Effects of cow-calf contact on feed intake, milk production and energy balance in dairy cows in early lactation

Effekter på foderintag, mjölkavkastning och energibalans hos mjölkkor som ger di parallellt med att de mjölkas i tidig laktation

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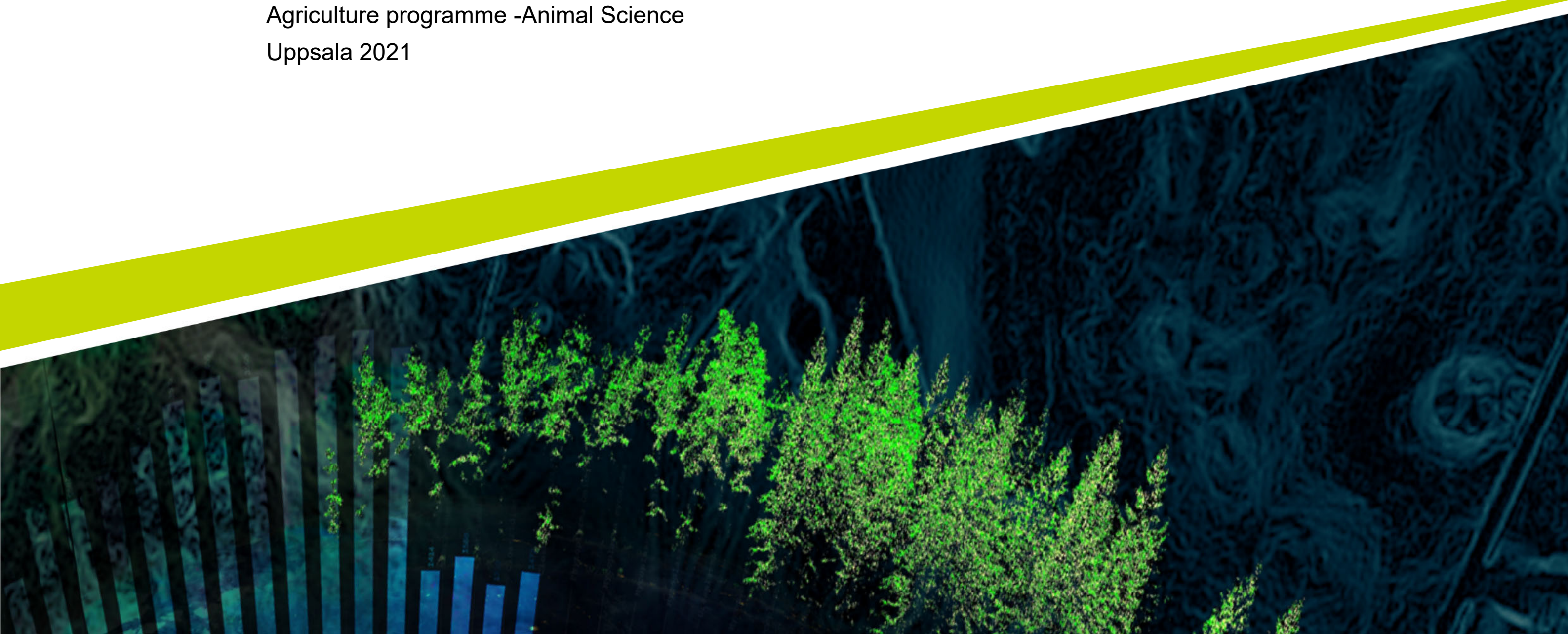

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Abstract

The objective of this study was to compare the production level, feed intake and energy balance in early lactation of non-suckled cows against cows in a cow-driven cow-calf contact (CCC) system. CCC systems have become interesting to evaluate as they are thought to provide a more natural behaviour for both the cow and calf, several studies have reported higher growth and better health in calves kept in these systems. Reports on how the cows' production and health are affected are fewer with varying results and often dependent on the type of contact system, type of feed and feeding management. There is also a need to evaluate potential difference in milking techniques, as the majority of reports are based on conventional machine milking and not from automatic milking systems (AMS). The production level on a farm is highly dependent on the animal's welfare and a high feed intake supporting high production and energy status, therefor, the relationship between these factors are interesting to evaluate in a whole day contact system.

Twenty-two treatment and nineteen control cows in different parities (12 vs. 6 first parity, 4 vs. 3 second parity, 6 vs. 7 older cows in treatment and control groups respectively), were included in the study between 4 and 50 days in milk (DIM). Daily observations of feed intake, feeding behaviour, milk yield from an automatic milking system (AMS) and body condition score (BCS) was collected, milk samples were taken every fortnight for analysing milk composition and blood samples were taken twice a week during the first two weeks postpartum for evaluation of non-esterified fatty acids. All cows were feed *ad libitum* of roughage from automatic feeders which enabled continuous recordings of the time spent eating and amount of eaten feed for each individual. Number of meals per day, visit duration, meal size, feeding rate and dry matter intake (DMI) was compared in order to study the feeding patterns between the two treatments. The results suggest that feed intake was similar in both treatments, while the treatment group had a more efficient feeding pattern. Treatment cows had fewer visits per day, longer meal durations, bigger meals, higher feeding rates and longer intervals between feedings. Harvested milk yield was significantly lower in the treatment group due to calf's milk intake, however, when estimating the energy corrected milk (ECM) (based on the energy mobilization) both treatments had a similar milk production. The BCS was more stable in the treatment group compared to the control cows, also non-esterified fatty acids (NEFA) values were lower in this group, indicating the that the control group was less able to adapt their feed intake to the lactation during this period. The study period included in this thesis was short and occurred during early lactation, therefor it will be important for future studies to investigate the entire lactation to confirm these findings and get a better picture of the cow's performance in these whole day CCC systems.

Keywords: Cow-calf contact system, dairy cow, suckling, feed intake, feeding pattern, milk production, energy balance

Sammanfattning

Syftet med denna studie var att jämföra produktionsnivån, foderintaget samt energibalansen i tidig laktation mellan kor i ett ko-kalvsystem med kor som enbart mjölkas. Intresset att utvärdera ko-kalvsystem har ökat då systemen anses ge möjlighet till ett mer naturligt beteende hos både ko och kalv, exempelvis vid digivning. Flera studier har rapporterat om högre tillväxt och förbättrad hälsa hos kalvar som vistats tillsammans med modern och haft möjlighet att dia (Bar-Peled *et al.* 1997; Meyer *et al.* 2006; Johnsen *et al.* 2016), däremot är de rapporterade effekterna på kons produktion få och ofta varierande då det finns flera aspekter som kan skilja sig åt i dessa system. Produktionsnivån på en mjölkgård är i hög grad beroende av en god djurhälsa och ett högt foderintag som stimulerar en hög mjölkavkastning utan att påverka energibalansen, därmed är det av intresse att undersöka dessa aspekter i ett ko-kalvsystem.

I studien inkluderades 22 behandlingskor och 19 kontrollkor i varierande laktationsnummer (12 vs. 6 förstakalvare, 4 vs. 3 andrakalvare, 6 vs. 7 äldre kor i behandlings- respektive kontrollgruppen), studien fortgick från dag fyra i laktationen till och med dag femtio. Dagliga observationer av foderintag, foderintagsmönster, mjölkavkastning från ett automatiskt mjölkningssystem (AMS) och hull samlades in tillsammans med mjölkprover varannan vecka samt blodprover som togs under fyra tillfällen första och andra veckan efter kalvning. Samtliga kor hade fri tillgång på grovfoder från automatiska fodertråg, dessa möjliggjorde att kontinuerliga data på foderintag kunde samlas in under hela försöket. Antal måltider per dag, besökstid, storleken på måltiden, äthastighet samt intaget av foder jämfördes mellan behandlingarna för att studera eventuella skillnader i foderintagsbeteendet. Resultatet tyder på att foderintaget var snarlikt i båda behandlingarna, dock antyder resultaten att behandlingskorna hade ett mer effektivt foderbeetende. Behandlingsgruppen åt färre måltider per dag, målen varade under en längre tid, de hade ett längre intervall mellan måltiderna hade en högre äthastighet. Mängden mjölk till mjölkningsroboten var signifikant lägre i behandlingsgruppen på grund av kalvarnas mjölkintag. Dock fanns ingen skillnad mellan grupperna i uppskattad energi korrigerad mjölk (ECM) (som baserats på energimobiliseringen). Behandlingskorna hade ett mer stabilt hull vilket bekräftades med lägre nivåer av icke-förestrade fettsyror (NEFA) i blodet jämfört med kontrollkorna, detta indikerar att kontrollkorna kan haft det svårare att anpassa foderintaget efter laktationen vid övergången från dräktighet till laktation. Studiens tidsperiod var kort och inkluderade endast de första sju veckorna i laktationen, framtida studier bör studera hela laktationen för att bekräfta resultaten i denna studie samt få en tydligare bild av hela laktationen och kornas produktion i ko-kalvsystem.

Nyckelord: ko-kalvsystem, mjölkko, digivning, foderintag, foder beteende, mjölkproduktion, energibalans

”There has been, and still are, very good reasons for separating calves at birth but there are also good reasons to study alternatives to this practice”
(Agenäs, 2017)

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Abbreviations

AMS	Automatic milking system
BCS	Body condition score
BHBA	Beta-hydroxybutyrate acid
CCC	Cow-calf contact
DIM	Days in milk
DMI	Dry matter intake
EB	Energy balance
eECM	Estimated Energy Corrected Milk
mECM	Measured Energy Corrected Milk
NEFA	Non-esterifed fatty acid
NE_L	Net Energy Lactation
SLU	Swedish University of Agricultural Science
VMS	Voluntary Milking System

1. Introduction

Common practise in Swedish dairy farms is to separate the dam and the calf shortly after birth. After separation, calves are usually kept in individual pens or calf hutches, restricting their movement and interactions with other animals. Milk is offered in buckets or through an artificial teat, which enables the calves to suckle. The amount of milk fed is often limited to roughly 10 % of the body weight, to stimulate an earlier intake of solid feeds (Khan *et al.* 2011; Pettersson *et al.* 2001). However, calves can consume twice as much milk when it is offered *ad libitum*. Studies where calves are fed high milk volumes or *ad libitum* have shown higher growth rates, (Appleby *et al.* 2001; Diaz *et al.* 2001; Jasper & Weary, 2002) improved feed efficiency and reduced incidence of disease (Diaz *et al.* 2001; Khan *et al.* 2011).

