Institut für Tierwissenschaften

The impact of automated, constant incomplete milking on energy balance, udder health, and subsequent performance in early lactation of dairy cows

Dissertation

zur

Erlangung des Grades

Doktorin der Agrarwissenschaften

(Dr. agr.)

der Landwirtschaftlichen Fakultät

der Rheinischen Friedrich-Wilhelms-Universität Bonn

von

Isabell Meyer, M.Sc.

aus Mechernich

Bonn, 2023

Referentin:	Prof. Dr. Dr. Helga Sauerwein
Korreferent:	Prof. Dr. Karl-Heinz Südekum
Tag der mündlichen Prüfung:	27.10.2023

Angefertigt mit Genehmigung der Landwirtschaftlichen Fakultät der Universität Bonn

Für Ewald † und Katharina Schick

Abstract

During the first weeks of lactation, dairy cows experience a negative energy balance (NEB), because the energy supply provided by feed intake can not cover the energy needed for maintenance and rising milk production at that time. To maintain milk production even during NEB, dairy cows mobilize body reserves mainly from fat. The non-esterified fatty acids (NEFA) released from the adipose depots represent a major energy source for the cow in early lactation. However, the capacity of the liver for full NEFA oxidation is limited, resulting in either an excess storage as triglycerides in the liver or incomplete oxidation, which further promotes ketogenesis. Insufficient metabolic adaption to NEB is associated with a higher incidence of metabolic disorders such as ketosis and fatty liver. Metabolic disorders are known to be an important risk factor for other diseases in early lactation and cause high costs due to the loss of performance and veterinary treatment. To modify the energy output with milk as one of the main variables affecting the energy balance (EB), incomplete milking (IM) to delay the increase in milk yield is one of the options to mitigate the NEB in early lactation. Our objectives were to test the effects of IM in early lactation on EB, metabolic status, udder health, and subsequent performance. An automated software module was used to facilitate the practical application, which enables an automatic cluster removal when a defined milk quantity (kg) instead of a defined milk flow (kg/min) is reached. Forty-six Holstein cows were equally allocated to either the treatment (TRT, n = 23; starting on days in milk (DIM) 8 ± 1.1) or the control group (CON, n = 23; conventional cluster removal at a milk flow rate < 0.3 kg/min). The amount of milk in the TRT group was clamped during IM to the milk yield of each cow 1 day before IM was started and held constant for 14 days. Thereafter, all cows were conventionally milked and records related to EB, performance, and udder health were continued up to 15 week of lactation. During the 2-week IM, on average 11.1% less milk were obtained from the TRT group than from the CON group. After IM, milk yield increased in the TRT group eliminating the group difference throughout the remaining observation period until week 15 of lactation. Udder health was not compromised during the entire observation period in all cows. The TRT cows tended to have less feed intake and also water intake than the CON cows during the 2-wk of IM. The extent of the NEB and the circulating concentrations of NEFA, ß-hydroxybutyrate, insulin-like growth factor-1 (IGF-1), and leptin did mostly not differ between the groups. The IM did not affect body condition. Our findings confirm the practicability of the milking software module for an automated, constant milk withdrawal to delay the increase in milk yield during the first weeks of lactation without affecting udder health and subsequent performance. However, it remains to be determined whether the absent effect on energy metabolism was due to the relatively stable energy status of the cows or to the relatively mild IM setting used herein. Further research is needed to understand the link between the degree and duration of IM and the adaptive mechanism of the organism in terms of milk synthesis, feed intake, and energy metabolism.

Kurzfassung

In den ersten Wochen der Laktation geraten Milchkühe in eine negative Energiebilanz (NEB), da die Energiezufuhr über die Futteraufnahme den Energiebedarf für den Erhaltungsbedarf und die steigende Milchproduktion in dieser Zeit nicht decken kann. Um die Milchproduktion auch während der NEB aufrechtzuerhalten, mobilisieren Milchkühe Körperreserven vorwiegend aus dem Fett. Die aus den Fettdepots freigesetzten nicht-veresterten Fettsäuren (NEFA) stellen eine wichtige Energiequelle für die Kuh während der Frühlaktation dar. Die Kapazität der Leber zur vollständigen NEFA-Oxidation ist jedoch während der NEB begrenzt, was entweder in einer übermäßigen Speicherung in Form von Triglyceriden in der Leber oder einer unvollständigen Oxidation mündet, wodurch die Ketonkörperproduktion weiter vorangetrieben wird. Eine unzureichende Anpassungsfähigkeit des Stoffwechsels an die NEB geht mit einer höheren Inzidenz von Stoffwechselstörungen wie Ketose und Fettleber einher. Stoffwechselstörungen sind bekanntermaßen ein bedeutender Risikofaktor für die Entstehung weiterer Krankheiten in der Frühlaktation und verursachen hohe Kosten aufgrund von Leistungseinbußen und tierärztlicher Behandlungen. Um die Energieabgabe mit der Milch als einer der Hauptvariablen des Energiesaldos (EB) zu modifizieren, stellt das unvollständige Melken (IM) eine Möglichkeit dar, den Anstieg der Milchleistung zu verzögern und das Ausmaß der NEB in der Frühlaktation zu verringern. Im Rahmen dieser Arbeit sollten die Auswirkungen des IM in der Frühlaktation auf die EB, den Stoffwechselstatus, die Eutergesundheit und die spätere Leistung untersucht werden. Für die praktische Anwendung wurde ein Softwaremodul eingesetzt, das eine automatische Melkzeugabnahme nach Erreichen einer bestimmten Milchmenge (kg) anstelle eines bestimmten Milchflusses (kg/min) ermöglicht. Es wurden 46 Holstein-Kühe entweder der Behandlungs- (TRT, n = 23) beginnend am Laktationstag (DIM) 8 ± 1,1 oder der Kontrollgruppe (CON, n = 23; konventionelle Melkzeugabnahme bei Milchfluss < 0,3 kg/min) zugeteilt. Die Milchmenge in der TRT-Gruppe wurde während des IM auf die individuelle Milchleistung der jeweiligen Kuh 1 Tag vor Beginn des IM festgesetzt und für 14 Tage konstant gehalten. Danach wurden alle Kühe konventionell gemolken und die Aufzeichnungen bezüglich EB, Leistung und Eutergesundheit wurden bis zur 15. Laktationswoche fortgeführt. Während des zweiwöchigen IM wurde von den TRT-Gruppe durchschnittlich 11,1 % weniger Milch gewonnen als von den CON-Gruppe. Anschließend nahm die Milchleistung in der TRT-Gruppe sofort zu, so dass kein Gruppenunterschied mehr bis zur 15. Laktationswoche bestand. Die Eutergesundheit war bei allen Kühen während des gesamten Beobachtungszeitraums nicht beeinträchtigt. In der zweiten Woche des IM neigten die TRT-Kühe zu einer geringeren Futter- und Wasseraufnahme als die CON-Kühe. Das Ausmaß der NEB und die zirkulierenden Konzentrationen von NEFA, ß-Hydroxybutyrat, Insulin-ähnlichen Wachstumsfaktor-1 (IGF-1) und Leptin unterschieden sich größtenteils nicht zwischen den Gruppen. Das IM hatte keinen Einfluss auf die Körperkondition. Insgesamt bestätigen unsere Ergebnisse die Praktikabilität des Melksoftwaremoduls für einen automatisierten, auf ein konstantes Niveau limitierten Milchentzug zur Verzögerung des Milchleistungsanstiegs in den ersten Laktationswochen, ohne dabei die Eutergesundheit und die anschließende Leistung zu beeinträchtigen. Es bleibt jedoch zu klären, ob die fehlende Auswirkung auf den Energiestoffwechsel auf die relativ stabile energetische Situation der Kühe oder auf die hier verwendete, relativ milde IM-Einstellung zurückzuführen ist. Weitere Untersuchungen sind erforderlich, um den Zusammenhang zwischen dem Ausmaß und der Dauer des IM und den Anpassungsmechanismen des Organismus in Bezug auf die Milchsynthese, die Futteraufnahme und den Energiestoffwechsel zu ergründen.

Table of contents

List of abbreviations

AA	Amino acid(s)
ACACA	Acetyl-co-enzyme A carboxylase α
BAX	B-cell lymphoma protein 2 associate x
BCS	Body condition score
BCL2	B-cell lymphoma protein 2
BFT	Back fat thickness
BHB	Beta (ß)-hydroxybutyrate
BW	Body weight
CSN2	β-casein
DHIA	Dairy Herd Improvement Association
DIM	Day(s) in milk
DMI	Dry matter intake
EB	Energy balance
ECM	Energy-corrected milk
FA	Fatty acids
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
IgG	Immunoglobulin G
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IM	Incomplete milking
LALBA	α-lactalbumin
MLD	*Musculus longissimus dorsi* diameter
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NE_L	Netto energy lactation

ODM	Once-daily milking
PRL	Prolactin
SCC	Somatic cell count
STAT5A	Signal transducer and activator of transcription 5A
STAT5B	Signal transducer and activator of transcription 5B
TNFα	Tumor necrosis factor-α
TRT	Treatment group (cows)
CON	Control group (cows)
wk	Week(s)

Chapter 1 Introduction

A negative energy balance (**NEB**) is characteristic of early lactation and is caused by the genetically prioritized mammary gland and the related precondition for high performance. The increase of milk secretion requires energy, often exceeding the increase in energy supply provided by feed intake. High milk yields infer high energy outputs with a corresponding metabolic load that requires a remarkable degree of adaptation. Insufficient metabolic adaptation may increase the risk of disease and negatively affect performance and productive life span (Drackley, 1999; Esposito et al., 2014).

For mitigating the NEB and thus the risk for such health disturbances, appropriate nutrition should prevent a drastic decline in voluntary feed intake, ensure rumen stability, supply additional energy, and (or) support intermediary metabolism to support pregnancy and lactation (Overton and Waldron, 2004; Jouany, 2006). Reducing the energy output via the milk is another option to balance the dairy cow's energy status. Several approaches for temporarily reducing milk yield were suggested, such as the use of pharmaceuticals (e.g., quinagolide) (Lacasse et al., 2019), modification of the dry period (Kok et al., 2019), reducing the miking frequency to once-daily milking (**ODM**) (Stelwagen et al., 2013), or incomplete milking (**IM**) at each milking (Lacasse et al., 2018). The latter is suggested as the most promising approach for reducing the metabolic load without compromising the productivity of dairy cows (Lacasse et al., 2018). For practical realization, automation is desirable. Therefore, suitable technical solutions must be easy to integrate into the milking routine on a farm and enable the application at the herd and the individual level without compromising udder health and subsequent performance.

1.1 The energy status during the transition from late gestation to early lactation in dairy cows

The transition from late gestation to early lactation represents an important and demanding period for the dairy cow. With the onset of milk production, major metabolic changes take place. Already a few weeks (**wk**) before calving, the dairy cow enters an energy and nutrient deficit, which is reflected in a NEB (Drackley, 1999) (Figure 1).

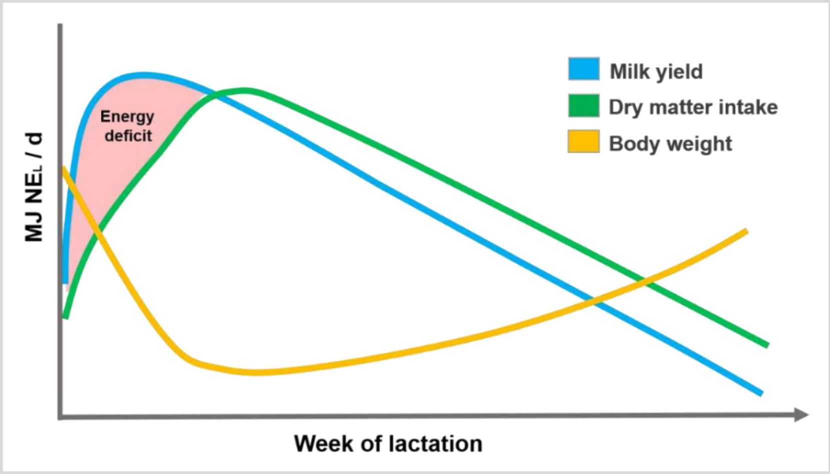


Figure 1. Unitless progression diagram showing variables tackled in the calculation of the energy balance (EB) in dairy cows: Energy intake via feeding and energy release for maintenance and milk production (modified according to Staufenbiel (1997)).

In general, the development of a NEB can be attributed to 4 main causes:

(1) The increasing nutrient requirement of fetal development during the exponential growth in the last trimester (Bell et al., 1995).

(2) Substantial growth and development of the mammary gland, and a change in energy and nutrient partitioning in favor of the mammary gland with the onset of lactation (Bauman and Currie, 1980; Ingvartsen, 2006).

(3) The "genetic drive" resulting from the genetic selection for high milk yields leads to a rapid increase of milk yield during the first wk of lactation until a peak is reached between wk 5 and 7 of lactation with declining yields thereafter (Akers, 2000; Ingvartsen and Andersen, 2000).

(4) Around calving, besides specific physical, metabolic, and endocrine changes (Ingvartsen and Andersen, 2000; Grummer et al., 2004), increased pro-inflammatory cytokine levels arising from subacute inflammation in various peripheral organs yield a decrease in voluntary feed intake (Kuhla, 2020). The maximum feed intake is reached between wk 8 and 22 of lactation (Ingvartsen and Andersen, 2000).

At the beginning of lactation, the energy and nutrient requirements increase; for instance, the mammary gland requires about 11 Mcal of energy, 140 g of protein, 23 g of Ca, 9 g of P, and 1 g of Mg to produce 10 kg of colostrum on the first day of lactation (Goff and Horst, 1997) and therefore exceeding the daily requirements for fetal development at the end of gestation (about 0.82 Mcal of energy, 117 g of protein, 10.3 g of Ca, 5.4 g of P, and 0.2 g of Mg) (Bell et al., 1995; Goff and Horst, 1997). The estimated glucose demand of Holstein cows 3 wk before calving is 1000 to 1100 g/d and increases up to 2500 g/d within 3 wk after calving (Drackley et al., 2001). To support lactation, regulatory processes ensure the metabolic priority of the mammary gland at the detriment of various other metabolic functions (Bauman and Currie, 1980). The mammary gland utilizes 83-97% of the ingested NE_L and metabolizable protein for milk synthesis, while only a small proportion remains for maintenance requirements. Systemic adaptations to NEB occur in tissues such as the liver, fat, muscle, mammary gland, and gut, requiring their metabolic regulation and coordination (Drackley, 1999; Herdt, 2000).

To maintain milk synthesis even during a NEB, the body's fat and protein reserves are mobilized (Kuhla et al. 2011). However, the mobilization of body protein is limited in quantity and duration. Already 5 wk postpartum, the mobilization of body protein ceases, whereas fat mobilization lasts up to 12 wk postpartum (Komaragiri and Erdman, 1997). The estimated mobilized amount of body reserves in early lactation ranges from 19 to 54 kg of fat, and up to 21 kg of protein (Komaragiri and Erdman, 1997; Chibisa et al., 2008). The intensity of tissue mobilization is also related to the body condition. Due to lower fat reserves, low-conditioned cows (Body condition score (**BCS**) of ≤ 2.5) appear to have more intense protein mobilization for energy and nutrient provision (Pires et al., 2013). In contrast, in high conditioned cows (BCS of ≥ 3.75), more rapid and excessive lipolysis occurs than in cows at moderate or lean condition (Pires et al., 2013).

The mobilization of fat reserves results in elevated plasma concentrations of non-esterified fatty acids (**NEFA**) and ketone bodies (ß-hydroxybutyrate (**BHB**), acetone, and acetoacetate) (Busato et al., 2002; Adewuyi et al., 2005). Several peripheral tissues (e.g., liver and muscle) can oxidize NEFA for energy supply. Moreover, the mammary gland can utilize the NEFA as a precursor for milk fat synthesis (Herdt, 2000). The capacity of the liver to oxidize the NEFA or to export them as very low-density lipoproteins is limited. Exceeding the oxidative capacity of the liver results in storage as triglycerides in the liver or incomplete oxidation of NEFA which further promotes ketogenesis (Goff and Horst, 1997). The mobilization of muscle tissue leads to enhanced amino acid (**AA**) release into blood. The AA can be assigned to different types (glucogenic, ketogenic) which define their utilization for milk protein synthesis, direct oxidation, hepatic gluconeogenesis, or ketogenesis. However, milk protein and protein synthesis can be limited by AA imbalance after calving (Kuhla et al., 2011; Sadri et al., 2023).

At the hormonal level, significant changes also occur in the peripartum period. The concentration of hormones such as growth hormone (**GH**) and glucocorticoids is increased, while the concentration of insulin, noradrenaline, leptin, thyroxine, triiodothyronine, and insulin-like growth factor-1 (**IGF-1**) is decreased. In addition, the insulin:GH ratio and insulin:glucagon ratio are decreased (Drackley et al., 2001; Adewuyi et al., 2005). Insulin plays a key role in energy metabolism. In early lactation, hypoinsulinemia is part of a series of coordinated changes that occur around the time of parturition to support lactation (Butler et al., 2003). During this time, insulin sensitivity decreases in peripheral tissues such as fat and muscle (Bell and Bauman, 1997), often leading to an insulin-resistant state (Chagas et al., 2009). Decreased insulin sensitivity promotes the catabolic processes in peripheral tissues and the nutrient flow to the largely insulin-independent and prioritized mammary gland (Bell and Bauman, 1997). Increased NEFA concentrations in plasma are associated with the amplification of catabolic processes. Prolonged elevated NEFA concentrations may, in turn, contribute to decreased insulin sensitivity of peripheral tissues (Pires et al., 2007).

The homorone IGF-1 is produced primarily by the liver in response to GH and the relationship between both hormones forms the basis of the GH-IGF-1-axis (Butler et al., 2003). A key metabolic signal in coupling the GH-IGF-1-axis represents the hormone insulin. Hypoinsulinaemia in early lactation is linked to the uncoupling of the GH-IGF-1-axis, which results in reduced secretion of IGF-1 (Butler et al., 2003). Already 2 wk before calving, the decline in the concentration of circulating IGF-1 is accompanied by a reduced plasma insulin concentration. In parallel, changes in plasma GH are opposed to those of IGF-I and insulin (Bell et al., 2000), thus stressing the catabolic, i.e., lipolytic function of GH on adipose tissue (Houseknecht et al., 1995). Besides the function of IGF-1 in energy homeostasis, it is also associated with the stimulation of mammary gland cell proliferation (Collier et al., 1993; Neville et al., 2002).

The adipocytokine leptin is secreted primarily by adipocytes and the circulating concentrations are proportional to adipose tissue mass (Ahima and Flier, 2000). The production of leptin is mainly regulated by insulin-induced changes in adipocyte metabolism and plays an essential role in regulating energy intake, storage, and release (Havel, 2002; Liefers et al., 2003; Chilliard et al., 2005). The plasma concentration of leptin drops close to parturition and remains depressed in early lactation (Sadri et al., 2011). Leptin is also associated with feed intake, body weight (**BW**), BCS, and adipocyte size (Locher et al., 2015). Further, it has been described that the plasma concentrations of leptin are positively correlated with those of insulin and glucose, and negatively correlated with GH and NEFA (Block et al., 2001).

In recent decades, studies have shown that the increased occurrence of production diseases (e.g., ketosis, fatty liver syndrome, hypocalcemia, metritis, displaced abomasum) in early lactation is related to the genetically driven demands of the metabolically prioritized mammary gland (Drackley, 1999; Duffield et al., 2009; LeBlanc, 2010). High milk yields infer high energy outputs with a corresponding metabolic load and thus require a remarkable degree of adaptation to maintain high milk production and stable health (Goff and Horst, 1997). The ability to adapt to NEB varies between individual animals (Kessel et al., 2008; van Dorland et al., 2009), an observation that has been termed “metabolic flexibility” in the review by Gross and Bruckmaier (2019). As a result of inadequate adaption, metabolic stress can occur with a negative impact on the immune system, fertility, milk yield, product quality, and overall well-being of the dairy cow (Bobe et al., 2004; LeBlanc, 2008; Esposito et al., 2014).

The analysis of blood parameters (NEFA, BHB, and glucose) is considered to be the standard for examining the metabolic condition and health status of the cow (Oetzel, 2004). Hypoglycemia is diagnosed when the blood glucose concentration drops below a pre-defined threshold of 2.2 or 2.5 mmol/L (Macmillan et al., 2017; Dubuc and Buczinski, 2018). High concentrations of circulating NEFA (> 400 µmol/L) are an indicator of intensive adipose tissue mobilization (LeBlanc, 2010) and hyperketonemia is diagnosed, when the BHB concentration in the blood rises above a threshold of 1.2 or 1.4 mmol/L (Oetzel, 2004; Dubuc and Buczinski, 2018; Raboisson et al., 2014).

Clinical signs of ketosis include decreased feed intake, reduced milk production, loss of BW, and may translate into impaired fertility (Bareille et al., 2003; Ospina et al., 2010). Ketosis is known to be associated with the occurrence of diseases such as fatty liver syndrome, displaced abomasum, retained placenta, metritis, mastitis, and laminitis (McArt et al., 2014; Suthar et al., 2013). This, in turn, elevates veterinary and treatment costs (Steeneveld et al., 2020; Cainzos et al., 2022) and enhances the risk of culling (Raboisson et al., 2014; Horváth et al., 2017).

1.2 Short digression: Potential factors involved in the regulation of milk production

The regulatory mechanisms of milk production were not part of the research question addressed in this thesis. Nevertheless, a short overview of this topic is provided. A comprehensive review regarding the regulation of the mammary gland was published by Weaver and Hernandez (2016).

Milk production is a continuous process. Within the first 6 to 8 h after milking (Davis et al., 1999), the intra-alveolar pressure increases to such an extent that capillary forces are exceeded, and the stapled milk flows from the alveoli via the milk ducts into the cistern. The milk production rate is relatively constant 12 h post milking. In the subsequent period from 12 to 16-18 h after milking, the production rate decreases slightly; thereafter, the production rate decreases more rapidly (Wheelock et al., 1966; Delamaire and Guinard-Flament, 2006; Stelwagen et al., 2008).

Besides a reduction in milk production rate, extended milking intervals are also associated with a drop in mammary blood flow (Delamaire and Guinard-Flament, 2006; Guinard-Flament et al., 2006; Guinard-Flament et al., 2007) and the loss of mammary barrier integrity resulting in the transfer of molecules from the blood to milk, and vice versa (Stelwagen, 2001; Guinard-Flament et al., 2011). The initiation of the loss of mammary barrier integrity seems to be influenced by the milking interval length (Stelwagen and Singh, 2014) and the degree of udder filling (Albaaj et al., 2018).

The milking stimulus leads to the release of hormones such as oxytocin, prolactin (**PRL**), and glucocorticoids into the bloodstream (Bruckmaier and Blum, 1998; Lacasse and Ollier, 2015; Ponchon et al., 2017). The importance of PRL in dairy ruminants was summarized in a review by Lacasse et al. (2016). They described that a “body of evidence” was generated for the galactopoietic role of PRL in dairy cows even though its importance was not acknowledged previously since bromocriptine, a dopamine receptor agonist that inhibits PRL release and galactopoesis in other (non-dairy) species, could not inhibit lactation in cows. However, a more recent dopamine agonist, quinagolide reduced milk production in dairy cows (Lacasse et al., 2016; Vanacker et al., 2017). The basal concentration of PRL in dairy cows is not clearly related to the level of milk production. However, PRL release caused by the milking process is correlated with the level of milk production (Lacasse et al., 2016).

As summarized in the review by Weaver and Hernandez (2016), besides milking management-related factors, autocrine and paracrine mechanisms at the level of the mammary epithelial cells appear to be linked to milk production regulation. Bioactive factors such as parathyroid hormone-related protein, various growth factors (transforming growth factor, IGF-1, epidermal growth factor), and serotonin are potential candidates for being involved in the regulatory mechanisms (Weaver and Hernandez, 2016). These potential candidates are synthesized in epithelial cells and also act upon them via receptors thus affecting growth, calcium homeostasis, and milk composition (Weaver and Hernandez, 2016). The underlying systems are still based on a combination of in vivo and in vitro experiments in different species, including ruminants (Weaver and Hernandez, 2016). However, the complexity of the system cannot yet be fully understood and described as a coherent system.

1.3 Opportunities of milking strategies as part of energy management in early lactation

To mitigate the NEB and thus the risk for health disturbances in early lactation, several approaches addressing the main variables in the equation to calculate the energy balance (**EB**) have been suggested. As far as nutritional intervention around parturition is concerned, several supportive options have been summarized (e.g., Overton and Waldron, 2004; Jouany, 2006; Esposito et al., 2014). The aim is to prevent a drastic decline in voluntary feed intake and ensure optimal rumen function and metabolic adaptions in glucose, fatty acid, and mineral metabolism to support pregnancy and lactation and avoid metabolic disorders after calving (Overton and Waldron, 2004; Jouany, 2006). These supportive options include a far-off and close-up nutritional scheme during the dry period to minimize overfeeding nutrients until 3 or 2 wk prepartum, followed by an increased nutrient supply to facilitate the metabolic adaptions and adaption of the rumen ecosystem to lactation (Overton and Waldron, 2004; Jouany, 2006;). The energy intake can be modified to some extent by dietary measures, e.g., by providing energy-dense and adequately structured diets (e.g., carbohydrate formulation of the diet; to some extent, added fat in diets). Supplements which support rumen stability, supply additional energy, and (or) support and improve intermediary metabolism (e.g., supplementation with glucogenic precursors such as propylene glycol; methyl donors such as choline, methionine, and lysine; specific fatty acids such as *trans*-10, *cis*-12 conjugated linoleic acid to induce a milk fat depression) can help mitigate the NEB (Overton and Waldron, 2004; Jouany, 2006). However, the occurrence of subacute inflammation in various peripheral organs, such as the uterus and mammary gland, as a response to tissue remodeling around calving cannot be overcome by nutritional intervention (Bradford et al., 2015; Kuhla, 2020). Tissue remodeling is accompanied by tissue damage;

both allow the entrance of microorganisms into these organs and trigger the response of the innate immune system (Kuhla, 2020). Subacute inflammation is associated with increased circulating pro-inflammatory cytokine levels; these cytokines (interleukin-1β (**IL-1β**), interleukin-6 (**IL6**), and tumor necrosis factor-α (**TNF-α**)) can cross the blood-brain barrier and are sensed by neurons located in the hypothalamus where they signal reducing feed intake (Ingvartsen and Andersen, 2000; Drackley et al., 2005; Kuhla, 2020).

The energy output via milk represents the other variable in the EB equation. Several approaches for temporarily reducing the energy output via the milk and therefore improving the cow's energy status were suggested: Inhibiting the secretion of the galactopoetic hormone PRL by administering dopamine agonists (e.g., quinagolide) (Lacasse et al., 2019). Proposed milking strategies for limiting milk production include omitting or shortening the dry period (Kok et al., 2019), thus lowering the peak milk yield, reducing the milking frequency to once daily (Stelwagen et al., 2013), or IM at each milking (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). The latter was suggested as the most promising approach to reduce metabolic stress without compromising the productivity of high-yielding dairy cows (Lacasse et al., 2018).

1.3.1 Prolactin inhibitors

The use of pharmaceuticals, such as dopamine agonists (e.g., quinagolide) that inhibit PRL release and therefore reduce milk secretion has been studied at drying-off (Ollier et al., 2015), early lactation (Vanacker et al., 2017), mid-lactation (Ollier et al., 2016), and the potential as a management tool in dairy husbandry has been reviewed by Lacasse et al. (2019).

