AGRONOMIC INTERNSHIP

Engineer school of Agrosup Dijon

Initial schooling

Impact of estruses and physical activity on milk yield of Holstein cows under extended lactation

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Summary

Thanks

Before to start this report, I would like to thank Charlotte Gaillard to give me the opportunity to make my internship in Denmark about a subject really useful for my future, and offer me the possibility to improve my knowledge on many subjects. I also would like to thank you Henrik Callesen, Jakob Sehested, Peter Løvendahl and Martin Tang Sørensen to help by giving me advices during this internship.

I also would like to thank the ANIS group for welcoming me during five months and helping me to improve my English.

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Presentation of Foulum Research Center

1. History

The research center of Foulum was founded in 1983. It covers a building area of about 100 000 square meters and 550 hectares of lands. It is the largest unit of the Faculty of Agricultural Sciences. This faculty is a part of Aarhus University since 2007, and owns 4 other research center: in Bygholm, Årslev, Flakkebjerg and Sorgenfri. Most of the researches in Foulum are about animal husbandry and plants, with an interdisciplinary area between ecology, animal husbandry and animal welfare.

2. Activities

Foulum center is divided into 5 departments:

The department of Food Science includes studies on all the food chain, from field to fork, as well as the health-promoting properties of foods and food constituents. The aim of the department is to increase the basic understanding of factors which have an influence on the production, in order to make this production competitive, sustainable and secure (with high quality foods).

The department of Agroecology studies several area, from the interactions between climate, soil, plants, animals and humans, in agricultural systems; to the advancement of health, sustainability and environmentally friendly production of foods, feed and energy crops. It contributes to increase the agricultural production of foods and energy in an environmentally friendly and sustainable way.

The department of Engineering conducts research of a high international standard. It is at the forefront with the development of new knowledge and technology-based solutions that can improve the quality of life by creating a greener environment and strengthen the competitiveness of Danish companies. In some other way it enhances the value of society.

The department of Molecular Biology and Genetics conducts research on molecular, genetic and physiological; on plants and animals. Modern techniques are used, including high-capacity sequencing. In parallel, they develop and implement new methods in statistical genetics, biostatistics and bioinformatics

The department of Animal Science carries out basic, strategic and applied research on nutrition, health and welfare of livestock, companion animals and humans. The department focuses on a sustainable livestock production, and the research and services therefore include development of new technologies. With these news technologies they want to improve production systems, documentation, surveillance and decision-support in relation with emissions measurements, food quality, animal health and animal welfare. It contains 7 different units: behavior and stress biology, reproductive biology, integrative physiology, animal nutrition and environment, epidemiology and management, immunology and microbiology, molecular nutrition and cell biology. I joined the unit of animal nutrition and environment for 5 months.

Close to Foulum there is also the Cattle Research Center (KFC). It is an experimental farm which belongs to farmers and has many connections with Foulum, especially with the department of animal science.

3. Strategies of the Animal Science department

The mission of the department of Animal Science is to conduct basic, strategic and applied research and innovation within the core areas of nutrition, physiology, cell biology, reproductive biology, immunology, ethology, disease prevention, epidemiology, and production and health management.

Animal husbandry focuses on the development of strategies to improve health, reproduction, welfare and quality without to forget the environment, climate, efficiency and economy. The objective is to obtain an animal production with high standards of animal welfare, nutrition, food quality and food security. For that they want to reduce medication and the impacts on the environment. One of the solutions is to work on optimizing nutrition. The researchers try to manipulate the diet of the livestock to reduce odor and gas emissions. The department concentrates on developing and improving methods and techniques to select and adjust feed to the physiological needs of the animals.

A second objective is to provide solutions for human nutrition and health issues, for example through the use of animal models. The researchers work on the biological effects of plant and animal-based foods with specific health-promoting characteristics at the cellular, organ and whole animal level. They investigate how nutrition, the microbial ecosystem, genotype and feed technology interact under a human angle. They also work on new and advanced models, at cell, organ and whole animal levels, to develop basic knowledge on the causal mechanisms of specific nutritional components and their positive and/or negative effects on health.

Some of the research of the Department is carried out in the cross-field between biology, sociology, economics and biometrics and generates new knowledge about the decision-making of livestock farmers. Theories and methods are developed for production and health management in the primary production and new concepts are developed for the sustainable livestock production systems of the future.

The department has also an eye on the future and expects to be able to combine input factors (nutrition, management, production system) optimized for the biological requirements of the animals with considerations for production efficiency, reproduction, product quality, health, behavior and the impact on environment and climate. Through a mutual adaptation of animals and production principles, they hope to improve livestock production in terms of welfare, where high food security and quality are combined with the minimum use of medication.

Introduction

1. General introduction

The REPROLAC project is a European project (Aarhus University 2014), with the aim of studying extended lactation and the different factors possibly having an influence on it like the breed, the parity and the level of feeding. This project is divided into two main packages, one where measurements are taken on dairy cows in Danish farms in activity, and another where data are recorded in an experimental farm, at Foulum Research Center. This last one studies the effect of individualized feeding strategies supporting extended lactation on Holstein cows. The aim is to test a new individual feeding strategy for the management of extended lactation and to compare it with a usual feeding strategy. It is expected that this new strategy will reduce the environmental impact of milk production, increase animal welfare and productivity without compromising milk quality. This report presents some analysis of the progesterone and activity data of this experiment. The aim was to determine the relationships between estrus periods and milk production of Holstein cows under extended lactation.

2. Background

2.1. Extended lactation

2.1.1. Definition

Extended lactation is characterized by a calving interval higher than the traditional 12 months, in our case of 18 months.

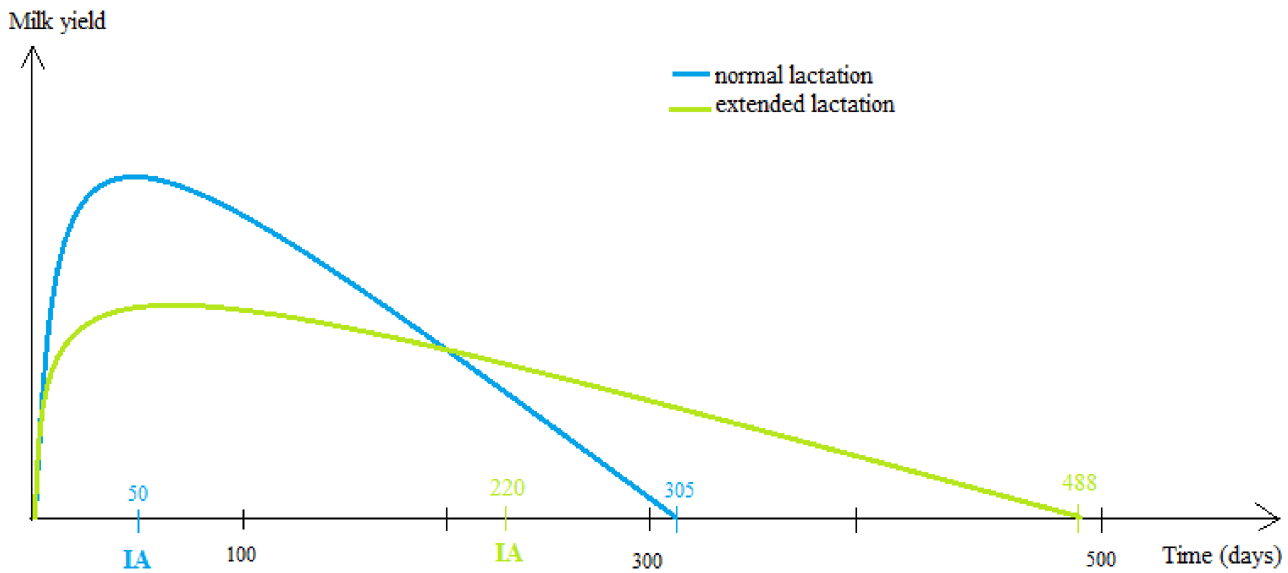

Figure 1: One representation of normal lactation (blue) vs extended lactation (green) and insemination date

Under traditional lactation, the insemination takes place between at 50 days while it is delayed under extended lactation (insemination between 200 and 250 days after calving for a 18 months extended lactation) (figure 1). In both systems the cows are dried for two months.

