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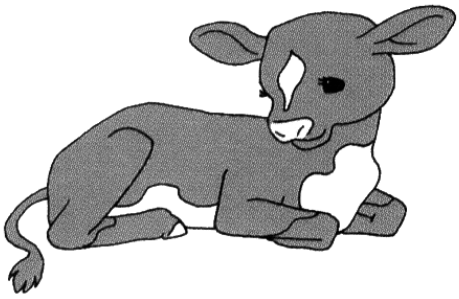
Effects of restricted suckling on abnormal behaviour, feed intake and weight gain in dairy calves, and udder health and milk let-down in dairy cows

Emma Gratte



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Studentarbete 26

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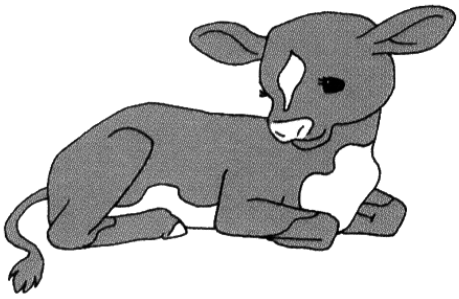

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Effects of restricted suckling on abnormal behaviour, feed intake and weight gain in dairy calves, and udder health and milk let-down in dairy cows

Emma Gratte

Degree project in Ethology, 20 credits (30 ECTS credits),
Master of Science Programme in Agriculture with a specialisation in Animal Science

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CONTENTS

1. SUMMARY

The purpose of this study was to increase our knowledge of how to raise healthy calves with no or few abnormal behaviours and favourable weight gain, along with cows with high milk yield and good udder health. The study investigated the effects of restricted suckling versus artificial milk feeding.

The study was carried out on a private dairy farm in Aguascalientes in Mexico. Twenty-two Holstein cow- and calf pairs were alternately allocated to two treatments where calves were artificially milk-fed in open buckets with floating nipple (A) or allowed to suckle the dam restrictively (R). The A-calves were fed whole milk from parlour twice a day, while the R-calves had the possibility to suckle for 30 minutes twice a day two hours after milking. Calves and cows were followed from birth of calf until weaning at eight weeks. Cows were milked three times a day. All calves had free access to hay, concentrate and water. Behavioural observations with 0/1-sampling at one minute intervals were made twice a week during week 1, 3, 5 and 7. All calves were weighed before milk feeding twice a week. R-calves were additionally weighed after suckling to estimate milk intake. California mastitis test was performed and time to milk let-down was noted on the cows once a week. Milk samples were taken from suckled cows and parlour milk. Statistical analysis was done with Analysis of variance with the Mixed effect model, Wilcoxon rank sum test, Chi^2-test, F-test and Pearson correlation.

Among the A-calves cross-sucking was present in 67% of the animals whereas only 20% of the R-calves performed this behaviour ($p<0.05$). In the majority (93%) of the cross-sucking recordings the calf was sucking the ear of another calf. The A-calves licked the interior significantly more than the R-calves ($p<0.05$). The A-calves had significantly more recordings on the behaviours ruminate, eat concentrate ($p<0.01$) and eat hay ($p<0.05$) compared to the R-calves. An average A-calf also consumed more than four times as much concentrate as an average R-calf (22 vs 5 kg). There were no differences in average milk intake or live weight gain (LWG), but the individual variation in LWG was significantly higher ($p<0.01$) in the R-group than in the A-group. Due to a higher fat- and dry matter content, the milk suckled by the R-calves had a higher content of metabolizable energy (ME) than the milk given to the A-calves (3.5 vs 2.9 MJ/kg). Consequently, the two groups ingested approximately the same total amount of ME during the rearing period and therefore they also used almost the same amount of ME per kg LWG (A-calves 40 vs R-calves 42 MJ/kg LWG). There were no differences in either udder health or milk let-down between A- and R-cows.

It is concluded that the investigated restrictive suckling system for dairy calves gave less abnormal behaviour, rumination, eating of hay and consumption of concentrate but greater individual variation in milk ME consumption and in LWG compared to artificial milk feeding.

SAMMANFATTNING

Syftet med denna studie var att öka vår kunskap om hur man föder upp friska kalvar med inga eller få onormala beteenden och fördelaktig tillväxt, jämte kor som har hög mjölkavkastning och god juverhälsa. Studien undersökte effekterna av restriktiv digivning jämfört med artificiell mjölkutfodring.

Försöket utfördes på en privat mjölkgård i Aguascalientes i Mexiko. Tjugotvå Holstein ko- och kalv-par fördelades växelvis på två behandlingar där kalvar blev artificiellt mjölk-utfodrade i hinkar med flytnapp (A) eller tillåtna att dia kon restriktivt (R). A-kalvarna utfodrades med helmjölk från mjölkgropen två gånger om dagen, medan R-kalvarna hade möjlighet att dia under 30 minuter två gånger om dagen två timmar efter mjölkning. Kalvar och kor följdes från kalvens födelse till avvänjning vid åtta veckor. Korna mjölkades tre gånger om dagen. Alla kalvar hade fri tillgång till hö, kraftfoder och vatten. Beteende-observationer med 0/1-registrering med en minuts intervall gjordes två gånger i veckan under vecka 1, 3, 5 och 7. Alla kalvar vägdes före mjölkutfodring två gånger i veckan. R-kalvar vägdes även efter digivning för att skatta mjölkintag. California mastitis test utfördes och tiden för mjölknedsläpp registrerades en gång i veckan. Mjölkprover togs från diade kor och mjölk från mjölkgropen. Statistisk analys gjordes med variansanalys (blandad modell), Wilcoxon ranksummetest, Chi^2-test, F-test och Pearson korrelation.

Bland A-kalvarna förekom ”cross-sucking” (d.v.s. sugande på varandra) hos 67% av djuren medan endast 20% av R-kalvarna utförde detta beteende ($p<0,05$). I majoriteten (93%) av registreringarna av ”cross-sucking” sög kalven på en annan kalvs öra. A-kalvarna slickade signifikant ($p<0,05$) mer på inredningen än R-kalvarna. A-kalvarna hade signifikant fler registreringar av beteendena idissla, äta kraftfoder ($p<0,01$) och äta hö ($p<0,05$) jämfört med R-kalvarna. En genomsnittlig A-kalv konsumerade också mer än fyra gånger så mycket kraftfoder som en genomsnittlig R-kalv (22 jämfört med 5 kg). Det fanns inga skillnader i genomsnittligt mjölkintag eller tillväxt, men den individuella variationen i tillväxt var signifikant högre ($p<0,01$) i R-gruppen än i A-gruppen. På grund av ett högre fett- och torrsubstansinnehåll, hade mjölken som diades av R-kalvarna ett högre innehåll av omsättbar energi än mjölken som gavs till A-kalvarna (3,5 jämfört med 2,9 MJ/kg). Följaktligen intog de två grupperna ungefär samma totala mängd omsättbar energi under uppfödningsperioden och därför använde de också nästan samma mängd omsättbar energi per kg tillväxt (A-kalvar 40 jämfört med R-kalvar 42 MJ/kg tillväxt). Det förekom inga skillnader vare sig i juverhälsa eller i mjölknedsläpp mellan A- och R-kor.

Slutsatsen är att det undersökta restriktiva digivningssystemet för mjölkraskalvar gav mindre onormalt beteende, idissling, konsumtion av hö och kraftfoder men större individuell variation i intaget av omsättbar energi från mjölk och i tillväxt jämfört med artificiell mjölkutfodring.

RESUMEN

El propósito de este estudio fue incrementar el conocimiento sobre la crianza de becerros saludables con pocos o ningún comportamiento anormal así como mejores ganancias de peso, junto con vacas con una alta producción lechera y buena salud de la ubre. El presente estudio comparo el efecto del amamantamiento restringido contra la crianza artificial con leche.

El estudio se llevo a cabo en una granja privada en el estado de Aguascalientes, México. Veintidós pares de vacas y becerros Holstein fueron asignados alternadamente a uno de los dos tratamientos; los becerros fueron criados artificialmente con leche en cubetas con un chupón flotante (A) ó se permitió a los becerros mamar de su madre en un sistema restringido (R). Los becerros-A fueron alimentados dos veces por día con leche del ordeño, mientras que a los becerros-R se les permitió mamar por 30 minutos dos veces al día, dos horas después del ordeño. La información fue colectada en vacas y becerros desde el parto hasta el destete de los becerros a las ocho semanas de edad. Las vacas fueron ordeñadas tres veces al día. Todos los becerros tenían libre acceso a heno, concentrado y agua. Las observaciones de comportamiento fueron realizadas dos veces por semana en las siguentes semanas de edad 1, 3, 5 y 7; con un sistema de muestreo 0/1 con intervalos de un minuto. Todos los becerros fueron pesados dos veces por semana antes de ser alimentados. Adicionalmente, los becerros-R fueron pesados después de mamar para estimar el consumo de leche. Una vez por semana se realizaron pruebas de mastitis de California y se registro el tiempo de eyección de la leche para cada vaca. Se tomaron muestras de leche de las vacas antes y después del amamantamiento y leche del ordeño. Se realizaron los siguientes análisis estadísticos, análisis de varianza con un modelo de efectos combinados, la prueba de suma de rangos de Wilcoxon, prueba de Chi^2, prueba de F y correlaciones de Pearson.

El 67% de los becerros-A presentaron cross-sucking mientras que solo 20% de los becerros-R presentaron este comportamiento ($p<0.05$). En la mayoría de los casos en que se presento el cross-sucking (93%) los becerros estaban mamando la oreja de otro becerro. Los becerros-A lamían significativamente mas el interior del corral que los becerros-R ($p<0.05$). Los becerros-A presentaron significativamente mas registros de los comportamientos rumiar, comer concentrado ($p<0.01$) y comer heno ($p<0.05$) que los becerros-R. Un becerro-A promedio consumió cuatro veces más concentrado que un becerro-R promedio (22 vs 5 kg). No hubo diferencias significativas en consumo de leche promedio y ganancias de peso vivo (LWG), pero la variación individual en LWG fue significativamente mayor ($p<0.01$) en el grupo-R que en el grupo-A. Debido a un mayor contenido de grasa y materia seca, la leche consumida por los becerros-R tuvo un mayor contenido de energía metabolizable (ME) que la leche consumida por los becerros-A (3.5 vs 2.9 MJ/kg). Debido a esto, los dos grupos ingirieron aproximadamente la misma cantidad de ME por kg de LWG (becerros-A 40 vs becerros-R 42 MJ/kg LWG). No hubo diferencias en la salud de la ubre o el tiempo de eyección de la leche entre las vacas de los grupos A y R.

Concluimos que el sistema de amamantamiento restringido para becerros en un sistema de producción de leche, trae como consecuencia una disminucion en comportamientos anormales, rumia, consumo de heno y consumo de concentrado pero una mayor variación individual en consumo de ME en leche y en LWG cuando se compara con la crianza artificial con leche.

2. INTRODUCTION

2.1 BACKGROUND

The dairy calf is often removed from the cow immediately or shortly post partum according to common husbandry practice (Webster, 1984) and instead of suckling the cow, the calf is artificially milk-fed by open bucket or through artificial nipple, e.g. nipple pail, nipple bottle or automatic feeding devices (Hafez and Bouissou, 1975; Davis and Drackley, 1998). On the contrast, it is common in many tropical countries to allow the calf to suckle the residual milk from the cow after milking, so called restricted suckling. The Zebu cow, which is used for milk and meat production in tropical countries, generally needs the presence of her calf to induce milk let-down (Das et al., 2000). Earlier studies of restricted suckling show less abnormal behaviour (Sambraus, 1980; Albright and Arave, 1997; Das et al., 2000) and better health and growth in calves (Fallon and Harte, 1980; Chamberlain, 1989; Das et al., 1999; Jung, 2001) as well as lower mastitis incidence in cows (Knowles and Edwards; 1983; Mejia, 1994).

