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The impact of short-term incomplete milking on dairy
cows in peak to mid-lactation

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„Die Dinge sind, wie sie sind“

-Aristoteles-

English abstract

The evolutionary-based genetic predisposition of dairy cows for milk production has been further enhanced by genetic selection and modern breeding programs. Resulting high milk production requires considerable energy supply, leading to a high lactation-induced metabolic load. Despite the fact that a high production level is only possible in healthy dairy cows, the vast need for energy and nutrients can be a burden to the cow, and high-yielding cows are more susceptible to certain diseases. In fact, in later lactation stages, dairy cows remain vulnerable to metabolic stress, especially when challenged by stressors, such as heat stress or insufficient feed supply. In this context, incomplete milking (IM) may be used to temporarily reduce milk production and mitigate the metabolic load of individual dairy cows.

In order to establish IM as a potential management strategy in peak- to mid-lactation dairy cows, comprehensive knowledge of its effects and potential limitations is essential. Therefore, the main aim of this dissertation was to implement an IM protocol and to investigate and characterize the effects on the lactation-induced metabolic load and the animal welfare of clinical healthy dairy cows. The experimental design included incompletely milked (INCL; $n = 23$, 93 ± 46 DIM) and completely milked (COMP; $n = 23$, 95 ± 49 DIM) multiparous dairy cows. For the INCL cows, IM was implemented using a software module, enabling earlier cluster removal after a predefined amount of milk (kg) has been collected, rather than based on the milk flow rate (kg/min). The IM protocol included a gradual reduction phase (first IM phase: 9% daily reduction to ~40% over 5.5 d), followed by 3 complete milkings, and a constant reduction phase (second IM phase: 40% reduction over 5.5 d). Concentrate allocation was based on the feed-to-yield system (depending on the milk yield). Blood samples were collected on d -9, -2, 6, 13, 20, 27 relative to the start of IM. The present study demonstrates that the IM protocol was successfully implemented and that the reduction in milk yield increased with the duration and intensity of IM. Consequently, INCL cows received less concentrate, reflecting lower energy requirements. The milk yield remained reduced shortly after IM but increased again with the resumption of continuous complete milking. The reduced milk yield affected the concentration of two metabolic indicators in serum: Free fatty acids decreased during both IM phases in INCL cows pointing to a lesser mobilization of body fat reserves, and insulin-like growth factor-1 concentrations were elevated during both phases, indicating a reduced lactation-induced metabolic load. At the same time, consistently good udder health and no evidence of impaired animal welfare were observed according to the evaluated indicators.

This dissertation provides initial insights into the application of an IM protocol and its effects on milk production, metabolic indicators, and animal welfare parameters in dairy cows during peak- to mid-lactation. In order to establish IM as a potential management strategy for these lactation stages, it should be further evaluated under actual stress conditions such as heat stress or insufficient feed supply in future studies.

Kurzfassung

Die evolutionsbedingte genetische Disposition von Milchkühen für Milchproduktion wurde durch genetische Selektion und moderne Zuchtprogramme weiter verstärkt. Die daraus resultierende hohe Milchproduktion erfordert jedoch eine beträchtliche Energiezufuhr und führt somit zu einer hohen laktationsbedingten metabolischen Belastung. Obwohl ein hohes Produktionsniveau nur von gesunden Milchkühen erreichbar ist, stellt der enorme Bedarf an Energie und Nährstoffen eine Belastung für die Kuh dar, wodurch hochleistende Tiere anfälliger für bestimmte Erkrankungen sind. Milchkühe sind selbst in späteren Laktationsstadien anfällig für metabolischen Stress – insbesondere, wenn sie durch Hitzestress oder eine unzureichende Futtermittellieferung zusätzlich belastet sind. In diesem Zusammenhang kann das unvollständige Melken (IM) potenziell eingesetzt werden, um die Milchproduktion vorübergehend zu reduzieren und somit die Stoffwechselbelastung einzelner Milchkühe zu mindern.

Um IM als potenzielle Managementstrategie bei Milchkühen in der Hoch- bis Mittellaktation zu etablieren, ist ein umfassendes Verständnis seiner Wirkung und möglicher Limitierungen erforderlich. Ziel dieser Dissertation war es daher, ein IM-Protokoll zu implementieren und dessen Auswirkungen auf die laktationsinduzierte metabolische Belastung sowie auf das Tierwohl klinisch gesunder Milchkühe zu untersuchen. Das Experiment umfasste unvollständig gemolkene (INCL; $n = 23$, 93 ± 46 Laktationstage) und vollständig gemolkene (COMP; $n = 23$, 95 ± 49 Laktationstage) mehrkalbige Milchkühe. Bei den INCL-Kühen wurde das IM mithilfe eines Softwaremoduls umgesetzt, welches eine frühere Abnahme der Melkzeuge nach Erreichen einer vordefinierten Milchmenge (kg) ermöglicht, anstatt wie üblich nach dem Milchfluss (kg/min). Das IM-Protokoll umfasste eine Phase der schrittweisen Reduktion (erste IM-Phase: tägliche Reduktion um 9 % bis auf ~40 % über 5,5 Tage), gefolgt von drei vollständigen Melkungen und einer konstanten Reduktionsphase (zweite IM-Phase: 40 % Reduktion über 5,5 Tage). Die Zuteilung des Kraftfutters erfolgt in Abhängigkeit der Milchleistung, Blutproben wurden an den Tagen -9, -2, 6, 13, 20 und 27 relativ zum Beginn des IM entnommen. Die vorliegende Studie zeigt, dass das IM-Protokoll erfolgreich umgesetzt wurde und die Reduktion der Milchleistung mit der Dauer und Intensität des IM zunahm. Dadurch wurde den INCL-Kühen weniger Kraftfutter zugeteilt, was den geringeren Energiebedarf der Kühe widerspiegelte. Die Milchleistung war auch noch kurz nach der IM Anwendung reduziert, stieg aber mit fortlaufendem vollständigem Melken wieder an. Die verringerte Milchleistung beeinflusste zwei metabolischen Indikatoren im Serum: Die Konzentration an freien Fettsäuren war während beider IM-Phasen bei den INCL Kühen reduziert, was auf eine geringere Mobilisation von Körperfettreserven hindeutet und die Konzentration des insulin-artigen Wachstumsfaktors 1 war in beiden Phasen erhöht, was auf eine verringerte laktationsinduzierte Stoffwechselbelastung hinweist. Gleichzeitig blieb die Eutergesundheit stabil und es zeigten sich anhand verschiedener Indikatoren auch keine Hinweise auf eine Beeinträchtigung des Tierwohls, trotz eines deutlichen Anstiegs der Euterfestigkeit bei den INCL Kühen.

Diese Dissertation liefert erste Erkenntnisse zur Anwendung eines IM-Protokolls und dessen Auswirkungen auf die Milchproduktion sowie auf metabolische und tierwohlbezogene Indikatoren bei Milchkühen in der Hoch- bis Mittellaktation. Um IM als potenzielle Managementstrategie zu etablieren, sollte es in zukünftigen Studien unter Belastungsbedingungen wie Hitzestress oder unzureichender Futtermittellieferung angewendet werden.

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List of abbreviations

AI	Artificial intelligence
ANS	Autonomic nervous system
BTV	Blue tongue virus
COMP	Complete milked cows
DIM	Days in milk
DMI	Dry matter intake
GLUT	Glucose transporter
HR	Heart rate
HRV	Heart-rate variability
IGF-1	Insulin-like growth factor-1
IM	Incomplete milking
INCL	Incomplete milked cows
MEC	Mammary epithelial cells
MIR	Mid-infrared
MO	Milking out
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
ODM	Once daily milking
PBMC	Peripheral blood monocular cells
PLF	Precision livestock farming
PRL	Prolactin
THI	Temperature-Humidity Index
TJ	Tight junctions

1. Introduction

Milk yield in modern dairy cows has increased almost linearly for decades, and there is currently no end to this trend in sight (Pulina et al., 2020). While this development improves production efficiency (Britt et al., 2018) and reduces the climate impact per kg of milk (Capper and Cady, 2020), it has also raised animal welfare concerns (Fleischer et al., 2001; Gross, 2024). In spite of that, only healthy cows can conceive their full genetic potential for milk production (Gross, 2022), yet high-yielding cows are at increased risk of various diseases (Fleischer et al., 2001; Boichard and Brochard, 2012). Thus, the burden of high milk production cannot be ignored, as several limiting factors, such as physiological constraints, environmental stressors, and management practices, impede the expression of genetic potential (Gross, 2023). This burden is particularly evident during the transition period, when extensive homeorhetic adaptations are required when the dairy cow has to shift from a non-lactating to a lactating state and to support increasing milk production (Bruckmaier and Gross, 2017), resulting in high energy requirements during a phase with low energy intake and thus the typically negative energy balance (**NEB**; Gross et al., 2011). During the transition period, disruptions of the homeorhetic processes, including infections or inadequate nutrition, can lead to metabolic diseases (Sordillo and Raphael, 2013). In this context, incomplete milking (**IM**) has been used to mitigate the metabolic load during early lactation (Carbennau et al., 2012; Morin et al., 2018; Meyer et al., 2024), by temporarily reducing milk yield (Penry et al., 2016; Deacon et al., 2022). However, little information is known about applying IM during peak- to mid-lactation, particularly regarding its effects on metabolism. Although metabolic prioritization of the mammary gland becomes less pronounced in established lactation compared to early lactation, due to more stable energy and nutrient homeostasis (Bauman and Currie, 1980; Mattmiller et al., 2011), energy demands remain high and physiologically relevant (Gross et al., 2011). As a result, dairy cows remain vulnerable to metabolic stress even in later lactation stages, particularly when challenged by lameness (Thomsen et al., 2023), inadequate nutrition (Zbinden et al., 2017), or heat stress (Tao et al., 2018). Heat stress, in particular, is a major concern of animal production systems, negatively affecting productivity, health, and welfare (Becker et al., 2020). In order to establish IM as a potential management strategy in peak- to mid-lactation, comprehensive knowledge of its effects and potential limitations are essential.

1.1 Impact of milk production on the dairy cow

1.1.1 Importance of evolutionary based prioritization of the mammary gland for modern dairy breeds

The modern dairy cattle breeds belong to the placental mammals, the largest group within the class Mammalia. A defining feature of mammals, and particularly placentals, is a lactation via specialized mammary glands, which entails significant survival advantages for both offspring and mother, but also results in problems for the dairy sector (Fig. 1). In cattle, calves are pseudo-monogastric in early life, depending entirely on milk as their only usable nutrient source. Beyond nourishment, colostrum intake is essential for establishing passive immunity, as immunoglobulins cannot cross the placental barrier in ungulates (Baumrucker et al., 2023). Given this dependency, start of lactation must be precisely timed to ensure timely colostrum provision for formation of passive immunity in the offspring. Accordingly, placental progesterone levels decline already before parturition, initiating lactogenesis and colostrum formation (Bruckmaier and Gross, 2017). Lactation has evolved as a metabolically prioritized process in mammals to ensure offspring survival, supported by extensive homeorhetic adaptations in the mother (Bruckmaier and Gross, 2017). Thereby, in cattle, the relatively mature state of calves at birth leads to rapid growth and increased nutrient demand, which in turn drives high milk production by the dam. If dietary energy intake cannot meet this rising demand, a NEB occurs. This triggers metabolic adaptations, including the mobilization of body reserves to support nutrient allocation to the mammary gland and accordingly milk synthesis (Gross and Bruckmaier, 2015). Such adaptations are physiologically normal, especially at the onset of lactation, allowing continued milk production despite limited feed intake (Ofstedal, 2012). The shape of the lactation curve in mammals reflects the nutritional demands of the offspring. As the calf matures and its gastrointestinal tract develops, it gradually shifts to external feed, reducing the dependency on milk. Consequently, the metabolic priority of the mammary gland declines over time (Guilloteau et al., 2009). In later lactation stages, the offspring can survive with little or no milk, further decreasing the importance of nutrient allocation to the mammary gland (Bruckmaier and Gross, 2017).

However, in modern dairy breeds, milk production exceeds by far the nutritional requirements of the offspring. The metabolic prioritization of the mammary gland has evolved during evolution and is genetically determined, enabling extensive homeorhetic adaptations to support lactation. Due to this genetic disposition, selective breeding programs aiming at in-

creasing milk production have been quite successful, resulting in high milk yields and correspondingly elevated energy demand for milk synthesis (Oltenacu and Algers, 2005). Moreover, modern breeding techniques have further accelerated genetic progress in milk yield (Fleming et al., 2018; Brito et al., 2021). Consequently, selection for milk production has favored cows partitioning energy and protein from the diet and body reserves towards the mammary gland to support milk synthesis instead of storage in body tissues (Veerkamp et al., 2003). The primarily selection for milk production has led to reduced reproductive success rates, animal health and fitness (Egger-Danner et al., 2015; Berry et al., 2016).

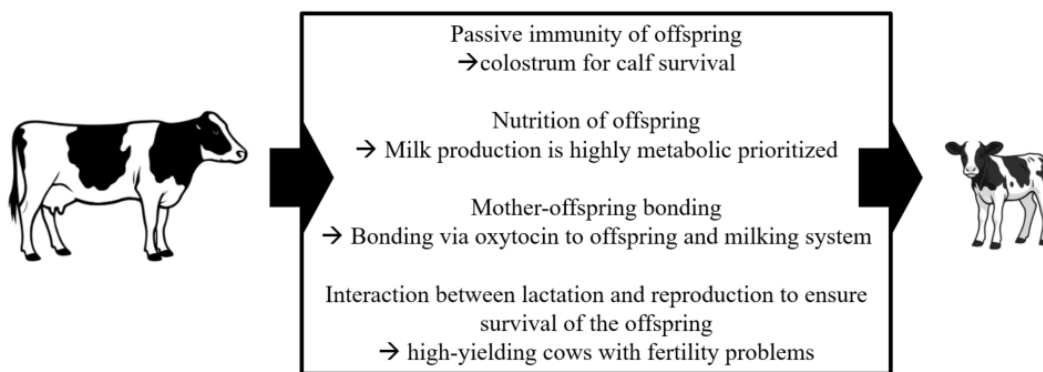


Figure 1. Own illustration of the major biological task of lactation to ensure maternal care of the offspring and resulting problems for the dairy sector (after Bruckmaier and Gross, 2017)

1.1.2 Homeorhetic control of milk production

The onset of lactation in dairy cows triggers a marked increase in the demand for nutrients such as glucose, fatty acids, and amino acids, which by far exceeds dietary intake (Bell, 1995; Drackley, 1999). Adaptive processes begin in late gestation and intensify around parturition, involving multiple tissues to support milk production (Bell and Baumann, 1997; Gross and Bruckmaier, 2017). These metabolic changes are governed by the principles of homeostasis and homeorhesis, as outlined by Baumann and Currie (1980). Homeorhetic regulation refers to coordinated, long-term adjustments in metabolism of tissues that support a specific physiological state – e.g., lactation – by altering tissue responsiveness to homeostatic signals (Baumann and Currie, 1980). Lactation, genetically determined and potentiated by breeding (see Chapter 1.1.1), involves significant shifts in endocrine functions. Changes in the control of metabolism were described by Kahn (1978) who suggested indicators of hormone response, responsiveness and sensitivity, which dramatically change in major metabolic tissues. The mammary gland,

adipose tissue, liver, and skeletal muscle undergo major reprogramming to prioritize milk synthesis (Bell, 1995; Nayeri and Stothard, 2016). Notably, dairy cows can remain in a NEB while milk yield continues to rise in early lactation, a condition termed “lactation-induced NEB” (Butler et al., 2003). This energy deficit is mainly compensated through extensive mobilization of adipose tissue (van der Drift et al., 2012; Mann et al., 2015) and skeletal muscle (Sadri et al., 2023). Hormonal adaptations underpin this mobilization: insulin, insulin-like growth factor-1 (**IGF-1**), leptin, and thyroid hormones decline, supporting metabolic shifts that redirect nutrients toward the mammary gland (Wankhade et al., 2017; Bruckmaier and Gross, 2017). Under normal homeostasis, insulin regulates glucose uptake in peripheral, insulin-dependent tissues (Brockman and Laarveld, 1986). Conversely, in early lactation, peripheral tissues become insulin-resistant (Bell and Baumann, 1997), insulin secretion is reduced (Butler et al., 2003), and glucose uptake in the mammary gland is facilitated primarily by the insulin-independent glucose transporter (**GLUT**)-1 (Gross et al., 2011). These adaptations reduce nutrient use by peripheral tissues and promote nutrient allocation to the mammary gland, supporting milk production (Bell and Baumann, 1997). Consistent with this, higher milk yields are associated with lower insulin concentrations (Zinicola and Bicalho, 2019). Changes in intermediary metabolism further enhance tissue mobilization and nutrient transfer to the mammary gland, reinforcing lactation as the body’s prioritized state (Baumann and Currie, 1980; Gross and Bruckmaier, 2019).

Only recently, the role of the immune system for the successful adaption of dairy cows to the transition period has started to be investigated (Mezzetti et al., 2024). Immune activation imposes high nutrient demands, particularly for glucose and amino acids, making it metabolically relevant for successful adaptation to increasing milk production. Immune cells rely exclusively on glucose (Sordillo et al., 2009), and cytokines trigger acute phase protein synthesis, which requires specific amino acids (Ceciliani et al., 2012). In this manner, the mammary gland and immune system compete for nutrients (Gross, 2023), increasing metabolic load and challenging adaptation. Consequently, immune suppression is common in early lactation, increasing disease risk (Wankhade et al., 2017). The abrupt metabolic shift during the transition period challenges the cow’s adaptive capacity and is linked to various disorders, including retained placenta, mastitis, ovarian cysts, claw diseases, and milk fever (Fleischer et al., 2001).

Although many health issues are associated with high performance levels (Gross, 2024), the exact relationship between high milk production and diseases remains unclear. Disruption of the fragile homeorhetic processes might lead to failed adaption and results in reduce perfor-

mance, health and metabolic disorders (Bertoni et al., 2009; Sordillo and Raphael, 2013). Unsurprisingly, the highest incidence of production diseases occurs during periods of maximum metabolic load, highlighting the critical role of milk production energy deficiency during homeorhetic adaptation (Mulligan and Doherty, 2008).

1.1.3 Homeostatic control of milk production

To support milk synthesis for nutrition of the offspring, the mammary gland operates largely independent of homeostatic control during early lactation (Bruckmaier and Gross, 2017). In later lactation, this metabolic burden decreases as milk becomes less critical for calf survival – for instance, when rumen development advances (Guilloteau et al., 2009) – but in modern dairy cattle, the milk yield still exceeds by far the nutritional requirements of the offspring. During advanced lactation, restrictive feeding of dairy cows results in reduced milk yield and less tissue mobilization compared to early lactation, reflecting a decline in metabolic priority and increased homeostatic regulation of milk production (Bauman and Currie, 1980; Gross et al., 2011; Gross and Bruckmaier, 2019). Homeostasis, as defined by Bauman and Currie (1980), involves maintaining internal physiological equilibrium. Despite such regulatory efforts, milk yield naturally declines after peak lactation, though this decline varies with lactation persistency, which is influenced by the number and activity of mammary epithelial cells (Capuco et al., 2003), as well as factors such as nutrition, metabolic oxidative stress, and pregnancy (Svennersten-Sjaunja and Olsson, 2005). During mid- and late-lactation, the expression of insulin-sensitive GLUT-4 increases in the mammary gland, indicating a shift toward homeostatic control (Mattmiller et al., 2011). Although insulin-independent GLUT-1 remains the most abundant transporter (Zhao et al., 1996), rising insulin levels and insulin sensitivity enhance glucose utilization in peripheral tissues (Bell and Baumann, 1997; Butler et al., 2003). In advanced pregnancy, milk production declines due to both fetal nutrient demands (Loker et al., 2009) and hormonal shifts that promote mammary gland regression (Zhao et al., 2019).

From a nutritional perspective, milk yield becomes limited by glucose and amino acid availability, physiological constraints such as intestinal absorption, intermediary metabolism, mammary nutrient uptake, environmental stressors, and animal health (Gross, 2022). During feed restriction in mid-lactation, milk yield response varies with days in milk (**DIM**) and genetic merit for milk production (Carlson et al., 2006; Gross et al., 2011). Nevertheless, even with reduced milk production, the resulting NEB is not fully mitigated (Gross et al., 2011; Pires et al., 2024), indicating limits to homeostatic compensation. The metabolic load, defined as the

burden imposed by the production of milk, increases with selective breeding for higher yields (Oltenacu and Broom, 2010). Notably, high genetic merit for milk yield persists even in later lactation when homeostatic control predominates (Gross et al., 2011; Bjerre-Harpøth et al., 2012). Of note, the effects of quantitative traits loci related to lactation performance are not constant throughout lactation, probably influenced by NEB in early lactation and by pregnancy in late lactation (Lu et al., 2020). Moreover, metabolic adaptations and gene expression profiles vary throughout lactation (Becker et al., 2021). However, other biological functions as milk production may also trigger homeorhetic adaptations, raising nutrient demands and modifying metabolism – for example, immune activation during inflammation (Bradford and Swartz, 2020) or coping mechanisms during heat stress (Bernabucci et al., 2010). When multiple processes require the same resources, competition arises under limited availability (Rendel, 1963). During NEB, lipolysis and gluconeogenesis are promoted, while immune function is suppressed, evidenced by reduced immunocytes and elevated inflammatory cytokines (Esposito et al., 2014). Thus, high milk production can become a physiological burden for modern dairy cows, which need to be addressed regarding animal health and welfare (Brito et al., 2021).

1.1.4 Environmental stress and the role of milk production

Stress originates externally and puts a strain on a biological system e.g., animal, dealing with it leads to expenditure of resources (Collier et al., 2017). During stress, the organism strives to maintain homeostasis, which is disrupted by the external challenge, through specialized adaptive responses aiming at preserving internal equilibrium. Environmental fluctuations influence physiological states and necessitate homeostatic adaptations. Thereby, individual cows vary in their resilience and metabolic adaptability to such conditions (Berghof et al., 2019; Poppe et al., 2020), with adaptive capacity also depending on lactation stage (Carlson et al., 2006; Gross et al., 2011). Coping with environmental stressors requires high energy expenditure (Collier et al., 2017). However, energy intake is limited by animal-based factors such as restricted rumen fill, fermentation, and passage rate (Gross, 2022), as well as external constraints like feed availability and quality. For instance, pasture-based diets typically support only up to ~30 kg/d milk yield (Bargo et al., 2003); higher yields require concentrate supplementation or mobilization of body reserves (Zbinden et al., 2017). Yet, increased concentrate intake has limitations, as it reduces rumination and salivary buffering capacity, impairing rumen function (Humer et al., 2018). Moreover, milk production is the primary driver of energy demand in dairy cows (Gross et al., 2011), and can vary depending on production related factors, e.g.,

social stress (Razzaghi et al., 2023) and environmental factors, e.g., heat stress (Baumgard and Rhoads, 2013). Also, the energy intake is influenced by these factors (Baumgard and Rhoads, 2013; Razzaghi et al., 2023). Social and heat stress can also trigger systemic inflammation, leading to cellular damage and increased metabolic load (Bradford et al., 2015). These stressors interact with production traits and may elevate the risk of production diseases (Schüpbach-Regula, 2024, in Gross, 2024). Such diseases originate from management deficiencies and are further exacerbated by genetic selection for high productivity, particularly in dairy cows (Nir, 2003). A well-known example is the negative genetic correlation between milk yield and fertility (Bedere et al., 2018). Disease development is also influenced by genetic predisposition, immune competence, age, breed, feeding practices, pathogen pressure and virulence, and environmental conditions (Schüpbach-Regula, 2024). Environmental stress affects nutrient partitioning and raises energy demands, potentially altering energy balance and lowering milk production (Razzaghi et al., 2023). Additionally, inflammation and immune activation lead to high glucose demand and reduced feed intake, causing NEB, extensive lipolysis, and associated disorders such as clinical/subclinical ketosis, mastitis, metritis, retained placenta, and reduced fertility (Horst et al., 2021). As milk yield and functional traits are often negatively genetically correlated, the focus of selection for milk production has compromised the genetic merit for health traits (Egger-Danner et al., 2015) and increased the environmental sensitivity of high-producing animals, i.e., the responsiveness of production traits to changing environmental conditions (Friggens et al., 2017). In recent years, health, fertility, and fitness traits have been incorporated into breeding values worldwide to address the challenges arising from selection for only higher milk yield. Multi-trait breeding programs now enable simultaneous improvement of milk production and other traits, e.g., fitness traits (Cole and van Raden, 2018; Brito et al., 2021).

1.1.5 Heat stress in lactating dairy cows and the role of milk production

Heat stress is recognized as the most significant environmental stressor affecting the dairy industry, leading to reduced productivity, impaired health, economic losses, and welfare concerns. In endothermic animals, heat stress arises when the thermal load exceeds the animal's capacity for thermoregulation. Thermal strain is defined as "any activation of organ system activities in response to thermal stress that causes sustained changes in the state of other, non-thermal regulatory systems" (IUPS Thermal Commission, 2001). As a result, adaptation to heat

stress involves multiple organ systems and behavioral changes (e.g., reduced feed intake, increased water intake, and decreased activity) to minimize heat production and enhance heat loss (Collier et al., 2017). Milk yield plays a key role in heat stress susceptibility, as higher metabolic rates – driven by milk production – lead to greater internal heat generation (Berman, 2011; Collier et al., 2017; Tao et al., 2020). The upper critical temperature of the thermoneutral zone decreases with increasing milk yield, making high-producing cows more vulnerable to heat stress (Tober, 2022; Fig. 2). In consequence, genetic selection for milk yield has reduced thermoregulatory capacity (Bohmanova et al., 2005; Santana et al., 2017), as shown by negative genetic correlations between milk production under thermoneutral and heat stress conditions (Sigdel et al., 2019).

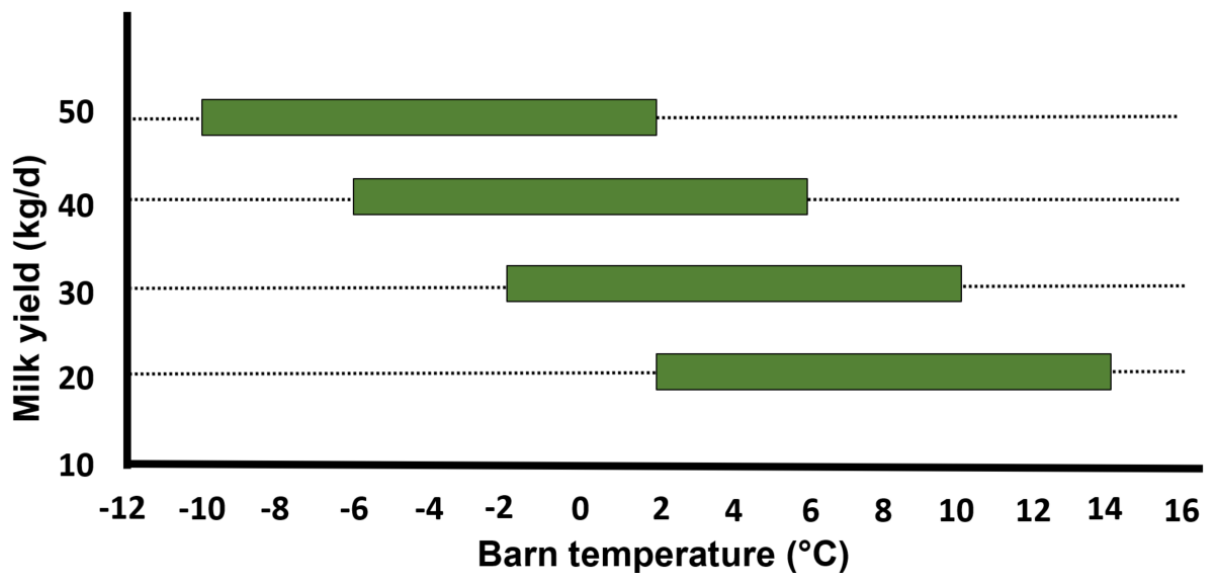


Figure 2. Own illustration of thermoneutral zones depending on milk yield level (after Tober, 2020).

To support high milk output, energy-demanding organs such as the mammary gland, liver, and digestive tract increase in size (Smith and Baldwin, 1974), leading to higher maintenance requirements and feed intake. However, increased feed intake further elevates heat production due to ruminal fermentation (Purwanto et al., 1990; Beatty et al., 2008; Bernabucci et al., 2014). Thus, modern high-yielding breeds are particularly susceptible to heat stress, especially when considering ongoing selection for higher milk production (Pulina et al., 2020). Importantly, climate change is expected to exacerbate heat stress, even in temperate regions (Henry et al., 2018). Already moderate ambient temperature rises can trigger heat stress in temperate climates, likely due to limited acclimatization (van Laer et al., 2015; Collier et al., 2019;

Pontiggia et al., 2023). The temperature-humidity-index (**THI**) is a common heat-stress indicator in dairy cows. The THI threshold for milk yield decline varies by region and climate. For example, cows in Arizona (USA) show milk yield losses when the THI exceeds 68, whereas in Central Europe, milk yield losses have been observed when the THI exceeds 62 (Zimbelmann et al., 2009; Hammami et al., 2013). Exceeding an individual cow's THI tolerance impairs thermal regulation, elevates body temperature, and compromises productivity and welfare (Zimbelmann et al., 2009; Becker et al., 2020). Meta-analyses confirm that the impact of THI on production traits depends on lactation stage and animal characteristics (Chen et al., 2024). Overall, the combination of heat stress intensity, duration, and physiological status determines its impact (Tao et al., 2020). Heat stress also reduces dry matter intake (**DMI**) and alters metabolism due to nutrient deficits, increasing the risk of metabolic disorders such as ketosis and acidosis (Collier et al., 2017). Reduced DMI is a behavioral adaptation to lower internal heat load (West, 2003) but also results in body weight loss (Baumgard et al., 2011). Importantly, decreased intake accounts for only ~50% of milk yield reduction, with altered post-absorptive metabolism responsible for the remainder (Wheelock et al., 2010). Recent research highlights the role of the gastrointestinal tract in heat stress pathology. Heat stress increases gut permeability, allowing diffusion of lipopolysaccharides and antigens into circulation, triggering inflammation (Baumgard and Rhoads, 2013; Oshima and Miwa, 2016; Fontoura et al., 2022). Although mechanisms of gut barrier disruption remain unclear, the induced immune activation affects metabolism by inducing hyperinsulinemia and altered glucose partitioning. By expressing insulin receptors, glucose is increasingly supplied to immune cells in response to heat stress-related elevated insulin concentrations (Baumgard and Rhoads, 2013; Kvidera et al., 2017). Heat stress also alters amino acid metabolism, increasing their mobilization to support heat shock proteins, acute phase responses, hepatic protein synthesis, and gluconeogenesis (Gao et al., 2017; Rius et al., 2019). These changes in post-absorptive metabolism, combined with reduced DMI, ultimately contribute to production losses during heat stress (Baumgard and Rhoads, 2013).

The metabolic dilemma during heat stress arises from the simultaneous reduction in nutrient intake and the increased energy demands for maintenance and immune function (NRC, 2001; Baumgard and Rhoads, 2013). High milk production during peak lactation is associated with elevated metabolic activity and heat generation, leading to a positive correlation between milk yield and hyperthermia (Zimbelmann et al., 2009). Moreover, the decline in feed intake due to heat stress is more pronounced in high-producing cows (Gauly and Ammer, 2020), with the greatest permanently milk yield losses observed in mid-lactation dairy cows (Tao et al.,

2018). This highlights peak- to mid-lactation as a particularly heat-sensitive period and underscores milk yield as a key factor influencing heat stress susceptibility. Consequently, effective mitigation strategies are essential to reduce the negative impacts of heat stress on productivity and animal welfare (Becker et al., 2020) and could include modulation of the milk production.

1.2 Incomplete milking of dairy cows and welfare implications

Incomplete Milking (**IM**) has been studied since the early 20th century (e.g., Woodward et al., 1936; Schmidt et al., 1964; Wheelock et al., 1965), establishing its fundamental principle: intentionally leaving residual milk in the udder by shortening milking duration reduces milk yield. The extent of this reduction depends on the intensity and duration of IM, and the effect can persist even after resuming complete milking (Schmidt et al., 1964). High milk volumes stored in the udder, independent of storage time, influence milk yield and composition (Albaaj et al., 2018). Therefore, milk removal is essential for continued secretion. While maintaining milking frequency, IM modifies residual milk volume, likely affecting secretion through auto- and paracrine signalling (Weaver and Hernandez, 2016).

However, public concerns for animal welfare increases (Clark et al., 2016), and addressing them is essential for maintaining a social license to operate (Clark et al., 2024, in Gross 2024). The IM procedure must not compromise animal welfare by leaving elevated amounts of residual milk in the udder. Animal welfare may be at risk during IM due to reduced udder health (Kuehnl et al., 2019; Bach et al., 2022) or increased udder pressure (Blau et al., 2019). The latter has been shown to reduce dairy cow welfare during abrupt dry-off (Bertulat et al., 2013; Vilar and Rajala-Schulz, 2020). Udder pressure can be assessed either by invasive intra-mammary pressure measurement (Blau et al., 2019) or by non-invasive external udder firmness measurement (Bertulat et al., 2012). Considering this, the implications of udder pressure must be carefully evaluated when applying IM. As animal welfare is multifactorial – encompassing animal functioning, emotional state, and the ability to perform natural behaviors (van Keyserlingk et al., 2009) – its assessment, particularly under practical conditions, remains challenging (Andreasen et al., 2013; Stygar et al., 2021). Non-invasive, animal-based stress indicators include heart rate variability (**HRV**; Kovacs et al., 2014) and behavioral assessments (Gleerup et al., 2015; Cerqueira et al., 2017), both of which have been applied to evaluate dairy cow welfare (Lindstädt et al., 2024).