There has been a growing interest from consumers and farmers in systems where cows and calves have contact (CCC) and cows are suckled in parallel to being machine milked (Agenäs, 2017; Busch *et al.* 2017). There are different types of contact systems, 1) whole day contact allowing unrestricted contact between the pair, 2) restricted suckling, allows daily contact for suckling only and 3) half day contact allowing contact between the pair either daytime or night-time (Sirovnik *et al.* 2020). Studies investigating the effect of a whole day CCC systems on dairy cows are scarce and often contradictory, especially studies exceeding early lactation. Bar-Peled *et al.* (1995) reported suckled cows to have a lower DMI and a more severe drop in BCS during the suckling period compared to two non-nursing cows. Yet, the suckled cows had the highest milk production when adding the calves milk intake. Johnsen *et al.* (2016) did not find any difference in DMI between cows being suckled and milked three times/d compared to cows only being milked three times/d. Decreases in harvested milk and milk fat during the suckling period have been reported, as an effect of the calf's milk intake (Johnsen *et al.* 2016; Meagher *et al.* 2019). The difference in milk yield have been reported to decrease or disappear post-weaning (review by Krohn *et al.* 2001; Flower & Weary, 2001; de Passillé *et al.* 2008).

However, there is limited information in literature about how CCC affect other breeds than Holstein, like the Swedish Red, and the effect of the cows being milked in AMS instead of a conventional cluster milking system (Johnsen *et al.* 2021). The aim of this study was to further investigate the cows' production when CCC is combined with AMS. For this investigation, the following questions were addressed, 1) will the feed intake or feeding pattern differ from each other depending on treatment, 2) what effects can be found on milk yield and milk composition when CCC cows are milked in a AMS, 3) will the energy balance be lower for cows that are kept in a whole day cow-calf contact system and 4) Do the Swedish Holstein and Swedish Red respond differently to CCC in combination with AMS in early lactation?

2. Literature review

2.1. Feed intake

Feed has a huge effect on the economy in dairy farms, yet a sufficient feed intake is essential to improve the milk production and at the same time keep a good body condition (Grant & Albright, 1995; Goff & Horst, 1997). Lactation curve models can be helpful to predict a cow's milk potential throughout the lactation and is of importance when designing feed rations (Hansen *et al.* 2006). Without sufficient nutrition a dairy cow cannot reach its genetic potential for her milk production (VandeHaar & St-Pierre, 2006).

The feed intake is often referred to as dry matter intake (DMI) which can be affected by a series of factors such as the animal, environment, dietary and management conditions, all of these will have an impact on the physical and metabolic regulation of the feed intake. Dietary factors include NDF content, palatability, nutritional value, and digestibility of the organic matter (Huhtanen *et al.,* 2007; Jensen *et al.,* 2016; Grant & Ferraretto, 2018). Animal factors can be body size and physiological state of the animal, milk production, parity, and days in gestation (Dado & Allen, 1994; Allen, 1996; Zom & Vuuren, 2012; Jensen *et al.,* 2016). Kertz *et al.* (1991) and Dado & Allen (1994) reported that first parity cows have a lower DMI compared to older cows, and that older cows increased their feed intake faster during early lactation.

The feed intake in early lactation is usually too low to meet the energy and protein requirements for the milk production (Bertics *et al.,* 1992; Drackley, 1999). The DMI postpartum is known to vary between individuals and are related to a series of factors as feed properties, DMI during the transitioning period, endocrine status, BCS as well as milk production (Grummer, 1995; Agenäs *et al.,* 2003; Garnsworthy, 2007). During the transition period the DMI decreases the last weeks prior to parturition (Grummer, 1995; Dann *et al.,* 1999; Ingvartsen & Andersen 2000). According to a study by Drackley (1999) the requirement for net energy lactation (NE_L) can exceed the feed intake by 25 % at the fourth day in lactation (DIM). The insufficient DMI limits the available energy sources for the metabolism in the animal during a critical time. Bar-Peled *et al.* (1995) reported cows being machine milked in addition to suckled three times/ d to have the lowest DMI compared to cows only being machine milked three or six times/d, respectively. Yet, these cows had the highest milk production. Both Bar-Peled *et al.* (1995) and Lupoli *et al.* (2001) found higher levels of oxytocin in both cow and calf during suckling compared to cows being machine milked. Oxytocin have previously been reported to affect the appetite control and suppress the voluntary feed intake of rats (Arletti *et al.,* 1989; Olson *et al.,* 1991). Studies examining the cows feed intake and BCS in CCC systems are limited and thereby this study explores new grounds.

2.1.1. Feeding pattern

Just as the mechanism behind the total DMI/ d may differ between cows, the feeding behaviour can also vary between individuals. The feeding behaviour can be affected by housing, management, milking system, feed properties (composition and physical characteristics), social hierarchy and interactions between animals (Sniffen *et al.,* 1993; Grant & Albright, 2001; Azizi *et al.,* 2010). Measuring individual feedings, that account for the social environment, is possible due to automatic recordings of the feed intake. It enables collection of detailed and quantitative data on feeding behaviour of each individual (Nielsen, 1999). Nielsen (1999) highlights the effect that the environment can create and thereby influence the measurements of the feeding behaviour. A cow's feed intake is typically split into a series of feeding events or "meals" throughout the day and separated by nonfeeding intervals. When provided with *ad libitum* feed, dairy cows spend approximately 3 to 5 hours eating, divided into 9-15 meals/ d, separated by 7 to 10 hours of nonfeeding intervals (ruminating) and approximately 30 minutes of drinking water (Forbes, 2007; Grant & Albright, 2000). Forbes (2007) describes a meal as "distinct eating periods, which may include short breaks, but which are separated by longer intervals". The identification of a meal criteria, the difference of an interval between meals or an interval within the meal, can be problematic to distinguish (DeVries *et al.,* 2003). The reason for an interval within a meal can be caused by many reasons, e.g. the cow leaves the feeding area either voluntarily or she is affected by another animal of higher rank, and thereby leaves the feeding area for a couple of minutes and then return. Tolkamp *et al.* (1998) and Langton *et al.* (1995) acknowledges some problematic aspects with the identification of a meal criteria, namely deletion of data before analysis due to the variation in length and the element of subjectivity when the intervals are chosen.

DeVries *et al.* (2003) examined the potential changes in feeding behaviour from early to peak lactation while defining meal criteria and determine the most repeatable feeding behaviours. A total of 21 cows with a milk production of 11 000 ± 2 916 (mean ± SD) kg per lactation were housed together in a free-stall barn, a TMR was feed from a feed alley. DeVries *et al.* (2003) determined the meal criterion by using the log_{10}-transformed frequency distribution. From the distribution two peaks were formed, one corresponding to intervals within a meal and the other one representing the intervals between meals. The distribution revealed where the intervals from the two distributions intersect. According to DeVries *et al.* (2003) the intersect occurred on average at 27.7 minutes, which was used as the pooled criterion. This meant that all visits with less than 27.7 minutes between them accounted for one meal, while if more than 27.7 minutes pasted between visits, the next visit became the start of a new meal. When using a pooled criterion instead of an individual meal criterion from each individual cow might result in loss of detail, since there can be a huge individual variation amongst the cows.

Dado & Allen (1994) studied the feed intake, time spent feeding and the number of meals consumed by six primiparous and six multiparous Holstein cows housed in tie-stalls. When all cows were studied the following results were found, they spent a mean of 301 minutes per day eating divided into 11 meals where one meal or a bout lasted for 28.8 minutes. The results differed slightly when dividing cows into parity. Primiparous cows spent a shorter time eating (284 vs. 314 minutes), had a lower feed intake per meal (1.8 vs. 2.5 kg DM), shorter eating bouts (25.9 vs. 31.1 minutes) and had a higher number of bouts/ d (11.3 vs. 10.8) compared to multiparous cows. The authors pointed out the variation in production, 28.7 kg/d and 37.5 kg/d respectively for primiparous and multiparous, as one of the main reasons behind the results.

2.2. Milk production

Historically, milk production has undergone a huge transformation. Looking back roughly 40 years in Sweden, less than 7 % of the dairy farms remains today. The number of animals used in the milk production is halved, however, the total milk yield has only decreased with approximately 16 % (Swedish Board of Agriculture, 2020). The reason for this is an increase in the average herd size and a genetic selection for a higher yield/ cow. The average annual production is 10 790 kg ECM for a Swedish Holstein and 9 910 kg ECM for Swedish Red (Table 1) (Växa Sverige, 2020). The lactation peak usually occurs at 40 to 60 days postpartum then followed by a daily decrease in milk yield until the cow is dried-off. The length and shape of the lactation curve is or can be affected by season, feed availability, physiological effects, health and BCS (Keown *et al.,* 1986; Garcia & Holmes, 2001; Huxley, 2013). It is this during this critical period, between partum and the lactation peak, that is most cows are affected by a negative energy balance.

The harvested milk yields from whole day CCC systems are often reported to be lower compared to the control cows. According to Lehmann *et al.* (2021) a calf with an average daily gain (ADG) of 1 200 g/d needs approximately 5.9 kg ECM (week 1-3), 9.2 kg ECM (week 4-7), 12.5 kg ECM (week 7-9) and 16.4 kg ECM (week 10-13) to meet the daily energy requirement if only consuming milk (ECM: 4.2 % fat, 3.4 % protein). The amount of milk a calf consume is influenced by the calf's age, the time the cow and calf spent together and milk availability and availability of other feeds and water (Lehmann *et al.* (2021). de Passillé *et al.* (2008) reported lower milk yields in nursing cows compared to cows only being machine milked, however when the calves milk consumption was added to the harvested milk yield no difference in total milk production between control cows and nursed cows was found during the first nine weeks of lactation. Bar-Peled *et al.* (1995) reported cows who were suckled in addition to machine milked three times/d had the highest milk production compared to non-nursing cows being machine milked three respectively 6 times/d. It is hypothesized that the degree of which the udders are emptied is linked to the increase in milk yield.

Table 1. Mean milk yield, fat %, protein % and kg ECM per lactation for SH and SR 2019 (Växa Sverige, 2020).