Depending on the type of cattle and the standard of the used technology, milk in Mexico is produced in either a specialized system (only producing milk), comprising 8% of the cows in Mexico, or a dual purpose system (producing both milk and meat), representing 67% of the cows. There is also a type of mix between the two systems, a semi-specialized production. In this system 25% of the Mexican cows are included, mainly cross-breds (Pulido, 2001). A previous project carried out in the tropical area of Mexico (El Clarin, Veracruz) during the summer of 2002 compared the effects of restricted suckling versus artificial milk feeding on dual purpose Zebu × Holstein cow- and calf pairs (Aspegren-Güldorff et al., 2003). The aim of the present study was to investigate how restricted suckling and artificial milk feeding affect behaviour and production of high-producing specialized Holstein cows and their calves in a temperate area of Mexico (La Escondida, Aguascalientes). Both studies are part of a project in the research of the Cow- and calf group within Food 21. The group works with questions concerning interrelationship between cow and calf at the beginning of the calf's life, a period that has a significant effect for the future of the animal. The aim of the research is to find methods to raise healthy calves with good weight gain and few or no abnormal behaviours along with cows of good milk yield and healthy udders.

2.2 BEHAVIOUR

2.2.1 Suckling behaviour

The terms concerning the act of suckling, used in this paper, are those adapted from Hall and co-workers (1988). *Suckling* is the infant's behaviour, e.g. recognizing a potential provider of milk, searching for and attaching to her teat, resulting in ingestion of milk. *Nursing* refers to the lactating mammal's activity when transferring milk to the young. *Sucking* is part of the suckling procedure and refers to the repetitive movements of the mouth that, through negative pressure on the teat or nipple, procure in the withdrawal of milk (Hall et al., 1988). The term *teat* is in this text used when referring to the actual teat of the cow and *nipple* when alluding to the artificial device.

After only a few minutes post partum a strong bond between cow and calf is established (Kilgour and Dalton, 1984) and if the calf is left with the cow it usually suckles the dam within six hours (Albright and Arave, 1997). Suckling of the calf's own dam almost always occurs in the normal opposite parallel position, while calves suckling an alien dam usually sneak up from behind (Waltl et al., 1995). Lidfors and her colleagues (1994) suggest three main phases during a suckling bout for beef cattle. The first step is the pre-stimulation: during a period of about one minute the calf performs sucking movements followed by release of teat and relatively high frequency of butting (Lidfors et al., 1994). Butting is when the calf vigorously butts the udder of the cow with its head (Hafez and Bouissou, 1975). The second phase is the milk intake: mainly rhythmic sucking movements of the same teat for an extended period and relatively low frequency of butting in the beginning, which is increasing towards the end. The third part is the post-stimulation: low frequency of butting together with sucking movements and releasing of teat in a declining rate until suckling bout finishes (Lidfors et al., 1994). The suckling bout lasts for about 10-12 minutes (Sambraus, 1980).

2.2.2 Abnormal behaviour

The main function of sucking seems to be obvious; to obtain milk. But also if the nutrition level is adequate, calves suck on each other- so called *cross-sucking*- or on the interior, if raised separated from the cow (de Passillé, 2001). The development of abnormal behaviour may start early, short bouts of tongue-rolling was observed within the first two weeks post partum in an experiment by Redbo (1992). It is assumed that there is a strong sucking motivation (de Passillé, 2001) which causes frustration in the case of sucking deprivation (Redbo, 1992; de Passillé, 2001). After a milk meal the calf is motivated to suck for a set amount of time (Gaboury and de Passillé, 1997). Deprivation may interfere with physiological functions such as digestion, involving insulin and cholecystokinin, as well as psychological satisfaction and therefore the welfare of the calf (de Passillé, 2001). Lidfors (1993) found that the ingestion of milk induces cross-sucking between calves. In a study by Margerison and co-workers (2003) non-suckling calves showed a peak of cross-sucking at one minute after milk feeding and the frequency of the behaviour declined linearly to almost zero at 13 minutes after the end of milk feeding. This suggests that the aroused motivation to suck wanes rather quickly (de Passillé, 2001). Once the calf is weaned from milk, a considerable drop in performance of cross-sucking is found (Lidfors, 1993; Krohn et al., 1999).

The drinking time of the calf affects its urge to suck (Hoyer and Larkin, 1954). In a study conducted by Krohn and co-workers (1999), the time spent on milk ingestion by suckling calves was eight times greater than that of calves fed in open buckets. To slow down the drinking rate, Hoyer and Larkin (1954) equipped the milk feeding bucket with a rubber nipple with a very small orifice. Calves drinking from an open bucket spent one to three minutes while those drinking from the nipple feeder spent 9-14 minutes. Cross-sucking was substantially reduced in the latter group (Hoyer and Larkin, 1954). Loberg and Lidfors (2001) concluded that access to a floating nipple with slow flow gave the lowest occurrence of cross-sucking and licking of interior in artificially milk-fed calves. Similar conclusions were drawn by de Passillé (2001). The later the calves are grouped together, the lower the frequency of cross-sucking tends to be (Albright and Arave, 1997).

Other ways to reduce cross-sucking is to give the calf a higher amount of milk, e.g. 5 litres compared to 1 or 2.5 litres, to offer hay immediately after the milk meal (Gaboury and de Passillé, 1997) or to leave the nipple bucket with the calf after milk intake (Jung and Lidfors, 2001). In an experiment by Gjestang (1983) calves housed in group-pens were confined to the

feed front and their own nipple bucket for three minutes after milk feeding with reduced cross-sucking as an effect. But even if the open bucket is equipped with a rubber teat, the duration of the milk intake generally is much shorter than the suckling bout on a cow (Redbo, 1992). Instead of all the management solutions to reduce abnormal behaviour, the simplest way might just be to let the calf suckle its own dam or another cow. Margerison and co-workers (2003) showed that cross-sucking was found more than five times as often in non-suckling as in suckling calves. In a study by Krohn and his colleagues (1999) non-suckling calves sucked the interior significantly more than the suckling calves.

2.3 FEED INTAKE

Early and frequent foraging behaviour, i.e. eating hay and concentrate, is important for an early development of the rumen of the calf (Roy, 1980; Davis and Drackley, 1998), which in turn is crucial if the calf is to be weaned to solid feed fairly early (Davis and Drackley, 1998). The most important factor for the level of concentrate intake is the milk intake (Olsson, 1981). A high milk intake will severely reduce the concentrate consumption (Roy, 1980; Davis and Drackley, 1998). Davis and Drackley (1998) recommend to feed calves a restricted amount of milk to encourage concentrate consumption. According to Roy (1980) the farmer should ensure that suckling calves have a daily milk intake of 5-9 litres of milk. Hay should be offered from week two and concentrate from week three. Artificially milk-fed calves, being weaned at eight weeks, should be given four litres of milk gradually decreasing to one litre at eight weeks of age. Like for suckling calves, hay should be offered from week two and concentrate from week three (Roy, 1980).

The intake of solid feed starts gradually. In a study conducted by Margerison and co-workers (2003) the calves hardly ate any hay at all during the first week after parturition and thereafter increased their intake to occupy more than 1% of their time at six weeks of age. No rumination occurred during week one, but the frequency of the behaviour increased steeply to reach a proportion of over 30% of the time at the age of six weeks. Further, artificially reared calves spent twice as much time consuming concentrate and consumed twice as much concentrate compared to suckling calves (Margerison et al., 2003). The artificially milk-fed calves in a study by Jonasen and Krohn (1991) consumed five times as much concentrate as the restrictively suckling calves. On the contrary, Franklin and her colleagues (2003) found no difference in concentrate consumption between bottle-fed calves and calves that had been allowed to suckle the dam for three days.

To estimate the milk intake of the suckling calf, one possibility is to depend on the transfer of tritiated water, via milk, from the cow to the suckling calf. The technique includes the use of deutarated water to determine the water turnover of the calf. However, this procedure relies on a steady-state system (Holleman et al., 1988), which might not always be the case with live animals, and is additionally relatively expensive. A simple and widely used way to estimate the milk intake of the calf, is to weigh the calf before and after suckling, the so called weigh-suckle-weigh method (Boggs et al., 1980; Sommerville and Lowman, 1980; Holloway et al., 1983; Beal et al., 1990; de Passillé et al., 1996). Jonasen and Krohn (1991) showed that suckling calves usually drink 7-10 kg milk per day. Martinsson (1983) demonstrated a milk intake of 9.4-11.3 kg during the first 40 days post partum in suckling calves, which were cross-breds between (Hereford × Swedish red and white cattle breed) × Charolais.

2.4 MILK COMPOSITION AND WEIGHT GAIN

The rate of weight gain and the development of gut tissues are dependent on the kind of feed offered to the calf from birth (Davis and Drackley, 1998). The concentration of milk fat, and consequently also the energy level, is higher in the residual milk than in the milk received during ordinary milking (Metz, 1987). Mejia (1994) found a 75% higher fat content in residual milk than in bucket milk. According to Chamberlain (1989), the milk obtained in the beginning of a milking session has a fat content of 1-2% while it is 7-9% in the end. This is due to the fact that the fat globules are not evenly distributed in the milk. The result will be that milk with high fat content tends to be left behind, while the milk received through the milking machine has a lower fat percentage (Chamberlain, 1989).

If artificially milk-fed calves are given milk from the milking parlour and restrictively suckling calves get to suckle the residual milk, and the amount of milk is the same, a higher weight gain rate can be expected in the group of suckling calves. Quite right, several reports have showed a higher rate of weight gain for restrictively suckling calves than for artificially milk-fed calves (Fallon and Harte, 1980; Knowles and Edwards, 1983; Metz, 1987; Jonassen and Krohn, 1991; Das 1999). As a contrast, Mejia (1994) reported a higher growth rate for artificially milk-fed calves than restrictively suckling calves at 75 days. There are also papers showing no difference between the two groups (Franklin et al., 2003).

2.5 UDDER HEALTH

Mastitis is an inflammatory reaction to bacterial or mechanical injury in the mammary gland of the lactating animal (Park and Jacobson, 1993). Predisposal factors are muddy environment, heat stress, unhygienic milking and over- and undermilking (Chamberlain, 1989). The consequences of mastitis may be decreased milk production and changed milk composition (Park and Jacobson, 1993). Most new infections of mastitis develop during the first month post partum and during the first three weeks of the dry period. The udder health is usually impaired with the age of the cow (Chamberlain, 1989). A number of studies have pointed at better udder health for nursing cows (Everitt et al., 1968; Knowles and Edwards; 1983; Krohn and Madsen, 1985; Chamberlain, 1989; Mejia, 1994). In a study by Krohn and co-workers (1990), where the cows were nursing the calf twice and milked twice a day during the first eight weeks of lactation, the suckled cows had a significantly lower incidence of subclinical mastitis than the control cows (Krohn et al., 1990). There may be two explanations for this. Firstly, the bactericidal saliva (Krohn et al., 1990; Ugarte, 1991) as well as the rubbing of the vigorous sucking of the calf (Rasmussen and Larsen, 1998) reduces the number of pathogens on the teat and secondly, the frequent udder emptying removes pathogens and thereby improves the udder health (Krohn et al., 1990; Ugarte, 1991). However, there are also reports showing that suckling had no effect on udder health status (Fulkerson et al., 1978).

2.6 MILK LET-DOWN

The removal of milk from the mammary gland requires adequate stimulation to result in the neurohormonal reflex that induces milk let-down. When the nerve-endings in the teat are

physically stimulated by the calf or milker, oxytocin is released into the blood stream from the pituitary gland. Oxytocin causes the myoepithelial cells surrounding the alveoli in the smaller ducts to contract vigorously and squeeze out milk into the milk reservoirs (Chamberlain, 1989; Park and Jacobson, 1993), where it can be removed by the negative pressure of the calf, milker or milking machine (Hafez and Bouissou, 1975). It has been proposed that the cow also can develop a conditioned reflex, associated with milking, and oxytocin is then released by stimuli like the sound or sight of her calf or the milking machine (Chamberlain, 1989). The cry of the young is an important auditory stimulus for the cow and it may result in an increased release of oxytocin (Albright and Arave, 1997). Suckling elicits a greater increase in oxytocin than milking (Akers and Lefcourt, 1982; Bar-Peled, 1995; Samuelsson and Svennersten-Sjaunja, 1996; Lupoli et al., 2001). According to a study by Tancin and co-workers (2001), primiparous cows responded to suckling with a higher level of oxytocin as compared with multiparous cows.