In this context, IM has been strategically applied to address key challenges in dairy production: improving energy balance in early lactation (Carbennou et al., 2012; Morin et al.,

2018; Meyer et al., 2024), reducing the risk of milk fever via increased blood calcium concentrations (Valdecabres et al., 2022), and improving the dry-off process (Martin et al., 2020; Müller et al., 2023). However, since increasing energy intake is limited in early lactation (Gross, 2022), reducing energy output via the mammary gland may help alleviate NEB. The IM has been explored for this purpose, though its effectiveness depends on protocol specifics and the metabolic status of the cow (Morin et al., 2018; Meyer et al., 2024). The IM may be particularly beneficial under conditions of elevated metabolic stress, which can occur not only in early but also in later lactation stages due to high milk yield and improved lactation persistency of modern dairy cows. At dry-off, high milk yields can cause discomfort and raise the risk of mastitis (Bertulat et al., 2013; Blau et al., 2019). To address this, automated IM procedures have been developed to gradually reduce milk yield and improve welfare and udder health (Martin et al., 2020; Müller et al., 2023). Although historical studies have established basic IM effects, milk yield responses vary depending on factors such as udder cistern size (Stelwagen, 2001; Albaaj et al., 2018), lactation stage (Martin et al., 2020; Meyer et al., 2024), mammary plasticity (Boutinaud et al., 2019), and intra-mammary signalling (Weaver and Hernandez, 2016). Thus, IM protocols must be evaluated case by case. Automated IM systems (Schmidt et al., 2020) offer standardized application and mitigate inconsistencies of manual IM (Morin et al., 2018; Deacon et al., 2022). However, effects of automated short-term IM during peak- to mid-lactation remain unexplored. While early lactation IM has shown metabolic benefits, its potential during later lactation is unclear. Although cows in established lactation may not require metabolic support, individual cows may benefit of IM under additional stressors such as heat stress or insufficient feed supply. Moreover, the potential of IM to improve animal welfare during periods of high milk output remains under-investigated.

2. Objectives and hypotheses

Evolutionary based genetic disposition and modern breeding programs determine the milk yield of dairy cows as important physiological function. Thereby, high milk production requires considerable energy supply, leading to high lactation-induced metabolic load. The IM has the potential to be a useful tool to strategically induce short-term reduced milk production to reduce metabolic stress. Of note, the effect of IM depends on several factors, including the applied intensity of the reduced milk withdrawal and the lactation stage. However, IM was applied in previous research in early lactation, late lactation, or using a half udder design, but its impact during peak- to mid-lactation is less well studied. In addition, the effect of different IM intensities and increased udder pressure on dairy cow welfare is not well understood. Welfare assessments are crucial to broaden our knowledge on the impact of IM on the dairy cow. Moreover, the effect of IM as strategy to alleviate heat stress induced acute metabolic stress is unknown. Consequently, the present study aimed to fulfil the research gaps by the following objectives:

- I. To implement a yield-dependent IM in dairy cows in peak- to mid-lactation using a software-module for automated earlier cluster removal, consisting of two phases: gradual IM (up to a reduction of ~40% over 5.5 d), followed by further 5.5 d in which this 40% reduction was maintained.
- II. To investigate the effect of short-term IM on milk yield, udder health, milk composition, feed intake, and selected metabolic parameters.
- III. To evaluate the effect of IM-induced changes in udder filling and firmness for assessing welfare using HRV and behavior records in the milking parlor and barn.
- IV. To implement IM during heat stress and to gain initial insights.

We hypothesized that limiting the amount of milk withdrawn by up to 40% using the software module would directly affect milk production, therefore reducing the energy requirement and lactation-induced metabolic load without compromising udder health, while milk production after the IM treatment would not be compromised. Additionally, we hypothesized that short-term IM in peak- to mid-lactation dairy cows would result in increased udder filling and firmness, without negatively affecting HRV and behavior records in the milking parlor or barn. Moreover, based on the hypothesized general effects of IM, we performed a preliminary pilot study applying short-term IM in heat-stressed dairy cows to alleviate associated metabolic stress.

3. Manuscript I

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Effects of automated short-term incomplete milking during peak to mid lactation on milk production, udder health, and selected metabolic parameters in dairy cows

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Abbreviations: The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

ABSTRACT

This study investigated the effects of automated short-term incomplete milking (IM) during peak- to mid-lactation on milk production, udder health, and selected metabolic parameters in multiparous Holstein cows. Forty-six cows (94 ± 47 DIM) were randomly assigned to the complete milking treatment (COMP; $n = 23$, 95 ± 49 DIM, lactation number 3.0 ± 1.3) or the incomplete milking treatment (INCL; $n = 23$, 93 ± 46 DIM, lactation number 2.6 ± 0.8). In INCL cows, IM was implemented using specialized software that precisely controlled milk withdrawal. Concentrate allocation was dynamically adjusted based on milk yield and DIM via transponder-controlled stations. All cows had ad libitum access to a partial mixed-ration and water via automated weighing troughs and bowls. The IM protocol included a gradual reduction phase (first IM phase: 9% daily reduction to ~40% over 5.5 d), followed by 3 complete milkings, and a constant reduction phase (second IM phase: 40% reduction over 5.5 d). No clinical signs or mastitis-relevant bacteria were observed, confirming udder health. In the wk prior to IM, milk yield (reference milk yield, REF) did not differ between treatments, with INCL cows achieving between 26 and 59 kg/d. Compared to REF, the milk yield of the INCL cows decreased by 19% (32.7 ± 1.54 kg/d) on the first d after the first IM phase and by 24% (30.7 ± 1.54 kg/d) on the first day after the second IM phase. After cessation of IM, milk yield increased again by 19% within 2 wk and stabilized further at REF level. Compared to the COMP cows, milk fat content of the INCL cows decreased by 21% and 43% with the onset of the first and second IM phases. Concentrate intake of INCL cows decreased by 65% from 5.4 ± 0.52 kg/d at the beginning of IM to 1.9 ± 0.39 kg/d at the end of IM due to the feed-to-yield feeding system, reflecting lower energy requirements, and increased thereafter. Udder health indicators remained stable during IM. In the INCL cows that completed the trial, SCC were consistently below mastitis thresholds, and no relevant pathogens were detected. Milk leakage occurred exclusively in INCL cows during IM, but did not compromise udder health. Serum non-esterified fatty acid concentrations decreased by 63% and 58% during the first IM and second IM phases, respectively, indicating reduced lipid mobilization. Concurrently, IGF-1 concentrations increased by 30% and 28% during the respective phases, indicating metabolic adaptations to the reduced energy demand. In summary, automated short-term IM performed on dairy cows in peak- to mid-lactation using specialized software was successful in temporarily reducing milk production and lactation-induced metabolic load without negatively affecting udder health or long-term performance.

Key words: Management strategy, milking strategy, metabolic adaptation, lactation performance.

INTRODUCTION

In recent decades, genetic selection has increased the milk production potential of dairy cows (Barkema et al., 2015), leading to extensive homeorhetic processes to meet the high energy demands of the mammary gland (Gross and Bruckmaier, 2019). As a result, high milk production requires a significant amount of energy, leading to metabolic stress in dairy cows (Drackley et al., 2005). Temporarily reducing milk withdrawal and thus milk production and energy requirements can alleviate metabolic stress and improve cow welfare, especially in cases of negative energy balance (NEB) or inflammation (Carbonneau et al., 2012; Lacasse et al., 2018; Morin et al., 2018). Alternative milking strategies such as adjusting the milking frequency (Lacasse et al., 2018), the milking interval (Matera et al., 2024), changing the milk flow-rate switch-point (Browne et al., 2024) or incomplete milking (IM; Martin et al., 2020; Müller et al., 2023; Meyer et al., 2024) offer the possibility to modulate milk production and improve cow welfare. Among these strategies, IM has gained attention for strategically reducing milk yield temporarily while maintaining milking stimuli, thereby alleviating metabolic stress (Carbonneau et al., 2012) without compromising udder health (Martin et al., 2020; Müller et al., 2023) or overall productivity (Krug et al., 2018; Meyer et al., 2024).

Incomplete milking intentionally leaves elevated volumes of residual milk in the mammary gland in order to reduce milk production while maintaining the milking frequency. However, practical application is limited by the labor-intensive nature of manual implementation (Morin et al., 2018; Bach et al., 2022). Milking systems equipped with specialized software modules (Schmidt et al., 2020) enable the precise implementation of IM protocols across various lactation stages, including dry-off and early lactation. In dry off, a gradual reduction in milk production is particularly beneficial to avoid discomfort from intramammary pressure and udder engorgement in high yielding cows (Bertulat et al., 2013; Silanikove et al., 2013). For example, Martin et al. (2020) reported that using the software module to reduce milk withdrawal by 5%/d for 10 d during a gradual IM program reduced milk yield by 35%, while udder health and SCC remained stable and teat morphology improved. Also, Müller et al. (2023) used the software module to achieve a 5% daily reduction in milk withdrawal, effectively suppressing milk yield within a wk by 22% without clinical mastitis, even in cows with minor pathogen infected udders.

Incomplete milking has been studied as a strategy to reduce metabolic stress and support energy balance during early lactation, alleviating NEB associated with inadequate energy intake without compromising cow health or long-term performance. Carbonneau et al. (2012) demonstrated that limiting milk withdrawal to one third of expected yield during the first 5 d postpartum reduced milk yield during treatment, but had no effect on milk yield, composition or feed intake in the following wk. Also, Morin et al. (2018) found that IM during the first 5 d of lactation reduced the prevalence of ketonemia and hyperketonemia in the postpartum period. Meyer et al. (2024) applied an IM protocol starting on d 8 postpartum and limiting milk withdrawal to the individual yield of each cow for 14 d, which reduced milk yield by 11.1% compared to the control treatment. Notably, no adverse effects on udder health were observed, and milk production recovered already in the wk after IM, confirming the feasibility of IM protocols to manage milk yield during early lactation without long-term effects on productivity or udder health.

During peak lactation, cows produce high milk yields that require considerable energy resources, resulting in high metabolic stress. Therefore, it was hypothesized that limiting the amount of milk withdrawn by up to 40% using the software module would directly affect milk production, reducing energy requirements and lactation-induced metabolic load, without compromising udder health, whereas milk production would remain stable in the long term. Despite previous research on IM during dry off and early lactation, its application during peak lactation is less well studied. The aim of this study was therefore to investigate the effects of gradual IM (up to a reduction of ~40% over 5.5 d), followed by a constant reduction in milk withdrawal (-40% for 5.5 d), on milk yield, udder health, milk composition, feed intake, and selected metabolic parameters in multiparous cows during peak- to mid-lactation. The two IM phases were divided by a milking out period to evaluate separately the effects of the first IM phase on milk production.

MATERIAL AND METHODS

All experimental procedures in this study were performed in accordance with ethical guidelines and approved by the competent authority (Office for Nature, Environment, and Consumer Protection [LANUV], North Rhine-Westphalia, Recklinghausen, Germany; reference number: 81-02.04.2022.A191/01). The experiment was conducted from September 2022 to April 2024 at the research station ‘Campus Frankenforst’ (Königswinter, Germany) of the University of Bonn.

Animals and Housing Conditions

Forty-six multiparous German Holstein dairy cows in peak- to mid-lactation (94 ± 47 DIM) were selected from a herd of approximately 60 dairy cows for this study. The cows were housed in a two-row open free-stall barn and fed a partial mixed ration (PMR) with an average energy content of 6.52 MJ NE_L/kg DM (Table 1) for ad libitum intake. The feed was delivered twice daily at 09:00 and 15:00, and feed intake was monitored using weighing troughs (Hokofarm Group B.V., Marknesse, the Netherlands). Concentrate was additionally provided in a restricted manner according to the individual milk yield and DIM with limited daily changes via separate feeding stations with transponder access (GEA Farm Technologies GmbH, Bönen, Germany). All cows had ad libitum access to clean water via automatic water bowls with weighing function (Hokofarm Group B.V.). The BW was measured twice daily after milking using electronic scales (Hokofarm Group B.V.). The function of all scales was routinely checked and calibrated to ensure accuracy and consistency in measurements. Milking was performed twice daily at 05:30 and 16:30 in a double four-line milking parlor (GEA Farm Technologies GmbH) with automatic cluster removal at <0.3 kg/min. The udders were cleaned with damp paper towels, followed by forestripping and mechanical pre-stimulation via the cluster vacuum. Milk flow, milking duration, and milk yield were automatically recorded for each cow during each milking using the Metatron C21 system (GEA Farm Technologies GmbH).

Table 1. Composition of the partial mixed ration (PMR) and its analysed characteristics

Item	Composition
Ingredient (% of ration DM)	
Corn silage	30.7
Grass silage	28.6
Concentrate mix ¹	16.6
Alfalfa silage	15.8
Beet pulp silage	6.7
Rapeseed meal	0.5
Mineral and vitamin mix ²	1.1
Analysed characteristics of the PMR	
Energy density (MJ NE _L /kg DM)	6.52
CP (g/kg DM)	139.6
ADF (g/kg DM)	248.2

¹Concentrate mix main ingredients: Corn; wheat; rapeseed meal, solvent-extracted; rapeseed meal, solvent-extracted and hydrothermally 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)

²Mineral and vitamin mix main ingredients: Ca, 18.0%; P, 4.0%; Na, 8.0%; Mg, 7.5%; vitamine A, 800000 I.E.; vitamine D3, 130000 I.E.; vitamine E, 3000 mg (Lexa TMR Top 18-4, Xaver Scheule GmbH, Kirchheim, Germany) and sodium bicarbonate (ETI SODA, Ankara, Turkey)

Experimental Design for Incomplete Milking

Between September 2022 and April 2024, multiparous cows in peak- to mid-lactation were examined for udder health by collecting aseptic quarter foremilk on two separate occasions. To qualify for enrolment in the trial, cows had to show no clinical signs of mastitis and have negative bacteriology results for all quarter samples, confirming udder health. Eligible cows were randomly assigned to either the complete milking treatment (COMP; $n = 23$, 95 ± 49 DIM, mean lactation number 3.0 ± 1.3) or the incomplete milking treatment (INCL; $n = 23$, 93 ± 46 DIM, mean lactation number 2.6 ± 0.8) with stratification by milk yield and milk fat content. The final number of cows per treatment was smaller than the number originally calculated by the *a priori* power analysis. This discrepancy was most likely due to the novel nature of the automated IM protocol, particularly its two-phased, software-controlled implementation in peak- to mid-lactation, for which no directly comparable studies were available to provide reliable estimates of effect size and variability for an accurate power calculation. The selected

sample size ($n = 23$ per treatment) was based on experimental considerations, including adherence to the 3R principles and significant treatment effects with medium to large effect sizes, ensuring sufficient sensitivity to detect biologically relevant differences.

For the INCL cows, IM was implemented using a software module developed by GEA (GEA Farm Technologies GmbH; based on Schmidt et al., 2020). This software enables cluster removal after a predefined amount of milk (kg) was collected, rather than based on the milk flow-rate (kg/min). The software allowed both a gradual stepwise reduction in milk withdrawal and the collection of a constant amount of milk. In the INCL cows, the IM protocol consisted of two distinct phases: a gradual reduction in milk withdrawal (first IM phase) and a phase of constant milk collection (second IM phase), divided by a milking out period, as shown in the study design (Figure 1a). In addition to controlling milk withdrawal, concentrate allocation based on the feed-to-yield system was also part of the study design for the INCL cows, as it meets the modified requirements. The concentrate allocation and intake followed the trend in milk yield, as shown in Figure 1b, and reflected the lower energy requirements associated with lower milk production. Prior to starting the IM protocol, all cows were milked conventionally, with cluster removal based on milk flow (<0.3 kg/min). The 14 d prior to the start of IM served as a control period. The software calculations for the IM protocol were based on the individual average milk yield in the 7 d preceding IM (referred to as reference milk yield = REF). During the first IM phase, milk withdrawal was gradually reduced in a curvilinear manner by approximately 9% per d over 5.5 d (until morning milking on d 6). At the end of this phase, milk withdrawal was reduced by approximately 40% compared to REF. This reduction in milk withdrawal was considered sufficient for the purpose of the study. A gradual approach was chosen to gradually increase the effect of IM, as it was considered gentler and more sustainable than an abrupt method. Following the first IM phase, three conventional milkings with cluster removal based on milk flow (<0.3 kg/min) were performed. During the first milking, the residual milk accumulated during IM was withdrawn from the udder, while during the second and third milking, the adaptation of the cows to the reduced milk withdrawal of the first IM phase was evaluated based on the milk yield achieved. This allows the interpretation of the effect of the first IM phase on milk production parameters separately, whereas the effect of the second IM phase is influenced by the first IM phase. In the second IM phase, the reduced milk withdrawal was maintained at the level of 40% achieved in the first IM phase for a further 5.5 d (until morning milking on d 6). After the second IM phase, conventional milking with cluster removal based on milk flow (<0.3 kg/min) was permanently reintroduced. At the first conventional milking after the second IM phase, the accumulated residual milk was withdrawn, and at the two

subsequent milkings, the adaptation of the cows to the IM protocol was assessed based on the milk yield achieved.

Throughout the study, COMP cows were milked conventionally, with cluster removal based on milk flow (<0.3 kg/min). The milk yield of both treatments (COMP and INCL) was monitored daily and compared throughout the trial period, which extended up to 6 wk after completion of the IM protocol.

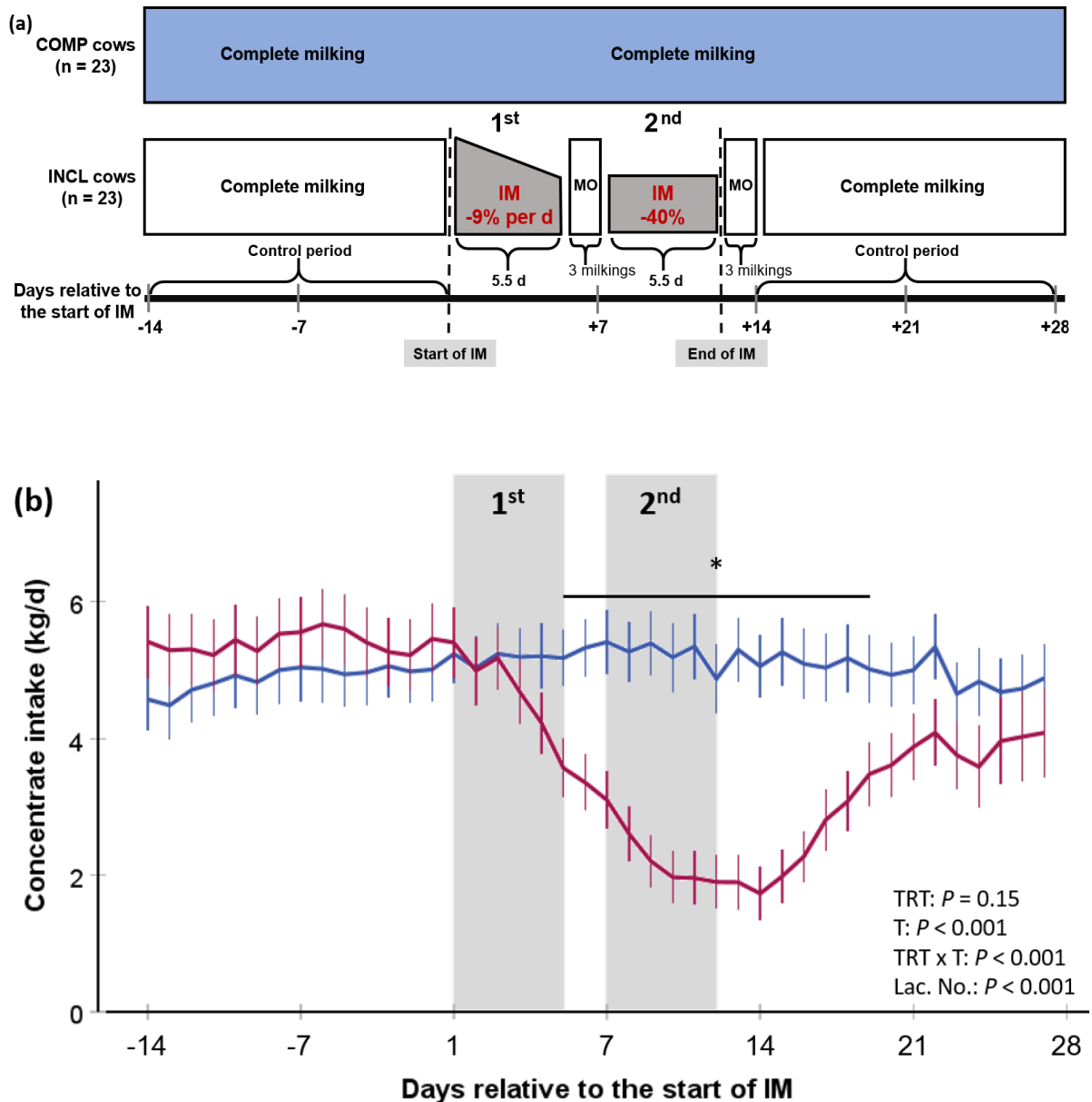


Figure 1. (a) Study design for incomplete milking (IM) of the INCL cows in peak- to mid-lactation (93 ± 46 DIM) through automatic cluster removal according to a predefined amount of milk using a software-module based on individual milk yield (%). The design included two

IM phases, indicated as first and second, separated by a milking-out (MO) period in which the conventional cluster removal (depending on milk flow (<0.3 kg/min)) was activated. (b) Effect of the study design and IM on daily concentrate intake (kg/d), of cows in peak- to mid-lactation, compared with conventionally milked cows: 14 d before start of IM, throughout IM (first and second phase) and 14 d after IM. Two treatments (TRT): blue line = complete milked cows (COMP, n = 23); red line = incomplete milked cows (INCL, n = 23); IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

Udder Health and Milk Leakage

Aseptic foremilk samples were taken from COMP and INCL cows in accordance with the recommendations of the DVG (Deutsche Veterinärmedizinische Gesellschaft e.V.; DVG 2009) to ensure udder health. Samples were taken at quarter level once a wk pre-IM and twice a wk during the two IM phases in the INCL cows, while the COMP cows continued to be milked conventionally. Additional foremilk samples from COMP and INCL cows were taken 1 and 2 wk post-IM to monitor udder health. Sampling was done after routine pre-milking treatment, including discarding the first strippings, disinfecting the teat ends with 70% alcohol and sampling into sterile vials with preservatives. Quarter-wise samples were analysed bacteriologically (bovicare GmbH, Bernau, Germany) and SCC was measured using the DCC Cell Counter (DeLaval GmbH, Glinde, Germany). Throughout the experiment, 2 cases of clinical mastitis occurred in both the COMP and INCL treatments; the affected cows were removed from the trial.

Milk flow at cluster removal in the INCL treatment was used as an indirect measure of udder filling during IM. A high milk flow at cluster removal indicated premature termination of milking process and a significant amount of residual milk in the udder. To monitor milk leakage, cows of the INCL and COMP treatments were observed in the milking parlor according to the protocol described by Gott et al. (2016). Each cow was visually inspected 10 to 15 s before fore-milking, after fore-milking, before attaching the cluster and after cluster detachment. Two assessments per wk took place in the wk pre-IM, during the first and second IM phase and in the wk post-IM. If milk leakage occurs at least at one teat, the cow was scored as

“positive”. Additionally, regular visual inspections of the boxes were carried out ~3 h after morning feeding to assess any milk leakage during the day.

Feed Analyses and Estimation of Energy Balances

Energy balance (MJ NE_L /d) was calculated to determine the initial energy status of the cows before the start of IM. Daily records were maintained of milk yield (kg/d), feed intake (kg/d; including concentrates and PMR), and BW (kg). Milk components were analysed at least once a wk and the energy output from the milk was calculated based on the daily milk yield using the following formula: Energy output in milk (MJ/d) = (0.385 x fat (%) + 0.242 x protein (%) + 0.165 x lactose (%) + 0.1) x milk volume (kg/d) (Susenbeth, 2018). Representative samples of the ration were taken twice weekly to determine DM content through oven-drying at 105°C (Memmert GmbH & Co. KG, Schwabach, Germany). DM content was corrected (DM_{cor}) for losses of volatiles during silage DM determination (DM_{cor} (%) = 2.08 + 0.975 x DM (%); Weißbach und Kuhla, 1995). The energy density (MJ NE_L/kg DM) of the PMR ingredients and concentrate was evaluated by a commercial laboratory (Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFÄ), Münster, Germany) to calculate the cow's energy intake.

Finally, the energy balance (EB, MJ NE_L/d) for each cow was calculated weekly according to the guidelines of the Gesellschaft für Ernährungsphysiologie (GfE, 2001), using the formula: EB (MJ NE_L/d) = Feed energy intake (MJ NE_L/d) - (NE_L requirement for maintenance (MJ/d = 0.293 x (kg of BW)^{0.75}) + requirement for conception (MJ/ d = (0,044 x e^(0.0165 x pregnancy^d))/ 0.29) + energy output in milk (MJ/d)).

Milk Analysis and ECM Calculation

Milk composition was analysed to further characterize the effects of IM. Therefore, whole milk samples were collected (Metatron; GEA Farm Technologies GmbH) on an evening milking and the following morning milking (milk amount of each milking was considered in the sample volume). Samples were taken once a wk pre-IM, on d 1 and 5 relative to the start of both the first and second IM phase, on the first d after each IM phase ended (during the second and third conventional milking after IM), and once a wk post-IM. Bronopol served as preservative and was added to the samples and milk fat, protein, and lactose were quantified by mid-

infrared spectroscopy and SCC was determined by flow cytometry (Labor- und Dienstleistungs GmbH & Co KG, Krefeld, Germany).

The ECM was calculated on the d when the milk composition was evaluated as ECM (kg) = (91.1 x fat (%) + 58.6 x protein (%) + 39.6 x lactose (%) /750) x milk amount (kg) (Sjaunja et al., 1984) for both the control periods and the IM phases. During the IM phases, the ECM only represents the milk that was withdrawn.

Selected Metabolic Parameters: NEFA and IGF-1

Non-esterified fatty acids (NEFA) and IGF-1 were analysed in serum samples to assess the effects of IM on the metabolism. Blood samples were collected from the *vena jugularis* on d -9, -2, 6, 13, 20, and 27 relative to the start of IM. Sampling took place directly after the morning milking prior to the morning feeding and 10 mL serum tubes with granules (Sarstedt AG & Co. KG, Nümbrecht, Germany) were used. Serum was separated via centrifugation at 2500 x g for 15 min at 15°C (Z 336 K, Hermle Labortechnik GmbH, Wehingen, Germany) and stored at -20°C until analysis. Frozen serum samples were transferred to the laboratory for photometric analysis of NEFA (Synlab.vet GmbH, Leverkusen, Germany). The IGF-1 concentration was quantified in serum using an enzyme immunoassay (Mediagnost GmbH, Reutlingen, Germany). The mean intra-assay CV was 2.49% and the inter-assay CV was 6.64%.

Statistical analysis

The data was analysed using SPSS Statistics Version 29.0 (IBM, SPSS Inc., Chicago, IL). Before the statistical analysis, data were screened for extreme outliers and plausibility by boxplots. Thereby, any technical issues with the barn equipment (e.g., weighing troughs) were addressed. Assumptions of parametric tests were evaluated visually using quantile-quantile plots and Box plots, as well as the Shapiro-Wilk test for normal distribution of model residuals, Levene's test for variance homogeneity, and heteroscedasticity or non-normality of model residuals was addressed using log-transformation. Linear mixed-effects models were fitted using the MIXED procedure with the REML method in SPSS, and alpha-error correction for multiple comparisons was applied using the Bonferroni method. Each model included treatment (INCL vs. COMP), time (relative to the start of IM), the interaction between treatment and time, and lactation number as fixed effects, with cow included as a random effect. In the NEFA and IGF-1 models, ECM groups (differentiated by >40 kg and <40 kg ECM/d as REF (rounded median))

were used as fixed covariate instead of lactation number. The covariance structure of all models was the variance components covariance structure. Model selection and covariance structure was based on the lowest Akaike information criterion (AIC). For SCC in foremilk samples, "udder quarter" was used as experimental unit and random factor instead of "cow". All models incorporated all trial periods. Single group comparisons were conducted using Student's t-test and the T-TEST procedure in SPSS, with effect sizes reported as Cohen's d (stated as "ES"). Cohen's d from 0.5–0.8 were considered medium, and those above 0.8 were considered large (Cohen, 1988). The Chi-square test was employed for the ordinally scaled variable "milk leakage" using the CROSSTABS procedure in SPSS, with effect size reported as Cramer's V and 0.1-0.3 were considered as medium and >0.5 as large (Cohen, 1988). All data are presented as mean \pm SEM. Statistical significance was set at $P \leq 0.05$.

RESULTS

Study Design-Induced Changes in Milk Yield and Concentrate Supply

No differences in milk yield in the wk prior to the start of IM (reference milk yield: REF (kg/d)) were observed between the COMP and INCL cows (Table 2). The INCL cows ranged in terms of REF from 26 to 59 kg/d. Reduced milk withdrawal was induced by IM and resulted in different milk yield trends between both treatments (Figure 2). After the first IM phase, 26.8 ± 1.20 kg was obtained at the first conventional milking (cluster removal at <0.3 kg/min), and 22.9 ± 1.01 kg after the second IM phase in the INCL treatment. For calculation of the average daily milk production of the INCL cows during both IM phases, the milk yield of the first conventional milking was included in the average daily milk yield of the corresponding IM phase. During the first IM phase, the average daily milk yield of INCL cows decreased by 23% compared to their REF, averaging 31.1 ± 0.35 kg/d ($P \leq 0.001$, ES = 1.73). In the second IM phase, the average daily milk yield of INCL cows decreased by 37% compared to their REF, averaging 25.2 ± 0.27 kg/d ($P \leq 0.001$, ES = 2.94). Further, the milk yield achieved on the first d after cessation of both IM phases was reduced compared to REF (Table. 2). This milk yield reduction was not correlated to the REF in INCL cows (after first IM phase: $r = 0.39$, $P = 0.07$; after second IM phase: $r = 0.38$, $P = 0.07$), but the REF was highly correlated with the milk yield on the first d after cessation of the first IM-phase ($r = 0.89$, $P \leq 0.001$) and the second IM phase ($r = 0.89$, $P \leq 0.001$). When conventional milking was permanently applied again, the

milk yield of INCL cows in the wk post-IM was 13.4% less compared to the REF ($P \leq 0.001$, ES = 1.68). Further, milk yield of INCL cows increased by 19% from 30.7 ± 1.5 kg/d on the first d after cessation of IM to 36.4 ± 1.59 kg/d in the second wk after IM ($P \leq 0.001$, ES = 2.23). From d 6 post-IM onwards, no further differences were observed between the COMP and INCL treatments (Figure 2). In contrast, the milk yield of the COMP cows remained stable at approximately 39 kg/d throughout the observation period (Figure 2).

The allocation of additional concentrate by separated feeding stations depended on milk yield and was therefore part of the study design. With the start of IM, the allocation and intake of concentrates in INCL cows decreased towards the end of IM and increased again after the end of IM (Figure 1b). The concentrate intake of INCL cows decreased by 65%, from 5.4 ± 0.52 kg/d at the beginning of IM to 1.9 ± 0.39 kg/d on the d IM ended ($P \leq 0.001$, ES = 0.771). Compared to COMP cows (5.2 ± 0.12 kg/d), concentrate intake of INCL cows was reduced by 64% at the end of IM and remained reduced during the first wk after IM (Figure 1b).

Table 2. Milk yields (means \pm SEM) related to the incomplete milking (IM) phases (first and second IM phase) in complete milked (COMP, n = 23) and incomplete milked cows (INCL, n = 23)

Item	COMP	SEM	INCL ¹	SEM	P-value	Effect size ²
Average milk yields (kg/d)						
REF ³	39.1	1.42	40.2	1.66	0.602	-0.16
first achieved milk yield ⁴	39.4	1.53	32.7	1.54	0.003	0.92
second achieved milk yield ⁵	38.6	1.38	30.7	1.54	< 0.001	1.12
first week post-IM	38.7	1.41	34.8	1.41	0.630	0.57
Changes in milk yield (%)						
REF to first achieved milk yield	0.9	0.02	-18.7	0.02	< 0.001	2.56
REF to second achieved milk yield	-0.8	0.02	-23.7	0.02	< 0.001	2.58

¹INCL was divided into 2 phases. During the first IM phase, daily milk withdrawal was gradually reduced by 9%/d for 5.5 d (resulting in ~40% reduction) compared to the REF. In the second IM phase, milk withdrawal was constantly reduced at 40% for another 5.5 d.

²Cohen's d was calculated, with 0.5 - 0.8 represents medium and >0.8 represents large effect size.

³Represents the milk yield (kg/d) in the wk pre-IM.

⁴First daily milk yield after the first IM phase.

⁵First daily milk yield after the second IM phase.