The lactation curve is plastic and is modified by management factors, allowing the extension of the lactation. Changing the shape of the lactation curve without reducing total milk yield involves an improvement of persistency rather than peak yield, that is the main key of extended lactation (Sorensen and Knight 2002). Persistency is defined as the rate of decline of milk production after peak yield (Togashi and Lin 2009). If the peak of milk production is low, the persistency should be improved because the needs of nutrients are lower than when the peak yield is high (Pryce, Veerkamp et al. 1997). In this case, he cows need a less energetic diet to produce milk. They can be feed with more forage, which is less expensive than concentrate (Dekkers, Ten Hag et al. 1998). For the primiparous, the lactation curve is less close to the curve of ingestion capacity than it is for the multiparous. As shown in the figure 2, peak milk yield is greater for the multiparous than the primiparous indicating that persistency should be better for the primiparous than for the multiparous (Dekkers, Ten Hag et al. 1998).

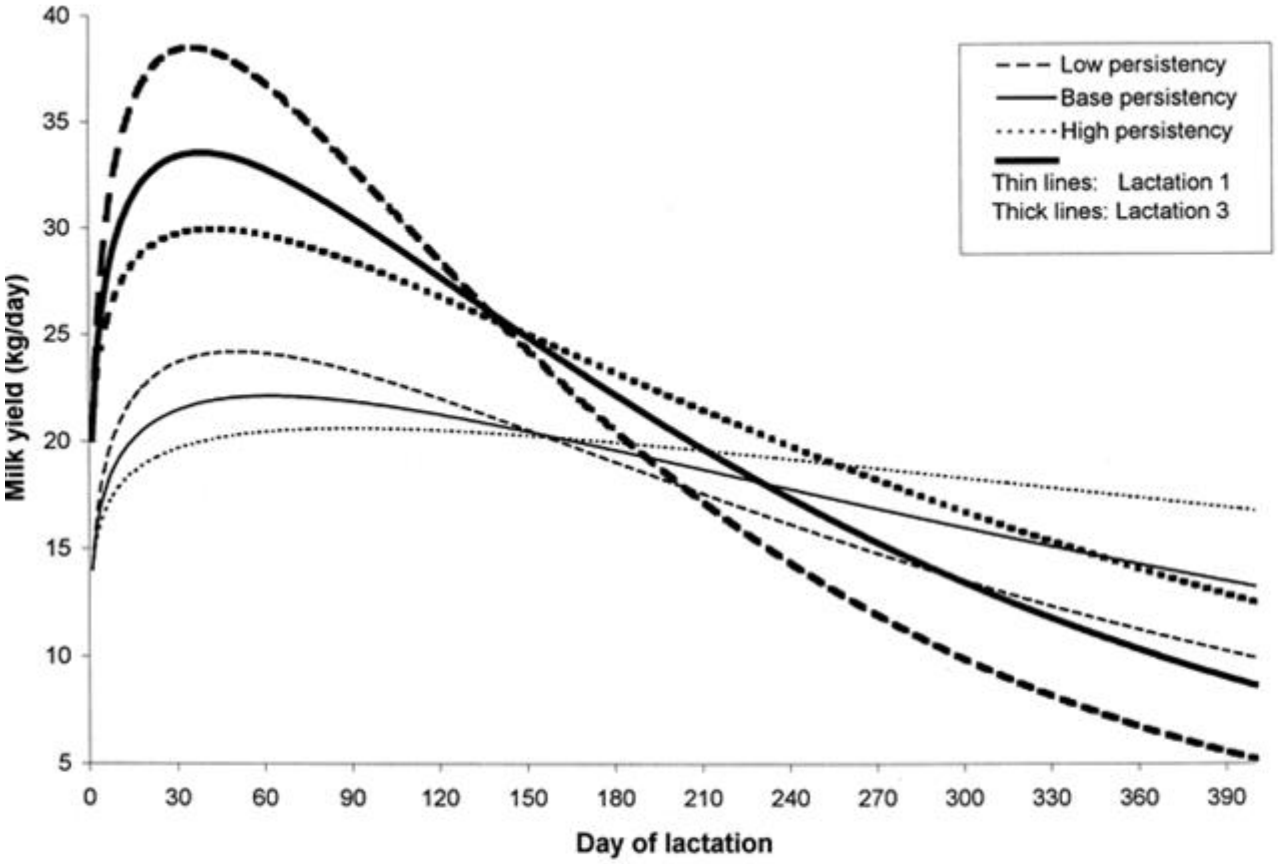

Figure 2: Shape of lactation curve for average producing cow in lactation 1 (thin lines) or lactation 3 (thick lines), for low, average, and high persistency (Dekkers, Ten Hag et al. 1998).

Decrease feed cost is one of the advantage of extended lactation, but there are others. According to one study, 65% of health problems occur around calving and in the first 45 days of lactation (Erb, Smith et al. 1984). Under extended lactation, there are only 2 lactations in 3 years instead of 3, so the risk of health problems is reduced by one third (Sorensen, Muir et al. 2008). Persistency is also correlated with body condition score (BCS). It is important to keep a BCS stable during the beginning of the lactation because a decrease in BCS would increase the peak of milk production and, consequently, decrease the persistency (Roche, Lee et al. 2007).

2.1.2. Why extended lactation is used in the REPROLAC project?

The REPROLAC project aims to use extended lactation to reduce the quantity of feed thanks to a lower rate replacement of heifers and dry cows. They also expect an increase of milk production thanks to more lactating days. This economic advantage should be combined with a decrease of emissions gases from the digestion of feed, like nitrous oxide and methane emission.

2.2. Estrus period

2.2.1. Definition

The estrus period is the period where a cow is ready to be inseminated. This period takes place every 21 days and it is characterized by different signs which permit to detect it. These signs can be hormonal (progesterone level) or behavioral (visual observations, activity).

The duration of estrus is variable between individuals implying more difficulties to detect estrus (Kerbrat and Disenhaus 2004), (Arney, Kitwood et al. 1994). The duration of estrus can be defined in the following way: onset of estrus is when the first estrus behavior is detected, and the end is when the last estrus behavior is detected (Van Vliet and Van Eerdenburg 1996). According to several studies, the duration of estrus is in average 13.7+/-6.7h (Van Vliet and Van Eerdenburg 1996) and is correlated with milk production (Arney, Kitwood et al. 1994, Yoshioka, Matsuda et al. 2007): when the level of milk production is high, the duration of estrus decreases. Above 39.5kg milk/day, each supplementary kg of milk produced induces a decrease of 1.6% in walking activity (López-Gatius, Santolaria et al. 2005). In average, the duration of estrus is higher in heifers than in cows, respectively 9.25h and 8.12h (Lovendahl and Chagunda 2010). Another study showed that cows with a lower duration of estrus have a higher intensity of estrus (Lopez, Satter et al. 2004). Intensity of estrus can be define in 3 levels: low intensity, when the cow is not mounted by another cow and not standing; medium intensity, when the cow is mounted by another cow but not standing; and high intensity, when the cow standing and is mounted by another cow (Garcia, Hultgren et al. 2011). For the high yielding cows, this correlation between duration of estrus and intensity is not true; a low duration of estrus induces a low intensity. For these cows, it is more difficult to detect estrus than for the others (Lopez, Satter et al. 2004). Moreover, the age of the cows influence the intensity of estrus. The number of lactation is also negatively correlated with the intensity of estrus activity (Lovendahl and Chagunda 2010).