The use of quinagolide in early lactation to reduce milk production in the first wk and to investigate if this treatment had a positive effect on energy metabolism and the immune system were the objectives of the study by Vanacker et al. (2017). Quinagolide was injected (2 mg; *i.m.*) in treatment cows (**TRT**; n = 11) for 4 days. The control group (**CON**; n = 11) received an injection of water. The first injection was given after calving, followed by 7 further injections every 12 h. In the TRT group, the milk production was lower from days in milk (**DIM**) 2 to 6, and no carry-over effect was observed afterward. The percentages of milk fat, protein, or lactose were not different between groups on DIM 5. Any differences in milk component contents were observed in the subsequent observation period (lactation wk 2 to 4), except for a trend towards greater protein contents in the TRT group. The treatment reduced metabolic stress and lowered dry matter intake (**DMI**) in the first lactation wk; the blood glucose and calcium concentrations were higher, and blood BHB concentration was lower in the TRT group. No differences occurred in the subsequent period. The NEFA, urea, and phosphorus concentrations in blood were not different between groups over the whole observation period (lactation wk 1 to 4). In addition, some aspects of the immune system were improved during the treatment period, e.g., the enhanced proportion of cells that entered oxidative burst in vitro and higher mitogen-induced proliferation of peripheral blood mononuclear cells incubated with serum from the TRT group (Vanacker et al., 2017).

From the study, it could be concluded that the injection of quinagolide in the first days after calving reduced milk production and enhanced the metabolic status of dairy cows without causing long-term milk yield reduction beyond the treatment period (Vanacker et al., 2017). Although the use of a quinagolide to mitigate the energy deficit in early lactation is promising (Lacasse et al., 2018), its use is not permitted in Europe. The cabergoline-based product Velactis was withdrawn from the Europe market in 2016 due to occurring symptoms similar to those of periparturient hypocalcemia in some cows (Lacasse et al., 2019).

1.3.2 Modifications of the dry period

The idea behind omitting or shortening (28 to 35 d) the dry period as an energy management strategy in dairy cows is to reduce postpartum milk production and, in terms of an omitted dry period, to postpone the onset of lactation to the period before calving when the cow can still meet the energy demand through feed intake (e.g., van Knegsel et al., 2013; Kok et al., 2019). These modifications of the dry period are associated with decreased postpartum milk production (Table 1) and increased milk protein content (Van Knegsel et al., 2013; Kok et al., 2019). The improvement of the energy status was reflected in the less NEB postpartum (Table 1) and, in terms of cows with an omitted dry period, in higher BW and BCS at the end of lactation (Kok et al., 2019). Moreover, both strategies are associated with decreased postpartum NEFA and BHB plasma concentrations, although the findings vary between studies. So far, elevated postpartum plasma glucose and insulin concentration were only reported after omitting the dry period (Kok et al., 2019).

Table 1. Kok et al. (2019) provided an overview of results from studies regarding the energy balance (EB, MJ NE_L/d) and the milk yield (kg/day) of multiparous (parity > 2) dairy cows in early lactation after a standard (55 to 60 days), short (28 to 35 days) or no (0 days) dry period (modified according to Kok et al. (2019)).

Study	n	Weeks after calving	Energy balance (MJ NEL/d) Dry period			Milk yield (kg/day) Dry period		
			Standard	Short	No	Standard	Short	No
Andrée O'Hara et al. (2018)	69	2 to 12	-8.8	10.9				
Chen et al. (2016b)	130	1 to 9	-36.4	-31.8	-28.9			
De Feu et al. (2009)	36	1 to 4	-13.7	11.5				
De Feu et al. (2009)	36	5 to 12	5.3	17.1				
Rastani et al. (2005)	65	1 to 10	-29.0	-17.0	2.9	43.4	39.9	36.3
Van Hoeij et al. (2017a)	123	1 to 3		-30.2	-8.1			
Van Hoeij et al. (2017a)[2]	123	4 to 7		-14.0	7.2 / -0.3			
Van Knegsel et al.[1] (2014)	167	1 to 14	-17.3	-7.7	5.3	44.1	39.2	34.8
Annen et al. (2004)[3]	69					47.7	46.6	43.4
Pezeshki et al. (2007)	71					35.6	36.7	
Santschi et al. (2011)	850					32.7	.32.7	

n = number of cows in the study.
[1] Studies are based on the same experimetn, where cows were subjected to a standard, short or no dry period for two subsequent lactations. Van Knegsel et al. (2014) report on the first lactation; Chen et al. (2016) report on the second lactation.
[2] Cows with no dry period were either fed a standard or a reduced level of concentrates (standard/reduced group).
[3] Cows were supplemented with bovine somatotropin (bST).

The impact of both strategies on feed intake is ambiguous; the feed intake increased or was not different in the early lactation of cows with a shortened or omitted dry period (Kok et al., 2019). Depending on the study, the somatic cell count (**SCC**) increased or was not different in cows with a modified compared to a standard dry period. No elevated incidence of clinical mastitis in the following lactation was observed in cows with a modified dry period (Kok et al., 2019). According to Kok et al. (2019), investigations concerning the behavior of cows with an modified dry period are limited to one study, in which a prolonged lying time before and after calving could only be found in cows with an omitted dry period (Kok et al., 2017).

To sum up, modifying the dry period improves the energy status of dairy cows postpartum, without compromising udder health and well-being (van Knegsel et al., 2013; Kok et al., 2019). However, these benefits are limited because both strategies are associated with a lack of recovery phase to regenerate the mammary gland parenchyma (Capuco et al., 1997; Capuco et al., 2003) and economic losses due to lower potential to achieve high milk yield in the subsequent lactation (Kok et al., 2019).

1.3.3 Reduction of the milking frequency

Reducing the milking frequency from twice-daily milking to ODM represents a postpartum milking strategy that allows for the regeneration of the mammary gland parenchyma through a dry period before calving but limits the energy output via extending the milking interval to 24 h in early lactation. Performing ODM for 1 to 7 wk in early lactation results in lower milk production (Table 2). Adverse carry-over effects were found beyond treatment but not in the next full lactation (Stelwagen et al., 2013; Lacasse et al., 2018). Concerning milk composition, elevated milk fat and protein concentrations and decreased lactose concentrations were observed in ODM cows (Stelwagen et al., 2013). The energy status could be improved as reflected through less BW and BCS losses, greater plasma glucose concentrations, and decreased plasma NEFA concentrations in ODM compared to twice-daily milked cows. However, the impact of ODM on BHB concentrations is ambiguous (Table 2) (Stelwagen et al., 2013; Lacasse et al., 2018).

Table 2. Lacasse et al. (2018) summarized the results of studies using once-daily milking (ODM) for several weeks beginning at calving for milk yield, energy balance (EB), body weight (BW), body condition score (BCS), and concentrations of blood glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHB) in dairy cows (Lacasse et al., 2018).

Study	Duration of 1x (weeks)	Reduction in milk yield (kg/d) during once-daily milking*	Effect on energy balance #			Effect on blood metabolites #		
			EB	BW	BCS	Glucose	NEFA	BHB
Rémond et al. (1999)	3	6.1 (20%)	+ +	NS	NS	+	-	NS
	6	11.3 (34%)	+ +	+ +	+	+	-	NS
Rémond et al. (2002)	3	7.0 (28%)	+ +	+ +	+ +	ND	ND	ND
McNamara et al. (2008)	4	7.8 (24%)	+ +	+	+ +	+ +	-	-
Schlamberger et al. (2010)	4	12.0 (38%)	ND	ND	+ +	+ +	- -	- -
O'Driscoll et al. (2012)	7	3.7 (17%)	ND	+ +	ND	ND	ND	ND
Phyn et al. (2014)	3	3.0 (14%)	ND	+	+	+ +	- -	ND
	6	4.5 (19%)	ND	+ +	+ +	+ +	- -	ND
Loiselle et al. (2019)	1	9.2 (31%)	ND	+ +	NS	+ +	- -	- -

* Relative to twice-daily milking.
# Relative to twice-daily milking: ++ = greater ($P < 0.05$), + = greater ($P < 0.1$), NS = not significantly different, − = lower ($P < 0.1$),− − = lower ($P < 0.05$), ND = not determined.

Limited information exists regarding the link between feed intake and milking frequency. The ODM is often applied in dairying systems based on pasture grazing, whereby the accurate recording of feed intake is difficult (Stelwagen et al., 2013). So far, studies with accurate feed intake recordings have reported reduced feed intake in ODM cows or no differences compared to twice-daily milked cows (Stelwagen et al., 2013). Udder health was not affected in terms of infection risk, but SCC increased in ODM cows. Stelwagen et al. (2013) suggested that a possible reason could be a mild inflammatory response which would be reflected both in an increase in polymorphonuclear leukocytes in the milk as well as in the activation of the innate immune system. In terms of well-being, no negative impact of ODM was observed on indicators for well-being, such as deviating walking stride, milk leakage, kicking behavior in the parlor, and reduced lying time (Stelwagen et al., 2013).

Reducing the milking frequency in early lactation is a strategy to limit postpartum milk production and therefore reduce NEB in early lactation. The negative impact of ODM on milk production is associated with economic losses; however, labor productivity can be improved due to reduced milking time (Stelwagen et al., 2013; Davis et al., 1999). Moreover, Lacasse et al. (2018) suggested that ODM could be a helpful strategy for pasture-based dairy farms where high-producing cows cannot be fed a high-concentrate diet.

1.3.4 Incomplete milking

On commercial farms, milking clusters are automatically removed once milk flow (kg/min) decreases below a defined threshold. After cluster removal, a certain amount of milk remains in the mammary gland. The amount of milk left behind depends on the threshold setting for cluster removal, which varies between manufacturers and ranges from 200 to 800 g/min (Cording et al., 2013). Already Woodward et al. (1936) observed that increasing degrees of milk remaining in the mammary gland are associated with diminished milk production. Moreover, Schmidt et al. (1964) and Wheelock et al. (1965) first mentioned that the combination of IM with a prolonged duration of milk stasis reinforced the restraining effect on milk production and persisted even after turning back to conventional milking routine when IM was repeated over several days (Schmidt et al., 1964). In the last two decades, the option of IM has received renewed attention and was considered for the first time as milking strategy to delay the increase in milk yield and limit energy loss in early lactation. The approach consists of a change in the degree of milk removal while keeping milking frequency, therefore maintaining the milking stimulus and, thus, the release of the related hormones (e.g., PRL).

An overview of IM studies conducted over the last two decades and the corresponding treatment designs are summarized in Table 3. In the further course, the parameters relevant to this study are described in the following order: (1) milk yield (including the basic principles of the study design), (2) milk composition, (3) udder health, (4) energy status, and (5) well-being.

(1) Milk yield

Carbonneau et al. (2012) were the first who investigated the approach of IM to migate the NEB in the early lactation of dairy cows. The impact of IM over 5 days in the colostrum phase on metabolic status, immune function, and subsequent performance was examined (Table 3). In total, 47 cows were allocated to three treatment groups. The CON group was milked twice a day. In the IM group, 6 to 14 L milk/d were harvested from DIM 1 to 5, with a daily increase of 2 L. The third group was left to nurse their calves until DIM 5, and ODM was conducted from DIM 3 to 5. From DIM 6 to 61, all cows were milked twice daily to study long-term effects on productivity and metabolic status. Over the entire IM treatment period, about 35.5% and 61.2% of milk of the CON group (27.3 ± 1.46 kg/d) were collected in the IM and nursing group, respectively. On a daily level, the amount of milk collected in the IM group was about 43, 35, 34, 38, and 36% of the control group on DIM 1 to 5. No carry-over effect of each treatment on milk production and energy-corrected milk (**ECM**) could be observed between wk 2 and 9, therefore the authors concluded that a short-term reduction of milk removal while maintaining the milking stimulus may be applied without compromising the subsequent milk performance (Carbonneau et al., 2012).

To transfer the IM concept of Carbonneau et al. (2012) to commercial farms (n = 13; n > 800 cows), Morin et al. (2018) conducted a randomized controlled trial from which several substudies emerged (Krug et al., 2018a; Krug et al., 2018b; Krug et al., 2018c) (Table 3). The substudy by Krug et al. (2018c) aimed to investigate the effect of IM in the colostrum phase on culling hazard and subsequent milk production and composition. Cows (n = 846) were randomly assigned to either a CON or TRT group. The IM protocol for the TRT group was adapted to individual milking management on the farm and deviated slightly from the one by Carbonneau et al. (2012). A total of 10, 12, and 14 L/d of milk was collected on DIM 1-3, 4, and 5, respectively. Daily milk production was recorded automatically via integrated software of the parlor or automatic milking system, manually once a wk by the producer, or monthly from records of the Dairy Herd Improvement Association (**DHIA**). Cows were followed until the end of lactation or culling. At DIM 1, the amount of milk that could be collected was limited by the actual amount of milk produced, as the CON group produced an average of only 6 ± 3.3 kg. In contrast, on DIM 2, 3, 4, and 5, the amount of milk obtained from TRT cows was about

48, 39, 42, and 47% of the control cows. From wk 2 to 44, no differences in milk weight and culling hazard were found between groups (Krug et al., 2018c). The ECM was lowered during wk 38 in the TRT group. No other differences were observed; thus, the authors concluded that IM in the colostrum phase has negligible effects on subsequent productivity (Krug et al., 2018c).

Furthermore, the study of Valldecabres et al. (2022) confirmed the uncritical use of the IM concept in the colostrum phase. There, IM was performed for 48 h on DIM 1 and 2. Cows (n = 90) were allocated to four groups: CON (12 h milking interval), ODM (24 h milking interval), TRT (incomplete milk removal; 3 L milk withdrawal at each milking; 12 h milking interval), and delayed milking (extended milking interval of 24 h followed by 2x milking with a 12 h milking interval). The respective milking strategy was conducted on DIM 1 to 2, and from DIM 3 onwards, all cows were milked twice daily. Besides the recording of milk yield after each milking (DIM 1 to 2), milk samples were collected in order to determine the SCC and the concentration of calcium and immunoglobulin G (**IgG**) in colostrum and transition milk. Conclusions about possible carry-over effects of treatments on performance were derived from records from the first month of DHIA testing.
Within 48 h after enrollment, milk yield was lowered to 38.1% (TRT), 59.3% (ODM), and 75.2% (delayed milking group) compared to the CON group (30.7 ± 1.5 kg) accompanied by lowered milk Ca concentration in all treatment groups on DIM 1 while exclusively persisting until DIM 2 in the TRT group. While no differences in IgG concentrations were found between the groups, TRT and ODM in the first milking, higher concentrations of IgG were detected in the milk from the TRT group in the following three milkings which might indicate an accumulation effect due to IM (Valldecabres et al., 2022). Results from the first month of DHIA testing did not indicate differences in milk yield and ECM between all groups. Regarding the concept of IM, Valldecabres et al. (2022) concluded that the milk Ca concentration can be affected through limited milk withdrawal without adversely affecting colostrum quality and subsequent productivity.

In order to investigate the cow's adaptation to increasing amounts of milk remaining in the mammary gland, the study by Albaaj et al. (2018) performed IM for a single milking interval. The impact of the repetition of IM was not considered; otherwise, as described by the authors, it would be difficult to differentiate the effect of the individual factor from another (Albaaj et al., 2018). The experimental design consisted of four different groups or rather of different degrees of milk withdrawal (100, 70, 40 and 0 %) conducted on a single morning milking event

(M0) at DIM 55 ± 9. Milk yield, composition, and SCC were analyzed at the following 7 milking events designated M1 to M7. Within the M0 + M1 milking interval, the milk production was decreased in a negative curvilinear manner, and the downregulation was proportional to the degree of IM (Albaaj et al., 2018). When 0, 40, or 70% of the expected milk yield were harvested, the milk production at M0 + M1 was approximately 30.7%, 12.7%, and 3.1% reduced compared to the 100% group (41.7 ± 1.26 kg/d). Negative carry-over effects after treatment in comparison to the 100% group (37.0 kg/d) only occurred for the 0 (-2.7 kg/d) and 40% (-2.0 kg/d) groups. Differences were leveled up after three subsequent milkings, indicating that the cow's productivity can recover after IM (Albaaj et al., 2018). In addition, Albaaj et al. (2018) initially combined the measurements of udder morphology with indicators for tight junction integrity to reflect the degree of udder filling. This section summarizes the findings, as the authors associated these parameters with milk production rather than well-being. The udder distension was determined 1 and 9 h after M0 by measuring the total teat end distance of the udder and increased in the M0 + M1 interval but to decreasing degrees for all treatments (Albaaj et al., 2018). The cisternal area as an indicator of cisternal capacity was assessed via four scans of the right rear quarter via ultrasonography. For the 0 and 40% treatments, within 1 h after the treatment, the cisternal area increased and exhibited no further increase after 9 h. According to Albaaj et al. (2018), the distension of the cistern at this stage was close to the maximum. Meanwhile, the lactose concentration in blood plasma was increased at 10 h and 4 h after M0 milking in the 0 and 40% group, indicating an earlier beginning of the mammary barrier integrity loss with an increasing amount of milk left in the udder (Albaaj et al., 2018). As negative carry-over effects on milk production only occurred when plasma lactose concentrations were elevated, the authors assumed a relationship between both factors (Albaaj et al., 2018). Altogether, Albaaj et al. (2018) concluded that the results further confirm the adverse effects of increasing milk accumulation on milk production; thereby, the combination with a prolonged duration of milk stasis appeared to have a more pronounced impact on milk secretion and possible carry-over effects than a sole factor.

The additional factor “repetition of IM” was included in the study conducted by Penry et al. (2017). The study investigated the effect of a long-term IM (DIM 5 to 47) on milk production rate (kg/h) and milk composition at the udder quarter level. A half-udder model was applied, wherein one udder half was milked completely (CON), while in the other half, approximately 30% of the total milk remained in the udder (TRT). All cows (n = 12) were milked twice daily. The milk yield ratio between the TRT and CON quarters on the first day after enrollment was determined to estimate the milk production per quarter and calculate the target amount of milk

(30%) remaining in the udder (Penry et al., 2017). Milk production was recorded daily, and milk composition and SCC were analyzed at the quarter level twice a wk. The target milk yield was recalibrated after each wk. On recalibration day, all quarters were milked completely to set the milk yield in the IM quarters. Across the entire IM period, 23% instead of the targeted 30% of milk remained in the TRT udder compared to the CON udder half. During IM, the average milk production rate (kg/h) in the TRT udder half was about 25% lower compared to the CON udder half (0.97 kg/h), which in turn, further supports the current comprehension of the connection between milk stasis and regulation of milk production. Moreover, the regulation of milk production occurs rather on a local than a systemic level, as the milk production of the CON udder half was not affected (Penry et al., 2017). No conclusions about possible adverse carry-over effects can be derived from the study, given that the development of milk production after IM was not reported.

As Kuehnl et al. (2019) assumed that an increase in milking frequency would partially compensate the reduced milk production due to IM, an additional variation in the factor “milking frequency” (2x vs. 3x) was implemented in the study design. Besides that, the experimental design was carried out according to Penry et al. (2017) (Table 3). While milk production at the quarter level was recorded daily, milk composition and SCC were analyzed twice a wk, and the recalibration of target milk yield took place at the end of each wk.
From DIM 5 to 47, the milk production rate (kg/h) in 2x CON half udder tended to be reduced by 8% compared to 3x CON half udder (1.97 ± 0.06 kg milk/h). The milk production rate of the TRT udder half was about 27% reduced compared to the CON udder half (1.09 ± 0.03 kg/h), supporting the assumption by Penry et al. (2017) of an existing connection between milk stasis and the regulation of milk production. Given that no interaction was detected between milking frequency and IM for milk production rate, Kuehnl et al. (2019) presumed that an increased milking frequency could not prevent the milk yield loss through IM. Productivity after IM was not recorded in the study of Penry et al. (2017) and Kuehnl et al. (2019), resulting in a lack of evidence for sustained effects on productivity after IM.

Recently, Deacon et al. (2023) investigated the mechanism underlying the modulation of milk production by IM in mid-lactation cows, intending to determine how IM affects mammary epithelial cell activity, cell number, and cell responsiveness to blood PRL. Accordingly, they were the first who examined the expression of genes and proteins related to mammary gland activity, PRL signaling, and apoptosis in milk and mammary gland biopsies from IM cows (Deacon et al., 2023). A half-udder model was applied for 4 wk in 8 mid-lactating cows (DIM

174 ± 45). All cows were milked twice daily, whereby the left udder half was milked completely (CON). In the right half, approximately 30% of the total milk remained in the udder (TRT), including a recalibration at the end of the second treatment wk. In the wk before and 12 days after treatment, the volume of milk collected after complete filling (26 h milk interval and i.m. injection of oxytocin) of the udder mammary gland was measured to determine the functional capacity of the mammary gland. Milk yield was measured daily at the quarter level. Milk samples were collected weekly to analyze milk composition and epithelial cell concentration and isolate milk fat globule RNA to determine the expression of genes related to mammary activity, PRL signaling, and apoptosis. At the end of the fourth wk after or rather the end of the treatment, mammary gland biopsies were taken from each rear quarter to determine the abundance and activation levels of proteins related to PRL signaling (Table 3) (Deacon et al., 2023). In the TRT half-udder, the milk production was 66% of the CON half-udder at the beginning of IM treatment and 53% at the end of IM treatment. The milk production in the CON half-udder remained constant over the entire treatment period. In milk fat, the mRNA abundance of genes related to milk synthesis (**CSN2** = β-casein; **LALBA** = α-lactalbumin; **ACACA** = acetyl-co-enzyme A carboxylase α) was lower in TRT udder halves. The total yield of epithelial cells in milk was not different between udder halves, but the concentration was higher in the TRT udder halves. Moreover, the mRNA abundance of genes involved in apoptosis, the B-cell lymphoma protein 2 (**BCL2**) to B-cell lymphoma protein 2 associate x (**BAX**) ratio, and the loss of mammary functional capacity (-2.61 ± 0.40 kg) were greater in the TRT udder half. Deacon et al. (2023) suggested an acceleration of involution in TRT udder halves, which further elucidates the association between IM-related decrease in milk production with a decrease in mammary cell activity and the number of secretory cells. Regarding the expression of genes involved in PRL signaling, in milk fat of the TRT udder halves the mRNA abundance of the short isoform of the PRL receptor gene tended to be lower, and of signal transducer and activator of transcription 5A und 5B (**STAT5A** and **STAT5B**) tended to decline in comparison to the CON udder halves. In the mammary biopsies, neither the PRL receptors nor the STAT protein and activation level were affected by IM. Only the ratio of the short isoform to long isoform of PRL receptor was lower in TRT udder halves and therefore confining the support of the assumption that mammary gland responsiveness to the PRL galactopoietic signal is modulated in response to IM (Deacon et al., 2023).

To sum up, performing IM over a single milking event (Albaaj et al., 2018) or a more extended period (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023) decreased milk production. In particular, the degree of milk accumulation appears to compromise milk production and to be reinforced by the milking interval and treatment length (Albaaj et al., 2018; Deacon et al., 2023); however, increasing the milking frequency seems insufficient to prevent the production loss through IM (Kuehnl et al., 2019). Albaaj et al. (2018) performed indirect methods to measure udder distension and tight junction integrity to elucidate the adaptation of the mammary gland to increasing degrees of IM. Factors related to accelerated involution of the mammary gland were only investigated in mid-lactating cows by Deacon et al. (2023). So far, no (Carbonneau et al., 2012; Valldecabres et al., 2022) or only negligible (Albaaj et al., 2018; Krug et al., 2018c) carry-over effects on subsequent milk yield development have been reported in early lactating cows. In mid-lactating cows, a persistent reduction in the functional capacity of the mammary gland has been observed after IM (Deacon et al., 2023). In order to investigate to what extent a longer IM treatment period in early lactation affects subsequent productivity, more information regarding the development of milk production after IM is necessary.

(2) Milk composition

The results investigating the effect of IM on milk composition are ambiguous between studies (Table 3), and deviating from the assumption by Wheelock et al. (1965) that changes in milk composition during incomplete milking are similar to those observed during extended milking intervals.

In studies from the past two decades, milk composition was analyzed either at each milking event (Albaaj et al., 2018), once a wk (Carbonneau et al., 2012; Deacon et al., 2023), twice a wk (Penry et al., 2017; Kuehnl et al., 2019), or monthly (Krug et al., 2018c; Morin et al., 2018). Milk samples were collected on udder (Carbonneau et al., 2012; Albaaj et al., 2018; Morin et al., 2018; Krug et al., 2018c) or quarter level (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023).

During the IM phase, a decrease (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023) or no change (Carbonneau et al., 2012) in lactose concentration in the TRT group/half-udder due to IM could be observed. In the study by Krug et al. (2018a), the development of milk lactose concentration was not analyzed. In the TRT group/half-udder, an increase (Kuehln et al., 2019), decrease (Carbonneau et al., 2012), or no change (Penry et al., 2017; Deacon et al., 2023) in milk fat concentration was reported. The milk protein concentration was not affected (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023) or increased (Carbonneau et al., 2012) between groups/half-udders. In addition, no interaction between the combined effect of IM and an increase in the milking frequency on milk composition was found by Kuehnl et al. (2019).

In the phase after IM, Carbonneau et al. (2012) observed that the milk fat concentration was higher in wk 2 to 3 and tended to be higher in wk 2 to 9 in the CON than in the TRT group. Only in wk 2 the milk protein concentration was higher in the TRT than in the CON group. The subsequent lactose concentration was not affected by IM (Carbonneau et al., 2012). In contrast, Krug et al. (2018a) could not find a difference between groups what concerns milk protein and fat concentration between wk 2 to 44. After conducting IM for a single milking event, Albaaj et al. (2018) observed decreased milk protein and lactose concentrations and increased fat concentration in the 40 and 0% group compared to the 100% group, whereby differences leveled up after 2 to 3 consecutive milkings.

Most studies have not tackled the causes of differences in the development of milk composition between studies. The development of lactose concentration was mainly discussed and related to the loss of tight junction integrity of the mammary gland (Penry et al., 2017; Kuehln et al., 2019; Deacon et al., 2023). Kuehnl et al. (2019) attempted to explain the different findings compared to the study of Penry et al. (2017) with a different sampling procedure, as Kuehnl et al. (2019) only analyzed samples from the cistern milk fraction. However, it remains unclear to what extent the diverse IM treatment design or sampling frequency may have affected the results.

(3) Udder health

Rising accumulation of milk in the udder may provide a growth medium for potentially harmful pathogens and could therefore impede udder health (Rovai et al., 2007). To control udder health, the SCC in milk during (Carbonneau et al., 2012; Penry et al., 2017; Kuehnl et al., 2019; Valldecabres et al., 2022) and after conducting IM (Carbonneau et al., 2012; Krug et al., 2018b; Albaaj et al., 2018; Valldecabres et al., 2022) was analyzed (Table 3).

In studies performing IM over several days or wk, no differences (Carbonneau et al., 2012; Valldecabres et al., 2022) or a slight increase (Penry et al., 2017; Kuehnl et al., 2019) of SCC in milk of TRT cows was observed. Thereby, the extent of the increase in SCC was relatively marginal; for instance, Penry et al. (2017) reported an increase from 26,3000 cells/mL in the CON udder half to 48,300 cells/mL in the TRT udder half.

After IM for several days, no change in SCC between groups could be detected (Valldecabres et al., 2022), while an increase of SCC in the CON group was reported by Carbonneau et al., (2012). After one single IM event, Albaaj et al. (2018) observed an increased SCC in the following five milkings for the 0 and 40% milk removal treatments. No reaction was observed for the 70% milk removal treatment; therefore, the authors presumed that triggering an inflammatory response might be related to the amount of milk remaining in the udder (Albaaj et al., 2018).