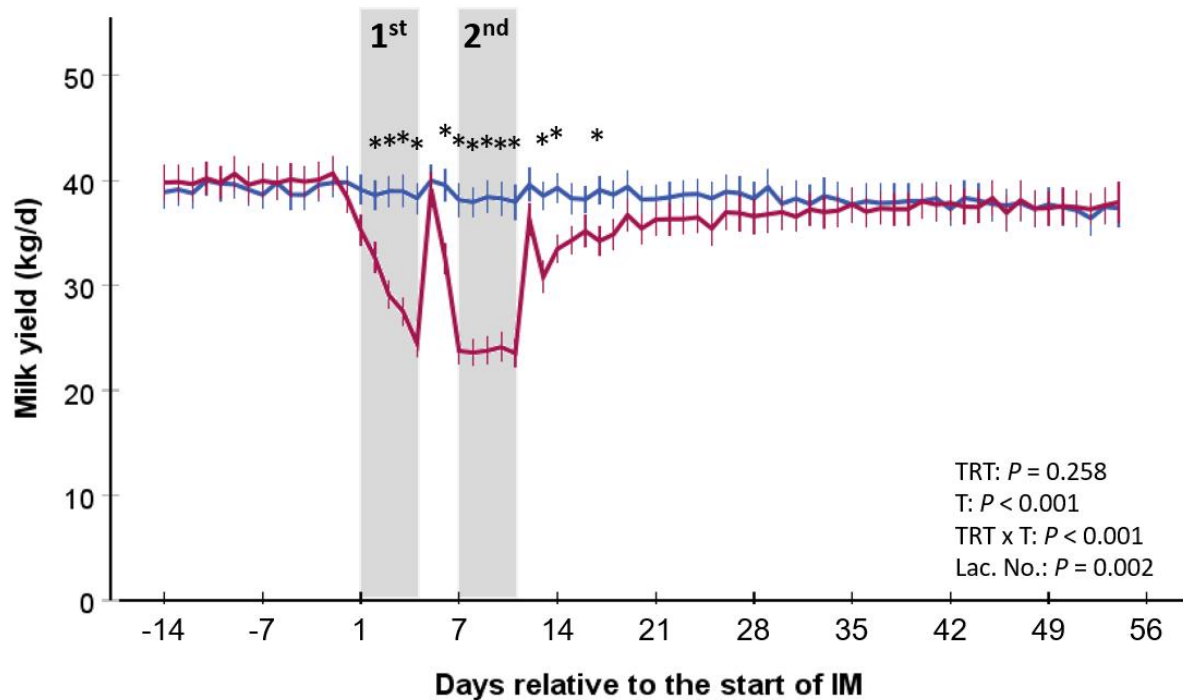


Figure 2. Effect of incomplete milking (IM), on daily milk yield (kg/d) of cows in peak- to mid-lactation, compared to conventionally milked cows: 14 d before start of IM, throughout IM (first and second phase) and 42 d after IM. Two treatments (TRT): blue line = complete milked cows (COMP, $n = 23$); red line = incomplete milked cows (INCL, $n = 23$). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

Udder Health and Milk Leakage

No mastitis-relevant pathogens or elevated SCC were detected in cows that completed the trial. Figure 3 illustrates the time course of SCC in quarter foremilk samples from COMP and INCL cows (during IM: COMP: $46.8 \pm 3.36 \times 10^3$ cells/mL; INCL: $40.5 \pm 2.79 \times 10^3$ cells/mL). Throughout the entire experiment, the SCC in both treatments remained well below the threshold for mastitis of 100×10^3 cells/mL, as defined by the DVG (2009) for healthy quarters (Figure 3).

The IM resulted in milk accumulation in the udder. The average last milk flow recorded at the time of cluster removal during both IM phases was 3.64 ± 0.07 kg/min in the INCL treatment (average of morning and evening milking). Thus, the last milk flow in the INCL

treatment remained well above the conventional cluster removal threshold of <0.3 kg/min (Figure 4).

Milk leakage occurred almost exclusively in INCL cows (Supplemental Table S1; <https://doi.org/10.6084/m9.figshare.28182815>). During IM, the number of milk leakages in the milking parlor (COMP: 3 cases; INCL: 37 cases; $P \leq 0.001$) and milk leakages in the boxes (COMP: 1 case; INCL: 29 cases; $P \leq 0.001$) of INCL cows were increased. Pre- and post- IM, no cases of milk leakage were observed in the INCL treatment (Supplemental Table S1; <https://doi.org/10.6084/m9.figshare.28182815>).

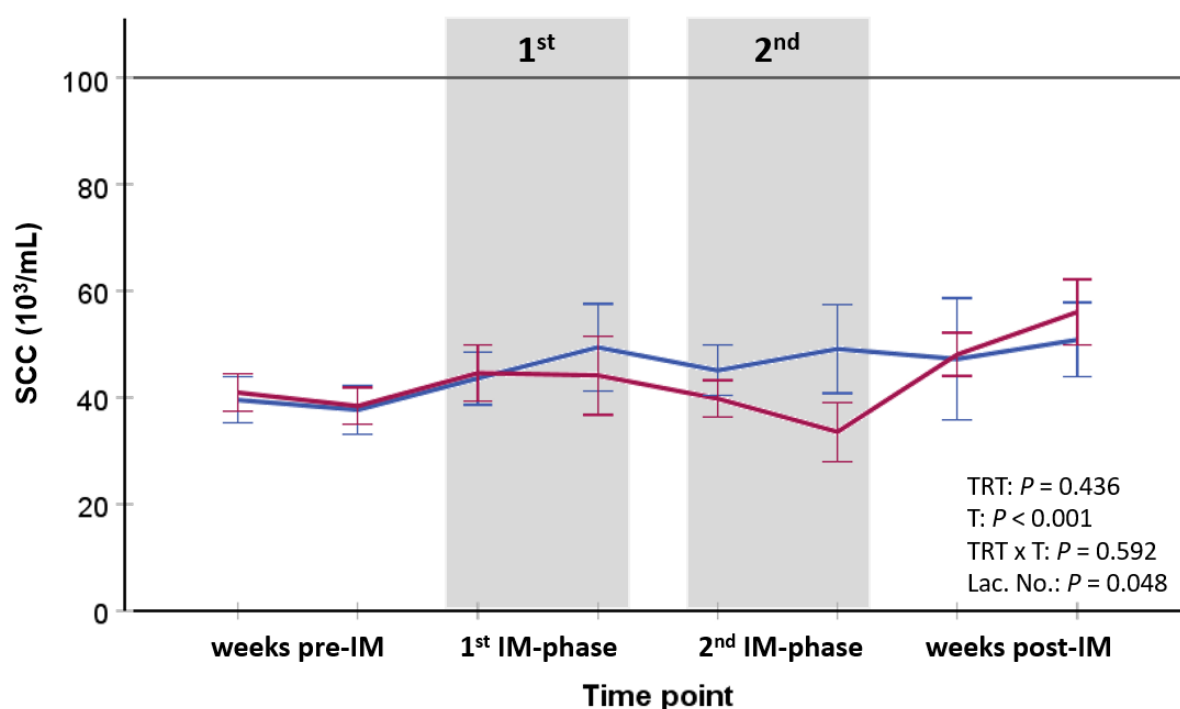


Figure 3. Effect of incomplete milking (IM), on SCC ($10^3/\text{mL}$) in foremilk samples of each quarter ($n = 184$ samples/ time point) of cows in peak- to mid-lactation, compared with conventionally milked cows: 2 wk pre-IM, throughout IM (first and second phase) and 2 wk post-IM. Two treatments (TRT): blue line = quarter of complete milked cows (COMP, $n = 92$); red line = quarter of incomplete milked cows (INCL, $n = 92$). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No. The horizontal line represents the upper SCC threshold according to the DVG (2009) for healthy quarters (100×10^3 cells/mL).

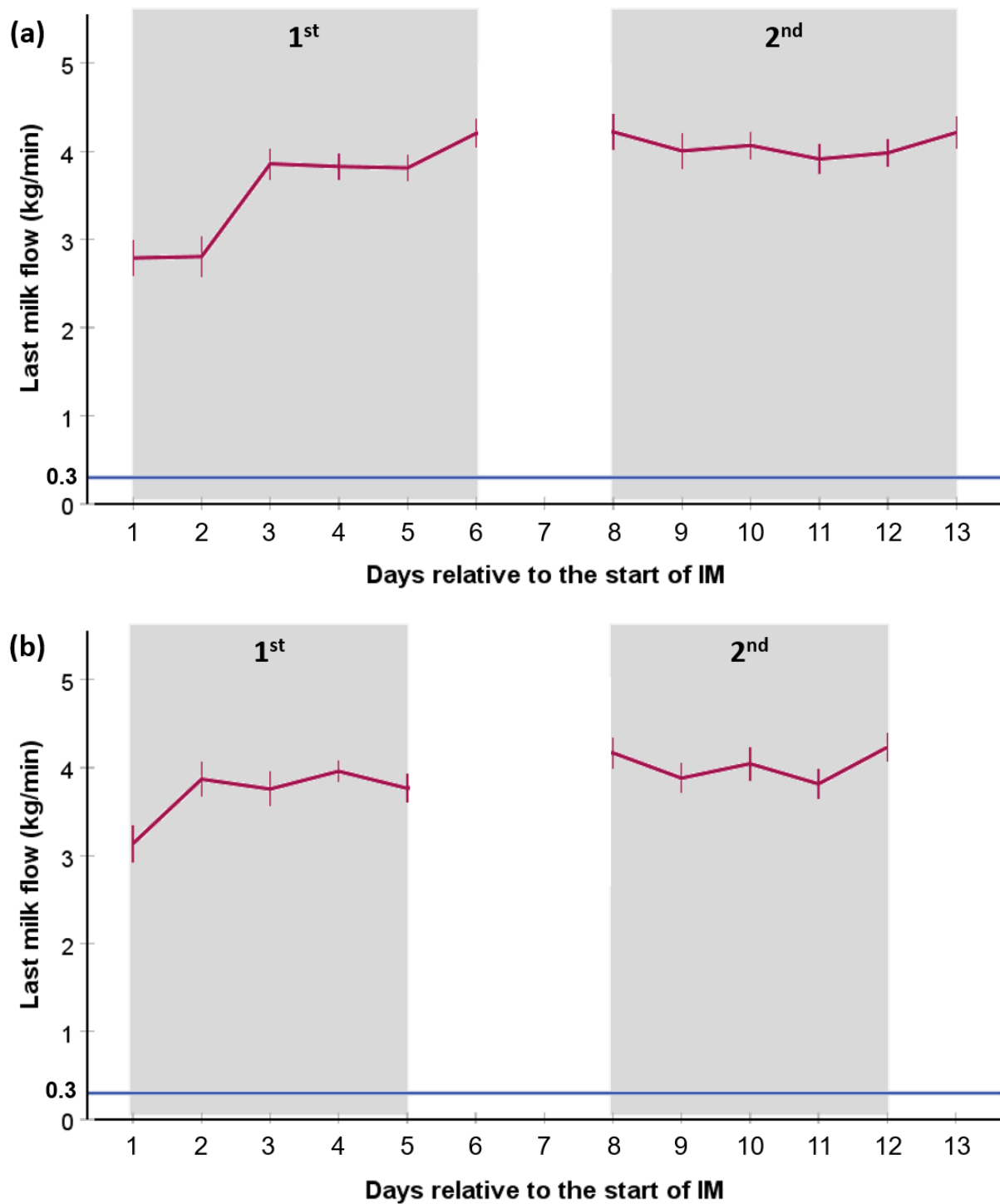
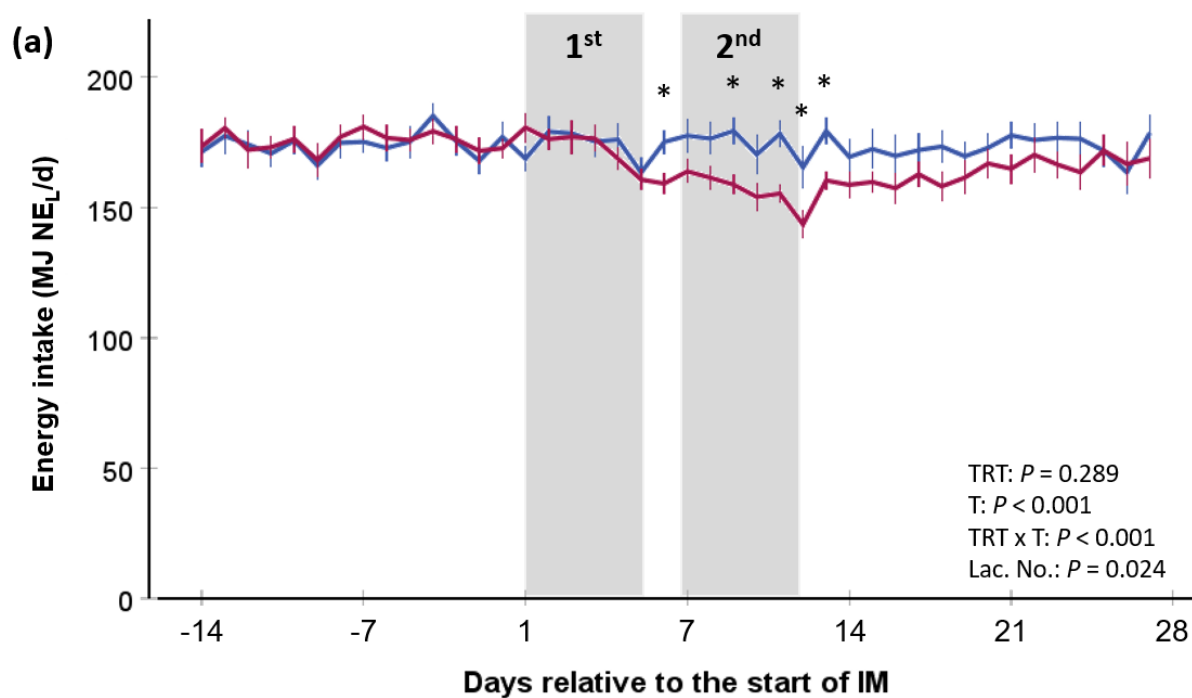


Figure 4. Effect of incomplete milking (IM) on the last milk flow (kg/min) measured in the milking unit (a) after morning milking and (b) after evening milking of cows in peak- to mid-lactation, compared with conventionally milked cows: throughout the first and second IM phase. Two treatments (TRT): blue line = complete milked cows (COMP, n = 23); red line = incomplete milked cows (INCL, n = 23). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data of INCL cows are presented as means \pm SEM, COMP cows are represented by the milk-flow based cluster removal (<0.3 kg/min).

Feed and Water Intake

The intake of the PMR remained the same in the COMP and INCL treatments throughout the experiment, with both maintaining an average feed intake of approximately 60 kg/d (Supplemental Figure S1a; <https://doi.org/10.6084/m9.figshare.28182815>). However, total DMI, which includes both the PMR and the concentrate, was reduced in the INCL cows during the second IM phase compared to the COMP cows (Supplemental Figure S1b; <https://doi.org/10.6084/m9.figshare.28182815>). The energy intake (MJ NE_L/d) is shown in Figure 5a. Pre-IM, there was no difference in energy intake (COMP: 174 ± 1.5 MJ NE_L/d; INCL: 175 ± 1.4 MJ NE_L/d; $P = 0.59$). While the COMP cows remained at a constant intake level, the energy intake of the INCL cows decreased over the course of IM, with 13% lower energy intake in the INCL cows compared to the COMP cows on the d IM ceased. When the conventional milking was activated again, the energy intake of the INCL cows increased, resulting in no further observable differences between treatments (Figure 5a). The water intake of the INCL cows decreased over the course of IM (Figure 5b). On average, the water intake during the second IM phase was 17% lower in the INCL cows (78.3 ± 1.75 kg/d) compared to the COMP cows (93.8 ± 2.11 kg/d). Differences between treatments persisted on the d after IM and on two additional d within the 2 wk following IM (Figure 5b).



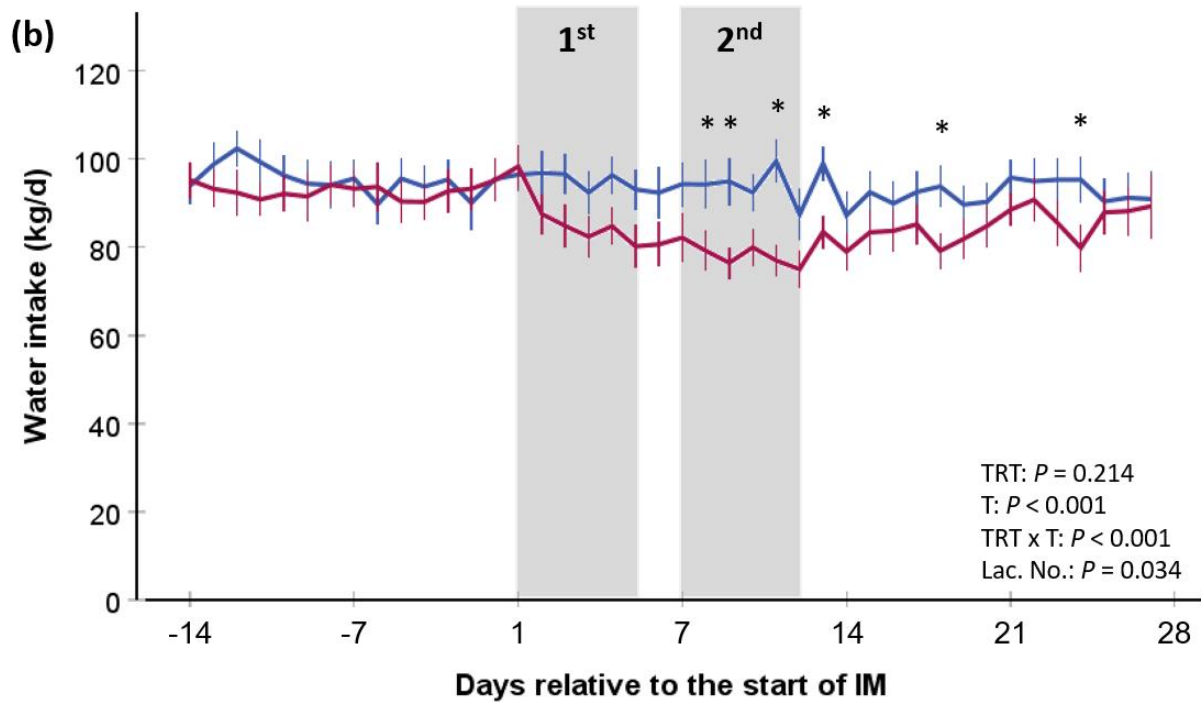


Figure 5. Effect of incomplete milking (IM) on daily (a) energy intake (MJ NE_L /d) and (b) water intake (kg/d) of cows in peak- to mid-lactation, compared with conventionally milked cows: 14 d before start of IM, throughout IM (first and second phase) and 14 d after IM. Two treatments (TRT): blue line = complete milked cows (COMP, n = 23); red line = incomplete milked cows (INCL, n = 23). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

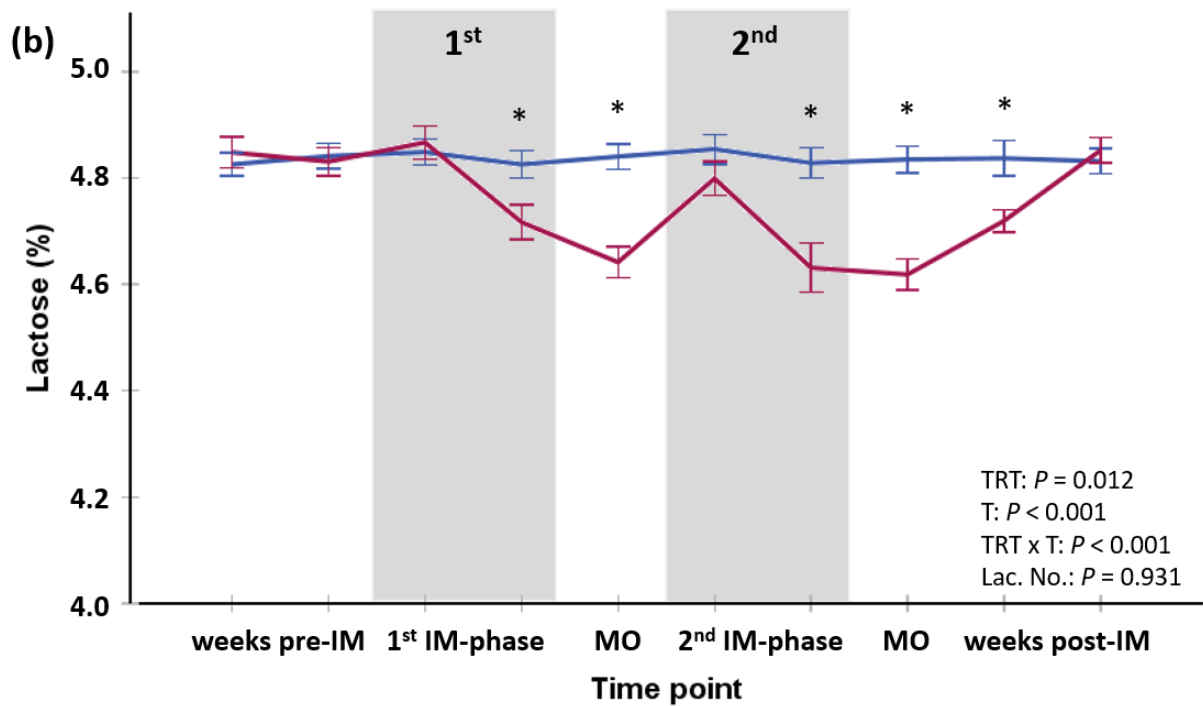
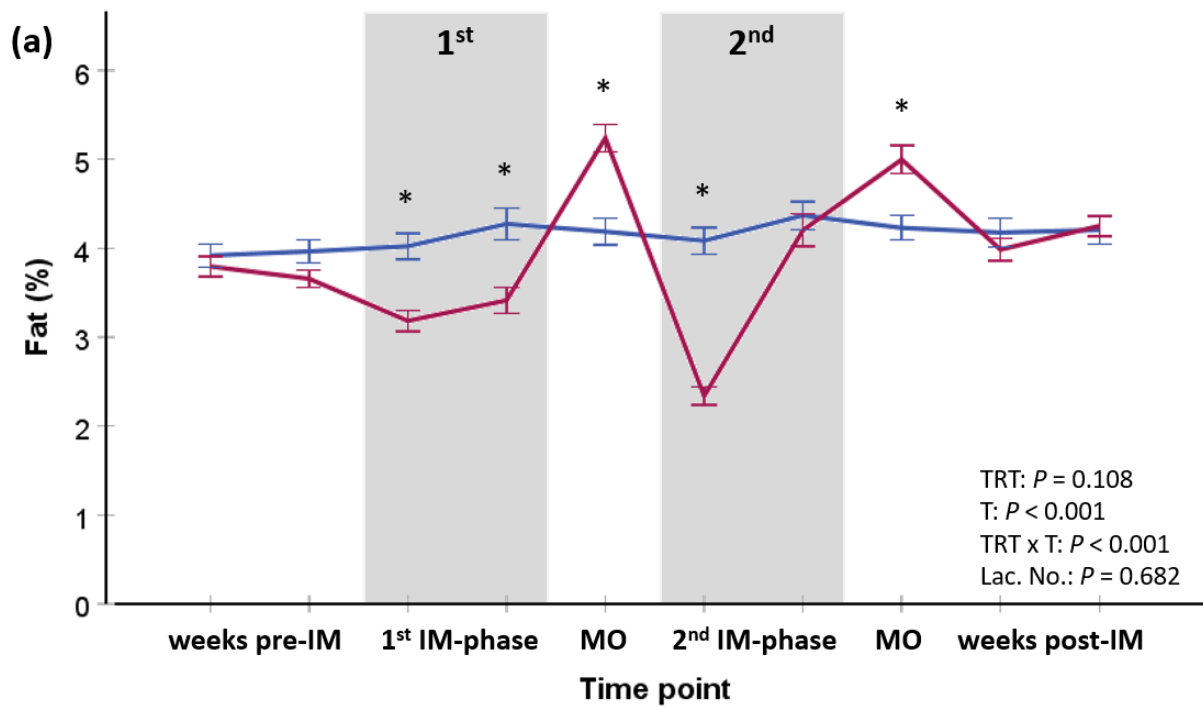
Milk Composition, Energy-Corrected Milk and Energy Balance

No differences in milk fat content between COMP and INCL cows were observed before and after IM (Figure 6a). Compared to the COMP cows, the milk fat content in the INCL cows decreased by 21% to $3.18 \pm 0.12\%$ with the onset of the first IM phase, and by 43% to $2.34 \pm 0.10\%$ with the onset of the second IM phase. Over the course of IM, the milk fat content of the INCL cows increased (Figure 6a). At the end of the first IM phase, the milk fat content of the INCL cows was still 20% less compared to the COMP cows, while at the end of the second IM phase, the milk fat content of the INCL cows was at the level of the COMP cows (COMP: $4.37 \pm 0.16\%$; INCL: $4.20 \pm 0.18\%$). The milk fat content of the INCL cows increased above the level of the COMP cows on the first d after IM ceased: by 20% to $5.24 \pm 0.15\%$

(COMP: $4.14 \pm 0.18\%$) after the first IM phase and by 15% to $4.99 \pm 0.16\%$ (COMP: $4.17 \pm 0.18\%$) after the second IM phase.

Despite the significant influence of time and treatment x time in the mixed model of milk protein content, no differences were observed between the INCL and COMP treatments at any time point (Supplemental Figure S2; <https://doi.org/10.6084/m9.figshare.28182815>). Throughout the experiment, the milk lactose content of the COMP treatment remained at a constant level (Figure 6b). Contrary, milk lactose in the INCL treatment was affected by IM and the lowest content were observed on the first d after the first IM phase ($4.64 \pm 0.03\%$) and the second IM phase ($4.62 \pm 0.03\%$), whereas the milk lactose content was 4% and 5% lower than in the COMP treatment, respectively. In the second wk post-IM, the milk lactose content increased in the INCL treatment and did not differ from the COMP treatment anymore (Figure 6b).

For ECM yield, differences between the COMP and INCL treatments were induced by IM (Figure 6c). The lowest ECM withdrawal was present at the end of the first IM phase and at the beginning of the second IM phase (Figure 5c). At the end of the first IM phase, 50% less ECM was withdrawn from the INCL cows compared to the COMP cows (COMP: 42.4 ± 1.39 kg/d vs. INCL: 21.0 ± 0.63 kg/d). At the beginning of the second IM phase, 54% less ECM was withdrawn from the INCL cows (COMP: 39.0 ± 1.42 kg/d vs. INCL: 18.1 ± 0.71 kg/d). Compared to the COMP cows, the ECM yield in INCL cows was reduced on the first d after IM ceased: by 9% after the first IM phase and 15% after the second IM phase. Furthermore, differences between the treatments after IM were limited to the first wk, with 12% lower ECM yield in the INCL treatment compared to the COMP treatment. Thereafter, INCL cows continued to increase their ECM yield and no further differences between were observed (Figure 6c). In the wk pre-IM, the average net energy balance of the COMP cows was 11 ± 2.9 MJ/d and of the INCL cows 13 ± 1.7 MJ/d ($P = 0.51$).



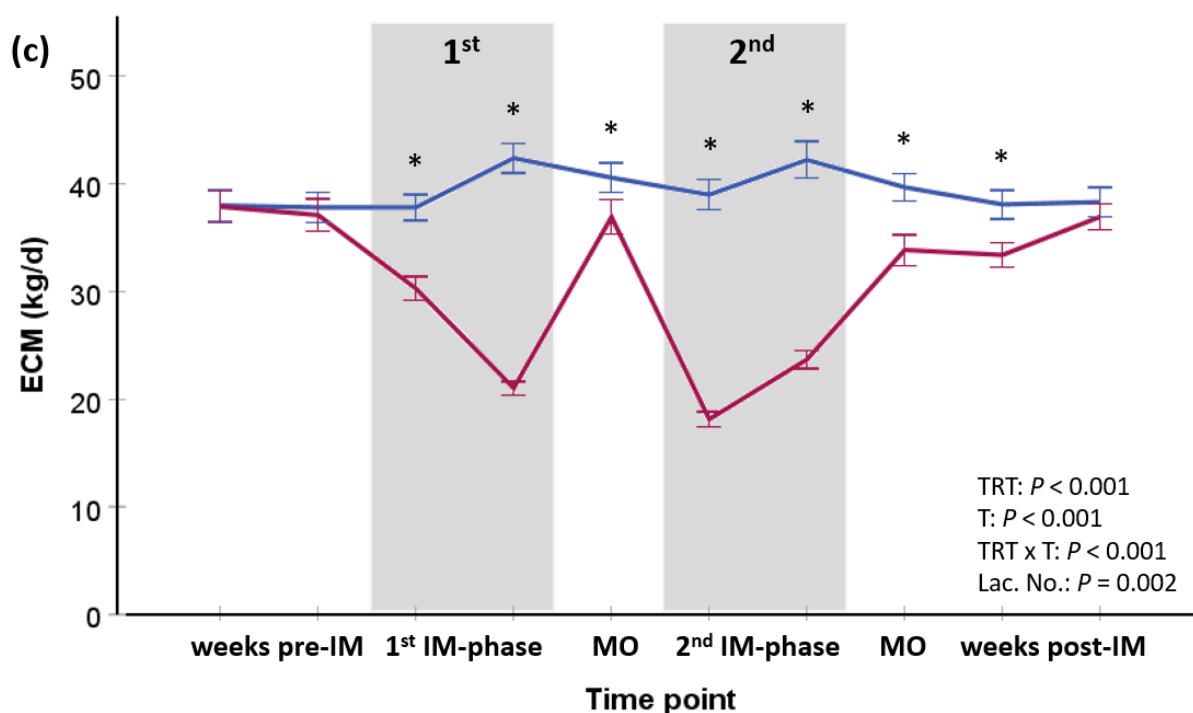


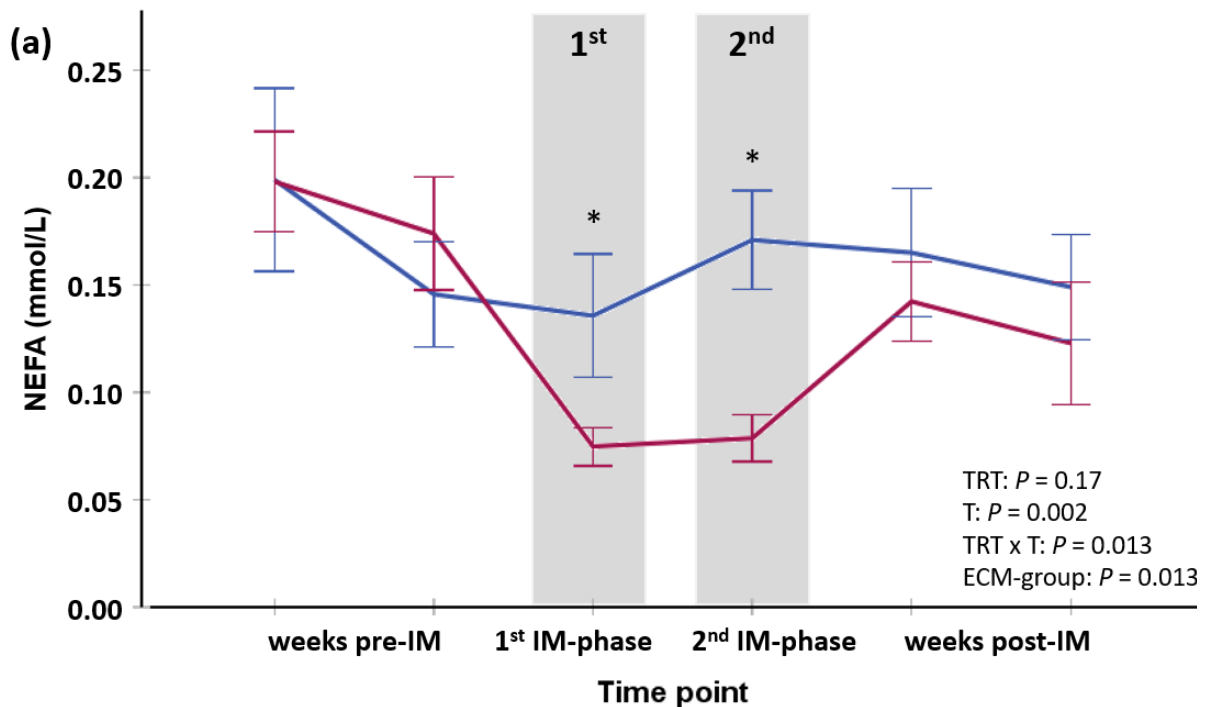
Figure 6. Effect of incomplete milking (IM) on (a) fat composition (%) and (b) lactose composition (%) of whole milk samples and the resulting (c) ECM yield (kg/d) of cows in peak- to mid-lactation, compared with conventionally milked cows: 2 wk pre-IM, throughout IM (first and second phase), on each first d after the first and second IM phase (milking out, MO) and 2 wk post-IM. Two treatments (TRT): blue line = complete milked cows (COMP, $n = 23$); red line = incomplete milked cows (INCL, $n = 23$). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

Selected Metabolic Parameters: NEFA and IGF-1

Serum NEFA concentrations are shown in Figure 7a. Pre-IM, no significant differences were observed between the COMP and INCL treatments. Affected by IM, NEFA concentrations of the INCL cows decreased from averaged 0.19 ± 0.02 mmol/L pre-IM by 63% to 0.07 ± 0.01 mmol/L during the first IM phase ($P \leq 0.001$, ES = 1.1). Also, in comparison to pre-IM, NEFA concentration of INCL cows decreased by 58% to 0.08 ± 0.01 mmol/L during the second IM phase ($P \leq 0.001$, ES = 0.86). Throughout IM, NEFA concentration in the COMP treatment (0.15 ± 0.02 mmol/L) was on average 47% greater compared to the INCL treatment. After IM, NEFA concentration in the INCL treatment increased and aligned with those of the COMP

treatment. Additionally, the covariate ECM group (<40 vs. >40 kg ECM/d prior to IM) significantly influenced the NEFA concentration (Figure 7a). Specifically, mean NEFA concentration for the <40 kg ECM/d group were 0.12 ± 0.02 mmol/L and for the >40 kg ECM/d group 0.21 ± 0.03 mmol/L in the wk pre-IM ($P = 0.02$, $ES = 0.814$).

The IM treatment resulted in elevated IGF-1 serum concentrations in INCL cows, with no initial differences between the COMP and INCL cows pre-IM (Figure 7b). As a consequence of IM, the IGF-1 concentration in the INCL treatment increased from 120.8 ± 7.93 ng/mL in the wk pre-IM by 30%, reaching 173.3 ± 8.34 ng/mL in the first IM phase ($P \leq 0.001$, $ES = 1.64$), and by 28% reaching 167.7 ± 10.22 ng/mL in the second IM phase ($P \leq 0.001$, $ES = 1.57$). On average, IGF-1 concentrations in the INCL treatment were 34% greater compared to the COMP treatment during IM (COMP: 113.3 ± 6.26 ng/ml). After IM ceased, IGF-1 concentration in the INCL treatment decreased and aligned with those of the COMP treatment. The covariate ECM group (<40 vs. >40 kg ECM/d in the wk prior to IM) significantly influenced the IGF-1 concentration (Figure 7b). Specifically, mean IGF-1 concentration in the <40 kg ECM/d group were 125.1 ± 6.86 ng/mL and in the >40 kg ECM/d group 102.2 ± 8.27 ng/mL in the wk pre-IM ($P = 0.039$, $ES = 0.647$).



