demand and a concurrent dip in peripartum feed intake (Grummer, 1993; Richards, 2011). Thus, a separate Close-UP diet can be formulated for cows close to parturition. The purpose of this section is to provide an overview of the adaptational considerations, which are thought to be included in the two-stage feeding strategy. These considerations are valuable in the reflections on when the periparturient cow is exposed to an intervention in diet from Far-OFF to Close-UP.

It is important to feed an energy-level in the early dry period that corresponds to the cow's needs. Dann et al. (2006) illustrated thoroughly how energy intake in Far-OFF period affects

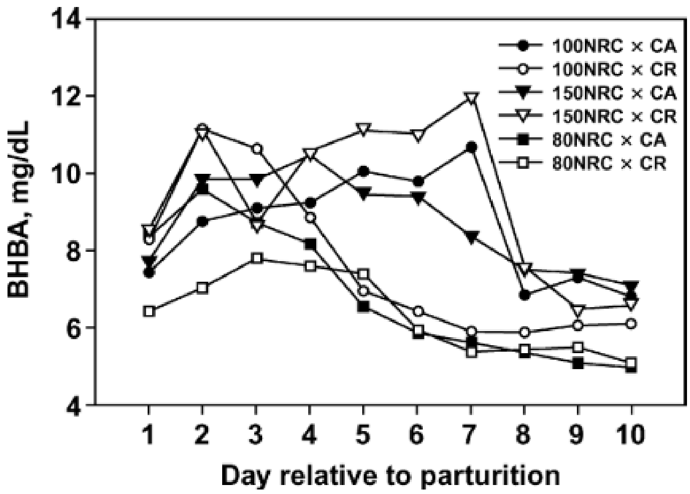

Figure 5 Least squares means for serum BHBA from 1 to 10 DIM for multiparous Holstein cows fed diets to meet 100% (100NRC), 150% (150NRC), or 80% (80NRC) of NRC requirements for NEL during the far-off dry period and a close-up diet at either ad libitum (CA) or restricted (CR) intake during the close-up dry period. For clarity, standard error bars have been omitted. The largest standard error for any treatment and day mean was 1.26 mg/dL. *(Dann et al., 2006)*.

periparturient metabolism and lactation in multiparous dairy cows. The dry cows were fed three different Far-OFF diets from dry off until -25 d relative to parturition. The Far-OFF dry cows were fed to meet national recommendations for net energy for lactation, NE_L, at ad libitum intake (100NRC), or with higher nutrient density (150NRC) or restricted intake at 80 % of requirements (80NRC). In the Close-UP period (-24 d to parturition) the cows received a Close-UP diet that also differed in two different feeding levels. The Close-UP diet was either fed ad libitum or was restricted to meet 80 % of requirements. The authors measured several indicators on metabolism throughout the dry period and the first 56 DIM. The overall conclusion regarding prepartum metabolism is that it was consistent with plane of nutrition. However, longer term carryover effects were observed as lactation was initiated. Cows that were overfed during the Far-OFF period (150NRC) and ad libitum fed during the Close-UP period, had the highest insulin level during the first 56 DIM. In the same period, these cows had the lowest energy balance, the highest body condition loss and the highest serum NEFA levels, which is unfavorable. Evaluating the first 10 DIM, cows that were overfed during Far-OFF period had lower DMI, lower energy balance and higher serum NEFA and BHBA concentration. Figure 5 illustrates that cows fed to meet their requirements in the Far-OFF period were faster to cope with elevated NEFA levels, as serum ketone levels decreased faster after parturition. Milk yield was not affected significantly by Far-OFF treatment but tended to be highest for cows fed 100NRC Far-OFF. Close-UP treatments were not found to affect serum NEFA concentrations postpartum (0-10 DIM), but Close-UP restricted cows had significantly higher NEFA concentrations prepartum (-7 d). Overall, the study demonstrates negative carryover effects of exceeding the requirements of the Far-OFF dry cow.

The overall aim of the Close-UP diet is to meet the cow's increasing demand for energy and protein (see Figure 2, Figure 3). This has sometimes been referred to as "steaming up" and the idea has been known since the 1930's (Boutflour, 1967). Although Dann et al. (2006) did not find any significant effects of Close-UP treatments on postpartum performance, the study did not investigate the possible consequences of not increasing the energy density in the Close-UP period in form of a negative control. Furthermore, the addition of starch-rich feedstuffs in the Close-UP diet have been found to have positive effects on NEFA mobilization postpartum. This is reported in a study by Minor et al. (1998) who found higher levels of plasma glucose, followed by lower levels of BHBA and NEFA when animals were fed a diet with high amounts of non-fiber carbohydrates. The authors also reported tendencies of higher milk yield and lower milk fat percentage postpartum, when cows received diets high in non-fiber carbohydrates from 19 days before expected parturition.

Another capacity of practicing a Close-UP diet is the possibility to apply milk fever preventative strategies by dietary modification. Of these strategies, one of the more well-known strategies is to decrease dietary calcium concentration in prepartum diets to prime the cow for increased calcium mobilization and absorption, by activating the calcium homeostatic mechanisms before parturition (Kichura et al., 1982; Wiggers et al., 1975). However, challenges may appear in achieving sufficiently low levels of dietary calcium in the diets that secure a daily calcium intake below 20 g with the commonly available feedstuffs (Thilsing-Hansen et al., 2002). A meta-analysis by Santos et al. (2019) reviewed another efficient strategy, referred to as the "low dietary cation anion difference principle" (DCAD-principle). The aim of this strategy is to bring the cow into a physiological stage of systemic/metabolic acidosis, by adding anionic salts to the diet. Goff & Horst (1998) added hydrochloric acid (HCl) to the prepartum diet as the anionic salt. The group monitored urine-pH from eight Jersey cows, to assess the success or failure of systemic acidification and found HCl to be efficient in reducing the pH of the urine 24 h after addition. This was linked to a systemic acidification and was followed by a simultaneous increase in calcium excretion in the urine. The results indicate that HCl as an anionic salt alters the homeorhetic mechanisms. Furthermore, the data showed significant decreases in milk fever incidences when HCl was used as the anionic salt in the prepartum DCAD-negative diet. The biological mechanisms behind the principle is not crystal clear, however Horst et al. (1997)

proposed that systemic acidification enhances stimulatory effects of PTH (see section 11). Applying this strategy requires accurate representative analysis of the DCAD balance of the feedstuffs, which may be challenging in the highly variable roughages.

To summarize; amongst other properties, two-stage feeding makes it possible to reduce the risk of overfeeding in the early dry period, which can be associated to longer term negative carryover effects in the transition cow. Moreover, bone mineral mobilization initiatives can be implemented with the aim of reducing incidences of milk fever. To secure the most optimal adaptation to lactation it appears of utmost importance to find a balance between the onset, duration and the intensity of the nutritional initiatives affecting the biological processes related to a successful adaptation to lactation. The goal is a cow with a high periparturient DMI, low risk of metabolic disorders and an onset of lactation optimized for high quality colostrum and a large milk production in the subsequent lactation.

Experimental study

3. Materials and methods

Animals, housing and treatments

The experiment was designed as a 2×2-crossover design, with duration of Close-UP period and feeding level as the two variables (Table 1). The research was undertaken in two subsequent periods, 1 and 2, on two commercial conventional dairy farms (herd A and B) in Denmark from June to December 2019. Duration of period 1 was 11 and 13 wk for herd A and B, respectively and the duration of period 2 was 15 and 13 wk, for herd A and B, respectively. Pregnant dry cows (n = 233) entering second or greater lactation, were randomly assigned to one of two treatments, either short (**S**) or long (**L**) Close-UP feeding duration. This was done according to their expected date of parturition, calculated as 281 days after insemination. Cows assigned for treatment S were fed a Close-UP diet for 14 d prior to parturition, while cows assigned for treatment L were fed the Close-UP diet 24 d prior to parturition. Cows were moved from Far-OFF to Close-UP feeding pens (transition pen (L) or calving pen (S)) on Mondays and Thursdays in herd A and on every second weekday in herd B. All S-cows were moved directly from the Far-OFF feeding pen to the calving area. Cows assigned to treatment L were moved into a transition pen at the predetermined date of moving. They were housed in this transition pen until 14 d prior to expected parturition. During this time these cows (L-group) received the Close-UP diet, in order to achieve the total of 24 days of feeding. After this, they were moved to the same calving pen as the S-group where they received the Close-UP diet. All cows were housed in free stall barns. Far-OFF cows were housed on slatted floor with cubicles, where Close-UP cows were housed on deep litter with straw bedding.

All cows were fed a similar Far-OFF diet from date of dry-off until initiation of their individual Close-UP period. Close-UP cows in each herd were fed different feeding levels during each experimental period as shown in Table 1. Herd A received a high feeding level (**H**) in period 1 and a control (**C**) diet in period 2. Herd B received the control diet (**C**) in period 1 and the high feeding level (**H**) in period 2. Close-UP diets (C or H) were fed until parturition. Thereby every dry cow was exposed to one of four treatments; SC, SH, LC or LH.

Table 1 Experimental design. Close-UP feeding level (CONTROL or HIGH) and Close-UP duration (short or long).

Herd	Experimental period 1	Experimental period 2
A	**HIGH** short | long	**CONTROL** short | long
B	**CONTROL** short | long	**HIGH** short | long

Both farms fed single-stage feeding before the experiment started. Cows were required to have been fed at least 15 d of the experimental Far-OFF diet in order to be enrolled in the experiment. Furthermore, only cows that had been exposed to Close-UP treatments were included. This is why 11 cows with early calvings or abortions (7 and 4 for herd A and B, respectively) were removed from the study. In addition, 15 cows were culled or died before parturition (8 and 7 for herd A and B, respectively). 207 cows remained in the study.