2.2.2. Visual observations

The main behavior signs are: “mounting another cow” or “being mounted by another cow”(success or attempts) (Esslemont, Glencross et al. 1980). Both signs are the most accurate indicators to detect estrus because they occur precisely the day of estrus (Kerbrat and Disenhaus 2004). The detection of estrus period is of major interest for the farmers as they will save time and money, but detection is not easy as the behavior signs (mounting) are, nowadays, rarely expressed (Lopez, Satter et al. 2004). A study reports that estrus detection rate decreases of 10% between 1985 and 2004 in Southeastern of US (Washburn, Silvia et al. 2002). Other behaviors should be considered like chin rest (Esslemont, Glencross et al. 1980), sniffing and licking the ano-genital region of another cow. It represents 27% of all sexual interactions during estrus. The increase of agonistic behavioral initiated by cow, mainly head-to-head, was also shown during estrus period. Moreover, during this period, a cow spends more time walking and less time lying (Kerbrat and Disenhaus 2004). The walking activity increases 8h before the beginning of estrus and stays high 5h after.

2.2.3. Activity

Activity can be measured in two ways. The first, and the oldest, method is the direct observation by the farmer. It requires time and become more difficult as the herds get bigger. The pedometer is the Nowadays; new technologies permit to measure and record the activity of big herds. Different systems are available like the pedometer, fixed on the leg of each cow, or the necklace, fixed around the neck of each cow. Both are recording continuously walking activity of the cow by registering the number of movements for a period of time (usually per hour). In addition, the necklace records the movement of the head.

Activity can be used to determined estrus period as it increases during estrus (Kiddy 1977) (Figure 3). However, some factors can affect its measurement leading to a less accurate and harder detection of estruses. For example, a relative humidity higher than 95% can decrease walking activity (Yániz, Santolaria et al. 2006). Moreover, the same study showed that when the lactation number increases, the activity decreases. The same observations were done regarding the quantity of milk produced: high yielding cows are less active than the other. Finally, the fertility of the cow also impacts walking activity: cows with high fertility level (measured as conception rate before 90 days postpartum) have high level of activity during estrus (Yániz, Santolaria et al. 2006).

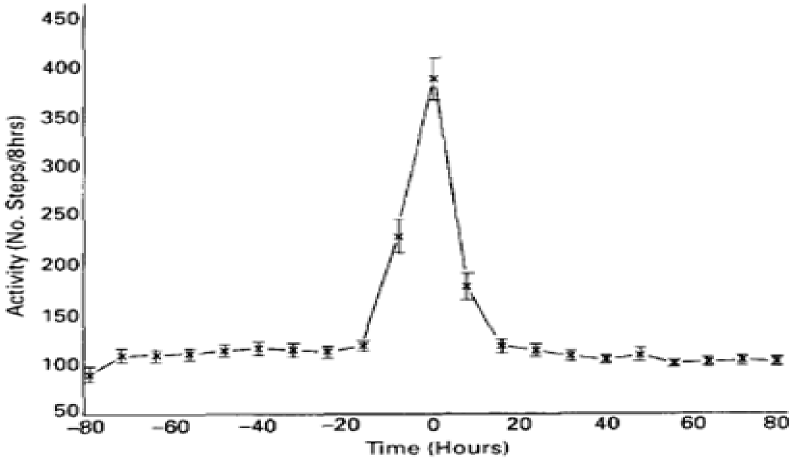

Figure 3: Mean activity score over the estrus period (Arney, Kitwood et al. 1994).

2.2.4. Progesterone

During lactation, milk progesterone concentration is variable regarding the sexual cycle of the cow, more precisely regarding the corpus luteum state (Hoffman, Hamburger et al. 1974). During estrus, the concentration of progesterone is low (< 4ng/ml). It increases the day after estrus during 9 or 10 days, decreases again 21 – 22 days later for non-pregnant cows. If the cow is pregnant, the concentration of progesterone stays high (Zaied, Bierschwal et al. 1979) (figure 4) because of the persistency of corpus luteum secreting progesterone. Progesterone values fluctuate regarding cows. In Hoffman and Hamburger study, cows are considered pregnant if progesterone concentration is above 11ng/ml the 20th day after insemination (Hoffman, Hamburger et al. 1974). For Zaied and Bierschwal, the threshold for pregnancy is 4 ng/ml at days 21 or 22 and at days 27 or 28 post-insemination (Zaied, Bierschwal et al. 1979).

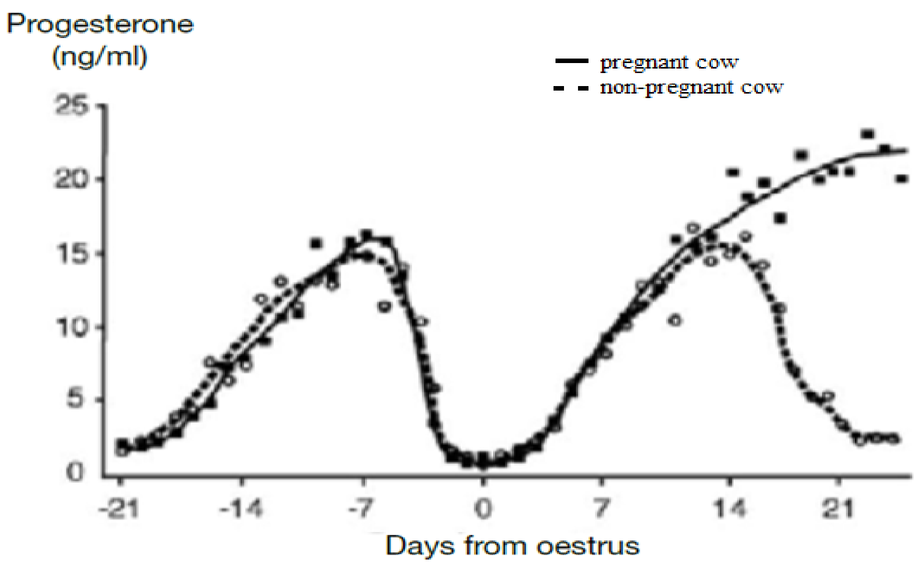

Figure 4: Milk progesterone profile for pregnant cow (full line) and non-pregnant cow (dotted line), (Delaval 2013).

Estrus cycle is considered as abnormal when the duration of anestrus postpartum (period of low concentration of progesterone before the first estrus) is below 12 days. The average length of anestrus postpartum is 30 days, but it is one more time very variable regarding cows (Mather, Camper et al. 1978). In this way, progesterone profile is also useful to detect fertility problems in dairy cows. Lamming and Darwash present 4 types of abnormal profiles. The first is the delay ovulation type I (DOV I) where the concentration of progesterone is below 3ng/ml during a period of more than 45 days postpartum. The second is delay ovulation type II (DOV II), the concentration of progesterone is below 3ng/ml during a period of more than 12 days after the commencement of luteal activity (CLA). The third is the persistency of corpus luteum type I (PCL I) where the concentration of progesterone is above 3ng/ml for more than 18 days during the first postpartum estrus cycle. The last one is the persistency of corpus luteum type II (PCL II), the progesterone is also above 3ng/ml for more than 18 days but during the subsequent postpartum estrus cycle (Lamming and Darwash 1998). This abnormal profiles increase the time between calving and first artificial insemination (AI) and so commencement of luteal activity. Cows with DOV II have less chance to conceive in all AI. If they have PCL I they have less chance to conceive in the first AI. PCL I and PCL II increase chance of retained fetal membrane (McCoy, Lennox et al. 2006). Abnormal progesterone profiles induce a high interval between postpartum and conception, a high number of inseminations per cow and a lower success in the first insemination (Lamming and Darwash 1998).

Progesterone can be measured in the blood or in the milk. In this study, progesterone was measured in the milk thanks to automatic milk samples and direct measurements in the milking robot.

Although the accuracy of estrus detection is better with the help of milk progesterone concentration than with visual detection (96% versus 84%) (Shemesh, Ayalon et al. 1978), it is better and easier to use both to improve the detection of estrus.