It generally takes about 45 seconds to induce milk let-down from the start of the stimulation (Chamberlain, 1989). For an optimal milk removal oxytocin has to be elevated during the entire milking (Bruckmaier and Blum, 1998). Bar-Peled and co-workers (1995) found that as long as high producing dairy cows were nursing their calves, there was a negative effect on the milk let-down in response to machine milking. Similar results were obtained by Krohn and his colleagues (1990). It is suggested that the cows voluntarily suppress milk ejection to ensure milk for their calves (Bar-Peled et al., 1995).

3. AIM

The aim of this study was to investigate the effect of restricted suckling versus artificial milk feeding in dairy calves and cows. The parameters of interest were abnormal, general and feeding behaviour and weight gain in calves, and udder health and milk let-down in cows.

The questions to be answered were:

1. Is there a difference in the amount of recordings on performing abnormal behaviour between the group of restrictively suckling calves and the group of artificially milk-fed calves?

   H_0: There is no difference in the amount of recordings on performing abnormal behaviour between the group of restrictively suckling calves and the group of artificially milk-fed calves.
   H_1: There is a difference in the amount of recordings on performing abnormal behaviour between the group of restrictively suckling calves and the group of artificially milk-fed calves.

2. Is there a difference between the group of restrictively suckling calves and the group of artificially milk-fed calves in the amount of recordings on performing active behaviour, represented by moving?

   H_0: There is no difference between the group of restrictively suckling calves and the group of artificially milk-fed calves in the amount of recordings on performing active behaviour, represented by moving.
   H_1: There is a difference between the group of restrictively suckling calves and the group of artificially milk-fed calves in the amount of recordings on performing active behaviour, represented by moving.

3. Is there a difference in the amount of consumed concentrate and/or the amount of recordings on eating concentrate between the group of restrictively suckling calves and the group of artificially milk-fed calves?

   H_0: There is neither a difference in the amount of consumed concentrate nor in the amount of recordings on eating concentrate between the group of restrictively suckling calves and the group of artificially milk-fed calves.
   H_1: There is a difference in the amount of consumed concentrate and/or the amount of recordings on eating concentrate between the group of restrictively suckling calves and the group of artificially milk-fed calves.

4. Is there a difference in weight gain between the group of restrictively suckling calves and the group of artificially milk-fed calves?

   H_0: There is no difference in weight gain between the group of restrictively suckling calves and the group of artificially milk-fed calves.
   H_1: There is a difference in weight gain between the group of restrictively suckling calves and the group of artificially milk-fed calves.

5. Is there a difference in udder health between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only?

   H_0: There is no difference in udder health between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only.
   H_1: There is a difference in udder health between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only.

6. Is there a difference in time to milk let-down between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only?

   H_0: There is no difference in time to milk let-down between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only.
   H_1: There is a difference in time to milk let-down between cows that are suckled by their calf and additionally milked and cows that are not suckled and milked only.

4. MATERIALS AND METHODS

4.1 ANIMALS

The study was carried out between May 19th and August 19th 2003 at La Escondida farm outside the city Aguascalientes in the centre of Mexico (semi-dry and temperate climate, 22°N, 102°W, altitude 1 900 m above sea level). The herd consisted of approximately 400 cows, mainly Holstein. Most of the cows were inseminated with Holstein, but heifers, in particular, were crossed with Jersey. Other breeds used in the breeding program were Red Holstein, Brown Swiss and Aberdeen Angus. The average yearly milk yield at the farm was about 8 000 kg, compared to 5 700 kg per cow and year in an average herd of intensive specialized production in Mexico (Ávila Téllez, 2002). Holstein cows giving birth between May 19th and July 9th 2003 were included in the experiment and cow- and calf pairs were allocated into two groups. For more information see 4.5 Experimental Design. The animals in the study are presented in Table 1.

Table 1. *Cow- and calf pairs participating in the study with cow-, lactation- and calf number, calving date, breed and sex of calf, treatment and annotations. Grey cell means that the animal was excluded from study*

Cow	Lact. No	Calf	Calv. Date	Breed	Sex	Treat.	Annotations
502	1	2198	19 May	H×J	F	A	
241	3	2199	20 May	H×J	F	R	Calf died 30 May, cow and calf excluded.
404	2	9642	27 May	H	M	R	
114	2	9643	27 May	H×RH	M	A	
243	3	2200	31 May	H	F	R	Cow mastitis 15 June, cow and calf excluded. Cow died later.
525	1	9644	1 June	H×J	M	R	Calf died 10 June, cow and calf excluded.
581	5	2201	3 June	H	F	A	Cow intestinal problems 4 July, cow excluded after 5 wks.
179	2	9645	3 June	H	M	R	Cow mastitis 22 June, cow excl. after 3 wks. Calf suckled 449.
315	3	9646	3 June	H×RH	M	A	Cow died 8 June, cow excluded.
75	2	9647	3 June	H	M	A	
348	1	2202	4 June	H×J	F	R	
625	4	9648	5 June	H	M	A	
103	2	2203	5 June	H	F	R	Calf died after weaning, 18 August.
591	2	9649	6 June	H×J	M	A	Calf died 10 June, cow and calf excluded.
536	2	9650	6 June	H	M	R	
17	5	2204	6 June	H	F	A	
673	2	9651	6 June	H	M	R	
658	1	2205	9 June	H×J	F	A	
449	2	2207	15 June	H×BS	F	R	Calf died 24 June, calf excluded. Cow nursed 9645.
140	2	2208	20 June	H	F	A	
369	3	9653	20 June	H	M	R	
142	1	2209	20 June	H×J	F	A	
1315	1	2211	25 June	H×J	F	R	
150	2	2212	25 June	H	F	A	
670	1	9656	28 June	H×J	M	A	Recorded for 7 wks
363	3	9657	28 June	H	M	R	Recorded for 7 wks
599	2	2218	9 July	H	F	R	Recorded for 5 wks

H=Holstein, J=Jersey, RH=Red Holstein, BS= Brown Swiss, F=Female, M=Male, A=Artificial milk feeding, R=Restricted suckling

4.2 HOUSING

The cows were kept in open paddocks with zero-grazing and were divided into groups according to milk production levels. The cows participating in the experiment were kept in the same paddock as newly parturated cows. The cow paddock measured 33 x 45 m^2 (Figure 1). There was a varying number of cows in the paddock, but usually about 35 cows. The ground in the paddock consisted of soil, was peat-like and mixed with faeces. It became rather muddy near the water tank, especially during rainfalls. To protect the cows from hot sun and heavy rain there was one bigger roof covering roughly one fourth of the paddock and one smaller roof along the side with feed. The feeding place had a concrete ground and there were 49 places to eat. When the cows were at the feeding place they were able to see the calf paddocks approximately 20 m ahead.

Figure 1. The cow paddock with the feeding place and the roof covering the cows from sun and rain. Photo: Emma Gratte

The common practice at the farm was to house calves in single pens, measuring 2.5 x 1.3 m^2, after the first intake of colostrum from the cow. The calves in the experiment were, from the beginning, kept in the same paddock (A) (Figure 2). When the number of calves exceeded 10 individuals, the calves were moved into two different paddocks, one for each treatment group. The sizes of the paddocks were 130 and 145 m^2 respectively (Figure 2). The two paddocks were symmetrically constructed and surrounded by sheep mesh, stone wall and wooden fence. There was a sheep mesh between the two paddocks that allowed physical contact between the two groups. The height of the fence was not below the height of 1.3 m anywhere. The calves were, just like the cows, protected from sun and rain by a roof covering about one fourth of each paddock. A milk-feeding pen, which was one of the wooden single pens in which the calves at the farm usually were housed, was used for the artificially milk-fed calves (A-calves) and situated in paddock A. The gates to the paddocks were made of metal and had an additional sheep mesh on the outside. The calves in the treatment with restricted suckling (R-calves) were let out to the suckling paddock through the gate (Figure 2).

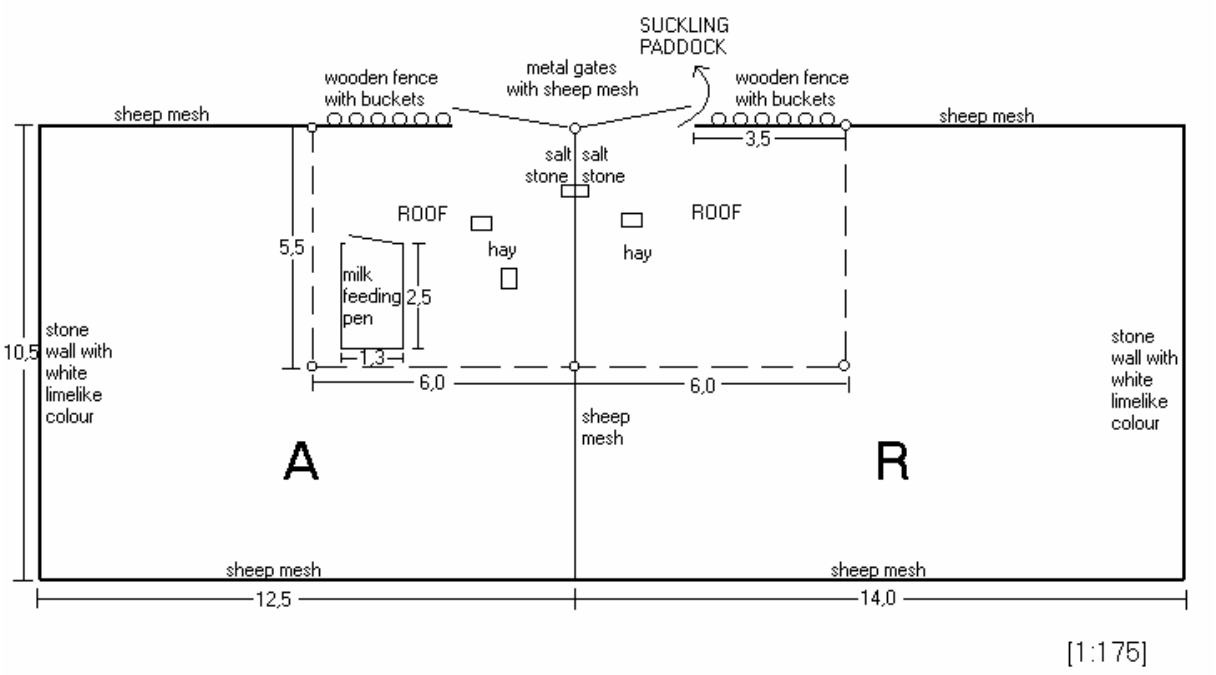


Figure 2. Illustration of the paddocks of the calves. The artificially milk-fed group (A-calves) were housed in the paddock named A and the restrictively suckling group (R-calves) in paddock R.

4.3 FEED

The cows were fed a total mixed ration of 23 kg DM a day, divided into three meals. The forage part, which constituted 50% of the diet, consisted of alfalfa hay, corn silage and green chop alfalfa. Corn, in different forms, covered most of the other part. The cows had free access to water.

4.3.1 Milk

Newborn calves, allocated to either group, were given colostrum from the mother in a plastic bottle with a rubber nipple as soon after parturition as possible. The calves were fed two litres of colostrum. Thereafter all calves were fed milk twice a day, around 0800 h and 1600 h, until week seven. During week eight the calves were fed milk only in the morning to get the calves to eat more concentrate and thereby prepare them for weaning. After week eight the calves were not given any milk at all. Weaning was made in groups on day 56 ± 2 days.

The A-calves were given fresh whole milk from the milking parlour. In the beginning they were fed with a nipple bottle, but as soon as they learned how to suck properly they were given milk in an open bucket with a rubber nipple (Albert Kerbl GmbH, Germany) (Figure 3a) floating on the milk, so that the calf had to suck the nipple in order to get milk. The A-calves were individually milk-fed in the milk feeding pen (Figure 2). The first two weeks all A-calves were given two litres of milk twice a day and as from week three they got three litres of milk twice a day. During week eight the A-calves were fed three litres of milk once a day. If the calf seemed sick and did not want to drink milk from the nipple of the bucket, the calf was fed milk from a nipple bottle instead.