Breed	Kg milk	Fat %	Protein %	Kg ECM
Swedish Holstein	10 551	4.11	3.52	10 790
Swedish Red	9 245	4.4	3.40	9 910

2.2.1. Increasing milk yield

Throughout the lactation the milk yield changes, it is a result of the intramammary mechanisms, the number of secretory cells and their metabolic activity (Stelwagen, 2001; Murney *et al.,* 2015). Several factors will affect the degree of which the udder is emptied, e.g., feeding and pre-stimulation (Johansson *et al.,* 1999), management and milking personnel (Rushen *et al.,* 1999). A well-studied management method to increase the efficiency in milk production is to increase the milking frequency (Bar-Peled *et al.,* 1995; Erdman & Varner, 1995; Stelwagen, 2001). An increase from twice daily to thrice or more have been shown to increase the milk yield (DePeters *et al.,* 1985; Erdman & Varner, 1995; Stelwagen, 2001; Hale *et al.,* 2003). DePeters *et al.* (1985) found no effect on the milk composition when increasing the milking frequency. Similar were later reported by Bernier-Dodier *et al.* (2010) who studied the effect of increased milking frequency on one of the udder-halves. When comparing the two udder-halves on the same cow the environment as well as genetical factors are minimized. One half being milked once daily was compared to the other half being milked thrice/ d, no effect was found in milk protein concentration, yet due to a higher milk yield in the quarters being milked thrice/ d the total protein yield increased with a higher milking frequency (Bernier-Dodier *et al.,* 2010).

Kuehnl *et al.* (2019) studied the effect of milk production rate when milking frequency increased, twice respectively thrice/ d. Twenty-two multiparous cows were included from 5 DIM and continued until 47 DIM, each cows udder-half was randomly assigned to be incompletely milked (30 % milk remained) and the other half was completely milked. Milk samples were taken twice/ w and resulted in a higher milk production rate for cows being milked three times/d (1.97 ± 0.06 vs. 1.81 ± 0.06 kg milk/h) compared to twice. The difference between the udder-halves revealed a lower milk production rate in the part being incompletely milked (0.80 ± 0.03 vs. 1.09 ± 0.03 kg milk/h). Kuehnl *et al.* (2019) also reported cows with a higher milking frequency increased in milk fat concentration. The higher milk fat in the incomplete milked halves were attributed to the sampling method, milk samples were taken from the cistern after approximately 70 % of the milk fraction was removed, and it is known that milk fat increases during milking (Ontsouka *et al.,* 2003).

According to Murney *et al.* (2015) and Hale *et al.* (2003) secretory cells are stimulated by the increase in milking frequency in early lactation, an increase in activity and proliferation of mammary cells results is a higher milk yield. Milk yield is also known to be influenced by the degree of which the mammary glands are emptied, hence the residual milk. When milk is accumulating in the alveoli, the intramammary pressure increases together with the concentrations of the protein that causes the feedback inhibitor of lactation **(**Stelwagen, 2001). There are many other complex regulatory processes such as loss of and leakage of tight junctions, apoptosis and hormone regulated processes that affects the milk synthesis and the milk production (Stelwagen, 2001; Bernier-Dooier *et al.,* 2010). Serotonin (5-hydroxytryptamine) is reported to be of importance in the mammary gland functions, in milk lipid and milk protein biogenesis as well as in essential cell biological processes (apoptosis and barrier permeability) (Matsuda *et al.,* 2004; Hernandez *et al.,* 2009; Marshall *et al.,* 2010; Hernandez *et al.,* 2012).

2.2.2. Milk composition

The main components in milk are, besides water, fat, proteins, lactose and minerals (Blum & Hammon, 2000). During milking the composition of the milk changes, concentrations of protein and lactose is relatively constant while the concentration of fat increases throughout the milk ejection (Sandoval-Castro *et al.,* 2000; Ontsouka *et al.,* 2003). This is due to the lower gravity of fat compared to water a larger part of the fat is found in the alveolar fraction of the udder (Ontsouka *et al.,* 2003). Ayadi *et al.* (2004) reported that without milk ejection up to 89% of the total fat yield was retained in the alveolar compartment. The udder of a dairy cow is divided into four quarters, as well as in a cisternal and an alveolar fraction in each quarter. Most of the milk is stored in the alveolar compartment (Bruckmaier & Wellnitz, 2008). Ayadi *et al.* (2004) reported that the rear quarters store an average of 34 % more cisternal milk than the front quarter. Similar observations were found in the alveolar milk, a larger amount was stored in the rear quarters than in the front quarters (Ayadi *et al.,* 2004). The cisternal fraction is first to be removed during milking or suckling, for the alveolar fraction to be ejected the hormone oxytocin is required. Oxytocin is released from the posterior pituitary when the udder or a teat is stimulated, the oxytocin induces alveolar contraction which leads to emptying of the alveoli (Vetharaniam *et al.,* 2003). This leads to a higher fat content at the end of the ejection, and therefore the timing of the suckling could influence the milk intake for the calf. Higher levels of oxytocin have been found in both the cow and the calf during suckling compared to machine milking and when milk was consumed from a bucket (Bar-Peled *et al.,* 1995; Lupoli *et al.,* 2001).

The milk fat is a highly valuable component in milk but also a variable component. The fatty acid composition can be affected by stage of lactation, season, nutrition and management, genetics, and others (Jensen *et al.* 1991; Palmquist *et al.*1993). Both milk yield and milk fat can be modulated short term by feed management, and both are prime economic aspects at dairy farms. The economical return at dairy farms largely depends on the feed conversion rate of the given diet to produce milk (Brun-Lafleur *et al.,* 2010; Sova *et al.,* 2013). Most of the milk fat originates from triglycerides and free fatty acids (FFA) in the blood (Bitman *et al.,* 1984), fatty acids are also created through *de novo* lipogenesis in the mammary glands, where the main carbon substrates for lipogenesis are products from the ruminal fermentation, acetate and beta-hydroxybutyrate (BHBA) (Palmquist *et al.,* 1969; Matamoros *et al.,* 2020).

2.3. Energy balance

The energy balance can be described as the difference between the net energy needed for maintenance and milk production and the net energy intake. Breeding and genetic selection for an increased milk yield have led to a larger difference between the cow's potential for a higher feed intake and the milk yield potential which put today's cows at a larger risk of a negative energy balance (NEB) (Berglund & Danell, 1987; Ingvartsen *et al.,* 2000). Most dairy cows are confronted with NEB during the transition period and early lactation, cows unable to adapt their feed intake to the milk production during this period are more prone to the negative effects (Berglund & Danell, 1987; Ospina *et al.,* 2010). The transition period, when a cow goes from nonlactating to lactating, is characterized by changes in the endocrine status, nutritional and immunological changes. These changes entail an increased risk for various health problems, both metabolic and infectious, such as ketosis, fatty liver, and milk fever (Goff & Horst, 1997; Drackley, 1999; Djokovic *et al.,* 2019). The health problems often originate or is related to management and feeding during the dry period. By adjusting the feed to the cow's condition during the dry period, the body condition score (BCS) can be kept at optimum (3.0-3.25 on a 5-point scale) to prevent additional risk of cows being over- or under-conditioned which could lead to implications at calving, impaired production and reproduction and metabolic problems (Roche *et al.,* 2009).

The endocrine status and insufficient energy intake stimulate the mobilization of body reserves to provide additional energy for the milk production (Patton *et al.,* 2006). The energy is mainly created from mobilization of fat from adipose tissue, glycogen from the liver and body protein (van Knegsel *et al.,* 2005). As a result of the excessive mobilization of adipose tissue, serum levels of non-esterified fatty acids (NEFA) are elevated (van Knegsel *et al.,* 2005). NEFA can further be oxidised to Acetyl-CoA or stored as tri-acyl glycerol (TAG) in the liver, which increases the risk of fatty liver (van Knegsel *et al.,* 2005). Acetyl-CoA will later be used to produce ketone bodies, as a consequent of an imbalance in the products needed for a normal Krebscycle. Ketone bodies is part of a normal response postpartum, but an excessive elevation in circulating ketone bodies (ketosis) is an indication of a poor adjustment to fulfil the energy requirement for the lactation (Grummer, 1993; Reist *et al.,* 2000; Herdt, 2000; Butler, 2003; Duffiled *et al.,* 2009).

2.3.1. Non esterified fatty acids & β-hydroxybutyrate

Both NEFA and the ketone body BHBA are common measurements to estimate the metabolic status, though both are normally present (Herdt, 2000). According to Mann *et al.* (2015) & Dann *et al.* (2006) there is an association between elevated concentrations of BHBA and NEFA postpartum, and reproductive problems as well as an increased risk for diseases as displaced abomasum and mastitis. McArt *et al.* (2013) stated that herds with excessively elevated concentrations of NEFA and BHBA suffer to a larger extent of negative subsequences such as poorer reproduction and lower milk yield. Caution should be taken when ketone bodies as BHBA are used as an interpretation of the energy balance or the nutritional status since these are also influenced by the carbohydrates from the feed, and its validity as an indicator of the energy balance should therefore be questioned (Ingvartsen *et al.,* 2003; DeFrain *et al.,* 2004).

Duffield *et al.* (2009) reported an association between high serum BHBA and a lower milk yield, greater milk fat percentage and less milk protein. The association between a lower milk protein and a higher milk fat is related to the increasing amount of circulating long-chained fatty acids, ketone bodies and NEFA (Hostens *et al.,* 2012; Jorjong *et al.,* 2014). Harrison *et al.* (1990) studied the energy balance in a high yielding group (10 814 kg) and an average yielding group (6 912 kg), in both groups the change in BHBA and NEFA followed a similar pattern. They were both higher during the first and second week postpartum, but NEFA concentrations were greater in the high yielding group during the first weeks (Harrison *et al.,* 1990). Just as high yielding cows tend to be more affected by a negative energy balance, Pryce *et al.* (1999) found a significant effect of parity on ketosis, mastitis, and milk fever. Threshold values varies depending on which symptom or disease that is studied and during which timeframe. Ospina *et al.* (2010) and Jorjong *et al.* (2014) classified NEFA ≥0.6 mmol/L as a critical value postpartum, while values above 1.0 mmol/L have been reported by LeBlanc *et al. (*2005) and Seifi *et al.* (2011) to be associated with a higher culling rate.