3. Objectives and hypothesis

The aim of this study was to determine the impact of estrus period and activity on milk production in cows under extended lactation. Some studies have already shown that estrus period has an impact on milk production in cows under the traditional 10 months lactation (Schofield, Phillips et al. 1991, Akdag, Cadirci et al. 2010). In our study, cows are under extended lactation and consequently had several estruses before being inseminated. In this case, we wanted to confirm the impact of estrus on milk yield, for cows under extended lactation, and determine if this impact was the same for all the estrus periods. Moreover, the effect of estrus on activity was studied.

First hypothesis: during estrus period, the milk production decreases. It had been found that, during estrus, cows spent less time to eat and more time to walk. Moreover, they had more agnostic comportments with other cows explaining the high activity and high consumption of energy during this period (Hurnik, King et al. 1975). If less time was spend to eat, less energy was available for milk production. Finally, it had been shown that, during the day of estrus, the milk production decreased compare to the days after and before estrus (Schofield, Phillips et al. 1991).

Second hypothesis: the effect of estrus on milk yield decreases over the lactation. Regarding the first hypothesis the estrus should have a negative effect on milk production, but this effect may decrease over the lactation as the estruses don't occur in the same conditions. After calving, the energy balance (EB) of the cow is negative so the cow uses her body reserves to satisfy the metabolic needs for milk production (Butler, Everett et al. 1981). The energy balance returned positive approximately 6 weeks after calving (Gilmore, Young et al. 2011). Regarding this, the first ovulation doesn´t occur in the same conditions than the others later in the lactation, so the impact of estruses on milk production should not be the same over the lactation.

Third hypothesis: cows with a general low activity have a higher milk production than cows with a high activity. The cows spend more time to walk during estrus (Hurnik, King et al. 1975) and, logically, less the cows are active less they consume energy. General activity can be an interesting cow characteristic influencing its general milk production and feed intake. So, a cow with a low general activity should have a higher milk production than a cow with a high activity rate, also the level of general activity could increase the activity during estrus.

Forth hypothesis: during estrus, daily activity is higher than during other "normal" days. With a 10 months lactation, many studies found an increase of activity during the 2 or 3 estruses (Schofield, Phillips et al. 1991, Lovendahl and Chagunda 2006, Lovendahl and Chagunda 2010). The aim is to see if it is also true in extended lactation and if the activity increases the same way for all the estruses

Fifth hypothesis: the general activity of the cow impacts the daily activity during estrus. If the daily activity increases during estrus and the general activity impact milk yield, it is possible that regarding the level of general activity, the daily activity during estrus is increasing differently. A cow with a high general level of activity would have a higher increase of daily activity during estrus than a cow with a low general level of activity.

Materials and Methods

1. Animals and housing

1.1. Animals and extended lactation

The experiment is currently running at the Danish Cattle Research Center at Aarhus University, AU-Foulum. It started in November 2012 and will end in December 2014. The Holstein cows were equally divided into two groups fed with different diets. All cows are following a 16 months extended lactation and are housed in the same pen. The cows are inseminated at minimum 220 days after calving, regarding the heat.

The cows were selected for the experiment according to different characteristics: parity, date of calving, milk yield. Only cows with a high level of production were selected because cows with a low milk yield have difficulties to handle an extended lactation.

1.2. Housing and milking

The 62 cows are housed in a loose system (420 m^2) with slatted floor, 62 cubicles with mattress and free access to water. Sawdust is distributed above each cubicle by a robot. The floor is scrubbed by a Lely Discovery robot. The cows have access to a milking robot (Delaval). They are milked at least twice a day. The robots are equipped with an electronic scale, to measure the body weight of each cow at each milking, and a Herd Navigator system, particularly to measure the progesterone in milk. Milk is sampled automatically in the milking robot for another part of the REPROLAC project.

2. Feeding strategies

For the REPROLAC project two diets are distributed to the cows after calving to support extended lactation. Dry periods are managed in the same way for both groups of cows. The basis of the ration is the same for the two groups: grass silage (mainly clover) and maize silage. The grass silage is a mixed between two years to avoid great nutritionals difference. Mineral supplement is the same for both ration and added in the mixed forage. Cows are receiving a partially mixed ration ad libitum, distributed 4 times per day, and 3kg of concentrate in the milking robot. The following rations are optimized thanks to NorFor system (Volden, 2011) for 10 000kg milk productions per year.

2.1. The group 1

"Group 1" is the control group. The same ration is given to the cows during all the lactation. They are receiving a mixed ration ad libitum and 3kg of concentrates per day in the milking robot. The concentrates are a mixture of dry sugar beet pulp, barley, rapeseed meal, and urea is added. For this diet, the ratio forage:concentrate is 60:40.

2.2. The group 2

"Group 2" is the experimental group. The ration contains more energy with a ratio forage:concentrate of 50:50. This ration is expected to give 15-20% extra energy compared with the ration of group 1. To increase energy in the ration they use wheat protected grain. Wheat grains are treated with sodium and reduce the degradability of starch in the rumen. This ration is given to the cows after calving until they reach a positive energy balance, estimated through individual live-weight measurements. When the daily live-weight change of a cow is null or positive on a 5 day average, and the cow is above 42 days of lactation; the cow is fed with the ration of group 1 (60:40 forage:concentrate).

3. The measures

3.1. Used for the study

3.1.1. Milk production

The quantity of milk is recorded at each milking thanks to the milking robot. Daily milk yield per cow are then calculated. These data are used to evaluate the impact of the feeding treatment on the milk yield, and, in this report, to evaluate the impact of estrus on milk yield.

3.1.2. Progesterone

Progesterone is measured in milk thanks to the Herd Navigator. Milk samples are directly collected by the milking robot, and analyzed by the Herd Navigator. The samples are not taken every day as it is costly, but the Herd Navigator is automatically building a graph close to the biologic reality without having regular values. A low concentration of progesterone indicates an estrus period. The software considers that the cow is in estrus period when the concentration is below 5ng/ml and alerts the farmer to inseminate 24-36 hours later. Few days after this period, measurements of progesterone are done again to know if the cow is pregnant (progesterone concentration stay high after day 30) or not. If the cow is not pregnant measurements of progesterone are taken few days before 21 days after the last heat. The software is also able to detect health problem like follicle cyst defined by a low level of progesterone maintained during at least 10 days, or luteal cyst defined by a high concentration of progesterone during at least 25 days (Delaval 2013).

3.1.3. Activity

Physical activity of the cows is recorded, thanks to neckbands (DeLaval, Tumba, Sweden), and send to the Delpro software (Delpro Delaval) for analysis. The software makes some activity graphs every day. The activity of the cow is compared to a basis activity, which allows the determination of an increase of activity. The software gives an alarm when the area between the curve of real activity of the cow and the curve of basis activity is high, to inform the farmer that the cow might be in estrus. The software provides daily information regarding the level of activity represented by "+" signs. One + indicates that the activity of the cow is almost normal but one peak is higher than the basis activity. The "+++" indicates that the number of activity peaks increases for this day compared to the basis activity and some peaks are higher than normal. When these "+" are surround by () it means that the increase of activity happened the day before.
Here, general activity was used to divide cows in three groups of activity: high, medium and low, by making an average of activity over the first 200 days of lactation. Also a daily activity was calculated for the days of estrus and 5 days before.

3.1.4. Visual observation

Visual observations are recorded every morning to improve estrus detection. Every morning, the farmers look at the activity alarm (++ signs). These information tell them which cows they have to look for because they were inseminated or in heat 21 days ago. Visual observations are recorded for these cows. The following signs are taken in account: transparent mucus, stride mucus, swell red, jump, stand, and blood.

3.1.5. Protocol for estrus detection

For this study, estruses were detected by looking manually at the progesterone curves and the visual observations.

First, a graph of progesterone was made for each cow (example Figure 5). The days where the progesterone was below 3ng/ml were used to define the estrus period.

Secondly, for each day contained in the estrus period, defined previously, the visual observations were associated to be sure the drop of progesterone matches with visual detected of estrus period by the farmers. It was always right, so visual observation could be used alone when there were not enough progesterone values to detect all estruses.