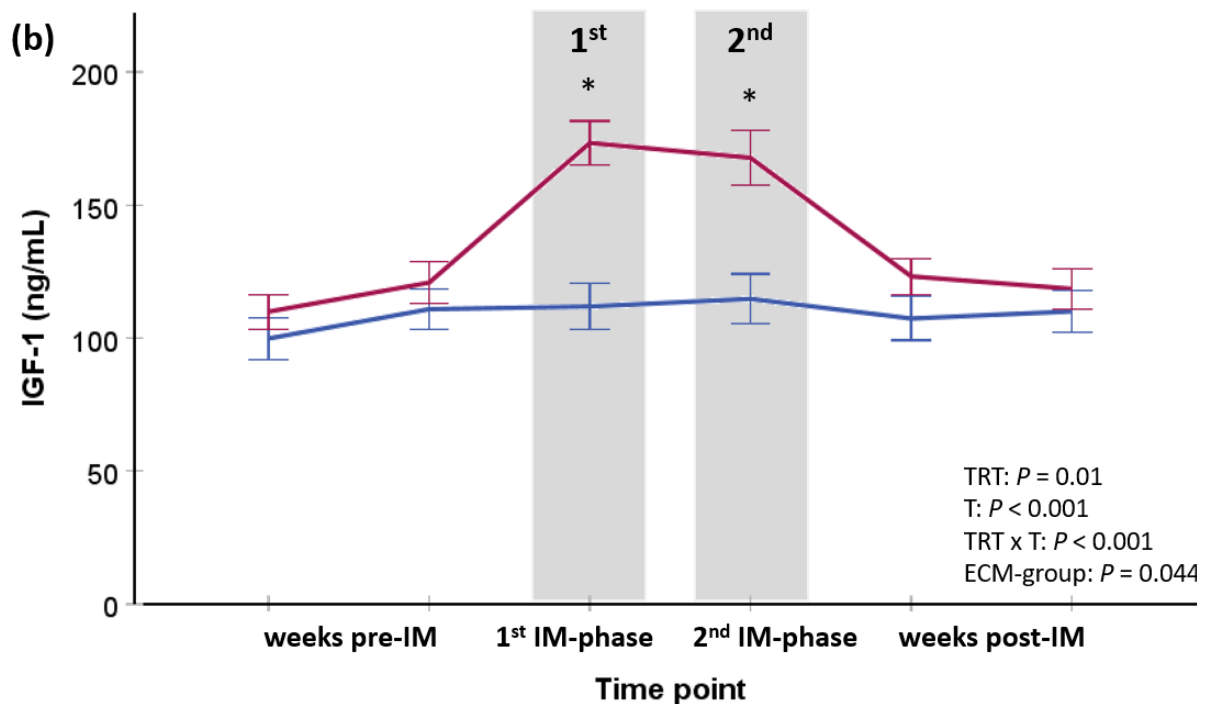


Figure 7. Effect of incomplete milking (IM) on blood serum concentrations of (a) fatty acids (FA; mmol/L) and (b) IGF-1 (ng/mL) of cows in peak- to mid-lactation compared with conventionally milked cows: 2 wk pre-IM, throughout IM (first and second phase) and 2 wk post-IM. Two treatments (TRT): blue line = complete milked cows (COMP, $n = 23$); red line = incomplete milked cows (INCL, $n = 23$). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the first IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the second IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. IM-Periods are shaded in grey. Data are presented as means \pm SEM, with differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of the covariate (<40 vs. >40 kg ECM/d in the wk prior to IM) as ECM-group.

DISCUSSION

This study aimed to investigate the effects of automated short-term IM on milk production, udder health, and selected metabolic parameters in multiparous German Holstein cows, hypothesizing that limiting milk withdrawal by up to 40% would reduce energy requirements for milk production and metabolic stress without compromising udder health or long-term milk yield. Previous research on IM has predominantly focused on early lactation (Carbonneau et al., 2012; Morin et al., 2018; Meyer et al., 2024), late lactation (Martin et al., 2020; Müller et al., 2023) or used experiments with a half udder design over extended periods (Penry et al., 2017; Kuehnl et al., 2019; Deacon et al., 2022). In this study, we investigated the feasibility of

short-term IM during peak- to mid-lactation. The IM was performed with a software module that automatically removed clusters once a predefined milk yield was reached to ensure controlled earlier cluster removal (Schmidt et al., 2020). Thereby, IM was divided in two phases by a milking out period (cluster removal <0.3 kg/min) to separately evaluate the effect of the first IM phase on milk production. Milk withdrawal was gradually reduced in the first IM phase and continuously reduced in the second IM phase, based on the REF. The procedure aimed to lower the cows' energy requirements by temporarily reducing milk production and thus reducing lactation-induced metabolic load without causing a long-term reduction in milk yield or negative effects on udder health.

Milk Yield

This study successfully implemented automated short-term IM using the software module of Schmidt et al. (2020), which facilitated earlier cluster removal. This preserves objectivity and avoids errors in the manual application of IM (Morin et al., 2018). The reduced milk withdrawal by IM led to an accumulation of milk in the udder, which was withdrawn during the first milking with conventional cluster removal (<0.3 kg/min), resulting in a high milk volume. Previous studies also reported increased milk volume directly after IM (Albaaj et al., 2018; Martin et al., 2020; Meyer et al., 2024), indicating milk stagnation in the udder. For cows in peak- to mid-lactation, the gradual reduction in milk withdrawal by 9 %/d over 5.5 d resulted in an 19% decrease in milk yield on the first d after IM compared to REF. Studies conducting IM also for 5 d but during the first days after parturition harvested about a third of the expected milk yield (Carbonneau et al., 2012) or a maximum of 14 L/d (Morin et al., 2018), and reported no effect on the milk yield immediately after IM. In the second IM phase of the present study, 40% less milk was withdrawn during a further 5.5 d. Compared to REF, IM resulted in a 24% lower milk yield on the first day after the second IM phase. Thus, with prolonged IM during the second phase, the milk yield of dairy cows in peak- to mid-lactation was further reduced.

The milk yield of the INCL cows after IM remained lower than that of the COMP cows by up to 5 d after IM; moreover, the milk yield of the INCL cows was 13% lower than that of the REF in the wk after IM. Over time, the milk yield of the INCL cows increased again, so that no lasting negative effect of the present IM protocol on long-term milk yield or lactation persistency of cows in peak- to mid-lactation can be assumed. Similarly, Carbonneau et al. (2012), Krug et al. (2018), and Meyer et al. (2024) found no long-term reduced milk yield after short-term or moderate IM during early lactation. In contrast, Penry et al. (2017) and Kuehnl et

al. (2019) applied a half-udder design with weekly calibrations and showed that 30% IM over 6 wk in early lactation led to a lasting 25% and 27% decrease in milk yield. Deacon et al. (2022) performed IM over 4 wk with the same design and intensity (30%) as Penry et al. (2017) and Kuehnl et al. (2019), but on cows in mid-lactation (DIM 174 ± 45). The milk yield of IM udder halves was reduced by 47% compared to fully milked halves at the end of IM, indicating a greater reduction in milk yield in mid-lactation cows compared to early lactation cows, despite shorter IM duration (Deacon et al., 2022). This is consistent with Charton et al. (2016), who reported faster recovery of milk yield in early lactation cows after a single extended 24-h milking interval, although lactation stage, milk yield level, and lactation number were not responsible for the overall variability in milk yield recovery. Reduced milk yield due to IM in Deacon et al. (2022) was also associated with reduced functional mammary capacity and reduced gene expression of milk synthesis-related genes like casein beta (*CSN2*), alpha lactalbumin (*LALBA*) and acetyl-CoA carboxylase alpha (*ACACA*) in IM-treated udder quarters. Compared to the study by Deacon et al. (2022), the cows in the present study had a shorter IM (2 x 5.5 d vs. 4 wk) and a lower DIM (93 ± 46 vs. 174 ± 45), which leads to different trends in milk yield after IM.

A previous study by Albaaj et al. (2018) investigated the influence of the amount of milk remaining in the udder regardless of the duration by applying IM during a single milking. Thereby, Albaaj et al. (2018) concluded that the amount of milk remaining in the udder is a crucial factor of IM. In the present study, the amount of milk withdrawn is determined by the REF minus the current reduction level (%). Consequently, the amount of milk remaining in the udder depends on the REF, with a larger volume of residual milk associated with a higher REF. Given the wide variation in REF of the INCL cows (26 - 59 kg/d), different response to IM might be expected. However, no significant correlation was found between the REF (i.e., the absolute milk volume left in the udder) and the decrease in milk yield following both IM phases. This suggests that the absolute volume of residual milk is not the sole factor influencing milk production in response to IM. Additionally, the adaptability (plasticity) of the mammary gland is influenced by stage of lactation, genetic factors, and health status (Boutinaud et al., 2019). In particular, the results from the experiments using a half-udder design suggest that the acute adaptation of milk production to a local factor (e.g. IM) is primarily promoted by autocrine-paracrine processes rather than systemic processes (Weaver and Hernandez, 2016; Deacon et al., 2022), but also the responsiveness of prolactin as a systemic factor appeared to be modulated by local factors (Lacasse et al., 2016).

Udder Health and Milk Leakage

This study included only udder-healthy cows (two cows per treatment were excluded). However, the absence of mastitis-related pathogens and the absence of increased SCC in the cows that completed the trial suggests stable good udder health. In a survey, Bach et al. (2022) pointed out the uncertainty of the participants regarding the effects of IM on udder health and attributed the concerns to the assumption that residual milk could serve as a substrate for pathogens. However, the present results contradict these concerns, as SCC levels remained consistently below the threshold of 100×10^3 cells/mL (DVG, 2009) in both the COMP and INCL treatments, even during the significant milk accumulation associated with IM. Studies on moderate IM with strict criteria for udder health (e.g., $SCC < 100 \times 10^3$ cells/mL and no positive bacteriological results prior to IM) consistently report no increase in SCC or mastitis risk (Martin et al., 2020; Meyer et al., 2024). Furthermore, studies have shown that IM does not negatively affect SCC or lead to clinical mastitis in cows infected with minor pathogens (Clarke et al., 2008; Müller et al., 2023). In contrast, a slight increase in SCC has been observed in studies with more intensive (30%) or prolonged (4 to 6 wk) IM, especially when less stringent criteria for udder health were applied (Penry et al., 2017; Kuehnl et al., 2019), such as $SCC < 240 \times 10^3$ cells/mL, as reported by Deacon et al. (2022). The risk for mastitis is influenced multifactorially, including hygiene and milking practices (Breen et al., 2009; Jamali et al., 2018).

Incomplete milking led to a considerable accumulation of milk in the udder, which was reflected in an increased flow-rate of the last milk at cluster removal. In the INCL treatment, the last milk flow rates (3.6 kg/min) in both IM phases were well above the conventional cluster removal threshold of 0.3 kg/min, indicating early cluster removal and a corresponding high residual milk yield. The last milk flow rate remained consistently high during the second IM phase, indicating a still elevated residual milk yield and a limited adaptation of milk production to IM, which contrasts with the observed reduced milk production immediately after IM. Despite the relatively stable final milk flow (kg/min), it is not recommended to increase the switch-off point for cluster removal to implement IM, as inter- and intra-individual variations in milk flow would lead to inconsistent application of the IM protocol. However, residual milk and increased intramammary pressure are associated with milk leakage (Katthöfer et al., 2024). Indeed, in this study, a significantly higher incidence of milk leakage was observed in the INCL treatment compared to the COMP treatment, both in the milking parlor and in the boxes. The milk leakage did not occur before or after IM, highlighting its association with the increased residual milk during IM. Previous studies have similarly associated milk leakage with high

residual milk volumes and intramammary pressure, especially in high yielding cows (Rovai et al., 2007; Bruckmaier and Wellnitz, 2008; De Prado-Taranilla et al., 2020). Although milk leakage increases the risk of mastitis as potential pathogens can enter through the teat canal (Rovai et al., 2007; Gott et al., 2016; De Prado-Taranilla et al., 2020), the absence of increased SCC or bacteriological results in cows that completed the trial suggests that the risk was effectively mitigated.

However, effective hygiene practices including frequent udder cleaning, regular milking (to flush the teat canal) and maintenance of box hygiene, likely mitigate this risk. Continued optimization of IM practices and stringent hygiene protocols remain essential to minimize these potential challenges.

Feed and Water Intake

In the present study, PMR intake in INCL cows remained constant during both IM phases. This is consistent with the results of Carbonneau et al. (2012) and Meyer et al. (2024), who observed no reduction in feed intake during IM in early lactation. The lower milk yield observed during IM in the present study resulted in a lower concentrate allocation due to the feed-to-yield system. Consequently, total DMI and corresponding energy intake decreased during the second IM phase. Reducing concentrate allocation during IM was essential to align feed intake with diminished energy needs, preventing overfeeding and reinforcing metabolic relief, as evidenced by favorable FA and IGF-1 responses. This adjustment ensured the strategy's practicality and efficacy. To mitigate possible negative effects of high separately fed concentrate levels on the rumen (Østergaard and Gröhn, 2000; Ingvarsten et al., 2001), concentrate administration was delayed and adjusted based on limited daily changes. The reduced milk yield during IM and the delayed increase in milk yield in INCL cows after IM, in addition to the direct effects of IM on the mammary gland and milk production, are probably related to the reduced concentrate allocation during the first wk after IM in the present study. This is relevant as concentrate intake positively influences DMI and milk production (Delaby et al., 2009; Lawrence et al., 2015). As the concentrate and energy intakes increased after IM, the milk yield of the INCL cows aligned with that of the COMP cows.

Among other factors, water intake is also influenced by DMI and milk production (Singh et al., 2022). In the current study, IM was associated with lower water intake, especially at the end of the second IM phase, which corresponded with lower DMI and milk production in INCL cows. Meyer et al. (2024) similarly reported lower water intake during the second wk of IM in early lactation.

Milk Composition, Energy-Corrected Milk and Energy Balance

Milk composition was analysed at the beginning of each IM phase, towards the end of the IM phases and on the d after cessation of IM, respectively. Within this measurement regime, we observed a high variation in milk fat content depending on the IM: In the INCL treatment, a decrease in milk fat content was initially observed in both IM phases, followed by an increase towards the end of the IM, with similar fat content in the INCL and COMP treatments at end of the second IM phase. After cessation of IM, the milk fat content was elevated in the INCL treatment. In contrast, in studies with longer IM duration and less variation, milk components were only measured once (Deacon et al., 2022; Meyer et al., 2024) or twice weekly (Penry et al., 2017; Kuehnl et al., 2019), which could explain the different outcomes of the effect of IM on milk fat content in these studies. In our study, the elevated milk fat content on the first d after IM indicates either fat accumulation in the udder or a content effect due to paralleled reduced milk yield. Since the milk fractions contain different fat levels (Ontsouka et al., 2003), it can be assumed that incomplete withdrawal of the fat-rich alveolar fraction leads to deviations in the milk fat content of the milk withdrawn, especially at the beginning of both IM phases.

Regarding milk protein and lactose, the results of this study are consistent with previous studies: The protein content remained stable in the milk sampled, while the lactose content decreased with or more intensive or longer duration of IM (Penry et al., 2017; Albaaj et al., 2018; Deacon et al., 2022). It is well known that compromised integrity of tight junctions facilitates paracellular transport, leading to an exchange between blood and milk components with elevated lactose content in the blood and a corresponding decrease in milk lactose content (Albaaj et al., 2018). Compromise tight junction integrity can be induced by milk accumulation (as induced by IM) that increases intra-alveolar pressure and reduces milk secretion (Stelwagen and Singh, 2014). Adaptations in milk production reflect the plasticity of the mammary glands, including changes in the activity and number of epithelial cells (Boutinaud et al., 2019). Such adaptive processes most likely occurred in the present study and led to an adjustment in milk production in the INCL treatment, with 12% lower ECM yield after IM compared to the COMP treatment. In addition, the reduced milk yield in the INCL cows was already observed after the first IM phase, indicating a reduced milk production and thus a lower energy requirement for milk synthesis already during IM. Due to the uncertainties in the amount of milk produced during IM and the high fluctuations in milk fat content, the EB were only calculated for the control period before IM. On average, INCL and COMP cows had a positive EB in the wk pre-IM, as expected for the DIM of INCL and COMP cows (Harder et al., 2019).

Selected Metabolic Parameters: NEFA and IGF-1

We hypothesized that IM would lead to adaptations in the mammary gland that would reduce the energy requirements for milk production and thus reduce metabolic load. Metabolic load is defined as the “burden imposed by the synthesis and secretion of milk” (Oltenucu and Broom, 2010) and to assess metabolic changes, we measured serum concentrations of NEFA and IGF-1. The NEFA levels indicate adipose tissue mobilization, NEB, and are associated with metabolic diseases in dairy cows (Ingvarstsen and Moyes, 2012; Moyes et al., 2013). Thereby, NEFA are also a reliable marker for NEB in the later stages of lactation (Bjerre-Harpøth et al., 2012). To evaluate the influence of milk production level on serum NEFA concentration, the average ECM yield of REF was categorized (distinguished between >40 kg/d and <40 kg/d ECM) and included as a covariate in the mixed model. Initial ECM level prior to the onset of IM phases influenced the NEFA concentration, with cows producing >40 kg ECM/d having higher NEFA concentrations, reflecting greater lactation-induced metabolic load. As a result of IM, NEFA concentrations in peak- to mid-lactation dairy cows decreased immediately compared to pre-IM and COMP treatment levels. Both the INCL and COMP cows had a positive EB prior to IM, as indicated by low NEFA Concentrations, thereby excluding any physiological imbalance, defined as deviation of physiological parameters from normal ranges (Moyes et al., 2013). The NEFA concentrations in the COMP treatment during IM remained within the typical range for this stage of lactation (Kennedy and Kuhla, 2023: 0.135 mmol/L; 89 ± 4.42 DIM). Despite the relatively low metabolic load in the present study, IM significantly reduced serum NEFA concentrations to 0.07 - 0.08 mmol/L. Similar NEFA concentrations (0.095 mmol/L) were observed in dairy cows in late lactation (293 ± 7.76 DIM) in the context of a positive EB characterized by high carbohydrate oxidation levels (Kennedy and Kuhla, 2023). A lower NEFA and BHB concentration (Carbonneau et al., 2012) as well as a lower incidence of metabolic disease (Carbonneau et al., 2012; Morin et al., 2018) have been reported in IM during the first 5 d post-partum. These results suggest that reduced milk withdrawal by IM can be used effectively during periods of elevated metabolic stress e.g. as induced by environmental stress (Wheelock et al., 2010; Razzaghi et al., 2023), thereby reducing the risk of physiological imbalance.

In addition, IM resulted in a significant increase in IGF-1 concentration in the INCL cows, indicating lower metabolic load (Zbinden et al., 2017) and suggesting an improved nutritional balance in INCL cows during IM (Wathes et al., 2021). Further, IGF-1 modulates nu-

trient partitioning (Gross and Bruckmaier, 2019), and is positively associated with fertility (Gobikrushanth et al., 2018; Constantin et al., 2023). Therefore, in addition to a lower metabolic load, further positive effects of an elevated IGF-1 concentration can be expected. Like the NEFA concentration, the level of ECM yield (distinguishing between >40 kg/d and <40 kg/d ECM) also influenced the IGF-1 concentration when included as a covariate in the statistical model. Thereby, lower IGF-1 levels were observed in the >40 kg ECM/d group, indicating higher metabolic load associated with higher milk yield. Meyer et al. (2024) observed no effect of IM on IGF-1 concentration in cows during early lactation. However, both Morin et al. (2018) and Meyer et al. (2024) expected a greater effect of IM during more severe NEB, which typically occurs in early lactation.

Under conditions such as insufficient feed intake relative to requirements, which can trigger physiological imbalance (Moyes et al., 2013) and compromise immune function (Mezzetti et al., 2020), reducing milk withdrawal by IM represents a promising approach to reduce lactation-induced metabolic load and immunosuppression in high-yielding dairy cows without compromising long-term productivity (Lacasse et al., 2018). In the present study, lower concentrate allocation in INCL cows reduced energy intake. However, highlighting the effectiveness of the IM protocol, IM in INCL cows resulted in a decrease in NEFA and an increase in IGF-1 concentrations in serum. As the changes in these metabolic parameters are related to more general aspects of metabolism (Bjerre-Harpøth et al., 2012; Wathes et al., 2021), further metabolic adaptations to IM are to be expected.

Limitation and Future Research Directions

Certain limitations should be considered when interpreting the results of this study. The sample size was relatively small, with only 23 cows per treatment, which may limit the generalizability of the results to a broader population. More frequent milk and blood sampling would allow a more detailed understanding of the effects of IM on both the mammary gland and metabolism of dairy cows. Despite these limitations, this study is the first to provide insight into the effects of short-term IM in dairy cows from peak- to mid-lactation, not only in terms of milk production but also in terms of metabolic parameters. Future research could refine the IM protocol, for example by testing it under different dietary strategies to assess its effects on milk fatty acid composition and de novo synthesis in the mammary gland. In addition, more comprehensive blood analyses could help to uncover potential metabolic shifts in detail. Investigating the effects of the IM protocol during periods of acute metabolic stress, e.g. triggered by

environmental stress, could further enhance our understanding of its potential as a tool to improve the health and welfare of dairy cows.

CONCLUSION

Short-term IM was applied to dairy cows in peak- to mid-lactation using a software module for automated earlier cluster removal after a predefined milk withdrawal. The IM up to 40% increased residual milk and temporarily reduced milk yield without affecting udder health or subsequent performance in INCL cows. The study showed that IM affected metabolic parameters in serum, with reduced NEFA and increased IGF-1 levels, suggesting a reduction in lactation-induced metabolic load and the potential for further metabolic adjustments. The IM strategy tested could help dairy cows manage challenges such as NEB, metabolic disorders, and heat stress, potentially improving animal health and welfare.

NOTES

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Nonstandard Abbreviations used: COMP = complete milked, EB = energy balance, IM = incomplete milking, INCL = incomplete milked; MO = milking out, NEB = negative energy balance, NEFA = non-esterified fatty acids, PMR = partial mixed ration, REF = reference milk yield; milk yield in the week before incomplete milking, TRT = treatment.

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4. Manuscript II

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Effects of automated short-term incomplete milking during peak to mid lactation on udder firmness, heart-rate variability, and behavior records in dairy cows.

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HIGHLIGHTS:

- Short-term incomplete milking resulted in increased udder filling and firmness
- Heart-rate variability indices showed no change in sympatho-vagal balance
- Behavior in the milking parlor and barn were not affected by incomplete milking
- Incomplete milking did not appear to compromise welfare of dairy cows

ABSTRACT

Incomplete Milking can strategically be used to induce short-term milk yield reduction, benefiting dairy cows' metabolism without affecting long-term productivity. However, while intermitted milking has been associated with elevated udder pressure and discomfort in dairy cows, incomplete milking's welfare effects remain less well studied. The aim of this study was to evaluate the effects of automated short-term incomplete milking on udder filling and udder firmness, as well as on the welfare of dairy cows, by assessing heart rate variability (HRV) and behavior recordings. Udder healthy, multiparous dairy cows in peak- to mid-lactation were randomly assigned to the complete milking treatment (COMP; $n = 23$, 95 ± 49 days in milk, lactation number 3.0 ± 1.3 (mean \pm SD)) or the incomplete milking treatment (INCL; $n = 23$, 93 ± 46 days in milk, lactation number 2.6 ± 0.8 (mean \pm SD)). The incomplete milking consisted of two phases: In the 1st phase, milk withdrawal was gradually reduced by 9%/day over 5.5 days, resulting in app. 40% reduction and was followed by 3 complete milkings. Subsequently, in the 2nd phase, the 40% reduction was maintained consistently for further 5.5 days. Milk yield did not differ between treatments before incomplete milking (COMP: 39.1 ± 1.42 kg/day; INCL: 40.2 ± 1.66 kg/day (mean \pm SEM) and a 43% reduced milk withdrawal was effectively achieved at the end of the 1st phase and throughout the 2nd phase. In INCL cows, udder filling increased, and udder firmness before milking was elevated on average by 18% during the 1st and by 22% during the 2nd phase, compared to COMP cows. After milking, udder firmness was on average 33% higher in INCL cows throughout both phases. Udder health was not affected by incomplete milking and it had no effect on HRV indices, which remained consistent between groups and over time ($P > 0.05$). No defense behavior was observed in the milking parlor, and behavior recordings in the barn showed no differences between treatments. This study demonstrates that, even though automated short-term incomplete milking resulted in increased udder filling and firmness – suggesting elevated residual milk and internal udder pressure –, incomplete milking does not indicate adverse effects on the welfare of dairy cows based on the indicators evaluated in this study. These findings support incomplete milking as a viable management strategy for dairy cows.

Key words: management strategy, milking strategy, welfare assessment, udder pressure

Nonstandard Abbreviations used: bpm = beats per minute, COMP = complete milking treatment, DIM = days in milk, IM = incomplete milking, INCL = incomplete milking treatment, ES = effect size, HRV = heart rate variability, Lac. No. = lactation number, MO = milking out, REF = reference milk yield; milk yield in the week before incomplete milking, SCC = somatic cell count, SOP = standard operating procedure, T = time, TRT = treatment.

1. INTRODUCTION

Concerns regarding animal welfare are increasing among both, consumers and other stakeholder groups (Ventura et al., 2015). In the dairy sector, this trend necessitates a reconsideration of current management practices (Weary and von Keyserlingk, 2017; EFSA Panel on Animal Health and Welfare (AHAW), 2023). Adjustments in milking routines, such as milking frequency, milk flow-rate switch-point and incomplete milking, are being considered to address challenges in the dairy sector (Martin et al., 2020; Silva Boloña et al., 2020; Williamson et al., 2022). The effect of incomplete milking relies on leaving an elevated amount of milk in the udder, resulting in reduced milk production (Albaaj et al., 2018; Deacon et al., 2023). Thereby, incomplete milking has been employed to enhance the dry-off process (Martin et al., 2020; Müller et al., 2023) and to mitigate negative energy balance and its adverse impact on dairy cows during early lactation (Carbonneau et al., 2012; Morin et al., 2018; Meyer et al., 2024). Most recently, incomplete milking was used to temporarily reduce milk production in dairy cows in peak- to mid-lactation, with beneficial effects on metabolism and no negative impact on udder health or long-term performance (Joest et al., 2025).

While incomplete milking presents advantages, potential drawback for animal welfare includes increased somatic cell count (SCC; Kuehnl et al., 2019) and elevated udder pressure (Blau et al., 2019). However, studies utilizing a software module for incomplete milking have not observed increased SCC when applied in early lactation or before dry-off (Martin et al., 2020; Meyer et al., 2024), even when applied to cows with udders infected by minor pathogens prior to dry-off (Müller et al., 2023). Further, no increase in SCC were reported during incomplete milking in mid lactation (Deacon et al., 2023). However, reduced milk withdrawal leads to increased udder pressure (Blau et al., 2019), but this has not yet been quantified in incomplete milked cows during peak- to mid-lactation, nor have the potential consequences for animal welfare been addressed. In contrast, concerns about elevated intra-mammary pressure and its implications for animal welfare have been raised during dry-off (Bertulat et al., 2013; Vilar and Rajala-Schultz, 2020), with the level of milk production at dry-off being considered as important factor (Zobel et al., 2013). Thus, high udder pressure and associated discomfort may also occur during incomplete milking in high-yielding dairy cows during peak lactation, but research on this issue is limited.

Assessing welfare in dairy cows poses challenges, whether using sensor technologies (Stygar et al., 2021) or behavioral assessment protocols (Andreasen et al., 2013). Data recorded by precision livestock farming tools and their interpretation are influenced by various factors,

such as causality, recording quality, and data analysis methods, affecting the quality of prediction. Thus, further validation and refinement of these systems are necessary to improve their accuracy (Lovarelli et al., 2020; Cavallini et al., 2025). It is therefore advisable to record several different parameters to obtain a comprehensive picture. Heart rate variability (HRV) is a sensitive, non-invasive, animal-based indicator of stress in dairy cattle (Kovács et al., 2014a; Linstädt et al., 2024), and behavioral responses such as stepping or kicking during milking are frequently used as indicators of discomfort (Rousing et al., 2004; Cerqueira et al., 2017). Based on HRV and behavior in the milking parlor, the routine milking procedures across various milking systems are generally considered non-stressful (Kézér et al., 2015; Kovács et al., 2019; Kovács et al., 2024). Further, it is established that behavior can be used to indicate already low stress levels, therefore considered as sensitive indicator (Weary et al., 2009; Gleerup et al., 2015). For instance, deviations in a cow's typical use of a mechanical brush can signal shifts in welfare status (Mandel et al., 2013; Keeling et al., 2016).

Research on the impact of incomplete milking and elevated udder pressure on dairy cows' welfare is limited. Thus, we aimed to evaluate the effects of automated short-term incomplete milking on udder filling and udder firmness, as well as on dairy cows' welfare by assessing HRV and behavior recordings. Our hypothesis was that short-term incomplete milking in peak- to mid-lactation cows would result in increased udder filling and firmness, with no adverse impact on HRV or the assessed behavior in the milking parlor and barn. This is the first study using a comprehensive approach, including HRV and behavior recordings, to evaluate the impact of incomplete milking on the welfare of dairy cows.

2. MATERIALS AND METHODS

The relevant authority approved the experiments of the present study (Office for Nature, Environment, and Consumer Protection [LANUV], North Rhine-Westphalia, Recklinghausen, Germany; reference number: 81-02.04.2022.A191/01) and all experimental procedures were performed in accordance with ethical guidelines.

2.1. Animals and Housing Conditions

All procedures were conducted from September 2022 to April 2024 at the University of Bonn's research station, "Campus Frankenforst" (Königswinter, Germany). The German Holstein dairy herd consisted of app. 60 animals which were housed in a two-row open free-stall

barn, with a maximum occupancy of 1:1 animal per cubicle. Only udder healthy multiparous cows in peak- to mid-lactation qualified for enrolment in the trial. Considering this, 46 dairy cows at 94 ± 47 days in milk (DIM; mean \pm SD) were selected for the experiments. Since we selected dairy cows at a specific lactation stage from a restricted herd size, we established several experimental units with a balanced number of cows per treatment group in each repeated experimental unit. Milking was performed twice daily at 05:30 h and 16:30 h in a double-four in-line milking parlour (GEA Farm Technologies GmbH, Bönen, Germany). Udder preparation included cleaning with a wet paper towel, forestripping and prestimulation via cluster vacuum. The conventional cluster take-off was set to <0.3 kg/min based on milk flow. The Metraton C21 (GEA Farm Technologies GmbH) recorded milk flow, milking duration and milk yield of each cow at every milking. Animals and housing conditions were recently described in more detail by Joest et al. (2025), as the present study is a follow-up of that article.

2.2. Experimental Design for Incomplete Milking

Cows were assigned randomly to either the complete milking treatment (COMP; $n = 23$, 95 ± 49 DIM, average lactation number 3.0 ± 1.3 (mean \pm SD)) or the incomplete milking treatment (INCL; $n = 23$, 93 ± 46 DIM, average lactation number 2.6 ± 0.8 (mean \pm SD)). Due to the novelty of the incomplete milking protocol, no directly comparable data were available for a reliable power calculation. Therefore, the a priori power analysis used to determine sample size was guided by experimental considerations, including the 3R principles and the expectation of pronounced treatment effects with larger effect sizes, to ensure sufficient sensitivity for detecting biologically relevant differences (Joest et al., 2025). In INCL cows, incomplete milking per milking was applied using a software module (Schmidt et al., 2020) allowing automated, controlled early cluster removal after a pre-defined milk volume was withdrawn. Thereby, the incomplete milking protocol was based on individual average milk yield and consisted of two phases divided by a milking out period, as shown in Figure 1. Prior to incomplete milking, conventional cluster removal (<0.3 kg/min) was applied on all cows. During the 1st phase, milk withdrawal was gradually reduced by 9% per day (d) over 5.5 d, resulting in an overall reduction of app. 40%. This gradual reduction was considered a more welfare-friendly approach for the animals compared to an abrupt method (directly inducing 40% incomplete milking). Following this initial phase, conventional cluster removal was reintroduced for three consecutive milkings. The first of these milkings was designated as the 1st "milking out", as it removed the accumulated milk. The subsequent two milkings were used to assess the milk yield achieved separately

of the 2nd phase. During the 2nd phase, milk withdrawal was maintained at a consistent reduction of 40% for additional 5.5 d. Upon completion of the incomplete milking protocol, conventional cluster removal based on milk flow was reinstated, with the first conventional milking referred to as the 2nd milking out. The COMP cows were permanently milked with the conventional cluster removal based on milk flow (<0.3 kg/min).

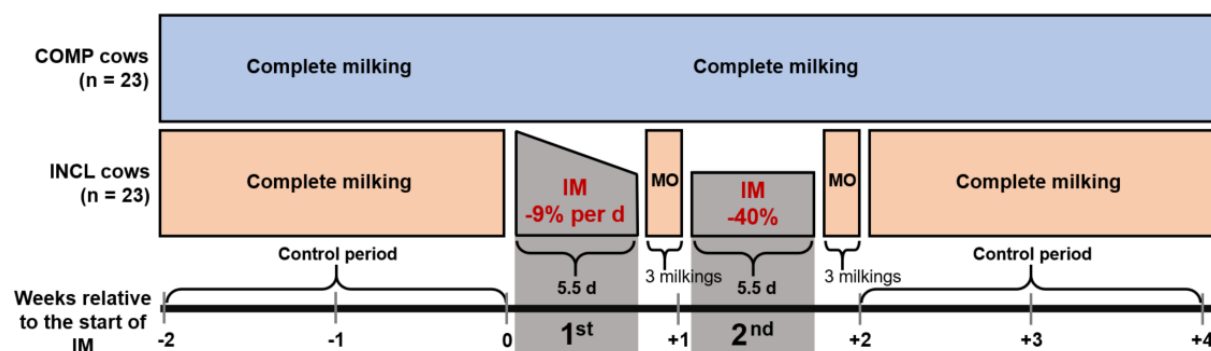


Figure 1. Study design for incomplete milking (IM) using a software-module based on individual milk yield (%) in incomplete milked cows (INCL; n = 23) compared to conventional milked (cluster take-off based on milk flow: <0,3 kg/min) control cows (COMP; n = 23). During the 1st IM phase, the amount of milk withdrawal was reduced in INCL cows by 9% per day for 5.5 days, resulting in total ~40% reduction. Before the 2nd IM phase, conventional cluster removal was activated for 3 milkings, with the first of these milkings defined as 1st “milking out” (MO). During the 2nd IM phase, the milk withdrawal was reduced in INCL cows by 40% in a constant manner for another 5.5 days. Conventional cluster removal was subsequently reactivated, and the first conventional milking was defined as 2nd MO.