2.3.2. Body Condition Score

Body condition score (BCS) is an estimation of the subcutaneous fat, and a useful management tool and indicator of the nutritional status on farms. The scoring system range from 1 (thin) to 5 (fat) with a 0.25-point increment, one unit of BCS corresponds to approximately 60 kg body weight (Volden & Nielsen, 2011). Changes in BCS reflects the metabolic status and body composition, changes during gestation and the transitioning period are especially important to notice. As previously mentioned, DMI and body reserves decreases prior and during early lactation which results in loss of body weight. When the weight is stabilized again and the cows gain in weight varies and depend on several factors such as genetics, breed, DMI, parity and milk yield (Pryce *et al.,* 2001; Koenen *et al.,* 2001). Koenen *et al.* (2001) and Pryce *et al.* (2001) reported the lowest body condition after calving at week 11 and 12 respectively, while Berglund & Danell (1987) suggested that minimum body weight was reached approximately 2 months after calving.

Both a low and high BCS after parturition has been reported to have a negative effect on the cow's milk production as well as the reproductive performance, e.g., delayed heat and lower conception rate at insemination which consequently leads to longer calving intervals (Heuer *et al.,* 1999; Reist *et al.,* 2000); Barletta *et al.,* 2017). Treacher *et al.* (1986) found cows with higher BCS at calving to have a lower DMI and lower milk yield than the thinner cows in early lactation. The over-conditioned cows in Treacher *et al.* (1986) also had higher weight losses due to mobilization of body reserves. Both Pryce *et al.* (2001) and Roche *et al.* (2007) reported similar results, cows with a higher BCS at calving were related to elevated plasma NEFA and BHBA concentrations and increased BCS loss. It is known that high yielding cow are at higher risk of NEB because the larger gap between energy intake and the energy requirement needed to cover milk production and maintenance, these cows tend to lose more in body weight (Kertz *et al.,* 1991).

3. Material & Method

This study was conducted by the Swedish University of Agricultural Science (SLU), at the Swedish Livestock Research Centre in Uppsala, Sweden. The experiment was legally conducted and approved by Uppsala Ethical Committee (ID: 5.8.18-18138/2019) and is divided into several batches. This study is based on batch 2 and aims to study the cow's milk yield, energy balance, feed intake and feeding pattern during early lactation.

3.1. General management and housing

Animals were selected after their expected calving date to get a narrow time frame and a similar age of the calves. Cows with health problems such as lameness, carriers of Staphylococcus aureus and nervous cows which lead to problematic milking's in the VMS were excluded. Breed was not taken into consideration in the group division and both primiparous and multiparous cows of Swedish Holstein (SH) (n = 19) and Swedish red (SR) (n = 22) were included. Cows given birth to heifers were prioritized for the cow-calf pairs to enable future studies on the heifer's milk production.

The study included 22 treatment cows-calf pairs and 19 control cows, but no control calves were included. The calves were born between 3rd of March and 15th of April 2020. All calves were born indoors in single pens, the cow-calf pairs spent the first 48-72 hours alone in the calving pens, thereafter they were moved to a loose housing system. Control cows were moved to the loose housing system within 24 hours postpartum. Both the control and treatment group were housed in the same area with cubicles and controlled cow-traffic. The cows had free access to roughage and water, the concentrate was individually regulated and offered in automatic dispensers in the stable and in the VMS (DeLaval VMS™ Classic). The treatment calves were kept in an enclosed contact area where the treatment cows had access when passing a selection gate. The selection gate either lead the cows to the contact area or to the VMS if the cows had milking permission, after milking they could once again pass though the same selection gate and get access to the contact area and calves. Milking permission differed between the groups, six and eight hours for the control and treatment group, respectively. The contact area was equipped with cubicles, automatic concentrate dispensers, water and a calf creep that only the calves had access to. The calf creep was equipped with roughage, concentrate, water and a laying area.

Table 2. Distribution of number of animals in each parity breed, and corresponding lactation group in treatments.

	Treatment	Control	Total (n)	Lactation group
Cows (n)	22	19	41	-
Parity 1 (n)	12	6	18	1
Parity 2 (n)	4	3	7	2
Parity 3 (n)	4	3	7	3
Parity 4 (n)	2	2	4	3
Parity 5 (n)	-	1	1	3
Parity 8 (n)	-	1	1	3
SH	11	8	19	-
SR	11	11	22	-

3.2. Feeders and measurements of feeding patterns

Ad libitum roughage was provided in BioControl's CRFI (Controlling and Recording Feed Intake) system (BioControl, Rakkestad, Norway) using feeding troughs on weight scales with transponder-controlled gates. The mean energy content in the roughage was 10.8 MJ/kg DM**,** mean crude protein 140 g/kg DM and mean neutral detergent fibre (NDF) was 458 g/kg DM throughout the study. The CRFI record each cow's individual feed intake, e.g., to study and evaluate the feed conversion ratio. When a cow approaches the system, the animal is identified by a transponder, once identified the gate is lowered and allows access to the feed. Cow number, start-time of the visit and the start-weight of the troughs were recorded. When the animal left the feeder, the gate closed and recorded the end-time and end-weight of the container. The CRFI data was continuously recorded and transferred to a computer for analysis. A visit was defined as the time spent by an individual with the head in one trough, consuming more than 0 grams of feed. The intervals between the visits were calculated from the end-time of a visit to the next start-time for the same individual. To determine if the next visit was part of previous meal or a start of a new meal a meal criterion was used. The meal criteria in this study was set to 28 minutes, based on results from DeVries *et al.* (2003) and Tolkamp *et al.*(1998). When analysing the results for feed per minute, data higher than 1000 g per min were considered outliers. These values could be a result of false data from the system or a cow tossing roughage. For the analysis of feeding rate, only observations over 0 g/ minute were included. The reason for a cow to consume 0 g of feed is likely explained by a too short visit, perhaps a cow of low social rank or that the cow simply chose not to consume any feed at a particular visit.

Concentrate was offered in automatic dispensers and in the VMS, the amount was individual and based on predicted yield, DIM, parity (first parity vs. older), and additional gestation. Two different concentrates were offered during the study, Komplett Norm 180 and Konkret Mega 28, (Lantmännen Lantbruk, Sweden). The composition of the concentrates differed mainly in crude protein (CP), crude fat (CF) and starch (S) content (CP:180 vs. 280 g/kg DM, CF:61 vs. 116 g/kg DM, S: 310 vs. 50 g/kg DM).

Table 3. Mean nutritional values in roughage during the study.

	1/3 – 31/3 - 2020	1/4 - 30/4 - 2020	1/5 – 14/5 - 2020
Roughage			
DM, g/kg feed	326	384	390
MJ ME, g/kg DM	10.9	10.8	10.8
Protein, g/kg DM	150	139	130
NDF, g/kg DM	434	450	490

3.3. Recording of production measures

At each milking, yield, time, milk flow, conductivity and blood were measured. Milk samples were taken every fortnight and were analysed for milk fat, milk protein, lactose, dry matter, urea, and somatic cell count. Daily milk yield measurements were corrected for an uneven number of milkings per day by calculating milk secretion rate per hour for the milkings performed during each individual day and multiplying this number with 24 hours, in order to reflect actual amount of milk synthesized per day for control cows. Energy balance was calculated according to Volden & Nielsen, (2011) (Appendix 1). The estimated ECM production was calculated using the change in BCS and mobilization of energy which later were used as an estimate to study each cow's energy balance according to the NorFor system (Volden & Nielsen, 2011). When the energy balance and the mobilization of energy was known another estimation of the remaining energy (that was available for the milk production) was calculated which resulted in an estimation of ECM for both treatments**.** Energy corrected milk (ECM, 3.14 MJ/kg) was calculated based on fat, protein and lactose content and calculated according to Sjaunja *et al.* (1990). For conversion of metabolizable energy (ME) to net energy (NE) 0.6 was used as the efficiency according to Kaasik (2010) and Volden & Nielsen, (2011).

ECM = Milk yield (kg/d) * (38.3 * fat (g/kg) + 24.2 * protein (g/kg) + 16.54 * lactose (g/kg) + 20.7) / 3140

A body condition score camera installed in the VMS recorded the cows BCS daily. Blood samples were drawn twice/w during the first and second week postpartum from each cow for NEFA and BHBA. The blood was sampled from the coccygeal vein*,* occasional samples hade to be drawn from the jugular vein. The blood samples were stored at -20 °C before analysis, an enzymatic colorimetric assay method for quantitative determination of non-esterified fatty acids (NEFA) in serum. The analysis was performed by HUV Analysis Laboratory, SLU, Department of Animal Nutrition and Management.

3.4. Statistical analyses

This study focused on the early lactation between DIM 4-50. DIM 4 were chosen since the cow-calf pair spent the first 48-72 hours together in the calving pen and to exclude the colostrum period. DIM 50 was chosen since all cows were let out on pasture on the 14^{th} of May, to avoid additional effects that a pasture has on the cows feed intake and production and to keep as many cows as possible with an even DIM. All statistical analysis were carried out using Mixed Procedure of SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA). When analysing the NEFA values in SAS enterprise guide any duplicates and high CV-values were excluded from the set. Treatment (treatment, control), cow nested within treatment was included as a repeated effect in the model, lactation group (1, 2, 3), DIM/ Week in lactation and breed (Swedish Holstein, Swedish red) were included as fixed effects in the statistical model**.** To reduce the risk of misleading results when a few animals is representing a whole parity, all cows with a parity of three and above was submerged due to the lower number of animals in lactation 4, 5 and 8 (Table 3). Parity one and two were kept separate due to factors such as growth. The new groups were named lactation group 1, 2 and 3. The lactation groups were used for analysing feed intake, energy balance and for the milk yield. Regarding DIM the individual days were kept for analysing milk yield, while DIM was submerged to represent lactation week after calving when analysing the energy balance and feed intake. Lactation week was used to create more representative values, since energy balance is a more long-term effect and feed intake have a considerable day-to-day and within-cow variation (Forbes, 2007). All relevant two-way interactions were tested and removed from the model if found unsignificant. Residuals were tested for normality and those who did not follow a normal distribution were logarithmic transformed whenever needed (e.g., feeding patterns, since time rarely is normally distributed**)** or square-root transformed (Feeding interval, Feed intake per meal, Meal duration). The normal distribution of data was controlled using the univariate procedure in SAS. Values in text are transformed P-values and least square means, calculated using the LSMEANS/PDIFF option. Statistical difference was determined following Tukey´s adjustment declared at $P \leq 0.05$. Values in figures are estimated from untransformed values and processed with Pivot tables (Excel). Effect of the treatments was in focus of this study.