Far-OFF and Close-UP diets

All Far-OFF cows were fed ad libitum Far-OFF diets mixed as Total Mixed Rations (TMR) fed every second day. The formulation of the diets was done depending on available grass silage on the farms which was mixed with straw, minerals and soy bean meal in herd A and red fescue straw, corn silage, minerals and soy bean meal in herd B (Table 2). Ration optimization was done according to Norfor (Volden, 2011). Parameters for optimization was organic matter digestibility (OMD) of 65 %, crude protein (CP) of 130 g/kg DM and energy content of 5,0 MJ net energy for lactation/kg DM (NE_{L20}/kg DM). Close-UP diets were mixed as TMR's in large batches approximately monthly. In total 6 (C) and 2 (H) batches were mixed for herd A, where 4 (C) and 5 (H) were mixed for herd B. The difference was due to varying numbers of participating cows and practical conditions. The batches were compacted in bunkers and stored under plastic until use. Aerobe stability tests were conducted to monitor secondary fermentation. Feed was fed directly from bunker silo and offered ad libitum. Close-UP diets (C, H) were both composited of corn silage, grass silage, rape seed meal, straw, dry cow minerals (Mosegaarden BoviFlex 23401 Close-UP Ammon) and magnesium chloride hexahydrate (MgCl). Hydrochloride acid (HCl), 36 %, was furthermore added to reduce DCAD-balance of the diets. In warmer weather conditions propionic acid and potassium sorbate were added to prevent secondary fermentation (Table 2). Formulation was done according to Norfor (Volden, 2011) and optimized for OMD = 75-80 %, CP = 130-145 g/kg DM, DCAD = -100 meq/kg DM, DM =

400-450 g DM/kg and 6.4-6.5 MJ NE_L/kg DM. Difference in feeding level between diet C and H was achieved by replacing 20 % of the corn silage DM with concentrates (rolled barley and sugar beet pulp meal (SBPM)). Thereby concentrate ratio on a DM basis was on average 19.0 % in diet C and 38.5 % in diet H. The substitution of corn silage with concentrates reduced the theoretical fill value of the diet H with 7-10 %. The ratio between rolled barley and SBPM was adjusted to obtain similar starch levels between diet C and H. The cows on treatment H were expected to increase their daily energy intake because of reduced fill value and increased concentrate amount in the diet (+20 % of TMR DM).

Table 2 Composition of prepartum diets in herd A and herd B. (Close-UP control = C, Close-UP high = H)

Item	Herd A			Herd B		
	Far-OFF	Close-UP **C**	Close-UP **H**	Far-OFF	Close-UP **C**	Close-UP **H**
DMI, formulated[1], kg/d	12.2	12.7	13.9	12.2	12.7	14.0
Net energy uptake[1], MJ/d	62.6	87.3	95.7	62.6	86.4	97.4
Fill value[1], FV/kg DM	0.51	0.37	0.34	0.51	0.39	0.33
Ingredient, % of DM						
Grass silage	59.2	20.7	18.4	22.8	13.0	10.0
Corn silage	-	57.7	42.0	30.2	65.1	47.5
Rolled barley	-	-	12.2	-	-	11.0
SBPM	-	-	9.5	-	-	11.0
Soy bean meal	6.4	-	-	9.6	-	-
Rape seed meal	-	19.0	15.5	-	19.0	17.8
Wheat straw	33.6	-	-	-	-	-
Red fescue straw	-	-	-	36.7	-	-
Minerals[2]	0.8	-	-	0.7	-	-
BoviFlex 23401[3]	-	0.4	0.4	-	0.4	0.4
$MgCl_2$	-	1.0	0.7	-	0.7	0.8
Propionic acid	-	0.7	0.7	-	1.1	1.1
HCl, 36 %	-	0.4	0.6	-	0.6	0.3
Potassium sorbate	-	0.1	-	-	0.1	0.1

[1]Estimated DMI, daily net energy uptake and fill value is calculated according to Norfor (Volden, 2011) based on the formulated diet.
[2]Contains (DM basis) 12 g Ca, 14 g Mg, 12 g Na, 4.000 mg Mn, 4.500 mg Zn, 1.500 mg Cu, 25 mg Co, 225 mg I, 50 mg Se, 600.000 IU vitamin A, 190.000 IU vitamin D_3 and 4000 IU vitamin E.
[3]Contains (DM basis) 1 g Ca, 338 g Na, 522 g Cl, 4 g S, 8.333 mg Mn, 10.000 mg Zn, 3.000 mg Cu, 67 mg Co, 50 mg Se, 900.000 IU vitamin A, 333.000 IU vitamin D_3 and 33.000 IU vitamin E.

Ingredient sampling, chemical analyses and indications on dry matter intake

Prior to feed ration optimization, roughages were sampled and analysed for chemical composition. Samples were dried at 60ºC for a minimum of 48 h. Determination of CP, NDF, in-vitro OMD, ash and sugar were done by FT-NIR-spectroscopy (NIRS™ DS 2500, FOSS A/S) (Kristensen, 2017) at Kvægbrugets Forsøgslaboratorium, Skejby, Denmark (KFL). Mineral composition was analysed by ICP-spectroscopy (PDR83) (Eurofins Agro Testing Denmark A/S). TMR samples of Far-OFF diets were collected on days of mixing of Close-UP diets. TMR-samples of Close-UP diets were collected directly after mixing of the specific batch. TMR samples of diets were analysed by FT-NIR-spectroscopy at KFL, following same procedure as for roughages. Mineral composition of Close-UP diets was analysed by ICP-spectroscopy.

Indications on DMI was based on the total amount of the C-diet and H-diet mixed in the two herds relative to the total number of feeding days (cows and Close-UP duration), the diets lasted.

Urine collection and analysis

Systemic acidification of the Close-UP dry cows was monitored through pH measurements of midstream urine spot samples (100 mL). These were collected randomly from different cows fed the Close-UP diet for more than 4 days. All Close-UP feed batches were represented in the monitoring. Samples were collected by stimulation of the perineal area. The samples were placed on ice and transported to KFL for pH-measuring. Within 12 hours from collection of the samples, they were heated to room temperature (18-20 ºC) and diluted (1:1) with deionized water. pH was measured using a glass-electrode, calibrated in the pH-range of 4 to 7.

Colostrum sampling and udder edema scoring

Samples of colostrum from the first milking after parturition were collected. The farm-personnel were instructed to sample 100 mL of colostrum from the well-stirred total first milk-out of colostrum. Samples were frozen immediately after collection. Number of hours from calving to milk-out, yield in first milk-out and the refractometric brix-value (ºBx) of the colostrum (measures the amount of light that is refracted/bent) were registered on-farm by farm-personnel. On both farms brix value of colostrum was analysed using digital refractometers. The samples were transported to KFL. After thawing, the samples were analysed with a refractometer (PAL-1, Atago, Food Diagnotics, Grenå) and scanned for protein, fat and lactose content using MilkoScan FT-120 (Foss A/S, Hillerød). Colostrum samples were furthermore analysed for content of alfa-tocopherol/vitamin E at Aarhus University. Data analysis that included the refractometric brix-value of colostrum was based on laboratory data to eliminate possible errors related to on-farm instruments.

Edema score of individual cows at time of first milk-out was determined using a visual, subjective method described by Swett et al. (1938). Degree of udder edema was scored and registered by farm-personnel instructed to use a 5-point scale (1 = no edema, 2 = slight edema in the base of the udder around the teats, 3 = moderate edema covering bottom half of the udder, 4 = severe edema covering the entire udder and 5 = very severe, with edematous tissue on brisket/thighs).

Birth weight measurements

Calves were weighed immediately after birth. Calves from herd B were fed colostrum prior to weighing. The amount of colostrum fed (kg), was subtracted from the weight records. Weight recordings of twin births (n = 4) were not used for data analysis.

Milk-yield recordings

Data on post-partum milk yield and milk components were collected from monthly yield controls on herd level. Herd B had an Automatic Milking System (AMS), thus recordings of daily milk production and fat- and protein content of milk were available on cow-level in this herd. Herd A had a milking parlour and had three daily milkings.

Health recordings

Incidences of clinical milk fever, retained placenta and metritis were registered by farm-personnel. Milk fever was characterized by a paralyzed fresh cow that responded to oral or intravenous administration of calcium immediately after treatment. Retained placenta was characterized by retention of fetal membranes 12 h after parturition and metritis was evaluated as number of medical treatments for the disease, registered in the Danish cattle database in combination with farm-personnel information. Ketosis was evaluated according to clinical registrations in the cattle-database.

Statistical analysis

Data were analysed as a randomized complete clock design with 2×2 factorial arrangement of treatments (duration of Close-UP period × feeding level) and cow as the experimental unit. For statistical analyses, the software programme R was used. Normality of residuals and homogeneity of variance were tested for continuous variables that were analysed after fitting the models.

Colostrum data and milk yield data from yield controls were analysed by 2-way analysis of variance (ANOVA) using the linear mixed effects models in R. The model was built with the fixed effects of duration of the Close-UP period (S vs. L), Close-UP feeding level (C vs. H), parity group (2nd lactation vs. older), experimental period (1 vs. 2), the interaction between duration and feeding level and the random effect of herd. Data were reported as LSM (Least Square Means) and statistical significances were declared at $P \leq 0.05$. Tendencies were declared when P-values $0.05 \leq P \leq 0.1$. When an interaction was significant, LSM comparisons were based on Tukey's multiple comparison test.

Data from previous monthly yield controls (2016-2020) were used calculate predicted milk yield on a given test-day. The model was fitted using the MIXED procedure of SAS and included fixed effects of parity, the interaction between parity and a Wilmink-correction factor and the interaction between parity and DIM. The random term included control year and the interaction between animal and parity. The Wilmink-correction factor, W, was calculated according to Wilmink (1987): $W = e^{-0.05*DIM}$.

Milk yield data and measurements of milk components from AMS in herd B were analysed by ANOVA using the linear mixed effects models in R. The model was built with the fixed effects of Close-UP duration (S vs. L), Close-UP feeding level (C vs. H), parity group (2nd lactation vs. older), experimental period (1 vs. 2), the interaction between duration and feeding level and the random effect of the animal.

Calf weight measurements were analysed by ANOVA using the linear mixed effects models in R. The model was built with the fixed effect of duration, feeding level, calf sex, calf breed, test period and the interaction between duration and feeding level.

A contingency table was constructed for count data (heath disorders). Possible differences between treatment groups were detected by a *Fisher's Exact Test*.

4. Results

Descriptive data of cows in the four experimental treatment groups are shown in Table 3. The actual days for the cows in the four Close-UP treatments were on average 12.8 (±5.4), 12.6 (±4.8) 23.0 (±5.1) and 23.1 (±6.4) days for SC, SH, LC and LH respectively. The periods were relatively shorter than predetermined.

Table 3 Descriptive data (mean, ± sd) on cows in the four treatment groups (n=207).