2.3. Degree of Udder Filling and Udder Firmness

The “degree of udder filling” was calculated based on Bruckmaier and Hilger (2001). Maximum storage capacity of the mammary gland was estimated by dividing the average daily milk yield during lactation week (wk) 5 and 6 of the respective lactation by 2 (assuming two daily milkings). The degree of udder filling for each milking is calculated by dividing the milk yield of the current milking by the maximum storage capacity. Calculations for udder filling were performed for the evening milkings during the wk before incomplete milking, for the 1st and 2nd milking out after incomplete milking (withdrawal of accumulated milk), and for the evening milkings in the wk after incomplete milking, stated as percentage. Calculation example: Estimated maximum storage capacity: 20 kg milk (based on a peak daily milk yield of 40 kg, divided by 2 due to twice-daily milking). Milk yield of a milking of the aforementioned occasions: 15 kg. Udder filling is calculated by dividing 15 kg by 20 kg, equals 0.75 or 75%.

The effect of incomplete milking on udder pressure was assessed by external udder firmness measurement. In both treatments, udder firmness was measured following the standard operating procedure of Bertulat et al. (2012) using a digital dynamometer (FMI-B30B5, 50 N; 0.15% accuracy; Alluris GmbH & Co KG, Freiburg, Deutschland). All measurements were performed by using the same measurement point on the boarder of the middle and lower third of the rear udder quarter, and conducted by the same operator, at each time point on each day: before premilking and immediately after milking. For each time point, two series of five measurements were conducted. A coefficient of variation of <10%, both overall and within individual measurement series, was used as the quality criterion. If the coefficient of variation exceeded this threshold, the values were discarded and the measurement was repeated. The arithmetic mean was used for further analysis. Measurements were taken once per wk during the two wk before incomplete milking, on d 2, 4, 6, relative to the start of the 1st and 2nd phase, and twice in the wk after incomplete milking.

2.4.Heart-Rate Variability

For HRV measurement, cows were equipped with an equine chest belt (Polar Equine Belt, Polar Electro Ltd., Finland) placed cranial around the thorax. The belt contains two electrodes which were placed proximal to the *articulatio cubiti* and on a level with the *scapula*. Ultrasound gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, NJ, USA) was applied at the electrode sites to improve conductivity (von Borell et al., 2007), ensuring optimal skin-electrode contact for data transmission. The Polar H7 sensor, attached to the chest belt, transmitted the RR-interval data to the Polar V800 (Polar Electro Ltd.), which was fixed to an additional collar on the cow. Recordings were conducted between the morning and evening milkings, considering the time frame between 08:00 h to 16:00 h for later analysis. During this period, the dairy cows were housed in a free-stall system that allowed them to express their natural behaviors under familiar conditions, while HRV was continuously recorded. HRV was measured twice in the wk before incomplete milking, throughout incomplete milking (1st and 2nd phase) and in the wk after incomplete milking. RR-interval data were transferred to a PC via Polar-specific software FlowSync 4.0 (Polar Electro Ltd.) and subsequently analyzed using Kubios HRV-Analysis software (Kubios Scientific; Biomedical Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland). Five-minute (min) recordings were analyzed following the recommendations of the Task force of the European and North American Society of Cardiology (1996). Frequency domain analysis was

performed using the fast fourier transformation method, with low frequency defined as 0.05 – 0.2 Hz and high frequency as 0.2 – 0.58 Hz, as recommended for cattle by von Borell et al. (2007). Trend components outside a range of three standard deviations were removed, the custom middle artifact correction was applied, and otherwise, the default settings of the Kubios software were used. A total of 30,336 5-min intervals were analyzed. Quality control excluded intervals exceeding corrected beats of >10% or three standard deviations, resulting in the exclusion of 9% of the intervals from further analysis. However, the average number of 5-min intervals of each cow per d was 87, representing average 7.26 h of recording per cow per d. The HRV indices from time-, frequency- and non-linear domains used in this study are described in Tab. 1 and average expression of the HRV indices of the two treatments were used for comparison.

TABLE 1 Definitions of heart rate variability (HRV) indices present in this study

Parameter	Definition
Time domain	
HR (beats/min)	Mean heart rate (beats/min)
SDNN (ms)	Standard deviation of inter-beat intervals
RMSSD (ms)	Root mean square of successive RR-intervals differences
RMSSD/SDNN	Ratio of RMSSD and SDNN
Frequency domain	
HF log	Log-transformed power (ms^2) of the high-frequency band: 0,2 - 0,58 Hz
Non-linear domain	
SD 1 (ms)	Standard deviation perpendicular to the line-of-identity in a Poincaré plot
SD 2 (ms)	Standard deviation along the line-of-identity in a Poincaré plot
SD2/SD1	Ratio of SD2 and SD1

High HRV represents high vagal tone. All included HRV indices are positive related to HRV, excepted for HR and SD2/SD1

2.5. Behavior Assessment

Behavioral observations were conducted in relation to milking (milking behavior) in the barn (general behavior) and the expression of functional behavior (brush use).

“Milking behavior” of COMP and INCL cows was assessed via direct observations following a standard operating procedure (SOP) protocol on two d per wk in the wk before incomplete milking, throughout incomplete milking (1st and 2nd phase), and in the wk after incomplete milking. Blinding of the observer to the treatment was not feasible, as the milking stall

display indicated allocation to the incomplete milking treatment by a red flashing light. However, strict adherence to the SOP protocol was maintained throughout the study to minimize potential observer bias. The observation started as the cow of interest enters the milking parlor. The milker performed the standardized milking procedure, as described in 2.1., with cluster removal either based on milk flow or after a predefined milk volume was withdrawn during incomplete milking in INCL cows. During the milking process, the observer was positioned in the milking pit and recorded the following behaviors: kicking during pre-milking, kicking during the attachment of the milking cluster and milking, as well as kicking off the cluster or notable restlessness. When any of these behaviors occurred, cows were scored as “positive”.

“General behavior” was assessed in a subset of cows (COMP: $n = 17$, INCL: $n = 14$). The assessment was conducted once per wk in the wk before and after incomplete milking, and twice weekly throughout incomplete milking (1st and 2nd phase), via direct observations. As in the milking parlor, blinding to the treatment was not feasible due to visible treatment differences in udder distensions resulting from incomplete milking. Therefore, strict adherence to the SOP protocol (adapted from Gleerup et al., 2015) was maintained to minimize potential bias. Observations took place after morning milking and prior to feeding. The ethogram was based on welfare indicators described in the literature (Gleerup et al., 2015; De Boyer des Roches et al., 2017; Durand et al., 2021). The behavioral assessment was validated in a preliminary study (data not shown), calculating observer reliability for each indicator. Only behaviors with a prevalence-adjusted bias-adjusted kappa (PABAK) of ≥ 0.6 were considered reliable (Landis and Koch, 1977) and were therefore included in this study. A detailed list of behaviors is provided in supplemental Tab. S1 (<https://doi.org/10.6084/m9.figshare.29143823>), with scoring scales ranging from 2 to 3. The same investigator conducted all the recording of the general behavior, as defined in the SOP protocol. For standardization of observations, cows had to be in a lying position at the start of behavioral assessment. Due to this constrain, not all cows were assessed at each time point, with varying number of excluded cows per time point. The mean number of excluded cows per time point and treatment was 5, exact numbers of observations per treatment and time point are stated in supplemental Tab. S2 (<https://doi.org/10.6084/m9.figshare.29143823>). The indicators "attention towards the surroundings", "head position – lying", "ear position", "facial expression", and "eyelid position" were evaluated from a distance, while the cow was not yet aware of the observer, thus capturing spontaneous behavior expression. The cow was then approached with an outstretched arm to evaluate the “response to approach”. Subsequently, the cow was encouraged to stand up and walk – either on its own or externally motivated using the low-stress stockmanship method if

necessary – allowing evaluation of “back position”, “head position – standing”, “tail position”, and “lameness”.

Due to temporary malfunction of the brushes during some experimental units, “brush use” was assessed in a reduced number of cows (COMP: $n = 13$, INCL: $n = 12$) once weekly in the wk before incomplete milking, throughout incomplete milking (1st and 2nd phase), and in the wk after incomplete milking. The barn was equipped with two brushes, one at each gable end, serving a herd of app. 60 dairy cows. Recordings were conducted using cameras installed at each brush (Schurr Gerätebau GmbH, Uttenweiler, Germany), and one 24-hour recording per week was evaluated. During this period, each cow had free access to the brush, and individual brush use was assessed manually by viewing the videos. Cows were identified based on their unique coat patterns, which was feasible due to the limited number of animals under observation at the same time. Brush use was defined as the activation of the brush and passive use for at least 5 s or active rubbing for at least 5 s, as defined by Weigele et al. (2018). A new instance of brush use was recorded when the brush had not been used for at least 1 min. Using the camera timestamps, the start and end times of each brushing event were documented, and the duration and frequency of brush use were calculated for each wk.

2.6. Statistical Analysis

SPSS Statistics Version 29.0 (IBM, SPSS Inc., Chicago, IL) was used for statistical analysis. Regarding parametric tests, normal distribution of the model residuals was evaluated using the Shapiro-Wilk test and visually inspection of quantile-quantile Plots, whereas homogeneity of variance was assessed with Levene’s test. Non-normality of model residuals or heteroscedasticity was addressed using log-transformation. For the degree of udder filling, udder firmness, HRV indices and brush use, linear mixed models were fitted with the MIXED procedure and the REML method in SPSS. Post-hoc comparisons were adjusted for the alpha-error using the Bonferroni method. Forward selection based on the lowest Akaike information criterion (AIC) value was used for model selection. As a result, each model included treatment (TRT; COMP vs. INCL), time (T; d), interaction between treatment and time (TRT x T), and lactation number (Lac. No.; two to six lactations) as fixed effect, with cow as a random effect, and variance components as covariance structure. Used models covered all time points before, during and after incomplete milking. Student’s t-test (T-TEST procedure in SPSS) was used for single within-treatment comparisons, with Cohen’s d (reported as “ES”) calculated as effect size (0.5-0.8 were considered medium, >0.8 were considered as large (Cohen, 1988)), for the

degree of udder filling and udder firmness, as well as for TRT comparisons for the heart rate. For the general behavior, as well as for milking behavior and brush use, data is presented using descriptive statistics only, due to low numbers of observations per time point. Presented data is given as mean \pm SEM (excepted if stated different and for the general behavior (proportion of observations)), with level of significance set at $P \leq 0.05$.

3. RESULTS

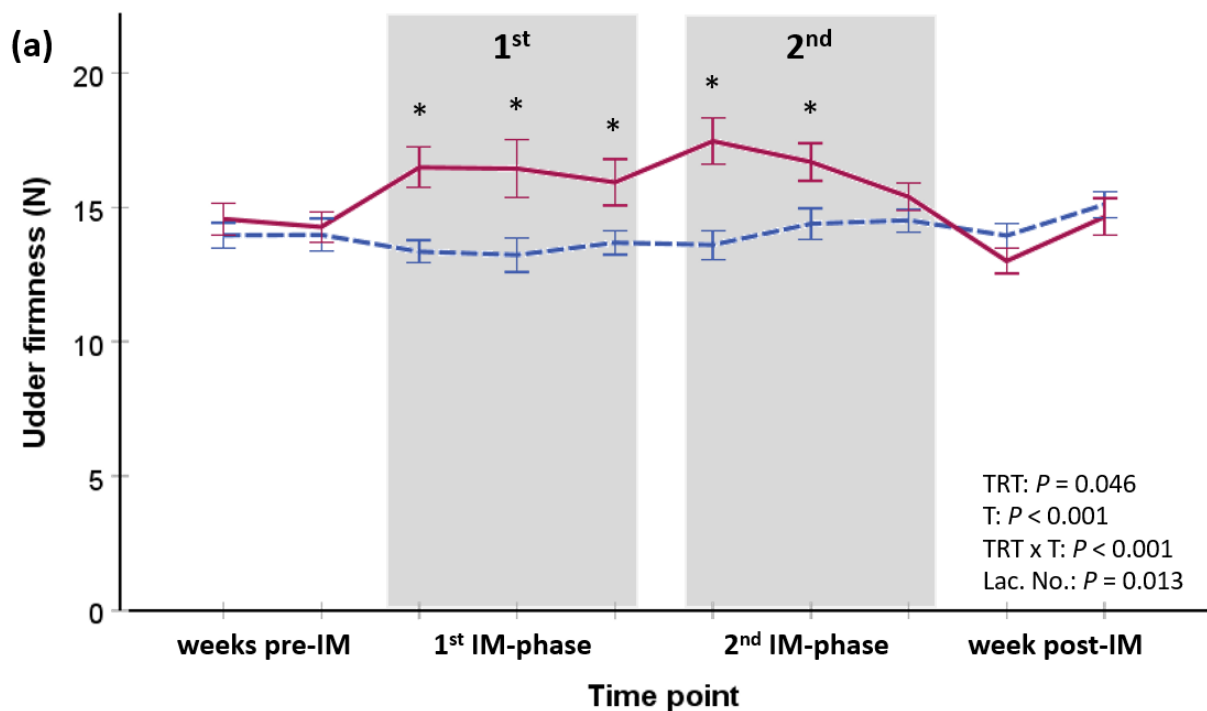
The milk yield in the wk before incomplete milking did not differ between the treatment groups (COMP: 39.1 ± 1.42 kg/d; INCL: 40.2 ± 1.66 kg/d; $P = 0.602$). The incomplete milking protocol was successfully implemented by the software. Thereby, relatively to the milk yield in the wk before incomplete milking, an average 43% reduced milk withdrawal by the end of the 1st phase and maintaining average 43% reduced milk withdrawal throughout the 2nd phase was achieved.

3.1. Degree of Udder Filling and Udder Firmness

Regarding udder filling before incomplete milking, no differences were observed between the COMP and INCL cows (COMP: $85 \pm 0.11\%$; INCL: $85 \pm 0.12\%$; $P = 0.937$). At the end of the 1st phase, the udder filling in INCL cows was elevated by 47% compared to before incomplete milking, averaging $128 \pm 4\%$ ($P \leq 0.001$, ES = 3.14). At the end of the 2nd phase, the udder filling in INCL cows was elevated by 28% compared to before incomplete milking, averaging $111 \pm 3\%$ ($P \leq 0.001$, ES = 2.14). Udder filling of the INCL cows was, on average, 38% higher than in the COMP cows at the end of both phase ($P \leq 0.001$). In the wk after incomplete milking, the udder filling of the INCL cows decreased by 14% compared to before incomplete milking, averaging $75 \pm 2\%$ ($P \leq 0.001$, ES = 1.84). Udder filling in COMP cows remained stable during the entire experimental period, averaging $86 \pm 0.4\%$.

In the wk before incomplete milking, udder firmness did not differ between the COMP and INCL cows, neither before nor after milking (Figures 2a-b). During the 1st phase, udder firmness before milking increased in the INCL cows, resulting in 18% higher firmness compared to the COMP cows (COMP: 13.4 ± 0.29 N; INCL: 16.3 ± 0.51 N). At the beginning of the 2nd phase, the udder firmness before milking in INCL cows was 22% higher than in COMP

cows. However, udder firmness before milking in INCL cows declined as the 2nd phase progressed, showing no difference from the COMP cows by the end of this phase or upon resumption of conventional milking (Figure 2a). Furthermore, udder firmness after milking in INCL cows increased over the 1st phase and remained elevated throughout the 2nd phase (Figure 2b). During incomplete milking, udder firmness after milking in INCL cows was on average 33% higher than in the COMP cows (COMP: 8.8 ± 0.12 N; INCL: 13.2 ± 0.23 N). Regarding the 2nd phase, udder firmness after milking in INCL cows (13.2 ± 0.23 N) was comparable to the udder firmness before milking in the COMP cows (13.8 ± 0.21 N; $P = 0.284$).



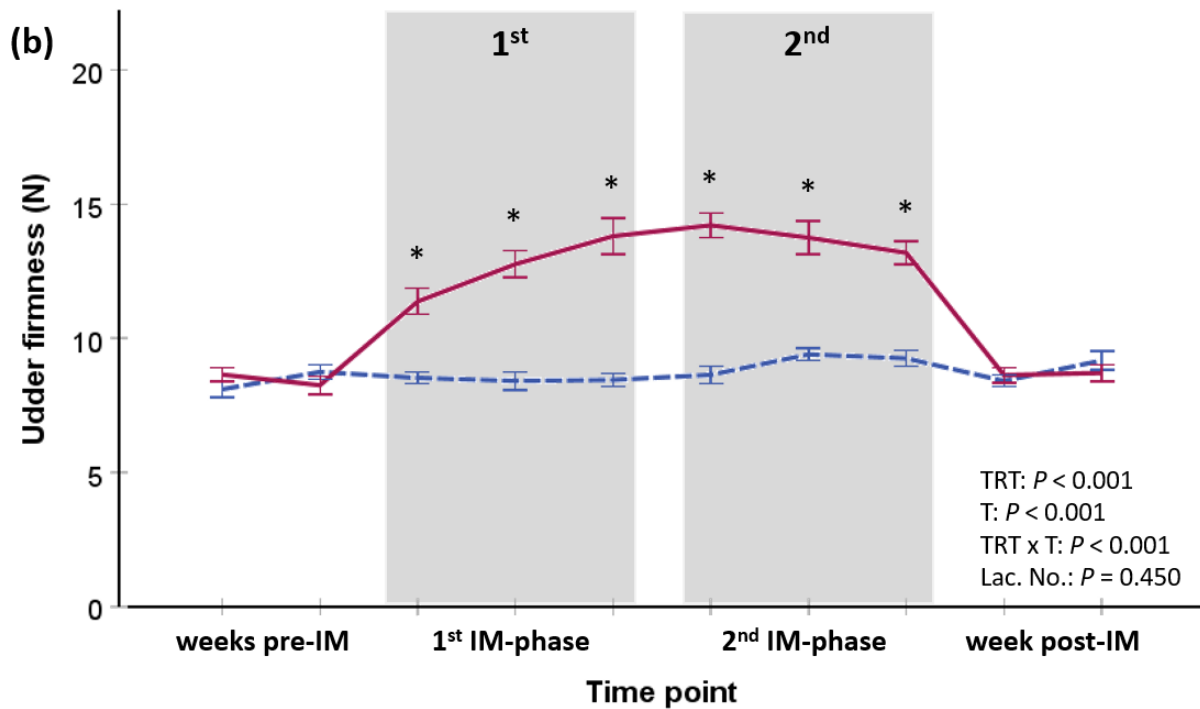
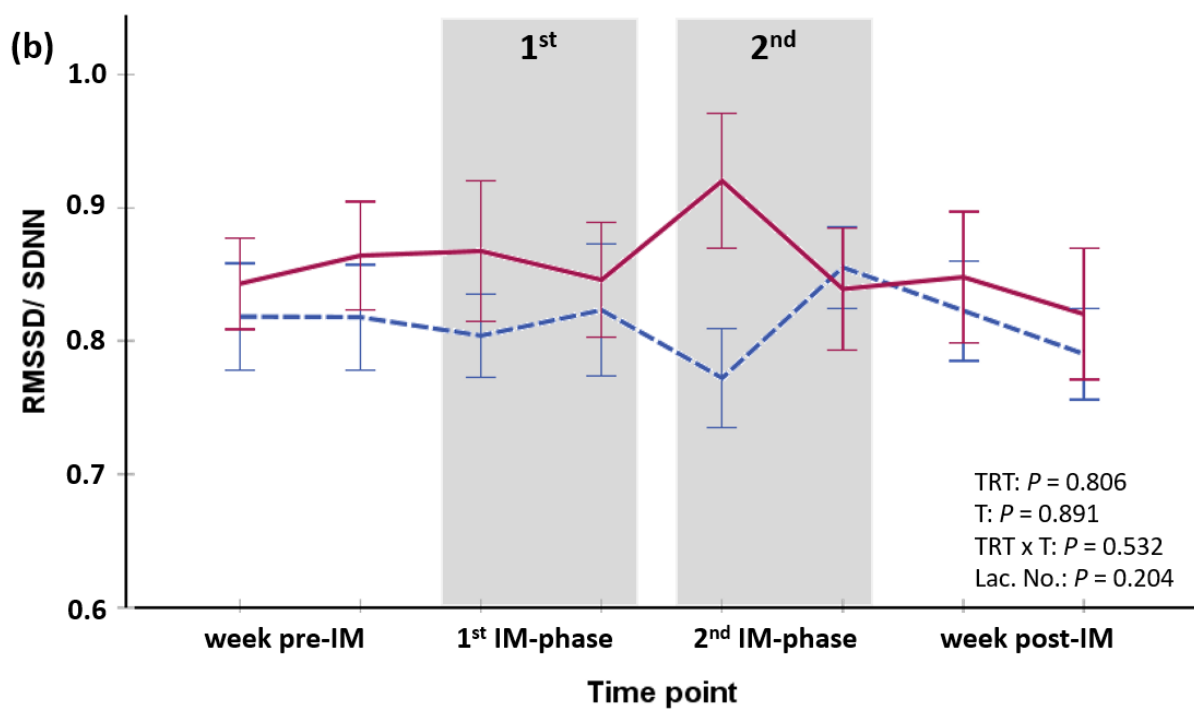
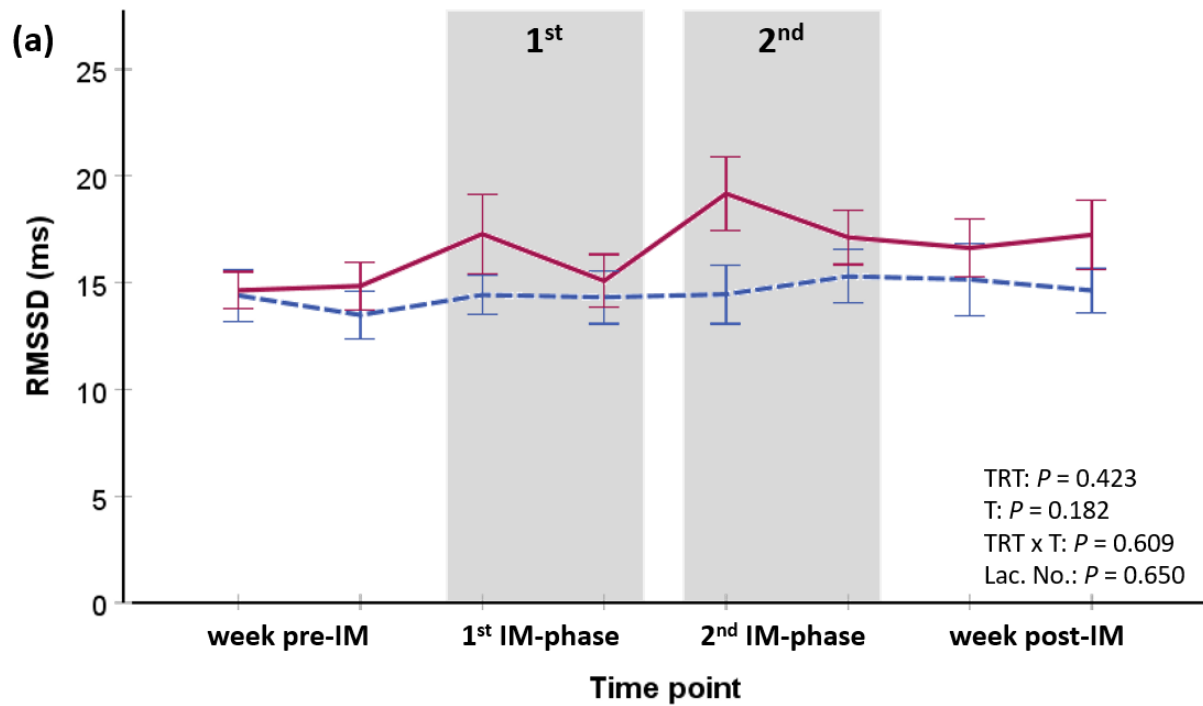


Figure 2. Effect of incomplete milking (IM) on udder firmness (N) (a) before milking and (b) after milking of cows in peak- to mid-lactation, compared with conventionally milked control cows: 2 weeks before, throughout (1st and 2nd phase) and in the week after IM. Two treatments (TRT): blue dotted line = complete milked cows (COMP, $n = 21$); red solid line = incomplete milked cows (INCL, $n = 20$). The IM of INCL cows was based on their individual milk yield levels in the week prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per day over 5.5 days, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained reduced consistently by 40% for additional 5.5 days. The TRT-periods are shaded in grey. Data are presented as means \pm SEM, with significant differences between TRT at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

3.2. Heart-Rate Variability

Heart rate did not differ between the COMP and INCL cows in the wk before incomplete milking (COMP: 81 ± 5 beats per minute (bpm); INCL: 81 ± 6 bpm; $P = 0.953$), throughout incomplete milking (COMP: 80 ± 6 bpm; INCL: 80 ± 7 bpm; $P = 0.847$) or in the wk after incomplete milking (COMP: 80 ± 5 bpm; INCL: 79 ± 7 bpm; $P = 0.215$). Figure 3a-d shows the root mean square of successive RR-intervals differences (RMSSD), RMSSD/standard deviation of inter-beat intervals (SDNN), log-transformed power of the high-frequency band (HF (log)), and standard deviation along the line-of-identity in a Poincaré plot (SD2)/standard deviation perpendicular to the line-of-identity in a Poincaré plot (SD1) of both treatment groups over time. For the calculated HRV indices, no effect of TRT (COMP vs. INCL) or TRT x T was evident. However, lactation number significantly influenced the SD2/SD1 ratio, with

higher values in cows in 4th and 5th lactation. Across the entire experimental period, mean values for RMSSD, RMSSD/SDNN, HF (log) and SD2/SD1 were as follows for COMP cows: 13.59 ± 1.31 ms, 0.77 ± 0.05 , 3.00 ± 0.14 ms and 2.93 ± 0.15 , and for INCL cows: 14.77 ± 1.59 ms, 0.79 ± 0.06 , 3.09 ± 0.17 ms and 2.94 ± 0.18 , respectively.



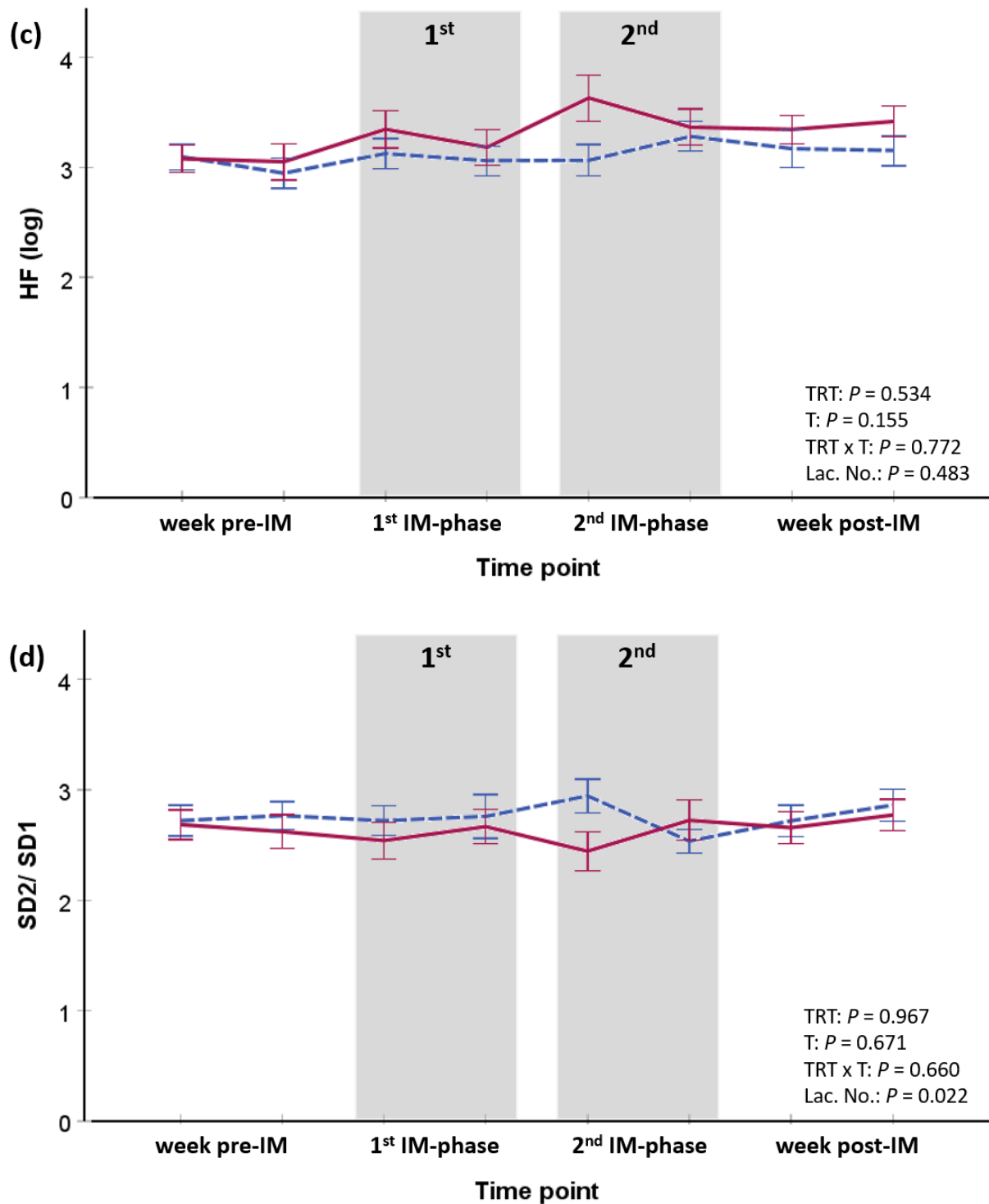


Figure 3. Effect of incomplete milking (IM) on heart-rate variability (HRV), expressed as (a) RMSSD (ms), (b) RMSSD/SDNN, (c) HF (log), and (d) SD2/SD1 of cows in peak- to mid-lactation, compared with conventionally milked control cows: in the week pre-IM, throughout IM (1st and 2nd phase) and in the week after IM. Two treatments (TRT): blue dotted line = complete milked cows (COMP, $n = 23$); red solid line = incomplete milked cows (INCL, $n = 23$). The IM of INCL cows was based on their individual milk yield levels in the week prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per day over 5.5 days, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained reduced consistently by 40% for additional 5.5 days. The TRT-periods are shaded in

grey. Data are presented as means \pm SEM, with interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

3.3. Behavior Assessment

For the milking behavior, each cow was assessed on two milkings per d of two d per wk, in the wk before, during, and after incomplete milking, in total 368 observation per treatment. No defense behavior such as kicking toward the milker during premilking or the milking cluster during milking was observed in assessed milkings in the COMP or INCL cows throughout the entire observation period.

Assessment of general behavior resulted in a low and variable number of observations per time point and TRT, which precluded inferential statistical analysis. The results of the assessment of the general behavior are shown in the supplemental Tab. S2 (<https://doi.org/10.6084/m9.figshare.29143823>). Numerically, no obvious differences in behavior expression were evident between the COMP and INCL cows during the experimental period, with no observable impact of incomplete milking on the assessed behavior. Across both treatment groups, cows commonly displayed a consistent combination of behaviors: Attentive toward the surroundings, high head while lying, ears back and high, attentive or neutral looking face with open eyelids.

The duration (min/d) and frequency (use/d) of brush use of the COMP and INCL cows are presented in Fig. 4a-b. Throughout the experimental period, the average brush use duration was 4.6 ± 0.61 min/d in COMP and 6.6 ± 0.90 min/d in INCL cows. Thereby, average brush use frequency per d was 2.9 ± 0.26 in COMP and 3.5 ± 0.25 in INCL cows, with sustainable variance between individual cows in both brush use traits in both treatments.