4. Results

4.1. Feed intake

No significant effect (*P*=0.92) was found on energy intake between the groups, LSMeans for the control group were (mean ± SE) 282.2 ± 2.25 MJ ME and 282.5 ± 1.98 MJ ME for the treatment group. However, energy intake was significantly affected by parity. Cows in first parity had a lower energy intake (mean ± SE) 239.0 ± 2.2 MJ ME/d) compared to cows in second parity (306 ± 3.1 MJ ME/d) and older cows mean of 305.7 ± 2.5 MJ ME/d. The difference between the first parity and the older cows was roughly 70 MJ ME/d.

The dry matter intake differed (*P*<0.0001) between the two treatments, (mean ± SE) 23.4 ±0.2 kg/d for control and 22.1 ± 0.17 kg/d for treatment, respectively (Figure 1), and a significant interaction of treatment*week was found (P=0.0006). The DMI plateaued in the treatment group at week 5, while the control group reached theirs in week 6, it was also in week 6 and 7 the largest difference in DMI was seen between the treatments (>3 kg). SH had the highest DMI in both groups (24.1 vs. 22.6 kg DM for SH and SR in the control group and 23.6 vs. 20.5 kg DM for SH and SR in the treatment group). Both the DMI from concentrates and roughage differed significantly between the treatments, however the difference was roughly 0.3 kg in both concentrates and roughage. The treatment group consumed a mean of 10.5 kg DM of concentrates, and the control group consumed a mean of 10.7 kg DM. Regarding the roughage, the treatment group consumed 13.3 kg DM and the control group consumed a mean of 13.0 kg DM of roughage.

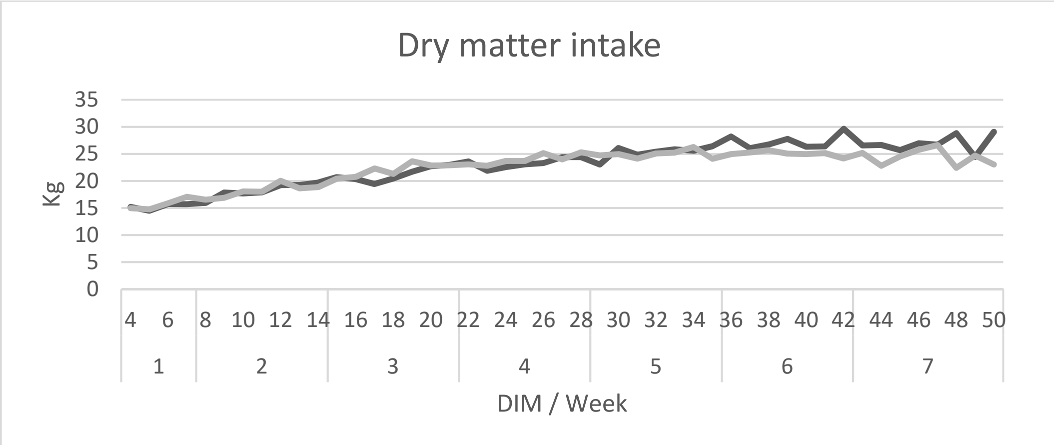

Figure 1. *Total dry matter intake for each lactation week, light grey represent treatment group and dark grey represents control group.*

4.1.1. Feeding interval & number of meals of roughage

In the feeding interval a significant effect (P<0.0001) was found for treatment and parity. Mean feeding interval for the control group were 150 min (2 h 30 min) and 183 min (3h 3 min) for the treatment group (Figure 2). The feeding interval increased with parity (152 min, 163 min and 185 min) for respectively parity 1, 2 and above 3. The interaction of treatment*breed was significant, as the two breeds in the treatment group differed significantly (173.4 vs. 193.0 min for SH and SR) while no significance was found between the breeds (150.7 vs. 149.8 min for SH and SR) in the control group. As seen in figure 2 the finding interval increases in the treatment group throughout the study while it decreases in the control group.

Regarding number of meals per day the results show that the treatment group had fewer visits to the Bio-control system. A significant effect (P<0.0001) was found of treatment and parity. The mean number of meals for the treatment group were (mean ± SE) 6.98 ± 0.08 and 8.10 ± 0.09 for the control group. The interaction of treatment*parity was also significant (*P*=0.0036). First and second parity in the treatment group had the same number of meals (7.5 ± 0.1) while parity 3 and above had 5.7 ± 0.1 visits/d. In the control group cows above second parity had similar number of meals (7.9 ± 0.2 and 7.3 ± 0.1) while first parity had 9.0 ± 0.1 visits/d.

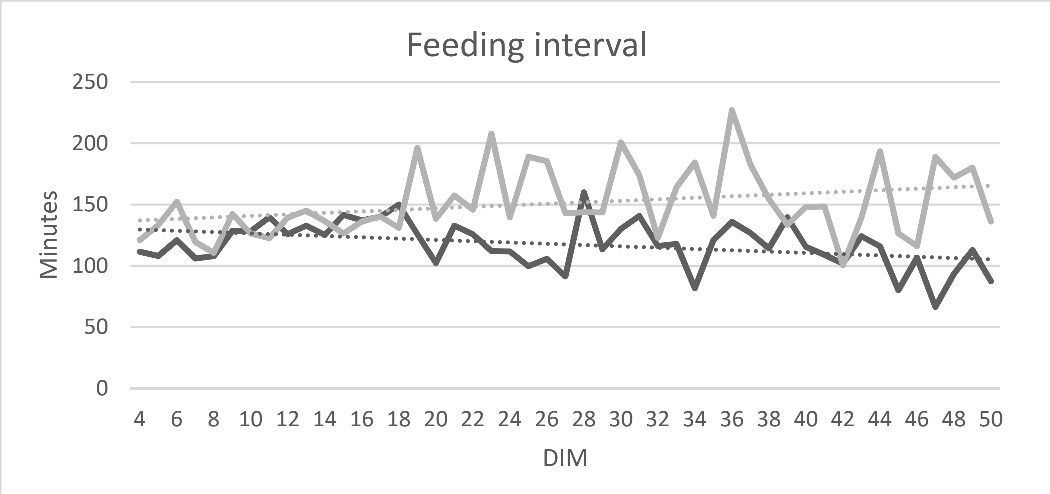

Figure 2. Feeding intervals between roughage feedings. Light grey represent treatment group and dark grey represents control group.

4.1.2. Meal duration & feed intake per meal

A significant effect was found of meal duration on treatment, breed, and parity. The mean time spent at the Bio-control system per meal were 18.84 min for the treatment group and 16.14 min for the control group (Figure 3). A significant interaction between treatment and breed showed that SRB in the control group spent shorter time per meal compared to SH, 14.5 min and 17.9 min respectively. While in the treatment group the opposite results were found, SR spent 19.6 min and SH spent 18.1 min.

The results on feed intake per meal (kg/meal) were significant ($P<0.0001$) for treatment and parity. The mean feed intake per meal was 3.3 kg for the control and 4.4 kg for the treatment group. Both breeds had a higher mean feed intake per meal in the treatment group (4.22 and 4.48 kg/ meal respectively for SH and SR) in the treatment group, compared to the control group (3.46 and 3.22 kg/ meal for SH respectively SR).

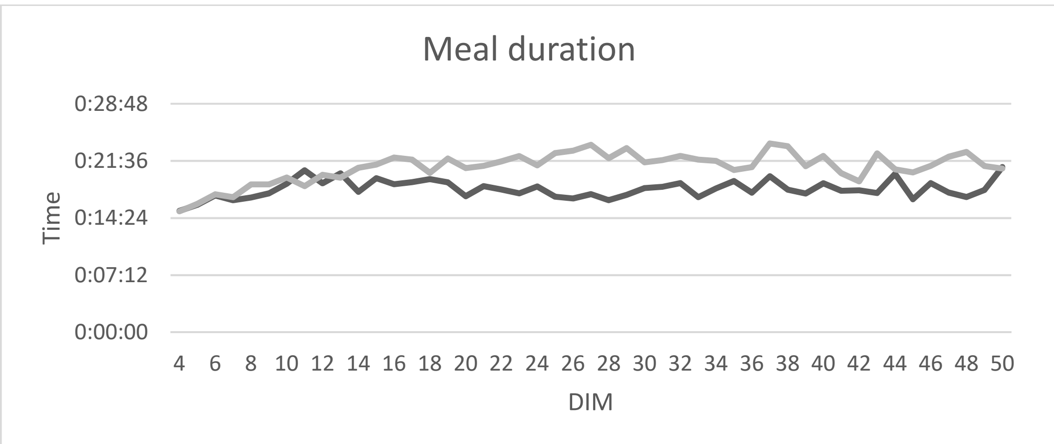

Figure 3. Minutes for mean meal duration. Light grey represent treatment group and dark grey represents control group.

4.1.3. Feeding rate

The treatment group had a significantly higher feeding rate compared to the control group. Mean value for the control group was 216 ± 0.8 g/min while the mean ± SE for the treatment group was 240 ± 0.7 g/min (Figure 4). Significant effects (P<0,0001) were found of treatment, parity, and breed. In the treatment group SH had a slightly higher feeding rate than SR (246 vs. 234 g/min), while in the control group SR had a higher feeding rate than SH (232 vs. 200 g/min).

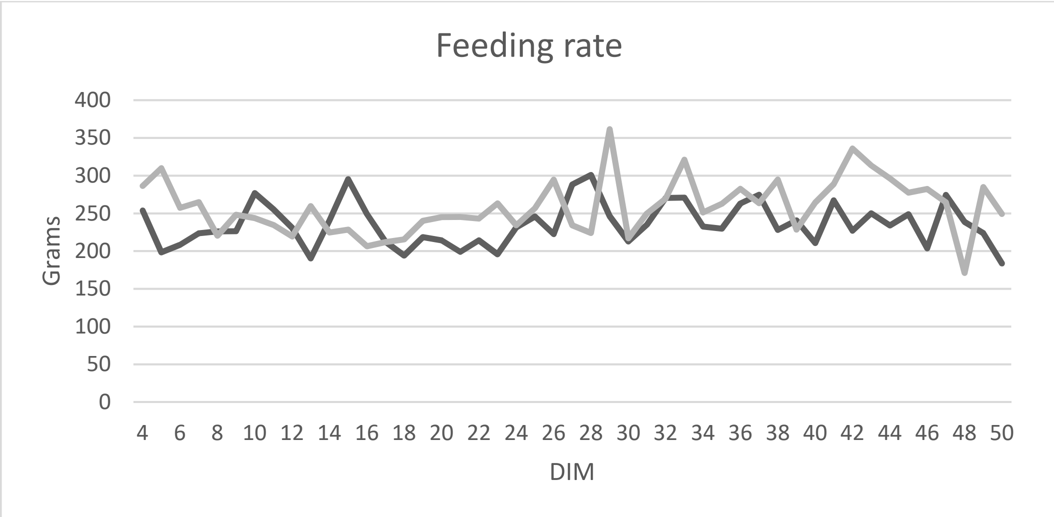

Figure 4. Feeding rate, feed intake per minute. Light grey represent treatment group and dark grey represents control group.