Third, the visual observations were classified regarding their intensity allowing the precise determination of the day of estrus. When a period contained a day with a visual observation "jump" this day was chosen as the date of estrus. It was the first criteria among all visual observations. If there was not "jump" observation, the observation "stand" was used to determine the date of estrus.

Forth, if there was no "jump" or "stand", the date with the greatest number of observation was used to define the day of estrus.

Fifth, if several days had the same amount of observations or if there was only "blood" observation, the date of estrus was determine as the date two days before "blood" observation. In fact when there is "jump" observation, it almost happened all the time two days before "blood" observation. If there is several days with the same amount of observation but no "blood" observation, the 21^{th} day after the last estrus is taken.

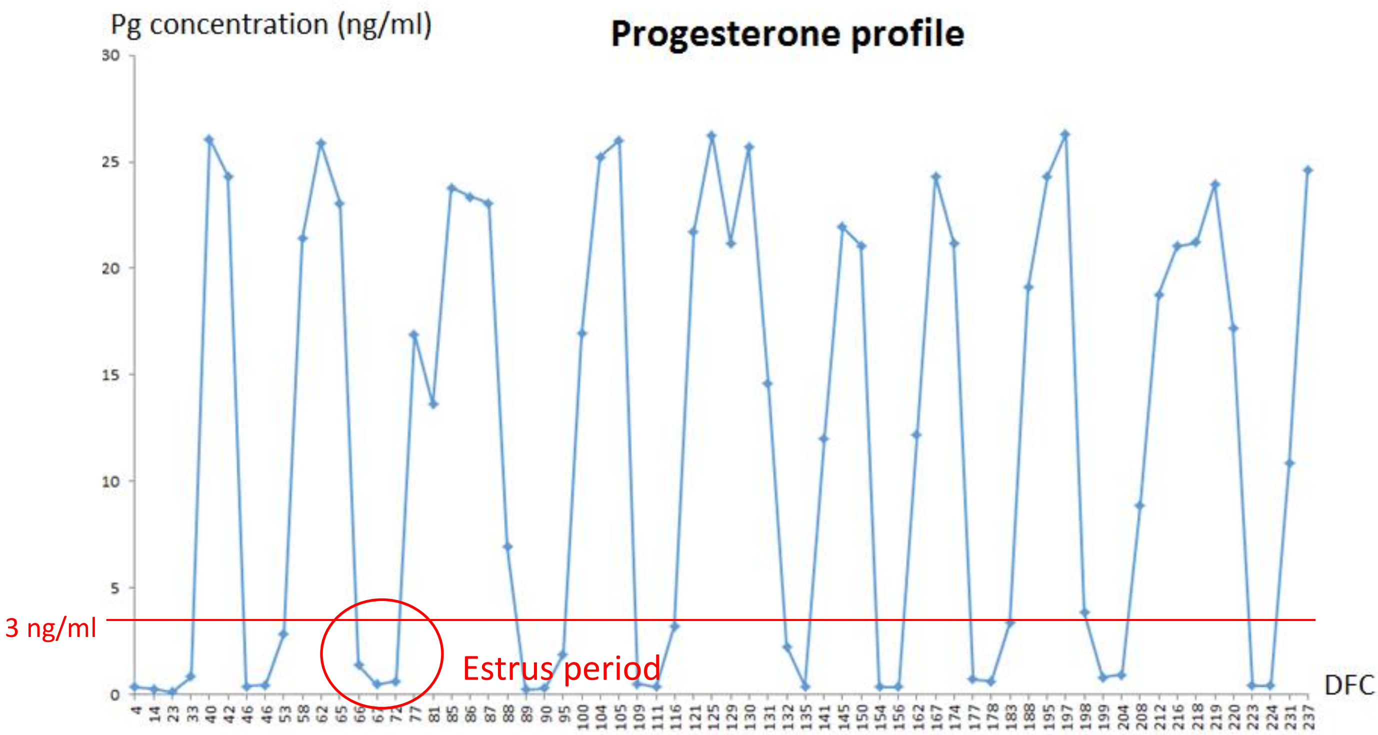

Figure 5: Progesterone profile

3.2. Used for the REPROLAC project

3.2.1. Feed intake

The cows have access to automatic feed bins with integrated scale device. Cows' identity is detected thanks to the necklace: when the cow puts the head in the feeder. The ID number of the cow and the weight of the feed before, and after, eating are recorded each time. Then, a daily feed intake is calculated for each cow.

3.2.2. Weight

The weight of the cow is assessed at each milking through an integrated scale in the milking robot. The scale takes 6 measurements of weight per second. Data are then cleaned up and a daily average weight per cow is then calculated.

3.2.3. Blood samples

Several plasma indicators of energy balance present are measured: glucose, NEFA (non-esterified fatty acids), BHBA (blood β-hydroxybutyrate), urea, acid uric, IGF-1 and insulin. Blood samples are taken every week from calving until week 12 and then every second week until week 36 (before insemination).

Blood samples are taken in the base of the tail, by veno-puncture. The cow is blocked in the cubicle and two tubes of blood are taken. One tube contains heparin and must be kept in the ice before centrifugation in the aim to collect plasma. The other tube allows blood to clot and, after centrifugation, to collect serum.

3.2.4. Mammary biopsies

The mammary biopsies are taken in some cows to see the effect of extended lactation on the mammary cells. The biopsies were taken after anaesthetizing the skin. Biopsies are stored in the fridge for 24 hours and then in a freezer at -80°C.

4. Statistical analysis

The R software was used for the statistical analysis. The Linear Mixed Model (lme function) was used for three things: to compare milk yield during estrus and during normal days; to compare impact of general activity on milk yield and to see the impact of general activity on activity during estrus. Daily milk yield (kg) was used as variable of the model, for the two first analysis, and the 4 following factors were taken into account: “nb of estrus” (from 1 to 9), parity (multiparous, primiparous), “event” (day of estrus, normal day) and “activity level” (high-medium-low). The “normal” milk yield was the milk yield mean of the 3 days that were 5 days before the days of estrus. For the thirds analysis, activity (movements/hour) was taken as variable and the factors are the same as previously. Activity was calculated as the mean of activity measurements during the day of estrus and 5 days before.

The model took all the cows randomly, and included the fact that we had repeated measurements on each by including a correlation (corAR1) between the measurements of a cow. The formula of this model is the following:

$$Y_{ijkl} = \mu + E_i + P_j + N_k + (EP)_{ij} +(EN)_{ik} + (PN)_{jk} + (EPN)_{ijk} + C_{ijl} + \varepsilon_{ijklm}$$

μ is the overall mean within cows, E is the event, milk during estrus or during normal days (i= estrus, normal), P is the parity (j=primiparous, multiparous), N is the number of estrus (k=1 to 9), $(EP)_{ij}$, $(EN)_{ik,}$ $(PN)_{jk}$, $(EPN)_{ijk}$ respectively illustrate the double and triple interactions, Cijl the random effect of the lth cow within i treatment and j parity, and εijklm is the random residual error.

Results

As the feeding treatment had no significant effect on milk production (p=0.6) or on activity (p=0.3), it was not included in the results. The following results were obtained with a data set of 62 cows.

1. Impact of estrus on milk production

It was hypothesized that milk production is lower the day of estrus than during normal days. To have a measurement of "normal" milk yield, a 3 days average of milk yield was done from 5 to 7 days before the day of estrus. As the cows were under extended lactation, the 9 first estruses were studied. The statistical results given by ANOVA are presented in table 1. Results were considered significant when the p-value was below 0.05. There was a significant interaction between the "nb of estrus" and "parity" (p<0.0001) and a significant effect of "event" on the milk production (p=0.0013).