The R-calves were allowed to suckle their dams (Figure 3b) two hours after the cows had been milked. Thus, the milk suckled by the R-calves both consisted of residual milk and the milk produced since the last milking two hours ago. The cows were brought from the cow paddock to the suckling paddock (26.5 x 12 m^2) situated right outside the paddocks of the calves (Figure 2). After this the R-calves were let out to their dams. As more calves were born, the number of suckling pairs in the paddock increased. Usually there where 12 cow- and calf pairs in the suckling paddock at the same time. Calves were kept to suckle their own mother as far as possible. If the cow did not have enough milk, which occurred occasionally, the calf was allowed to suckle another cow with remaining milk, after her own calf had finished suckling. From the moment the R-calves were let out from their paddock to the cows in the suckling paddock, they were allowed to suckle during a maximum of 30 minutes. If the calf showed signs of bloat, with remarkable swollen stomach, it was not allowed to suckle more than 15 minutes due to risk of impaired condition of the calf. The calf was then put back into the calf paddock, but it was still possible for the cow and calf to have physical contact above the fence even though the calf could not suckle the cow. Cows were prevented from having contact with the A-calves. When 30 minutes had passed, R-calves were put back into their paddock. After that the cows were brought back to the cow paddock. If an R-calf was observed to suckle poorly, for example if the calf was weak, it was given fresh whole milk from the milking parlour in a bottle.

(a) (b)

Figure 3. The group of (a) A-calves (artificially milk-fed calves) were fed in buckets with a floating rubber nipple (Albert Kerbl GmbH, Germany) and (b) R-calves (restrictively suckling calves) were allowed to suckle their dams for 30 minutes. Photo: Carlos E. Hernández, Emma Gratte, Albert Kerbl GmbH, Germany

4.3.2 Concentrate

The calves were given concentrate *ad libitum*. Concentrate was refilled every morning and if necessary also in the evening. To avoid wasting concentrate the calves were given an amount of concentrate that would leave approximately 300 g for the following morning. The concentrate was given in buckets (three in the group of A-calves and two in the group of R-calves) which were put up on the outside of the wooden fence (Figure 2). To protect the concentrate from rain, a white plastic can (50 x 40 x 40 cm) with one side cut away was put around every bucket. Thus, the calf had to put its head into the can to be able to eat concentrate from the bucket. The concentrate was bought in by the farm and consisted mainly of steam flake corn. Three similar types of concentrate were used, the first one for 25 days, the second one for 18 days and the last one for 26 days. The concentrate had an estimated metabolizable energy (ME) content of 12 MJ per kg concentrate. Concentrate was given with start on day 24 in the experiment, which implied that the three oldest calves did not get access to concentrate until the age of three weeks.

4.3.3 Hay, water and salt

All calves had *ad libitum* access to hay (alfalfa). The hay was put in white plastic cans with one side cut away and the cans (two in the group of A-calves and one in the group of R-calves) were put on the ground. Hay was refilled every morning and also through out the day if necessary.

Water was given in buckets put up on the wooden fence beside the buckets with concentrate (Figure 2). Each group had free access to water in an increasing number of buckets (around six at weaning time). Water was refilled after milk feeding in the morning, in the afternoon and, if necessary, also in the evening.

From day 41 in the experiment all calves had free access to a salt stone. One salt stone in each calf paddock was put upon a lying white plastic can, to keep it from getting dirty on the ground and to put it in the same level as the head of the calf.

4.4 MANAGEMENT

4.4.1 Milking

The cows were milked three times a day, at 0600 h, 1400 h and 2200 h, in a 8 x 2 unit herringbone parlour (Westfalia). The group of cows to be milked were moved to a collecting paddock outside the milking parlour. The roofed collecting parlour was equipped with sprinklers and fans if there was a need to cool the cows down during a hot day. Cows were then let in to the milking parlour.

Since there were quite a few milkers employed at the farm, the cows were slightly differently treated by different milkers. The main practice was to prepare two in the parlour consecutive cows for milking by turns. The teats were pre-dipped in a cleaning liquid, thereafter they were dried with a cloth. Before attachment of the milking cluster, the cows were foremilked a few squirts in a vessel to detect abnormal milk. If no milk ejection occurred the cow was treated intramuscularly with oxytocin. Another possibility to get more control over milking if needed was to press a button on the machine milking display to keep the milking organ from automatically dropping off the udder when there was low flow from all teats. This way the milking organ kept working until someone pressed the button again. After milking, the udder was checked and all teats were post-dipped in a bactericidal liquid. The type of liquid varied with the weather forecast, when rainy weather approached a thicker and stronger liquid was put on to cover the teats. Liquids used were for example chlorohexidin and iodine.

According to the routines at the farm, the cows were dried off either two months before calving or when the milk yield was less than six litres. Preventive treatment against mastitis in the form of dry cow therapy, i.e. intramammary infusion of antibiotics, was used during this period.

4.4.2 Paddocks

The cow paddock was cleaned when needed with a scraper pulled by a tractor. The manure and mud were taken away. The paddocks of the calves were cleaned a few times. Manure was taken away and slaked lime was spread out wherever needed to help dry out and prevent growth of bacteria in wet areas (Figure 3a). The fence was checked regularly to be intact and to keep the right height (not below 1.3 m). The paddocks were also kept free from any litter that might have blown inside the paddock.

4.4.3 Calf health

As a standard procedure the newborn calves at the farm were sprayed with a bactericidal and insecticidal liquid (Lepecid, Chlorpirifos 2.5%, Farmatec, Spain) where the umbilical cord had been. They were also orally given bovine ecolizer (*Escherichia coli* antibodies, Grand Laboratories Inc, U.S.). All calves were checked for diarrhoea every day. If a calf suffered from diarrhoea, the first treatment step was to mix the milk (A-calves) or some water (R-calves) with Diakur (lecithin coated orange fibre and electrolytes, Boehringer Ingelheim GmbH, Germany) and give it orally. If the calf did not recover, one of the veterinarians at the farm examined the calf as a second step. Usually the calf was then given Vedifloxacina (10%, Vedi de México, S.A. de C.V.) intramuscularly. The veterinarians could also prescribe electrolytes in the form of Lactolyte (Laboratorios Virbac México, S.A de C.V.) to be mixed with water and given orally about two hours after milk feeding. If the calf was very sick it was given electrolytes and mucosal protection through an esophagial tube. To reduce temperature as well as potential pain the calves were given Dipironas (Paracetamol, Tornel Laboratorios, México) intramuscularly. All calves were also vaccinated with an i. m. injection of Micotil (Elanco Animal Health, U.S.) against bovine respiratory disease complex (BRD).

4.5 EXPERIMENTAL DESIGN

Holstein cows parturating between May 19th and July 9th 2003 were included in the study (Table 1) with an exception for cows giving birth to twins, sick or dead calves. Within the primiparous and multiparous cows, the cow- and calf pairs were alternately allocated to the two treatments shown in Table 2. Since the heifers were inseminated with Jersey, the cross-bred calves also were distributed evenly between the treatment groups as a consequence. Cow- and calf pairs were followed from calving to weaning, a total of eight weeks (56 ± 2 days).

Table 2. *The different treatments of the cow- and calf pairs in the study*

Treatment	Calf	Cow
A	Artificial milk-feeding with whole milk in open buckets with floating nipple	No contact allowed between cow and calf
R	Restricted suckling of dam for 30 minutes two hours after milking of dam	Restricted nursing of calf for 30 minutes two hours after milking

Three R-calves and one A-calf died around one week of age. As a result three of the dams were excluded as well. The fourth, an R-cow (cow no 449), became 'foster mother' and nursed one of the R-calves (calf no 9645) that had lost its dam. There were four cows excluded due to sickness, two R-cows and two A-cows. Of those, one R-cow (cow no 179) and one A-cow (cow no 581) was included in the study until they got sick, i.e. three and five weeks, respectively. The reason for this is firstly, due to lack of animals the study could not afford to lose some valuable data and secondly, the time mentioned is enough to make a sufficient number of recordings to contribute to the results of the group. The calves of the excluded artificial cows could still remain in the project while one calf of the R-cows had to be excluded and the other one got a 'foster mother' as described above.

A total of 22 calves participated in the study, 12 A-calves (seven females and five males) and 10 R-calves (four female and six males). Accordingly, the total number of cows included in the experiment was 22. Of those cow- and calf pairs, one A-pair (no 670/9656) and one R-pair (no 363/9657) could only be followed for seven weeks and one R-pair (no 599/2218) only for five weeks due to the termination of the experiment. See Table 1 for more information.

4.6 RECORDINGS

4.6.1 Behavioural observations

Studies of the general behaviour of the calves were made in the evening at least two hours after the calves had been milk-fed, i.e. with a start between 1830 h and 2000 h. Calves were observed as focal animals during 60 minutes each and the observer watched one or two calves at the same time. Behaviour was recorded with 0/1-sampling at one minute intervals using a stopwatch. This means that if any of the behaviours defined in Table 3 was performed, then one mark was made in the protocol in the column for that specific behaviour on the row for that minute. When one mark had been made for a behaviour, it could not be marked again within the same minute. However, several different behaviours could be marked during the same minute. The number of marks for each behaviour for each calf during each observation study was summed up. Observation of general behaviour was made when the calf was one, three, five and seven weeks old. Each calf was studied twice a week, around the fifth and seventh day in the week depending on when the calf was born. Abnormal behaviour was defined as cross-sucking, licking interior and tongue-rolling. Active behaviour was represented by moving. Eating concentrate, eating hay and ruminating were regarded as foraging behaviours.

Figure 4. Behavioural observation. All recording work was shared among three persons. Photo: Carlos E. Hernández

Table 3. *Definitions of behaviours recorded in the evening*

Behaviour	Definition
Lie	Lying down in different positions
Stand	Standing up and being still
Move	Moving; walking, running, jumping
Social	Sniffing, rubbing against, pushing head against head, or mounting another calf
Eat hay	Chewing or ingesting hay
Eat concentrate	Chewing or ingesting concentrate
Drink	Swallowing water from water bucket
Ruminate	Regurgitating bolus of feed that was swallowed earlier, performing chewing movements with the mouth
Sniff interior	Nostrils inhaling air and muzzle being close to interior e.g. fence, pole, milk feeding pen
Lick self	Touching own body with tongue in repeated movements
Lick other calf	Touching body of another calf with tongue, either once or with repeated movements
Lick interior	Touching interior e.g. fence, pole, milk feeding pen with tongue
Cross-sucking	Sucking on any body part of another calf
Tongue-rolling	Tongue stretched out of the mouth and moved in a circulating way, or rolled around inside the mouth with half open mouth
Other	Any other interesting behaviours

4.6.2 Weighing of concentrate

After milk feeding in the morning the residuals of concentrate in the buckets were weighed using a manual scale. The concentrate that looked fresh and additional new concentrate were weighed out. Since the purpose was to give the calves free access to concentrate, it was tried to give the two treatment groups amounts resulting in about 300 g leftovers per group for the following morning. If there was a missing value the consumed amount of concentrate was estimated to be the average of the day before and the following day in that group.

4.6.3 Weighing of calves

Calves were weighed directly after birth. They were then weighed *before* milk feeding in the morning, on day three and day six each week up to and including week eight. During weighing each calf was weighed four times and an average was calculated. In five cases (calf no 2201, 2205, 9656, 9657 and 2218) the weaning weight was calculated due to missing weighing values. The weight gain of the calf was then assumed to be the average weight gain in the treatment group during the same period. The scale (Tru-test Pro II, New Zealand) had an accuracy of one tenth of a kg and was powered by a car battery. The complete scale consisted of a plate of wood upon two metal bars, the bars were connected to the display of the scale (Figure 5).

Figure 5. Weighing of calves. Photo: Emma Gratte

The milk intake of suckling calves was estimated by the weigh-suckle-weigh method. The R-calves were additionally weighed *after* suckling in the morning and again *before* and *after* suckling in the afternoon. This was done on day six each week to estimate weekly milk intake of the R-calves from birth to weaning. Due to the termination of the study the milk intake could not be estimated for week eight for three calves (calf no 2211, 9657 and 2218). In those cases the milk intake was assumed to be the calculated average of the estimated intakes of the rest of the calves in the same treatment group. Accordingly, the missing weighing values for week six and seven were calculated in the same way for the calf (calf no 2218) which was only followed for five weeks.