Table 4. Daily feeding patterns for both treatments.

	Feed intake/ d (kg DM)[a]	Number of meals (n)[b]	Feeding interval (min)[b]	Meal duration (min)[b]	Feeding rate (g/min)[b]	Feed intake/ Meal (kg)[b]
Control	23.4	8.1	150	16.1	215.7	3.3
Treatment	22.1	7.0	183	18.8	240.0	4.4
P1*	19.3	8.3	152	13.6	218.1	2.8
P2**	25.4	7.8	163	19.5	218.6	4.1
P3***	25.6	6.5	185	19.8	246.9	4.7
SH	23.8	7.8	162	18.0	222.9	3.8
SR	21.5	7.3	170	16.9	232.8	3.8

* = first parity, ** = second parity, *** = third parity and above

a = roughage and concentrate intake, b = roughage intake only

4.2. Milk yield

The results are based on the milk yield harvested in the AMS, a total of 1557 observations were registered for milk yield. The mean ± SE milk yield for the control group was 34.2 ± 0.32 kg/ d and 19.0 ± 0.26 kg/d for the treatment group (Figure 5). A significant effect (P<0,0001) was found of treatment, parity and breed. When analysing the effect between breeds a significant effect (P<0,0001) was found in the treatment group, while no effect could be found between the breeds in the control group. ECM was based on milk samples taken approximately every fortnight, resulting in fewer values compared to the daily milk yields, 274 and 1557 observations, respectively. Calculations of ECM were based on one sample per occasion and the (corrected) milk yield for the corresponding day. The large variation in ECM is probably a result of few observations together with a large variation in milk fat and abnormal milk yields from the treatment group. The mean (±SE) ECM was 37.5 kg ± 0.95 kg for the control group and 20.3 kg ± 0.85 kg for the treatment group. Significant effects (P<0.001) were found of treatment and lactation group and (P 0.007) of breed, while DIM had no significant effect. No interaction was found in ECM between treatment and breed.

Table 5. Least square mean ± standard error of harvested milk yield, ECM, estimated ECM (eECM) and composition of treatment and control cows.

	Harvested milk		
	Treatment	Control	Significance[a]
Milk yield (kg/d)	19.0 ± 0.3	34.2 ± 0.3	***
ECM (kg/d)	20.3 ± 0.8	37.5 ± 0.9	***
eECM (kg/d)	42.9 ± 0.4	43.7 ± 0.4	NS
Fat (%)	4.14 ± 0.2	4.63 ± 0.2	*
Protein (%)	3.67 ± 0.05	3.63 ± 0.06	NS
Lactose (%)	4.57 ± 0.05	4.78 ± 0.06	**

[a]Level of significance for treatment difference: NS, not significant; *p < 0.05; ** p < 0.01; *** p < 0.001

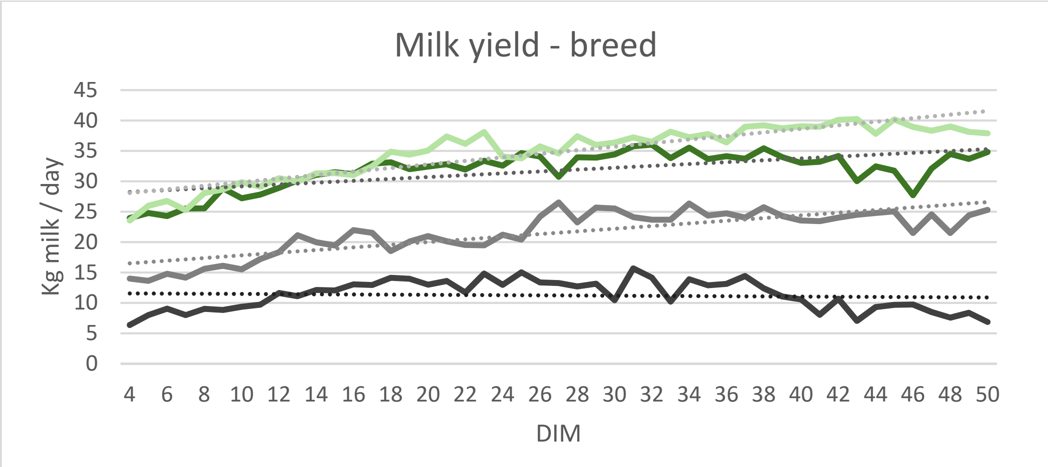

Figure 5. Milk yield for Swedish Holstein and Swedish red in both treatments. Light green represents SH and dark green represents SR in the control group. Light grey represents SH and dark grey represents SR in the treatment group.

This study does not include the amount of milk suckled by the calves nor calf growth and thereby the actual milk production in the treatment group remains unknow. As seen in Figure 6 the estimated ECM for both groups does not differ significantly between control and treatment. The estimated ECM means were similar in both treatments, 43.7 ± 0.44 kg and 42.9 ± 0.37 kg for the control group respectively the treatment group. A significant effect (P<0.0001) was found of lactation week, parity and breed.

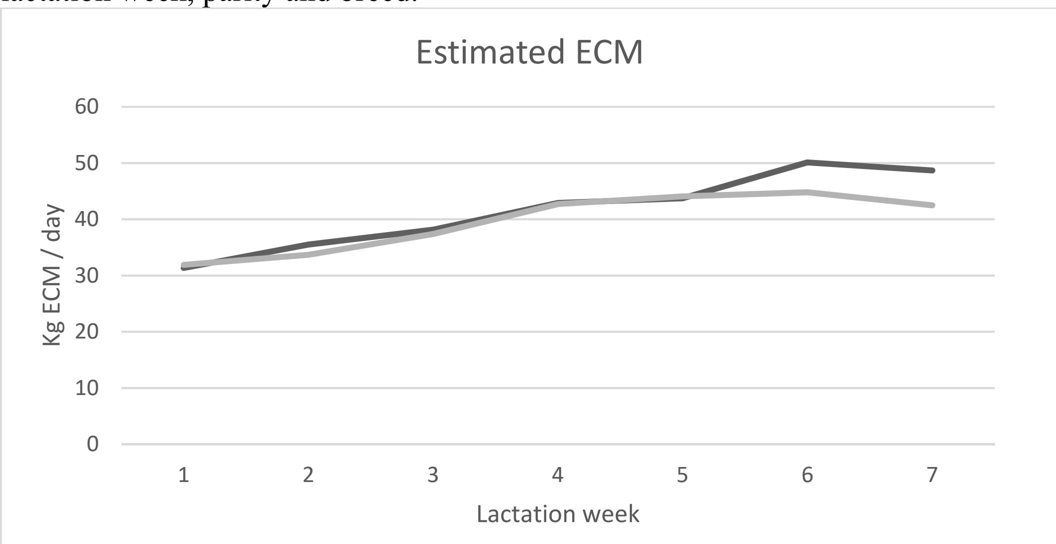

Figure 6. Estimated ECM in each lactation week. Light grey represent treatment group and dark grey represents control group.

4.2.1. Milk composition

For milk fat a total of 286 observations were used. The treatment group had a lower mean (±SE) in milk fat compared to the control group, § However, the control group had less of a variation in milk fat percentage compared to the treatment group (Figure 7). The milk fat varied between 2.7 % and 8.7 % for the control group and 18.8 and 0.12 % for the treatment group. A significant effect was found of treatment (P=0.043), and lactation group (P=0.018) and a tendency of DIM (P=0.051). Mean protein concentration were 3.62 % and 3.67 % respectively for the control and treatment group, no significant effect was found on milk protein. A significant effect (P=0.0058) was found of treatment on milk lactose. The mean values for lactose were 4.8 % for the control group and 4.6 % for the treatment group.

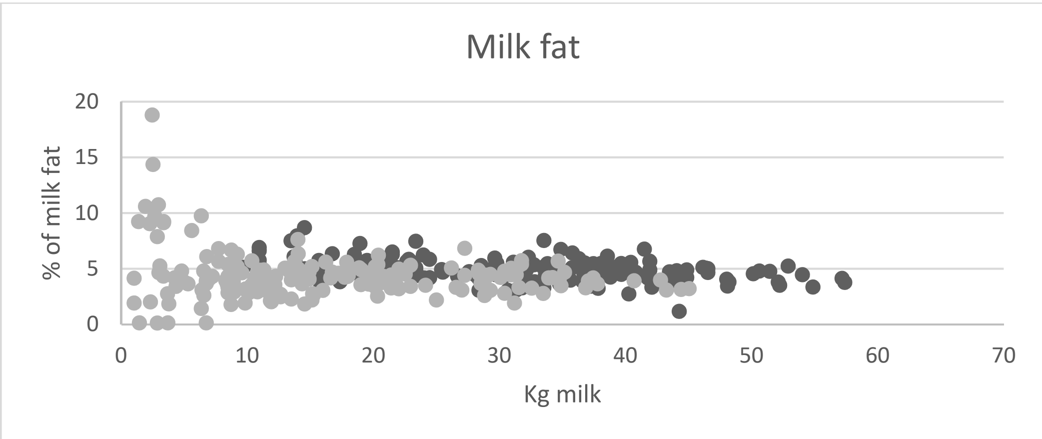

Figure 7. Milk fat content, difference between treatments. Light grey represents treatment group and dark grey represent the control group.