In another substudy (Krug et al., 2018b) of the already mentioned randomized controlled trial (10 to 14 L milk/d from DIM 1 to 5) (Table 3), at approximately DIM 11 and 18, the SCC in milk was measured at the quarter level to investigate the effect of IM in the colostrum period on the subsequent risk of mastitis in dairy cows (n = 878). The threshold for SSC indicating intramammary infection in quarters was set at ≥200,000 cells/mL and without intramammary infection at <100,000 cells/mL (Krug et al., 2018b). Quarters of the initial samples (DIM 11) were tested again 1 wk later (DIM 18) to calculate intramammary infection incidence and elimination rate. In addition, the producers recorded clinical mastitis events. Krug et al. (2018b) reported that applying IM during the colostrum phase (DIM 1 to 5) increased the odds of a decrease in SCC from 11 to 18 DIM but did not affect the odds of an increase in SCC in the same period. Within the first 90 DIM, the IM treatment did not affect clinical mastitis incidence in dairy cows (Krug et al., 2018b), which supports the assumption that performing IM for several days or wk does not lead to compromised udder health.

To conclude, the implementation of IM for several days (Carbonneau et al., 2012), wk (Penry et al., 2017; Kuehnl et al., 2019), or one single milking interval (Albaaj et al., 2018) caused no or only a minor increase in SCC. Penry et al. (2017) assumed that the differences in SCC between groups could be due to the increased dilution of somatic cells in the CON udder halves. In contrast, Albaaj et al. (2018) presumed a mild inflammatory reaction in response to IM. No information was provided regarding the bacterial status of the udder of enrolled cows, which would have been helpful for a better interpretation. Moreover, IM in the colostrum phase has not been associated with impairment of udder health in the subsequent observation period (Carbonneau et al., 2012; Krug et al., 2018b; Valldecabres et al., 2022). Whether an IM treatment lasting for several wk affects the subsequent udder health has not yet been investigated.

(4) Energy status

So far, only three studies (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022) investigated the effect of IM in the colostrum phase on the energy status of the cows (Table 3). As already described, Carbonneau et al. (2012) harvested 6 L to 14 L milk in the first 5 DIM with a 2 L increase each day (n = 47 cows). From DIM 6 to 61, all cows were milked twice daily. Blood samples were collected by tail venipuncture on d -21, 2, 4, 14, 21, and 28 relative to calving. The DMI and water intake were recorded daily for the entire observation period and from DIM 8 to 61, respectively. The cow's body weight was recorded on DIM 2, 8, 28, and 61. The DMI, water intake and BW did not differ between groups from day 2 to 61. Within the IM period and lasting until DIM 21, the glucose concentration increased while the BHB concentration decreased in the TRT compared to the CON group. The NEFA concentration was lower in TRT compared to the CON group, but no carry-over effect occurred in the subsequent observation period. Furthermore, Carbonneau et al. (2012) reported a numerical difference of cows that met the criteria for subclinical and clinical ketosis; distributed by group (CON, TRT), 13% (2/15) and 6% (1/16) of cows met the criteria for subclinical ketosis, and 47% (7/15) and 6% (1/16) of cows met the criteria for clinical ketosis, respectively. Therefore, Carbonneau et al. (2012) concluded that IM in the colostrum phase represents a successful approach to stabilize the EB beyond treatment and thus might reduce the incidence of metabolic disorders in early lactation.

The initial study of the randomized controlled trial by Morin et al. (2018) aimed to investigate the impact of IM (DIM 1 to 5) on BCS variation, BHB concentration, and prevalence of hyperketonemia (BHB ≥1.4 mmol/L) for defined DIM categories (1 to 3, 4 to 7, 8 to 17, and 18 to 26 DIM) on commercial farms. Weekly blood samples were collected from each cow (n = 838) during the first 3 wk of lactation beginning 36 h after calving. Variation in BCS was calculated by the difference between the BCS assessment of lactation wk 1 and 7. No association was observed between IM treatment and BCS loss. Only in DIM category 4 to 7 the geometric mean of BHB concentration was lower in the TRT group (0.66 (95% confidence interval = 0.60, 0.73) mmol/L) compared to the CON group (0.79 (0.72, 0.87) mmol/L). Overall, the BHB concentration was low in both groups, and the geometric mean exceeded the threshold for hyperketonemia in none of the categories, therefore Morin et al. (2018) suspected that the treatment effect would be greater in herds with a more extended NEB. The predicted prevalence of hyperketonemia was reduced in the TRT group during DIM categories 4 to 7 and 8 to 17 and was, respectively, 4.6 (2.0, 10.0) and 13.4% (8.4, 20.0) for the TRT group versus 10.7 (5.6, 19.3) and 19.4% (13.0, 27.9) for the CON group (Morin et al., 2018). As a possible reason for the low prevalence of subclinical ketosis, Morin et al. (2018) presumed a well-functioning transition management of enrolled commercial farms. Morin et al. (2018) concluded, along with Carbonneau et al. (2012), that IM in the colostrum period is an effective strategy to reduce ketonemia and the prevalence of hyperketonemia in the early postpartum period.

The effect of a short-term IM treatment (3 L milk/ milking; DIM 1 to 2) on the blood BHB concentration was investigated by Valldecabres et al. (2022). At DIM 3 and 11, blood samples were collected from the coccygeal vessel. The BHB concentration did not differ between IM (TRT) compared to twice daily milked (CON) cows; therefore, Valldecarbes et al. (2022) suggested that the findings further elucidate the relevance of the treatment length in addition to the degree of IM. However, a numerical difference in the proportion of cows with subclinical ketosis (blood BHB >1.2 mmol/L) was observed between CON (42%) and TRT (18%) cows at DIM 3 and 11 (Valldecabres et al., 2022).

In conclusion, implementing IM in the colostrum phase can improve the energy status of cows beyond the treatment period (Carbonneau et al., 2012; Morin et el., 2018). Besides the degree of milk accumulation and the duration of milk stasis, the extent of the IM repetition appears to influence the findings (Valldecabres et al.,2022). To date, no study examined the effect of performing IM after the colostrum phase on the energy status of cows in early lactation.

(5) Well-being

Milk accumulation in the udder is related to increased intra-mammary pressure and thus could affect the well-being of the cow. As previously summarized, Albaaj et al. (2018) examined parameters indicating udder distension and udder filling, but did not link these to well-being. So far, only the study by Krug et al. (2017) has investigated indicators of well-being concerning IM (Table 3). In the first 5 DIM a maximum of 10 to 14 L milk were collected, and lying behavior was recorded as an indicator of discomfort due to increased intra-mammary pressure. The cows (n = 32) were equipped with acceleration data loggers attached to the left hind leg. The lying time was not shortened during the IM period. Moreover, adverse effects were observed neither on lying bouts nor on lying bout duration. Based on the findings of the study, the assumption that a short-term IM in early lactation might lead to elevated intra-mammary pressure was refuted (Krug et al., 2017). However, as this is the only study investigating well-being concerning IM, it is even more important to consider this aspect in further studies.

Altogether, the previously summarized studies further support the assumption that the combination of the degree of IM, the duration of milk stasis, and the repetition of periods of milk stasis influence the findings (Carbonneau et al., 2012; Albaaj et al., 2018; Penry et al., 2017; Kuehnl et al., 2019). So far only three studies aiming at improving the NEB in early lactation and addressing the metabolic consequences of applying IM in the colostrum phase (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). In addition, the knowledge about potentially sustained effects of IM on performance and udder health beyond the time of IM treatment is limited.

From a technical point of view, the IM approaches targeting the amount of milk rather than the milk flow as steering criterion for cluster removal were impeded by the need of manual intervention (Krug et al., 2018a; Krug et al., 2018b; Krug et al., 2018c; Morin et al., 2018). Manual cluster removal enhances the potential for errors in accurate cluster removal after reaching the target milk yield and implies a substantial workload for the producer (Morin et al., 2018). Moreover, the onset of IM during the first days of lactation, when colostrum and the following milkings are not considered as salable milk, requires more effort outside of the normal milking routine.

For a practicable realization, an automated software module (Schmidt et al., 2020) was used, that enables an automatic cluster removal after a defined milk quantity (kg) instead of a defined milk flow (kg/min) is reached. This software was developed and has been applied for a gradual dry-off in the study of Martin et al. (2020) and was used for the first time in early lactation in our study. Our objective was to delay the increase in milk yield during the first wk of lactation and mitigate the NEB by limiting the amount of milk withdrawn to the volume harvested when the milk is no longer separated (DIM 8 ± 1.1).

Table 3. Overview of studies investigating the effect of incomplete milking (IM) in dairy cows from the last two decades (including number of animals, observation period, duration of IM treatment, IM treatment design and examined parameters). The authors in the table are sorted according to the year of publication.

Author	Number of animals	Observation period	Duration of treatment	Incomplete milking (IM[1]) treatment design	Examined parameters
Carbonneau et al., 2012	n = 47 multiparous	DIM 1 to 61	DIM* 1 to 5	**Treatment design**: - Milking of 6, 8, 10, 12, and 14 L/d - Whole-udder level - Milking parlor **Milking frequency and interval:** - 2x → 11:13	- **Milk production:** Daily, from DIM 1 to 61 - **Milk composition:** Weekly; Composite sample from a.m. and p.m. milking (Milk protein, fat, lactose and SCC[2]) - **Feed samples:** Weekly; Samples were pooled by groups at 4-wk[#] intervals for composition analysis - **Feed intake:** Daily, from DIM 1 to 61 - **Water intake:** Daily, from DIM 8 to 61 - **Body weight:** On DIM 2, 8, 28 and 61 - **Blood samples:** - On day 21 prepartum and DIM 3, 4, 14, 21, and 28 by tail venipuncture; Analysis of serum BHB[3], serum phosphorous, plasma urea, plasma glucose, plasma NEFA[4], serum cortisol, serum haptoglobin, and peripheral blood mononuclear cells - On DIM 2, 5, and 61 by jugular venipuncture; Analysis of concanavalin A-stimulated peripheral blood mononuclear cell proliferation, Interleukin-4, and blood cell populations (polymorphonuclear leukocyte, total white blood cells and lymphocytes)
Penry et al., 2017	n = 12 parity not specified	DIM 5 to 47	DIM 5 to 47	**Treatment design**: - Approx. 30% milk remaining in the gland including a weekly recalibration - Half-udder design - Milking parlor	- **Milk production:** Daily, at the quarter level - **Milk composition:** Twice a wk, at the quarter level; Testing for milk fat, protein, lactose, solids nonfat, milk urea nitrogen - **Udder health:** Twice a wk, at the quarter level; Measurement of the SCC

				Milking frequency and interval: - 2x → 12:12	
Krug et al., 2017	n = 32 multiparous	DIM 2 to 14	DIM 1 to 5	**Treatment design**: - Milking of 10, 10, 10, 12, and 14 L/d - Whole-udder level - Milking in parlor **Milking frequency and interval:** - Depending on the farm management - 2x → 12:12 3x → 8:8:8	- **Resting behavior:** Daily, via acceleration data loggers; Attached to the left hind leg above the metatarsophalangeal joint of cows Analyzing the daily duration of lying time (h/d), lying bout frequency (bouts/day), and mean duration of lying bouts (min/bout)
Morin et al., 2018	n = 838 multiparous	Divided into 4 categories: - DIM 1 to 3 - DIM 4 to 7 - DIM 8 to 17 - DIM 18 to 26	DIM 1 to 5	**Treatment design**: - Milking of 10, 10, 10, 12, and 14 L/d - Whole-udder level - Milking in parlor and with automated milking systems **Milking frequency and interval:** - Depending on the farm management (2x or 3x) - Milking interval was not specified for each farm	- **Milk production:** Daily, in 6/13 Farms (automated) and once a wk in 7/13 Farms (manually) for the first 4 wk of lactation; Afterwards monthly through DHIA[5] testing - **Blood Samples:** Weekly, for the first 3 wk after calving via venipuncture of the coccygeal vessels; Analyze BHB concentration - **BCS:** In wk 3 to 4 prepartum and in lactation wk 1 and 7 - **Health events:** Recorded manually by the farmer (dystocia, clinical hypocalcemia, and retained placenta)

Krug et al., 2018c	n = 846 multiparous	Lactation wk 2 to 44	DIM 1 to 5	**Treatment design**: - Milking of 10, 10, 10, 12, and 14 L/d - Whole-udder level - Milking in parlor and with automated milking systems **Milking frequency and interval:** - Depending on the farm management (2x or 3x) - Milking interval was not specified for each farm	- **Milk production:** Daily, in 6/13 Farms (automated) and once a wk in 7/13 Farms (manually) from wk 1 to 4; Afterwards monthly through DHIA testing until the end of lactation or culling - **Milk composition:** Monthly through DHIA records (milk fat and protein concentration, in %) - **Culling date:** Monthly through DHIA records
Krug et al., 2018b	n = 878 multiparous	Divided into 3 categories: - DIM 11 to 18 - DIM 35 - DIM <90	DIM 1 to 5	**Treatment design**: - Milking of 10, 10, 10, 12, and 14 L/d - Whole-udder level - Milking in parlor and with automated milking systems **Milking frequency and interval:** - Depending on the farm management (2x or 3x) - Milking interval was not specified for each farm	- **Intramammary infection incidence and elimination:** On DIM 11.5 ± 2.3 and 18.5 ± 2.2 (Mean ± SD), Quarter-milk samples, 2 to 8 h after morning milking; - **Clinical mastitis**: Recorded by farmers via electronic health records - **Reproductive tract health:** On DIM 32.9 ± 2.9, Cows were tested for purulent vaginal discharge, Endometrial cytology for smear and for leukocyte esterase test

Krug et al., 2018a	n = 853 multiparous	Divided into 5 categories: - DIM 1 to 21 - DIM 22 to 43 - DIM 44 to 65 - DIM 66 to 87 DIM >87	DIM 1 to 5	**Treatment design**: - Milking of 10, 10, 10, 12, and 14 L/d - Whole-udder level - Milking in parlor and with automated milking systems **Milking frequency and interval:** - Depending on the farm management (2x or 3x) - Milking interval was not specified for each farm	- **Blood Samples:** Weekly, for the first 3 wk after calving; Venipuncture of the coccygeal vessels; Analysis of blood BHB concentration - **Luteal activity:** In wk 5 and 7; Blood sample; Measurement of progesterone serum concentration - **Time to pregnancy:** Reproductive information's from computerized record systems on farm (herd use of ovulation synchronization program and timed artificial insemination, calving dates, voluntary waiting period, artificial insemination dates, DIM at pregnancy, and culling dates)
Albaaj et al., 2018	n = 16 n = 12, multiparous and n = 4 primiparous	In total 7 days 4-d sampling (M0 to M7) 3-d washout	DIM 55 ± 9 Applied for a single morning milking event (M0)	**Treatment design**: - 0%; 40%; 70% and 100% milk removal - Expected milk yield for the 40% and 70% was based on the average milk yield of the four-preceding morning milking's - Whole-udder level - Milking parlor **Milking frequency and interval:** - 2x → 9.5:14.5	- **Milk production:** Daily, recorded at each milking - **Milk composition:** Each milking from DIM 1 to 5 (Milk protein, fat and lactose concentration, in %) - **SSC:** From DIM 1 to 4, at each milking - **Udder distension:** At 1 and 9 h after treatment; Measurements of the total distance between the ends of the 4 teats - **Cisternal capacity:** At 1 and 9 h after treatment; Evaluation of cisternal area by ultrasonographic scan - **Blood samples:** At 1 h before and 4, 7, and 10 h after treatment; Collected from the coccygeal vein; Measurement of plasma lactose concentration

Kuehnl et al., 2019	n = 22 multiparous	DIM 5 to 47	DIM 5 to 47	**Treatment design**: - Approx. 30% milk remaining in the gland including a weekly recalibration - Half-udder level - Milking parlor **Milking frequency and interval:** - 2x or 3x - Milking interval was not specified ("relatively constant")	- **Milk production:** Daily, recorded at the quarter level - **Milk composition:** Twice a wk (third and seventh day from a wk), at the beginning of a milking event, analyzed at the quarter level; testing for milk fat, protein, lactose, solids nonfat, milk urea nitrogen - **Udder health:** Twice a wk (third and seventh day from a wk), at the beginning of a milking event, analyzed at the quarter level; testing for SCC
Valldecabres et al., 2022	n = 90 multiparous	DIM 1 to 2 DHIA records representative for the first month of lactation	DIM 1 to 2	**Treatment design**: - 3 L/milking event - Whole-udder level - Milking parlor **Milking frequency and interval:** - 2x → 12:12	- **Milk production while treatment:** Recorded at each milking for the first 48 h on whole-udder level - **Milk production and milk composition after treatment:** Data from the first monthly DHIA records - **Udder health:** At each milking for the first 48 h; Determined on whole-udder level. - **Blood samples:** Every 4 h up to 48 h and at DIM 3: Collected from the coccygeal vein → Analysis of total plasma calcium, phosphorus, and magnesium; DIM 3 and 11: Collected from the coccygeal vein → Analysis of BHB concentration in blood - **BCS:** Once at enrollment

Deacon et al., 2023	n = 8 multiparous	Divided into 3 categories: - 2 wk before IM (started from DIM 174 ± 45) - 4 wk while IM - 2 wk after IM	Started from DIM 188 ± 45	**Treatment design**: - Approx. 30% milk remaining in the gland including a recalibration at the end of wk 2 - Half-udder design **Milking frequency and interval:** - 2x → 12:12	- **Milk production:** Recorded daily at the quarter level while IM - **Milk composition:** Weekly at the quarter level; Testing for milk fat, protein, lactose, SCC (not addressed in the study), and epithelial cell concentrations - **Aseptic milk samples for isolation of milk fat globule RNA**: Weekly at the quarter level; Expression of genes related to mammary activity (CSN2 = β-casein; LALBA = α-lactalbumin; ACACA = acetyl-co-enzyme A carboxylase α), PRL signaling (Long and short isoform of prolactin receptor; Suppressors of cytokine signaling 3 = SOCS3; Signal transducer and activator of transcription 5A und 5B = STAT5A and STAT5B), and apoptosis (BCL2 and BAX) via quantitative reverse-transcription PCR. - **Mammary gland functional capacity:** 2 wk before and 2 wk after the IM; Evaluation through the measurement of the volume of milk harvested after complete filling of the gland (26-h) with a final intramuscular injection of oxytocin (20 IU) - **Mammary gland biopsy**: 12 days after finishing IM treatment; From each rear quarter, western blot analysis was conducted to determine abundance of the short and long isoforms PRL receptors. The abundance or activation of signal transducer and activator of transcription proteins (= STAT 5A, 5B, 3) via ELISA procedure

[*]DIM: Day(s) in milk; [#]wk: week(s); [1]IM: Incomplete milking; [2]SCC: Somatic cell count; [3]BHB: ß-hydroxybutyrate; [4]NEFA: Non-esterified fatty acids; [5]DHIA: Dairy Herd Improvement Association

Chapter 2 Objectives

In the present study, we aimed to perform an automated, constant IM to delay the increase in milk yield during the first wk of lactation. A software module (Schmidt et al., 2020) was used, which allows automatic cluster removal after reaching a defined amount of milk (kg/d). At the end of treatment, the conventional, milk-flow dependent (0.3 kg/min) automatic cluster removal was reactivated. The IM started when the milk was no longer separated and antibiotic residues from dry-off were no longer detectable (DIM 8 ± 1.1). The degree of milk removal at enrollment was not allowed to increase but was kept constant for 2 wk and corresponded to the cow's individual milk yield 1 day before the experiment started.

It was hypothesized that IM stabilizes the energy status of dairy cows in early lactation, without compromising udder health and performance thereafter. For testing this hypothesis, the following records, samples, and measurements were performed:

- **Energy status:** Blood samples to analyze the NEFA, BHB, IGF-1, and leptin concentration (wk relative to calving -3, -2, 1 to 7), estimation of the EB (wk after calving 1 to 15), based on the records the DMI and water intake (wk after calving 1 to 15), BW (wk after calving 1 to 15), BCS (wk relative to calving -3, -1, 2, 4, and 6), back fat thickness (**BFT**), and muscle diameter (*M. longissimus dorsi,* **MLD**) via ultrasound imaging (wk relative to calving -3, -1, 2, 4, and 6).

- **Performance:** Milk yield (days relative to the start of IM 0 to 21; wk after calving 1 to 15), milk composition (wk after calving 1 to 15), peak yield, and day of reaching peak yield.

- **Udder health and indicators of udder filling:** SCC at the quarter level (wk after calving 1 to 7), cytobacteriological examinations of aseptic fore milk samples at the quarter level (wk after calving 1 and 4), udder firmness as indirect indicator for intramammary pressure (wk after to calving 1 to 4), observation of milk leakage before and after fore milking (wk after calving 1 to 4), last milk flow before automated cluster removal (days relative to the start of IM 0 to 21), and milking time (days relative to the start of IM 0 to 21).

Chapter 3 Manuscript

INTERPRETIVE SUMMARY

The impact of automated, constant incomplete milking on energy balance, udder health, and subsequent performance in early lactation of dairy cows. *By Meyer et al.* Reducing the amount of milk withdrawn at each milking is one of the options to mitigate the negative energy balance (NEB). To facilitate the implementation of incomplete milking (IM), an automated system, first developed as a tool for dry-off, was used. When clamping the milk withdrawn to the amount obtained at DIM 8 for 2 wk, udder health was maintained, milk yield and composition until wk 15 were not altered, and cows tended to have less feed intake than normally milked cows during the 2-wk of IM, leaving EB largely unaffected during the 2-wk of IM and thereafter.

Journal of Dairy Science; https://doi.org/10.3168/jds.2023-23777

The impact of automated, constant incomplete milking on energy balance, udder health, and subsequent performance in early lactation of dairy cows

I. Meyer[1], E. Haese[1], K.-H. Südekum[1], H. Sauerwein[1], and U. Müller[1*]

[1]Institute of Animal Science, University of Bonn, 53115 Bonn, Germany.

[*]Corresponding author: Ute Müller; Institute of Animal Science, University of Bonn, Katzenburgweg 7, 553115 Bonn, Germany, +49 228 735112, ute-mueller@uni-bonn.de

ABSTRACT

Incomplete milking (IM) is one way of mitigating the negative energy balance (NEB) that is characteristic for early lactation and may increase the risk for disease. Our objectives were to test the effects of IM in early lactation on energy balance (EB), metabolic status, udder health, and subsequent performance. To facilitate the practical application, an automated system was used to remove the milking clusters once a pre-defined amount of milk is withdrawn. Forty-six Holstein cows were equally allocated to either the treatment (TRT, starting on 8 DIM) or the control group (CON; conventional cluster removal at milk flow rate < 0.3 kg/min). Milk removal in the TRT group was limited to the individual cow`s milk yield 1 d before IM started and held constant for 14 d. Thereafter, all cows were conventionally milked and records related to EB, performance, and udder health were continued up to 15 wk of lactation. During the 14 d of IM, on average 11.1% less milk were obtained from the TRT cows than from the CON cows. Thereafter, milk yield increased in the TRT group eliminating the group difference throughout the remaining observation period until wk 15 of lactation. The TRT cows tended to have less DMI and also water intake than the CON cows. The extent of the NEB and the circulating concentrations of fatty acids, BHB, IGF-1, and leptin did mostly not differ between the groups. The IM did not affect body condition. Udder health was maintained over the entire observation period in all cows. Our results demonstrate the applicability of the automated cluster removal for limiting milk withdrawal to a defined amount in early lactation. However, it remains to be determined whether the absent effect on energy metabolism was due to the relatively stable energy status of the cows or to the relatively mild IM setting used herein.

Key words: incomplete milk removal, energy status, management strategy

INTRODUCTION

The negative energy balance (**NEB**) during early lactation results from the gap between the energy needed for maintenance and milk secretion, and the energy supply provided by feed intake that is not covering the needs at that time. For sustaining the increasing milk yields during the first weeks of lactation, dairy cows have to mobilize body reserves, mainly fat. The release of fatty acids (**FA**) from the adipose depots is reflected by increased circulating FA concentrations. During the NEB, the capacity of the liver for FA oxidation is limited, and incomplete FA oxidation can result in metabolic diseases such as ketosis, and fatty liver (Drackley et al., 2005; Esposito et al., 2014). For mitigating the NEB and thus the risk for such health disturbances, the main variables affecting the energy balance (**EB**) can be tackled: energy intake can be modified to some extent by dietary measures, e.g., by providing palatable, energy-dense and adequately structured diets. Supplements which support rumen stability, supply additional energy, and (or) improve intermediary metabolism can be helpful in mitigating the NEB (Jouany, 2006; McGuffey, 2017). However, voluntary feed intake is negatively associated with the increased pro-inflammatory cytokine levels arising from subacute inflammation in various peripheral organs shortly before or after calving; these cytokines reach the hypothalamus where they signal reduction in feed intake (Kuhla, 2020), a phenomenon which cannot be overcome by dietary intervention. The other significant variable in the early-lactation EB concerns the energy output via milk. Several approaches for temporarily reducing milk yield were suggested: Inhibiting the secretion of the galactopoetic hormone prolactin (**PRL**) by administering dopamine agonists (Lacasse et al., 2019), by omitting or shortening the dry period (Kok et al., 2019) thus lowering the peak milk yield, by reducing the milking frequency to once daily as reviewed by Stelwagen et al. (2013), or by incomplete milking (**IM**) at each milking. The latter was achieved by removing only a defined amount of milk from the mammary gland (e.g., Carbonneau et al., 2012; Krug et al., 2018a; Krug et al., 2018b). Lacasse et al. (2018) assumed that IM is the most promising approach to reduce metabolic stress without compromising the productivity of high-yielding dairy cows. Studies aiming at improving the NEB and the concomitant metabolic consequences in early lactation by IM were largely limited to the colostrum phase (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). In addition, the knowledge about potentially sustained effects of IM beyond the time of IM treatment is limited. The IM approaches targeting the amount of milk rather than the milk flow as steering criterion for cluster removal were impeded by the need for manual intervention. Manual cluster removal enhances the potential for errors in accurate cluster removal after reaching the target milk yield and implies a substantial workload (Morin et al., 2018). Moreover, the onset of IM during the first days of lactation, when colostrum and the following milkings are not considered as salable milk, requires more effort outside of the normal milking routine. With this background, we aimed at starting IM not before the second week after parturition when the milk is no longer separated. Moreover, for targeting cluster removal when a defined amount of milk is withdrawn rather than using milk flow rate, we aimed at applying a software module that was initially developed for drying cows off by reducing the amount of milk withdrawn per milking (Martin et al., 2020; Schmidt et al., 2020). Our objective was to delay the increase in milk yield during the first weeks of lactation by limiting the amount of milk withdrawn to the volume milked at the beginning of the second week in milk over the two following weeks. We hypothesized that a 2-wk automated and constant IM would mitigate the NEB, without compromising udder health and performance thereafter.

MATERIALS AND METHODS

The animal experimental procedures (July 2020 to April 2022) were approved by the relevant authority (State Office for Nature, Environment, and Consumer Protection (LANUV), North Rhine-Westphalia, Recklinghausen, Germany; 81-02.04.2019.A404) and were carried out at the Campus Frankenforst, Faculty of Agriculture, University of Bonn.

Animals and housing

Forty-six pluriparous German Holstein cows were selected from the 60-cow herd, and randomly allocated to either the control group (CON, n = 23; average lactation number 3.5 ± 1.6) or the treatment group (TRT, n = 23; average lactation number 3.1 ± 1.5; mean ± SD). All cows were housed in a free-stall barn with slatted concrete floor and cubicles. During the first 15 weeks of lactation, all lactating cows were fed a partial mixed ration with an average energy content of 6.2 MJ NE_L/kg DM (Supplemental Table 1). The diet was provided for ad libitum intake after each of the two milking times (05:30 and 16:30). In addition, a dairy compound feed was provided through separate feeding stations (GEA Farm Technologies GmbH, Bönen, Germany) via transponder access according to DIM and milk yield. Feed intake was recorded daily via weighing troughs (Hokofarm Group B.V., Marknesse, The Netherlands). The cows had ad libitum access to fresh water and water intake was recorded by water weighing bowls (Hokofarm Group B.V.). After each milking, BW were recorded via an electronic scale (Hokofarm Group B.V.).