	Short		**Long**	
Item	**C**	**H**	**C**	**H**
Number of cows	59	42	59	47
Number of 2nd parity cows	30	14	24	23
Number of older cows (>2nd parity)	29	28	35	24
Gestation length, days	278.8 ±5.3	278.6 ±4.4	279.4 ±5.0	278.2 ±4.7
Far-OFF duration, days	40.5 ±5.4	43.9 ±6.8	32.4 ±11.6	31.6 ±8.7
Close-UP duration, days	12.8 ±5.4	12.6 ±4.8	23.0 ±5.1	23.1 ±6.4
Dry period length, days	53.4 ±7.9	56.6 ±6.9	55.3 ±14.0	54.7 ±9.8

Diet composition

Chemical composition and nutritional characteristics for prepartum diets are shown in Table 4. Formulation criteria were partly achieved in prepartum diets. In Far-OFF diets, OMD was higher than formulated in herd B (~ +5 %) and CP content was lower than formulated in herd A. CP variation was high in both herds. This was because of variation in CP-content of grass silage. Energy content of the Far-OFF diet in herd B was highest (5.14 (± 0.15) vs. 4.59 (± 0.26)). This was mainly because of higher OMD. Chemical composition of Close-UP diets generally corresponded to the formulations. However, the OMD's were similar for the two feeding levels in herd A (78.2 (±0.8) and 77.6 (±0.6) for C and H-diet, respectively), whereas the OMD of the C-diet was relative lower than the H-diet in herd B (74.4 (±1.1) vs. 78.4 (±0.7)). In addition, DCAD-value of Close-UP diets were lower than formulated. This was due to variation in DCAD-value of grass-silages.

Table 4 Chemical composition (mean ± sd) and nutritional characteristics of prepartum diets in herd A and B and the Close-UP treatments (Control = C, High = H).

Item	Herd A			Herd B		
	Far-OFF	Close-UP **C**	Close-UP **H**	Far-OFF	Close-UP **C**	Close-UP **H**
DMI[1], kg/d	12.5	13.6	15.3	12.5	13.2	14.6
Energy intake[1], MJ/d	57.40	87.93	100.20	64.30	86.59	94.46
DM, g/kg	387.5 ±13.9	348.8 ±13.3	439.4 ±9.4	487.5 ±27.9	364.8 ±9.5	460.3 ±27.6
Ash, g/kg DM	76.6 ±10.4	64.9 ±3.6	60.8 ±2.7	68.4 ±5.4	62.7 ±4.4	57.4 ±4.6
Starch, g/kg DM	-	179.0 ±14.0	201.9 ±7.3	85.8 ±16.4	160.9 ±4.4	185.7 ±20.2
Crude protein, g/kg DM	116.1 ±15.5	137.9 ±6.5	143.2 ±4.7	137.5 ±14.9	140.0 ±10.1	145.1 ±5.1
Sugar, g/kg DM	-	33.4 ±11.2	27.1 ±5.6	19.5 ±11.8	18.2 ±7.4	19.4 ±4.1
Crude fat, g/kg DM	20.0 ±2.7	47.5 ±1.7	41.5 ±1.8	27.7 ±1.5	50.5 ±2.0	46.0 ±1.2
NDF, g/kg DM	538.1 ±30.5	376.1 ±6.1	339.0 ±12.8	492.6 ±18.1	394.2 ±4.4	353.6 ±12.5
OMD, % of OM	66.8 ±2.8	78.2 ±0.8	77.6 ±0.6	70.2 ±1.7	74.4 ±1.1	78.4 ±0.7
Calcium, g/kg DM	-	4.4 ±0.3	4.5 ±0.2	-	4.6 ±0.0	5.3 ±0.4
Phosphorus, g/kg DM	-	3.3 ±0.2	3.8 ±0.2	-	3.9 ±0.1	3.6 ±0.3
Magnesium, g/kg DM	-	2.9 ±0.0	3.0 ±0.2	-	3.0 ±0.0	3.1 ±0.3
NE_L20, MJ/kg DM	4.59 ±0.26	6.48 ±0.07	6.55 ±0.08	5.14 ±0.15	6.57 ±0.03	6.47 ±0.05
DCAD[2], meq/kg DM	-	-107 ±16	-123 ±13	-	-173 ±22	-141 ±26

[1]Based on estimates (see 3. Materials and Methods).

[2] DCAD, meq/kg DM = $\left(\left(\frac{K}{39.1}+\frac{N}{23}\right)+\left(\frac{Cl}{35}+\frac{2*S}{32}\right)\right)*1000$

Indications on DMI and status on metabolic acidification in Close-UP dry period

Cows in herd A had an apparently DMI of 13.6 and 15.3 kg DM for the C- and H-diet respectively, whereas cows in herd B had an apparently DMI of 13.2 and 14.6 kg DM for the C- and H-diet respectively. Thus, cows fed the H-diet had on average a 11.5 % greater daily net energy intake compared to cows on the C-diet. Cows on C-diet and H-diet in herd A had a daily net energy intake of 87.93 MJ and 100.20 MJ respectively, and cows on C- and H-diet in herd B had a daily net intake of 86.59 MJ and 94.46 MJ.

pH-measurements of urine from cows fed the different Close-UP batches indicated successful systemic acidification of the cows. Urine-pH from cows from herd A was on average 5.62 (±0.2) and 5.73 (±0.2) for herd B, during the entire experimental period.

Colostrum yield and composition

Analyses of colostrum data were based on 182 samples. First milking of colostrum happened on average 6.1 (±5.0) h after parturition. Colostrum yield did not differ between feeding levels, but long duration of Close-UP period negatively affected colostrum yield at first milk-out ($P = 0.03$) (Table 5). Yield of colostrum was reduced with 1.4 L or 17.4 % in the L-group compared to the S-group. Duration of Close-UP period alone did not affect the measured colostrum quality parameters (protein, fat, lactose and alfa-tocopherol). However, a tendency for an effect of duration was observed on total protein yield, with lower total protein yield at first milk-out in the L-group ($P = 0.06$). Significantly higher refractometric brix-values were observed for older cows, compared to cows in their second lactation, for all treatment groups ($P < 0.01$). Feeding level per se did not affect any measured colostrum components. However, interactions between duration and feeding level were observed for brix-value ($P = 0.02$), protein percentage ($P = 0.04$) and content of alfa-tocopherol ($P = 0.01$). Cows fed the H-diet in the S-group had a higher brix-value than cows fed the H-diet in the L-group, but no significant difference appeared in brix-value of cows fed the C-diets. Pairwise comparisons of protein percentages showed significant difference between feeding levels within the L-group. The analysis of alfa-tocopherol was based on 58 samples. Pairwise comparisons of alfa-tocopherol content showed significant difference between feeding levels within the L-group ($P < 0.001$).

Table 5 Effect of Close-UP duration (DUR; S, L) and feeding level (FEED; C, H) on colostrum yield and composition and calf weight measurements (LS means ±SEM).

	Short		Long		P-value[1]		
Item	**C**	**H**	**C**	**H**	*DUR*	*FEED*	*DUR × FEED*
Colostrum							
Yield, L	8.3 ±0.7	7.7 ±0.8	6.7 ±0.7	6.6 ±0.8	0.03	0.66	0.69
Brix-value, ºBx	22.1^{ab} ±1.2	23.2^{b} ±1.2	22.8^{ab} ±1.1	21.1^{a} ±1.2	0.19	0.98	0.02
- *2nd parity*	20.9 ±1.1	22.0 ±1.2	21.5 ±1.2	19.9 ±1.2	-	-	-
- *>2nd parity*	23.3 ±1.2	24.5 ±1.2	24.0 ±1.2	22.4 ±1.2	-	-	-
Protein, pct.	12.97^{ab} ±0.9	13.39^{ab} ±1.0	13.45^{b} ±0.9	12.03^{a} ±0.9	0.29	0.53	0.04
Protein yield, kg	0.969 ±0.08	0.971 ±0.09	0.864 ±0.07	0.796 ±0.08	0.06	0.78	0.66
Fat, pct.	3.30 ±0.36	3.56 ±0.39	3.26 ±0.34	3.63 ±0.39	0.99	0.27	0.88
Lactose, pct.	3.42 ±0.11	3.39 ±0.11	3.38 ±0.10	3.56 +0.11	0.34	0.24	0.11
Alfa-tocopherol, µg/g milk fat	208.80^{ab} ±25.38	214.46^{ab} ±24.35	264.37^{b} ±22.91	173.80^{a} ±23.14	0.53	0.01	0.01
Calves							
Birth weight, kg	42.8 ±2.8	40.6 ±2.9	42.3 ±2.8	42.2 ±2.9	0.96	0.06	0.25

^{ab} LSM in the same row with different superscripts differ at $P < 0.05$; those with identical superscripts do not differ.
[1]Effect of treatments from the ANOVA (DUR = duration; FEED = feeding level; DUR× FEED = interaction between DUR and FEED).

Calf weight measurements (Table 5) showed no effect of duration of Close-UP period, but high feeding level tended (P = 0.06) to decrease birth weight. When a pairwise comparison was conducted, no significant effect was present. Twin-births occurred in 3.3 % of the parturitions. Birth weights of twins were not included in the analysis.

Health disorders and udder edema score

Incidences of health disorders and distribution of udder edema scores are presented in Table 6. No statistical differences of health disorders were observed in the four treatment groups. Total incidence rate of milk fever was 0.96 % during the experimental period and only 2 cases were reported; both from herd A. Incidence rate of retained placenta was 5.3 and numerically most cases were observed in the LH-group (8.5 %). One cow with retained placenta had twin-birth. Three cows that had retained placenta also showed metritis. Metritis was observed among 6.3 % of the cows in the study. Clinical registrations of ketosis showed 3.4 % incidence rate.

Udder edema score was reported for 172 cows in the study. 97.1 % of all cows were graded score 1 or 2 (Table 6). No significant differences between treatment groups were noted for score 1, 3, 4 and 5. Fewer cows were scored 2, when fed the H-diet, compared to the C-diet, however this pattern is not observed for other scores of udder edema and the number of cows with this score was low.

Table 6 Incidences of milk fever, retained placenta, metritis and ketosis in cows between experimental treatments and distribution of cows with udder edema score (1-5) between treatment groups.