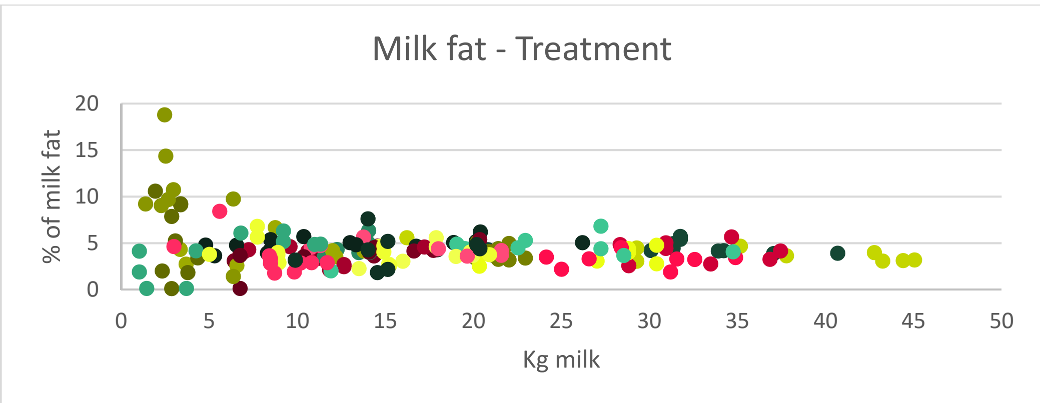

Figure 8. Individual cow's milk fat in the treatment group, each colour represents an individual.

Table 6. Mean milk fat of treatment and control groups.

Effect	Treatment	Breed	Lactation group	Estimate	SE	Sig.
Treatment	Control			4,63	0,2	<0.0001
Treatment	Treat			4,14	0,2	<0.0001
P1*			1	4,87	0,2	<0.0001
P2**			2	4,12	0,3	<0.0001
P3***			3	4,17	0,2	<0.0001
Breed		SH		4,26	0,2	<0.0001
Breed		SR		4,51	0,2	<0.0001

* = first parity, ** = second parity, *** = third parity and above

4.3. Energy balance

4.3.1. Body Condition Score

A significant effect was found in treatment, week, lactation group, breed and in an interaction between treatment and breed (P<0.0001). Mean BCS for the control group was 3.3 ± 0.01 and 3.5 ± 0.01 for the treatment group. The significant effect of lactation group was found between 1 and 2 and 1 and 3 but not between 2 and 3 the least affected while both lactation group 2 and 3. Lactation group 1 had the lowest change during the study, while the other two had a greater loss.

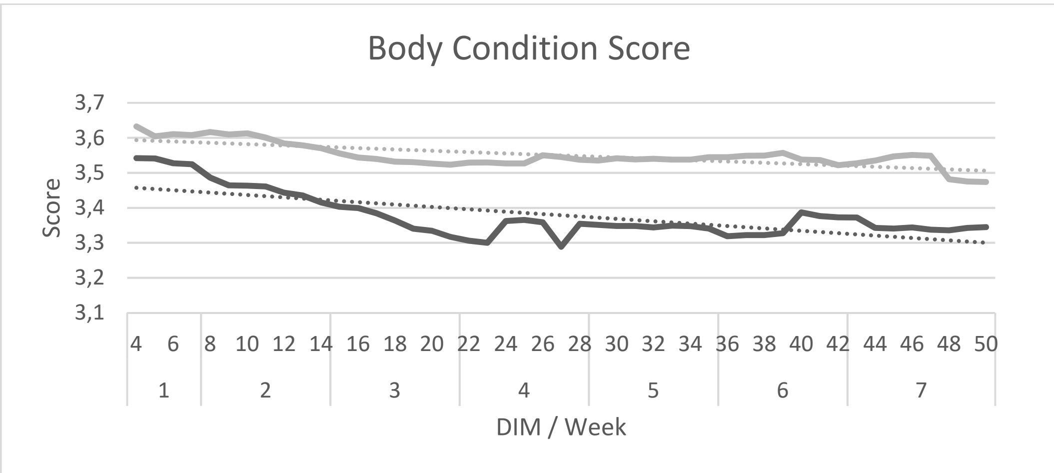

Figure 9. Body Condition Score difference between treatments. Light grey represents treatment group and dark grey represent the control group.

4.3.2. Non-esterifed Fatty Acids

The mean values of NEFA for the control group were 0.47, a variation between 0.12 and 1.24 were found. The mean values for the treatment cows were 0.37 with a variation between 0.08 and 1.32. Significant effects were found of lactation group (P<0.0001) and week (P=0.0012). An interaction between treatment and week were also significant (P=0.0414). During the first week postpartum both control and treatment cows had similar mean values 0.49 and 0.47 respectively, the second week the values for the control group were higher than in the treatment group, 0.44 and 0.26, respectively.

Figure 10. Non-esterified fatty acids, difference between treatments during the first two weeks postpartum. Light grey represents treatment group and dark grey represent the control group.

5. Discussion

5.1. Feed intake

One of the worries, apart from loss of saleable milk, is that cows that are both suckled and machine milked might have a too low feed intake to cover their milk production. Bar-Peled *et al.* (1995) found cows in CCC to have the highest milk production compared to cows not being suckled, but their feed intake did not cover the energy requirement and thereby had a larger change in BCS. This was not seen in the study presented here. Energy intake in the two treatment groups did not differ, however, significant effects of treatment, breed and parity were found on DMI, where the control group had the highest DMI throughout the study. The significant difference in DMI was seen in both concentrates and in roughage intake, however the mean difference were small between the treatments, as only 0.3 kg DM differed in both concentrates and in roughage. When analysing the concentrate intake (data not shown), it was confirmed that both treatments had a similar concentrate intake, which means the difference in DMI is likely a result of different roughage intakes. One possibility could be that the treatment group had a higher roughage intake until DIM 30 in the study, at that point the control group had reached the same DMI and continued to increase to a higher DMI than the treatment group. Also, the DM and NDF content in the roughage gradually increased during the study (Table 4), meaning the control group consumed more feed with a higher DM and NDF content which could explain why the two treatments had a similar energy intake but a different DMI. Also, there was a slightly skewered distribution of parity between treatments, but parity was included in the model to counteract this effect. Kertz *et al.* (1991) and Dado & Allen (1994) reported younger cows to produce less milk and to have a lower feed intake. This is in line with results from this study, where first parity cows had a lower energy intake of approximately 69.5 MJ ME less compared to multiparous cows.

5.1.1. Feeding pattern

The feeding patterns only regard the roughage intake. The results indicates that the treatment group had a more efficient feeding pattern (Table 5) which might be related to the maternal behaviour among these cows (Johnsen *et al.,* 2016), however maternal behaviours were not assessed in this study. During the 24 hours, they have the same need for feed intake, rumination, resting and social behaviours as the control cows. It is possible that the higher feeding rate is necessary for the treatment cows to be able to reach their daily feed intake on fewer meals. The eating pattern might have been different if cows were able to eat roughage with the calf by their side and it would be interesting to test that as well. To our knowledge, the current study is the first to evaluate how the cows' feeding pattern is affected by CCC.

The chosen length of a meal criteria could have affected the feeding pattern in this thesis, e.g., a longer meal criterion would result in a lower number of meals/d. However, according to Tolkamp *et al.* (2000) and DeVries *et al.* (2003) the total time for the daily feed intake should not be affected by this. According to Nielsen (1999) the total daily feed intake would only differ through changes in number of meals/d and feed intake per meal. Since the same meal criterion (28 minutes) were chosen for both treatments the results between the two treatments could have been affected. As DeVries *et al.* (2003) reported, a pooled meal criterion could result in loss of detail. If an individual criterion would have been used in this study, one group could have had several intervals within a meal, as they would be more influenced by each individual feeding pattern. For example, if one individual would have several shorter intervals within a meal, yet shorter than 28 minutes, these would all account for one meal and thereby affect the number of meals per day.

The individual transponders together with the CRFI provide detailed information regarding the cows feed intake, however these does not provide information whether feed is consumed or tossed outside the container. Outliers in the feeding rate were therefore removed to minimise the risk of tossed feed affecting our conclusion. The possibilities to compare results on eating time with other studies is limited due to differences in type of feed, feed properties as well as feeding places per cow and cow traffic. Dado & Allen (1994) found high producing multiparous cows to consume more feed/ meal with a higher feeding rate, consume more DMI with fewer number of meals/d. This was in a tie-stall with no feeding competition between the animals, yet the results are in agreement with the results from this study. What is interesting is that the treatment group adopted a feeding pattern more similar to these high producing multiparous cows, regardless of parity.

5.2. Milk yield

As expected, harvested milk was lower from the treatment group as they were suckled by their calves and some by more than one calf. Thereby, there was an individual variation of daily milk yields from the treatment group (data not shown), which is in line with the results from Johnsen *et al.* (2021). It is important to keep in mind that control calves received 6 L of whole milk per day from the tank milk, and for a fair comparison this should be deducted from the milk yield in the milking unit for the control cows. When this is considered the actual difference in saleable milk between control and treatment was around 9 kg/d during the first 50 DIM (Figure 5). According to Lehmann *et al.* (2021) a calf with an ADG of 1200 g/d, same as calves in this study, needed approximately 6 to 12.5 kg ECM between week 1-9 to meet the energy requirement. If calves in this study consumed 9 kg more milk per day than control calves their energy intake in this study was higher than the theoretical requirements for the shown ADG. One of the possibilities for a system like this to be sustainable for the farmer is if the calves have a high ADG and are healthier. This could lead to a decreasing age at first calving with a higher body weight and higher milk yield in first parity (Bar-Peled *et al.,* 1997; Meyer *et al.,* 2006; VandeHaar & St-Pierre, 2006; Moallem *et al.,* 2010; Khan *et al.,* 2011; Johnsen *et al.,* 2016). Though, this have to be confirmed by future studies were this is evaluated and where their solid feed intakes are taken into consideration throughout the entire upbringing to establish if the total feed consumption is higher or not.

Another possibility is if the milk yield would increase postweaning, either resulting in a longer lactation period or in a higher total milk yield compared to control cows. The loss of harvested milk during the suckling period is in line with results from previous studies on CCC systems (Bar-Peled *et al.,* 1995; Mendoza *et al.,* 2010; Barth, 2020). Bar-Peled *et al.* (1995), Krohn (2001) and de Passillé *et al.* (2008) reported lower milk yields in nursing cows compared to cows only being machine milked, however when the calves milk consumption was added to the harvested milk no difference in total milk production between treatments was found during the first nine weeks of lactation. A review by Meagher *et al.* (2019) concluded that studies with free contact in CCC systems did not seem to increase the total milk yield throughout the lactation, due to the loss of milk during the suckling period. However, in restricted CCC systems similar milk yield or even increased milk yields both during the suckling period and in total milk yield have been reported (Meagher *et al.,* 2019). The reason behind a possible increase in total milk production is most likely a higher milking frequency in early lactation (Bar-Peled *et al.,* 1995; Erdman & Varner, 1995; Stelwagen, 2001; Bernier-Dodier *et al.,* 2010). As seen in figure 5 the milk yield increased for both breeds in the control group, while only SH increased their milk yield in the treatment group. If this was a coincidence in this study or if the SR is less suited for a CCC system needs to be further investigated in future studies.