Supplemental Table 1. Weighted average of ingredient and chemical composition of the ration from the late dry period (wk 3 ante partum to calving) and early lactation (wk 1 to 15 of lactation) for control cows (CON, *n* = 23) and treatment cows (TRT, *n* = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started.

Item	Close-up period	Early lactation
Ingredient (% of ration DM)		
Corn silage	27.7	24.6
Grass silage	28.1	24.9
Alfalfa silage	7.7	6.8
Beet pulp silage	8.3	7.3
Brewers grain silage	2.0	1.8
Sugar beet pulp, dehydrated	1.0	0.9
Mineral mix[1]	0.3	0.2
Cattle salt[2]	0.05	0.04
Concentrate mix[3]	16.9	15.8
Dairy compound feed[4]	7.9	17.6
Analyzed composition of the partial mixed ration *		
NE_L (MJ/kg DM)	6.21	
Ash (g/kg DM)	96.1	
CP (g/kg DM)	115.9	
ADF (g/kg DM)	185.0	

* Analyzed chemical composition does not include the concentrate mix and dairy compound feed, these were additionally provided through separate feeding stations (GEA Farm Technologies GmbH, Bönen, Germany) via transponder access according to the individual lactation stage and milk yield.

[1] Composition per kilogram product: Ca, 160 g; P, 40 g; Na, 90 g; Mg, 80 g; Zn, 8000 mg; Mn, 4200 mg; Cu, 1500 mg; Co, 45 mg; I, 150 mg; Se, 45 mg; vitamin A, 900000 IE; vitamin D3, 135000 IE; vitamin E, 3000 mg; Biotin, 6 mg (BoVital TOP, Biomin Animal Nutrition GmbH, Getzsersdorf, Austria).

[2] Composition per kilogram product: Ca, 4.8 g; Na, 387 g; Mg, <0.1 g; Zn, 1.3 mg; Mn, <1.0 mg; Cu, <3.0 mg; Co, <0.05 mg; Se, 0.02 mg (Ralinger Salz Handels GmbH, Föhren/Trier, Germany).

[3] Main ingredients: Corn; wheat; rapeseed meal, solvent-extraced; rapeseed meal, solvent-extratced and hydro-thermally treated; corn gluten feed; sugar beet vinasse; wheat gluten feed; sugar beet molasses; corn vinasse (Deuka Landliebe 224-S, gek., Deutsche Tiernahrung Cremer GmbH & Co. KG, Neuss, Germany).

4 Main ingredients: Wheat gluten feed; barley; wheat bran; sunflower meal, solvent-extracted; sunflower husk; corn gluten feed; corn vinasse; sugar beet vinasse (Deuka Landliebe 183, gek., Deutsche Tiernahrung Cremer GmbH & Co. KG, Neuss, Germany).

A double-four in-line milking parlor was used (Classic 300 cluster and ClassicPro 21-27 liners; GEA Farm Technologies GmbH) with 62 pulses/min, 64:36 pulsation ratio, and a vacuum level of 40 kPa. The threshold for automatic, milk flow-dependent cluster removal was set at 0.3 kg/min. The pre-milking routines of cleaning with a wet paper towel (UdderoClean;

Albert Kerbl GmbH, Buchbach, Germany) and forestripping were followed by mechanical prestimulation (300 pulses/min during the first 30 s). Milk yield, milking duration, and last milk flow in the measuring unit at the moment of cluster removal were recorded automatically at the udder level for each cow during each milking (Metatron P21; GEA Farm Technologies GmbH). The value obtained for the last milk flow in the measuring unit at the moment of triggering cluster removal is composed of the set threshold for milk flow-dependent cluster removal and additional milk that accumulates in the milk draining components of the milking unit preceding the measuring unit due to the delay time. The lactation preceding the current investigation was terminated by drying off the cows at 52 ± 9 d before expected calving and applying an intramammary antimicrobial treatment (Orbenin Extra, Zoetis Deutschland GmbH, Berlin, Germany) and an internal teat sealer (Orbeseal, Zoetis Deutschland GmbH) after the last milking.

Experimental design

During the first 8 ± 1.1 DIM, all cows were milked in the same manner i.e., with cluster removal at milk flow rate < 0.3 kg/min. The cows were allocated to either the control (CON) or the treatment (TRT) group by stratifying for udder health in the preceding lactation, calving date, and lactation number. The TRT cows were milked using the software module (Schmidt et al., 2020) by which the milking clusters are removed after obtaining a defined amount of milk (kg) until d 21 ± 1.1 of lactation. We hypothesized that the TRT cows would adapt their milk production to the lower demand during the 14 d treatment period and would thus have a less negative EB, without udder health restrictions. By setting a constant milk quantity for the IM according to the animal-specific performance level 1 d before IM was started, we could take the individual`s milk yield into account, and thus exert the same relative impact on all cows. The software module was originally developed for facilitating dry-off (Martin et al., 2020) and was integrated into the milking computer (Metatron C21; GEA Farm Technologies GmbH) on the farm. The constant amount of milk (kg) used during the 2-week IM period corresponded to the cow`s individual milk yield 1 d before the IM started. At the end of IM, the conventional automatic cluster removal at < 0.3 kg/min was reactivated and records of milk yield and feed intake were continued and evaluated for this study until wk 15 of lactation.

Milk composition and energy-corrected milk

At the udder level, milk samples (proportionate total composite milking) were collected weekly (equivalent to the routine monthly milk recording samples) at consecutive evening and morning milkings, and were analyzed for milk fat, protein, and lactose (Landeskontrollverband Nordrhein-Westfalen e.V., Krefeld, Germany).

The ECM (kg; 4% fat and 3.4% protein) was calculated individually for each week according to the equation of Tyrrell and Reid (1965): ECM = Milk (kg) × [(0.38 x fat (%) + 0.21 × protein (%) + 1.05)/ 3.28].

Udder health and indicators of udder filling

For udder health control, the SCC was assessed weekly in quarter foremilk samples (DCC cell counter; DeLaval GmbH, Glinde, Germany) in the first 7 weeks of lactation. During wk 1 and 4, foremilk samples from both groups were subjected to cytobacteriological examination according to the recommendations of the German Veterinary Society (DVG, 2009) at a commercial laboratory (Milchtierherden-Betreuungs- und Forschungsgesellschaft mbH (MBFG), Wunstorf, Germany).

During the first 4 weeks of lactation, both groups were observed twice per week for milk leakage based on the protocol by Gott et al. (2016): one investigator stood in the milking parlour and observed each study animal standing in the milking parlor visually for 10 to 15 s before fore-milking, after fore-milking before attaching the milking clusters and after cluster removal. Milk leakage was recorded as “yes” or “no” at the quarter level. The cow was scored as “yes”, if at least one leaking teat was observed.

The udder firmness was assessed for estimating the filling state of the udders. The measurements were performed twice per week before attaching the milking clusters by using a digital dynamometer (FMI-B30B5, 50 N, 0.15% accuracy; Alluris GmbH & Co. KG, Freiberg, Germany). The device was equipped and the measurement was performed considering the standard operation procedure implemented by Bertulat et al. (2012). Herein one representative measurement point located on the border between the lower third and middle third of the rear udder quarter was used. The measurement was performed and readouts were transferred in real time to the computer using an advice related software (FMI_Connect 3.02, Alluris GmbH & Co. KG). The output value represented the arithmetic mean (N) and the coefficient of variation (%)

of two consecutive measurement series. Values with a coefficient of variation exceeding 10% were discarded and the measurement repeated.

Feed analyses and estimation of energy balance

For calculating the EB, the energy content of the feed was estimated first: samples of the partial mixed ration were taken twice per week over the entire experimental period. The DM content was determined by oven-drying (Memmert GmbH & Co. KG, Schwabach, Germany) at 105°C overnight. The DM was corrected (DMcor) for the loss of volatiless during drying according to Weißbach and Kuhla (1995): DMcor (%) = 2.08 + 0.975 × DM (%). Concentrate samples were taken from each batch at delivery and stored at −20°C. Analysis of DM content (%; VO (EG) 152/2009, III, A) and estimation of energy concentration (MJ NE_L/kg DM; GfE, 2001) were done by a commercial laboratory (Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) North Rhine-Westphalia, Münster, Germany).

The EB (MJ NE_L/d) was calculated individually for each week according to the Gesellschaft für Ernährungsphysiologie (GfE, 2001): Feed energy intake (MJ NE_L/d) minus the NE requirement for maintenance (MJ/d = 0.293 × (kg BW)$^{0.75}$), and the energy output in milk (MJ/d = (38.5 × fat (%) × 0.01 + 24.2 × protein (%)× 0.01 + 16.5 × lactose (%)× 0.01 + 0.1) × (milk amount (kg)) (Susenbeth, 2018).

Body condition score, and measurements of back fat thickness and muscle diameter

The BCS (Edmonson et al., 1989), average back fat thickness (**BFT**) and measurement of the average M. longissimus dorsi diameter (**MLD**) were measured every second week starting 3 weeks before the expected calving date until 6 wk thereafter. The BFT measurement was done above the ML, right side and perpendicular to the spinal cord between the 12th and 13th rib and at the 5th lumbar vertebra (Bruckmaier et al., 1998a; Bruckmaier et al., 1998b) by ultrasound (LOGIQ V2; GE Healthcare GmbH, Solingen, Germany) using an endolinear probe (5-10 MHz; LK760-RS, GE Healthcare GmbH) at 9 MHz and 7 cm depth. Ultrasound images were stored immediately for later analysis (IC Measure 2.0.0.245, The Imaging Source Europe GmbH, Bremen, Germany). Image analysis allowed for assessing not only BFT but also MLD.

Blood sampling and analyses

Blood was obtained from the V. jugularis in wk 3 and 2 before calving and weekly during the first 7 wk after calving. Blood was collected into tubes containing coagulation activator (9 mL; S-Monovette, Sarstedt AG & Co. KG, Nümbrecht, Germany). After collection, samples were left to rest for 30 min at room temperature. Samples were centrifuged at 2500 rpm for 15 min at 15°C (Z 366 K; HERMLE Labortechnik GmbH, Wehingen, Germany). Subsequently the serum was removed and stored at -20°C for later analyses. Non-esterified fatty acid (**NEFA**) and BHB concentrations were measured in serum by using an automatic photometric analyzing system (Eurolyser; Type VET CCA, Salzburg, Austria) at the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany. Serum leptin was analyzed with an enzyme immunoassay validated for bovine samples (Sauerwein et al., 2004). The mean intra-assay coeffcient of variation (CV) was 7.37%, and the inter-assay CV was 9.97%. An enzyme immunoassay (Mediagnost GmbH, Reutlingen, Germany) was used for the quantitative determination of IGF-1 in serum. The mean intra-assay CV was 3.17%, and the inter-assay CV was 5.15%.

Statistical data analysis

Statistical analyses for all data were performed using SPSS 28 (IBM, Armonk, NY). Before analysis, raw data on a daily basis were checked for extreme outliers. Outliers in milk yield, feed intake, water intake, BW, milk yield, and milk composition were defined as deviating more than 3 SD from the mean. In addition, the plausibility of data was checked and eventually corrected for technical failures that occurred during the trial (e.g., temporary loss of function of the weighing troughs or the scale). Data were analyzed by calculating the data within 2 or 4 periods of weeks or days (before calving and before start of the treatment; with treatment and after treatment), depending on the availability of data from before calving and data on day or week level. Data for each period were tested for normal distribution, normal distribution of the residuals of each variable, and the homogeneity of variance with the Levene’s test. Using a linear mixed model and Bonferroni correction within periods for multiple comparisons, data were analyzed as repeated measures with ’treatment group’ (treatment) and ’time’ (week(s) or day(s) relative to calving) and their interaction as fixed effects. ‘Cow’ was used as random factor. For the udder health control by the SCC of foremilk samples, ’udder-quarter’ was considered as random factor instead of ‘cow’. As covariance structure, the variance components covariance structure was used. Student’s t-test was carried out for the variables ‘time of peak’ and ’peak yield’, and the Chi square test was performed for ’milk leakage’ for each week. All data are presented as means with SEM. The level of significance was set at $P \leq 0.05$ and a trend was defined at $0.05 < P \leq 0.10$.

RESULTS

Milk production

One day before starting the IM, milk production was not different between the groups (Table 1). Figure 1a shows the time course of daily milk yields during the two weeks IM-period. The average amount of milk (kg/d) from CON cows was greater than from TRT cows, starting from d 4 of IM (Figure 1a). While milk yield remained constant in the TRT group, that of the CON group continued to increase over the 14 d of IM, resulting in 11.1% less milk in the TRT group than in the CON group during IM. Milk yields during the entire period from IM and the subsequent time until wk 15 of lactation are shown as weekly averages in Figure 1b. When IM ceased, milk production of the TRT cows immediately reached the level of the CON cows (Figure 1, Table 1) increasing by 18.6% above the yield from the preceding last day of IM. At the second day of conventional milking, daily milk yield decreased ($P \leq 0.001$) again by 3.2 kg in average to 36.5 kg ± 1.12 in the TRT cows, but remained unchanged in the CON cows (P = 1.000; Figure 1a). No further group differences in milk yield were observed both on a daily and a weekly basis after the IM treatment (Table 1 and Fig. 1 b). From wk 4 to 15 after IM, average milk yield was 39.2 kg/d ± 0.23. Neither time of peak nor peak yield were affected by the treatment (Table 1).

Table 1. Milk yields (means ± SEM) related to the automated and constant incomplete milking (IM) period in control cows (CON, *n* = 23) and treatment cows (TRT, *n* = 23).

Item	TRT[1]	SEM	CON	SEM	P-value
Start milk yield (kg)	32.3	0.99	32.8	1.41	0.788
Average milk yield (kg/d) in wk 1 of IM	31.8	0.91	34.4	0.98	0.052
Average milk yield (kg/d) in wk 2 of IM	32.3	0.94	37.5	1.01	≤ 0.001
Milk yield (kg/d) one d after IM	39.7	1.09	37.3	1.13	0.133
Average milk yield (kg/d) in wk 1 after IM	37.4	1.22	38.9	1.23	0.376
Average milk yield (kg/d) in wk 4 to 15 after IM	38.3	0.28	40.1	0.36	0.246
Time of peak[2] (DIM)	52.3	4.06	50.8	4.32	0.804
Peak yield[3] (kg/d)	43.9	0.80	45.8	1.29	0.209

[1]Milked incompletely twice daily from d 8 ± 1.1 until d 21 ± 1.1 of lactation; the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started.

[2]Day with highest milk yield achieved within the first 100 DIM.

[3]Milk yield at the time of peak yield.

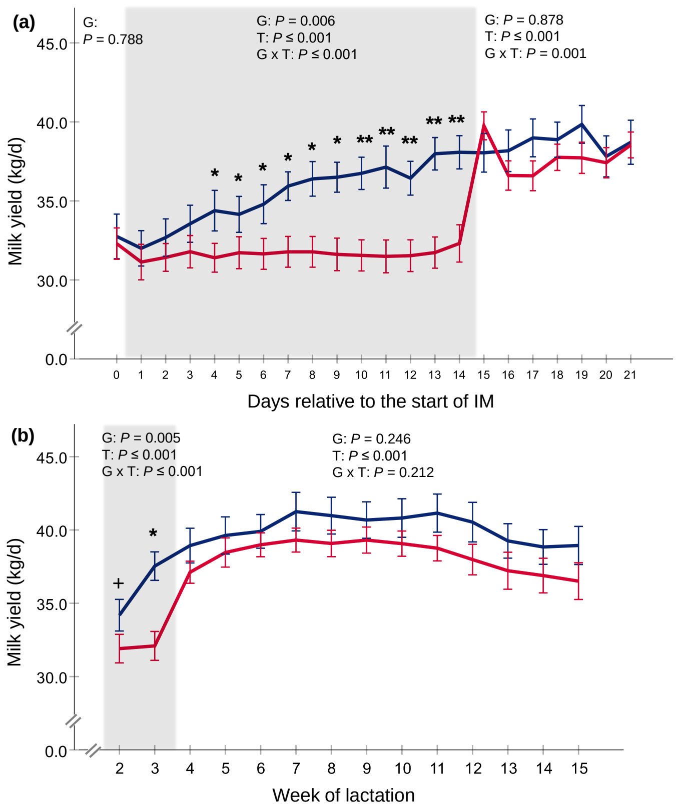


Figure 1. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation as compared to conventional milking on (a) daily milk yield over the first 21 d relative to the start of IM and (b) weekly average of milk yield over the first 15 wk of lactation. Blue line = control cows (CON, $n = 23$); red line = treatment cows (TRT, $n = 23$). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$), or ** ($P < 0.001$) at a given timepoint, respectively. Trends ($P \leq 0.10$) for differences between the groups at a given time point are indicated by +.

Milk composition and energy-corrected milk

Milk composition as well as fat, protein, and lactose yields are summarized in Supplemental Figure 1 a - f. Milk fat concentration was not affected by IM, neither during nor after the treatment (Supplemental Figure 1a). Milk fat yield during IM tended to be 1.13-fold higher in the CON than in the TRT group ($P = 0.074$). For milk protein content, no group difference was found during the IM-Period (Supplemental Figure 1c). After IM, there were no differences between the groups. Protein yield differed between groups during IM with 1.15-fold more protein in the CON than in the TRT group (Supplemental Figure 1d). After the IM, no further group difference was observed. The milk lactose concentrations and yields differed between groups and there was a group × time interaction during the IM phase. The group difference in lactose concentrations was limited to the *second* week of IM, with 0.15% ± 0.05 higher contents in CON than in TRT (Supplemental Figure 1e). Within the groups, the concentration remained constant during IM in TRT and increased from 4.73 % ± 0.04 to 4.82 % ± 0.04 in CON as substantiated by the time x group interaction. After IM, the lactose concentration did not further differ between the groups except a trend for a group by time interaction (*P* = 0.099) with greater values in CON as compared to TRT in wk 4 (*P* = 0.085) and wk 13 (*P* = 0.077) of lactation (Supplemental Figure 1e). Milk lactose yields tended and were higher in CON than in TRT in the first and the second week of IM, respectively. After IM, no differences were noted (Supplemental Figure 1f).

Figure 2 shows the time course of ECM yields (kg/d) until wk 15 of lactation. Approximately 12% less ECM were milked from TRT than from CON cows during the 2 wk of IM (32.8 kg/d ± 0.91 vs 37.3 kg/d ± 0.87). Time was significant during IM only for the CON animals (*P* = 0.014) increasing their ECM yield from 36.3 kg/d ± 1.35 to 38.5 kg/d ± 1.25. After switching back to conventional milking, ECM did not differ between groups and averaged 38.3 kg/d ± 0.20 (Figure 2).

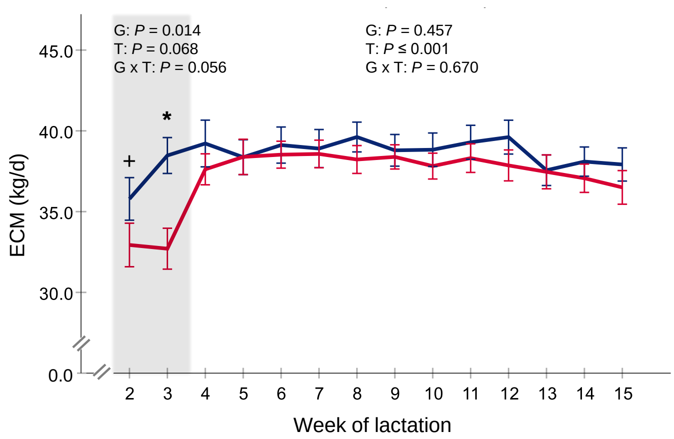
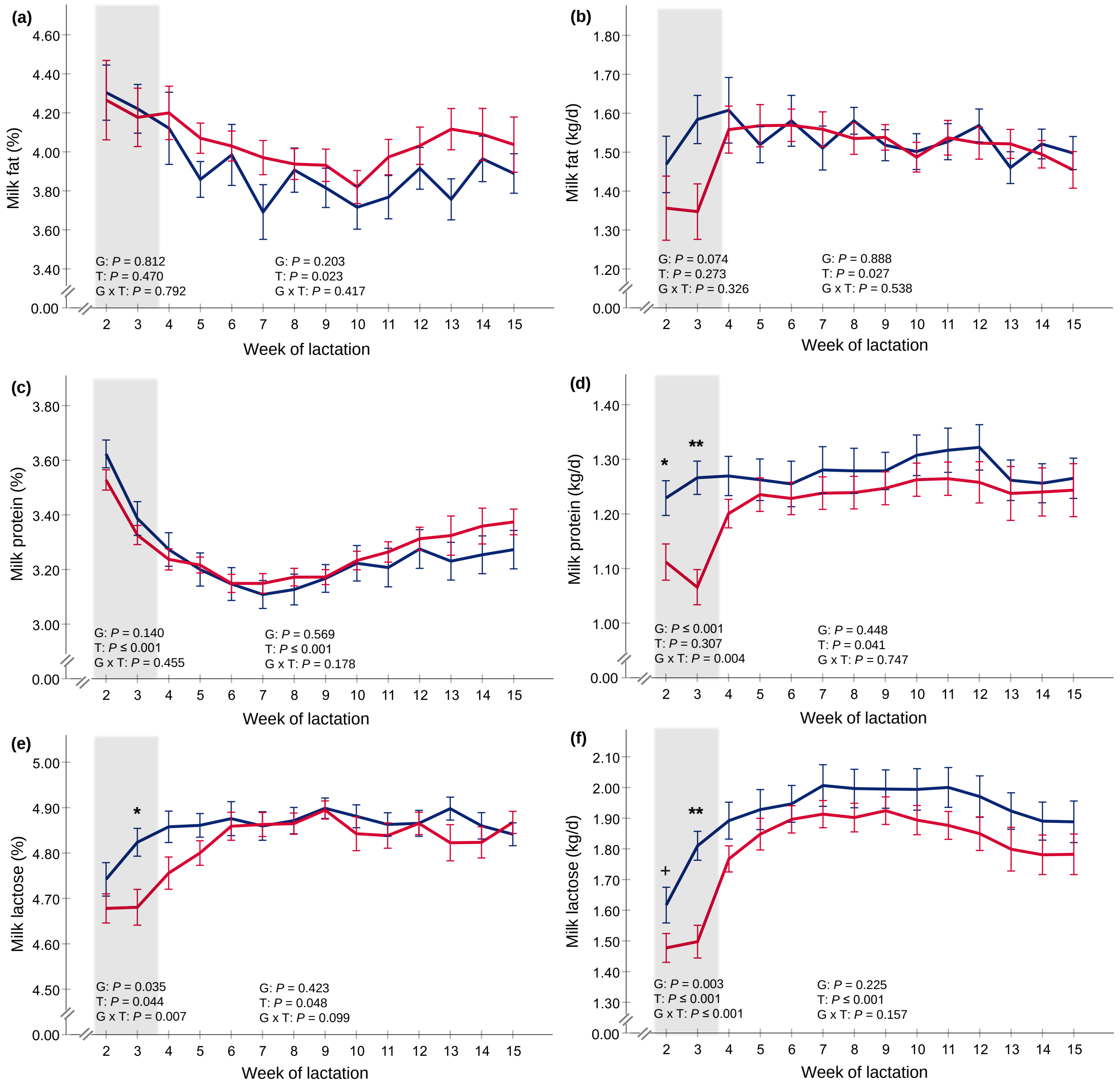


Figure 2. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on ECM yield over the first 15 wk of lactation. Blue line = control cows (CON, *n* = 23); Red line = treatment cows (TRT, *n* = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * (*P* ≤ 0.05) or ** (*P* < 0.001), and + designates trends (*P* ≤ 0.10) at a given time point, respectively.



Supplemental Figure 1. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on (a) fat concentration, (b) fat yield, (c) protein concentration, (d) protein yield, (e) lactose concentration and (f) lactose yield over the first 15 wk of lactation. Blue line = control cows (CON, n = 23); Red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The IM period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$) or ** ($P < 0.001$), and + designates trends ($P \leq 0.10$) at a given time point, respectively.

Udder health, indicators of udder filling, and milking duration

Neither elevated SCC nor mastitis-relevant bacteria were detected in the foremilk quarter samples of both groups. Figure 3 shows the time course of SCC in the quarter milk samples from both groups. At any time, the values were well below the threshold for mastitis (SCCLog$_{10}$/mL < 2.00) as defined by the German Veterinary Association (DVG, 2009).

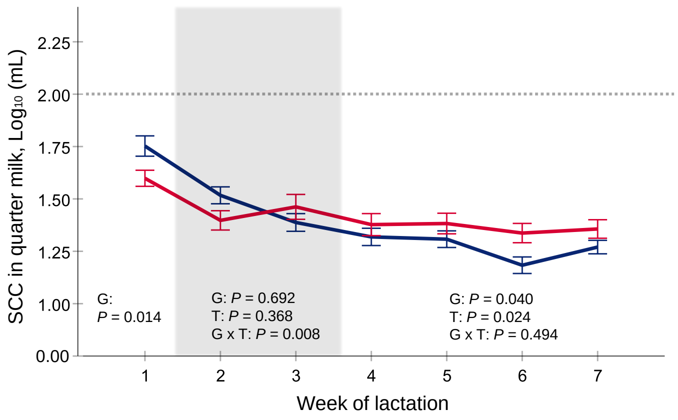


Figure 3. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on the time course of SCC (log$_{10}$/mL) in foremilk samples of each quarter (n = 184 samples/timepoint) over the first 7 wk of lactation. Blue line = control cows (CON, *n* = 23); Red line = treatment cows (TRT, *n* = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The IM period is highlighted with a grey background. The horizontal dashed line designates the upper SCC threshold for healthy quarters, i.e., 100,000 cells/mL (DVG, 2009). Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$), ** ($P < 0.001$), and + ($P \leq 0.10$) at a given time point, respectively.

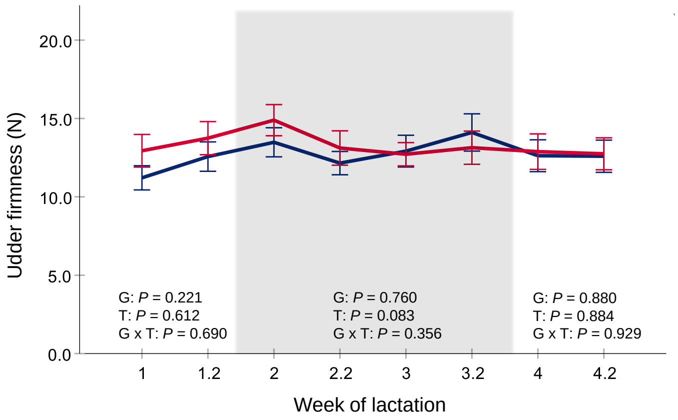
Cases of milk leakage occurred in both groups before and after fore-milking (Supplemental Table 2). Before fore-milking, at the second assessment in the first week of IM, more cases of milk leakage occurred in TRT (7 cases) than in CON (1 case). After fore-milking, at both assessments in the second week with IM, also more cases of milk leakage occurred in TRT (6 and 5 cases) than in the CON cows (1 and 0 cases), respectively (Supplemental Table 2). After milking, milk leakage was not observed in either group.

Supplemental Table 2. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation as compared to conventional milking on number of cases of milk leakage recorded twice a wk before fore-milking, after fore-milking and after milking from lactation wk 1 to 4 in control cows (CON, *n* = 23) and treatment cows (TRT, *n* = 23).