		Short		**Long**		
Item	Incidence (no./no.)	**C**	**H**	**C**	**H**	*P*-value[1]
Milk fever, %	1.0 (2/207)	0.0	0.0	3.4	0.0	0.3
Retained placenta, %	5.3 (11/207)	5.1	4.8	3.4	8.5	0.7
Metritis, %	6.3 (13/207)	8.5	2.4	6.8	6.4	0.7
Ketosis, %	3.4 (7/207)	0.5	0.0	2.0	1.0	0.3
Udder edema score	Proportion (no./no.)					
Score 1, %	87.8 (151/172)	82.2	92.3	83.0	95.2	0.17
Score 2, %	9.3 (16/172)	15.6	7.7	12.8	0.0	0.04
Score 3, %	2.3 (4/172)	2.2	0.0	4.2	2.4	0.90
Score 4, %	0.6 (1/172)	0.0	0.0	0.0	2.4	0.47
Score 5, %	0.0 (0/172)	0.0	0.0	0.0	0.0	-

[1] Effect of treatments from *Fisher's Exact Test.*

Lactation performance

Post-partum lactation performance parameters (0-50 DIM), based on monthly yield controls, are shown in Table 7. Average daily ECM-yield (0-50 DIM) increased with 3 kg or 6.7 % from test-period 1 to test-period 2 (P = 0.01). Duration of the Close-UP period did not significantly affect any of the evaluated lactation performance parameters (energy corrected milk yield (ECM), fat percentage, protein percentage, FP-ratio or somatic cell count (SCC)). However, a tendency for elevated SCC was observed in the L-group, compared to the S-group. Lactation performance responded positively to increased feeding level of Close-UP diets. ECM-yield was higher for the

H-groups compared to the C-groups (*P* = 0.05). Cows fed the H-feeding level had an increased milk yield of 1.7 kg or 3.5 % compared to the cows on C-feeding level. Furthermore, milk yield increased with parity. Cows on H-feeding level also had decreased concentration of protein in the milk (*P* = 0.03) and tended to have increased FP-ratio (P = 0.06). Concentration of fat was unaffected by the treatments. Evaluating actual test-day milk yield relative to predicted yield, feeding level again showed effect on post-partum performance, since the relative ECM-yield was greatest for cows fed the H-diets, compared to cows fed the C-diets (P = 0.02).

Table 7 Effect of Close-UP duration (DUR; S, L) and feeding level (FEED; C, H) on lactation performance (0-50 DIM) of cows in the four treatment groups (LSM ±SEM). Data are based on monthly recordings on cow level.

	Short		**Long**		*P*-value[1]		
Item	**C**	**H**	**C**	**H**	*DUR*	*FEED*	*DUR × FEED*
0-50 DIM							
ECM, kg	45.4 ±2.25	48.4 ±2.39	45.8 ±2.25	46.1 ±2.36	0.49	0.05	0.22
· *2nd parity*	44.9 ±2.31	47.9 ±2.50	45.3 ±2.34	45.6 ±2.42	-	-	-
· *>2nd parity*	45.9 ±2.32	48.9 ±2.41	46.3 ±2.29	46.6 ±2.42	-	-	-
Fat, %	4.17 ±0.10	4.27 ±0.13	4.31 ±0.10	4.31 ±0.12	0.39	0.49	0.63
Protein, %	3.53 ±0.05	3.38 ±0.07	3.56 ±0.05	3.46 ±0.06	0.43	0.03	0.63
FP- ratio	1.18 ±0.03	1.27 ±0.04	1.23 ±0.03	1.26 ±0.04	0.48	0.06	0.33
Relative ECM-yield[2]	0.982 ±0.022	1.060 ±0.027	0.990 ±0.022	1.009 ±0.025	0.47	0.02	0.21
SCC, 1000/mL	121 ±44	162 ±53	257 ±44	162 ±51	0.09	0.38	0.15

[1]Effect of treatments from the ANOVA (DUR = duration; FEED = feeding level; DUR× FEED = interaction between DUR and FEED).
[2]Actual ECM-yield relative to predicted ECM-yield based on Wilmink-model.

Data from AMS in herd B supported the results that were based on yield controls. AMS data confirmed the increasing effect of high Close-UP feeding level on ECM-yield when evaluating the period 0-45 DIM (Table 8). The same data also showed that high feeding level significantly affected ECM-yield in early lactation (0-20 DIM) (P > 0.01) but not later in lactation (20-45 DIM) (P = 0.12). FP-ratio was unaffected by the treatments. Despite no statistical interactions were observed between Close-UP period duration and feeding level, Figure 6 Means of daily ECM yield relative to days after parturition. Left: Short Close-UP duration. Right: Long Close-UP duration. Plots are based on raw average milk yield from cows in the four treatment groups in herd B. illustrates that the difference in daily ECM-yield of C-cows compared to H-cows in early lactation (0-20 DIM) appear numerically larger for cows in the S-group compared to cows in the L-group.

Table 8 Effect of Close-UP duration (DUR; S, L) and feeding level (FEED; C, H) on lactation performance of cows in the four treatment groups in herd B (LSM ±SEM). Data are based on daily milk yield registrations from AMS.

	Short		**Long**		*P*-value[1]		
Item	**C**	**H**	**C**	**H**	*DUR*	*FEED*	*DUR × FEED*
Milk yield							
ECM, kg (0-45 DIM)	42.0 ±1.2	46.3 ±1.0	43.2 ±1.3	44.6 ±1.0	0.70	0.01	0.21
ECM, kg (0-20 DIM)	37.6 ±1.3	42.8 ±1.0	39.1 ±1.2	41.2 ±1.0	0.75	<0.01	0.17
ECM, kg (20-45 DIM)	45.1 ±1.4	48.1 ±1.1	45.7 ±1.4	46.3 ±1.1	0.54	0.12	0.34
FP-ratio (0-45 DIM)	1.16 ±0.03	1.11 ±0.02	1.13 ±0.03	1.13 ±0.02	0.96	0.38	0.26

[1]Effect of treatments from the ANOVA (DUR = duration; FEED = feeding level; DUR× FEED = interaction between DUR and FEED).

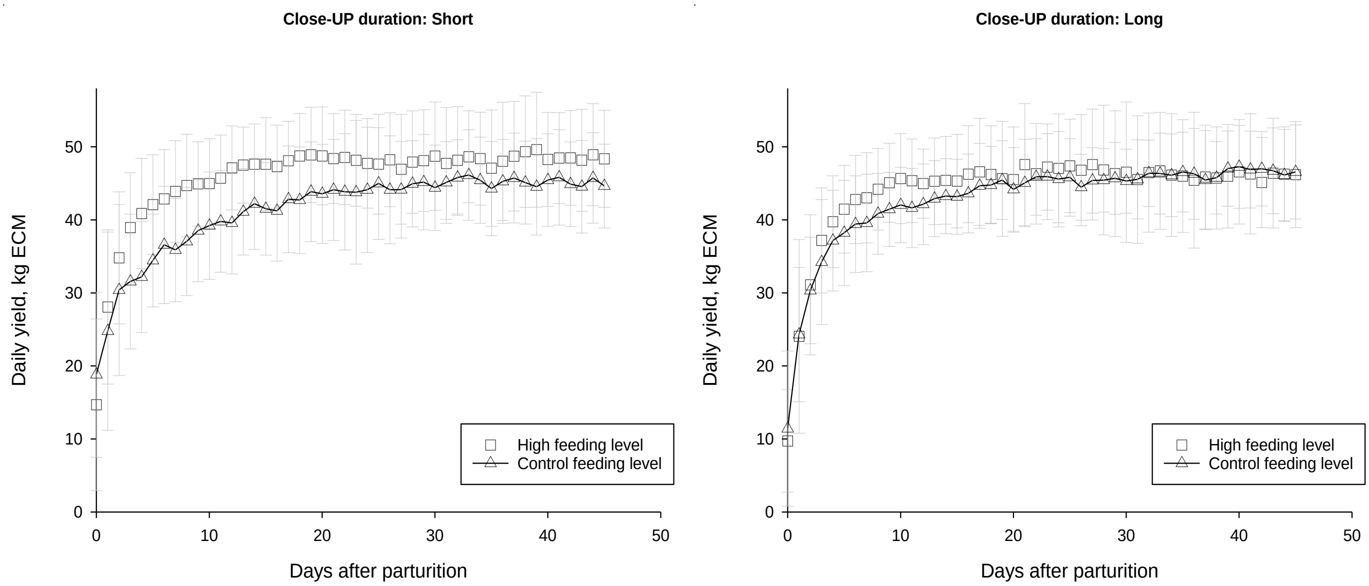

Figure 6 Means of daily ECM yield relative to days after parturition. Left: Short Close-UP duration. Right: Long Close-UP duration. Plots are based on raw average milk yield from cows in the four treatment groups in herd B.

5. Discussion

The overall goal of this study was to investigate if the duration of Close-UP feeding affected postpartum performance parameters of transition cows. In addition, the aim was to investigate possible effects of feeding level on the same performance parameters. There are several underlying drivers for the success of postpartum performance should; a central one being to ensure an optimized DMI around and after parturition. It was not the aim to evaluate the daily DMI on cow-level and neither the cow-level DMI on days relative to parturition, nevertheless, the results of this study could potentially be used to reflect on how DMI around parturition could be affected by the treatments. The results of the present study showed that duration of Close-UP periods along with the feeding level of Close-UP diets affected different performance parameters, such as colostrum yield- and quality and lactation performance, whereas other parameters remained unaffected.

Before evaluating the results of the present study, it is necessary to consider underlying factors that might have influenced or interacted with the treatments and thereby the results. Therefore, parameters that might affect DMI should be considered. Two parameters that might affect DMI are 1) the duration and 2) the degree of systemic acidification of the Close-UP cows, achieved by negative DCAD-balance of prepartum diets. A study by Lopera et al. (2018) revealed that reducing the DCAD-balance from -70 meq/kg DM to -180 meq/kg DM the last 21 d of gestation linearly reduced daily DMI with 1.1 kg. In addition, the study also demonstrated that there were no advantages of extending the duration of DCAD-negative feeding to 42 days prior to calving compared to 21 days, as longer duration decreased the milk yield in the subsequent lactation. These findings were confirmed by Zimpel et al. (2018), who furthermore found that the DMI depression was because of metabolic acidification of the cow, and not a palatability issue related to the addition of acidogenic products or salts containing Cl to the diet. In contrast, the use of HCl as the anionic salt to reduce DCAD in a study by Goff & Horst (1998), actually increased prepartum DMI. Several researchers agree that DCAD-negative feeding causes metabolic acidification that reduces urine-pH, as excess acids are excreted in the urine (Goff & Horst, 1997; Vagnoni & Oetzel, 1998; Zimpel et al., 2018). Urine pH-measurements in the present study indicated successful metabolic acidification of the Close-UP cows. The cows in the study by Lopera et al. (2018) decreased their daily DMI when fed a diet with DCAD-value of -180 meg/DM. These cows had a urine pH below 6.0, and the researchers found no benefits of a further decrease. The urine-pH of the cows in the present study were on average 5.62 (±0.2) for herd A and 5.73 (±0.2) for herd B. With DCAD-values in Close-UP diets from ranging from -107 (±16) to -141 (±26) meg/kg DM, there might be a risk that the daily DMI's were perhaps affected, although HCl was the source for anionic salts. Further analyses and measurements of total acid excretion in the urine would be needed to determine the total degree of acidification. This precondition that DCAD-negative feeding was practised during the experiment, should be taken into consideration when evaluating the results.