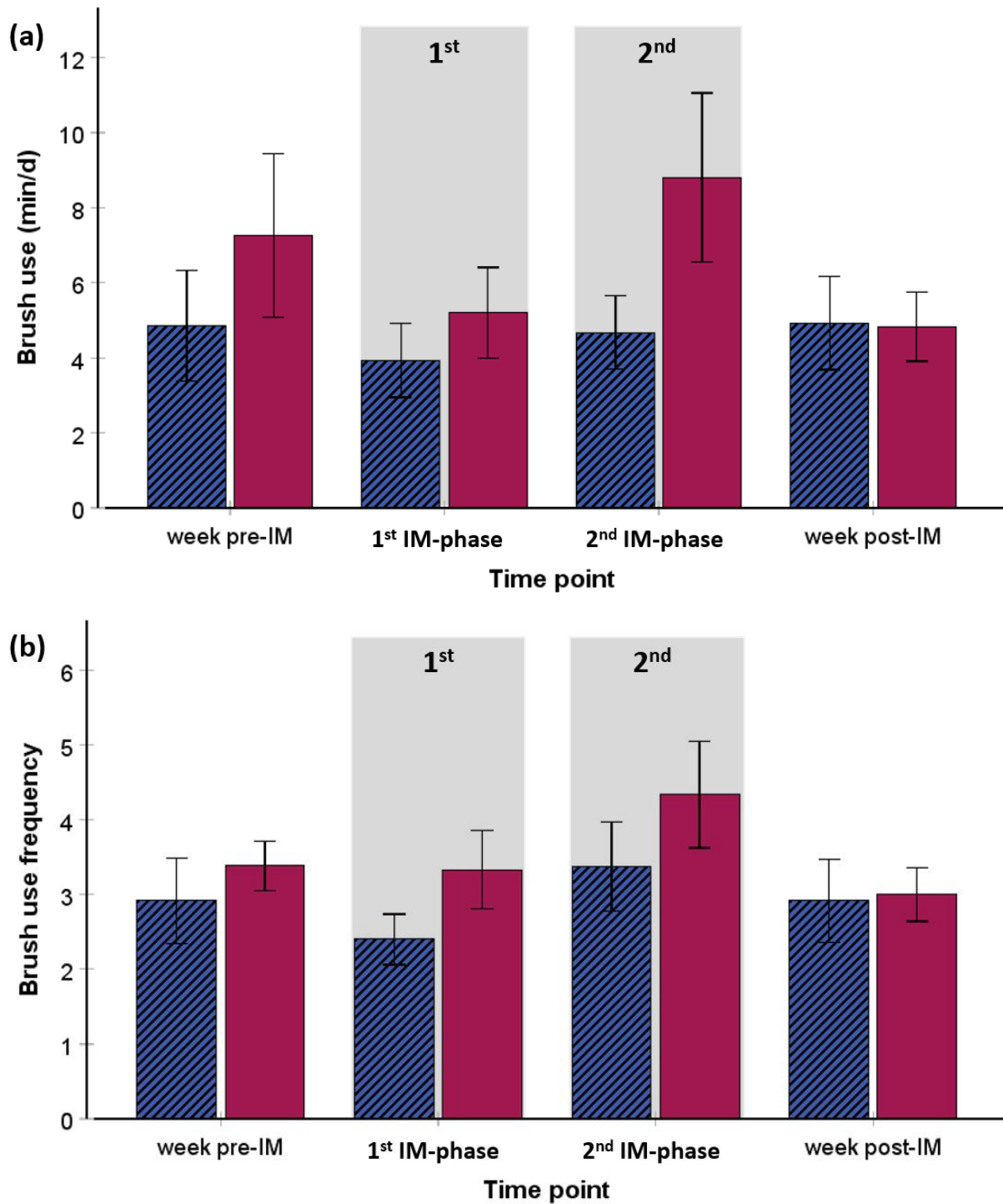


Figure 4. Effect of incomplete milking (IM) on brush use (a) duration (min/d) and (b) frequency (use/d) of cows in peak- to mid-lactation, compared with conventionally milked control cows: in the week pre-IM, throughout IM (1st and 2nd phase) and in the week after IM. Two treatments (TRT): blue dashed bars = complete milked cows (COMP, n = 12); red solid bars = incomplete milked cows (INCL, n = 13). The IM of INCL cows was based on their individual milk yield levels in the week prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per day over 5.5 days, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained reduced consistently by 40% for additional 5.5 days. The TRT-periods are shaded in grey. Data are presented as means \pm SEM,

with interactions between TRT and T are represented as TRT x T and the influence of lactation number as Lac. No.

4. DISCUSSION

The incomplete milking procedure is increasing residual milk and subsequent udder filling, thereby leading to greater udder firmness. This, in turn, may negatively impact animal welfare. The aim of this study was to evaluate the effects of automated short-term incomplete milking on the welfare of dairy cows during peak- to mid-lactation by assessing HRV and behavior recordings. Previously, we published the effects of the incomplete milking protocol on milk production, udder health, and selected metabolic parameters, demonstrating a temporary reduced milk production and a decreased lactation-induced metabolic load, without negatively affecting udder health (Joest et al., 2025). The incomplete milking protocol was successfully implemented in peak- to mid-lactation dairy cows averaging app. 40 kg/d before incomplete milking. The settings of the incomplete milking protocol resulted in average 43% reduced milk withdrawal by the end of the 1st phase and throughout the 2nd phase.

4.1. Degree of Udder Filling and Udder Firmness

Peak milk yield is considered an indicator of the udder's storage capacity, as proposed by Bruckmaier and Hilger (2001). Corresponding udder filling is calculated by dividing the actual milk yield by the estimated storage capacity. In our study, udder filling exceeded 100% at the end of both incomplete milking phases, indicating high amounts of milk stored in the udder that surpassed the assumed storage capacity in INCL cows. For context, using the method of Bruckmaier and Hilger (2001), udder filling of a typical morning milking in peak lactation would also exceed the assumed storage capacity. However, the exceedance observed in later lactation and the increase compared to COMP cows suggest substantial milk accumulation in the udders of INCL cows during incomplete milking. Gleeson et al. (2007) found, that elevated residual milk and the resulting intramammary pressure is associated with discomfort in dairy cows. Discomfort is a negative affective state, less than pain, which contains physical, physiological, and mental effects and results in avoidance attempts (Franchii et al., 2024). Additionally, discomfort in livestock animals can affect productivity (Endris and Feki, 2021). A measure of udder discomfort is udder firmness, which is a non-invasive measured proxy for intramammary pressure (Bertulat et al., 2012; Blau et al., 2019). The udder firmness in the present

study was significantly increased in INCL cows and an increased udder firmness might have negative effects on animal welfare. For example, Bertulat et al. (2013) investigated udder firmness after abrupt dry-off and reported, regardless of milk yield, the highest udder firmness at d 2. Thereby, they found the faecal glucocorticoid concentration to peak on d 3 after dry-off, suggesting a link between increased udder firmness and stress during dry-off. During the 1st phase of the present study, the gradual increase of the intensity of incomplete milking resulted in lower milk withdrawal with higher milk accumulation and corresponding udder filling, thereby elevating udder firmness before and after milking. Similarly, Tucker et al. (2007) observed increased udder firmness in mid-lactation cows during the transition from twice daily milking to once daily milking. In addition, ongoing milk accumulation during extended milking intervals was associated with increased udder firmness, as reported by Blau et al. (2019), and with behavioral changes and pathological signs, as noted by Kohler et al. (2016). Although these findings align with the results of our study, the literature remains inconsistent. In contrast, Meyer et al. (2024) found no impact of mild incomplete milking on udder firmness before milking in early-lactation dairy cows, with firmness levels comparable to those of the COMP cows in the present study. By the end of the 2nd phase, udder firmness before milking had declined, suggesting an adaptation to incomplete milking. However, udder firmness after milking remained elevated in the INCL cows throughout incomplete milking. During the 2nd phase, udder firmness after milking in INCL cows was comparable to the firmness before milking of the COMP cows, indicating ongoing milk accumulation and associated high intramammary pressure.

4.2. Heart-Rate Variability

The elevated udder pressure and the altered milking routine associated with incomplete milking might induce stress in dairy cows. Stress is originated by internal or external stressors and is a threat to the organism. As adaptations to stress involves physiological alterations (von Borell, 2001; Linstädt et al., 2024), we assessed the impact of incomplete milking on the autonomous nervous system (ANS) using HRV. The HRV indices used in this study specifically reflects either the vagal tone like RMSSD and HF (log) (von Borell et al., 2007; Shaffer and Ginsberg, 2017) or sympatho-vagal balance like RMSSD/SDNN and SD2/SD1 (Task force of the European and North American Society of Cardiology, 1996; Kovács et al., 2015). Previous research highlights the sensitivity of high-producing dairy cows to technological stress and pathological loads, reflected by changes in HRV (Kovács et al., 2014a). Herein, reduced HRV

indicates a decrease in vagal tone or an increase in sympathetic dominance, suggesting elevated physiological stress level (von Borell et al., 2007; Kovács et al., 2014a). In this study, no effects on HRV indices across all domains were observed, indicating stable vagal tone during incomplete milking. This suggests that incomplete milking, including associated increased udder filling and udder firmness, was not perceived as a negative stressor. Gygax et al. (2008) and Kovács et al. (2024) observed HRV depending on milk yield, with lower RMSSD in high-yielding cows. Therefore, the reduced milk production in INCL cows may mitigate the potential negative effect of increased udder pressure due to incomplete milking. However, measuring HRV in dairy cows under practical field conditions remains challenging. Kovács et al. (2024) observed no differences in the HRV during rectal examination, despite the latter being considered a stressor for dairy cows (Stojkov et al., 2015; Kovács et al., 2016; Giese et al., 2018). In contrast, other studies have demonstrated stress responses during rectal examinations using HRV measurements (Kovács et al., 2014b; Kovács et al., 2016). This inconsistency in the literature might reflect difficult HRV measurement under field conditions. However, in the present study, we did not monitor the respective activities of the cows, and we were therefore unable to account for varying levels of physical activity. The importance of physical activity in the interpretation of HRV under free-moving conditions has been demonstrated by, for example, Kézér et al. (2017) and Oishi et al. (2018). The absent of changes in HRV between the treatments may reflect the difficulties of accurate measurements under field conditions (Kovács et al., 2024). One potential approach to mitigate these issues is to focus on HRV during milking, when the cow is standing. This approach avoids challenges associated with HRV measurement during physical activity and allows for the analysis of the earlier termination of milking during incomplete milking. However, other factors must be considered when interpreting HRV during milking, such as the oxytocin-induced increase in vagal activity (Uvnäs-Moberg and Petersson, 2005). Furthermore, combining HRV analysis with glucocorticoid measurements may enhance sensitivity, as it is generally recommended to assess multiple physiological parameters. In our study, we combined HRV measurements with behavior recordings. Since no effects were detected in either parameter, the analysis of glucocorticoids might strengthen the analysis of the effects of IM on animal welfare.

4.3. Behavioral Assessment

To get a comprehensive picture, behavioral observations were conducted, as alterations in behavior can be found as an adaptation to stress (von Borell, 2001). During incomplete milking, dairy cows exhibited no antagonistic behavior in the milking parlor, as evident by the absence of kicking behavior. Previous research has shown behavioral changes in the milking parlor when udder discomfort was present, such as elevated stepping frequency during overmilking (Cerqueira et al., 2017), and increased milking cluster kick-offs in dairy cows with udder edema (Okkema et al., 2023). Further, Browne et al. (2024) measured leg movement under different cluster removal settings and reported reduced rear leg movement with increasing the milk flow-rate switch-point from 0.2 to 0.8 kg/min, indicating improved welfare (Browne et al., 2024).

For the behavior recorded in the barn, too, no effects of incomplete milking could be observed. In the present study we used various parameters, which were described to indicate negative stress with regard to udder problems in dairy cows. For example, De Boyer des Roches et al. (2017) found deviations in ear position, cows' attention towards the surrounding and tail position after experimental *E.coli* infection of one udder quarter with clinical signs of mastitis and systemic inflammation. Caplen and Held (2021) reported altered behavior and increased salivary serum amyloid A in dairy cows with subclinical mastitis (SCC >200 × 1,000 cells/mL) compared to udder healthy control cows (SCC <100 × 1,000 cells/mL), further suggesting a link between udder discomfort and changed behavior. The elevated udder pressure induced by incomplete milking in the present study is most likely less severe than the patho-physiological response observed in De Boyer des Roches et al. (2017) or Caplen and Held (2021), which may explain the lack of behavioral alterations of the INCL cows. Moreover, the selected behaviors may not have been suitable to detect a potential change in welfare related to incomplete milking. Bach et al. (2015) observed, that udder discomfort results in reduced lying time. This parameter was not measured in the present study, however, Krug et al. (2017) showed that short-term incomplete milking in early lactation does not reduce lying time. Furthermore, the SOP protocol of the general behavior recording required a standardized starting point, with cows had to be in a lying position to participate. This led to varying numbers of observations per time point. This limitation should be taken into account when interpreting the results and could be addressed in future studies through the use of video recordings and AI-supported behavioral assessments. In the present study, blinding was not feasible due to visible treatment-related signs. Consequently, potential sources of bias include observer and confirmation bias. To minimize these

risks, assessments were standardized through strict adherence to SOP protocols. To further reduce subjective bias, behavioral observations were complemented by objective, automated measurements (HRV). Nonetheless, the absence of blinding remains a recognized limitation of this study.

An additional behavioral record in the barn was brush use, a highly prioritized activity that tends to decrease when energy availability is limited or the required effort increases (Mandel et al., 2013; McConnachie et al., 2018). Due to technical issues with the brushes, only a subset of cows could be examined for brush use. This limitation should be considered when interpreting the results. Based on the available recordings, no differences in brush use duration or frequency were observed between the COMP and INCL cows in a descriptive analysis, although both parameters showed high individual variation. Jensen et al. (2023) investigated behavioral changes in a cross-over design including changed milking frequency and feeding level prior to dry-off. The changed feeding regime reduced brush use, whereas changes in milking frequency had no such effect (Jensen et al., 2023). Regarding the effect of subclinical mastitis on brush use, Caplen and Held (2021) reported no significant reduction in brush use in cows with subclinical mastitis compared to udder healthy cows, based on a 24-hour video recording. Altogether, these findings might suggest that brush use is not sensitive enough to detect stress associated with udder discomfort or incomplete milking. However, assuming sufficient sensitivity, as found in certain studies (Mandel et al., 2013; Keeling et al., 2016), missing differences in brush use between treatments regardless of missing statistical analysis indicated that the cows were not negatively affected by incomplete milking.

4.4. Limitation of the study

Certain limitations should be considered when interpreting the results of this study. The sample sizes for general behavior and brush use were limited due to experimental constraints, and observer blinding of the behavior assessments was not feasible, as described in the respective chapters. Consequently, statistical analyses were not performed for these variables due to insufficient statistical power. Furthermore, the inclusion of additional physiological stress indicators, such as glucocorticoids, would provide a more comprehensive understanding of the effects of incomplete milking on animal welfare. Despite these limitations, this study is the first to apply a broad, integrative approach to examine the potential impact of incomplete milking on dairy cow welfare, by quantifying the udder firmness, using HRV as physiological indicator, and behavior recordings. Future research strategies, discussed in the respective sections, should

aim to further elucidate the effects on animal welfare to evaluate the potential of incomplete milking as a viable management practice.

5. CONCLUSIONS

The incomplete milking protocol applied in this study resulted in increased udder filling and udder firmness. However, no changes were present due to incomplete milking in HRV indices or in the behavior recordings assessed in the milking parlor and barn. Thus, despite elevated udder filling and udder firmness, incomplete milking did not appear to compromise the welfare of dairy cows, based on the indicators evaluated in this study. These findings suggest that incomplete milking is a viable management strategy in dairy cows. Future studies, including the assessment of cortisol levels and extended HRV measurements, might help to comprehensively investigate potential stress responses associated with incomplete milking.

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5. Effects of incomplete milking applied during summer on dairy cows in peak lactation

Heat stress is a major concern in animal production, resulting in reduced milk production, impaired reproductive performance, and compromised animal health and welfare (Polsky and von Keyserlingk, 2017; Becker et al., 2020). This necessitates the development of mitigation and adaptation strategies to alleviate the impact of heat stress in the context of climate change (West, 2003; Gauly and Ammer, 2020; Chen et al., 2024). Thereby, high-yielding dairy cows are especially vulnerable to heat stress because the milk production-related metabolic performance is associated with heat production, leading to a higher heat load (Purwanto et al., 2009; Zimbelmann et al., 2009; Baumgard and Rhoads, 2013). One potential strategy to assist cows in coping with heat stress may involve an adapted milk yield, given the significant role of milk yield for the occurrence of heat stress (Bernabucci et al., 2010; Tober, 2022) and for the consequences of heat stress on e.g., the metabolism (Tao et al., 2020). Introducing IM has been demonstrated to temporarily reduce milk production (Albaaj et al., 2018; Joest et al., 2025), lactation-induced metabolic load (Joest et al., 2025), and metabolic stress, particularly during NEB and inflammation (Carbonneau et al., 2012; Lacasse et al., 2018; Morin et al., 2018), without negatively affecting udder health (Martin et al., 2020; Müller et al., 2023; Joest et al., 2025) or long-term productivity of dairy cows (Krug et al., 2018; Meyer et al., 2024; Joest et al., 2025), making IM a potential strategy to address the negative consequences of heat stress.

Considering this, the aim of this pilot study was to implement the IM protocol – first used in Joest et al. (2025) – in heat-stressed dairy cows in peak-lactation and to investigate the effect on the milk yield, energy intake, and selected metabolic parameters.

MATERIAL AND METHODS

A pilot study was conducted between July and August 2024 and cows were selected based on DIM and udder health. Due to the experimental requirements, fewer cows could be selected from the herd than expected. Clinical healthy cows in peak-lactation were assigned either to the complete milking treatment (HCOMP; $n = 3$, DIM: 46 ± 16 , average lactation number: 2.3 ± 0.3) or incomplete milking treatment (HINCL; $n = 3$, DIM: 62 ± 14 , average lactation number: 2.3 ± 0.3). The aim of the IM was to serve as a preventative measure, implemented prior to the onset of severe heat stress conditions. The IM was initiated in response to

predicted heat stress based on the weather forecast (www.wetter-online.de). Unexpectedly, the weather changed, so that no severe heat stress occurred during the application of IM. The THI after the IM phases approximately corresponds to the conditions under which IM was intended to be applied (Fig. 1a).

Of note, high incidence of the bluetongue virus (**BTV**) infections in the region (Friedrich Loeffler-Institute, Greifswald, Germany) and within the herd itself (Campus Frankenforst, Königswinter, Germany) were present during the experiments. Despite clinical signs of BTV infections were absent in the selected cows, subclinical infections cannot be excluded, further supported by elevated haptoglobin serum concentrations ($\mu\text{g/mL}$) in some individuals (Tab. 1).

Cows of the HINCL treatment were milked incompletely during two IM phases (detailed description in Joest et al. (2025)). During the 1st IM phase, milk withdrawal was reduced by 9%/d for 5.5 d, resulting in ap. 40% reduced milk withdrawal. During the 2nd IM phase, milk withdrawal was consistently reduced by 40% for further 5.5 d. Due to the spontaneous initiation of IM, only one control week pre-IM was conducted. The data collection (e.g., milk yield, feed intake), as well as the blood sampling and analysis were carried out as described in Joest et al. (2025). Blood samples were taken on d -2, 6, 13, 20, and 27 relative to the start of IM. Frozen serum samples were transferred to the laboratory for photometric analysis of non-esterified fatty acids (NEFA; Synlab.vet GmbH, Leverkusen, Germany). The insulin-like growth factor-1 (IGF-1) concentration was quantified in serum using an enzyme immunoassay (Mediagnost GmbH, Reutlingen, Germany). The mean intra-assay CV was 2.49% and the inter-assay CV was 6.64%. Additionally, Haptoglobin ($\mu\text{g/mL}$) was analyzed by an in-house developed ELISA in serum (Hiss et al., 2004), with a mean intra-assay CV of 12.1% and a mean inter-assay CV of 11.8%. Furthermore, the ambient temperature and humidity levels were recorded using four climate data loggers (Gemini Data Loggers, Chichester, United Kingdom) strategically positioned throughout the barn. The THI was calculated in accordance with NRC (1971). On two occasions per wk, in the wk before, during both IM phases, and in the wk after IM, the breath rate (breaths per min) was counted visually and the rectal temperature ($^{\circ}\text{C}$) was measured after evening milking. The data of the pilot study was not statistically analyzed, as the number of cows included in the study was too small.

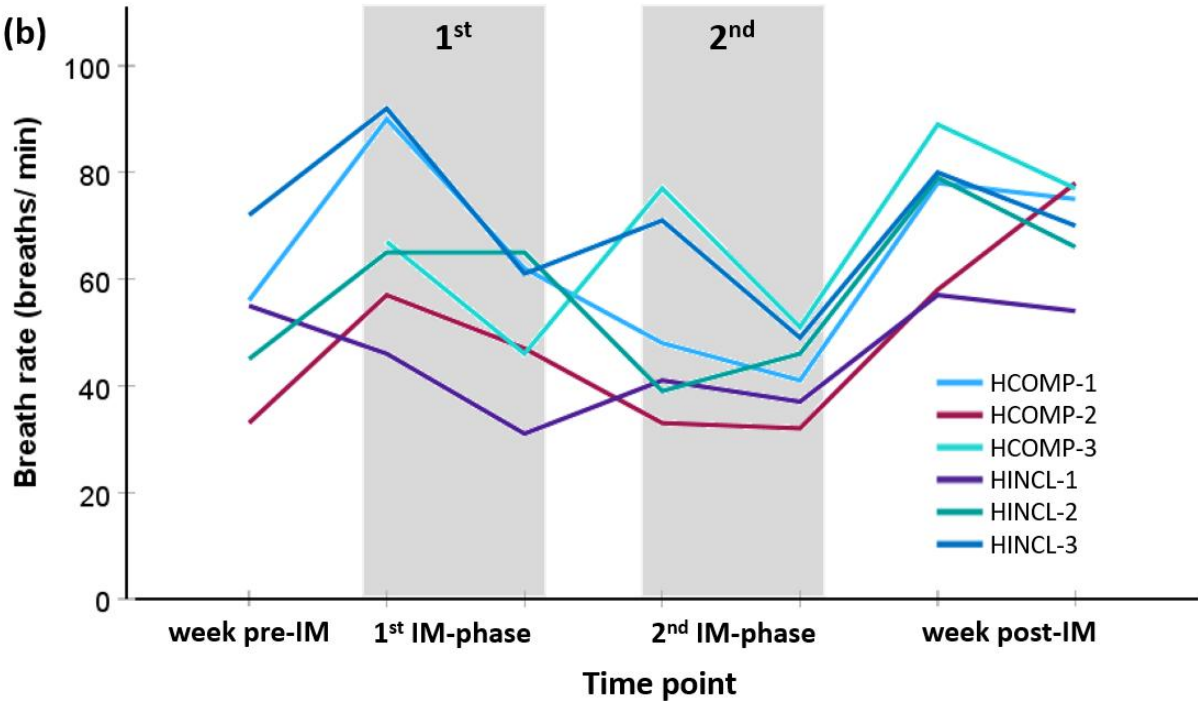
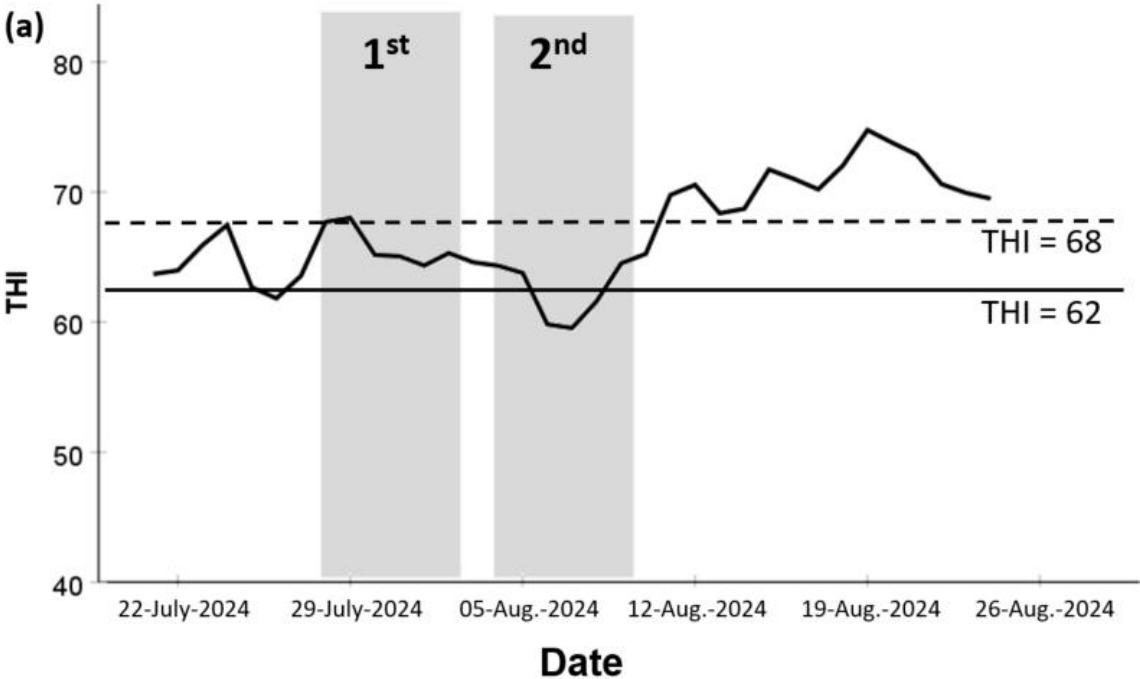
Table 1: Haptoglobin concentration in serum ($\mu\text{g/mL}$) relative to the start of incomplete milking (IM) of dairy cows in peak-lactation compared with conventionally milked control cows: 1 wk pre-IM, throughout IM (1st and 2nd phase) and 2 wk post-IM. Two treatments (TRT): complete milked cows (HCOMP, $n = 3$) and incomplete milked cows (HINCL, $n = 3$)).

Cow	Time points relative to the start of IM				
	wk pre-IM	1 st IM-phase	2 nd IM-phase	wk post-IM	
HCOMP 1	46.0	73.8	77.8	86.6	89.2
HCOMP 2	43.5	134.0	67.2	66.8	75.4
HCOMP 3	42.3	60.0	63.8	75.4	63.0
HINCL 1	38.1	2660.0	54.1	44.0	42.9
HINCL 2	46.3	245.2	70.0	84.0	494.3
HINCL 3	39.1	139.8	79.9	70.4	74.6

RESULTS

In the present pilot study, IM was implemented in dairy cows during peak-lactation. Initiation of IM in HINCL cows was based on weather forecast; the average daily THI was 65 ± 0.56 during the 1st IM phase, 63 ± 0.89 during the 2nd IM phase and 70 ± 0.46 in the wk post-IM (Fig.1a). Breath rate (breaths/min) and rectal temperature ($^{\circ}\text{C}$) are illustrated in Fig.1b+c. The breath rate varied between 31 and 92 breaths/min in HINCL cows and 33 and 90 breaths/min in HCOMP cows during IM. Concurrently, rectal temperature was especially high (41°C) in HINCL-2 at the second measurement during the 1st IM phase. Further, the rectal temperature of HINCL-3 stands out. The milk yield of HINCL cows pre-IM varied between 24.1 and 48.9 kg/d, and the energy balance between -43.8 and -1.4 MJ NEL/d. In HCOMP cows, milk yield varied between 32.2 and 43.4 kg/d pre-IM, and the energy balance varied between -41.8 and -8.2 MJ NEL/d. The IM protocol was successfully implemented in HINCL cows and the milk yield of HCOMP cows remained constant, as no clear trend was visible (Fig. 2). Haptoglobin concentration was noticeably elevated in one HINCL cow during the 1st IM-phase (Tab. 1). Total energy intake was relatively low in two HINCL cows at the end of the 1st IM-phase compared to HCOMP cows (Fig.3). The concentration of NEFA in serum exhibited considerable variation, as shown in Fig. 4a. The NEFA concentration showed high variation in both groups during the 1st IM phase (HCOMP: 0.35 – 0.51 mmol/L, HINCL: 0.16 – 0.61 mmol/L), a low variation at a lower level during the 2nd IM-phase (HCOMP: 0.11 – 0.18 mmol/L, HINCL: 0.1 – 0.12 mmol/L), and was followed by an increase in both groups (Fig. 4a). Prior to IM, the serum concentrations of IGF-1 were comparable in the two treatments (Fig.4b; HCOMP: 81.2 – 126.8 ng/ml, HINCL: 97.9 – 138.9 ng/ml). During the 1st IM-phase,

the IGF-1 concentration was comparable in both groups to pre-IM. However, during the 2nd IM phase, the highest IGF-1 concentrations were found in HINCL cows, varying between 166.93 and 302.9 ng/mL; IGF-1 varied in HCOMP cows from 105.1 to 147.2 ng/mL.



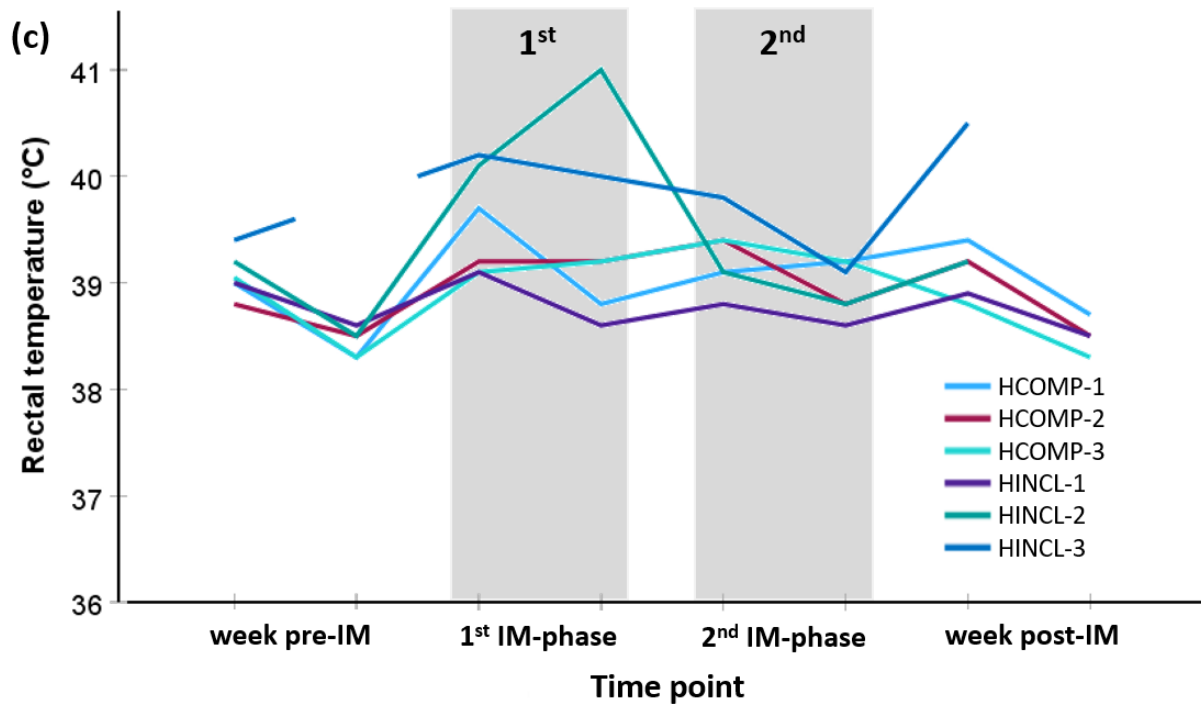


Figure 1. (a) Temperature-Humidity-Index (THI) during the experiments, with the solid line indicating a THI of 62 and the dotted line representing THI of 68. The effect of incomplete milking (IM) on (b) breath rate (breaths/min) and (c) rectal temperature (°C) of cows in peak-lactation, compared to conventionally milked control cows: 1 wk pre-IM, throughout IM (1st and 2nd phase) and 1 wk post-IM. Two treatments (TRT): complete milked cows 1-3 (HCOMP 1-3, n = 3), incomplete milked cows 1-3 (HINCL 1-3, n = 3), in different colours (indicated in the legend). The IM of HINCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. Treatment periods are shaded in grey.

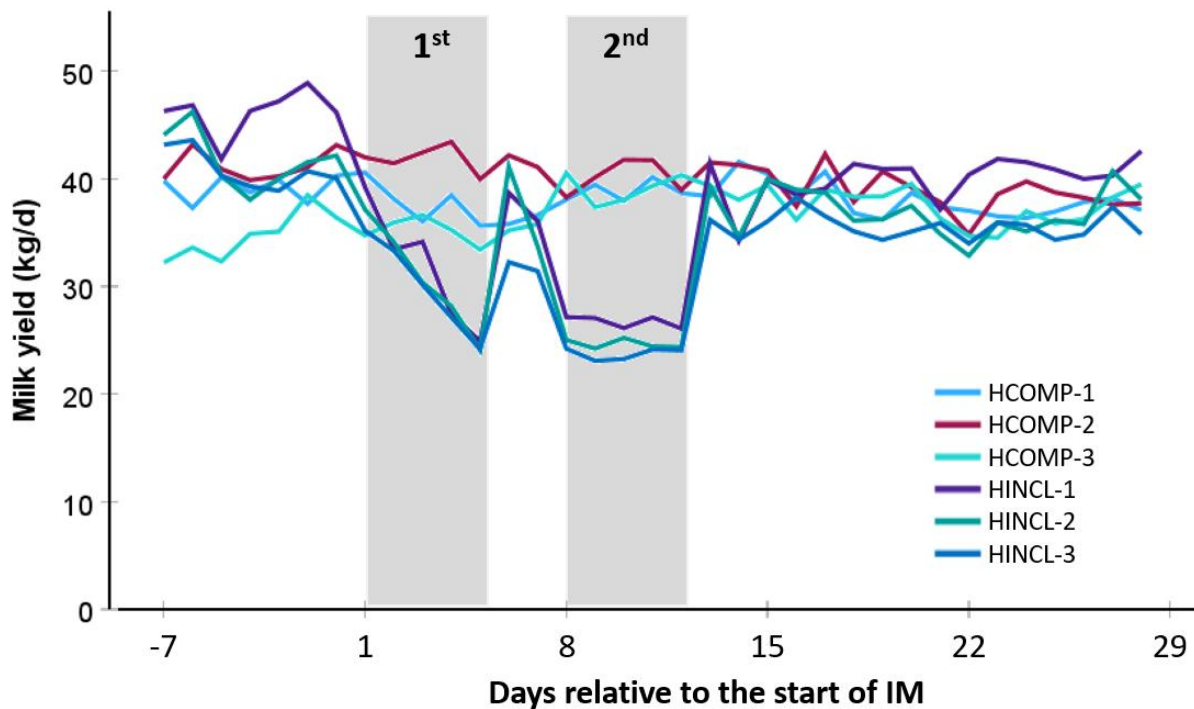


Figure 2. Effect of incomplete milking (IM), on daily milk yield (kg/d) of cows in peak-lactation, compared to conventionally milked control cows: 7 d before start of IM, throughout IM (1st and 2nd phase) and 14 d after IM. Two treatments (TRT): complete milked cows 1-3 (HCOMP 1-3, n = 3), incomplete milked cows 1-3 (HINCL 1-3, n = 3), in different colours (indicated in the legend). The IM of HINCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. Treatment periods are shaded in grey.