Milk leakage / Period	Measurement no.	No. of cases		*P*-Value
		TRT[1]	CON	
Before fore-milking				
Week before IM	1	0	1	0.312
	1.2	3	2	0.636
Wk 1 of IM	2	6	3	0.265
	2.2	7	1	0.020
Wk 2 of IM	3	8	4	0.179
	3.2	8	7	0.753
Week after IM	4	4	6	0.475
	4.2	0	1	0.312
After fore-milking				
Week before IM	1	0	1	0.312
	1.2	0	1	0.312
Wk 1 of IM	2	3	1	0.295
	2.2	2	1	0.550
Wk 2 of IM	3	6	1	0.040
	3.2	5	0	0.018
Week after IM	4	1	1	1.000
	4.2	0	2	0.148
After finishing milking				
Week before IM	1	0	0	-
	1.2	0	0	-
Wk 1 of IM	2	0	0	-
	2.2	0	0	-
Wk 2 of IM	3	0	0	-
	3.2	0	0	-
Week after IM	4	0	0	-
	4.2	0	0	-

[1]The TRT cows were milked incompletely twice daily from d 8 ± 1.1 until d 21 ± 1.1 of lactation.
The amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started.

Udder firmness before milking did not differ between the groups during the entire observation period (Supplemental Figure 2). The last milk flow in the measuring unit at the moment of triggering cluster removal was 2.87-fold higher in the TRT than in the CON cows (Figure 4a). After switching back to conventional milking, no further differences were observed (Figure 4a). Before the start of IM, milking took the same time in both groups (Figure 4b). During IM, the CON cows had 1.37-fold longer (*P* = 0.001) milking times than the TRT cows (7.53 min ± 0.13 vs. 5.49 min ± 0.10). In the week after IM, milking time was again the same in both groups (average of both groups: 7.74 min ± 0.14; Figure 4b).



Supplemental Figure 2: Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on udder firmness before milking. The measurement was performed twice a wk, over the first 4 wk of lactation. Blue line = control cows (CON, *n* = 23); Red line = treatment cows (TRT, *n* = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The IM period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$) or ** ($P < 0.001$), and + designates trends ($P \leq 0.10$) at a given time point, respectively.

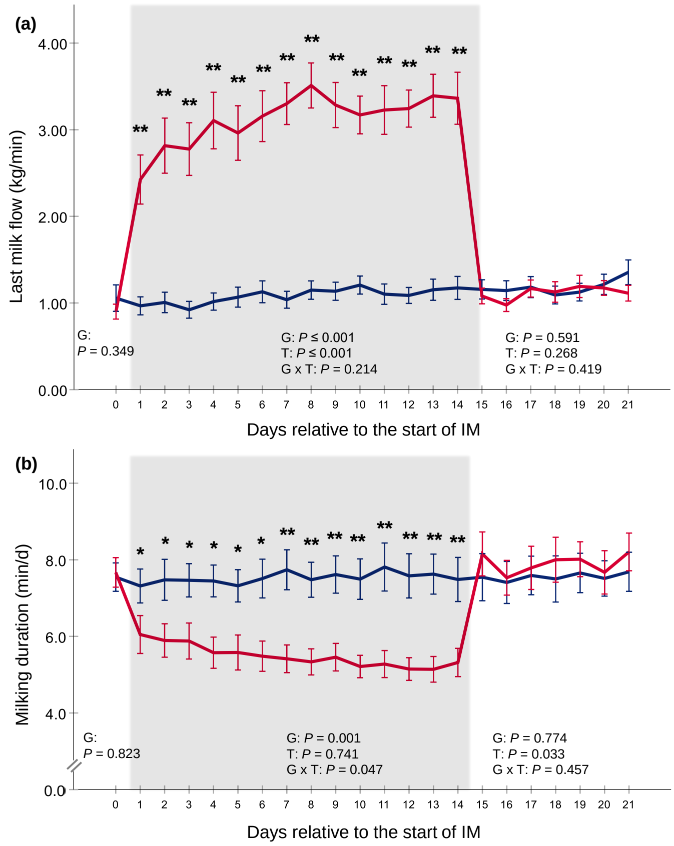


Figure 4. Effect of automated and incomplete milking (IM) in wk 2 and 3 of lactation on (a) last milk flow per day, and (b) daily milking duration over the first 21 d relative to the start of IM. Day 0 is the d before starting the treatment. Blue line = control cows (CON, *n* = 23); Red line = treatment cows (TRT, *n* = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$), ** ($P < 0.001$), and + ($P \leq 0.10$) at a given time point, respectively.

Indicators of the energetic status

The DMI in the CON cows tended to be higher than in the TRT group during the IM period (Figure 5 a). In the first IM week, both groups had similar DMI (*P* = 0.286; CON 21.8 kg/d ± 0.34; TRT 21.3 kg/d ± 0.32). Both groups increased their DMI in the subsequent week, without further group differences and averaging 25.9 kg/d ± 0.11 (Figure 5 a). For water intake, there was a group x time interaction (*P* = 0.006) during IM due to increased water intake by the CON cows in contrast to constant intakes in the TRT cows. Distinct group differences were observed in the second week of IM: the CON cows drank more water than the TRT cows (CON 88.4 L/d ± 1.96; TRT 79.6 L/d ± 1.78). In the subsequent period, no further differences between groups were observed (average of both groups: 85.0 L/d ± 0.40; Figure 5 b).

Throughout each period of the experiment, BW was not different between groups (CON: 662 kg/d ± 3.9; TRT: 660 kg/d ± 3.9; Figure 5 c). When all time points were considered in the statistical model, time was significant for BCS, BFT, and MLD (Supplemental Figure 3 a to 3c), but treatment was insignificant; only for BFT, a trend (*P* = 0.055) for greater values in CON cows was noted. When considering BFT in the period before calving, CON cows had approximately 0.32 cm thicker back fat than TRT cows (*P* = 0.043; 1.44 cm ± 0.11 vs. 1.12 cm ± 0.08), whilst no difference between groups occurred in the period after calving (average of both groups:1.10 cm ± 0.039; Supplemental Figure 3 b). The CON cows exhibited the most pronounced decrease from wk 3 before calving (1.32 cm ± 0.14) to wk 6 after calving (1.11 cm ± 0.10) by 0.21 cm, whereas BFT in TRT cows declined by only 0.03 cm (1.02 cm ± 0.10 to 0.99 cm ± 0.10). The BCS declined from wk 3 before to wk 6 after calving by about 0.27 in both groups (3.38 ± 0.05 to 3.11 ± 0.05; Supplemental Figure 3 a). In both groups MLD declined by about 0.51 cm from the period before to after calving (average of both groups: 5.63 cm ± 0.09 to 5.12 cm ± 0.08 Supplemental Figure 3 c).

The calculated EB during the IM period was not influenced by group but by time (Figure 5 d). Altogether, the two weeks of constant IM lead to 14.4 MJ /d less energy output with milk in TRT as compared to CON cows (120.9 MJ /d). The DMI in TRT cows tended to increase less than in the CON cows during IM, and the energy intake of TRT cows was approximately 5.4 MJ NE_L/d below that of CON cows (150.7 MJ NE_L/d). Considering that the NE requirement for maintenance did not differ between groups (average of both groups: 38.0 MJ/d), around 9.0 MJ NE_L/d could be saved in TRT cows during the entire period of IM. In general, the EB during that time was hardly negative, with a nadir in the second week of CON cows that was not undercut in the entire observation period. The EB increased with time in both groups without group differences throughout the observation time ($P = 0.664$); the average EB of both groups between wk 4 and 15 of lactation was 8.75 MJ NE_L/d ± 0.71.

The circulating concentrations of NEFA, BHB, IGF-1, and leptin did not differ between the groups in each of the periods recorded (Figure 6 a to d).

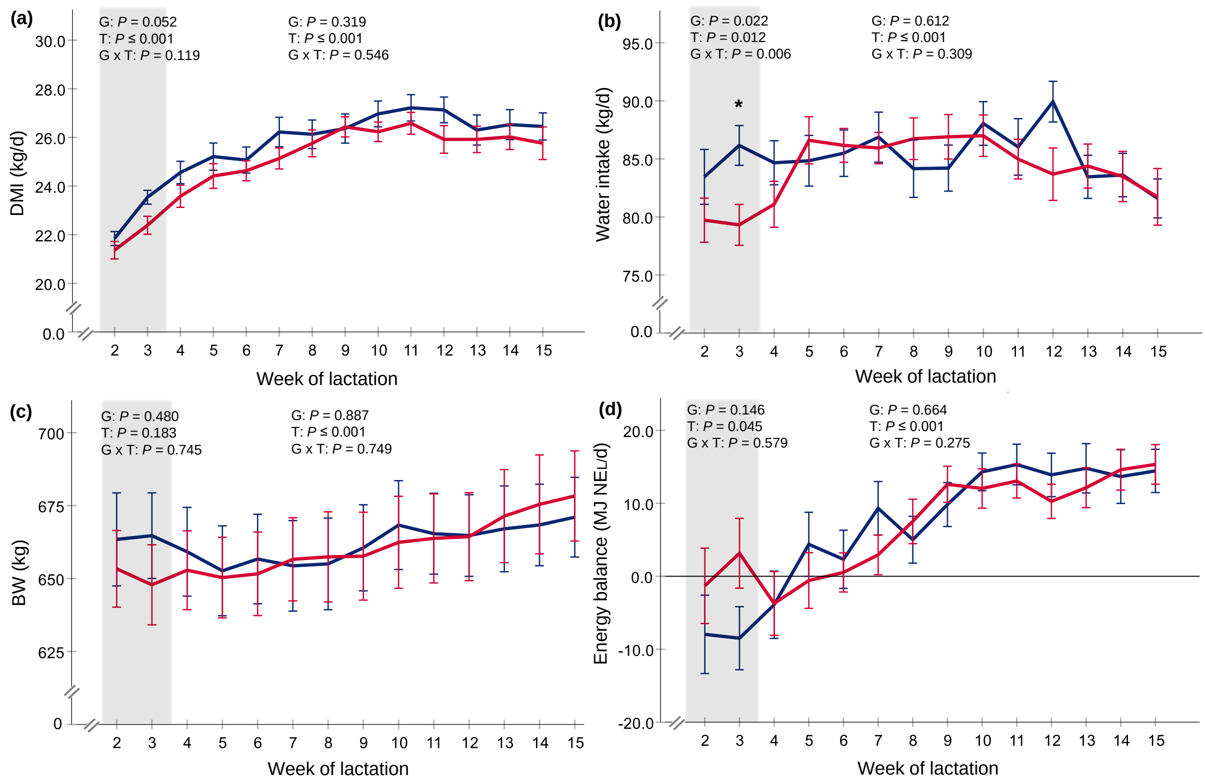
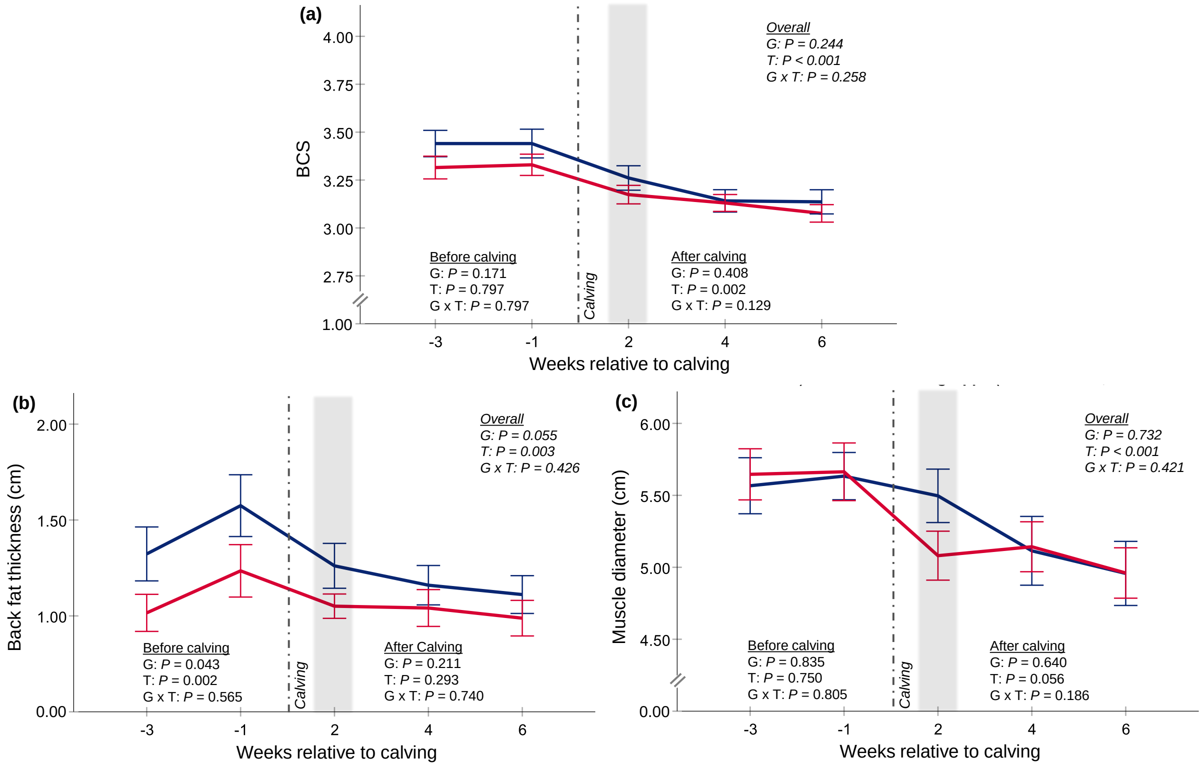


Figure 5. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on (a) DMI, (b) water intake, (c) BW over the first 15 wk of lactation, and (d) energy balance (EB) over the first 15 wk of lactation. Blue line = control cows (CON, $n = 23$); Red line = treatment cows (TRT, $n = 23$). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a grey background. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$), ** ($P < 0.001$), and + ($P \leq 0.10$) at a given time point, respectively.



Supplemental Figure 3: Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on the course of (a) BCS (b) back fat thickness (BFT) and (c) muscle diameter of the musculus longissimus dorsi (MLD). The measurements were performed every other wk, starting from wk -3 to 6 relative to calving. Blue line = control cows (CON, n = 23); Red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The IM period is highlighted with a grey background. The vertical dashed line illustrates calving. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$) or ** ($P < 0.001$), and + designates trends ($P \leq 0.10$) at a given time point, respectively.

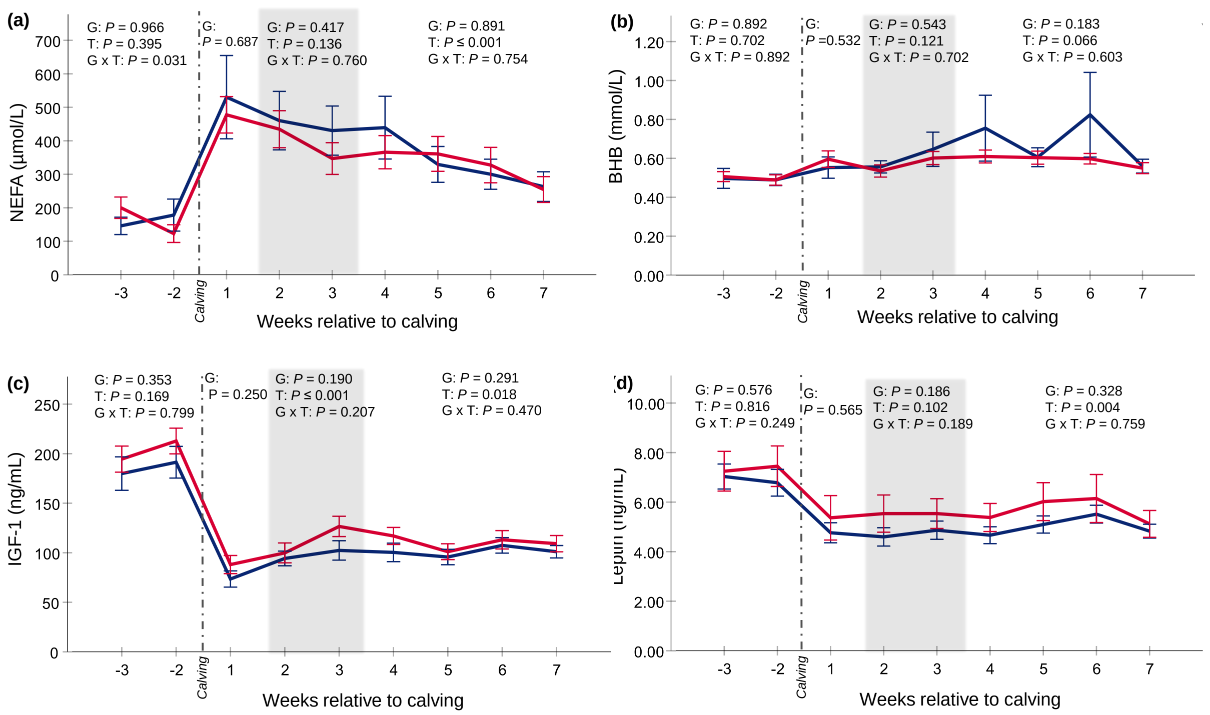


Figure 6. Effect of automated incomplete milking (IM) in wk 2 and 3 of lactation on serum concentrations of (a) non-esterified fatty acids (NEFA), (b) BHB, (c) IGF-1, and (d) leptin over the first 15 wk of lactation. Blue line = control cows (CON, n = 23); Red line = treatment cows (TRT, n = 23). In TRT, the amount of milk (kg) harvested was limited to the amount of milk collected 1 d before IM was started. The treatment period is highlighted with a greay background. The vertical dashed line designates calving. Data are presented as means ± SEM. Differences between the groups are indicated with asterisks: * ($P \leq 0.05$), ** ($P < 0.001$), and + ($P \leq 0.10$) at a given time point, respectively.

DISCUSSION

To mitigate the energy deficit in early lactation of pluriparous cows, we used the IM approach thus modifying the energy output with milk as one of the main variables affecting EB. We selected DIM 8 for the start of IM since we aimed at integrating it into the normal milking routine when cows do not have to be milked separately any more. According to the legislation in most countries, the mammary secretions during the colostrum phase are excluded in the definition of salable milk, for example by stressing that milk has to be "practically free from colostrum" as in the USA (FDA, 1973, 21 C.F.R. § 131.110(a)) or by explicitly banning colostrum and transition milk from the the first DIM from sale (e.g. 5 days in Germany (§ 18, (2) Milchverordnung)). Moreover, depending on the length of the preceding dry period, the maximal residue concentration of antibiotics applied for dry cow therapy may still be exceeded during the first d of milking (Bachmann et al., 2018), thus requiring separation of the milk. However, the greater practicability of starting the IM only after the first week of lactation is offset by the loss of salable milk. To minimize the latter as well as the risk for possible adverse effects that may result from IM, such as increased intra-mammary pressure (Krug et al., 2017; Blau et al., 2019), increasing SCC (Penry et al., 2017; Albaaj et al., 2018; Kuehnl et al., 2019), and irreversible decline in productivity known from once-daily milking (**ODM**; Stelwagen et al., 2013; Lacasse et al., 2018), we performed a 2-wk constant IM to achieve a smooth delay in the increase in milk yield during the first weeks of lactation. For practical realization, a software module, first developed as a tool for dry-off (Martin et al., 2020; Schmidt et al., 2020), was used that allows individual automatic cluster removal after reaching a defined amount of milk instead of a defined milk flow.

Milk production

In the present study, besides daily milk yield, the last milk flow rate in the measuring unit at the moment of triggering milk yield-dependent cluster removal was recorded to reflect IM and indicate the possible subsequent adaptation of the mammary gland. During the IM period, the average last milk flow was elevated to about 3.1 kg/min in TRT cows, reflecting the remaining udder filling due to the IM and was well above common cluster removal thresholds as applied for practical or scientific purposes (Krawczel et al., 2017; Wieland et al., 2020). Given that the value of the last milk flow remained relatively constant during the period of IM in TRT cows, we assumed that milk production was largely maintained and not adapted to the lesser milk removal. So far, only two studies (Penry et al., 2017; Kuehnl et al., 2019) have captured the development of milk production during IM in early lactating cows. In both studies, from DIM 5 to 47, a constant percentage of milk was removed (targeting approximately 30% of milk remaining in the gland) using a half-udder design. The milk production rate was calculated once per week when the TRT udder half was milked completely to recalibrate the estimate of the target milk yield. Contrary to our findings, a decrease in milk production rate in TRT udder halves of approximately 25% (Penry et al., 2017) and 27% (Kuehnl et al., 2019) was observed during the period of IM. Moreover, Deacon et al. (2023) recently applied the IM concept of Penry et al. (2017) for 4 wk in mid-lactating cows. Although they observed a decline in milk production, the mammary gland adapted quickly to IM, with milk production reaching a new level within a few days (Deacon et al., 2023).

After switching back to conventional milking with milk flow-dependent automatic cluster removal, we observed that milk production of the TRT cows reached the level of the CON cows immediately, increasing by 18.6% above the yield from the preceding last day of IM. The increase in milk yield on the first day of conventional milking could be attributed to an accumulation of residual milk that was retained in the mammary gland due to IM, an effect that has also been observed in previous studies (Albaaj et al., 2018; Martin et al., 2020). Besides the accumulation effect, an additional feature of milk stasis caused by IM (Albaaj et al., 2018; Deacon et al., 2023) or extended milking intervals (Stelwagen et al., 2013; Lacasse et al., 2018) can be reduced productivity beyond treatment. However, we observed no further group differences in daily and weekly milk yield from the second day of conventional milking onwards. Moreover, we could not find a difference in the day of reaching peak yield and peak yield between groups. Therefore, milk losses due to IM were limited solely to the treatment period, suggesting that a 2-wk constant IM while maintaining the overall milking routine may be used without compromising subsequent performance.

Investigations of potentially adverse carry-over effects on performance after IM during the colostrum phase showed either no (Carbonneau et al., 2012; Valldecabres et al., 2022) or only negligible (Krug et al., 2018b) effects on subsequent milk production. In studies applying IM for several weeks in early lactation (Penry et al., 2017; Kuehnl et al., 2019), the consequences for the post-treatment milk yields were not reported. However, Deacon et al. (2023) determined the mammary functional capacity of TRT udder halves after terminating a 4-wk IM in mid-lactation cows (targeting approximately 30% of milk remaining in the gland). They observed a greater loss of mammary functional capacity in the TRT udder half, suggesting an irreversible loss of mammary tissue due to IM (Deacon et al., 2023).

The cause of the variation in findings between our and previous studies may be due to the differences in experimental design concerning the targeted amount of milk remaining in the gland, the treatment duration, and (or) the phase of lactation in which the IM was tested. In future studies, various degrees of IM should be combined with different treatment lengths to identify the range initiating the sustained modulation of milk production. Moreover, a better understanding of the underlying adaption processes on the functional level remains to be determined. Since a body of evidence was generated for the galactopoietic role of PRL in dairy cows (Lacasse et al., 2016), Deacon et al. (2023) were the first, who determined the abundance and activation levels of proteins related to PRL signaling in mammary gland biopsies obtained in the week after completing IM. As only negligible effects of IM on PRL signaling were observed, these results constrain the assumption of a modulatory effect of IM on mammary gland responsiveness to the galactopoietic signal of PRL (Deacon et al., 2023). Aside from PRL, other autocrine-paracrine mechanisms (e.g., parathyroid hormone-related protein, transforming growth factor, IGF-1, epidermal growth factor, and serotonin) which are involved in the functional adaption of the mammary gland to IM (Weaver and Hernandez, 2016) remain to be investigated.

Milk composition and energy-corrected milk

In general, ECM and milk yield from the TRT cows differed by a similar extent (by 12 and 11 %, respectively) from the CON cows during the IM period. After switching back to conventional milking, group differences in ECM were levelling off, thus supporting that milk production including composition were not decreased beyond the treatment period. For lactose, a largely consistent decrease in the concentration was reported during IM in our and in previous studies (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023). The milk lactose content after IM has only been reported in the study by Carbonneau et al. (2012) and was not different between the groups from wk 2 to 9 of lactation. We also observed no group differences in lactose content after IM. In most studies (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023), the decrease in milk lactose content was mainly related to the loss of tight junction integrity of the mammary gland and the related efflux of lactose from milk into blood, which may be caused by increasing intra-mammary pressure as a consequence of IM (Stelwagen and Singh, 2014). The decreased milk lactose content during the period of IM observed in our study may also be explained by reduced lactose synthesis (Grala et al., 2011) and lower mammary uptake of glucose (Guinard-Flament et al., 2007) as already described in the context of extended milking intervals (Stelwagen et al., 2013), though we did not assess blood lactose concentrations.

The causes for differences in milk composition between studies could be, besides differences in the degree and duration of IM, a varying sampling procedure. Kuehnl et al. (2019) pointed to the importance of milking fraction to explain the differences between their and another study using the same experimental design (Penry et al., 2017). Likewise, the varying milk sampling frequency between studies ranged that from once (Carbonneau et al., 2012; Deacon et al., 2023) or twice per week (Penry et al., 2017; Kuehnl et al., 2019) to once per month (Krug et al., 2018b), could account for the different outcomes.

Udder health, indicators of udder filling, and milking duration

During the period of IM, we found neither signs of impaired udder health nor increased SCC in TRT cows. After IM, the SCC in TRT cows increased but did not exceed the threshold of 100.00 cells/mL milk per quarter as an indicator of inflammation (DVG, 2009). Furthermore, no positive bacteriological findings were detected in any of the cows during the first 7 wk of lactation, thus supporting that a 2-wk constant milk withdrawal does not impede udder health. Similar results were obtained in studies in which IM was applied during the first days of lactation (Carbonneau et al., 2012; Krug et al., 2018a; Valldecabres et al., 2022). All these studies consistently reported no increase of the incidence of clinical mastitis due to IM, but the impact on SCC differed between studies. Conducting IM for a single milking interval (Albaaj et al., 2018) or several weeks (Penry et al., 2017; Kuehnl et al., 2019) lead to a slight increase in SCC during IM. Penry et al. (2017) assumed that the differences in SCC between groups could be due to the increased dilution of SCC in the completely milked udder half. In contrast, Albaaj et al. (2018) presumed an inflammatory reaction in cows as a response to increased milk accumulation. However, the differences could also be due to the aforementioned variation in IM settings across studies.

Milk leakage is a symptom of compromised teat sphincter function that may occur during all stages of lactation and is associated with an increased risk of mastitis (Rovai et al., 2007; Gott et al., 2016). The occurrence of milk leakage is farm-specific and ranged between 0 and 36% of cows within a farm (Schukken et al., 1990). We decided to control milk leakage before and after fore-milking when the amount of milk stored in the udder due to IM and the resulting increased pressure on the teat sphincter was assumed to be greatest, and after milking to indicate whether rising milk accumulation could reinforce milk leakage even though the milking process has already been finished. The greater portion of cows with milk leakage in the TRT group, both before (30%) and after fore-milking (24%) is in line with Gleeson et al. (2007), who reported that 30% of ODM cows showed milk leakage in early lactation. Milk leakage can be caused by multiple factors, such as stage of lactation (Bruckmaier and Hilger, 2001; Klaas et al., 2005), rising internal milk pressure (Persson Waller et al., 2003; Bruckmaier and Wellnitz, 2008), anatomical characteristics (e.g., udder storage capacity; Bruckmaier and Hilger, 2001), and functional traits (e.g., milk flow profiles; Klaas et al., 2005; Rovai et al., 2007). However, no cases of milk leakage were observed in either group after completing milking, indicating that the milk left in the mammary gland immediately after milking due to constant milk withdrawal did not exceed the closing forces of the teat sphincter of cows.