<u>Colostrum yield and -quality and birth weight measurements</u>

An established criterion for the success or failure of the dry period is colostrum yield and -quality. In the present study, colostrum yield decreased with 1.4 kg when cows were exposed to a long Close-UP duration compared to a short duration (Table 5). Few experiments have investigated the effect of Close-UP period duration and feeding level on colostrum parameters. However, the decreased yield in the L-groups in this study, stands in contrast to the findings of Farahani et al. (2017), who found tendencies for decreased colostrum yield when cows fed a Close-UP diet for 10 days were compared to cows fed the diet for 21 days. Moreover, Lopera et al., (2018) found no differences in colostrum yield when cows were fed DCAD-negative Close-UP diets for either 21 or 42 days prepartum. Despite the opposing results in present study, the production level of colostrum at first milk-out corresponded to the levels achieved in the two experiments.

Brix-value is often used as an on-farm tool for indication of protein content in colostrum (Bielmann et al., 2010; Silva-del-Río et al., 2017). The protein content of colostrum indicates the content of immunoglobulins; essential for the immunisation of the neonatal calf. Protein percentages of colostrum were increased by high feeding level in the L-group, but unaffected in the S-group (Table 5). However, what reflects the total efficiency of the cow's colostrogenesis, is the total protein yield at first milk-out, where both yield and its quality is evaluated. Total protein yield tended to decrease in cows with long exposure to Close-UP period and was unaffected by feeding level. Nevertheless, total protein levels in this study corresponds to protein levels in colostrum found by others (Lopera et al., 2018; Richards, 2011; Weich et al., 2013). This might indicate that other factors could influence protein yield in colostrum, such as the source of vitamin D (Martinez et al., 2018).

Several studies have demonstrated that colostrum yield and its components depend on the time of milk-out relative to parturition (Godden et al., 2019; Silva-del-Río et al., 2017). By evaluating the total protein yield of colostrum, possible dilution effects of increased yield on colostrum components are eliminated. However, yield of colostrum at first milk-out increased by 0.2 L/h in this dataset. This could have confounded the observed effect of duration on colostrum yield. To counteract this, a correction was made to account for this association between yield and milk-out time. However, this correction did not change the results of the present study.

Content of fat and lactose was unaffected by the treatments (Table 5). The lactose percentage corresponded to the levels found by Lopera et al. (2018), while the fat percentages were lower compared with other findings (Lopera et al., 2018; Mann et al., 2016). Cows in these studies produced less colostrum compared to the present study, which could indicate a dilution effect of the observed fat percentages. Although the numerical decrease in fat percentage from C- to H-treated cows was insignificant, the difference might indicate that the fat percentage in colostrum was influenced by the relatively high energy level of both Close-UP diets. In addition, there is a risk that the sampling technique affected the results.

Maternal vitamin E status can be reflected in the alpha-tocopherol content of colostrum. Vitamin E is essential for the neonates (Zanker et al., 2000) and maternal vitamin E status might furthermore be associated with the of immunoglobulin concentrations in colostrum (Lacetera et al., 1996). One can hypothesize that the longer conservation or storage of premixed Close-UP feed in batches might have decreased the content of vitamin E in feed. Despite the interaction between duration and feeding level on alpha-tocopherol in colostrum (Table 5) it seemed that the conservation method did not harm the vitamin E content of the feed. However, this was not tested systematically in this experiment.

Although a significant effect of duration was observed for the colostrum yield, the treatments did not have large quantitative effects on yield nor quality. The responses in decreased yield of colostrum when cows were fed the Close-UP diet for 23 (±5.7) d, might indicate that these cows may have had a more decreased DMI immediately prior to calving, compared to cows exposed to the short duration. This was also indicated by Weich et al., (2013), although Lopera et al. (2018) suggested that prepartum DMI depression was more likely to be caused by level of DCAD than duration of DCAD-negative feeding. The more severe DMI depression suggested for the L-group could possibly also be caused by stress related to hierarchy and the move from the transition pen to the calving area. The possible decreased DMI could coincide with time of milk synthesis that is initiated a few days prior to calving (Akers, 2002; Capuco et al., 1997).

Another mechanism for the decreased colostrum yield in the L-group might be that longer exposure to DCAD-negative feeding has disrupted prolactin signalling (Yang et al., 2013) or inhibited the GH/IGF-1 axis important for mammary development (Challa et al., 1993). These authors found that mouse cell cultures transfected with human prolactin and GH receptors, were disrupted by acidosis of pH 6.8 (Yang et al., 2013). Challa et al. (1993) found that metabolic acidosis could inhibit GH secretion in rats. Therefore, it can reasonably be speculated that prolonged exposure to DCAD-negative feeding could affect mammogenesis either by inhibited prolactin signalling, important for initiation of lactogenesis (Akers, 2002), or by affecting the IGF-1/GH axis influencing the nutrient balance and mammary blood flow (Barrington et al., 2001).

Calf birth weight measurements (Table 5) showed no significant difference between treatment groups. This was similar to results from Farahani et al. (2017), who compared 21 d Close-UP period with 10 d Close-UP period. According to Prior & Laster (1979) total fetal growth rate of bovine fetuses is highest around day 230-235 of gestation and is not affected by maternal dietary energy level. This could explain why birth weights in this experiment were unaffected by the treatments.

Health disorders and udder edema

Another important indicator of the success or failure of dry cow nutrition and management is the incidence rate of specific transition diseases. In this present study, incidences of milk fever, retained placenta, metritis and ketosis were evaluated in each treatment group (Table 6). As the total incidence rate of milk fever during the experiment was below 1%, the different treatments could not be associated to the incidences of milk fever. Urine pH-measurements indicated successful metabolic acidification, which is known to prevent clinical and subclinical incidences of milk fever (DeGaris & Lean, 2008; Goff & Horst, 1998; Santos et al., 2019). Retained placenta occurred in 5.3 % of the cows in the study and metritis occurred in 6.3 %. Incidence rates were not significantly different between treatment groups. This is in agreement with the results found by Lopera et al. (2018), although the incidence rates were higher in their study; 14.0 % and 15.9 % for retained placenta and metritis, respectively. Hypocalcemia is considered a gateway disease that increases the risk for developing other postparturient diseases (Martinez et al., 2012; Rodríguez et al., 2017). Therefore, eliminating the risk of hypocalcemia may have reduced the incidences of retained placenta and metritis in this case. Three of thirteen cows treated for metritis had a retained placenta, which is known to be a risk factor for metritis (Giuliodori et al., 2013). As uterine disorders are multifactorial, significant differences between treatment groups were not expected in present study. However, studies agree that increasing the energetic status of the cow around parturition decreases the risk for metritis (Huzzey et al., 2007; Pérez-Báez et al., 2019). In the present study no effect was observed with different feeding levels (nor durations), perhaps because both feeding levels were relatively high.

Incidence of ketosis was unaffected by the different treatments and the total incidence rate was 3.4 %. Registrations were based on cases where cows displayed clinical signs of ketosis. As not all cows were systematically health-checked early in lactation, there is a risk that some cows with ketosis were not detected. Based on this, the energy- and thus the mobilization status of the fresh-cows in the herds should be evaluated via the FP-ratio in milk.

An issue of concern was that prolonged exposure to energy-dense diets and/or increased feeding level would increase the number of cows with severe udder edema. Scorings of udder edema were based on subjective evaluations, which might have confounded the result. However, in this study, neither Close-UP feeding duration nor feeding level affected udder edema (Table 6). This is in agreement with a study by Mashek & Beede (2001), who found no effect of feeding energy-dense diets for the last 3 or 6 weeks prepartum.

<u>Lactation performance</u>

Lactation performance was affected by feeding level of Close-UP diets, but no effects of duration of Close-UP feeding were observed in the experiment (Table 7). Average daily milk yield from 0-50 DIM showed that cows fed the H-diet had a 3.5 % higher ECM yield, compared to cows fed the C-diet in the Close-UP period. This was supported by the increased relative ECM-yield in the H-group, which showed the same significant effect of feeding level. The relative ECM yield reported test-day ECM-yield relative to the Wilmink-predicted ECM-yield. Fat percentages of milk was unaffected by treatments. Although protein percentages were significantly lower in the H-group compared to the C-group, the quantitative difference in protein percentage was small between H- and C- treatment (0.12 %-point). Evaluating the FP-ratio of the milk, the FP-ratio tended to increase with increased feeding level. This is perhaps not surprising, given that H-fed cows also yielded more milk. The FP-ratio can indirectly indicate energy status of cows early in lactation as FP-ratio of milk is expected to increase with increased mobilization of adipose tissue. Thereby FP-ratio can be used as an indicator for the risk of developing ketosis (Heuer et al., 1999). A survey in Danish herds suggest a threshold for critical FP-ratio of 1.42-1.43 for large breeds in early lactation at 4-30 DIM (Trinderup et al., 2010). FP-ratios in the present study were evaluated throughout 0-50 DIM, which might have decreased the average estimate compared to a shorter interval. Still, the observed FP-ratios in the present study did not indicate risk for ketosis. FP ratios averaged 1.24 ±0.23 and the highest FP-ratio of the 75 % lowest observations was 1.36.

AMS-data, where daily measurements of milk production during 0-45 DIM were available from herd B, reflects a similar yield response when cows were fed the H-diet compared to the C-diet (Table 8). These results must be considered with caution, because of the confounding effect of test period in this case. Based on yield controls, ECM-yield increased from test period 1 to test period 2. This increase could be caused by several other factors affecting the herds, such as postpartum feeding, season etc. However, considering the first 45 days of lactation via AMS-data, the ECM-yield was on average 2.85 kg or 6.6 % higher in the H-group compared to the C-group. Despite the confounding effect of test-period, these results interestingly demonstrated that the difference between the two groups was largest during early lactation (0-20 DIM) and the statistical difference diminished as lactation proceeded (Table 8, Figure 6).