Factors/Interactions	NumDF	F-value	P-value
Event	1	4.91	**0.0013**
Nb of estrus	8	10.78	<0.0001
Parity	1	13,31	<0.0001
Nb of estrus*Parity	8	3.17	**<0.0001**
Event*Parity	1	0.58	0.25
Event*Nb of estrus	8	1.31	0.36

Table 1: Statistics results testing the impact of estrus on milk production

The interaction indicated that primiparous produced less milk than the multiparous (Figure 6). The lsmean indicated no significant difference of milk yield between all the estrus for the primiparous, but these differences were significant for the multiparous. Milk production of the multiparous decreased over the lactation compare to primiparous (Figure 6). Primiparous produced less milk since the beginning of the lactation, but the production was more stable according to the figure 6. At the end of the lactation, from estrus 7, milk yield between primiparous and multiparous was not significantly different.

Milk yield during estrus day was lower than milk yield during "normal" days (p=0.0013), whatever the parity. Estrus impacted milk production by reducing it.

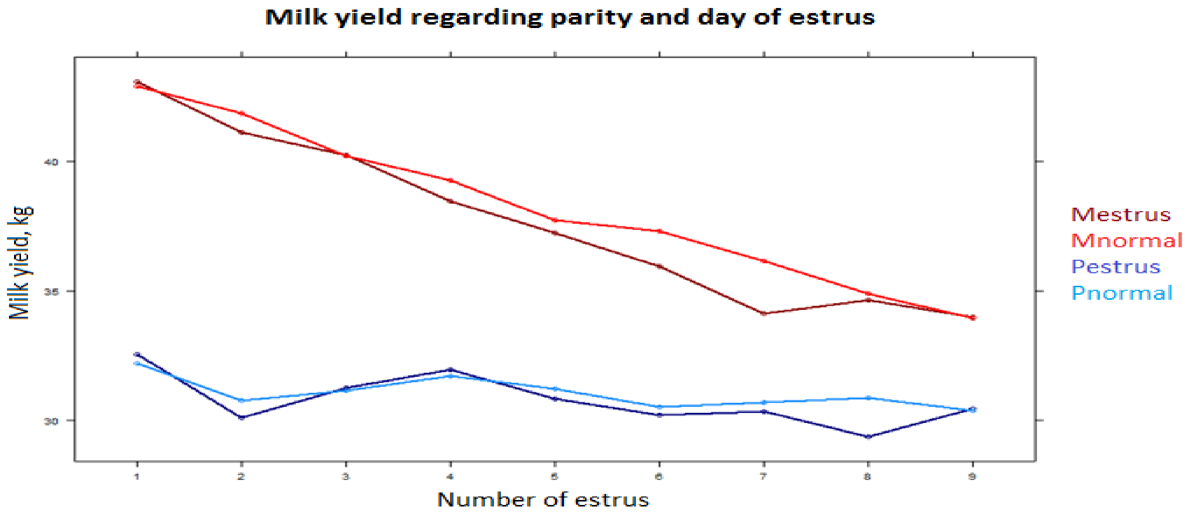

Figure 6: Milk yields for the 9 estrus days (dark blue and brown lines) and the days before estrus (red and light blue), regarding parity (M=multiparous, P=primiparous)

2. Impact of estrus at different time on milk production

The intensity of the effect of estrus on milk yield was also studied. According table 1, there was no interaction between estrus/normal with the two other variables (p=0.25 and p=0.36). This indicated that the decrease of milk production during estrus was always the same for the 9 first estruses, for multiparous and primiparous. In average, the decrease of milk yield during estrus was around 0.51kg±0.16 per estrus day.

3. Impact of general activity on milk production

To determine the impact of the intensity of general activity on milk production, cows were divided into three groups: cows with a high activity (20 first cows with the higher level of activity), cows with a low level of activity (20 cows with the lowest level of activity, and cows with a medium level of activity (the other cows). The results are presented in table 2.

Factors/Interaction	NumDF	F-value	P-value
Activity level	2	0.09	0.92
Event	1	22.8	<0.0001
Parity	1	34.1	<0.0001
Nb of estrus	8	31.6	<0.0001
Activity level*Event	2	4.1	**0.0164**
Parity*Nb of estrus	8	7.9	**<0.0001**

Table 2: Statistics results testing impact of activity intensity (high, medium and low) on milk production

As found previously, there was an interaction between “parity” and “nb of estrus” ($p<0.0001$). The primiparous cows produced less milk than the multiparous cows.

A second interaction, between “activity level” and “event” ($p=0.0164$) showed that activity during estrus was different than activity in normal days regarding the level of activity. The Activity level was not influenced by the parity so graphs regarding the level of activity and the event were plotted (Figure 7) and the lsmeans analyzed. Milk production was lower during estrus for the highest active cows and the lowest active cows (respectively $p=0.007$ and $p=0.048$); the estrus curves of these two groups were below the normal curves. The difference of milk yield was 0.77kg±0.27 for the “high” cows and 0.87kg±0.27 for the “low” cows. There was no difference for the “medium” cows ($p=0.999$), the two curves crossed each other many times. Finally, the milk yield was similar for the 3 levels of activity, in estrus or normal days.

To conclude, the general activity of a cow didn’t impact milk yield.

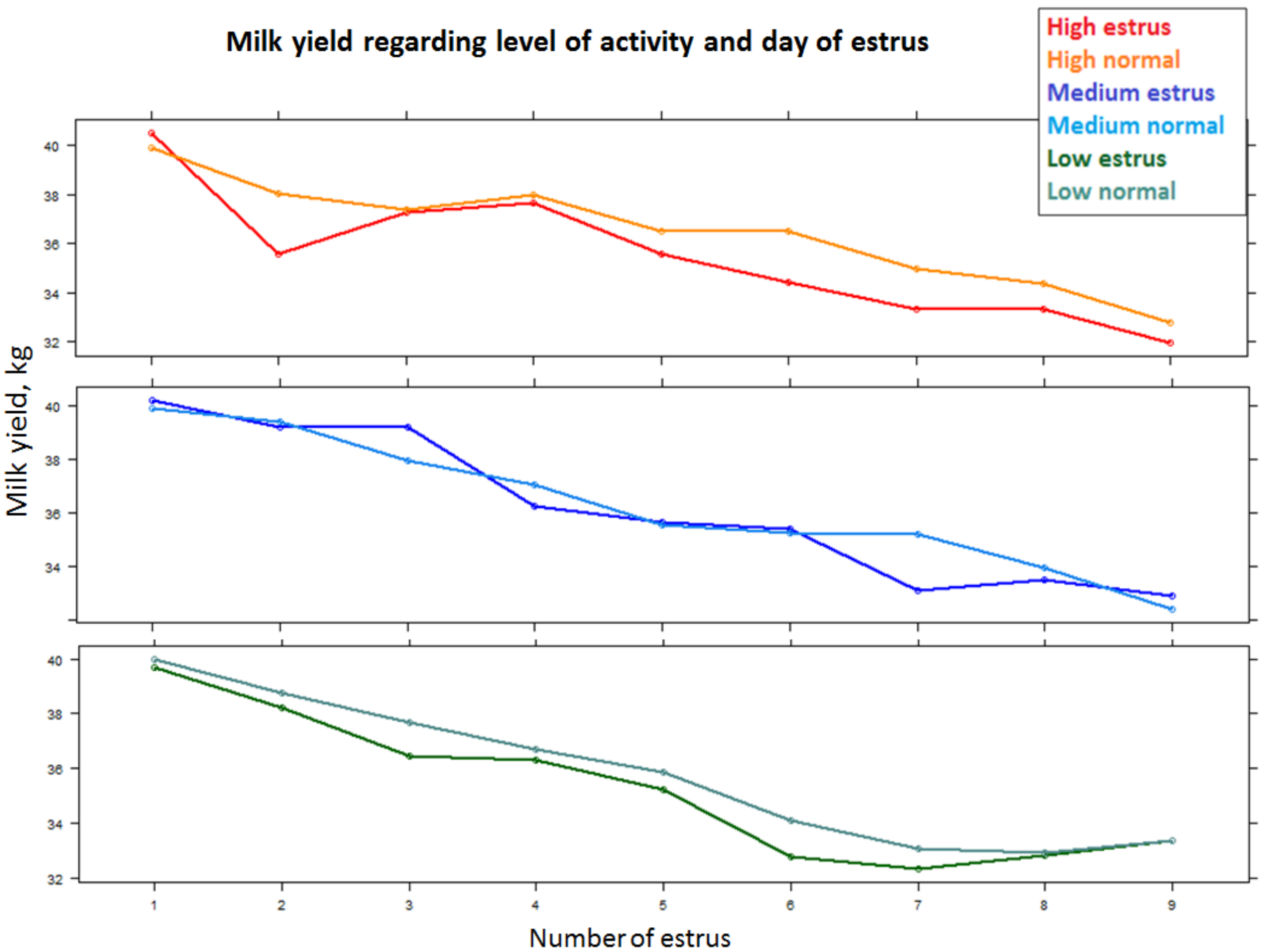

Figure 7: Milk yield for 3 levels of activity: high (red, orange), medium (blue, turquoise-blue) and low (green, greengray) and day of estrus (estrus) and normal days (normal)