4.6.4 Milk samples

Milk samples were taken from the restrictively nursing cows once a week. Before suckling in the morning, the teats of the cows were cleaned with a cloth to remove potential post-dip left-overs. The teats were then foremilked a few squirts before the milk samples were taken. One test tube was used for each teat sample. This was done with the cows still in their paddock.

After the suckling period of 30 minutes milk samples were taken again. Those samples were taken with the cows in the suckling paddock.

Four samples of milk from different periods of the experimental period were taken from the milking parlour. All milk sample test tubes were prepared with bronopol and directly put in the refrigerator while waiting for being analysed.

4.6.5 Estimate of udder health

California mastitis test (CMT) was performed on the cows in the study before the morning milking once a week, around day seven. After the teats had been cleaned and dried, a few squirts of milk were foremilked from each teat. Then three to five squirts from each teat were collected in a CMT-pad, one cup per teat. Thereafter Diagmastin (Laboratorios Sanfer), a special CMT-solution, was put to each sample. The CMT reaction was classified according to the Scandinavian scoring system 1 to 5, used by Klastrup and Schmidt Madsen (1974). A sample estimated to 1 is negative (good udder health) and a sample estimated to 3 and over, a

distinct slime, is positive (Table 4). The California mastitis tests on the cows was performed by three persons, but if any hesitation about the classification appeared, we helped each other.

Table 4. *The Scandinavian scoring system for CMT, the equivalent U.S. score and the corresponding cell count (Klastrup and Schmidt Madsen, 1974)*

Scandinavian scoring CMT class	Equivalent U.S. CMT score	Corresponding cell count system range
1	Negative	<200 000
2	Trace (suspicious)	150 000-500 000
3	1 (suspicious)	400 000-1 500 000
4	2 (positive)	800 000-5 000 000
5	3 (positive)	>5 000 000

A record of all cows treated for mastitis and other diseases were kept regularly. Except for the preventive dry cow therapy, the cows suffering from mastitis were treated with e.g. Excenel (Ceftiofur 5%, Pfizer Inc).

4.6.6 Milk let-down

Once a week, either during morning or afternoon milking, the time to milk let-down was recorded. If a CMT was to be taken from the cow or if the cow was given an injection of oxytocin by the milkers before milking, no clocking was performed at that time.

The following times were noted: when the udder was touched to dry the teats with a cloth or to foremilk, when the milking cluster was put on the first teat and when the milk flow reached 0.3 kg per minute according to the display of the milking machine. The reason for this particular amount of milk was that 0.3 was the first step after 0.0 at the display. The time when the cluster was taken off automatically was also noted.

Figure 6. The herringbone milking parlour. Photo: Sofie Fröberg

4.7 ANALYSES

4.7.1 Milk samples

Some of the milk samples of the suckling milk had become sour or mouldy due to extended storing time, and could consequently not be analysed. The rest of the milk samples were analysed for fat, protein and lactose with infrared spectroscopy and a value for the dry matter (DM) content was given (FMA2001, Miris AB, Sweden). Some of the fresh analysed samples from *before* suckling did not have corresponding samples from *after* suckling, since it sometimes was hard to get any milk out of the teats after suckling. Those samples were not included when calculating average values for the suckling milk. Of the analysed samples left, only teats that had actually been suckled during that suckling occasion were included in calculations. Since the behaviour and suckling bouts of the calves were studied during suckling as an additional part, even though these observations are not included in this degree project, it was no problem to sort out which teats had been suckled and which had not. After this thinning out, 21 milk sample pairs remained. Those were from six different cows, alternating teats and different periods, and the average values were judged to be representative milk for the whole group of restrictively nursing cows.

4.7.2 Milk let-down

To calculate the time for milk let-down and the duration of milking (milking time) for further statistical analysis, the notified times on the protocol was used. The time to milk let-down was defined as the time between first touch of teat, e.g. drying with cloth or foremilking, and a milk flow of 0.3 kg per minute. The milking time was defined as from the moment the milking cluster was put on until it was taken off.

4.8 STATISTICAL METHODS

All the recordings from the protocols were transferred to Excel sheets. These were imported to SAS version 8.2 (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA, 1999-2001) where mean values, standard error and statistical tests were done. Wilcoxon rank sum test was used to test the treatment differences on the behaviour drink water. Chi^2-test was used to test the difference between treatments on both number of calves performing cross-sucking and California mastitis test of cows. All other behaviours were tested with Analysis of variance with the Mixed effect model to test the effect of treatment (A or R), week (1, 3, 5 or 7), sex (male or female), breed (Holstein or Holstein x Jersey) and treatment*week. Analysis of variance with the Mixed effect model was also used to test the effect of treatment (A or R), week (1, 2, 3, 4, 5, 6 or 7) and time of milking (morning or afternoon) on time to milk let-down and milking time. MINITAB® Statistical Software (Minitab Inc., State College, PA, USA, 2003) was used to make a box-plot to show the effect of week on time to milk let-down. F-test was used to test the difference in variance between treatments on live weight gain.

Pearson correlation was used to test correlations between:

- cross-sucking and social behaviour
- cross-sucking and lick interior
- lick interior and sniff interior
- lick interior and social behaviour

5. RESULTS

5.1 BEHAVIOUR

There were significant differences between the treatment groups on four of the recorded behaviours (Table 5).

Table 5. *Mean number of recordings ± S.E.M. per hour and p-values of effect of treatment and age of behaviours significantly different between A-calves (artificially milk-fed calves) and R-calves (restrictively suckling calves) (Analysis of variance)*

Behaviour	A-calves	R-calves	Treatment effect p-value	Age effect p-value
Ruminate	3.93 ± 0.73	1.25 ± 0.30	0.001	0.0003
Eat concentrate	2.49 ± 0.41	1.06 ± 0.21	0.006	0.0005
Lick interior	7.05 ± 0.85	4.29 ± 0.60	0.037	<0.0001
Eat hay	7.12 ± 0.83	4.08 ± 0.98	0.014	<0.0001

Seven of the recorded behaviours did not show a significant difference when comparing the two groups (Table 6).

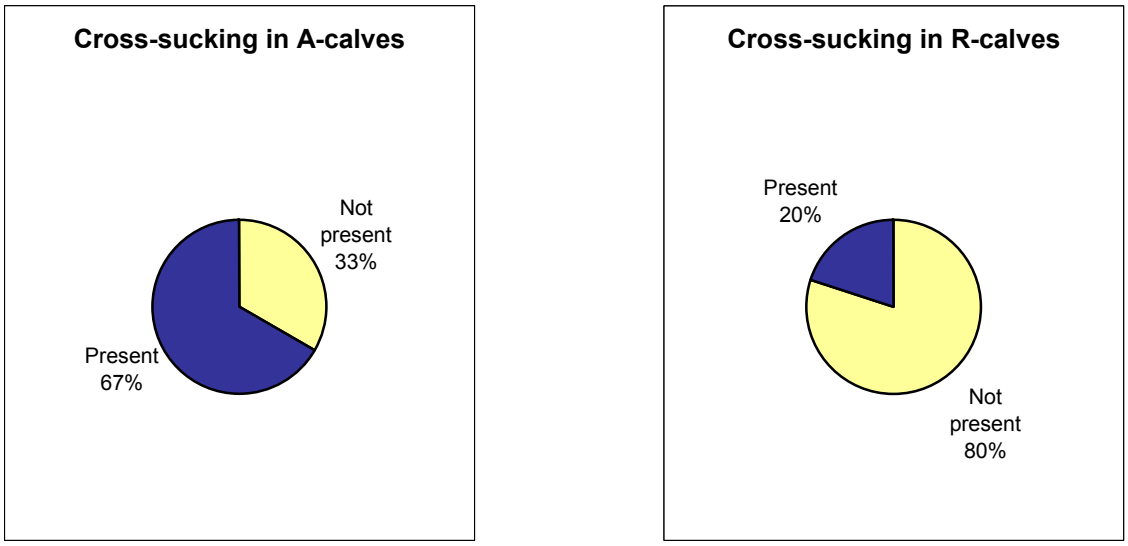
Table 6. *Mean number of recordings ± S.E.M. per hour and p-values of effect of treatment and age of behaviours* not *significantly different between A-calves (artificially milk-fed calves) and R-calves (restrictively suckling calves) (Analysis of variance)*

Behaviour	A-calves	R-calves	Treatment effect p-value	Age effect p-value
Move	12.16 ± 0.94	12.44 ± 1.23	0.34	<0.0001
Sniff interior	6.71 ± 0.86	6.81 ± 0.58	0.34	0.0028
Lie	31.90 ± 1.74	34.75 ± 1.68	0.55	<0.0001
Stand	29.51 ± 1.64	27.03 ± 1.66	0.65	<0.0001
Lick itself	12.01 ± 0.80	10.97 ± 1.11	0.71	<0.0001
Social	8.74 ± 0.92	8.11 ± 1.33	0.79	<0.0001
Lick other calf	2.17 ± 0.35	1.60 ± 0.42	0.85	0.0044

5.1.1 Abnormal Behaviour

The A-calves appeared to perform more cross-sucking (1.03 ± 0.33) than the R-calves (0.06 ± 0.04). However, cross-sucking was not normally distributed and was not performed by all calves. Therefore it was not tested with the Analysis of variance, but with Chi^2-test. Cross-sucking was performed by significantly more A-calves than R-calves (Figure 7). Cross-sucking did not occur during week one in any of the groups. In the group of A-calves the

behaviour was most frequently found during week three and five, while in the group of R-calves there was a slight increase in the occurrence during week five and seven.



(a) (b)

Figure 7. Presence of cross-sucking in the group of (a) artificially milk-fed calves and (b) restrictively suckling calves ($p<0.05$, $Chi^2 = 4.79$, Chi^2-test).

In the group of A-calves, there was a strong positive correlation between cross-sucking and social behaviour ($r=0.83$, $p<0.001$). There was also a tendency of a positive correlation between cross-sucking and lick interior ($r=0.56$, $p=0.059$). R-calves were not tested since there were too few occurrences of cross-sucking.

In the absolute majority of the cross-sucking recordings the calf was sucking the ear of another calf. Other regions that were subjected to cross-sucking were belly and mouth (Table 7).

Table 7. *Per cent of cross-sucking recorded on different body parts in total and by A- and R-calves respectively*

Body part	% cross-sucking in total	A-calves	R-calves
Ear	93	94	80
Belly	3	2	20
Mouth	2	2	0
Other	2	2	0
	100	100	100

There was a significant effect of treatment ($p<0.05$, $F=4.41$) on 'lick interior' (Table 5). The A-calves licked the interior significantly more than the R-calves. There was a significant positive correlation between 'lick interior' and social behaviour ($r=0.79$, $p<0.01$) and between 'lick interior' and 'sniff interior' ($r=0.66$, $p<0.05$). The behaviour 'lick interior' occurred in both groups during all weeks of recording. There was a significant effect of calf age ($p<0.0001$, $F=10.98$) on 'lick interior'. Both groups had an increase in the behaviour with age, even though it increased more among the A-calves than the R-calves (Figure 8). Tongue-rolling was not recorded in any group during any of the observations.

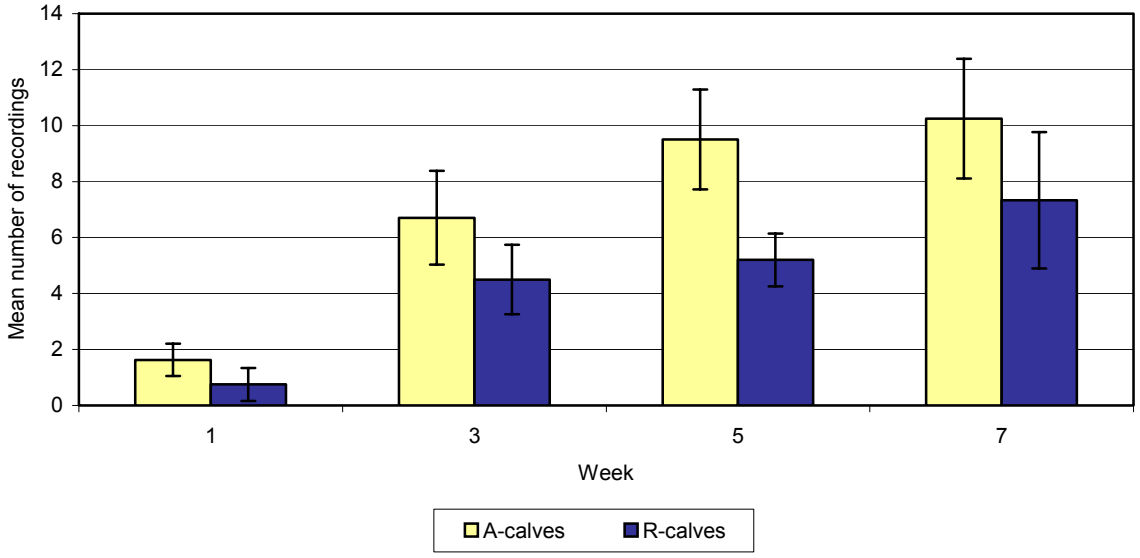


Figure 8. Mean number of recordings (± S.E.M.) per hour of the behaviour 'lick interior' in the group of A-calves (artificially milk-fed calves) and the group of R-calves (restrictively suckling calves) week 1, 3, 5 and 7.