Differences in feeding level was achieved by replacing corn silage with concentrates to reduce fill value and thereby increase daily DM- and energy intake. Estimates on daily DMI might confirm the expected difference in feeding level between treatment C and H. Cows fed the H-diet ate 1.6 kg DM/day more on average when compared to cows fed the C-diet, and therefore had an 11.5 % greater daily energy intake (Table 4). Thereby, the obtained energy intakes in the Close-UP period exceeded the recommendations suggested by Norfor (Volden, 2011). The cows on the C-diet had a net energy intake of app. 156 % of these requirements while cows on the H-diet had a net energy intake of app. 174 % of the requirements. The increased energy intake might have helped to reduce the prepartum dip in DMI that has been found to occur prior to parturition (Grummer, 1995; Hayirli et al., 2002; Richards, 2011). Doepel et al. (2002) fed Close-UP diets using two levels of energy, low or high. While the authors did not detect any differences in prepartum feed intake between the two groups, feed intake after calving was significantly higher for the cows fed the Close-UP diet with high energy level. These cows also had lower plasma NEFA concentrations, indicating an improved energy status. However, the increased postpartum feed intake did not lead to any increase in milk production in the study. Similar findings are also reported by Rabelo et al. (2003), who found increased postpartum DMI but no increase in milk production, when cows were fed a high energy Close-UP diet. Based on the findings in literature, it can be speculated that a similar mechanism could have occurred in the present study, where cows fed the H-diet in the Close-UP period might have had an increased postpartum feed intake. In this case, the cows responded with an increased milk yield. Tendencies for slightly increased FP-ratio in milk indicated higher mobilization in these cows. The improved milk yield and tendencies for higher mobilization could suggest that high Close-UP feeding level primed the cows for increased metabolism after parturition, which led to higher production. Additional measurements of biological parameters before and after calving would be needed in order to elucidate the mechanisms.

Contrarily, the literature also suggests that increasing the energy density of prepartum diet as parturition approaches is not necessary, as a greater decline in DMI along with energy intake has been observed prior to parturition (Ingvartsen et al., 1997; Minor et al., 1998; Olsson et al., 1998). Although the present experiment did not find any significant effects of duration of Close-UP feeding on postpartum milk production, it cannot be rejected that the success or failure of increasing energy density of diets is a matter of timing and avoiding the risk of overfeeding in early dry period (Dann et al., 2006). This aspect was not tested in the abovementioned studies.

Avoidance of intramammary infections (IMI) is important for the probability of the completion of another lactation. A risk factor for IMI is, among others, elevated SCC (Breen et al., 2009). In this study the treatments did not significantly affect SCC in milk. However, a tendency for increased SCC in milk from cows exposed to long Close-UP period was observed (Table 7). A review by O'Rourke (2009) suggested that the major impact of nutrition on udder health is via suppression of the immune system. In the present study, the treatments did not affect any health parameters, indicating that no suppression of the immune status of the cows was present.

6. Conclusion

This study demonstrated that the duration of Close-UP feeding and the feeding level in the Close-UP period affected different postpartum performance parameters. Dry cows exposed to a short Close-UP period (12.8 ±5.2 d) had higher colostrum yield and tended to have higher total protein yield at first milk-out compared with cows exposed to a long Close-UP period (23.0 ±5.7 d). Therefore, the hypothesis that long duration of Close-UP feeding would improve colostrum yield and quality can be rejected. Feeding level of Close-UP diets did not per se influence any parameters related to colostrum and its quality. Neither duration nor feeding level significantly affected birth weight measurements of calves.

The four treatments did not have any detectable effects on health status (milk fever, retained placenta and ketosis). pH-measurements of urine revealed that Close-UP cows were metabolically acidified, which might have had a positive effect on calcium status at parturition, since only 2 out of 207 cows showed clinical signs of milk fever. Udder edema scores were not affected by the treatments.

Postpartum milk production was unaffected by the duration of the Close-UP-period, but high Close-UP feeding level led to increased ECM-yield in the subsequent early lactation. Fat to protein ratio in milk tended to increase when cows were exposed to the high feeding level, which could support the increased milk production. Therefore, the hypotheses that long duration would negatively affect subsequent milk production, and high feeding level would improve subsequent milk production can be rejected and confirmed, respectively.

Evaluating the results across the different criteria for success or failure of Close-UP treatments in this experiment, the study suggests a short Close-UP period with a high feeding level to be a superior Close-UP strategy.

7. Perspectives

Several subjects for discussion in this thesis were based on assumptions that concerned changes in DMI before and after parturition. Daily measurements of DMI on both a cow- and day-to-day level were not possible to monitor in the current study, though it would have strengthened the basis for the discussion. In future studies it would be beneficial/ideal to carry out such measurements, although the practicality of this degree of monitoring may pose challenges on commercial farms. Rumination monitoring can perhaps be used as an indicator. Additionally, weekly body weight measurements of the cows would assist in unfolding the changes.

In future studies, it would be interesting to investigate the effects of further increases in feeding level of Close-UP diets. What is the upper limit? A further increase in energy intake prepartum would perhaps prime the cow for increased yield after parturition. This might also increase the risk for transition diseases, if preventive strategies are not managed immaculately. The question that remains is; when will an increased yield match the increased risk for diseases?

Negative DCAD-value of feed during the entire Close-UP period was a precondition for the present study. To avoid possible depressing effects on DMI caused by DCAD-negative feeding, it would be interesting to investigate the effects of feeding a transition diet between the Far-OFF diet and the conventional DCAD-negative Close-UP diet. This transition diet should strive for a neutral DCAD-value (DCAD = 0 meq./kg DM) and could be followed by a acidogenic diet to be fed until parturition.

Possible epigenetic effects of dry cow feeding strategies could also be considered in future experiments. It can be speculated that fetal programming of fetuses may occur and that nutritional strategy for the dam may affect the calf and the future cow. Are calves born from dams on a high feeding level more tolerant to high serum insulin levels, than calves from dams on lower prepartum feeding level? These are questions, a long-term study should strive to answer.

8. References

Adewuyi, A.A., E. Gruysi & F.J.C.M. van Eerdenburg (2005): Non esterified fatty acids (NEFA) in dairy cattle. A review. *Veterinary Quarterly*, Vol. 27:3, pp. 117–126.

Akers, R.M. (2002): Endocrine, Growth Factor, and Neural Regulation of Mammary Function. *Lactation and Thee Mammary Gland*. First edit., Iowa State Press, pp. 165–198.

Andersen, J.B., T.G. Madsen, T. Larsen, K.L. Ingvartsen & M.O. Nielsen (2005): The Effects of Dry Period Versus Continuous Lactation on Metabolic Status and Performance in Periparturient Cows. *Journal of Dairy Science*, Elsevier, Vol. 88:10, pp. 3530–3541.

Barrington, G.M., T.B. McFadden, M.T. Huyler & T.E. Besser (2001): Regulation of colostrogenesis in cattle. *Livestock Production Science*, Vol. 70:1–2, pp. 95–104.

Beever, D.E. (2006): The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. *Animal Reproduction Science*, Elsevier, Vol. 96:3–4, pp. 212–226.

Bergman, E.N. (1971): Hyperketonemia-Ketogenesis and Ketone Body Metabolism. *Journal of Dairy Science*, Vol. 54:6, pp. 936–948.

Besser, T.E. & C.C. Gay (1994): The Importance of Colostrum to the Health of the Neonatal Calf. *Veterinary Clinics of North America: Food Animal Practice*, Elsevier, Vol. 10:1, pp. 107–117.

Bielmann, V., J. Gillan, N.R. Perkins, A.L. Skidmore, S. Godden et al. (2010): An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *Journal of Dairy Science*, Elsevier, Vol. 93:8, pp. 3713–3721.

Bobe, G., J.W. Young & D.C. Beitz (2004): Invited Review: Pathology, Etiology, Prevention, and Treatment of Fatty Liver in Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 87:10, pp. 3105–3124.

Boutflour, R. (1967): *The high yielding daiy cow*, Crosby Lockwood & Son Ltd.

Brandon, M.R., D.L. Watson & A.K. Lascelles (1971): The mechanism of transfer of immunoglobulin into mammary secretion of cows. *Aust. J. Exp. Biol. Med. Sci*, p. 613.

Breen, J.E., A.J. Bradley & M.J. Green (2009): Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in united Kingdom dairy cows. *Journal of Dairy Science*, Vol. 92:7, pp. 3106–3115.

Butler, J.E. (1983): Bovine immunoglobulins: An augmented review. *Veterinary Immunology and Immunopathology*, Elsevier, Vol. 4:1–2, pp. 43–152.

Capuco, A.V., R.M. Akers & J.J. Smith (1997): Mammary Growth in Holstein Cows During the Dry Period: Quantification of Nucleic Acids and Histology. *Journal of Dairy Science*, Elsevier, Vol. 80:3, pp. 477–487.

Challa, A., R.J. Krieg, M.A. Thabet, J.D. Veldhuis & J.C.M. Chan (1993): Metabolic acidosis inhibits growth hormone secretion in rats: Mechanism of growth retardation. *American Journal of Physiology - Endocrinology and Metabolism*, Vol. 265:4 28-4, available at:http://doi.org/10.1152/ajpendo.1993.265.4.e547.

Contreras, L.L., C.M. Ryan & T.R. Overton (2004): Effects of Dry Cow Grouping Strategy and Prepartum Body Condition Score on Performance and Health of Transition Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 87:2, pp. 517–523.

Convey, E.M. (1974): Serum Hormone Concentration in Ruminants during Mammary Growth, Lactogenesis, and Lactation: A Review. *Journal of Dairy Science*, available at:http://doi.org/10.3168/jds.S0022-0302(74)84986-6.

Corbett, R.B. (2002): Influence of days fed a close-up dry cow ration and heat stress on subsequent milk production in western dairy herds. *Journal of Dairy Science*, Vol. 85 (suppl:, pp. 191–192.

Dann, H.M., N.B. Litherland, J.P. Underwood, M. Bionaz, A. D'Angelo et al. (2006): Diets During Far-Off and Close-Up Dry Periods Affect Periparturient Metabolism and Lactation in Multiparous Cows. *Journal of Dairy Science*, Vol. 89:9, pp. 3563–3577.

DeGaris, P.J. & I.J. Lean (2008): Milk fever in dairy cows: A review of pathophysiology and control principles. *Veterinary Journal*, W.B. Saunders, Vol. 176:1, pp. 58–69.