For addressing intra-mammary pressure that is associated with discomfort and pain for the cow (Tucker et al., 2007), we also assessed udder firmness as a proxi for udder filling and intra-mammary pressure. We observed no group differences in udder firmness before milking, suggesting that a 2-wk IM does not cause discomfort for the cows. Krug et al. (2017) investigated the impact of IM on lying behavior as an indicator of discomfort due to increased intra-mammary pressure. During the IM period, they collected a maximum of 10 to 14 L milk in the first 5 DIM, and lying behavior was recorded via acceleration data loggers attached to the hind leg of cows. Neither lying time was shortened nor adverse effects were observed on lying bouts or lying bout duration during the IM period (Krug et al., 2017).

Labor costs are a key factor in the decision-making process on commercial farms concerning changes in the milking strategy (Stockdale, 2006). Thus, reducing the duration of milking can improve labor efficiency and also reduces the mechanical load of the teat tissue due to shortened cluster attachment time (Martin et al., 2020; Wieland et al., 2020). During IM, milking time was about 2 min shorter for TRT than for CON cows, and increased to the same level as in CON cows after IM in our study. Edwards et al. (2013) observed that approximately 1.33 min per cow could be saved when changing the milk flow rate thresholds from 0.2 kg/min to 0.8 kg/min. Even though reducing the milking frequency to ODM halves the required milking time, it is accompanied by an irreversible loss of productivity thereafter (Stelwagen et al., 2013). In addition to the time savings for the milking process, we could diminish extra effort outside of the regular milking routine through the software-induced automation of cluster removal after obtaining a defined amount of milk. In contrast, milk-flow dependent cluster removal requires manual intervention by the producer and enhances the potential for errors in accurate cluster removal after reaching the target milk yield (Morin et al., 2018). Accordingly, our findings confirm the applicability of the automated cluster removal that is further associated with less milking time needed during IM.

Indicators of the energetic status

In the present study, DMI was hardly compromised, with approximately 21.8 kg DM/d already in the second lactation week of CON cows thus exceeding the values typical for this period (Ingvartsen and Andersen, 2000; Grummer et al., 2004). However, DMI in TRT cows did not increase as rapidly as in CON cows; similarly, water intake was less in the TRT cows thus confirming the association between DMI and water intake (Kramer et al., 2009; Kume et al., 2010). After IM all group differences were diminished. In contrast, ODM studies with accurate DMI recordings have reported both reduced DMI in ODM cows or no differences compared to twice-daily milked cows (Stelwagen et al., 2013); for example, McNamara et al. (2008) suggested that the reduced milk withdrawal during IM periods induces an adaptation of feed intake. In future studies, DMI should be recorded during the IM phase to determine whether this phenomenon of decreased feed intake recurred. Moreover, the underlying mechanisms involved in the DMI regulation of IM cows remain to be investigated.

In order to maintain milk synthesis even during a NEB, body fat and protein reserves are mobilized (Kuhla et al., 2011) what in turn leads to losses of BW and body condition (Pires et al., 2013). When reducing the milking frequency to once daily, an improvement of the energetic status was reflected in an attenuated loss of BW and BCS in ODM when compared to twice-daily milked cows, as summarized by Stelwagen et al. (2013). Similar to Carbonneau et al. (2012), who harvested 6 - 14 L milk/d in the first 5 DIM, we observed in our study no group differences in BW. Moreover, the IM treatment did not affect BCS, BFT, and MLD. Likewise, Morin et al. (2018) observed no differences in BCS between the groups after removing only a maximum of 10 - 14 L milk in the first 5 DIM of cows. It is known that fatter cows are mobilizing more body resevers when entering lactation (Pires et al., 2013); however, the trend for greater BFT in the CON cows ante partum was not related to greater BFT losses after calving in the present study.

In a review, Lacasse et al. (2018) summarized the efficacy of milking management strategies, e.g., the use of dopamine agonists to inhibit PRL release, prepartum milking, ODM, or IM, for reducing the susceptibility to metabolic and infectious diseases. The strategy of IM turned out as the most promising approach for reducing metabolic stress without compromising the productivity of high-yielding dairy cows (Lacasse et al., 2018). So far, studies aiming at improving the metabolic situation in early lactation by IM were largely limited to the colostrum phase (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022). In contrast, we we decided to start the 2-wk IM in the second week after parturition when the milk is no longer separated and limited the energy output with milk by clamping the amount of milk withdrawn to a constant level. In general, we observed that the EB during that time was hardly negative, with a nadir of only -8.5 MJ NE_L/d in the second lactation week of CON cows and thus was above the values that are typical for early lactating cows (e.g., Vries and Veerkamp, 2000; Weber et al., 2013). However, we are the first providing data about the calculated EB in the context of IM treatment, whereas previous IM studies have focused solely on the analysis of blood metabolites to reflect the energy status of cows due to IM (Carbonneau et al., 2012; Morin et al., 2018; Valldecabres et al., 2022).

In general, the metabolic condition of both groups from our study was hardly changing during the observation period. During the first 7 wk of lactation, the average blood concentrations of NEFA and BHB remained within the range considered as healthy. Only 3 cases of hyperketonemia (blood BHB >1.2 mmol/L) in CON cows and 0 cases in TRT cows were observed in the period after calving. We also observed no group differences in the circulating concentrations of NEFA, BHB, IGF-1, and leptin in each of the periods recorded. The energy status of both groups seemed hardly challenged by the onset of lactation and thus the effects of IM might have been more pronounced in a situation of a more NEB. Likewise, Valldecabres et al. (2022) found no group difference in blood concentration of BHB when removing only 3 L milk/milking on the first 2 DIM compared to twice daily milked cows. However, a small numerical difference in the proportion of cows with subclinical ketosis (blood BHB > 1.2 mmol/L) was observed between CON (42%) and TRT (18%) cows at 3 and 11 DIM (Valldecabres et al., 2022). They suggested that the findings further elucidate the relevance of the degree and duration of IM (Valldecabres et al., 2022). In contrast, the preceding study of Carbonneau et al. (2012), who harvested a maximum of 6 - 14 L milk in the first 5 DIM of cows (n = 47), observed that within the treatment period and lasting until 21 DIM, the glucose concentration increased while the BHB concentration decreased in the TRT compared to the CON group. The NEFA concentration was also lower in the TRT compared to the CON group but showed no carry-over effect beyond the IM period. Furthermore, both Carbonneau et al. (2012) and Morin et al. (2018), reported the number of cows with subclinical and clinical ketosis was smaller in cows that underwent IM. Morin et al. (2018) suggested that the treatment effect would be greater in herds with more NEB and interpreted the low prevalence of subclinical ketosis was due to the well-functioning transition management of the enrolled commercial farms (Morin et al., 2018). Nevertheless, they and also Carbonneau et al. (2012) concluded that IM in the colostrum period is an effective strategy to reduce the prevalence of hyperketonemia in the early postpartum period. Altogether, the degree of milk accumulation and the duration of milk stasis seems to influence the findings and stronger effects can be expected in herds with a more extended NEB.

CONCLUSION

Our findings confirm the applicability of the milking software module used herein for delaying the increase in milk yield during the first weeks of lactation through a 2-wk automated, constant milk withdrawal. When IM ceased, milk production of the TRT cows immediately reached the level of the CON cows, and udder health was not compromized over the entire observation period in all cows. The EB, as well as body condition and several blood metabolites, remained largely unaffected, as the TRT cows tended to eat less than the CON cows during the period of IM. It remains to be determined whether the absent effect on energy metabolism was due to the relatively stable energy status of the cows or to the relatively mild IM setting used here. Further research is needed to understand the link between the degree and duration of IM and the adaptive mechanism of the organism in terms of milk synthesis, feed intake, and energy metabolism.

ACKNOWLEDGMENTS

This study was partly conducted by members of the Center of Integrated Dairy Research (CIDRe) of the University of Bonn, Germany. The research project was funded by the Inter-Departmental Center of Sustainable Agriculture of the Ministry of Agriculture and Consumers' Affairs of the State of North Rhine-Westphalia, Germany. We thank the cattle team of the Campus Frankenforst for their help to carry out the experiment on the dairy cows. We also thank Thomas Blees, Barbara Heitkönig, Katharina Kustwan and Karin Strack from the Institute of Animal Science of the University of Bonn, and the laboratory staff of the Institute of Animal Nutrition of the Friedrich-Loeffler-Institute in Braunschweig, for their help in conducting the laboratory analyses from this experiment.

ORCIDS

Isabell Meyer: https://orcid.org/0000-0002-2631-4425

Eva Haese: https://orcid.org/0000-0001-7795-1750

Karl-Heinz Südekum: https://orcid.org/0000-0002-8147-1060

Helga Sauerwein: https://orcid.org/0000-0002-6905-4053

Ute Müller: https://orcid.org/0000-0002-0929-8195

REFRENCES

Albaaj, A., P. G. Marnet, C. Hurtaud, and J. Guinard-Flament. 2018. Adaptation of dairy cows to increasing degrees of incomplete milk removal during a single milking interval. J. Dairy Sci. 101(9):8492–8504. https://doi.org/10.3168/jds.2018-14451.

Bachmann, J., C. Helmschrodt, A. Richter, W. Heuwieser, and S. Bertulat. 2018. Residue concentration of cefquinome after intramammary dry cow therapy and short dry periods. J. Dairy Sci. 101(8):7540–7550. https://doi.org/10.3168/jds.2017-13826.

Bertulat, S., C. Fischer-Tenhagen, A. Werner, and W. Heuwieser. 2012. Technical note: validating a dynamometer for noninvasive measuring of udder firmness in dairy cows. J. Dairy Sci. 95(11):6550–6556. https://doi.org/10.3168/jds.2012-5370.

Blau, U., L. Zanini, and R. M. Bruckmaier. 2019. Intramammary pressure and udder firmness during a 72-h interruption of milking to simulate dry-off, with and without feed restriction. J. Dairy Sci. 102(8):7548–7555. https://doi.org/10.3168/jds.2019-16333.

Bruckmaier, R., E. Lehmann, D. Hugi, H. Hammon, and J. Blum. 1998a. Ultrasonic measurement of longissimus dorsi muscle and backfat, associated with metabolic and endocrine traits, during fattening of intact and castrated male cattle. Livest. Prod. Sci. 53(2):123–134. https://doi.org/10.1016/S0301-6226(97)00162-0.

Bruckmaier, R. M., L. Gregoretti, F. Jans, D. Faissler, and J. W. Blum. 1998b. Longissimus dorsi muscle diameter, backfat thickness, body condition scores and skinfold values related to metabolic and endocrine traits in lactating dairy cows fed crystalline fat or free fatty acids. Zentralbl. Veterinaermed. A 45(6-7):397–410. https://doi.org/10.1111/j.1439-0442.1998.tb00842.x.

Bruckmaier, R. M., and M. Hilger. 2001. Milk ejection in dairy cows at different degrees of udder filling. J. Dairy Res. 68(3):369–376. https://doi.org/10.1017/S0022029901005015.

Bruckmaier, R. M., and O. Wellnitz. 2008. Induction of milk ejection and milk removal in different production systems. J. Anim. Sci. 86(13 Suppl):15–20. https://doi.org/10.2527/jas.2007-0335.

Carbonneau, E., A. M. de Passillé, J. Rushen, B. G. Talbot, and P. Lacasse. 2012. The effect of incomplete milking or nursing on milk production, blood metabolites, and immune functions of dairy cows. J. Dairy Sci. 95(11):6503–6512. https://doi.org/10.3168/jds.2012-5643.

Deacon, A.-M., R. Blouin, C. Thibault, and P. Lacasse. 2023. Mechanism underlying the modulation of milk production by incomplete milking. J. Dairy Sci. 106(1):783–791. https://doi.org/10.3168/jds.2022-22164.

Deutsche Veterinärmedizinische Gesellschaft (DVG). 2009. Leitlinien Entnahme von Milchproben unter antiseptischen Bedingungen und Isolierung und Identifizierung von Mastitiserregern. 2nd ed.; Verlag Deutsche Veterinärmedizinische Gesellschaft Service GmbH, Gießen, Germany.

Drackley, J. K., H. M. Dann, N. Douglas, N. A. J. Guretzky, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Ital. J. Anim. Sci. 4(4):323–344. https://doi.org/10.4081/ijas.2005.323.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A Body Condition Scoring Chart for Holstein Dairy Cows. J. Dairy Sci. 72(1):68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Edwards, J. P., J. G. Jago, and N. Lopez-Villalobos. 2013. Milking efficiency for grazing dairy cows can be improved by increasing automatic cluster remover thresholds without applying premilking stimulation. J. Dairy Sci. 96(6):3766–3773. https://doi.org/10.3168/jds.2012-6394.

Esposito, G., P. C. Irons, E. C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144(3-4):60–71. https://doi.org/10.1016/j.anireprosci.2013.11.007.

Gesellschaft für Ernährungsphysiologie (GfE). 2001. Empfehlung zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG Verlag.

Gleeson, D. E., B. O'Brien, L. Boyle, and B. Earley. 2007. Effect of milking frequency and nutritional level on aspects of the health and welfare of dairy cows. Animal. 1(1):125–132. https://doi.org/10.1017/S1751731107658030.

Gott, P. N., P. J. Rajala-Schultz, G. M. Schuenemann, K. L. Proudfoot, and J. S. Hogan. 2016. Intramammary infections and milk leakage following gradual or abrupt cessation of milking. J. Dairy Sci. 99(5):4005–4017. https://doi.org/10.3168/jds.2015-10348.

Grala, T. M., C. V. C. Phyn, J. K. Kay, A. G. Rius, M. D. Littlejohn, R. G. Snell, and J. R. Roche. 2011. Temporary alterations to milking frequency, immediately post-calving, modified the expression of genes regulating milk synthesis and apoptosis in the bovine mammary gland. Proc. N.Z. Soc. Anim. Prod. 71:3–8

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. Vet Clin North Am Food Anim Pract. 20(3):447–470. https://doi.org/10.1016/j.cvfa.2004.06.013.

Guinard-Flament, J., E. Delamaire, P. Lamberton, and J. L. Peyraud. 2007. Adaptations of mammary uptake and nutrient use to once-daily milking and feed restriction in dairy cows. J. Dairy Sci. 90(11):5062–5072. https://doi.org/10.3168/jds.2007-0259.

Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83(7):1573–1597. https://doi.org/10.3168/jds.S0022-0302(00)75029-6.

Jouany, J.-P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim. Reprod. Sci. 96(3-4):250–264. https://doi.org/10.1016/j.anireprosci.2006.08.005.

Klaas, I. C., C. Enevoldsen, A. K. Ersbøll, and U. Tölle. 2005. Cow-related risk factors for milk leakage. J. Dairy Sci. 88(1):128–136. https://doi.org/10.3168/jds.S0022-0302(05)72670-9.

Kok, A., J. Chen, B. Kemp, and A. T. M. van Knegsel. 2019. Review: Dry period length in dairy cows and consequences for metabolism and welfare and customised management strategies. Animal. 13(S1):s42-s51. https://doi.org/10.1017/S1751731119001174.

Kramer, E., E. Stamer, J. Spilke, G. Thaller, and J. Krieter. 2009. Analysis of water intake and dry matter intake using different lactation curve models. J. Dairy Sci. 92(8):4072–4081. https://doi.org/10.3168/jds.2008-1957.

Krawczel, P., S. Ferneborg, L. Wiking, T. K. Dalsgaard, S. Gregersen, R. Black, T. Larsen, S. Agenäs, K. Svennersten-Sjaunja, and E. Ternman. 2017. Milking time and risk of over-milking can be decreased with early teat cup removal based on udder quarter milk flow without loss in milk yield. J. Dairy Sci. 100(8):6640–6647. https://doi.org/10.3168/jds.2016-12312.

Krug, C., T. J. DeVries, J.-P. Roy, J. Dubuc, and S. Dufour. 2017. Incomplete Milking in Early Lactation Does Not Affect Dairy Cows Resting Behaviors: Results from a Randomized Controlled Trial. Front. Vet. Sci. 4:66. https://doi.org/10.3389/fvets.2017.00066.

Krug, C., P.-A. Morin, P. Lacasse, J.-P. Roy, J. Dubuc, and S. Dufour. 2018a. Effect of incomplete milking during the first 5 days in milk on udder and reproductive tract health: Results from a randomized controlled trial. J. Dairy Sci. 101(10):9275–9286. https://doi.org/10.3168/jds.2018-14713.

Krug, C., P.-A. Morin, P. Lacasse, D. E. Santschi, J.-P. Roy, J. Dubuc, and S. Dufour. 2018b. A randomized controlled trial on the effect of incomplete milking during the first 5 days in milk on culling hazard and on milk production and composition of dairy cows. J. Dairy Sci. 101(5):4367–4377. https://doi.org/10.3168/jds.2017-14021.

Kuehnl, J. M., M. K. Connelly, A. Dzidic, M. Lauber, H. P. Fricker, M. Klister, E. Olstad, M. Balbach, E. Timlin, V. Pszczolkowski, P. M. Crump, D. J. Reinemann, and L. L. Hernandez. 2019. The effects of incomplete milking and increased milking frequency on milk production rate and milk composition1. J. Anim. Sci. 97(6):2424–2432. https://doi.org/10.1093/jas/skz113.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: implications for insufficient feed intake of transition dairy cows. Animal. 14(S1):s65-s77. https://doi.org/10.1017/S1751731119003124.

Kuhla, B., G. Nürnberg, D. Albrecht, S. Görs, H. M. Hammon, and C. C. Metges. 2011. Involvement of skeletal muscle protein, glycogen, and fat metabolism in the adaptation on early lactation of dairy cows. J. Proteome Res. 10(9):4252–4262. https://doi.org/10.1021/pr200425h.

Kume, S., K. Nonaka, T. Oshita, and T. Kozakai. 2010. Evaluation of drinking water intake, feed water intake and total water intake in dry and lactating cows fed silages. Livest. Sci. 128(1-3):46–51. https://doi.org/10.1016/j.livsci.2009.10.012.

Lacasse, P., S. Ollier, V. Lollivier, and M. Boutinaud. 2016. New insights into the importance of prolactin in dairy ruminants. J. Dairy Sci. 99(1):864–874. https://doi.org/10.3168/jds.2015-10035.

Lacasse, P., N. Vanacker, S. Ollier, and C. Ster. 2018. Innovative dairy cow management to improve resistance to metabolic and infectious diseases during the transition period. Res. Vet. Sci. 116:40–46. https://doi.org/10.1016/j.rvsc.2017.06.020.

Lacasse, P., X. Zhao, N. Vanacker, and M. Boutinaud. 2019. Review: Inhibition of prolactin as a management tool in dairy husbandry. Animal. 13(S1):s35-s41. https://doi.org/10.1017/S1751731118003312.

Martin, L. M., H. Sauerwein, W. Büscher, and U. Müller. 2020. Automated gradual reduction of milk yield before dry-off: Effects on udder health, involution and inner teat morphology. Livest. Sci. 233:103942. https://doi.org/10.1016/j.livsci.2020.103942.

McGuffey, R. K. 2017. A 100-Year Review: Metabolic modifiers in dairy cattle nutrition. J. Dairy Sci. 100(12):10113–10142. https://doi.org/10.3168/jds.2017-12987.

McNamara, S., J. J. Murphy, F. P. O'Mara, M. Rath, and J. F. Mee. 2008. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. Livest. Sci. 117(1):70–78. https://doi.org/10.1016/j.livsci.2007.11.013.

Morin, P.-A., C. Krug, Y. Chorfi, J. Dubuc, P. Lacasse, J.-P. Roy, D. E. Santschi, and S. Dufour. 2018. A randomized controlled trial on the effect of incomplete milking during early lactation on ketonemia and body condition loss in Holstein dairy cows. J. Dairy Sci. 101(5):4513–4526. https://doi.org/10.3168/jds.2017-13151.

Penry, J. F., E. L. Endres, B. de Bruijn, A. Kleinhans, P. M. Crump, D. J. Reinemann, and L. L. Hernandez. 2017. Effect of incomplete milking on milk production rate and composition with 2 daily milkings. J. Dairy Sci. 100(2):1535–1540. https://doi.org/10.3168/jds.2016-11935.

Persson Waller, K., T. Westermark, T. Ekman, and K. Svennersten-Sjaunja. 2003. Milk leakage--an increased risk in automatic milking systems. J. Dairy Sci. 86(11):3488–3497. https://doi.org/10.3168/jds.S0022-0302(03)73953-8.

Pires, J. A. A., C. Delavaud, Y. Faulconnier, D. Pomiès, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. J. Dairy Sci. 96(10):6423–6439. https://doi.org/10.3168/jds.2013-6801.

Rovai, M., M. T. Kollmann, and R. M. Bruckmaier. 2007. Incontinentia lactis: physiology and anatomy conducive to milk leakage in dairy cows. J. Dairy Sci. 90(2):682–690. https://doi.org/10.3168/jds.S0022-0302(07)71551-5.

Sauerwein, H., U. Heintges, M. Hennies, T. Selhorst, and A. Daxenberger. 2004. Growth hormone induced alterations of leptin serum concentrations in dairy cows as measured by a novel enzyme immunoassay. Livest. Prod. Sci. 87(2-3):189–195. https://doi.org/10.1016/j.livprodsci.2003.08.001.

Schmidt S, B. Linius, B. Maaßen-Francke, U. Müller, W. Büscher, and L. M. Martin. 2020. Method for reducing the individual-specific milk production of milk-producing animals United States Patent Application Publication: US Pat. No. 2020/0281151 A1.

Schukken, Y. H., F. J. Grommers, D. van de Geer, H. N. Erb, and A. Brand. 1990. Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 1. Data and risk factors for all cases. J. Dairy Sci. 73(12):3463–3471. https://doi.org/10.3168/jds.S0022-0302(90)79045-5.

Stelwagen, K., C. V. C. Phyn, S. R. Davis, J. Guinard-Flament, D. Pomiès, J. R. Roche, and J. K. Kay. 2013. Invited review: reduced milking frequency: milk production and management implications. J. Dairy Sci. 96(6):3401–3413. https://doi.org/10.3168/jds.2012-6074.

Stelwagen, K., and K. Singh. 2014. The role of tight junctions in mammary gland function. Mammary Gland Biol. Neoplasia 19(1):131–138. https://doi.org/10.1007/s10911-013-9309-1.

Stockdale, C. R. 2006. Influence of milking frequency on the productivity of dairy cows. Aust. J. Exp. Agric. 46(7):965. https://doi.org/10.1071/EA05308.

Susenbeth, A. 2018. Der Energiebedarf von Milchkühen heutiger Rassen. Tagungsband optiKuh. LfL-Schriftenreihe (2/2018):40-43.

Tucker, C. B., D. E. Dalley, J.-L. K. Burke, and D. A. Clark. 2007. Milking cows once daily influences behavior and udder firmness at peak and mid lactation. J. Dairy Sci. 90(4):1692–1703. https://doi.org/10.3168/jds.2006-577.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48(9):1215–1223. https://doi.org/10.3168/jds.S0022-0302(65)88430-2.

Valldecabres, A., R. B. Lopes, A. Lago, C. Blanc, and N. Silva-Del-Río. 2022. Effects of postpartum milking strategy on plasma mineral concentrations and colostrum, transition milk, and milk yield and composition in multiparous dairy cows. J. Dairy Sci. 105(1):595–608. https://doi.org/10.3168/jds.2021-20590.

Vries, M. J. de, and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83(1):62–69. https://doi.org/10.3168/jds.S0022-0302(00)74856-9.

Weaver, S. R., and L. L. Hernandez. 2016. Autocrine-paracrine regulation of the mammary gland. J. Dairy Sci. 99(1):842–853. https://doi.org/10.3168/jds.2015-9828.

Weber, C., C. Hametner, A. Tuchscherer, B. Losand, E. Kanitz, W. Otten, S. P. Singh, R. M. Bruckmaier, F. Becker, W. Kanitz, and H. M. Hammon. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96(1):165–180. https://doi.org/10.3168/jds.2012-5574.

Weißbach, F., and S. Kuhla. 1995. Substances losses in determining the dry matter content of silage and green fodder: Arising errors and possibilities of correction. Übersichten zur Tierernährung, 23:189-214.

Wieland, M., D. V. Nydam, W. Heuwieser, K. M. Morrill, L. Ferlito, R. D. Watters, and P. D. Virkler. 2020. A randomized trial to study the effect of automatic cluster remover settings on milking performance, teat condition, and udder health. J. Dairy Sci. 103(4):3668–3682. https://doi.org/10.3168/jds.2019-17342.

Chapter 4 General discussion and perspectives

In the present study, we confirmed the applicability of the software module for performing a 2-wk milk withdrawal that was automatically limited to delay the increase in milk yield during the first wk of lactation. The subsequent performance was not affected in the TRT cows, and udder health was not compromised over the entire observation period in both groups. The TRT cows tended to have less feed intake and also water intake than the CON cows during the 2-wk of IM. The extent of the NEB and the circulating concentrations of NEFA, ß-hydroxybutyrate, insulin-like growth factor-1 (IGF-1), and leptin did mostly not differ between the groups. The IM did not affect body condition.

Previous IM studies to improve NEB have been largely limited to the colostrum phase (DIM 1 to 5). The respective authors used a defined milking protocol (6 or 10 to 14 L milk/d), resulting in less than 50% of the milk of CON cows being obtained from TRT cows during the period of IM (Carbonneau et al., 2012; Morin et al., 2018). The energy metabolism could be improved (Carbonneau et al., 2012; Morin et al., 2018), but body condition was not affected by IM (Morin et al., 2018). However, short-time IM for 48 h (DIM 1 to 2; 6 L milk/d) did not ease the metabolic situation (Valldecabres et al., 2022), suggesting that besides the degree and duration of milk accumulation, the treatment length appears to be crucial for affecting the energy status due to IM. Likewise, depending on the combination of the factors mentioned above, subsequent milk production could be irreversibly compromised by IM (Albaaj et al., 2018; Deacon et al., 2023); therefore, we aimed for a constant milk removal, representing a relatively mild IM setting as compared to previous studies (11.1% vs. > 50% of milk remaining in the gland), in combination with a more extended treatment length of 14 d. This IM approach could ease energy metabolism and prevent potential production losses thereafter. For reasons of practicability, we used the milk yield of each cow 1 day before IM started as the default setting without the need for any manual calculations.

Conducting IM with manual cluster removal during the first days of lactation, when colostrum and the following milkings are not considered as salable milk, implies a substantial workload outside of the normal milking routine and enhances the potential for errors in accurate cluster removal after reaching the target milk yield (Morin et al., 2018). With this background, we selected DIM 8 for the start of IM to integrate the automated IM approach into the normal milking routine when cows separate milking is no longer necessary. However, the greater practicability of starting the IM only after the first wk of lactation is opposed by the loss of salable milk. Accordingly, we assumed that with milk removal being automatically clamped to a constant amount, the costs incurred for salable milk which is not harvested and for the extra effort outside the milking routine could be reduced and would be accompanied by benefits associated with potential time savings for the milking process and with energetic relief by IM, thus preventing diseases such as ketosis in early lactation.

Ketosis is one of the most prevalent diseases associated with severer NEB in early lactation and an important risk factor for developing other diseases in early lactation that cause high costs due to production losses and veterinary treatment (Duffield et al., 2009; LeBlanc, 2010; Suthar et al., 2013). However, the estimated costs of ketosis vary considerably due to varying factors in determining the costs, as assessed in the systematic review of Cainzos et al. (2022). The variation in estimated costs derives from several factors, such as the definition of ketosis (e.g., sample, threshold, testing period, and testing frequency), production system (e.g., organic vs. conventional, pasture grazing vs. stable housing), geographic location (e.g., currency, farm structure, and infrastructure), farm features (e.g., herd composition, milk production levels, milk price, culled cows' value, culling costs, feed costs, and veterinary costs), and estimated inputs (costs and losses) that are more or less considered in the included studies (Cainzos et al., 2022). From the included publications, the summarized estimated costs range from €19 to €812 per case of ketosis and between €3.60 and €39 per cow and year on the farm level (Cainzos et al., 2022).