5.1.2 Foraging behaviour

There was a significant difference between treatments ($p<0.01$, $F=7.67$) for 'eat concentrate' (Table 5). The A-calves had a higher number of recordings of 'eat concentrate' than the R-calves. There was also an effect of calf age ($p<0.001$, $F=6.31$). The calves did hardly eat any concentrate at all during week one, while the behaviour occurred more often during week three, five and seven in both groups, particularly in the group of A-calves (Figure 9).

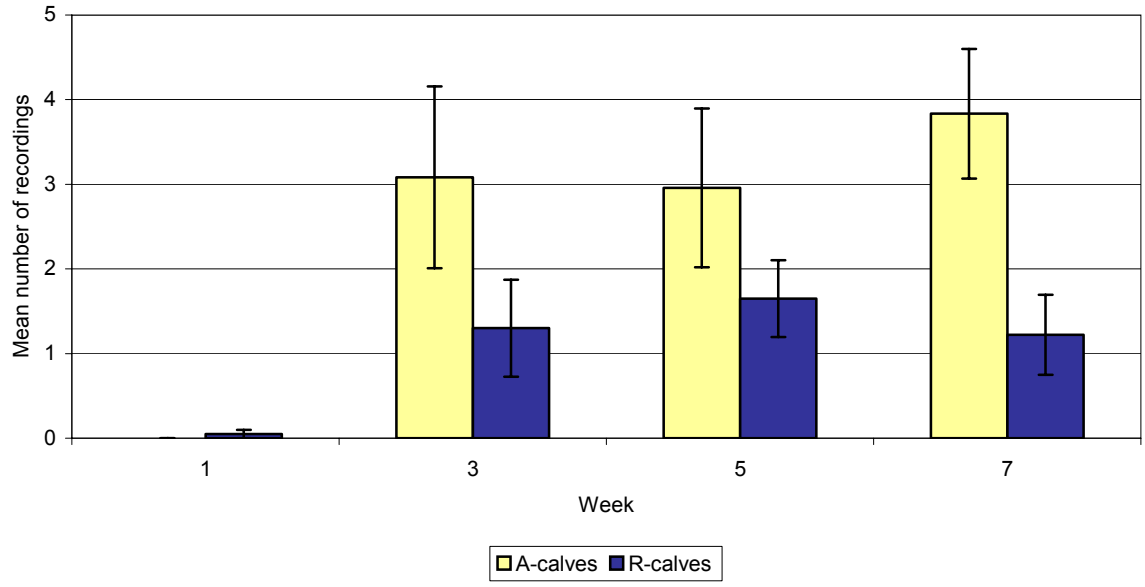


Figure 9. Mean number of recordings (± S.E.M.) per hour of the behaviour 'eat concentrate' in the group of A-calves (artificially milk-fed calves) and the group of R-calves (restrictively suckling calves) week 1, 3, 5 and 7.

Correspondingly, the A-calves had significantly more recordings ($p<0.05$, $F=6.24$) of 'eat hay' than the R-calves (Table 5). There was also an effect of calf age ($p<0.0001$, $F = 16.00$), almost no calves ate any hay during week one. The behaviour was more frequently recorded during week three in both groups. The number of recordings of 'eat hay' increased even more during week five and seven, especially in the group of A-calves (Figure 10).

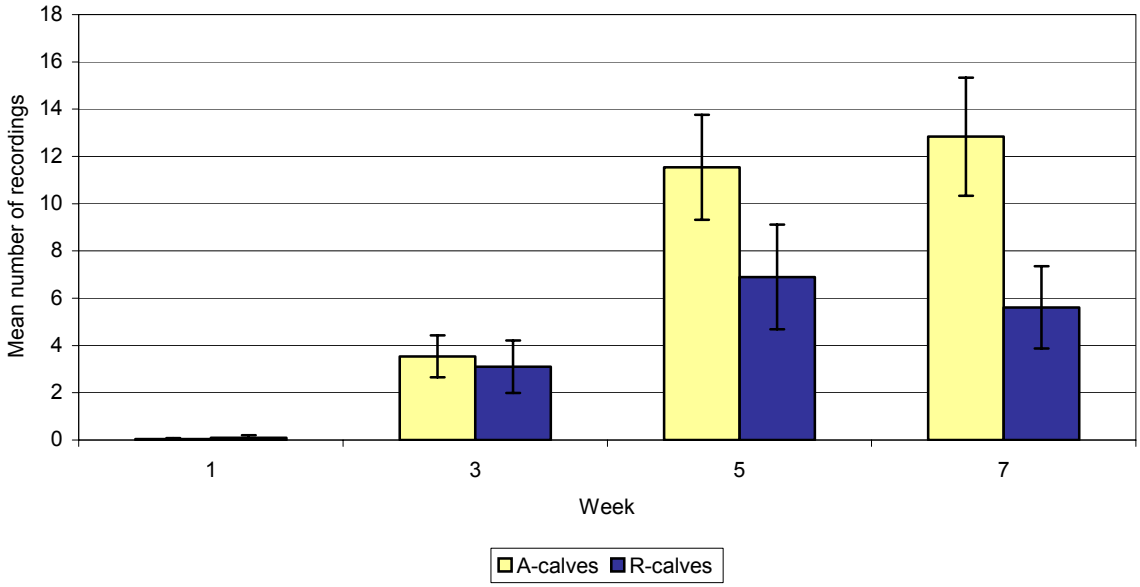


Figure 10. Mean number of recordings (± S.E.M.) per hour of the behaviour 'eat hay' in the group of A-calves (artificially milk-fed calves) and the group of R-calves (restrictively suckling calves) week 1, 3, 5 and 7.

There was a significant effect of treatment ($p<0.01$, $F=10.86$) on the behaviour 'ruminate' (Table 5). The A-calves performed more rumination than the R-calves. There was also an effect of calf age ($p<0.001$, $F=6.72$). Rumination did not occur in any of the groups during week one. The number of recordings then increased during week three, five and seven in the group of A-calves, while there was no particular increase in the group of R-calves (Figure 11). There was further an effect of the interaction between treatment and week ($p<0.05$, $F=3.45$).

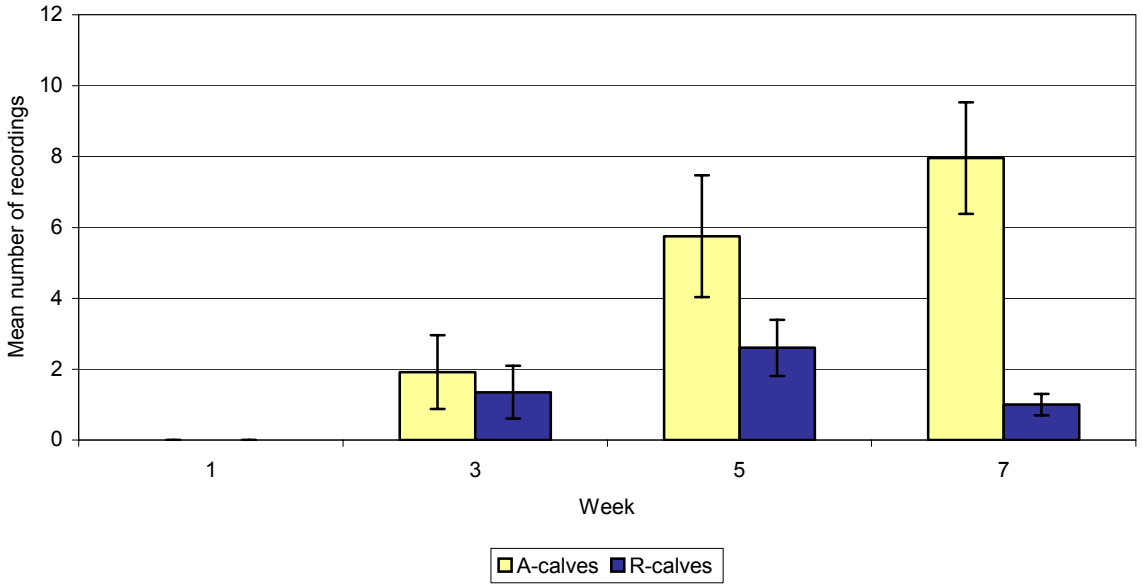


Figure 11. Mean number of recordings (± S.E.M.) per hour of the behaviour 'ruminate' in the group of A-calves (artificially milk-fed calves) and the group of R-calves (restrictively suckling calves) week 1, 3, 5 and 7.

The behaviour 'drink water' was not normally distributed. Therefore it was not tested with the Analysis of variance but with a non-parametric test. A tendency of an effect of treatment ($p<0.1$, Wilcoxon rank sum test) for 'drink water' was found. R-calves (1.47 ± 0.29) tended to have a higher number of recordings of the behaviour than A-calves (0.86 ± 0.18). Although not statistically tested, there seemed to be a difference in the behaviour 'drink water' with age. There was a small increase in number of recordings during week five in the group of A-calves. The group of R-calves had a bigger increase during the same week.

5.1.3 Other behaviours and effects of breed and sex

Except for those behaviours already mentioned under 5.1.1 Abnormal Behaviour and 5.1.2 Foraging behaviour, there was a significant effect of calf age on the following behaviours: 'stand' ($p<0.0001$, $F=23.38$), 'lie' ($p<0.0001$, $F=23.03$), 'social' ($p<0.0001$, $F=16.12$), 'move' ($p<0.0001$, $F = 14.08$), 'lick self' ($p<0.0001$, $F=10.13$), 'sniff interior' ($p<0.05$, $F=4.89$) and 'lick other calf' ($p<0.05$, $F=4.55$). The behaviours 'stand', 'move', 'lick self', 'sniff interior', 'lick other calf' and performance of social behaviour increased with age of calf. On the contrary, with increasing age of calf there was a decreasing number of recordings of 'lying'.

An effect of breed was found in the following behaviours: 'social' ($p<0.0001$, $F=17.01$), 'move' ($p<0.01$, $F=8.70$), 'ruminate' ($p<0.05$, $F=6.56$) and 'sniff interior' ($p<0.05$, $F=5.33$). There were significantly more recordings on Holstein × Jersey calves performing social behaviour, moving and sniffing interior compared to pure Holstein calves. The Holstein × Jersey calves also seemed to perform more cross-sucking than pure Holstein calves, even though this was not statistically tested. Pure Holstein calves performed more rumination than Holstein × Jersey calves. There was no significant effect of sex on any of the behaviours tested.

5.2 WEIGHT GAIN

5.2.1 Concentrate consumption

An average A-calf consumed 21.6 kg of concentrate during the eight weeks of study, while an average R-calf ate 4.8 kg. In other words, an A-calf consumed more than four times as much concentrate from birth to weaning as an R-calf.

5.2.2 Milk intake

All A-calves got the same allowance of milk, 287 kg, during the period from birth to weaning. It was distributed as described in 4.3.1 Milk. One litre of milk was assumed to be equal to one kg of milk. According to the weigh-suckle-weigh method, the average intake of milk among the R-calves was 285.8 ± 2.7 kg. As showed in Figure 12 the individual intake varied a lot.