Dias, F.M. & F.R. Allaire (1982): Dry Period to Maximize Milk Production Over Two Consecutive Lactations. *Journal of Dairy Science*, Vol. 65:1, pp. 136–145.

Doepel, L., H. Lapierre & J.J. Kennelly (2002): Peripartum Performance and Metabolism of Dairy Cows in Response to Prepartum Energy and Protein Intake. *Journal of Dairy Science*, Elsevier, Vol. 85:9, pp. 2315–2334.

Douglas, G.N., T.R. Overton, H.G. Bateman, H.M. Dann & J.K. Drackley (2006): Prepartal Plane of Nutrition, Regardless of Dietary Energy Source, Affects Periparturient Metabolism and Dry Matter Intake in Holstein Cows. *Journal of Dairy Science*, Elsevier, Vol. 89:6, pp. 2141–2157.

Drackley, J.K. (1999): Biology of Dairy Cows During the Transition Period: the Final Frontier? *Journal of Dairy Science*, Elsevier, Vol. 82:11, pp. 2259–2273.

Van Es, A.J.H. (1978): Feed evaluation for ruminants. I. The systems in use from May 1977-onwards in The Netherlands. *Livestock Production Science*, Elsevier, Vol. 5:4, pp. 331–345.

Farahani, T.A., H. Amanlou & M. Kazemi-Bonchenari (2017): Effects of shortening the close-up period length coupled with increased supply of metabolizable protein on performance and metabolic status of multiparous Holstein cows. *Journal of Dairy Science*, Vol. 100:8, pp. 6199–6217.

Florini, J.R., D.Z. Ewton & S.A. Coolican (1996): Growth Hormone and the Insulin-Like Growth Factor System in Myogenesis. *Endocrine Reviews*, Vol. 17:5, pp. 423–480.

Friggens, N.C., J.B. Andersen, T. Larsen, O. Aaes & R.J. Dewhurst (2004): Priming the dairy cow for lactation: a review of dry cow feeding strategies. *Animal Research*, Vol. 53:2004, pp. 453–473.

Fronk, T.J., L.H. Schultz & A.R. Hardie (1980): Effect of Dry Period Overconditioning on Subsequent Metabolic Disorders and Performance of Dairy Cows. *Journal of Dairy Science*, Vol. 63:7, pp. 1080–1090.

Giuliodori, M.J., R.P. Magnasco, D. Becu-Villalobos, I.M. Lacau-Mengido, C.A. Risco et al. (2013): Metritis in dairy cows: Risk factors and reproductive performance. *Journal of Dairy Science*, Elsevier, Vol. 96:6, pp. 3621–3631.

Godden, S.M., J.E. Lombard & A.R. Woolums (2019): Colostrum Management for Dairy Calves. *Veterinary Clinics of North America - Food Animal Practice*, Vol. 35:3, pp. 535–556.

Goff, J.P. (2008): The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. *The Veterinary Journal*, W.B. Saunders, Vol. 176:1, pp. 50–57.

Goff, J.P. & R.L. Horst (1997): Effects of the Addition of Potassium or Sodium, but Not Calcium, to Prepartum Rations on Milk Fever in Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 80:1, pp. 176–186.

Goff, J.P. & R.L. Horst (1998): Use of Hydrochloric Acid as a Source of Anions for Prevention of Milk Fever. *Journal of Dairy Science*, Elsevier, Vol. 81:11, pp. 2874–2880.

Goff, J.P., K. Kimura & R.L. Horst (2002): Effect of Mastectomy on Milk Fever, Energy, and Vitamins A, E, and β-Carotene Status at Parturition. *Journal of Dairy Science*, Elsevier, Vol. 85:6, pp. 1427–1436.

Gröhn, Y., L.-A. Lindberg, M.L. Bruss & T.B. Farver (1983): Fatty Infiltration of Liver in Spontaneously Ketotic Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 66:11, pp. 2320–2328.

Grummer, R.R. (1993): Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 76:12, pp. 3882–3896.

Grummer, R.R. (1995): Impact of changes in organic nutrient metabolism on feeding the transition dairy cow Impact of Changes in Organic Nutrient Metabolism Cowl Feeding the Transition Dairy. *Journal of Animal Science*, pp. 2820–2833.

Guy, M.A., T.B. McFadden, D.C. Cockrell & T.E. Besser (1994): Regulation of Colostrum Formation in Beef and Dairy Cows. *Journal of Dairy Science*, available at:http://doi.org/10.3168/jds.S0022-0302(94)77241-6.

Hadsell, D.L., R. Baumrucker & R.S. Kensinger (1992): Effects of elevated blood insulin-like growth factor-I secretions during the colostrum phase. *Journal of Endoccrinology*, Vol. 137:, pp. 223–230.

Hammer, D.K., B. Kickhöfen & H. Malchow (1969): Preferential Adsorption of a Single Bovine IgG Type by Isolated Epithelial Cells of the Mammary Gland. *Protides of the Biological Fluids*, Pergamon, pp. 663–668.

Hayirli, A., R.R. Grummer, E.V. Nordheim & P.M. Crump (2002): Animal and Dietary Factors Affecting Feed Intake During the Prefresh Transition Period in Holsteins. *Journal of Dairy Science*, Elsevier, Vol. 85:12, pp. 3430–3443.

Hernández-Castellano, L.E., L.L. Hernandez & R.M. Bruckmaier (2019): Review: Endocrine pathways to regulate calcium homeostasis around parturition and the prevention of hypocalcemia in periparturient dairy cows. *Animal*, pp. 330–338.

Hernández-Castellano, L.E., L.L. Hernandez, S. Weaver & R.M. Bruckmaier (2017): Increased serum serotonin improves parturient calcium homeostasis in dairy cows. *Journal of Dairy Science*, Elsevier, Vol. 100:2, pp. 1580–1587.

Heuer, C., Y.H. Schukken & P. Dobbelaar (1999): Postpartum Body Condition Score and Results from the First Test Day Milk as Predictors of Disease, Fertility, Yield, and Culling in Commercial Dairy Herds. *Journal of Dairy Science*, Elsevier, Vol. 82:2, pp. 295–304.

Holst, B.D., W.L. Hurley & D.R. Nelson (1987): Involution of the Bovine Mammary Gland: Histological and Ultrastructural Changes. *Journal of Dairy Science*, Elsevier, Vol. 70:5, pp. 935–944.

Horst, R.L. (1986): Regulation of Calcium and Phosphorus Homeostasis in the Dairy Cow. *Journal of Dairy Science*, Elsevier, Vol. 69:2, pp. 604–616.

Horst, R.L., J.P. Goff & T.A. Reinhardt (1997): Calcium and vitamin D metabolism during lactation. *Journal of Mammary Gland Biology and Neoplasia*, Vol. 2:3, pp. 253–263.

Hurley, W.L. (1989): Mammary Gland Function During Involution. *Journal of Dairy Science*, Elsevier, Vol. 72:6, pp. 1637–1646.

Huzzey, J.M., D.M. Veira, D.M. Weary & M.A.G. von Keyserlingk (2007): Prepartum Behavior and Dry Matter Intake Identify Dairy Cows at Risk for Metritis. *Journal of Dairy Science*, Elsevier, Vol. 90:7, pp. 3220–3233.

Ingvartsen, K.L., J. Foldager & O. Aaes (1997): Effect of prepartum TMR energy concentration on feed intake milk yield and energy balance in dairy heifers and cows. *Journal of Dairy Science*, Vol. 80 (Suppl.:, pp. 211–211.

Janovick, N.A.A., Y.R.R. Boisclair & J.K.K. Drackley (2011): Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. *Journal of Dairy Science*, Elsevier, Vol. 94:3, pp. 1385–1400.

Johannsen, U., S. Menger, R. Staufenbiel & H. Klukas (1993): The morphology and function of the liver in high performance cows 2 weeks post partum. *Dtsch. Tierarztl. Wochenschr.*, Vol. 100:, pp. 177–181.

Kemler, R., H. Mossmann, U. Strohmaier, B. Kickhöfen & D.K. Hammer (1975): In vitro studies on the selective binding of IgG from different species to tissue sections of the bovine mammary gland. *Euopean Journal of Immunology*.

Kichura, T.S., R.L. Horst, D.C. Beitz & E.T. Littledike (1982): Relationship between prepartal dietary calcium and phosphorus, vitamin D metabolism, and parturient paresis in dairy cows. *Journal of Nutrition*, Vol. 112:3, pp. 480–487.

Kok, A., A.T.M. van Knegsel, C.E. van Middelaar, B. Engel, H. Hogeveen et al. (2017): Effect of dry period length on milk yield over multiple lactations. *Journal of Dairy Science*, Elsevier, Vol. 100:1, pp. 739–749.

Kristensen, N.B. (2017): SEGES NIR kalibreringer. *LandbrugsInfo*, available at: https://www.landbrugsinfo.dk/kvaeg/foder/kmp/sider/kv-17-4072-seges-nir-kalibreringer.aspx (accessed 15 January 2020).

Lacetera, N., U. Bernabucci, B. Ronchi & A. Nardone (1996): Effects of selenium and vitamin E administration during a late stage of pregnancy on colostrum and milk production in dairy cows , and on passive immunity and growth of their offspr ... *American Journal of Veterinary Research*, Vol. 57:, pp. 1776–1780.

Larson, B.L. (1992): Immunoglobulins of the mammary secretions. *Advanced Dairy Chemistry*. P.F. Fox e., Vol. 1, Elsevier Applied Science, London, pp. 231–254.

Larson, B.L., H.L. Heary & J.E. Devery (1980): Immunoglobulin Production and Transport by the Mammary Gland. *Journal of Dairy Science*, Elsevier, Vol. 63:4, pp. 665–671.

Lean, I.J., R. Van Saun & P.J. DeGaris (2013): Energy and protein nutrition management of transition dairy cows. *Veterinary Clinics of North America - Food Animal Practice*, Vol. 29:2, pp. 337–366.

Lopera, C., R. Zimpel, A. Vieira-Neto, F.R.R. Lopes, W. Ortiz et al. (2018): Effects of level of dietary cation-anion difference and duration of prepartum feeding on performance and metabolism of dairy cows. *Journal of Dairy Science*, Elsevier, Vol. 101:9, pp. 7907–7929.

Madsen, T.G. & M.O. Nielsen (2003): Næringsstofomsætning i ekstrahepatiske væv. *Kvægets Ernæring Og Fysiologi*, pp. 419–462.