Labor cost savings could enlarge the benefits of the automated IM treatment due to shortened milking time during the IM period. In the present study, we could reduce the milking time by about 2 min per cow at each milking during the IM period; however, the magnitude of time saved per milking during IM was not affecting the total milking troughput in the current experiment due to the milking parlor dimension on the farm (row milking parlor with front exit). The time of one batch (row) of cows spent in the parlour is determined by the animal with the longest milking time. This contrasted with the associated expenses due to IM, such as the incurred costs for the purchase of the software, ranging between €3 and €18 per cow depending on herd size (personal communication GEA sales persons), and the losses per cow of about 4.5 kg ECM/d of salable milk not harvested due to IM treatment. It was not possible to perform the treatment earlier, i.e., during the colostrum period to reduce losses of salable milk due to technical limitations. The software module can only be applied when the milk flows through the measuring unit; therefore, its application is not feasible when cows have to be milked separately during the colostrum phase or pharmaceutical treatment.

In the present study, the benefits of IM were limited. Both the energy status and the body condition remained largely unaffected by IM; therefore, we can not yet recommend implementing the here-used IM setting on commercial farms to modulate the energy status of the dairy cows in early lactation. The potential connection between reduced milk withdrawal and energy metabolism remains to be investigated. The finding of reduced feed intake may indicate that the cows were not able to reduce the mobilization of their own body reserves by keeping feed intake at the previous level. However, based on our data, suggestions on the physiological mode of action are very limited. As a next step, we could use the machine learning approaches used in the study by Ghaffari et al. (2020) to identify potential adaption types to IM from our collected data of the last milk flow before cluster removal during IM as an indicator for udder filling and milk production and combine those with milk production data as well as the targeted blood metabolites to explore possible connections thereof. Furthermore, as mentioned in the manuscript, various degrees of IM should be combined with different treatment lengths in future studies to identify the range initiating the relief of energy metabolism and the sustained modulation of milk production to prevent production losses. For this purpose, a constant degree of IM (e.g., 20%, 30%, 40%, 50% of milk remaining in the gland), combined with a treatment length of 5 to 7 days, would be an appropriate methodical approach. Shorten or extending the treatment length beyond the previously mentioned days cannot be recommended so far, given that an effect on energy metabolism was first observed after 5 days of IM (Carbonneau et al., 2012; Morin et al., 2018) but not after 2 days of IM (Valldecarbes et al., 2022). In turn, a treatment length beyond 7 days requires weekly recalibration days to adjust the IM setting to rising milk production in early lactation (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2023). This would imply extra effort for the producer and thus reduce practicability.

To extend the application range and the benefits of conducting IM in early lactation, additional blood varibles associated with disorders after calving should be investigated. For instance, besides energy deficit, the risk of suffering from Ca and P deficiency is also elevated in early lactation (Drackley et al., 2005; Venjakob et al., 2017). Hypocalcemia promotes the risk of developing further diseases, and increases the associated costs for veterinary treatment (McArt and Oetzel, 2015; Venjakob et al., 2017). Indeed, evidence from previous studies exists that IM treatment can ease Ca and P metabolism (Carbonneau et al., 2012; Valldecabres et al., 2022); However, the impact on reducing the milk fever incidence remains to be further investigated and confirmed by subsequent IM studies.

When considering the feed supply, both groups were offered the same amount of a partial mixed ration and concentrate that was not corrected for individual milk production during the first 3 wk of lactation. We observed that the DMI in TRT cows did not increase as rapidly as in CON cows during IM. As already described in the discussion, this phenomenon has been observed in ODM studies (McNamara et al., 2008), but the findings between studies are inconsistent (Stelwagen et al., 2013). It remains to be determined whether feed intake is affected by IM treatment or whether factors involved in regulating milk synthesis and (or) energy metabolism could be interrelated to yield a decrease in feed intake. Several reviews have provided an overview of factors associated with feed intake regulation (Ingvartsen and Andersen, 2000; Sartin et al., 2010; Kuhla, 2020). Nevertheless, the complexity of the system of feed intake regulation cannot yet be fully understood and described as a coherent system. Candidates associated with feed intake regulation are, besides the already mentioned pro-inflammatory cytokines (Kuhla, 2020), humoral signals due to pregnancy, adipose tissue mass, metabolic load sensed by the liver, gastrointestinal peptides, and hormones that may activate the central neural pathways resulting in the down-regulation of intake (Ingvartsen and Andersen, 2000). The involved factors have in common that they are (in)directly sensed by specific neurons located in the hypothalamus to yield an increase or decrease in feed intake (Ingvartsen and Andersen, 2000; Sartin et al., 2010; Kuhla, 2020). Potential explanations for the decreased feed intake from our collected data are limited. However, a first approach would be to identify factors from the literature associated with the respective regulatory cascade mentioned previously. For instance, a potential candidate that could be analyzed in the blood is the neurotransmitter serotonin which has already been investigated in the context of regulating milk synthesis (Hernandez et al., 2008; Weaver and Hernandez, 2016), energy metabolism (Laporta et al., 2015), and has also been associated with a decrease in feed intake (Ingvartsen and Andersen, 2000).

Chapter 5 Summary

The onset of lactation is marked by the metabolic priority of the mammary gland which results in partitioning energy and nutrients towards milk production and is caused by the "*genetic drive*" resulting from the genetic selection for high milk yields. Due to metabolic, physical, and hormonal changes around calving, the increase in voluntary feed intake is delayed relative to the increase in milk production during the first weeks of lactation. Therefore, the energy and nutrient supply provided by feed intake is commonly not covering the needs for milk production and maintenance at that time and thus results in a negative energy balance (NEB). To maintain milk production even during NEB, the cows mobilize body reserves mainly from adipose tissue. The mobilization of adipose depots results in elevated circulating concentrations of non-esterified fatty acids (NEFA). The capacity of the liver to oxidize NEFA during early lactation is limited; therefore, exceeding the oxidative capacity of the liver results in storage as triglycerides in the liver and incomplete oxidation of NEFA that is associated with increased ketogenesis. Metabolic disorders, such as ketosis and fatty liver, are known to be important risk factors for other diseases in early lactation and can cause high costs due to the loss of performance and veterinary treatment. To mitigate the NEB and thus the risk for health disturbances in early lactation, several approaches affecting the main variables in the equation to calculate the energy balance (EB) have been suggested. The energy intake can be modified to some extent by dietary measures, and a delay in the increase in energy output with milk can be implemented by several milking strategies (omitting or shortening the dry period, reducing the milking frequency, and incomplete milk removal) or by administering dopamine receptor agonists (e.g., quinagolide) to reduce the secretion of the galactopoietic hormone prolactin.

Incomplete milking (IM) by removing only a defined amount of milk from the mammary gland at each milking to delay the increase in milk yield is one of the options to mitigate the NEB in early lactation. So far, previous studies aiming at improving the energy status in early lactation by IM were limited to the colostrum phase. Moreover, studies that examined the consequences on milk composition and production by IM beyond the colostrum phase are limited concerning the knowledge about potentially sustained effects of IM on performance and udder health beyond the time of IM treatment. From a technical point of view, the IM approaches targeting the amount of milk rather than the milk flow as steering criterion for cluster removal were impeded by the need for manual intervention that, in turn, enhances the potential for errors in accurate cluster removal after reaching the target milk yield and requires more effort outside of the normal milking routine. Therefore, we used an automated software module to facilitate the practical application, which enables an automatic cluster removal after a defined milk quantity (kg) instead of a defined milk flow (kg/min) is reached. Our objectives were to test the effects of the automated, constant IM in early lactation on EB, metabolic status, udder health, and subsequent performance.

Forty-six pluriparous German Holstein cows were allocated to either the control group (CON, n = 23) or the treatment group (TRT, n = 23) by stratifying for udder health in the preceding lactation, calving date, and lactation number. We selected days in mik (DIM) 8 ± 1.1 for the start of IM since we aimed at integrating it into the normal milking routine when cows do not have to be milked separately any more. The amount of milk in the TRT group was clamped during IM to the milk yield of each cow 1 day before IM was started and held constant for 14 days. At the end of treatment (DIM 21 ± 1.1), the conventional, milk-flow dependent (0.3 kg/min) automatic cluster removal was reactivated.

During the study period, we recorded performance-related parameters, such as milk yield (days relative to the start of IM 0 to 21; weeks after calving 1 to 15), milk composition (weeks after calving 1 to 15), and elaborated the data in terms of peak yield, and day of reaching peak yield. The milking time (days relative to the start of IM 0 to 21) was recorded at each milking to examine potential time savings for the milking process during IM. For udder health control, the somatic cell count was assessed in foremilk samples at the quarter level (weeks after calving 1 to 7). Aseptic foremilk samples were subjected for cytobacteriological examinations at the quarter level (weeks after calving 1 and 4). Both groups were observed twice per week for milk leakage before and after fore milking (weeks after calving 1 to 4). In terms of estimating the filling state of the udder due to IM, we assessed the udder firmness via a hand-held dynamometer (weeks after calving 1 to 4) and recorded the last milk flow in the measuring unit at the moment of triggering cluster removal at each milking (days relative to the start of IM 0 to 21).

To reflect the energy status of the cow due to IM, we estimated the energy balance (weeks after calving 1 to 15), based on the records the dry matter intake and water intake (weeks after calving 1 to 15). We also obtained blood samples to ameasure the concentrations of NEFA, ß-hydroxybutyrate (BHB), insulin-like growth factor-1 (IGF-1), and leptin concentration (weeks relative to calving -3, -2, 1 to 7). Moreover, for assessing the body condition, we recorded the body weight after each milking via an electronic scale (weeks after calving 1 to 15), assessed the BCS (weeks relative to calving -3, -1, 2, 4, and 6) by a trained investigator, and measured the back fat thickness and muscle diameter (*M. longissimus dorsi*) via ultrasound imaging (weeks relative to calving -3, -1, 2, 4, and 6).

The software module worked well during the IM study period without errors or data losses. During the 14 days of IM, on average 11.1% less milk were obtained from the TRT cows than from the CON cows. The average last milk flow rate in the measuring unit at the moment of triggering milk yield-dependent cluster removal was elevated to about 3.1 kg/min in TRT cows, reflecting the udder filling during IM. Given that the value of the last milk flow remained relatively constant during the period of IM in TRT cows, we assumed that milk production was largely maintained and not adapted to the lesser milk removal. Thereafter, milk yield and the last milk flow of the TRT cows immediately reached the level of the CON cows, eliminating the group difference throughout the remaining observation period until week 15 of lactation. Milk composition was largely unaffected, besides the group difference in lactose concentrations in the second week of IM, that were sustained during the first week after IM. The decrease in milk lactose content could be related to the loss of tight junction integrity of the mammary gland, which may be caused by increasing intra-mammary pressure due to IM. Udder health was not compromised over the entire observation period in all cows. The captured cases of milk leakage indicated that the milk left in the mammary gland immediately after milking due to IM did not exceed the closing forces of the teat sphincter of cows. Likewise, the udder firmness as a proxi for udder filling and intra-mammary pressure associated with discomfort was not increased by IM over the entire observation period. During IM, milking time was about 2 min shorter for TRT than for CON cows and increased to the same level as in CON cows after IM. However, the magnitude of time saved per milking during IM was not affecting the total milking troughput in the current experiment due to the milking parlor dimension on the farm (row milking parlor with front exit). The time of one batch (row) of cows spent in the parlour is determined by the animal with the longest milking time.

During the 14 days of IM, the EB was 9.0 MJ NEL/d less negative in the TRT cows during the entire period of IM, given that voluntary feed intake and thus the energy intake of TRT cows tended to increase less than in the CON cows during IM. The calculated savings by IM mentioned above were neither reflected in terms of body condition losses nor in the circulating NEFA, BHB, IGF-1, and leptin in the blood that did mostly not differ between the groups. However, it remains to be determined whether the absent effect on energy metabolism and body condition was due to the relatively stable energy status of the cows or to the relatively mild IM setting used herein.

Altogether, the findings that emerged from the present thesis confirm the practicability of the milking software module for an automated, constant IM for delaying the increase in milk yield during the first weeks of lactation without affecting udder health and subsequent performance. Further research is needed to understand the link between the extent and duration of IM and the adaptive mechanism in terms of milk synthesis, feed intake, and energy metabolism. Accordingly, to implement the IM approach as a feasible concept on commercial farms for modulating the energy status of dairy cows in early lactation, the effect of varying IM settings on the energy status and subsequent performance at both the herd and individual level should first be examined by follow-up studies. Moreover, the feed intake during IM should be recorded in future studies to determine whether this phenomenon of decreased feed intake recurred. To extend the application range and, thus, the benefits of IM, additional blood variabes (e.g., Ca and P) associated with disorders after calving should be examined in future studies. To assess the economic viability of IM, labor costs and costs incurred due to IM have to be opposed to the potential benefits of IM for ease the cows' energy status during early lactation.

Chapter 6 Zusammenfassung

Der Beginn der Laktation ist gekennzeichnet durch eine hohe Priorisierung bei der Verteilung von Energie und Nährstoffen zugunsten der Milchdrüse, bedingt durch den genetischen *"drive*" infolge der Selektion auf hohe Milchleistung. Aufgrund der metabolischen, physischen und hormonellen Veränderungen rund um das Kalben, erfolgt der Anstieg der freiwilligen Futteraufnahme zeitlich verzögert zum Anstieg der Milchproduktion in den ersten Laktationswochen. Folglich deckt die Energie- und Nährstoffzufuhr durch die Futteraufnahme zu diesem Zeitpunkt nicht den Bedarf für die Milchproduktion und Erhaltung, was sich in einer negativen Energiebilanz (NEB) widerspiegelt. Um die Milchsynthese auch während einer NEB aufrecht erhalten zu können, mobilisieren die Kühe Körperreserven, hauptsächlich aus dem Fettgewebe. Die Mobilisierung der Fettdepots führt zu einem Anstieg der zirkulierenden Konzentrationen an nicht veresterten Fettsäuren (NEFA) im Blut. Die Kapazität der Leber zur vollständigen NEFA-Oxidation ist jedoch während der NEB begrenzt, was in einer übermäßigen Speicherung in Form von Triglyceriden in der Leber und der unvollständigen Oxidation mündet, wodurch die Ketogenese gefördert wird. Eine unzureichende Anpassungsfähigkeit des Stoffwechsels an die NEB geht mit einer höheren Inzidenz von Stoffwechselstörungen wie einer Ketose und Fettleber einher. Stoffwechselstörungen sind bekanntermaßen ein bedeutender Risikofaktor für die Entstehung weiterer Krankheiten in der Frühlaktation und verursachen hohe Kosten aufgrund von Leistungseinbußen und tierärztlichen Behandlungen. Um die NEB und damit das Risiko für Gesundheitsstörungen in der Frühlaktation zu mindern, gibt es mehrere Ansätze, welche die wichtigsten Variablen in der Gleichung zur Berechnung des Energiesaldos (EB) beeinflussen. Die Energieaufnahme kann bis zu einem gewissen Grad durch Fütterungsmaßnahmen verbessert werden. Die Verzögerung des Anstiegs der Energieabgabe mit der Milch kann über verschiedene Melkstrategien (z.B. die Modifikation der Trockenstehzeit, die Verringerung der Melkfrequenz oder die unvollständige Milchentnahme) oder durch Verabreichung von Dopaminrezeptor-Agonisten (z. B. Quinagolid) zur Verringerung der Sekretion von dem galatopoetischen Hormon Prolaktin erreicht werden.

Das unvollständige Melken, bei dem nur eine bestimmte Milchmenge bei jedem Melken aus der Milchdrüse entnommen wird, stellt eine Möglichkeit dar, den Anstieg der Milchleistung zu verzögern und das Ausmaß der NEB in der Frühlaktation zu verringern. Bisherige Untersuchungen zur Verbesserung der energetischen Situation in der Frühlaktation durch IM beschränkten sich auf die Kolostrumphase. Zudem sind Studien, die die Auswirkungen von IM auf die Milchzusammensetzung und -produktion auch außerhalb der Kolostrumphase untersuchten, hinsichtlich des Wissens über potenziell anhaltende Effekte von IM auf Leistung und Eutergesundheit über den Zeitpunkt der IM-Behandlung hinaus begrenzt. Aus technischer Sicht waren die IM-Ansätze, die auf die Milchmenge statt auf den Milchfluss als Steuerungsgröße für die Melkzeugabnahme abzielten, durch die Notwendigkeit manueller Eingriffe eingeschränkt, was wiederum das Fehlerpotenzial bei der genauen Melkzeugabnahme nach Erreichen der Zielmilchmenge erhöht und einen zusätzlichen Aufwand außerhalb der normalen Melkroutine erfordert. Für eine praktische Umsetzung wurde daher in dieser Studie ein Softwaremodul eingesetzt, das eine automatische Melkzeugabnahme nach Erreichen einer bestimmten Milchmenge (kg) anstelle eines bestimmten Milchflusses (kg/min) ermöglicht. Im Rahmen dieser Arbeit sollten die Auswirkungen des IM in der Frühlaktation auf die EB, den Stoffwechselstatus, die Eutergesundheit und die spätere Leistung untersucht werden.

Es wurden 46 pluripare deutsche Holstein-Kühe entweder der Kontrollgruppe (CON, n = 23) oder der Behandlungsgruppe (TRT, n = 23) zugeteilt, wobei die Eutergesundheit in der vorangegangenen Laktation, das Abkalbedatum und die Laktationsnummer berücksichtigt wurde. Für den Beginn des IM wählten wir den 8 ± 1,1 Laktationstag, da die Maßnahme in die normale Melkroutine integriert werden sollte, bei der die Kühe nicht mehr separat gemolken werden. Die Milchmenge in der TRT-Gruppe wurde während des IM an die individuelle Milchleistung der jeweiligen Kuh 1 Tag vor Beginn des IM festgesetzt und für 14 Tage konstant gehalten. Nach Beendigung des IM (Laktationstag 21 ± 1,1) wurde die konventionelle, milchflussabhängige (0,3 kg/min) automatische Melkzeugabnahme wieder aktiviert.

Während des Untersuchungszeitraums erfassten wir Leistungsparameter wie die Milchleistung (Tage relativ zum Beginn des IM 0 bis 21; Wochen 1 bis 15 nach der Kalbung) und die Milchzusammensetzung (Wochen 1 bis 15 nach der Kalbung). Zudem werteten wir die Daten in Bezug auf die Spitzenleistung und den Tag des Erreichens der Spitzenleistung aus. Zudem wurde die Melkdauer bei jedem Melken aufgezeichnet (Tage relativ zum Beginn des IM 0 bis 21). Zur Kontrolle der Eutergesundheit wurde die somatische Zellzahl in Viertelanfangsgemelksproben gemessen (Wochen 1 bis 7 nach der Kalbung). Zudem wurden aseptische Viertelanfangsgemelksproben zytobakteriologisch untersucht (Wochen 1 und 4 nach der Kalbung). Beide Gruppen wurden zweimal wöchentlich vor und nach dem Vormelken hinsichtlich des Auftretens von „Milch laufen lassen“ beobachtet (Wochen nach der Kalbung 1 bis 4). Zur Abschätzung des Euterfüllstandes wurde die Euterfestigkeit durch ein digitales

Druckkraftmessgerät während des IM gemessen (Wochen 1 bis 4 nach der Kalbung) und der letzte Milchfluss in der Messeinheit zum Zeitpunkt der Abnahme der Melkzeugabnahme bei jedem Melken aufgezeichnet (Tage relativ zum Beginn des IM 0 bis 21).
Zur Erfassung der energetischen Situation der Kuh infolge des IM, schätzten wir das Energiesaldo (Wochen 1 bis 15 nach der Kalbung), erfassten die Trockenmasse- und Wasseraufnahme (Wochen 1 bis 15 nach der Kalbung) und entnahmen Blutproben zur Messung der NEFA-, ß-Hydroxybutyrat- (BHB), Insulin-ähnlichen Wachstumsfaktor-1- (IGF-1) und Leptinkonzentrationen (Wochen -3, -2, 1 bis 7 relativ zur Kalbung). Um die Körperkondition zu beurteilen, erfassten wir das Körpergewicht nach jedem Melken mittels einer elektronischen Waage (Wochen 1 bis 15 nach der Kalbung), bestimmten den BCS (Wochen -3, -1, 2, 4 und 6 relativ zur Kalbung) und ermittelten zeitgleich die Rückenfettdicke und den Muskeldurchmesser (*M. longissimus dorsi*) mittels Ultraschallaufnahmen.

Während des gesamten Untersuchungszeitraums arbeitete das Softwaremodul fehlerfrei und ohne Datenverluste. In den 14 Tage des IM wurde von der TRT-Gruppe durchschnittlich 11,1 % weniger Milch entnommen als von den CON-Gruppe. Die durchschnittliche letzte Milchflussrate in der Messeinheit zum Zeitpunkt der Abnahme der Melkzeuge war bei den TRT-Kühen auf etwa 3,1 kg/min erhöht, was die Euterfüllung während der IM widerspiegelt. Da der Wert des letzten Milchflusses während der IM bei den TRT-Kühen relativ konstant blieb, nehmen wir an, dass die Milchproduktion nicht an den verringerten Milchentzug angepasst wurde. Anschließend erreichten die TRT-Kühe hinsichtlich der Milchleistung und des letzten Milchflusses unmittelbar das Niveau der CON-Kühe, so dass der Gruppenunterschied bis zur 15. Laktationswoche eliminiert wurde. Die Milchzusammensetzung blieb weitestgehend unbeeinflusst, mit Ausnahme des Gruppenunterschieds in der Laktosekonzentration während der zweiten Woche des IM, der noch in der nachfolgenden Woche anhielt. Der Rückgang der Milchlaktosekonzentration könnte mit einem Verlust der Tight Junction-Integrität der Milchdrüse zusammenhängen, der durch den zunehmenden Druck in der Milchdrüse infolge des IM verursacht gewesen sein könnte. Die Eutergesundheit war während des gesamten Beobachtungszeitraums bei allen Kühen nicht beeinträchtigt. Zudem deuteten die erfassten Fälle von Kühen, die Milch laufen ließen darauf hin, dass während des IM der Verschluss des Schließmuskels der Zitze ausreichend war. Auch die Euterfestigkeit als Indikator für den Euterfüllstand und den Euterinnendruck, der bei Erhöhung mit Unwohlsein für die Kuh verbunden ist, war während des gesamten Beobachtungszeitraums nicht auffällig. Die Melkzeit bei den TRT-Kühen war während des IM etwa 2 min kürzer als bei den CON-Kühen und lag nach dem IM wieder auf dem gleichen Niveau wie bei den CON-Kühen. Das Ausmaß der Zeitersparnis pro

Melkvorgang durch das IM war allerdings durch die vorhandenen Reihenmelkstand mit Frontauslass auf dem Versuchsbetrieb für die Gesamtverweildauer der Kühe einer Reihe irrelevant.

Das EB war bei den TRT-Kühen während der 14 Tage der IM um etwa 9,0 MJ NEL/d weniger negativ, weil die freiwillige Futter- und damit die Energieaufnahme der TRT-Kühe während des IM tendenziell langsamer anstieg als bei den CON-Kühen. Jedoch spiegelten sich die zuvor genannten Energieeinsparungen durch das IM weder in dem Maß der Körperkonditionsverluste noch in den zirkulierenden NEFA-, BHB-, IGF-1- und Leptinwerten im Blut wider. Es bleibt jedoch zu klären, ob die fehlende Auswirkung auf den Energiestoffwechsel auf die relativ stabile energetische Situation der Kühe oder auf die hier verwendete relativ milde IM-Einstellung zurückzuführen ist.

Insgesamt bestätigen die Erkenntnisse aus der vorliegenden Arbeit die Praktikabilität des Melksoftwaremoduls für einen automatisierten, konstanten Milchentzug zur Verzögerung des Milchleistungsanstiegs in der ersten Laktationswoche, ohne dabei die Eutergesundheit und die anschließende Milchleistung zu beeinträchtigen. Weitere Untersuchungen sind erforderlich, um den Zusammenhang zwischen dem Ausmaß und der Dauer des IM und den Anpassungsmechanismen des Organismus in Bezug auf die Milchsynthese, die Futteraufnahme und den Energiestoffwechsel zu ergründen. Um den IM-Ansatz als praktikables Konzept zur Modulation der energetischen Situation von Milchkühen in der Frühlaktation auf kommerziellen Betrieben zu implementieren, sollten dementsprechend zunächst die Auswirkungen verschiedener IM-Einstellungen auf die energetische Situation und die nachfolgende Leistung sowohl auf Herden- als auch auf Einzeltierebene durch weitere Studien untersucht werden. Des Weiteren sollte in künftigen Studien die Futteraufnahme während der IM aufgezeichnet werden, um zu überprüfen, ob sich das Phänomen der verringerten Futteraufnahme absichern läßt. Zur Erweiterung des Anwendungsbereichs und damit des Anwendungsnutzens des IM sollten in weiteren Studien zusätzliche Blutparameter untersucht werden, die ebenfalls im Zusammenhang mit Problemen in der Frühlaktation stehen (z.B. Ca und P). Zur Beurteilung der wirtschaftlichen Tragfähigkeit des IM sind die Arbeitskosten und die Kosten, die durch das IM entstehen, dem Nutzen des IM zur Entlastung der energetischen Situation der Kühe in der Frühlaktation entgegenzustellen.

Chapter 7 References

Adewuyi, A. A., E. Gruys, and F. J. C. M. van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. The veterinary quarterly 27(3):117–126. https://doi.org/10.1080/01652176.2005.9695192.

Ahima, R. S., and J. S. Flier. 2000. Leptin. Annual review of physiology 62:413–437. https://doi.org/10.1146/annurev.physiol.62.1.413.

Akers, R. M. 2000. Selection for milk production from a lactation biology viewpoint. Journal of dairy science 83(5):1151–1158. https://doi.org/10.3168/jds.S0022-0302(00)74981-2.

Albaaj, A., P. G. Marnet, C. Hurtaud, and J. Guinard-Flament. 2018. Adaptation of dairy cows to increasing degrees of incomplete milk removal during a single milking interval. Journal of dairy science 101(9):8492–8504. https://doi.org/10.3168/jds.2018-14451.

Bareille, N., F. Beaudeau, S. Billon, A. Robert, and P. Faverdin. 2003. Effects of health disorders on feed intake and milk production in dairy cows. Livestock Production Science 83(1):53–62. https://doi.org/10.1016/S0301-6226(03)00040-X.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. Journal of dairy science 63(9):1514–1529. https://doi.org/10.3168/jds.S0022-0302(80)83111-0.

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. Journal of mammary gland biology and neoplasia 2(3):265–278. https://doi.org/10.1023/A:1026336505343.

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. The Proceedings of the Nutrition Society 59(1):119–126. https://doi.org/10.1017/s0029665100000148.

Bell, A. W., R. Slepetis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. Journal of dairy science 78(9):1954–1961. https://doi.org/10.3168/jds.S0022-0302(95)76821-7.

Block, S. S., W. R. Butler, R. A. Ehrhardt, A. W. Bell, M. E. van Amburgh, and Y. R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. The Journal of endocrinology 171(2):339–348. https://doi.org/10.1677/joe.0.1710339.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited Review: Pathology, Etiology, Prevention, and Treatment of Fatty Liver in Dairy Cows. Journal of dairy science 87(10):3105–3124. https://doi.org/10.3168/jds.S0022-0302(04)73446-3.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. Journal of dairy science 98(10):6631–6650. https://doi.org/10.3168/jds.2015-9683.