Also, a more complete milk removal and thereby less residual milk has a positive effect on the total milk production (Bar-Peled *et al.,* 1995; Bruckmaier & Blum, 1998). This might be the reason for a greater effect reported from studies where the calf could suckle, as their suckling leads to an increased udder stimulation and milking frequency. In this study the calves could suckle throughout the day, whenever the cow was in the contact area, meaning the treatment cows would have a higher milking frequency compared to the control cows. Barth (2020) reported that suckled cows never reached the same production level post weaning as the control group**.** Barth *et al.* (2020) suggested that this was an effect of a decrease in milk secretion due to the calves' incomplete milk removal together with a disturbed oxytocin release during machine milking. Therefore, it would be interesting to see how the total milk production throughout the whole lactation is affected by the calf's milk consumption.

5.2.1. Estimation of total milk yield (ECM)

When estimating ECM it is assumed in this study that changes in BCS can be translated to energy mobilization (NEL_{mob}) and thereby this energy could be used for milk production. The difference between the estimated ECM and the measured ECM was 6.2 kg/d for the control group and 22.4 kg/d for the treatment group. The estimation was slightly overrated for the control group and thus the same assumption can probably be made regarding the eECM for the treatment group. If this is correct, that both groups had a similar eECM, it enhances the chance for a high milk production after weaning (De Passillé *et al.,* 2008; Mendoza *et al.,* 2009; Johnsen *et al.,* 2016), which was not examined in present study but interesting for future research to investigate further.

In both treatments, SH had a higher eECM (45.0 and 43.8 kg) compared to SR (41.2 and 41.0 kg eEMC), this is in agreement with the national level and Koenen *et al.* (2001). The eECM were similar in both treatments until lactation week 6 where the control group kept increasing while the treatment group reached a plateau in week 7. The significant interaction between treatment*week could be an effect of the lactation curve and how it is affected by treatment. The difference at the end of this study was mirrored in the feed intake by both groups, since the eECM were calculated using feed intake and available energy the difference was no surprise.

5.2.2. Milk Composition

The lower fat content in the treatment group is in agreement with Bar-Peled *et al.* (1995), Fröberg *et al.* (2007), Mendoza *et al.* (2010), Johnsen *et al.* (2016) and

Barth, (2020). Fröberg *et al.* (2007) suggested that this was caused by the calves emptying of the residual high fat content milk. In Bar-Peled *et al.* (1995), Fröberg *et al.* (2007) and Mendoza *et al.* (2010) calves could suckle after milking, in Johnson *et al.* (2016) the calves where only kept together with the dame during night-time and in Barth (2020) the calves were divided in three groups (whole day contact, night-time contact and 15 min contact prior to milking). Interestingly, all these studies reported a lower milk fat content regardless of when the calf suckled. Another possible explanation to the lower milk fat content, than loss of fat to the calf through removal of residual milk, may be that the total milk yield is higher and that the milk therefore has a lower dry matter content. In this study the calves have been able to suckle residual milk and thereby consume a higher amount of ECM than amount of kg milk. When non-suckled cows are machine milked and the residual milk with a higher fat content remains in the udder it will be milked during the next milking, resulting in all the fat will eventually end up in the tank milk, whereas milk with the high fat content now is consumed by the calves after milking.

The large variation in milk fat content, as seen in the treatment group (Figure 7, 8), could be related to the interval between a suckling and the machine milking, as well as the milk let down when machine milked. If a low milk yield with a high fat content is harvested from a cow, it indicates that the milk comes from the alveolar fraction and that the cow had an easy milk let down due to the lower yield (Ontsouka *et al.,* 2003). This could be an effect of recent suckling prior to the machine milking. While, a low milk fat percentage and a low yield, suggests the cow has a less effective milk let down and is mainly consisting of milk from a cisternal fraction.

Milk protein content did not differ between the treatments, yet a tendency for a significant difference in milk lactose was found. Reports on how milk lactose content is affected by cow-calf contact systems is rear. According to Ontsouka *et al.* (2003) both milk fat and lactose increased in the alveolar fractions, while milk protein can decrease at the end of milking, however, no significant decrease was found until 75 % of the cisternal fraction was removed. The influence of CCC system on the milk protein is somewhat conflicting in earlier reports, overall, milk protein is seldomly affected regardless of feed or milking techniques (Ontsouka *et al.,* 2003; Ferneborg *et al.,* 2107). Bar-Peled *et al.* (1995), Mendoza *et al.* (2010) and Fröberg *et al.* (2007) all reported no difference in milk protein content between treatments, while Barth, (2020) reported higher milk protein content in the treatment group during the suckling period.

5.3. Energy balance

The more pronounced drop in BCS in the control group is intriguing (Figure 9). As it indicates that CCC may have had an effect on energy balance. This could be related a higher DMI or a lower total milk yield or both in the CCC cows. Other findings on BCS in the current study, like lower BCS with higher parity, were in agreement with literature (Harrison *et al.,* 1990; Pryce *et al.,* 1999; 2001). The lower BCS in higher parity is often explained by higher milk yield and more pronounced negative energy balance. This is in agreement with the results on NEFA in current study. The treatment group started out with the highest levels in NEFA at the first sampling, then dropped 0.3 units between the first and third sampling, while the control group stayed at the same value during the same time. It would have been interesting to analyse the NEFA values for a longer period instead of only two weeks as in this study. However, neither control nor treatment cows had what is considered problematic NEFA values in this study (LeBlanc *et al.,* 2005; Ospina *et al.,* 2010; Jorjong *et al.,* 2014).

The two treatments started out with a difference in mean in BCS which might have had an impact on the outcome of final BCS. However, the more acute drop would likely still be present and confirmed by the higher concentrations of NEFA in the control group. This suggests that the control group were less able to adjust their feed intake during these first weeks in lactation (Ospina *et al.,* 2010) and that the treatment cows as well as cows in lower parity used less of their body reserves to support the milk production. This study did not investigate body conformation or feed intake during the gestation, which can have a strong influence the cow's capacity to adjust during the transition period (Grummer, 1995; Dann *et al.,* 1999; Drackley, 1999). In future research it would be interesting to investigate this more thoroughly and for a longer duration to find a more concreate reason for why the treatment cows was better adjusted to this critical state.

6. Conclusion

A whole day CCC system significantly reduced the harvested milk yield. However, the estimated total ECM indicated no significant difference in the production level, which is of importance for the total milk yield throughout the whole lactation. Therefore, the degree of loss in sealable milk should depend on the length of the suckling period, and the difference in how much the calves suckle during a day vs. the amount of milk that the calves would normally receive per day. The treatment group had a lower milk fat content, a tendency of lower milk lactose, yet no difference in milk protein was found between treatments. This indicates that there were treatment cows who mainly were milked of their cisternal milk fraction. The CCC system had no effect on energy intake/d, but the treatment cows seem to have a more efficient feeding pattern. Whether a more efficient feeding pattern was related to the maternal behaviour or other social behaviours needs to be investigated in future research. Also, treatment cows were less affected by a negative energy balance, which was shown in lower NEFA values and a lesser change in BCS compared to the control group. Significant effect of breed was found on DMI, energy intake, BCS, milk yield (only in the treatment group), ECM, estimated ECM, feeding rate, feeding interval, meal duration and on meals/ d. Since there are many factors that could have affected these results (e.g., uneven distribution of animals in treatments, few animals, different breeds, and parity), further studies investigating total milk yield and energy balance in cow-calf contact systems are needed to confirm these findings.

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9. Appendix 1.

Calculations of energy balance and mobilisation and deposition for lactation cows

Factor a shows the mobilisation (BW) in BW change in early lactation.
a= 30 for SR parity >1
a= 20 for SR parity 1
a= 36 for SH parity >1
a= 27 for SH parity 1

BW_mob = a * (1+(2 * ((BCS_calv - 3,5)/ 3.5)))
b= 0,04 + 0.05 * BW_mob - 0,305 * (BCS_calv-BCS_end) * 2
c= (b/ (2,4207/-7,3955)) + 0,151 * (-(BCS_calv-BCS_end) * 2 * 2,55)

BW_ change_mobdep = (BW_mob + b*sqrt (DIM)*log (DIM)+c*((log (DIM)) **2)) - (BW_mob+b*sqrt (DIM-1) * log (DIM-1)+c*((log(DIM-1))**2))

If bw_change_mobdep => 0 then NEL_dep= BW_change_mobdep * 31,0)
If BW_change_mobdep =< 0 then NEL_mob = 1*BW_change_mobdep*24,8

NEL_dep is the energy requirement for deposition (MJ/d); NEL_mob is the energy supply from body reserve mobilization (MJ/d); NEL is the energy intake.

NE requirements (MJ/d) for growth in primiparous cows related to BW and daily gain. (The NE req. for the deposition of 1kg body tissue is 31 MJ. A weight loss of 1kg body tissue supplies the cow with 24.8 MJ NEL, due to 80% conversion efficiency).

BW > 400 → NELgain = 4,4
BW > 450 → NELgain = 4,5
BW > 550 → NELgain = 4,7
BW > 650 → NELgain = 4,8
Parity > 2 then NELgain = 0

NEL_bal is the energy balance in %

Maint MJ=0,29256 * ($BW^{0,75}$) * 1.1 ←1.1 =constant for lose housing systems

NEL_dep → ProdMJ = NEL_intake → MJ intake - maint MJ – NEL_gain + NEL_mob
NEL_mob → ProdMJ = NEL_intake → MJ intake – maint MJ - NELgain - NEL_dep

NEL_diff = NEL_mob → NEL_needed = NEL_intake + NEL_mob

Estimated ECM = (prod MJ) / 3.14

NEL_bal = (NEL * 100)/ (NE_maint + NEL_milk + NEL_gain + NE_gest + NEL_dep – NEL_mob)

ProdMJ = NEL_intake - maint MJ – NEL_gain - NEL_mob + NEL_dep