Mann, S., F.A. Leal Yepes, T.R. Overton, A.L. Lock, S.V. Lamb et al. (2016): Effect of dry period dietary energy level in dairy cattle on volume, concentrations of immunoglobulin G, insulin, and fatty acid composition of colostrum. *Journal of Dairy Science*, Elsevier, Vol. 99:2, pp. 1515–1526.

Mann, S., F.A.L. Yepes, T.R. Overton, J.J. Wakshlag, A.L. Lock et al. (2015): Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. *Journal of Dairy Science*, Elsevier, Vol. 98:5, pp. 3366–3382.

Martinez, N., C.A. Risco, F.S. Lima, R.S. Bisinotto, L.F. Greco et al. (2012): Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *Journal of Dairy Science*, Elsevier, Vol. 95:12, pp. 7158–7172.

Martinez, N., R.M. Rodney, E. Block, L.L. Hernandez, C.D. Nelson et al. (2018): Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Lactation performance and energy metabolism. *Journal of Dairy Science*, Elsevier, Vol. 101:3, pp. 2544–2562.

Mashek, D.G. & D.K. Beede (2001): Peripartum Responses of Dairy Cows Fed Energy-Dense Diets for 3 or 6 Weeks Prepartum. *Journal of Dairy Science*, Elsevier, Vol. 84:1, pp. 115–125.

Minor, D.J., S.L. Trower, B.D. Strang, R.D. Shaver & R.R. Grummer (1998): Effects of Nonfiber Carbohydrate and Niacin on Periparturient Metabolic Status and Lactation of Dairy Cows. *Journal of Dairy Science*, Elsevier, Vol. 81:1, pp. 189–200.

Nielsen, N.I., N.C. Friggens, M.G.G. Chagunda & K.L. Ingvartsen (2005): Predicting Risk of Ketosis in Dairy Cows Using In-Line Measurements of β-Hydroxybutyrate: A Biological Model. *Journal of Dairy Science*, Elsevier, Vol. 88:7, pp. 2441–2453.

Nielsen, N.I. & H. Volden (2011): 9. Animal requirements and recommendations. In: Volden, H. (Ed.), *NorFor - The Nordic Feed Evaluation System*, pp. 85–112.

NRC (1985): *Ruminal nitrogen usage*. *National Academy Press,* Washington DC, USA.

O'Rourke, D. (2009): Nutrition and udder health in dairy cows: A review. *Irish Veterinary Journal*, Vol. 62:4, pp. 15–20.

Olsson, G., M. Emanuelson & H. Wiktorsson (1998): Effects of different nutritional levels prepartum on the subsequent performance of dairy cows. *Livestock Production Science*, available at:http://doi.org/10.1016/S0301-6226(97)00022-5.

Pérez-Báez, J., C.A. Risco, R.C. Chebel, G.C. Gomes, L.F. Greco et al. (2019): Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part I. Calving disorders and metritis. *Journal of Dairy Science,* American Dairy Science Association, Vol. 102:10, pp. 9138–9150.

Prior, R.L. & D.B. Laster (1979): Development of the Bovine Fetus. *Journal of Animal Science*, Vol. 48:6, pp. 1546–1553.

Rabelo, E., R.L. Rezende, S.J. Bertics & R.R. Grummer (2003): Effects of Transition Diets Varying in Dietary Energy Density on Lactation Performance and Ruminal Parameters of Dairy Cows. *Journal of Dairy Science,* Elsevier, Vol. 86:3, pp. 916–925.

Ramberg, C.F., E.K. Johnson, R.D. Fargo & D.S. Kronfeld (1984): Calcium homeostasis in cows, with special reference to parturient hypocalcemia. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, Vol. 15:5, available at:http://doi.org/10.1152/ajpregu.1984.246.5.r698.

Raundal, P. & J. Nielsen (2020): Tal om kvæg - Kodødelighed. *LandbrugsInfo*, available at: https://www.landbrugsinfo.dk/Kvaeg/Tal-om-kvaeg/Sider/KododHist.aspx (accessed 18 January 2020).

Reinhardt, T.A., J.D. Lippolis, B.J. McCluskey, J.P. Goff & R.L. Horst (2011): Prevalence of subclinical hypocalcemia in dairy herds. *The Veterinary Journal*, W.B. Saunders, Vol. 188:1, pp. 122–124.

Reynolds, C.K., P.C. Aikman, B. Lupoli, D.J. Humphries & D.E. Beever (2003): Splanchnic

Metabolism of Dairy Cows During the Transition From Late Gestation Through Early Lactation. *Journal of Dairy Science*, Elsevier, Vol. 86:4, pp. 1201–1217.

Richards, B.F. (2011): Strategies to decrease incidence of fatty liver in dairy cows.

Roberts, C., I. Reid, G. Rowlands & A. Patterson (1981): A fat mobilisation syndrome in dairy cows in early lactation. *The Veterinary Record*, Vol. 108:, pp. 7–9.

Rodríguez, E.M., A. Arís & A. Bach (2017): Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. *Journal of Dairy Science*, American Dairy Science Association, Vol. 100:9, pp. 7427–7434.

Rowland, G.N., C.C. Capen, D.M. Young & H.E. Black (1972): Microradiographic evaluation of bone from cows with experimental hypervitaminosis D, diet-induced hypocalcemia, and naturally occurring parturient paresis. *Calcified Tissue Research*, Vol. 9:1, pp. 179–193.

Rulquin, H. & R. Vérité (1993): The effects of bovine somatotropin injections on mammary blood flow in dry and lactating dairy cows. *Annales de Zootechnie*, Vol. 42:2, pp. 209–209.

Santos, J.E.P., I.J. Lean, H. Golder & E. Block (2019): Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. *Journal of Dairy Science*, American Dairy Science Association, Vol. 102:3, pp. 2134–2154.

van Saun, R.J. & C.J. Sniffen (2014): Transition Cow Nutrition and Feeding Management for Disease Prevention. *Veterinary Clinics of North America - Food Animal Practice*, Elsevier Inc, Vol. 30:3, pp. 689–719.

Silva-del-Río, N., D. Rolle, A. García-Muñoz, S. Rodríguez-Jiménez, A. Valldecabres et al. (2017): Colostrum immunoglobulin G concentration of multiparous Jersey cows at first and second milking is associated with parity, colostrum yield, and time of first milking, and can be estimated with Brix refractometry. *Journal of Dairy Science*, American Dairy Science Association, Vol. 100:7, pp. 5774–5781.

Sjaastad, Ø. V., O. Sand & K. Hove (2010a): 20. Lactation. *Physiology of Domestic Animals*. 2nd editio., Scandinavian Press, Olso.

Sjaastad, Ø. V., O. Sand & K. Hove (2010b): 7. Bone Tissue and Mineal Metabolism. *Physiology of Domestic Animals*. 2nd editio., Scandinavian Press, Olso, pp. 259–278.

Smith, K.L., L.A. Muir, L.C. Ferguson & H.R. Conrad (1971): Selective Transport of IgGl into the Mammary Gland: Role of Estrogen and Progesterone. *Journal of Dairy Science*, available at:http://doi.org/10.3168/jds.S0022-0302(71)86130-1.

Smith, K.L. & D.A. Todhunter (1982): The physiology of mammary gland during the dry period and the relationship to infection. *21 Annual Meeting National Mastitis Council*, Louisville, KY, p. 87.

Strudsholm, F., O. Aaes & H.L. Hyttel (2016): Antal nykælvere med mælkefeber er højest hos ældre køer og jersey. *LandbrugsInfo*, pp. 1–7.

Swett, W.W., C.A. Matthews & R.R. Graves (1938): Nature of the Swelling in the Udder of a Cow at Calving Time. *Journal of Dairy Science*, Elsevier, Vol. 21:11, pp. 713–723.

Team, R.C. (2017): R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Thilsing-Hansen, T., R.J. Jørgensen & S. Østergaard (2002): Milk fever control principles: A review. *Acta Veterinaria Scandinavica*, Vol. 43:1, pp. 1–19.

Trinderup, M., O. Aaes, R. Thøgersen & K. Helle Sloth (2010): *Kvæginfo 2107: Sundhedsovervågning Baseret På Fedt-Protein Forholdet Ved Første Ydelseskontrol Efter Kælvning*.

Vagnoni, D.B. & G.R. Oetzel (1998): Effects of Dietary Cation-Anion Difference on the Acid-Base Status of Dry Cows. *Journal of Dairy Science*, Elsevier, Vol. 81:6, pp. 1643–1652.

Vickers, L.A., D.M. Weary, D.M. Veira & M.A.G. von Keyserlingk (2013): Feeding a higher forage diet prepartum decreases incidences of subclinical ketosis in transition dairy cows. *Journal of Animal Science*, Vol. 91:2, pp. 886–894.

Volden, H. (2011): *NorFor - The Nordic feed evaluation system*. edited by Volden, H., EAAP - European Federation of Animal Science.

Weich, W., E. Block & N.B. Litherland (2013): Extended negative dietary cation-anion difference feeding does not negatively affect postpartum performance of multiparous dairy cows. *Journal of Dairy Science*, Elsevier, Vol. 96:9, pp. 5780–5792.

White, H.M. (2015): The role of TCA cycle anaplerosis in ketosis and fatty liver in periparturient dairy cows. *Animals*, Vol. 5:3, pp. 793–802.

Wiggers, K.D., D.K. Nelson & N.L. Jacobson (1975): Prevention of Parturient Paresis by a Low-Calcium Diet Prepartum: a Field Study,. *Journal of Dairy Science,* Elsevier, Vol. 58:3, pp. 430–431.

Wilmink, J.B.M. (1987): Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livestock Production Science*, Vol. 16:4, pp. 335–348.

Yang, N., C. Liu, A.R. Peck, M.A. Girondo, A.F. Yanac et al. (2013): Prolactin-Stat5 signaling in breast cancer is potently disrupted by acidosis within the tumor microenvironment. *Breast Cancer Research*, Vol. 15:5, available at:http://doi.org/10.1186/bcr3467.

Zanker, I., H. Hammon & J. Blum (2000): beta-Carotene, retinol and alpha-tocopherol status in calves fed the first colostrum at 0-2, 6-7, 12-13 or 24-25 hours after birth. *International Journal for Vitamin and Nutrition Research*, Vol. 70:6, pp. 305–310.

Zimpel, R., M.B. Poindexter, A. Vieira-Neto, E. Block, C.D. Nelson et al. (2018): Effect of dietary cation-anion difference on acid-base status and dry matter intake in dry pregnant cows. *Journal of Dairy Science,* American Dairy Science Association, Vol. 101:9, pp. 8461–8475.