Bruckmaier, R. M., and J. W. Blum. 1998. Oxytocin release and milk removal in ruminants. Journal of dairy science 81(4):939–949. https://doi.org/10.3168/jds.S0022-0302(98)75654-1.

Busato, A., D. Faissle, U. Küpfer, and J. W. Blum. 2002. Body condition scores in dairy cows: associations with metabolic and endocrine changes in healthy dairy cows. Journal of veterinary medicine. A, Physiology, pathology, clinical medicine 49(9):455–460. https://doi.org/10.1046/j.1439-0442.2002.00476.x.

Butler, S. T., A. L. Marr, S. H. Pelton, R. P. Radcliff, M. C. Lucy, and W. R. Butler. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. The Journal of endocrinology 176(2):205–217. https://doi.org/10.1677/joe.0.1760205.

Cainzos, J. M., C. Andreu-Vazquez, M. Guadagnini, A. Rijpert-Duvivier, and T. Duffield. 2022. A systematic review of the cost of ketosis in dairy cattle. Journal of dairy science 105(7):6175–6195. https://doi.org/10.3168/jds.2021-21539.

Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. Journal of dairy science 80(3):477–487. https://doi.org/10.3168/jds.S0022-0302(97)75960-5.

Capuco, A. V., S. E. Ellis, S. A. Hale, E. Long, R. A. Erdman, X. Zhao, and M. J. Paape. 2003. Lactation persistency: insights from mammary cell proliferation studies. Journal of animal science 81 Suppl 3:18–31. https://doi.org/10.2527/2003.81suppl_318x.

Carbonneau, E., A. M. de Passillé, J. Rushen, B. G. Talbot, and P. Lacasse. 2012. The effect of incomplete milking or nursing on milk production, blood metabolites, and immune functions of dairy cows. Journal of dairy science 95(11):6503–6512. https://doi.org/10.3168/jds.2012-5643.

Chagas, L. M., M. C. Lucy, P. J. Back, D. Blache, J. M. Lee, P. J. S. Gore, A. J. Sheahan, and J. R. Roche. 2009. Insulin resistance in divergent strains of Holstein-Friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. Journal of dairy science 92(1):216–222. https://doi.org/10.3168/jds.2008-1329.

Chibisa, G. E., G. N. Gozho, A. G. van Kessel, A. A. Olkowski, and T. Mutsvangwa. 2008. Effects of peripartum propylene glycol supplementation on nitrogen metabolism, body composition, and gene expression for the major protein degradation pathways in skeletal muscle in dairy cows. Journal of dairy science 91(9):3512–3527. https://doi.org/10.3168/jds.2007-0920.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. Domestic animal endocrinology 29(1):3–22. https://doi.org/10.1016/j.domaniend.2005.02.026.

Collier, R. J., M. F. McGrath, J. C. Byatt, and L. L. Zurfluh. 1993. Regulation of bovine mammary growth by peptide hormones: involvement of receptors, growth factors and binding proteins. Livestock Production Science 35(1-2):21–33. https://doi.org/10.1016/0301-6226(93)90179-L.

Cording, F., M. Hoedemaker, and V. Krömker. 2013. Einfluss der Menge loser Restmilch in Milchdrüsen auf die Entstehung klinischer Mastitiden bei Milchkühen. Tierarztl Prax Ausg G 41(06):379–386. https://doi.org/10.1055/s-0038-1623193.

Davis, S., V. Farr, and K. Stelwagen. 1999. Regulation of yield loss and milk composition during once-daily milking: a review. Livestock Production Science 59(1):77–94. https://doi.org/10.1016/S0301-6226(98)00204-8.

Deacon, A.-M., R. Blouin, C. Thibault, and P. Lacasse. 2023. Mechanism underlying the modulation of milk production by incomplete milking. Journal of dairy science 106(1):783–791. https://doi.org/10.3168/jds.2022-22164.

Delamaire, E., and J. Guinard-Flament. 2006. Longer milking intervals alter mammary epithelial permeability and the Udder's ability to extract nutrients. Journal of dairy science 89(6):2007–2016. https://doi.org/10.3168/jds.S0022-0302(06)72268-8.

Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? Journal of dairy science 82(11):2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3.

Drackley, J. K., H. M. Dann, N. Douglas, N. A. J. Guretzky, N. B. Litherland, J. P. Underwood, and J. J. Loor. 2005. Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science 4(4):323–344. https://doi.org/10.4081/ijas.2005.323.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. Journal of dairy science 84:E100-E112. https://doi.org/10.3168/jds.S0022-0302(01)70204-4.

Dubuc, J., and S. Buczinski. 2018. Short communication: Cow- and herd-level prevalence of hypoglycemia in hyperketonemic postpartum dairy cows. Journal of dairy science 101(4):3374–3379. https://doi.org/10.3168/jds.2017-13773.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. Journal of dairy science 92(2):571–580. https://doi.org/10.3168/jds.2008-1507.

Esposito, G., P. C. Irons, E. C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Animal reproduction science 144(3-4):60–71. https://doi.org/10.1016/j.anireprosci.2013.11.007.

Ghaffari, M. H., A. Jahanbekam, C. Post, H. Sadri, K. Schuh, C. Koch, and H. Sauerwein. 2020. Discovery of different metabotypes in overconditioned dairy cows by means of machine learning. Journal of dairy science 103(10):9604–9619. https://doi.org/10.3168/jds.2020-18661.

Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. Journal of dairy science 80(7):1260–1268. https://doi.org/10.3168/jds.S0022-0302(97)76055-7.

Gross, J. J., and R. M. Bruckmaier. 2019. Invited review: Metabolic challenges and adaptation during different functional stages of the mammary gland in dairy cows: Perspectives for sustainable milk production. Journal of dairy science 102(4):2828–2843. https://doi.org/10.3168/jds.2018-15713.

Grummer, R. R., D. G. Mashek, and A. Hayirli. 2004. Dry matter intake and energy balance in the transition period. The Veterinary clinics of North America. Food animal practice 20(3):447–470. https://doi.org/10.1016/j.cvfa.2004.06.013.

Guinard-Flament, J., E. Delamaire, P. Lamberton, and J. L. Peyraud. 2007. Adaptations of mammary uptake and nutrient use to once-daily milking and feed restriction in dairy cows. Journal of dairy science 90(11):5062–5072. https://doi.org/10.3168/jds.2007-0259.

Guinard-Flament, J., E. Delamaire, S. Lemosquet, M. Boutinaud, and Y. David. 2006. Changes in mammary uptake and metabolic fate of glucose with once-daily milking and feed restriction in dairy cows. Reproduction, nutrition, development 46(5):589–598. https://doi.org/10.1051/rnd:2006030.

Guinard-Flament, J., Y. Gallard, and H. Larroque. 2011. Lactose in blood plasma and the ability of dairy cows to tolerate once-daily milking in terms of milk loss and milk recovery. Journal of dairy science 94(7):3446–3454. https://doi.org/10.3168/jds.2010-4081.

Havel, P. J. 2002. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Current Opinion in Lipidology 13(1):51. https://doi.org/aspx.

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. The Veterinary clinics of North America. Food animal practice 16(2):215-30, v. https://doi.org/10.1016/S0749-0720(15)30102-X.

Hernandez, L. L., C. M. Stiening, J. B. Wheelock, L. H. Baumgard, A. M. Parkhurst, and R. J. Collier. 2008. Evaluation of serotonin as a feedback inhibitor of lactation in the bovine. Journal of dairy science 91(5):1834–1844. https://doi.org/10.3168/jds.2007-0766.

Horváth, J., Z. Tóth, and E. Mikó. 2017. The Analysis of Production and Culling Rate With Regard to the Profitability in a Dairy Herd. Advanced Research in Life Sciences 1(1):48–52. https://doi.org/10.1515/arls-2017-0008.

Houseknecht, K. L., D. A. Dwyer, D. P. Lanna, and D. E. Bauman. 1995. Effect of somatotropin on adipose tissue metabolism: ontogeny of the enhanced response to adrenergic challenge in the lactating cow. Domestic animal endocrinology 12(1):105–113. https://doi.org/10.1016/0739-7240(94)00013-Q.

Ingvartsen, K. L. 2006. Feeding- and management-related diseases in the transition cow. Animal Feed Science and Technology 126(3-4):175–213. https://doi.org/10.1016/j.anifeedsci.2005.08.003.

Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. Journal of dairy science 83(7):1573–1597. https://doi.org/10.3168/jds.S0022-0302(00)75029-6.

Jouany, J.-P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Animal reproduction science 96(3-4):250–264. https://doi.org/10.1016/j.anireprosci.2006.08.005.

Kessel, S., M. Stroehl, H. H. D. Meyer, S. Hiss, H. Sauerwein, F. J. Schwarz, and R. M. Bruckmaier. 2008. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. Journal of animal science 86(11):2903–2912. https://doi.org/10.2527/jas.2008-1016.

Kok, A., J. Chen, B. Kemp, and A. T. M. van Knegsel. 2019. Review: Dry period length in dairy cows and consequences for metabolism and welfare and customised management strategies. Animal an international journal of animal bioscience 13(S1):s42-s51. https://doi.org/10.1017/S1751731119001174.

Kok, A., R. J. van Hoeij, B. J. Tolkamp, M. J. Haskell, A. T. M. van Knegsel, I. J. M. de Boer, and E. A. M. Bokkers. 2017. Behavioural adaptation to a short or no dry period with associated management in dairy cows. Appl. Anim. Behav. Sci. 186:7–15. https://doi.org/10.1016/j.applanim.2016.10.017.

Komaragiri, M. V., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. Journal of dairy science 80(5):929–937. https://doi.org/10.3168/jds.S0022-0302(97)76016-8.

Krug, C., T. J. DeVries, J.-P. Roy, J. Dubuc, and S. Dufour. 2017. Incomplete Milking in Early Lactation Does Not Affect Dairy Cows Resting Behaviors: Results from a Randomized Controlled Trial. Frontiers in veterinary science 4:66. https://doi.org/10.3389/fvets.2017.00066.

Krug, C., P.-A. Morin, P. Lacasse, J.-P. Roy, J. Dubuc, and S. Dufour. 2018a. A randomized controlled trial on the effect of incomplete milking during the first 5 days in milk on reproductive performance of dairy cows. Journal of dairy science 101(12):11330–11341. https://doi.org/10.3168/jds.2018-14909.

Krug, C., P.-A. Morin, P. Lacasse, J.-P. Roy, J. Dubuc, and S. Dufour. 2018b. Effect of incomplete milking during the first 5 days in milk on udder and reproductive tract health: Results from a randomized controlled trial. Journal of dairy science 101(10):9275–9286. https://doi.org/10.3168/jds.2018-14713.

Krug, C., P.-A. Morin, P. Lacasse, D. E. Santschi, J.-P. Roy, J. Dubuc, and S. Dufour. 2018c. A randomized controlled trial on the effect of incomplete milking during the first 5 days in milk on culling hazard and on milk production and composition of dairy cows. Journal of dairy science 101(5):4367–4377. https://doi.org/10.3168/jds.2017-14021.

Kuehnl, J. M., M. K. Connelly, A. Dzidic, M. Lauber, H. P. Fricker, M. Klister, E. Olstad, M. Balbach, E. Timlin, V. Pszczolkowski, P. M. Crump, D. J. Reinemann, and L. L. Hernandez. 2019. The effects of incomplete milking and increased milking frequency on milk production rate and milk composition1. Journal of animal science 97(6):2424–2432. https://doi.org/10.1093/jas/skz113.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: implications for insufficient feed intake of transition dairy cows. Animal an international journal of animal bioscience 14(S1):s65-s77. https://doi.org/10.1017/S1751731119003124.

Kuhla, B., G. Nürnberg, D. Albrecht, S. Görs, H. M. Hammon, and C. C. Metges. 2011. Involvement of skeletal muscle protein, glycogen, and fat metabolism in the adaptation on early lactation of dairy cows. Journal of proteome research 10(9):4252–4262. https://doi.org/10.1021/pr200425h.

Lacasse, P., and S. Ollier. 2015. The dopamine antagonist domperidone increases prolactin concentration and enhances milk production in dairy cows. Journal of dairy science 98(11):7856–7864. https://doi.org/10.3168/jds.2015-9865.

Lacasse, P., S. Ollier, V. Lollivier, and M. Boutinaud. 2016. New insights into the importance of prolactin in dairy ruminants. Journal of dairy science 99(1):864–874. https://doi.org/10.3168/jds.2015-10035.

Lacasse, P., N. Vanacker, S. Ollier, and C. Ster. 2018. Innovative dairy cow management to improve resistance to metabolic and infectious diseases during the transition period. Research in veterinary science 116:40–46. https://doi.org/10.1016/j.rvsc.2017.06.020.

Lacasse, P., X. Zhao, N. Vanacker, and M. Boutinaud. 2019. Review: Inhibition of prolactin as a management tool in dairy husbandry. Animal an international journal of animal bioscience 13(S1):s35-s41. https://doi.org/10.1017/S1751731118003312.

Laporta, J., S. A. E. Moore, S. R. Weaver, C. M. Cronick, M. Olsen, A. P. Prichard, B. P. Schnell, T. D. Crenshaw, F. Peñagaricano, R. M. Bruckmaier, and L. L. Hernandez. 2015. Increasing serotonin concentrations alter calcium and energy metabolism in dairy cows. The Journal of endocrinology 226(1):43–55. https://doi.org/10.1530/JOE-14-0693.

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. The Journal of reproduction and development 56 Suppl:S29-35. https://doi.org/10.1262/jrd.1056S29.

LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: a review. Veterinary journal (London, England 1997) 176(1):102–114. https://doi.org/10.1016/j.tvjl.2007.12.019.

Liefers, S. C., R. F. Veerkamp, M. F. W. te Pas, C. Delavaud, Y. Chilliard, and T. van der Lende. 2003. Leptin concentrations in relation to energy balance, milk yield, intake, live weight, and estrus in dairy cows. Journal of dairy science 86(3):799–807. https://doi.org/10.3168/jds.S0022-0302(03)73662-5.

Locher, L., S. Häussler, L. Laubenthal, S. P. Singh, J. Winkler, A. Kinoshita, Á. Kenéz, J. Rehage, K. Huber, H. Sauerwein, and S. Dänicke. 2015. Effect of increasing body condition on key regulators of fat metabolism in subcutaneous adipose tissue depot and circulation of nonlactating dairy cows. Journal of dairy science 98(2):1057–1068. https://doi.org/10.3168/jds.2014-8710.

Macmillan, K., I. López Helguera, A. Behrouzi, M. Gobikrushanth, B. Hoff, and M. G. Colazo. 2017. Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows. Research in veterinary science 115:327–331. https://doi.org/10.1016/j.rvsc.2017.06.019.

Martin, L. M., H. Sauerwein, W. Büscher, and U. Müller. 2020. Automated gradual reduction of milk yield before dry-off: Effects on udder health, involution and inner teat morphology. Livestock Science 233:103942. https://doi.org/10.1016/j.livsci.2020.103942.

McArt, J. A. A., D. V. Nydam, G. R. Oetzel, and C. L. Guard. 2014. An economic analysis of hyperketonemia testing and propylene glycol treatment strategies in early lactation dairy cattle. Preventive veterinary medicine 117(1):170–179. https://doi.org/10.1016/j.prevetmed.2014.06.017.

McArt, J. A. A., and G. R. Oetzel. 2015. A stochastic estimate of the economic impact of oral calcium supplementation in postparturient dairy cows. Journal of dairy science 98(10):7408–7418. https://doi.org/10.3168/jds.2015-9479.

McNamara, S., J. J. Murphy, F. P. O'Mara, M. Rath, and J. F. Mee. 2008. Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. Livestock Science 117(1):70–78. https://doi.org/10.1016/j.livsci.2007.11.013.

Morin, P.-A., C. Krug, Y. Chorfi, J. Dubuc, P. Lacasse, J.-P. Roy, D. E. Santschi, and S. Dufour. 2018. A randomized controlled trial on the effect of incomplete milking during early lactation on ketonemia and body condition loss in Holstein dairy cows. Journal of dairy science 101(5):4513–4526. https://doi.org/10.3168/jds.2017-13151.

Neville, M. C., T. B. McFadden, and I. Forsyth. 2002. Hormonal regulation of mammary differentiation and milk secretion. Journal of mammary gland biology and neoplasia 7(1):49–66. https://doi.org/10.1023/A:1015770423167.

Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. The Veterinary clinics of North America. Food animal practice 20(3):651–674. https://doi.org/10.1016/j.cvfa.2004.06.006.

Ollier, S., F. Beaudoin, N. Vanacker, and P. Lacasse. 2016. Effect of reducing milk production using a prolactin-release inhibitor or a glucocorticoid on metabolism and immune functions in cows subjected to acute nutritional stress. Journal of dairy science 99(12):9949–9961. https://doi.org/10.3168/jds.2016-11711.

Ollier, S., X. Zhao, and P. Lacasse. 2015. Effects of feed restriction and prolactin-release inhibition at drying-off on susceptibility to new intramammary infection in cows. Journal of dairy science 98(1):221–228. https://doi.org/10.3168/jds.2014-8426.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. Journal of dairy science 93(8):3595–3601. https://doi.org/10.3168/jds.2010-3074.

Overton, T. R., and M. R. Waldron. 2004. Nutritional Management of Transition Dairy Cows: Strategies to Optimize Metabolic Health. Journal of dairy science 87:E105-E119. https://doi.org/10.3168/jds.S0022-0302(04)70066-1.

Penry, J. F., E. L. Endres, B. de Bruijn, A. Kleinhans, P. M. Crump, D. J. Reinemann, and L. L. Hernandez. 2017. Effect of incomplete milking on milk production rate and composition with 2 daily milkings. Journal of dairy science 100(2):1535–1540. https://doi.org/10.3168/jds.2016-11935.

Pires, J. A. A., C. Delavaud, Y. Faulconnier, D. Pomiès, and Y. Chilliard. 2013. Effects of body condition score at calving on indicators of fat and protein mobilization of periparturient Holstein-Friesian cows. Journal of dairy science 96(10):6423–6439. https://doi.org/10.3168/jds.2013-6801.

Pires, J. A. A., A. H. Souza, and R. R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. Journal of dairy science 90(6):2735–2744. https://doi.org/10.3168/jds.2006-759.

Ponchon, B., X. Zhao, S. Ollier, and P. Lacasse. 2017. Relationship between glucocorticoids and prolactin during mammary gland stimulation in dairy cows. Journal of dairy science 100(2):1521–1534. https://doi.org/10.3168/jds.2016-11490.

Raboisson, D., M. Mounié, and E. Maigné. 2014. Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review. Journal of dairy science 97(12):7547–7563. https://doi.org/10.3168/jds.2014-8237.

Rovai, M., M. T. Kollmann, and R. M. Bruckmaier. 2007. Incontinentia lactis: physiology and anatomy conducive to milk leakage in dairy cows. Journal of dairy science 90(2):682–690. https://doi.org/10.3168/jds.S0022-0302(07)71551-5.

Sadri, H., M. H. Ghaffari, and H. Sauerwein. 2023. Invited review: Muscle protein breakdown and its assessment in periparturient dairy cows. Journal of dairy science 106(2):822–842. https://doi.org/10.3168/jds.2022-22068.

Sadri, H., M. Mielenz, I. Morel, R. M. Bruckmaier, and H. A. van Dorland. 2011. Plasma leptin and mRNA expression of lipogenesis and lipolysis-related factors in bovine adipose tissue around parturition. Journal of animal physiology and animal nutrition 95(6):790–797. https://doi.org/10.1111/j.1439-0396.2010.01111.x.

Sartin, J. L., J. A. Daniel, B. K. Whitlock, and R. R. Wilborn. 2010. Selected hormonal and neurotransmitter mechanisms regulating feed intake in sheep. Animal an international journal of animal bioscience 4(11):1781–1789. https://doi.org/10.1017/S1751731110001497.

Schmidt, G. H., R. S. Guthrie, and R. W. Guest. 1964. Effect of Incomplete Milking on the Incidence of Udder Irritation and Subsequent Milk Yield of Dairy Cows. Journal of dairy science 47(2):152–155. https://doi.org/10.3168/jds.S0022-0302(64)88608-2.

Schmidt S, B. Linius, B. Maaßen-Francke, U. Müller, W. Büscher, and L. M. Martin. 2020. Method for reducing the individual-specific milk production of milk-producing animals United States Patent Application Publication: US 2020/0281151 A1.

Staufenbiel, R. 1997. Konditionsbeurteilung von Milchkühen mit Hilfe der sonographischen Rückenfettdickenmessung. Praktische Tierarzt 78: 87-92.

Steeneveld, W., P. Amuta, F. J. S. van Soest, R. Jorritsma, and H. Hogeveen. 2020. Estimating the combined costs of clinical and subclinical ketosis in dairy cows. PloS one 15(4):e0230448. https://doi.org/10.1371/journal.pone.0230448.

Stelwagen, K. 2001. Effect of Milking Frequency on Mammary Functioning and Shape of the Lactation Curve. Journal of dairy science 84:E204-E211. https://doi.org/10.3168/jds.S0022-0302(01)70219-6.

Stelwagen, K., V. C. Farr, G. D. Nicholas, S. R. Davis, and C. G. Prosser. 2008. Effect of milking interval on milk yield and quality and rate of recovery during subsequent frequent milking. Livestock Science 114(2-3):176–180. https://doi.org/10.1016/j.livsci.2007.04.021.

Stelwagen, K., C. V. C. Phyn, S. R. Davis, J. Guinard-Flament, D. Pomiès, J. R. Roche, and J. K. Kay. 2013. Invited review: reduced milking frequency: milk production and management implications. Journal of dairy science 96(6):3401–3413. https://doi.org/10.3168/jds.2012-6074.

Stelwagen, K., and K. Singh. 2014. The role of tight junctions in mammary gland function. Journal of mammary gland biology and neoplasia 19(1):131–138. https://doi.org/10.1007/s10911-013-9309-1.

Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. Journal of dairy science 96(5):2925–2938. https://doi.org/10.3168/jds.2012-6035.

Valldecabres, A., R. B. Lopes, A. Lago, C. Blanc, and N. Silva-Del-Río. 2022. Effects of postpartum milking strategy on plasma mineral concentrations and colostrum, transition milk, and milk yield and composition in multiparous dairy cows. Journal of dairy science 105(1):595–608. https://doi.org/10.3168/jds.2021-20590.

van Dorland, H. A., S. Richter, I. Morel, M. G. Doherr, N. Castro, and R. M. Bruckmaier. 2009. Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. Journal of dairy science 92(5):1924–1940. https://doi.org/10.3168/jds.2008-1454.

van Knegsel, A. T. M., S. G. A. van der Drift, J. Cermáková, and B. Kemp. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: a systematic review. Veterinary journal (London, England 1997) 198(3):707–713. https://doi.org/10.1016/j.tvjl.2013.10.005.

Vanacker, N., S. Ollier, F. Beaudoin, R. Blouin, and P. Lacasse. 2017. Effect of inhibiting the lactogenic signal at calving on milk production and metabolic and immune perturbations in dairy cows. Journal of dairy science 100(7):5782–5791. https://doi.org/10.3168/jds.2017-12570.

Venjakob, P. L., S. Borchardt, and W. Heuwieser. 2017. Hypocalcemia-Cow-level prevalence and preventive strategies in German dairy herds. Journal of dairy science 100(11):9258–9266. https://doi.org/10.3168/jds.2016-12494.

Weaver, S. R., and L. L. Hernandez. 2016. Autocrine-paracrine regulation of the mammary gland. Journal of dairy science 99(1):842–853. https://doi.org/10.3168/jds.2015-9828.

Wheelock, J. V., J. A. F. Rook, and F. H. Dodd. 1965. The effect of incomplete milking or of an extended milking interval on the yield and composition of cow's milk. Journal of Dairy Research 32(3):237–248. https://doi.org/10.1017/S0022029900018604.

Wheelock, J. V., J. A. F. Rook, F. H. Dodd, and T. K. Griffin. 1966. The effect of varying the interval between milkings on milk secretion. Journal of Dairy Research 33(2):161–176. https://doi.org/10.1017/S0022029900011833.

Woodward, T. E., R. P. Hotis, and R. R. Graves. 1936. Incomplete milking in relation to milk production and udder troubles in dairy cows. USDA Technical Bulletin No. 522.

Chapter 8 Acknowledgements/Danksagung

Mein ganz besonderer Dank gilt Frau Prof. Dr. Dr. Sauerwein und Herrn Prof. Dr. Südekum, die mir die Möglichkeit gegeben haben am Institut für Tierwissenschaften zu promovieren. Ihre Türe, oder nach dem Ausbruch der Pandemie stellvertretend der Bildschirm, stand für meine Fragen immer offen. Die Möglichkeit, zusätzliche Parameter ins Projekt zu integrieren und zu untersuchen, sowie die Freiheiten, die sie mir während des gesamten Forschungsprojektes gewährten, hat maßgeblich zum Gelingen dieser Arbeit beigetragen. Ebenfalls danke ich Frau Dr. Müller für die Möglichkeit, das USL-Projekt bearbeitet haben zu dürfen.

Herrn Dr. Morteza Hosseini-Ghaffari und Frau Dr. Stephanie Hiss-Pesch möchte ich besonders danken. Ihre Türen standen für meine Fragen immer offen und auch die Diskussionen bei einer Tasse Kaffee waren wertvoller Input für meine Arbeit. Zudem gilt ein ganz großer Dank an Frau Dr. Eva Haese. Durch ihre Unterstützung bei der praktischen Umsetzung des Projekts auf dem Campus Frankenforst konnten die Versuche erfolgreich durchgeführt werden. Für die interessanten und hilfreichen Gespräche bin ich Ihnen sehr verbunden. Bedanken möchte ich mich auch bei den Mitarbeitern auf Frankenforst für die Vor- und Nachsorge der Versuchstiere und die unendliche Geduld bei der Probenentnahme während des Melkens.

Iris Gockel-Boehner (+🐾) war nicht nur bei den zahlreichen Probennahmen unverzichtbar, sondern ist auch als Freundin aus meinem Leben nicht mehr wegzudenken. Danke für deine Unterstützung und deine Warmherzigkeit in jeglicher Lebenssituation! Vielen lieben Dank an Barbara Heitkönig, Thomas Blees, Katharina Kustwan, Inga Hofs, Karin Strack, und die Azubis, die mir über die Laborarbeit hinaus eine große Hilfe waren. Ich werde die vielen lustigen Momente und Ausflüge, inklusive Feierabendfitness im Siebengebirge oder am Grill, nicht vergessen. Ebenso ein herzliches Dankeschön an Christian Post und Lena Dicks. Als „Office-Partner" habt ihr mit mir die Höhen und Tiefen einer Promotion durchlebt. Auch danke ich für die zahlreichen Diskussionen, Ratschläge und Gespräche.

Vielen Dank an meine Familie für den Glauben an mich und die Möglichkeit zu studieren und damit auch zu promovieren. Zum Schluss möchte ich zwei ganz besonderen Menschen danken: Sebastian und Lena. Ich bin froh, dass es euch in meinem Leben gibt, ihr immer für mich da seid, und mir den Rücken stärkt!

Chapter 9 Publications and contributions to conferences

Derived from this doctorate thesis:

- Meyer, I., E. Haese, K.H. Südekum, H. Sauerwein, and U. Müller. 2023. The impact of automated, constant incomplete milking on energy balance, udder health, and subsequent performance in early lactation of dairy cows. Journal of Dairy Science. https://doi.org/10.3168/jds.2023-23777.
- Meyer, I., E. Haese, K.H. Südekum, H. Sauerwein, and U. Müller. 2022. Automated, incomplete milking as energy management strategy in early lactation of dairy cows. Abstract; 5th -8th of September 2022, 73rd Annual Meeting of the European Federation of Animal Science (EAAP) in Porto, Portugal.