4. Impact of activity during estrus

Daily activity during estrus and 5 days before estrus ("normal" day) was studied. The results indicated a triple interaction between "parity", "nb of estrus" and "event" (p=0.0393). To simplify the interpretation of this result, the data were split regarding parity and then regarding the event (estrus/normal). It gave the following results (Table 4):

	Factors/Interactions	NumDf	F-value	P-value
Primiparous	**Nb of estrus**	8	2.48	**0.013**
	Event	1	111.5	**<0.0001**
Multiparous	Nb of estrus	8	3.11	0.0019
	Event	1	197.3	<0.0001
	Nb of estrus*Event	8	2.16	**0.029**
Estrus	Nb of estrus	8	0.96	0.47
	Parity	1	0.017	0.90
Normal	**Nb of estrus**	8	5.21	**<0.0001**
	Parity	1	0.34	0.56

Table 3: Statistics results testing impact of parity and event on daily activity (estrus and 5 days before)

For the primiparous, activity was different regarding the event (p<0.0001): during estrus activity is higher (20 movements/hour) than during normal days.

For the multiparous there was an interaction between "nb of estrus" and "event" (p=0.029). It showed that activity seems to be higher during estrus (Figure 8); but this difference was only significant from estrus 4th to 7th.

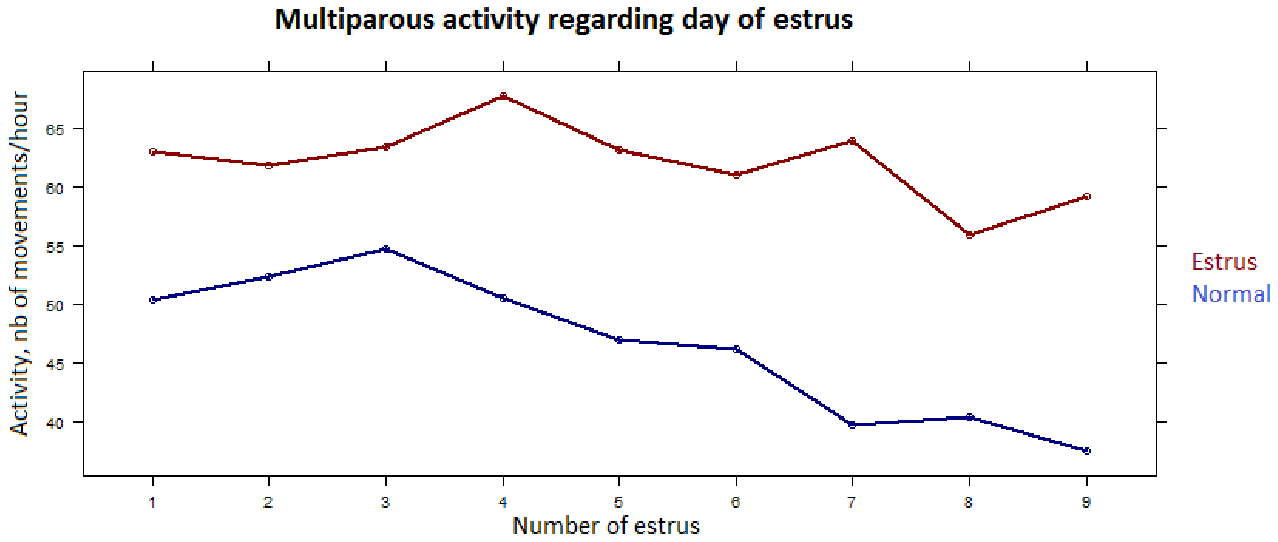

Figure 8: Activity for the 9 estrus days (brown lines) and the days before estrus (dark blue), for the multiparous

The activity, during estrus or normal days, was the same for the primiparous and for the multiparous because there was no impact of parity (p=0.90, p=0.56).

The activity was stable during estrus (p=0.47), maintaining an average of 63 movements per hour. During normal days, the daily activity of the cow was stable at the beginning (around 52 movement/hours), then it decreased regularly after the cycle 3 to finally reach an average of 40 movements/hour from cycle 7 to 9. In general, the daily activity was higher during estrus than normal days, for both parity. It was stable during estrus all over the lactation but decreased in normal days.

5. Impact of different levels of general activity on estrus

In this part we want to see if the level of general activity of cow impacts the activity during estrus. It was hypothesized that cows with a general high level of activity will have higher daily activity during estrus than cows with a low general level of activity.

As previously, the results indicated a triple interaction between "nb of estrus", "parity" and "event" (p=0.033), so the data set was split regarding parity, and then event. The statistic results are presented in table 5.

	Factors/Interactions	NumDf	F-value	P-value
Primiparous	**Activity level**	2	16.6	**0.0002**
	Event	1	96.5	**<0.0001**
Multiparous	**Activity level**	2	78.9	**<0.0001**
	Event	1	164.1	**<0.0001**
Estrus	**Activity level**	2	54.2	**<0.0001**
	Parity	1	0.003	0.95
	Nb of estrus	8	1.07	0.38
Normal	**Activity level**	2	123	**<0.0001**
	Parity	1	3.7	0.059
	Nb of estrus	8	4.8	**<0.0001**

Table 4: Statistics results testing impact of parity, event and level of activity on daily activity (estrus and 5 days before)

As expected, in general, the general "activity level" significantly impacts activity (p<0.0001; p=0.0002). The cows with "high" level of activity were more active during the day than the medium group and the low group. For both parity, the activity during estrus is higher than during normal days (p<0.0001). There was no interaction "event:activitylevel" (p=0.12) so the increase of activity during estrus was similar for all cows, whatever their general level of activity.

The level of general activity didn't impact the intensity of activity during estrus for this study.

Discussion

As expected primiparous produced less milk than multiparous. The udder of primiparous is still developing until the 3rd lactation with a peak at the 2nd lactation (Stastny and Pasek 1985). That can explain why it is not possible for the primiparous to produce the same amount of milk than multiparous which have an udder more developed.

In our study, the milk yield during estrus decreased compared to normal days as shown in many other studies in traditional 10 months lactation (Horrell, Macmillan et al. 1985, Blanchard, Kenney et al. 1987, Schofield, Phillips et al. 1991, Akdag, Cadirci et al. 2010). According to these studies there are different explanations. The decrease of milk yield during estrus can be correlated with the decrease of feed intake, inducing less energy to produce milk, and the increase of estrogen in milk and blood (Akdag, Cadirci et al. 2010). In fact the increase of estrogen is responsible of estrus behavioral, such as mounding, stand; which increases cow's activity and decreases energy available for producing milk (Lopez, Bunch et al. 2002). According to Horrel, the reduction of milk yield is more related to a diminution of milk excretion (the cow doesn't give completely its milk) rather than a decrease of milk production (Horrell, Macmillan et al. 1985). This idea is also supported by Schofield, Phillips *et al*, who says that milk production after the day of estrus is greater than normal (Schofield, Phillips et al. 1991).