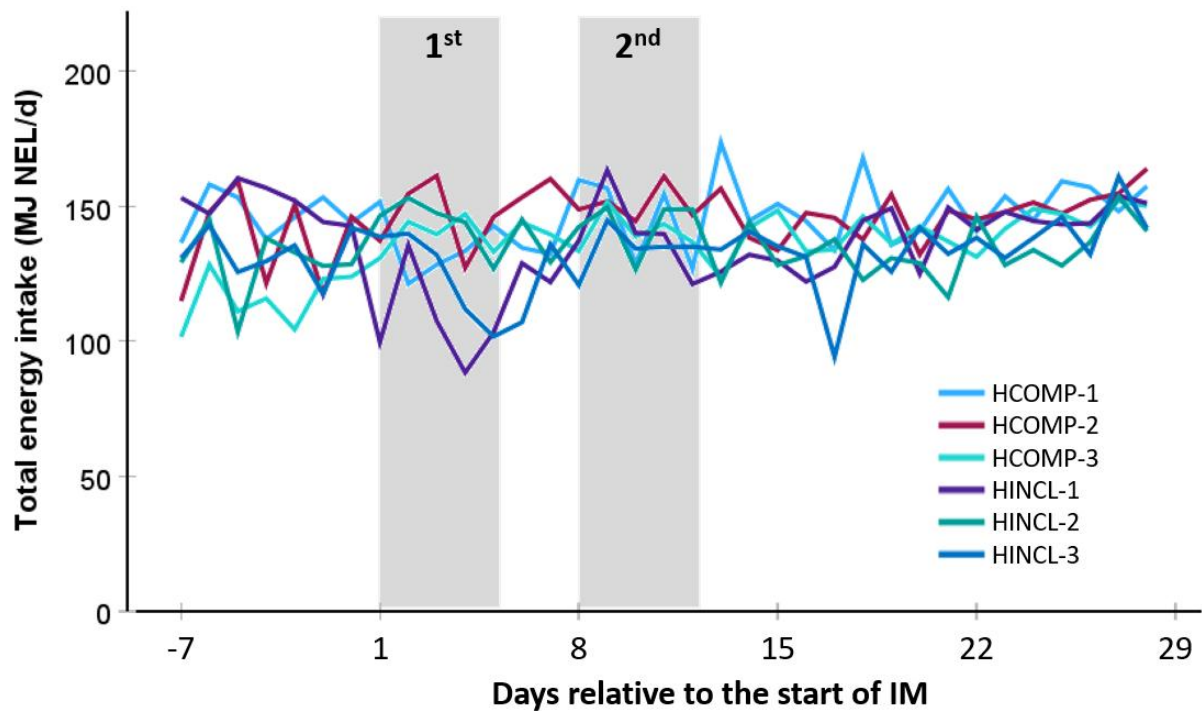


Figure 3. Effect of incomplete milking (IM) on daily total energy intake (MJ NEL/d) of cows in peak-lactation, compared with conventionally milked control cows: 7 d before start of IM, throughout IM (1st and 2nd phase) and 14 d after IM. Two treatments (TRT): complete milked cows 1-3 (HCOMP 1-3, n = 3), incomplete milked cows 1-3 (HINCL 1-3, n = 3), in different colours (indicated in the legend). The IM of HINCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. Treatment periods are shaded in grey.

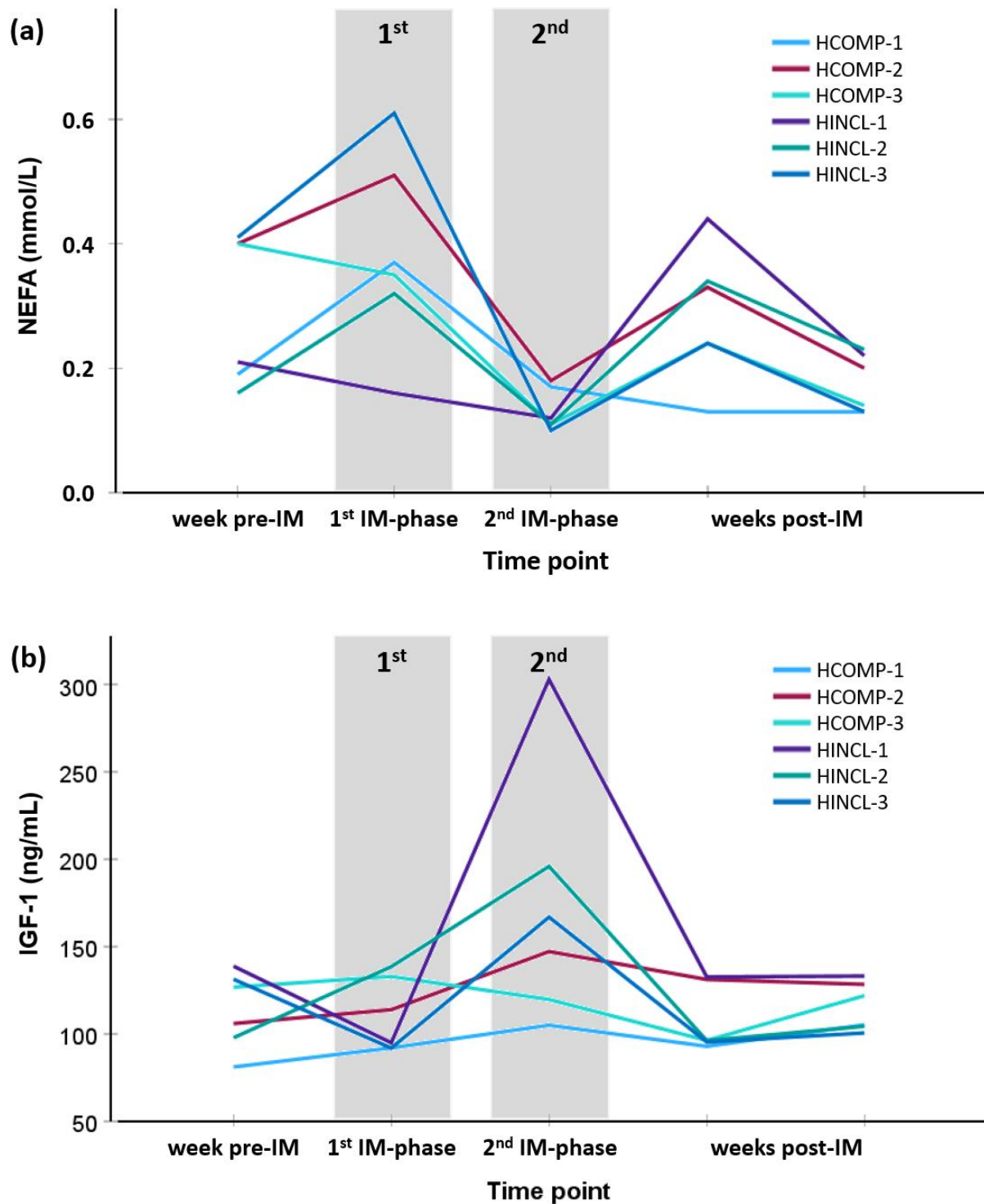


Figure 4. Effect of incomplete milking (IM) on blood serum concentrations of (a) non-esterified fatty acids (NEFA; mmol/L) and (b) IGF-1 (ng/mL) of cows in peak-lactation compared with conventionally milked control cows: 1 wk pre-IM, throughout IM (1st and 2nd phase) and 2 wk post-IM. Two treatments (TRT): complete milked cows 1-3 (HCOMP 1-3, n = 3), incomplete milked cows 1-3 (HINCL 1-3, n = 3), in different colours (indicated in the legend). The

IM of HINCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. Treatment periods are shaded in grey.

The aim of this pilot study was to implement the IM protocol in heat-stressed dairy cows in peak-lactation and to investigate the impact of the IM protocol on the milk yield, energy intake, and selected metabolic parameters. Considering the reduced lactation-induced metabolic load observed in cows milked with the IM protocol in Joest et al. (2025), we hypothesized that IM in heat-stressed dairy cows would reduce metabolic stress. This pilot study was designed to investigate the initial effects of IM in dairy cows under heat stress.

However, the informative value of this pilot study on the use of IM to mitigate heat stress and its negative effects in dairy cows is limited by the small sample size and by the unexpected change of the weather conditions during the experiments. As a result, no severe heat stress was present during IM. Moreover, the selected cows were potentially affected by BTV. Measuring of BTV antigens or antibodies would have provided additional insights into the occurrence of BTV infections.

Overall, this pilot study on the further use of IM in dairy cows is limited by the factors mentioned above, making it impossible to provide a valid answer to the targeted research question. Future research should focus on applying IM during periods of severe heat stress, although practical implementation may be challenging, as it was in the current study. To achieve this, both, the IM protocol and the study design should be adapted to the prevailing conditions. Using heatable rooms or electric blankets might help to achieve controlled heat conditions. Research is required to evaluate the extent to which the IM protocol can support dairy cows during heat stress and other metabolic challenges, such as infections.

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6. General Discussion

This doctoral thesis aimed to implement and investigate the effects of automated short-term IM on German Holstein dairy cows in peak- to mid-lactation. We hypothesized, that IM would affect the lactation-induced metabolic load, without negatively affecting udder health, animal welfare, or long-term milk production. Therefore, we applied automated short-term IM with a gradually reduced milk withdrawal of 9 %/d for 5.5 d (resulting in app. 40% decreased milk withdrawal in total) and consistently 40% reduced milk withdrawal over another 5.5 d. The effects of IM on the milk yield trend, milk composition, as well as NEFA and IGF-1 concentrations in serum were analyzed to assess the effects of IM on the energy demand for milk production and the lactation-induced metabolic load. Further, to estimate the effects of IM on animal welfare, udder health indicators, udder firmness, HRV, and behavior recordings were analyzed. In addition, the IM protocol was tested in heat-stressed dairy cows in a pilot study to assess whether IM can reduce the negative effects of heat stress.

6.1 Effect of incomplete milking on milk production

The genetic predisposition for milk production in all mammals ensures species survival. In cattle, the relatively mature state of the newborn (calves) at birth results in a high milk demand early in life and a corresponding high milk yield of the dam. In modern dairy cows, high milk yields have been further enhanced through selective breeding and genetic selection, increasing the nutrient requirements of the calf several-fold. Milk production is determined by multiple factors, including genetics, nutrition, and environmental conditions (Gross, 2023). The shape of the lactation curve – the chronic change in milk yield over time – is driven by the metabolic activity and number of secretory mammary epithelial cells (Akers, 2017), as well as nutrient supply to the mammary gland (Gross, 2023; Zeng et al., 2024). Milk production is genetically programmed to provide nutrition for the offspring, while milk withdrawal by the calf or milking machine also further shapes the production of milk. Low withdrawal levels and high milk production result in high amounts of residual milk and high udder pressure, representing a local feedback factor at the udder level (Stelwagen et al., 2013; Albaaj et al., 2018). Further, residual milk might activate auto-paracrine processes leading to adaption of the milk production to the current requirements (Weaver and Hernandez, 2016). Consequently, IM can function as a local feedback factor, with its impact depending on the IM intensity and on the plasticity of the mammary gland, which allows milk production to adapt to changing conditions on a local level (Weaver and Hernandez, 2016; Deacon et al., 2023). This plasticity is mediated

by changes in epithelial cell activity and number, influenced by lactation stage, health, and genetic background (Capuco et al., 2003; Boutinaud et al., 2019). Plasticity can be described through resistance (tolerance to disturbance while maintaining function) and resilience (capacity to recover post-disturbance), both driven by local adaptive responses and may also describe the ability to adapt to environmental constraints (Friggens et al., 2010). Furthermore, prolactin (**PRL**) as a systemic factor has also been shown to be modulated by local feedback factors, thereby integrating both local and systemic mechanisms in the regulation of milk yield (Lacasse et al., 2019).

In the present study, the IM protocol successfully reduced milk withdrawal by 43% at the end of the 1st and throughout the 2nd IM phase. The IM protocol resulted in increased udder filling and udder firmness, indicating increased intra-mammary pressure and representing a local feedback-factor shaping the milk yield. The protocol included milking-out (**MO**) periods of three milking times to assess the effects of each IM phase separately. In future applications, shorter milking-out periods to relieve udder pressure without confounding effects on milk yield may be applied. Further, the duration and degree of reduction may be adjusted and may distinguish from the present one. Using the present IM protocol, in which the normal milking frequency of twice per day is maintained, PRL is released from the pituitary gland in response to the nervous stimulation of the udder and supports maintaining lactation and milk yield (Lacasse et al., 2016). In contrast, a reduced milking frequency lowers PRL release due to decreased udder stimulation and increases residual milk as well as intramammary pressure as a result of less frequent milk withdrawal, thereby leading to reduced milk secretion (Bruckmaier and Blum, 1996; Stelwagen et al., 2013). Next to milking frequency, milk accumulation varies depending on milking interval length and degree of milk removal. Increasing the milking frequency and shortening the corresponding milking interval led to less milk accumulated in the udder and has been shown to increase milk production (Wall and McFadden, 2012; Akers, 2017). Thus, the amount of residual milk is strongly affecting the milk yield (Albaaj et al., 2018). In the present study, the daily milk yields pre-IM varied from 26 to 59 kg/d, which might have affected the milk yield responses. However, the milk yield pre-IM was highly correlated with the milk yield after IM. Short-term IM reduced the milk yield by 23% (1st phase) and 37% (2nd phase), with ongoing IM further decreasing production, consistent with earlier studies (Penry et al., 2016; Kuehnl et al., 2017; Deacon et al., 2023). Lactation stage also modulates the response to IM, as mammary gland resistance and metabolic priority decline over the course of lactation (Baumann and Currie, 1980, Boutinaud et al., 2019).

An effect of the IM protocol on the teat tissue is also likely. Martin et al. (2020) reported shortened milking time and altered internal teat morphology during IM, potentially strengthening the teat canal as a defense barrier. Further, Reinemann and Mein (2018) highlighted the benefits of frequent milking in maintaining clean teat canals with low bacterial count at the teat end and reducing infection rates during lactation compared to the dry period. However, frequent milk leakage reported in this study suggested impaired teat sphincter function under elevated intramammary pressure. The dogma that residual milk is directly leading to mammary infections, persists and complete milking of cows is thus still recommended (Cording et al., 2013). Challenging this dogma, the SCC in foremilk samples remained low during IM and its milk accumulation, indicating good udder health. Besides the milk components presented in Manuscript 1, the SCC in whole milk samples was additionally measured at the same occasions by flow cytometry (Labor- und Dienstleistungs GmbH & Co KG, Krefeld, Germany). During the MO, the 2nd and 3rd complete milking after each IM phase are included. During the MO after the 1st IM phase, the SCC in whole milk samples of INCL cows was $111 \pm 22.9 \times 10^3$ cells/mL, thus elevated by 396% compared to COMP cows ($P = 0.014$). Further, during the MO after the 2nd IM phase, the SCC in whole milk samples of INCL cows was $132 \pm 22.2 \times 10^3$ cells/mL, thus elevated by 452% compared to COMP cows ($P = 0.02$). During all other occasions, the SCC in whole milk samples remained well under the threshold of 100×10^3 cells/mL for healthy udders (DVG, 2009). The SCC in whole milk samples was still elevated in INCL cows compared to COMP cows on the first occasion after the MO of the 2nd IM Phase (COMP: $21 \pm 7.2 \times 10^3$ cells/mL; INCL: $51 \pm 7.7 \times 10^3$ cells/mL; $P = 0.02$); thereafter, no differences between treatments were evident (Fig. 3).

During a single milking, the SCC decreases with the onset of milking and increases with ongoing duration, with highest values at the end of milking (Herve et al., 2017). Also, the milking process itself induce mammary epithelia cell (MEC) exfoliation through oxytocin induced mechanic force on the alveoli, detectable during milking (Herve et al., 2018). It can be hypothesized that due to IM, SCC-rich alveolar milk is only partly extracted, leading to potential SCC accumulation in the udder. Further, mechanic forces of udder pressure could induce the same effect on MEC as oxytocin, leading to tissue damage and higher MEC cells in milk, contributing to high SCC concentration found. Thereby, the number of MEC and their metabolic activity determines the milk yield (Capuco et al., 2003), with disruption of mammary epithelium leading to less proportion of apoptotic cells in milk, indicating involuntary loss of functional MEC due

to forces on the mammary epithelium (Herve et al., 2018). Disruption of the mammary epithelium in the present study can be assumed by increasing SCC in whole milk samples beside stable good udder health and reduced milk lactose concentration.

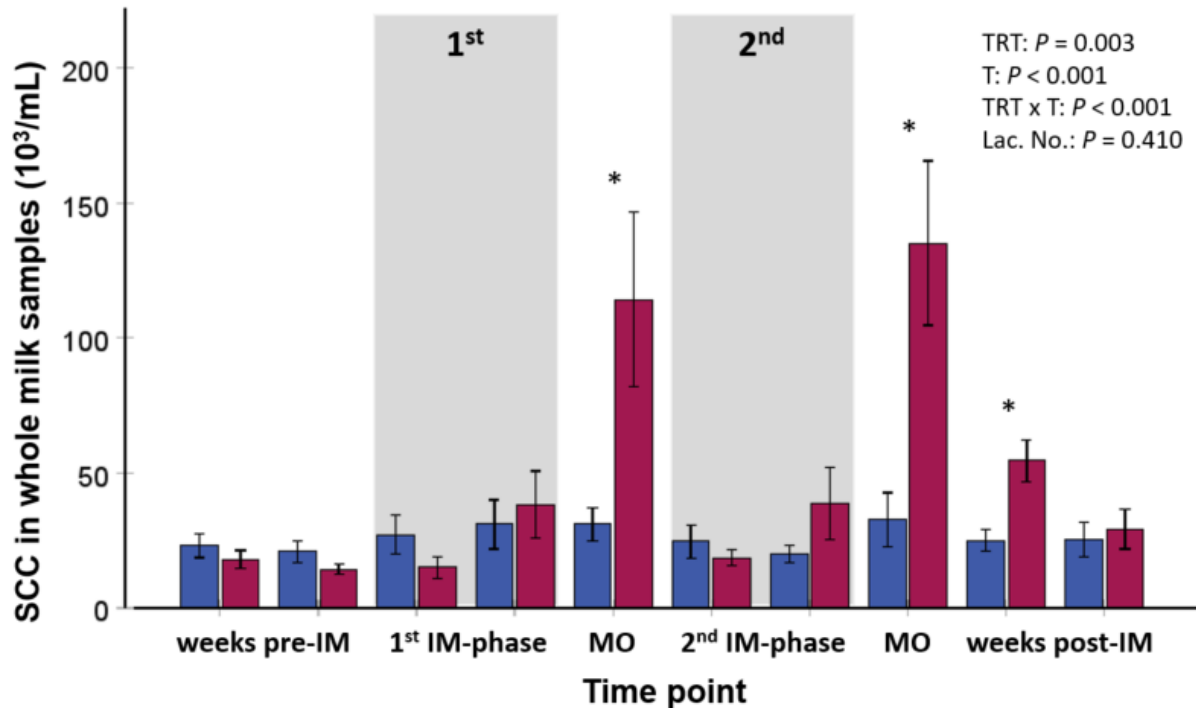


Figure 3. Effect of incomplete milking (IM) on the somatic cell count (SCC) in whole milk samples of cows in peak- to mid-lactation, compared with conventionally milked control cows: 2 wk pre-IM, throughout IM (1st and 2nd phase), on each first d after the 1st and 2nd IM phase (milking out, MO) and 2 wk post-IM. Two treatments (TRT): blue bars = completely milked cows (COMP, $n = 23$); red bars = incompletely milked cows (INCL, $n = 23$). IM of INCL cows was based on their individual milk yield levels in the wk prior to IM initiation. In the 1st IM phase, milk withdrawal (kg) was reduced by 9% per d over 5.5 d, resulting in a total reduction of ~40%. In the 2nd IM phase, milk withdrawal (kg) remained consistently reduced by 40% for additional 5.5 d. Treatment periods are shaded in grey. Data are presented as means \pm SEM, with differences between treatments at a specific time point (T) indicated by an asterisk ($*P \leq 0.05$). Interactions between treatment and time point are represented as TRT x T and the influence of lactation number as Lac. No.

Elevated blood lactose and reduced milk lactose observed in IM studies support impaired tight junction integrity and efflux between the compartments blood and milk (Stelwagen, 2001; Albaaj et al., 2018). Additional reasons for reduced lactose concentration as reaction to a local feedback factor such as IM might be less expression of enzymes regulating lactose synthesis (Grala et al., 2011) and lower mammary uptake of glucose, and therefore less lactose synthesis (Guinard-Flament et al., 2007). Nevertheless, reduced tight junction (TJ) integrity

and reduced effectiveness of the blood-milk barrier leads to exchange between these two compartments (Wellnitz and Bruckmaier, 2021). Applying different milking intervals, Stelwagen et al. (2008) reported reduced rate of milk secretion as the lactose concentration in blood increased, indicating milk yield regulation by TJ integrity. Further, Albaaj et al. (2018) applied different complete milking degrees (0%, 40%, 60%, 100%) and reported highest sensitivity to milk accumulation, i.e., highest milk loss, in cows showing the earliest increase in blood lactose and therefore earliest loss of TJ integrity. Thereby, internal udder pressure and TJ integrity is modulated by individual udder capacity and ability for milk storage (Stelwagen, 2001). Residual milk induced udder pressure is most likely one major factor affecting short-term milk production adaption to IM, with lactose being the mediator. Kobayashi et al. (2020) demonstrated in an in-vitro model the importance of TJ integrity by reporting the consequences of disrupted TJ on cellular milk synthesis. They reported reduced expression of certain genes, e.g., signal transducer and activator of transcription 5 (STAT-5), and activity of milk synthesis related enzymes. Moreover, lactose influenced milk synthesis in a dose related manner, when present at the basolateral side of the mammary epithelium membrane, further underpinning the important role of lactose in acute milk production regulation and the importance of the TJ integrity for milk production. This is also shown by the affected synthesis of milk components, with reduced protein, fat and lactose yields during IM (Deacon et al., 2023).

However, changes in milk fat concentration observed in INCL cows may have resulted from harvesting different proportion of milk fractions, since the fat concentration varies between different milk fractions (Ontsouka et al., 2003; Rico et al., 2014). Also, modest changes in the milk fatty acid profile were reported during a single milking (Rico et al., 2014). Altered milk fatty acid concentrations can affect milk processing properties (Leitner et al., 2013) and the analysis of milk fatty acid profiles should therefore to be considered in future IM studies.

Overall, milk production is largely self-regulated at the level of the mammary gland, involving mechanical feedback, auto-, paracrine signaling, and systemic influences such as PRL, serotonin, parathormone-related protein, growth hormone, IGF-1, and thyroid hormones (Capuco et al., 2001; Weaver and Hernandez, 2016; Lacasse et al., 2019). In our study, reduced milk yield during the 2nd IM phase and in the days following IM may have been amplified by decreased concentrate allocation under a feed-to-yield system. With resumption of complete milking and increased concentrate supply, the milk yield returned to pre-IM levels. In summary, IM apparently acts on local feedback factors reducing milk production through intramammary pressure, disrupting TJ integrity and likely altered epithelial activity, but the exact mechanisms by which IM affects milk production remain unknown. While this may relieve the metabolic

load by reducing energy demands for milk synthesis, high residual milk can compromise udder physiology and animal welfare.

6.2 Incomplete milking: Animal welfare perspective

The IM procedure reduces milk production depending on IM intensity and animal characteristics. Since high milk yields are only possible in healthy cows (Gross, 2023), and the link between high production and disease remains under investigation (Fleischer et al., 2001; Gross, 2024), permanent IM is not necessary. Instead, IM may be applied temporarily to support health under specific conditions. However, interventions designed to improve health should not introduce stress. To evaluate potential implications of IM, we assessed welfare indicators including udder health, udder firmness, HRV, and behavior records.

Experience from the field underline the importance of complete milking to maintain milk yield, and also as a tool to cure udder infections by removing pathogens. This assumption leads to reservation about the IM method, as the residual milk may introduce udder infections (Bach et al., 2022). In contrast, our study found no increase in SCC in foremilk samples during IM and no clinical mastitis was observed, in line with previous studies applying different IM intensities in different lactation stages (e.g., Müller et al., 2023; Mayer et al., 2024).

Udder firmness, a reliable non-invasive proxy for intramammary pressure (Bertulat et al., 2012; Blau et al., 2019), increased in IM cows, reflecting residual milk accumulation rather than infection. Similar results were reported during once daily milking (**ODM**; Tucker et al., 2007) and with extended milking intervals (Blau et al., 2019), and have been associated with discomfort (Bertulat et al., 2013). Thus, for applying IM as a tool to support animal health, evaluating the effects of IM on animal welfare is essential. Our short-term IM protocol reduced milk withdrawal by up to 43%. While this reduced the metabolic load of the dairy cows, high amount of residual milk could induce stress. Stress may not always cause visible welfare impairments, but stress can be a threat to which the organism must adapt, resulting in physiological and behavioral adaptations (von Borell, 2001). A validated measure of the sympathovagal balance and stress in cattle is the HRV (von Borell et al., 2007; Kovács et al., 2014). The RR-intervals were recorded between morning and evening milkings to calculate HRV indices to assess chronic stress responses to the increased milk accumulation and udder firmness, as discussed at the beginning of this chapter. We found no significant deviations in HRV indices, with stable heart rate (**HR**) and vagal activity, indicating no stress in response to IM. Our study is, to our knowledge, the first to examine HRV in response to IM over this time frame. Of note,

HRV comparisons remain difficult due to methodological and farm-level variation (Frei et al., 2022). Previous research investigating the milking process itself has shown improved vagal tone during milking in both herringbone (Kézér et al., 2015) and rotary milking system (Kovács et al., 2019), and only slightly reduced HRV in automatic milking systems (Gygax et al., 2008), when compared to baseline values. In this regard, the effect of oxytocin on HRV was discussed (Kovács et al., 2019). Future IM studies should focus on the HRV during milking, particularly at the moment of earlier cluster removal, when milk withdrawal is terminated, to further investigate the effect on the welfare of dairy cows. Generally, HRV measurements of dairy cows under practical field conditions remain challenging. High artefact rates can be attributed to dry or shifted electrodes, a known issue when using elastic belts (Mohr et al., 2002; Hagen et al., 2005; Gygax et al., 2008). These artefacts may contribute to the significant variation in HRV between individuals (Oishi et al., 2018), as also noted in the present study. Further, physical activity plays a crucial role in the analysis of HRV under free-moving conditions (Kézér et al., 2017; Oishi et al., 2018) and consideration of physical activity to correct the HRV was not possible in this study. In accordance with that, Kézér et al. (2017) identified various factors influencing HRV in dairy cows: lying time, size of the farm and activity, with larger-scale farms and activity leading to higher HR and sympathetic activity. Similarly, HRV measurements after parturition showed greater sympathetic dominance in cows with inflammatory conditions, resulting in decreased HRV (Huang et al., 2024). Despite the reduced metabolic load associated with IM in the present study (Joest et al., 2025), no improved HRV due to IM was present.

For a broader perspective, behavioral recordings complemented HRV data, since these are sensitive indicators to detect changes in welfare (Weary et al., 2009; Gleerup et al., 2015). No avoidance or defense behaviors were recorded in the parlor and no differences in behavior recordings in the barn or brush use between treatments. This suggests no adverse welfare effects, although the sensitivity to detect subtle IM-related stress may be limited. Future studies should include additional stress indicators such as measuring cortisol or cortisol metabolites, as they are well-established for assessing stress (Rutherford, 2002) and may be useful for evaluating potential stress associated with IM. Various sample types can be utilized for assessing glucocorticoids, including feces (Idris et al., 2024), saliva (Giaretta et al., 2023), milk (Gellerich et al., 2015) and hair (Grelet et al., 2022), while assessing the glucocorticoid metabolites in feces together in combination with other physiological and behavioral measures, is suggested to be the most promising approach to evaluate chronic stress (Morrow et al., 2002). Previous work shows variable glucocorticoid responses to udder pressure (Tucker et al., 2007; Bertulat

et al., 2013), highlighting multifactorial influences on the glucocorticoid concentrations (Putman et al., 2018; Cattaneo et al., 2023). However, the precise impact of different degrees of elevated udder pressure on the cow's welfare remains unclear. Assuming only low effect sizes of IM on behavior records and high standard deviations, the statistical power of the present study is low considering the relatively small number of cows included in each variable for animal welfare assessment. In future studies, a higher number of observations is needed to truly examine the effects of IM on animal welfare. Furthermore, artificial intelligent (AI)-supported behavior assessment might (i) increase the number of evaluated behaviors, especially behaviors patterns, and (ii) increase the number of observed individuals and number of observations per animal, increasing detection of small welfare effects. Taken together, our results suggest that short-term IM does not impair welfare in clinically healthy cows during peak- to mid-lactation, considering the welfare indicators assessed herein. However, the effects of IM on animal welfare under metabolic or heat stress conditions remain unclear.

6.3 Incomplete milking as a tool to support animal health

Under suboptimal conditions – such as heat stress, insufficient energy intake, or infections – high milk production can impose a burden on the cow. This arises from the limited availability of key resources (e.g., glucose, amino acids) and the competition between the mammary gland and other essential functions, including immunity, reproduction, and body reserves. The risk of health disorders and culling increases with higher milk yield (Rilanto et al., 2020), and high metabolic load may contribute to production diseases (McArt and Neves, 2020; Macmillan et al., 2021). Nevertheless, persistently high milk yields are only achievable in healthy cows, indicating that high production does not inherently constitute a burden (Gross, 2023). During lactation, milk synthesis may demand more than four times the energy required for maintenance (own data; NRC, 2001). Thus, reducing milk production can conserve energy, when the possibility to modulate milk yield is feasible. Milk yield can be adjusted through ODM (Stelwagen et al., 2013), endocrine interventions such as PRL inhibitors (Lacasse et al., 2019), or IM (Chapter 6.1). In the present study, the IM protocol reduced the milk yield by 23% during the 1st IM phase (54.6 kg less milk harvested) and 37% during the 2nd IM phase (90 kg less), relative to baseline production (40.2 kg/d). Assuming no changes in milk composition or post-IM yield and a milk price of 0.50 €/kg (German average, first half of 2025), the estimated economic loss per cow was 27.3 € in the 1st IM phase and 45 € in the 2nd IM phase. Reduced concentrate consumption during and shortly after IM must also be considered, as it offsets part

of these costs. To justify IM for practical use, its value under different challenging conditions for the dairy cows requires further evaluation. Particularly, the economic assessment of potential welfare and health benefits remains a key challenge.

6.3.1 Incomplete milking as a tool to support animal health in early lactation

During the transition period, dairy cows face low energy intake and high energy demands to support both milk production and immune activation associated with parturition (Bradford et al., 2015), resulting in a high risk of disease. Ketosis can develop when energy deficits trigger excessive fat mobilization, leading to the accumulation of ketone bodies in blood (McArt et al., 2015). Beyond direct health effects, metabolic disorders such as ketosis reduce milk yield, impair reproduction, increase veterinary costs, and elevate the culling risk (McArt et al., 2013). To mitigate these risks, Morin et al. (2018) restricted milk withdrawal to 10 - 14 kg/d during the first 5 d p. p., reporting lower ketonemia and reduced hyperketonemia prevalence. Meyer et al. (2024) applied constant milk withdrawal of the milk yield at d 7 p. p. for 14 d, resulting in 11.1% lower milk withdrawal in IM cows but no detectable effects on NEB or metabolic parameters. Valdecabre et al. (2022) used IM to improve mineral balance, limiting withdrawal to 3 L per milking during the first two d p. p., observing slightly higher plasma Ca concentrations. Collectively, these findings suggest that IM can reduce both energy and mineral requirements in early lactation, thereby supporting metabolic health during the transition period. Elevated production increases metabolic load and the risk of production-related disorders (Rilanto et al., 2020). Thereby, genetic correlations indicate an unfavorable association between milk yield and disease susceptibility (Koeck et al., 2014). Cows experiencing NEB and high NEFA concentrations in early lactation show immunosuppression, such as reduced peripheral blood mononuclear cells (**PBMC**) proliferation and diminished oxidative burst of polymorphonuclear leukocytes, making them more vulnerable to infections (Vernacker et al., 2019). Elevated NEFA also impair PBMC proliferation and cytokine secretion (Vanacker et al., 2022). Such increases occur not only in early lactation (Bruckmaier and Gross, 2019) but also under insufficient feed supply (Zbinden et al., 2017; Vernacker et al., 2019).

In the present study, the IM protocol reduced NEFA concentrations, despite already low baseline values, suggesting potentially greater benefits under higher NEFA concentrations. Additionally, increased IGF-1 concentrations indicate that IM may support energy and protein

balance (Wathes et al., 2021). These findings underline the potential of IM to improve metabolic health by lowering milk production demands, highlighting possible applications for managing production-related disorders in the dairy industry.

6.3.2 Incomplete milking as a tool to support animal health during insufficient nutrient intake

Insufficient nutrient intake can result from low dietary energy density (Zbinden et al., 2017), restricted digestive capacity (Gross et al., 2022), cytokine-induced appetite suppression during inflammation (Kuhla, 2020), a sharp rise in milk yield (Bruckmaier and Gross, 2017), and also feed scarcity due to climate change (Gauly and Ammer, 2020). Reduced intake has been linked to health disorders such as ketosis and lameness across lactation stages, with greater impact in multiparous cows (Gonzalez et al., 2008; Siberski-Cooper et al., 2023). Mastitis, milk fever, ketosis, and lameness are associated with reduced feed intake and milk yield, with an estimated loss of 1.94 kg milk per kg decrease in DMI (Bareille et al., 2003). Dairy cows in energy deficit show impaired immunity and higher disease susceptibility (Ster et al., 2012; Gross, 2023), with high-yielding cows that prioritize milk production metabolically being at higher risk (Gross, 2023). Glucocorticoids, released during stress, further suppress feed intake, PRL, and milk yield (Ponchon et al., 2017; Ma et al., 2023). Increasing the energy density of the ration by increasing the proportion of starch-based concentrates in dairy diets must be restricted (Humer et al., 2018), as adequate rumen activity requires sufficient physically effective fiber with lower energy content (NRC, 2001; GfE, 2024).