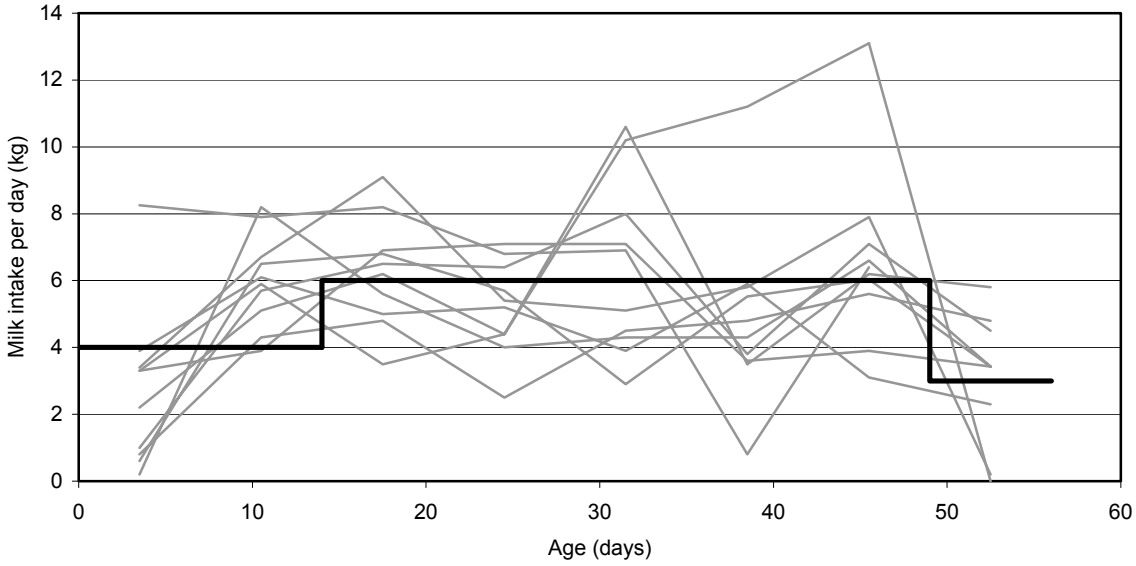


Figure 12. Milk intake per day, from birth to weaning, of individual R-calves (restrictively suckling calves) showed with thinner grey lines and any A-calf (artificially milk-fed calf) presented in form of a thicker black line.

5.2.3 Live weight gain

The live weight gain (LWG) from birth to weaning in the two groups were very similar, 26.1 ± 1.4 kg for an average A-calf and 26.2 ± 3.9 kg for an average R-calf (Figure 13). The A-calves had an average daily weight gain of 0.472 kg per day and the R-calves of 0.476 kg per day. However, the treatment effect on individual variation in LWG was greater in the group of R-calves than in the group of A-calves. The variance quotient was significant ($p<0.01$).

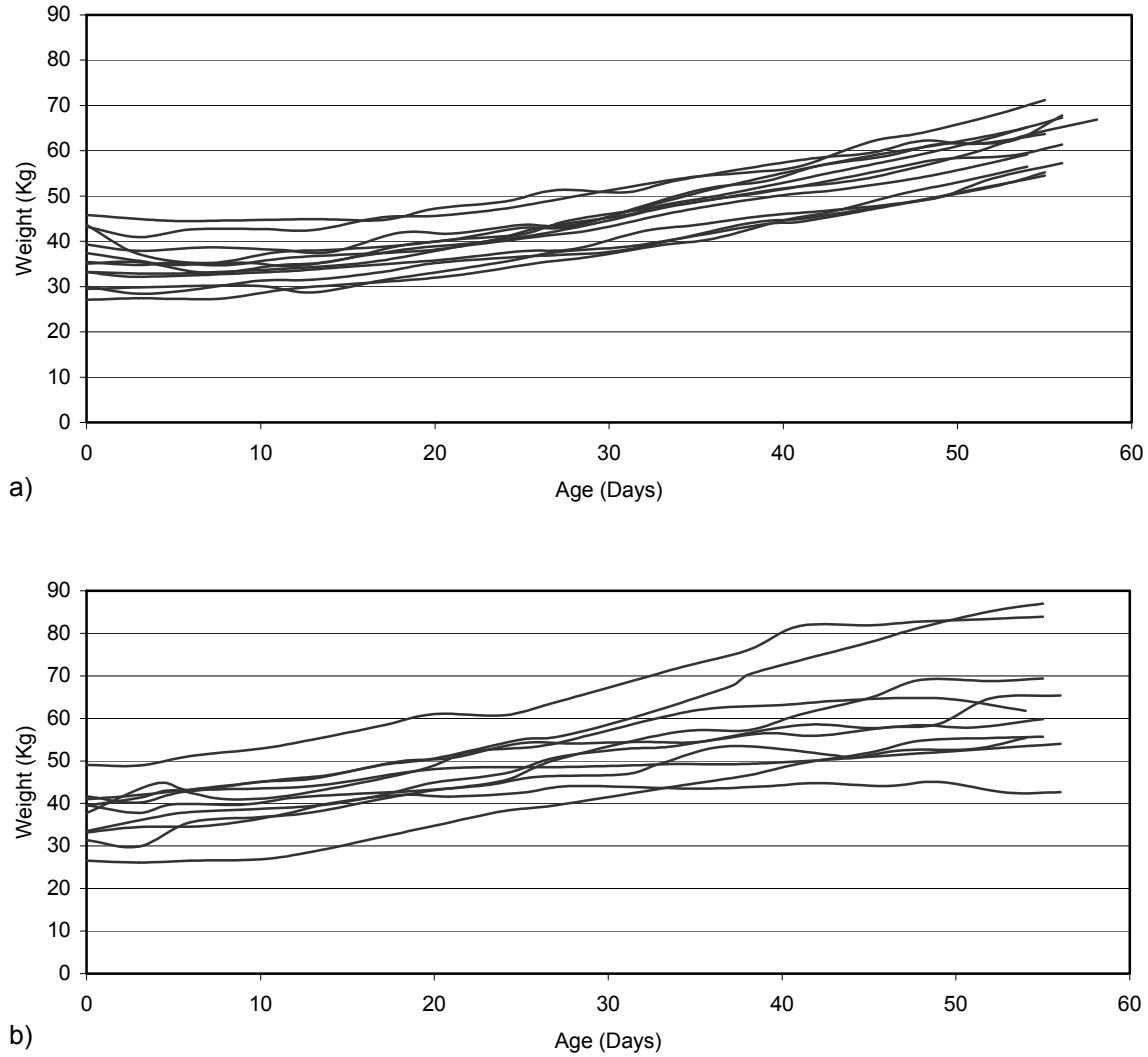


Figure 13. Live weight gain from birth to weaning in the group of (a) A-calves (artificially milk-fed calves) and (b) R-calves (restrictively suckling calves). Each line shows an individual calf's weight gain.

5.2.4 Energy conversion

The results of the analysed milk samples from the R-cows and the milking parlour are presented in Table 8.

Table 8. *Results of analyses of samples of suckling milk from the R-cows (restrictively suckled cows) and milk from the milking parlour*

	Fat (%)	Protein (%)	Lactose (%)	Dry Matter (%)
Milk before suckling	*3.99*	*2.85*	*4.92*	*12.49*
Milk after suckling	*8.12*	*2.55*	*4.64*	*15.88*
Average suckling milk	6.05	2.70	4.78	14.19
Average parlour milk	4.16	3.12	4.72	12.81

The ME content of milk was calculated according to Tyrrell and Reid (1965):

Gross energy (GE) = 0.00924 * (41.84 * Fat% + 22.29 * SNF% - 25.58)
ME = 0.92 * GE

where solids-non-fat (SNF) = Dry Matter (DM)% - Fat%. According to these calculations an A-calf got 2.90 MJ ME per kg parlour milk and, due to a higher fat- and dry matter content, an R-calf got 3.48 MJ ME per kg suckling milk. If these energy contents are multiplied with the respective intake of milk during the experimental period, an A-calf got 833 MJ ME from milk and an R-calf got 994.

The respective concentrate intake of the calves during the experimental period multiplied with the ME level of 12 MJ per kg concentrate makes 259 MJ ME for an A-calf and 57 MJ ME for an R-calf.

Thus, the total ME intake during the experimental period from milk and concentrate gave an A-calf 1092 MJ and an R-calf 1051 MJ. Since the hay intake could not be measured, it was not included in the total energy ingestion. If the total ME intake is divided with the weight gain for the experimental period, we get the energy conversion. It was 42 MJ ME per kg LWG for the A-calves and 40 for the R-calves.

5.3 UDDER HEALTH

5.3.1 California mastitis test

A comparison of the results of the CMT scores of cows in the two treatment groups, during week two and seven, showed only small differences. During week two, 12.8% of the A-cows and 12.5% of the R-cows had CMT results classed as 3 to 5, which implies poor udder health. Five weeks later, the udder health had become worse in both groups. The proportion of cows with a CMT score between 3 and 5 had increased to 20.5% among the A-cows and to 21.9% among the R-cows. If the CMT scores of all eight weeks were included, there was no statistical difference ($p>0.1$) between treatment groups if classes were merged (1 to 2 and 3 to 5). However, if classes were treated separately (1, 2, 3, 4 and 5) there was a tendency ($p=0.082$, $Chi^2 = 8.29$, Chi^2-test) to a better udder health in the group of R-cows (Figure 14).

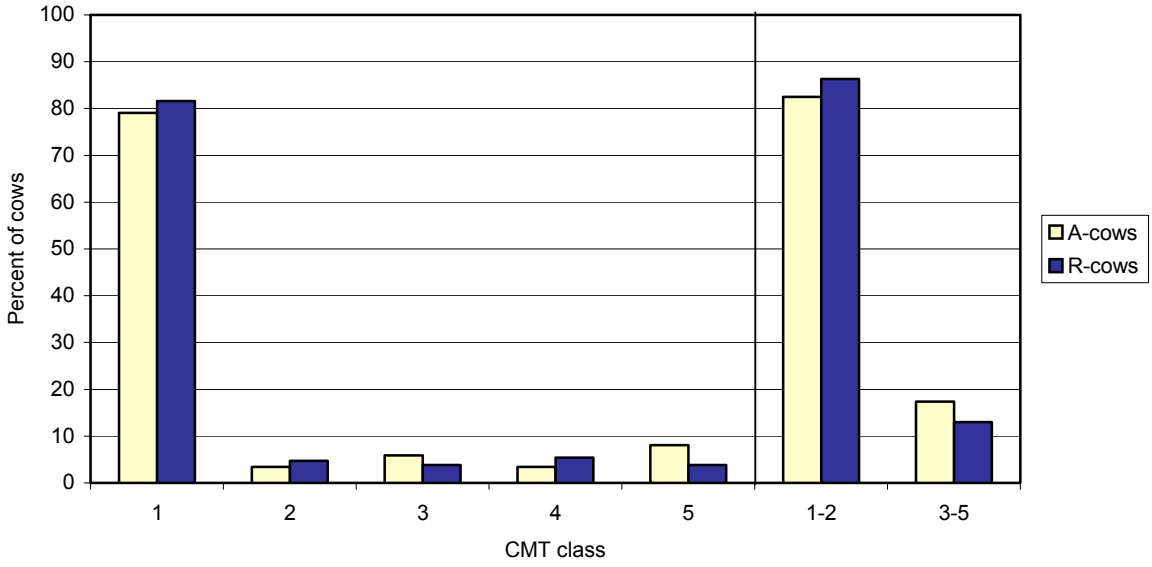


Figure 14. Result of CMT scores of A-cows (non-suckled cows) and R-cows (restrictively suckled cows). Columns show both separated and merged CMT classes when all eight weeks are included.

5.3.2 Incidence of mastitis

In both treatment groups mastitis was most common within the three weeks following parturition. In the group of A-cows 45% were treated for mastitis while it was 55% in the group of R-cows. No analysis was done on which type of mastitis bacteria that had infected the cows.

5.4 MILK LET-DOWN

5.4.1 Time to milk let-down

There was no effect on the time to milk let-down, either of treatment or of time of milking. The average time to milk let-down was 68 seconds for the R-cows and 61 seconds for the A-cows. In contrast, there was a significant effect of week ($p<0.001$, $F=3.93$). There was an increased time to milk let-down with increased time after parturition, except for week eight (Figure 15).

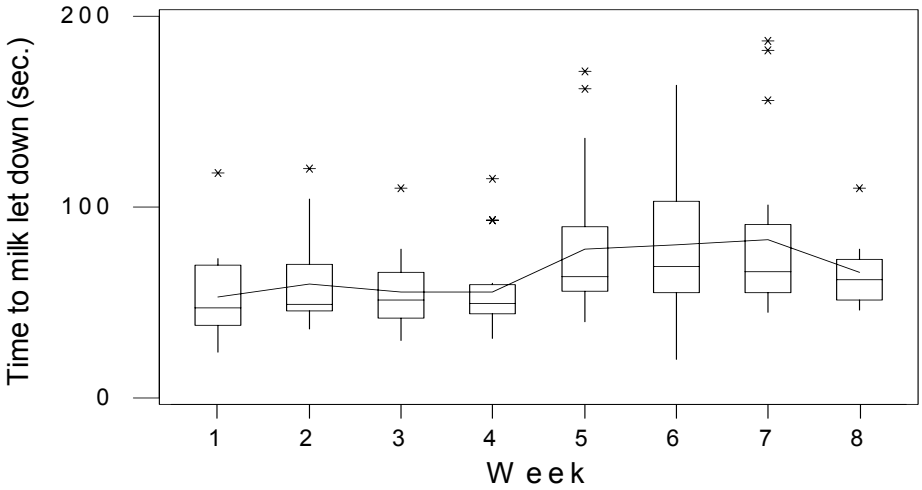


Figure 15. Box-plot showing time to milk let-down, week 1 to 8 after parturition. The line shows mean values, the horizontal lines show q_3, q_2 (median) and q_1, whiskers show min and max within inner fences and asterisks show outliers.

5.4.2 Milking time

Significant effects of treatment ($p<0.05$, $F=6.85$) and time of milking ($p<0.01$, $F=14.61$) were found for milking time. The average milking time of the R-cows was 5 minutes and 30 seconds, while it was 4 minutes and 46 seconds for the A-cows. The milking session in the afternoon was, on average, 1 minute and 10 seconds faster than the one in the morning.

5.5 MISCELLANEOUS RESULTS

All calves, no matter which treatment, got diarrhoea one time or more during the observation period. It usually occurred during the first week of life. A few calves did not recover. Four calves, three R-calves (calf no 2199, 9644, 2207) and one A-calf (calf no 9649), died of dehydration in connection to diarrhoea, one had an additional pneumonia (calf no 2207). Two cows died, an R-cow (cow no 243) due to intestinal problems and mastitis and an A-cow (cow no 315) due to clostridial infection (Table 1).

The R-cows appeared to have a strong bond to their calves. When the suckling period was over and the calves had been taken back to the calf paddock, it happened more than once that a cow, in a non-supervised moment, went through the fence to the paddock of the R-calves.

Both R-cows and, in particular, A- and R-calves learned quickly when it was time for milk feeding. The calves were standing along the gate to the paddock and the R-cows were standing at the feeding place, watching the calves and vocalizing.

6. DISCUSSION

This study shows that calves that have been artificially milk-fed in open buckets with a floating nipple (A-calves) perform more abnormal behaviours, such as cross-sucking and licking of interior, than calves that have been able to suckle their dam (R-calves). This is in agreement with earlier observations (Krohn et al., 1999; Margerison et al., 2003). Even though a sufficient level of nutrients was supplied to the artificially milk-fed calf, the instinctive need for sucking was not satisfied. Compared to the suckling calf the sucking time is diminished by 90% in the artificially milk-fed calf (Sambraus, 1980). A possible way to reduce the occurrence of cross-sucking and licking of interior, according to our study, is to give the calf restricted access to the cow at least 30 minutes twice a day. The majority of the A-calves performed cross-sucking, while the major part of the R-calves did not.

In more than 90% of the observations, the target of the cross-sucking was the ear. This is substantially more than in previous studies. In one study, 65% of the cross-sucking was directed at the belly and only 20% at the ear (Loberg and Lidfors, 2001) and in another experiment, the mouth was subjected in 40% of the observations and the ear in 35% (Lidfors, 1993). In a third study, 80% of the cross-sucking was directed at the mouth and 10% at the ear among the suckling calves, while 80% was directed towards the udder and scrotum and 10% at the ear among the artificially milk-fed calves (Margerison et al., 2003). One reason for the calves to suck on each other's ears might be that the ear is one of the most easily accessible body parts for sucking. The belly was the other region subjected to cross-sucking among the R-calves, even though there were not many observations. An explanation, as proposed by Margerison and her colleagues (2003), could be that the calves that are used to suckling have learned to search for milk in that region of another animal.

The positive correlations between performing abnormal behaviour, acting social and sniffing interior show that it seemed to be the most social and curious calves that were most prone to cross-sucking and licking of interior. Generally, it was noticed that both groups of calves were relatively occupied with licking the interior. As a matter of fact, both treatment groups spent as much time on licking the interior as on eating hay. Wiepkema and co-workers (1987), who studied veal calves, found that the occurrence of different abnormal behaviours had different developments over time. They suggested that deprivation of sucking can constitute a conflict during the first 10 weeks of the calf's life and that this stress could result in abnormal biting and licking. Another type of conflict, which could involve tongue-rolling, can arise if there is an absence of roughage during the following 10 weeks of the calf's life (Wiepkema et al., 1987). That might explain why we did not observe any tongue-rolling among our calves. It is more common in older animals and further, our calves had free access to hay.

The calves started to eat hay and concentrate after the age of one week, which is in accordance with the literature (Roy, 1980; Margerison et al., 2003). In a study of Holstein and Jersey calves by Swanson and Harris (1958), a few calves began rumination as early as five days post partum. All Holstein and all but one Jersey were ruminating by the age of four weeks (Swanson and Harris, 1958). No calves in our study were observed ruminating before one week of age. From week three the behaviour became more and more frequent, particularly among the A-calves. The A-calves ate four times as much concentrate as the R-calves. Earlier work have demonstrated artificially milk-fed calves to eat two (Margerison et al., 2003) and five times (Jonasen and Krohn, 1991) as much as restrictively suckling calves. The A-calves in our study also spent much more time on performing foraging behaviours like eating hay and concentrate. Those foraging characteristics are crucial for the calf to stay healthy with a good weight gain both before and especially after weaning (Roy, 1980). Thus,

the A-calves should be better off since they quickly learned to eat considerable amounts of concentrate and hay. After weaning all calves in the study were put in single pens to make sure they had a sufficient intake of concentrate and were enough developed to let them out in a bigger group of calves. When visiting the weaned calves in the single pens after the end of the study, we noticed that the A-calves seemed to be in a better condition with a shinier coat. However, no data was collected. Calculations based on Olsson (1981) and Olsson (pers. comm.) show that the milk intake of the calves would be enough for a weight gain, from birth to weaning, of 34 kg for the A-calves and 38 kg for the R-calves. The average weight gain in the two treatment groups was only 26 kg, though. The poor weight gain could for example be due to high infection pressure, extreme weather changes and big paddock surfaces, all resulting in more energy consumption for maintenance and play and less for weight gain. Further research needs to be carried out to see how well artificially milk-fed and suckling calves held in group manage after weaning.

Although the average milk intake of the R-calves during the eight weeks was similar to the allowance of the A-calves, the R-calves obtained more metabolizable energy (ME) from the same amount of milk. That is due to the fact that the milk suckled by the R-calves contained 45% more fat, had a 10% higher dry matter content and consequently contained 20% more ME than the parlour milk given to the A-calves. The calves in the different treatment groups had similar energy conversion, but used different sources of energy. The A-calves got a considerable part, about 30%, of the energy from concentrate, while the R-calves only got 5%. A reasonable suggestion is that the R-calves got enough energy from the milk and thus they were not as motivated as the A-calves to eat concentrate.

The average LWG during the study period of eight weeks was the same in the two treatment groups. That is in line with Franklin and colleagues (2003), even though those calves were only allowed to suckle for three days. A variety of articles (Fallon and Harte, 1980; Knowles and Edwards, 1983; Metz, 1987; Jonassen and Krohn, 1991; Das, 1999) report a higher growth rate for restrictively suckling calves. Mejia (1994) demonstrated the opposite, artificially milk-fed calves had a higher rate of weight gain at 75 days. But after 175 days there was no difference between the groups (Mejia, 1994). The average daily weight gain of all calves in our study was about 0.47 kg. Jonasen and Krohn (1991) demonstrated an average daily gain of 0.63 kg (bucket-fed) and 1.20 kg (suckling) during the first six or eight weeks in dairy calves. In a study by Knowles and Edwards (1983), Friesian cross-breds had an average daily gain of 0.35 kg (bucket-fed) and 0.47 kg (suckling) during the first 10 weeks. Friesian bull calves had a weight gain of 0.53 kg (artificially nipple-fed) and 0.63 kg (suckling) between the age of 1 and 12 weeks (Fallon and Harte, 1980). The relatively low daily weight gain in our calves is to be put in contrast to the fact that all calves suffered from diarrhoea at one time or another. The graphs of the weight gain of individual A-calves were more parallel and coherent than the divergent ones of the R-calves, showing a significant individual variation. There was a huge difference between individual R-calves, the heaviest weighed 87 kg at weaning while the lightest weighed 43 kg. A more uniform group of calves implies easier management, for example when it comes to deciding feeding ration, ensuring that all calves get to eat and deciding time for slaughter.

The udder health was about the same in both groups of cows, as showed by Fulkerson and co-workers (1978). However, several studies have displayed better udder health for nursed cows (Everitt et al., 1968; Knowles and Edwards, 1983; Krohn and Madsen, 1985; Chamberlain, 1989; Krohn et al., 1990; Mejia, 1994). It should be mentioned that the cows were suckled by more than one calf in some of the studies. The udder health was impaired with time post partum in both groups and half of the cows in the experiment got mastitis. The cows got the infection within three weeks post partum, which is in accordance with Chamberlain (1989)

who asserts mastitis to develop during the first month. In general the udder health among the cows was poor, 17% of the A-cows and 13% of the R-cows had California mastitis tests (CMT) classed as 3 to 5. Contributing factors could have been that the paddocks were muddy especially during the heavy rainfalls and further, the cows were often overmilked since a high vacuum was left on working long after the teat was milked.

In contrast to the results of Krohn and his colleagues (1990) and Bar-Peled and co-workers (1995), there was no difference in time to milk let-down between the R- and A-cows. What we did find, was a difference in milking time. That might indicate that the R-cows to some extent withhold the milk when machine milked, as described by Bar-Peled and co-workers (1995). The tendency to slower let-down of milk with week post partum could have its explanation in the transition of the udder, from the newly parturated cow's heavily filled udder which is almost bursting with milk to a relatively ordinary milk-producing udder. The cows in our study let the milk down slower than in the literature, 61 (A-cows) and 68 seconds (R-cows) compared to 45 seconds (Chamberlain, 1989). This time difference indicates that the cows in our study let down milk for the artificial oxytocin which was randomly injected when the cow entered the milking parlour or after attachment of the milking cluster. It would be interesting to investigate how the regular use of oxytocin at the farm affects the long-term milk let-down of the cows. Some work in this matter has been done by Bruckmaier (2003), who asserts that a permanent use of oxytocin could result in addiction to this treatment as well as in reduced milk ejection.

7. CONCLUSIONS

The following conclusions were drawn:

1. The group of artificially milk-fed calves performed more abnormal behaviour than the group of restrictively suckling calves. H_0 could be rejected.

2. No difference was detected in the amount of recordings on performing active behaviour, represented by moving, between the group of restrictively suckling calves and the group of artificially milk-fed calves. H_0 could not be rejected.

3. The group of artificially milk-fed calves consumed more concentrate and had more recordings on eating concentrate than the group of restrictively suckling calves. H_0 could be rejected.

4. No difference was detected in weight gain between the group of artificially milk-fed calves and the group of restrictively suckling calves, though the latter had greater individual variation. H_0 could not be rejected.

5. No difference was detected in udder health between suckled and non-suckled cows. H_0 could not be rejected.

6. No difference was detected in time to milk let-down between suckled and non-suckled cows. H_0 could not be rejected.

It is concluded that the investigated restricted suckling system gave less abnormal and foraging behaviour but no difference in weight gain in dairy calves and no difference in udder health and milk let-down in dairy cows in a temperate area in Mexico.

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