Nutrient shortages also arise when feed quality and quantity do not meet individual energy requirements. Climate change exacerbates this by altering feed availability and quality, with effects varying by region, system, and year (Gauly and Ammer, 2020). In hot and dry areas, lower crude protein and digestible organic matter have been reported (Craine et al., 2010). At the same time, modern high-yielding dairy breeds require optimal nutrition to sustain milk output. Ration of only high-quality forage supports ~30 kg milk/day, and higher yields are associated with elevated NEFA and reduced IGF-1, indicating metabolic stress under inadequate feeding (Kolver et al., 2003; Zbinden et al., 2017). Feeding strategies to deal with climate change induced insufficient feeding include supplementation with saturated fatty acids (Wang et al., 2010), functional oils (Ghizzi et al., 2018), or vitamins (Zimbelmann et al., 2010). Furthermore, the rising global demands for food are expected to limit the availability of concen-

trates and cereals for dairy cow nutrition due to increasing competition with human consumption (Eisler et al., 2014). Consequently, modern dairy cow nutrition must develop strategies to formulate adequate rations that meet the milk yield–driven demands of dairy cows, for example by replacing human-edible fractions with high-quality by-products (Karlsson et al., 2018). In addition, climate-driven increases in infectious disease risk (Vercruyssen et al., 2018) will further challenge dairy production, underscoring the need for preventive strategies.

Regulation of the energy demand for milk production through IM may alleviate metabolic stress during insufficient nutrient intake, as milk production requires large amounts of glucose and amino acids – nutrients that are limited in dairy cows (Gross, 2022). In our study, IM reduced NEFA and increased IGF-1, indicating metabolic relief. Lower milk withdrawal reduced concentrate allocation under the feed-to-yield system, resulting in decreased total DMI. This adjustment-aligned energy intake with reduced requirements, prevent overfeeding and reinforce metabolic relief, as evident by the favorable NEFA and IGF-1 responses. The PMR intake remained unaffected, raising the question of whether IM influences appetite. Feed intake is driven by gut capacity and the metabolic demand (Albornoz et al., 2023). Since IM reduces energy requirements for milk synthesis, this may lower intake. Moreover, high concentrate proportions often reduce PMR DMI (Allen, 2023), while reduced concentrate allocation may have stimulated PMR intake, explaining the stable PMR consumption observed. Thus, effects of IM on appetite require further studies.

Another strategy to reduce milk production is PRL inhibition using dopamine agonists. Prolactin inhibition has been shown to temporarily reduce milk yield during different lactation stages, alleviate metabolic perturbations, and mitigate immunosuppression during nutritional stress (Ollier et al., 2014, 2016; Lacasse et al., 2019). For example, feed-restricted mid-lactation cows treated with PRL inhibitors experienced less severe NEB, lower NEFA and BHB, and higher glucose serum concentration without compromising long-term productivity. At dry-off, PRL inhibition reduced new intramammary infections, reflecting improved immune status (Ollier et al., 2015). However, the method requires repeated injections (e.g., quinagolide twice daily for 4 d; Ollier et al., 2016), raising welfare concerns. The effects of PRL inhibition are comparable to IM, which also reduces milk yield and alleviates metabolic stress (Carbennau et al., 2012; Martin et al., 2020). In studies with similar DIM, IM induced milk yield reductions are equal to or greater than those observed with PRL inhibition, alongside favorable metabolic responses (lower NEFA and higher IGF-1 serum concentrations). Hence, IM offers a practical, non-pharmacological and non-invasive alternative with similar benefits. Overall, it can be hypothesized, that IM may help cows to cope with heat stress and insufficient nutrient supply by

reducing metabolic load, supporting immune function, and lowering the risk of secondary diseases, but further studies are needed. Secondary diseases may occur as a direct result of another, primary diseases or stressor. For instance, heat stress results in suppressed innate immune function, which increase the risk of e.g., metritis or mastitis, as secondary diseases (Jingar et al., 2014; Steele, 2016). As a response, they can reduce milk yield and reproductive performance, increase veterinary costs, and impair animal welfare (Becker et al., 2020).

6.3.3 Incomplete milking as a tool to support animal health during heat stress

Climate change, characterized by global warming and increasingly frequent weather extremes, poses major risks to ecosystems, food security, and livestock production. Dairy farming is particularly vulnerable, as rising average temperatures and changes in rainfall distribution challenge both feed production and animal performance (Hammami et al., 2013; Horton et al., 2016). Climate change-related increases in hot days, average temperatures, and heat waves will dramatically elevate the number of heat stress days in dairy cows and challenge agricultural production (IPCC, 2014). Feeding strategies aim to counteract reduced DMI during heat stress by increasing dietary energy density, for instance through fat supplementation (West, 2003; Staples, 2007). These strategies are still limited by the reduced DMI of dairy cows during heat stress (Spiers et al., 2004). Thereby, heat stress can affect cows at all lactation stages, reducing feed intake (Bernabucci et al., 2014), increasing metabolic maintenance requirements (NRC, 2001; Baumgard and Rhoads, 2013), imposing additional demands for immune system activation (Baumgard and Rhoads, 2013), and finally also elevating morbidity and mortality (Laporta and Skibel, 2024). Reduced feed intake, combined with immune activation and altered post-absorptive metabolism, drives lower milk yield during heat stress (Wheelock et al., 2010; Baumgard and Rhoads, 2013). Retrospective studies confirm that elevated THI correlates with increased NEB (Moore et al., 2024); thus, the higher the THI, the higher the metabolic stress.

Moreover, high producing dairy cows are more sensitive to heat stress (Becker et al., 2020), and milk yield losses occur at a lower temperature threshold in high-producing cows compared with lower-yielding cows (Berman, 2005). As a primary response, dairy cows reduce DMI to decrease metabolic heat production (Becker et al., 2020), and alterations of the post-absorptive metabolism occur (Baumgard and Rhoads, 2013), with carbohydrates are preferentially utilized in peripheral tissues of dairy cows (Baumgard and Rhoads, 2012). Consequently, insufficient nutrient supply to the mammary gland restricts the production of large amounts of

milk (Rhoads et al., 2009). Therefore, dairy cows adapt their milk yield to heat stress to a certain extent. However, despite reduced milk yield, dairy cows most certainly enter a state of NEB during heat stress (Shwartz et al., 2009). Moreover, selection for greater genetic milk production potential has increased metabolic heat production as well as the cows' sensitivity to environmental stress (Collier et al., 2012; Gauly and Ammer, 2020; Shephard and Maloney, 2023). Under controlled severe heat stress, milk yield consistently declines, with cows in mid-lactation (with the highest milk yield) suffering the greatest losses (Tao et al., 2020). Milk yield losses of early-lactation cows were less pronounced (Tao et al., 2020). It can be hypothesized, that dairy cows in early lactation may suffer more severely from heat stress, due to already existing NEB and impaired immune status (Lamp et al., 2015; Chen et al., 2024). The higher metabolic prioritization of milk production in early lactation may result in an inadequate adaptation of milk yield to heat stress, thereby increasing health risks (Gernand et al., 2019), and identifying dairy cows in early lactation as a particularly relevant target group for heat stress mitigation strategies. By reducing milk production, IM may lower the metabolic heat load, facilitating thermoregulation, and reduce metabolic stress in dairy cows during heat stress.

High NEFA and low glucose serum concentrations are observed during the hottest hours of the day, while insulin concentrations rise, lowering circulating carbohydrate and lipid metabolite concentrations in serum (Shehab-El-Deen et al., 2010; Baumgard and Rhoads, 2013). Further, insulin becomes uncoupled from glucose concentrations as a further consequence of heat stress (Wheelock et al., 2010). Heat stress triggers whole-body protein breakdown to meet immune and inflammatory demands (Rius, 2019). Moreover, reduced microbial protein synthesis in the rumen during heat stress further promotes muscle catabolism, as indicated by increased plasma 3-methylhistidine (Kamiya et al., 2006; Gao et al., 2017). Glucose and amino acids are limiting substrates used in vast amounts under heat stress for both, milk production and the immune system (Gross, 2022). Therefore, IM may act as a glucose- and amino acid-sparing strategy. By lowering milk yield, IM reallocates glucose and amino acids, which can be used for immune functions (Kvidera et al., 2017). This effect is also supported by increased IGF-1 serum concentrations during IM, which is positively associated with energy and protein balance as well as immunity and functional traits (Wathes et al., 2021).

Our IM protocol may have further reduced metabolic heat load. Combined with the reduced milk yield, concentrate allocations were adjusted. As DMI declined in IM cows and digesting and processing nutrients generates metabolic heat, this may have further reduced the heat load in IM cows (Baumgard and Rhoads, 2013). Importantly, high concentrate diets under heat stress can promote selective feeding, reduce rumen pH, and induce rumen acidosis (Miller-

Cushon et al., 2019; Neubauer et al., 2020). Thus, reduced concentrate allocation during IM may provide an additional protective effect.

The useful primary effect of IM is to temporarily reduce milk production – when needed. In this study, IM reduced milk yield by 23% during the 1st IM phase and 37% during the 2nd IM phase, relative to the COMP cows. The effect was intensified with prolonged and more frequent IM. Even after IM phases, reduced yield was evident, suggesting carryover effects. As climate change increases the frequency of heat waves, the question arises whether IM should be applied repeatedly. Repeated use could amplify the effects of IM, though timing, lactation stage, and seasonal acclimatization to heat stress must be considered. Cows require 2 - 7 weeks to adapt biochemically and morphologically to heat stress (Blackshaw and Blackshaw, 1994; Collier et al., 2019; Shephard and Maloney, 2023). Thus, the first heat wave of the year may be more harmful than later ones. This emphasizes the need to refine IM recommendations according to acclimatization status.

To reduce heat stress and its negative effects on the dairy cow, a range of mitigation strategies – management, housing, feeding, and breeding – have been proposed (Gauly and Ammer, 2020). In this study, we proposed and discussed IM as a potential future mitigation strategy to reduce heat stress. Moreover, we tested it in a pilot study. Due to the small number of cows and the likely confounding effects of BTV infections, the expected metabolic responses observed in the first study were not evident in the pilot study. Moreover, the implementation of IM during heat stress based on the weather forecast was not fully successful, as severe heat stress was not present during the IM application. Future studies with larger cohorts and extended analyses are needed to fully characterize the effects of IM on heat stressed dairy cows and to validate its potential as a tool to enhance animal welfare under stressful conditions.

The studies included in this doctoral thesis describe the successful implementation of automated short-term IM in dairy cows in peak- to mid-lactation. Various measurements were used for a broad analysis of the effects of the IM protocol. The results of the studies indicate short-term adaption of milk yield and energy demand towards reduced lactation-induced metabolic load. More comprehensive analyses in serum and also in milk and other tissues involved in energy metabolism would provide a deeper understanding of the IM-induced effects. However, the IM protocol did not compromise udder health or animal welfare based on the indicators used, although milk leakage was more frequent and udder firmness was increased. Alternative approaches are needed to deepen our understanding of the effects of IM on the welfare

of high-yielding dairy cows, as this study includes, to our knowledge, the only assessments of the effects of IM during peak- to mid-lactation on animal welfare. Additional assessment of glucocorticoids may provide valuable further insights. The current studies focused on the implementation of IM and the general effects. Application of IM during stressful conditions (NEB/insufficient nutrition, heat stress, metabolic disorders, lameness) as discussed in this thesis, might be a suitable management tool to help the cow to cope with these challenges, besides, analysis of direct use of IM will be necessary. Notably, the pilot study represented an initial effort to test the IM protocol under stressful conditions, such as heat stress. However, due to the unsuccessful implementation during severe heat stress, unintended interference of BTV infection and the small sample size, the study could not provide a valid answer to the targeted research question. The key question remains when to initiate IM in the context of metabolic disorders, heat stress, or general NEB. The most promising strategy is a preventive application, starting before the onset of stress. However, this requires early detection, which is challenging since many diseases develop subclinical without clear physiological manifestations (e.g., ketosis; Berge and Vertenten, 2014). Similarly, fixed thresholds for thermal indices are unsuitable (Choi et al., 2024), and the benefits of IM must be clearly demonstrated. Data from precision livestock farming (**PLF**), combined with information from milk mid-infrared (**MIR**) spectrometry analyses (Benedet et al., 2019; Costa et al., 2024), may provide valuable inputs for AI-based decision systems to identify when IM should be applied. Such software-supported IM protocols could offer dairy farmers a practical and rapid tool to preserve and improve cow health and welfare, although the optimal timing of implementation remains to be defined as well as the IM intensity.

7. Summary

The evolutionary-based genetic predisposition of dairy cows for milk production has been further enhanced by genetic selection and modern breeding programs, making lactation a central physiological function. High milk production, however, requires substantial energy input and consequently imposes a significant lactation-induced metabolic load. Although high productivity is only achievable in healthy cows, the immense demand for energy and nutrients can strain the animal and increase susceptibility to various diseases. Therefore, the burden associated with high milk yield cannot be overlooked, as dairy cows remain prone to metabolic stress even in later lactation stages, particularly when exposed to additional stressors such as heat stress or insufficient feeding.

In this context, incomplete milking (IM) offers the opportunity to temporarily reduce milk yield and alleviate the metabolic load of individual cows. Fundamental principles of IM were already defined in the early 20th century: intentionally leaving residual milk in the udder by shortening milking duration reduces milk yield. The extent of this reduction depends on the intensity and duration of IM, as well as the lactation phase and other cow-individual factors. High milk volumes stored in the udder, independent of storage time, influence the synthesis of milk likely through auto- and paracrine signaling. Using the milk yield reducing effect, IM has been strategically applied to address key challenges in dairy production. Applying IM has improved the energy balance during early lactation, reduced the risk of milk fever by increasing blood calcium concentrations, and improved the dry-off process by reducing the milk yield. Further, the general effects have been explored in experiments using a half-udder design over extended periods. Despite previous research on IM before dry off and early lactation, its application during peak- to mid- lactation is less well studied. Moreover, its potential to improve animal welfare during periods of high milk output and stress remains under-investigated.

In order to establish IM as a potential management strategy in peak- to mid-lactation, comprehensive knowledge of its effects and potential limitations is essential. Therefore, the aim of this study was to investigate the effects of a short-term IM protocol on the dairy cow using a software module that allowed precise control of milk withdrawal. The experiments included in this dissertation focused on three main aspects: (i) to implement yield-dependent IM in dairy cows in peak- to mid-lactation using a software module for automated earlier cluster removal; (ii) to limit the amount of milk withdrawal by up to 40% to directly reduce the milk production, thereby reducing the energy requirements for milk production and the lactation-induced metabolic load without causing a long-term reduction in milk yield; (iii) furthermore, the assessment of animal welfare indicators to deepen the understanding of the effects of IM on dairy cows. The application of IM in dairy cows can only be justified if both udder health and overall animal welfare are not compromised by IM and the associated increase in residual milk, which can potentially result in high udder pressure and discomfort. The direct consideration of animal welfare is also essential to ensure the social license to operate.

To explore the effects and potential limitations of the designed IM procedure, the animal experiment included 46 multiparous German Holstein dairy cows in peak- to mid-lactation, showing no clinical signs of mastitis and having negative bacteriology results for all quarter samples, confirming udder health. Eligible cows were randomly assigned to either the complete milking treatment (COMP; $n = 23$, 95 ± 49 DIM, mean lactation number 3.0 ± 1.3) or the

incomplete milking treatment (INCL; $n = 23$, 93 ± 46 DIM, mean lactation number 2.6 ± 0.8), with stratification by milk yield and milk fat content. For the INCL cows, IM was implemented using a software module enabling cluster removal after a predefined amount of milk (kg) was collected, rather than based on the milk flow rate (kg/min). The IM protocol included a gradual reduction phase (first IM phase: 9% daily reduction to ~40% over 5.5 d), followed by 3 complete milkings and a constant reduction phase (second IM phase: 40% reduction over 5.5 d). The IM protocol was divided into two phases to separately evaluate the effect of the first IM phase on milk production. The gradual reduction of the first IM phase was considered a more welfare-friendly approach for the animals compared to an abrupt method (directly inducing 40% IM). In parallel, the COMP cows were milked completely using cluster removal depending on the milk flow rate (<0.3 kg/min) during the entire experimental period. Further, the evaluation of udder health, milk composition, feed intake, and selected metabolic parameters in serum (NEFA and IGF-1) in both groups was complemented by the assessment of animal welfare indicators such as udder health, udder firmness, heart-rate variability, and behavior recordings. Concentrate was provided in a restricted manner according to individual milk yield and DIM with limited daily changes via separate feeding stations. In addition to controlling milk withdrawal, concentrate allocation based on the feed-to-yield system was also part of the study design for the INCL cows, as it meets the modified requirements. In this way, concentrate allocation and intake followed the trend in milk yield. Additionally, a pilot study was carried out using $n = 3$ cows in both groups, aiming to gain first insights into the effects of the IM protocol on heat-stressed cows. Of note, the explanatory power of the pilot study is limited by the small number of animals and the potential confounding effects of BTV infections.

Compared with the milk yield prior to IM (varying between 26 and 59 kg/d in INCL cows), the milk yield of INCL cows decreased with ongoing IM duration and increasing IM intensity during the IM application. The milk yield was still reduced shortly after cessation of IM, but with ongoing complete milking, the milk yield of INCL cows reached the level of the COMP cows' milk yield. Since few IM studies exist and these differ in lactation stage, milk yield level, and IM duration and intensity, the comparison of the effects of the IM protocol of the present study with other experiments can hardly be done. Regarding udder health while being milked incompletely, udder health indicators remained stable, as also reported in other studies using the software module for controlled IM in early lactation and before dry-off. Despite this, udder firmness increased in INCL cows during the IM treatment. Increased udder firmness and udder pressure have been associated with stress in dairy cows. Therefore, the risk of IM being a stressor can be raised. In contrast, all assessed animal welfare indicators showed no differences between treatments, indicating no adverse effects of the IM protocol on the welfare of dairy cows as an important requirement to use IM as a management strategy.

In this context, the effects of IM on the milk yield trend, milk composition, and NEFA and IGF-1 concentration were analyzed to assess the effect on the energy demand for milk production and the lactation-induced metabolic load. Moreover, during IM, the concentrate intake was reduced in INCL cows due to the feed-to-yield system, reflecting lower energy requirements. Highlighting the effectiveness of the IM protocol, the NEFA concentration decreased during both IM phases, indicating reduced lipid mobilization. Concurrently, IGF-1 concentrations increased during both phases, indicating improved energy balance due to a reduced

energy demand. As the changes in these metabolic parameters are related to more general aspects of metabolism, further metabolic adaptations to IM are to be expected. Nevertheless, further studies are needed using IM during actually challenging times such as heat stress or insufficient nutrition, to confirm the effects. In this regard, a pilot study aiming to gain first insights into the effects of the IM protocol on heat-stressed cows was restricted by the difficult implementation of the IM protocol during heat stress, the limited number of animals, and potential confounding effects of BTV infections. The expected metabolic responses observed in the first study were not evident in the pilot study. Moreover, its potential to improve animal welfare during periods of high milk output and additional stressors (e.g., heat stress or insufficient feeding) requires further analysis, including the assessment of cortisol levels and extended HRV measurements, which might help to comprehensively investigate potential stress responses associated with IM.

Our results show that automated short-term IM up to 40% performed on dairy cows in peak- to mid-lactation using specialized software was successful in temporarily reducing milk production and lactation-induced metabolic load without negatively affecting udder health or long-term performance. Thereby, the IM protocol increased the residual milk significantly without affecting animal welfare, considering the indicators used in this study. These fundamental results support IM as a management strategy. Future applications of the IM strategy might include using variable durations of the gradual and constant IM periods, as well as shorter milking-out periods, to only reduce udder pressure and avoid inflammation without confounding effects on milk yield. Although healthy cows in established lactation may not require metabolic relief, individual cows during periods of high milk output may benefit from IM when challenged by additional stressors such as heat stress or insufficient feeding, since the IM protocol temporarily reduces milk yield and the corresponding lactation-induced metabolic load. For upcoming research activities, the results presented in this doctoral thesis provide fundamental insights into the effects of short-term IM on milk production, metabolism, and animal welfare in dairy cows during peak- to mid-lactation, and will support to design future studies aiming to investigate IM as a tool to help cows cope with metabolic stressors.

8. Zusammenfassung

Die evolutionsbedingte genetische Disposition von Milchkühen für die Milchproduktion wurde durch genetische Selektion und moderne Zuchtprogramme weiter verstärkt, wodurch die Laktation eine zentrale physiologische Funktion einnimmt. Eine hohe Milchproduktion ist mit einem erheblichen Energieaufwand verbunden und führt folglich zu einer ausgeprägten, laktationsbedingten metabolischen Belastung. Obwohl eine hohe Produktivität nur von gesunden Kühen realisierbar ist, können der immense Bedarf an Energie und Nährstoffen das Tier belasten und die Anfälligkeit für verschiedene Erkrankungen erhöhen. Daher darf die Belastung, die mit einer hohen Milchleistung einhergeht, nicht außer Acht gelassen werden. Somit sind Milchkühe auch in späteren Laktationsstadien anfällig für metabolischen Stress – insbesondere, wenn zusätzliche Stressoren wie Hitzestress oder eine unzureichender Futtermittellieferung hinzukommen.

Vor diesem Hintergrund bietet das Incomplete Milking (IM; unvollständiges Melken) die Möglichkeit, die Milchleistung zeitweise zu reduzieren und dadurch die laktationsbedingte metabolische Belastung einzelner Kühe zu verringern. Grundprinzipien des IM wurden bereits zu Beginn des 20. Jahrhunderts beschrieben: Durch das absichtliche Belassen von erhöhten Restmilchmengen im Euter mittels verkürzter Melkdauer lässt sich die Milchleistung reduzieren. Das Ausmaß dieser Leistungsreduktion hängt von der Intensität und Dauer des IM sowie von der Laktationsphase und individuellen tierbezogenen Faktoren ab. Große im Euter verbleibende Restmilchmengen beeinflussen die Milchbildung, vermutlich über auto- und parakrine Mechanismen. Durch Nutzung dieser Milchleistung mindernden Effekte wurde IM strategisch zur Bewältigung von Herausforderungen der Milchviehwirtschaft eingesetzt. So verbesserte IM die Energiebilanz in der Früh-laktation, verringerte das Risiko für Milchfieber durch die Erhöhung der Blutkalziumkonzentration und erleichterte den Trockenstellprozess durch Reduktion der Milchleistung. Darüber hinaus wurden generelle Effekte des IM über längere Zeiträume mit Halb-Euter-Design Studien untersucht. Trotz bisheriger Forschung zum IM vor dem Trockenstellen und zu Beginn der Laktation ist der Einsatz in der Hoch- bis Mittellaktation bislang weniger gut untersucht. Zudem ist das Potenzial vom IM zur Verbesserung des Tierwohls während Phasen hoher Milchleistung und zusätzlicher Belastungen bislang weitgehend unerforscht.

Um IM als potenzielle Managementstrategie in der Hoch- bis Mittellaktation zu etablieren, ist ein umfassendes Verständnis der Effekte und potenziellen Limitationen erforderlich. Ziel der vorliegenden Studie war daher die Untersuchung der Auswirkungen eines kurzfristigen IM-Protokolls bei Milchkühen, welches unter Verwendung eines Softwaremoduls angewendet wurde, das eine präzise Steuerung der Milchabgabe ermöglicht. Die im Rahmen der Dissertation durchgeführten Experimente konzentrierten sich auf drei Hauptaspekte: (i) die Implementierung eines leistungsabhängigen IM bei Milchkühen in der Hoch- bis Mittellaktation mittels eines Softwaremoduls zur automatisierten früheren Abnahme des Melkzeugs; (ii) eine Begrenzung der entnommenen Milchmenge um bis zu 40 % um die Milchleistung unmittelbar zu reduzieren und somit den Energiebedarf für die Milchproduktion sowie die laktationsinduzierte metabolische Belastung zu verringern, ohne eine langfristige Reduktion der Milchleistung zu verursachen; sowie (iii) die Erhebungen von Tierwohlindikatoren, um das Verständnis der Auswirkungen von dem IM auf Milchkühe zu vertiefen. Das IM bei Milchkühen kann nur angewandt werden, wenn sowohl die Eutergesundheit als auch das allgemeine Tierwohl durch IM und die damit verbundene höhere Restmilchmenge – mit potenziell erhöhtem Euterdruck und Unbehagen – nicht beeinträchtigt werden. Die direkte Berücksichtigung vom Tierwohl ist zugleich essenziell, um die gesellschaftliche Akzeptanz der Anwendung zu sichern.

Um diese Aspekte zu untersuchen, umfasste das Experiment 46 mehrkalbige Deutsche Holstein Milchkühe in der Hoch- bis Mittellaktation, die keine klinischen Anzeichen einer Mastitis aufwiesen und bei denen für alle Viertelproben negative bakteriologische Befunde vorlagen, was eine intakte Eutergesundheit bestätigte. Die geeigneten Tiere wurden zufällig entweder dem vollständigen Melken (COMP; $n = 23$, 95 ± 49 Laktationstage, mittlere Laktationsnummer $3,0 \pm 1,3$) oder dem unvollständigen Melken (INCL; $n = 23$, 93 ± 46 Laktationstage, mittlere Laktationsnummer $2,6 \pm 0,8$) zugeteilt, wobei eine Stratifikation nach Milchleistung und Milchfettgehalt erfolgte. Bei den INCL-Kühen wurde das IM Protokoll mittels eines Softwaremoduls umgesetzt, welches die Abnahme des Melkzeugs nach Erreichen einer vordefinierten Milchmenge (kg) ermöglichte, anstatt – wie üblich – auf Grundlage der Milchflussrate (kg/min). Das IM-Protokoll bestand aus einer Phase mit gradueller Reduktion (erste IM-Phase: tägliche Reduktion um 9 % auf ~40 % über 5,5 Tage), gefolgt von drei vollständigen Melkungen, und einer Phase mit konstanter Reduktion (zweite IM-Phase: 40 % Reduktion über weitere 5,5 Tage). Das Protokoll wurde in zwei Phasen unterteilt, um den Effekt der ersten IM-Phase auf die Milchproduktion separat bewerten zu können. Die graduelle Reduktion wurde als mehr tierwohlgerechter Ansatz im Vergleich zu einem abrupten Übergang (sofortige Reduktion auf 40 %) angesehen. Parallel dazu wurden die COMP-Kühe während des gesamten Versuchszeitraums vollständig gemolken, wobei die Abnahme des Melkzeugs anhand der Milchflussrate ($< 0,3$ kg/min) erfolgte. Darüber hinaus wurden in beiden Gruppen die Milchezusammensetzung, die Futteraufnahme sowie ausgewählte metabolische Parameter im Serum (NEFA und IGF-1 Konzentration) untersucht und durch die Erfassung weiterer Tierwohlindikatoren wie die Eutergesundheit, Euterfestigkeit, Herzfrequenzvariabilität und Verhaltensbeobachtungen ergänzt. Kraftfutter wurde in begrenztem Umfang gemäß individueller Milchleistung und Laktationsstadium über separate Futterstationen bereitgestellt, wobei tägliche Änderungen limitiert waren. Neben der Kontrolle der Milchabgabe war die kraftfutterbasierte Fütterung nach dem „feed-to-yield“-System ebenfalls Teil des Studiendesigns bei den INCL-Kühen, wobei die Kraftfutteraufnahme dem Trend der Milchleistung folgte und so dem modifizierten Bedarf entsprach. Zusätzlich wurde eine Pilotstudie mit jeweils $n = 3$ Kühen in beiden Gruppen durchgeführt, um erste Erkenntnisse zu den Effekten des IM-Protokolls bei hitzestressierten Kühen zu gewinnen. Die Aussagekraft der Pilotstudie ist durch die begrenzte Tieranzahl sowie den potenziellen Störeinfluss von BTV-Infektionen eingeschränkt.

Im Vergleich zur Milchleistung vor Beginn der IM-Anwendung (zwischen 26 und 59 kg/Tag bei den INCL-Kühen) sank die Milchleistung der INCL-Kühe mit zunehmender Dauer und steigender Intensität des IM. Kurz nach Beendigung des IM war die Milchleistung weiterhin reduziert, näherte sich jedoch mit fortlaufendem vollständigem Melken wieder dem Niveau der COMP-Kühe an. Da nur wenige Studien zum IM existieren und diese sich hinsichtlich Laktationsstadium, Milchleistung der Versuchstiere sowie Dauer und Intensität der IM-Protokolle unterscheiden, ist ein direkter Vergleich der Effekte des hier verwendeten IM-Protokolls auf die Milchleistung mit anderen Untersuchungen kaum möglich. Bezüglich der Eutergesundheit während des unvollständigen Melkens blieben die entsprechenden Eutergesundheits-Indikatoren stabil, was auch mit Ergebnissen anderer Studien übereinstimmt, in denen das Software-Modul für das kontrollierte IM in der Früh-laktation bzw. vor dem Trockenstellen eingesetzt wurde. Die Euterfestigkeit nahm während des IM zu. Eine erhöhte Euterfestigkeit sowie ein gesteigerter Euterdruck werden mit Stress bei Milchkühen in Verbindung gebracht, sodass ein Risiko besteht, dass IM Stress bei den Milchkühen hervorrufen könnte. Im Gegensatz dazu zeigten jedoch alle erhobenen Tierwohlindikatoren keine Unterschiede zwischen den beiden

Behandlungen, was darauf hinweist, dass sich IM – unter den Bedingungen dieses Studiendesigns – nicht nachteilig auf das Wohlergehen der Kühe auswirkte und damit eine wesentliche Voraussetzung für seine Anwendung als Managementstrategie erfüllt ist.

In diesem Kontext wurden die Effekte des IM auf den Verlauf der Milchleistung, die Milchzusammensetzung sowie die Konzentrationen von NEFA und IGF-1 im Serum analysiert, um den Einfluss auf den Energiebedarf für die Milchproduktion und die laktationsbedingte Stoffwechselbelastung zu bewerten. Weiterhin wurde während der IM-Phasen bei den INCL-Kühen die Kraftfutteraufnahme aufgrund des „feed-to-yield“-Systems reduziert, was den geringeren Energiebedarf der INCL Kühe widerspiegelt. Die Wirksamkeit des IM-Protokolls zeigte sich insbesondere darin, dass die NEFA-Konzentration während beider IM-Phasen verringert war, was auf eine reduzierte Lipidmobilisierung hinweist. Gleichzeitig stieg die IGF-1-Konzentration während beider IM Phasen an, was eine verbesserte Energiebilanz infolge eines geringeren Energiebedarfs signalisiert. Da die Veränderungen dieser Stoffwechselfparameter mit weitfassenden Vorgängen des Stoffwechsels assoziiert sind, sind weitere metabolische Anpassungen durch das IM zu erwarten. Dennoch sind weiterführende Studien erforderlich, in denen weitere Stoffwechselfparameter untersucht werden und in denen IM unter tatsächlich Stress-Bedingungen wie Hitzestress oder eine unzureichende Futtermittelversorgung angewendet wird, um die beobachteten Effekte zu bestätigen. In diesem Zusammenhang wurde eine Pilotstudie durchgeführt, die erste Einblicke in die Auswirkungen des IM-Protokolls auf hitzestressste Kühe ermöglichen sollte. Die Umsetzung des IM-Protokolls unter Hitzestressbedingungen erwies sich jedoch als schwierig; zudem war die Anzahl an Tieren begrenzt, und potenzielle Störeinflüsse durch BTV-Infektionen konnten nicht ausgeschlossen werden. Die erwarteten metabolischen Reaktionen, die in der ersten Studie beobachtet wurden, traten in der Pilotstudie nicht auf. Somit besteht Bedarf, das Potenzial von IM das Tierwohl während Phasen hoher Milchleistung und zusätzlicher Belastungen (z. B. Hitzestress oder unzureichende Futtermittelversorgung) zu verbessern, in weiteren Studien zu untersuchen. Diese sollten unter anderem die Bestimmung von Cortisolkonzentrationen im Serum sowie erweiterte HRV-Messungen umfassen, um potenzielle Stressreaktionen des IM einhergehen zu untersuchen.

Unsere Ergebnisse zeigen, dass ein kurzzeitiges IM von bis zu 40 %, durchgeführt bei Milchkuhen in der Hoch- bis Mittellaktation mithilfe einer spezialisierten Software, erfolgreich war, um die Milchproduktion und die laktationsbedingte Stoffwechselbelastung vorübergehend zu reduzieren – ohne negative Auswirkungen auf die Eutergesundheit oder die langfristige Milchleistung. Das IM-Protokoll führte dabei zu einer signifikanten Erhöhung der Restmilchmenge, ohne das Tierwohl zu beeinträchtigen, wie die in dieser Studie erhobenen Indikatoren zeigen. Diese grundlegenden Ergebnisse unterstützen den Einsatz von IM als Managementstrategie. Zukünftige Anwendungen des IM könnten eine variable Dauer der graduellen und konstanten IM-Phase sowie ein kürzeres Ausmelken umfassen, um den Euterdruck zu reduzieren und Entzündungen zu vermeiden, ohne den Effekt auf die Milchleistung zu beeinträchtigen. Auch wenn gesunde Kühe während der etablierten Laktation keine Entlastung des Stoffwechsels benötigen, könnten einzelne Tiere in Phasen hoher Milchleistung von IM profitieren – insbesondere dann, wenn zusätzliche Belastungen wie Hitzestress oder eine unzureichende Futtermittelversorgung vorliegen. In solchen Fällen kann IM die Milchleistung und die damit verbundene laktationsinduzierte Stoffwechselbelastung vorübergehend verringern. Für zukünftige Forschungsaktivitäten liefern die in dieser Dissertation dargestellten Ergebnisse wichtige Erkenntnisse zu den Auswirkungen des kurzzeitigen IM auf die Milchproduktion, den Stoffwechsel und das Tierwohl von Milchkuhen. Sie werden künftige Studien zur Nutzung des IM als Managementstrategie zur Reduktion von metabolischem Stress bei Milchkuhen unterstützen.

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