In our study, the drop of milk yield during estrus is around 0.5kg of milk, which is small compare to other studies. For example in Kuczaj's study with cows under 10 months lactation, the decrease of milk yield is an average of 2.9kg during estrus (Kuczaj 2007). Cows under extended lactation seem to lose less milk during estrus than cows in 10 months lactation; even if during extended lactation cows have several estruses. Meanwhile, we found that the milk yield didn't drop during estrus for the cows with general "medium" activity. Removing these cows from our estimation of milk lost during estrus, with the "high" and "low" groups, the drop of milk is around 0.82kg, still below the values found for cow under 10 months lactation.

The lack of significant difference between the milk during estrus and normal days for the medium cows can be explained by the large variations of activity in this group. Because of the way that we built the groups, some medium cows are really close to the high group, and some other are really close to the low group.

The activity is significantly higher during estrus than during normal day, as it has previously been found several times (Schofield, Phillips et al. 1991, Lovendahl and Chagunda 2006, Micinski, Zwierzchowski et al. 2010). In our study, for primiparous cows, the activity is clearly higher during estrus compared to normal days; and for multiparous almost the same (significant from 4th to 7th cycles).

Some studies found that activity is different between the primiparous and multiparous (Lovendahl and Chagunda 2006, Brzozowska, Lukaszewicz et al. 2014). Løvendahl and Chagunda showed that parity had a strong effect on activity. The youngest cows are more active than the older cows (Lovendahl and Chagunda 2006). Another study shows that primiparous in normal days are more active than other cows. The hierarchy in the herd can explain it as the old cows eat first, take the best place to sleep and prevent the younger cows to eat or sleep properly (Brzozowska, Lukaszewicz et al. 2014). Our results didn't show a difference of activity between primiparous and multiparous. It can be explain by the number of cows. In our study we have only 62 cows (with 18 primiparous) again 426 and 132 for the two other studies (Lovendahl and Chagunda 2006, Brzozowska, Lukaszewicz et al. 2014). It is possible that our sample is not big enough to show the difference of parity.

It has been found that physical activity in normal days followed the lactation curve over the lactation. Activity increased until the peak of lactation and then decreased slowly until the end of the lactation (Lovendahl and Chagunda 2006). In our experiment we saw only the decrease of activity, which can be explain by the fact that our scale of time is the number of estrus. This is not enough precise compare to a daily scale and prevents us to see the increase of activity at the beginning of the lactation.

Conclusion

Estruses during a 16 months extended lactation impacted milk yield, the physical activity increases during estrus and the milk yield decreases, like in a 10 months lactation. Even so, the drop of milk production during estrus on extended lactation appeared to be lower than the one in 10 months lactation giving advantages to delay rebreeding. The drop of milk is always the same during all the several estruses as well as the increase of activity. Finally the general level of activity of a cow didn’t influence milk yield.

These results will be submitted for publication soon: “Effect of estrus and activity on milk yield of Holstein cows under extended lactation”, Gaillard Charlotte, Barbu Hélène, *Callesen Henrik, Vestergaard Mogens*

References:

Akdag, F., et al. (2010). "Effect of estrus on milk yield and composition in Jersey cows." Bulgarian Journal of Agricultural Science **16**(6): 783-787.

Arney, D. R., et al. (1994). "The increase in activity during oestrus in dairy cows." Applied Animal Behaviour Science **40**(3–4): 211-218.

Blanchard, T., et al. (1987). "Relationship of declines in grain consumption and milk-yield to estrus in dairy-cattle." Theriogenology **28**(4): 407-415.

Brzozowska, A., et al. (2014). "Locomotor activity of dairy cows in relation to season and lactation." Applied Animal Behaviour Science **156**: 6-11.

Butler, W. R., et al. (1981). "The relationships between energy-balance, milk-production and ovulation in postpartum holstein cows." Journal of Animal Science **53**(3): 742-748.

Dekkers, J. C. M., et al. (1998). "Economic aspects of persistency of lactation in dairy cattle." Livestock Production Science **53**(3): 237-252.

Erb, H. N., et al. (1984). "Rates of diagnosis of 6 diseases of holstein cows during 15 days and 21 days intervals." American Journal of Veterinary Research **45**(2): 333-335.

Esslemont, R. J., et al. (1980). "Quantitative study of pre-ovulatory behavior in cattle (British friesian heifers)." Applied Animal Ethology **6**(1): 1-17.

Garcia, E., et al. (2011). "Intensity of oestrus signalling is the most relevant indicator for animal well-being in high-producing dairy cows." Veterinary medicine international **2011**: 540830.

Gilmore, H. S., et al. (2011). "An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior of high-yielding Holstein-Friesian dairy cows." Journal of Dairy Science **94**(7): 3510-3526.

Hoffman, B., et al. (1974). "Determination of progesterone in milk applied for pregnancy diagnosis in the cow." Theriogenology **2**(1-2): 21-28.

Horrell, R. I., et al. (1985). "Changes in milk yield and composition at estrus in dairy cows." Journal of Dairy Research **52**(1): 9-16.

Hurnik, J. F., et al. (1975). "Estrous and related behaviour in postpartum Holstein cows." Applied Animal Ethology **2**(1): 55-68.

Kerbrat, S. and C. Disenhaus (2004). "A proposition for an updated behavioural characterisation of the oestrus period in dairy cows." Applied Animal Behaviour Science **87**(3–4): 223-238.

Kiddy, C. A. (1977). "Variation in physical activity as an indication of estrus in dairy cows." Journal of Dairy Science **60**(2): 235-243.

Kuczaj, M. (2007). "The changes of daily milk yield of cows before and after estrus." Acta Scientiarum Polonorum - Zootechnica **6**(2): 29-36.

Lamming, G. E. and A. O. Darwash (1998). "The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows." Animal Reproduction Science **52**(3): 175-190.

López-Gatius, F., et al. (2005). "Walking activity at estrus and subsequent fertility in dairy cows." Theriogenology **63**(5): 1419-1429.

Lopez, H., et al. (2002). "Estrogen concentrations in milk at estrus and ovulation in dairy cows." Animal Reproduction Science **72**(1-2): 37-46.

Lopez, H., et al. (2004). "Relationship between level of milk production and estrous behavior of lactating dairy cows." Animal Reproduction Science **81**(3–4): 209-223.

Lovendahl, P. and M. G. G. Chagunda (2006). Assessment of fertility in dairy cows based on electronic monitoring of their physical activity.

Lovendahl, P. and M. G. G. Chagunda (2010). "On the use of physical activity monitoring for estrus detection in dairy cows." Journal of Dairy Science **93**(1): 249-259.

Mather, E. C., et al. (1978). "Assessment of ovarian activity in the postpartum dairy cow by use of a milk progesterone assay." Theriogenology **10**(2–3): 119-129.

McCoy, M. A., et al. (2006). "Milk progesterone profiles and their relationship with fertility, production and disease in dairy cows in Northern Ireland." Animal Science **82**: 213-222.

Micinski, J., et al. (2010). "Locomotor activity and daily milk yield of dairy cows during the perioestrous period in successive lactations." Journal of Agrobiology **27**(2): 111-119.

Pryce, J. E., et al. (1997). "Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle." Animal Science **65**: 353-360.

Roche, J. R., et al. (2007). "Relationships among body condition score, body weight, and milk production variables in pasture-based dairy cows." Journal of Dairy Science **90**(8): 3802-3815.

Schofield, S. A., et al. (1991). "Variation in the milk production, activity rate and electrical impedance of cervical mucus over the oestrous period of dairy cows." Animal Reproduction Science **24**(3-4): 231-248.

Shemesh, M., et al. (1978). "Milk progesterone measurement in dairy cows: Correlation with estrus and pregnancy determination." Theriogenology **9**(4): 343-352.

Sorensen, A. and C. H. Knight (2002). "Endocrine profiles of cows undergoing extended lactation in relation to the control of lactation persistency." Domestic Animal Endocrinology **23**(1-2): 111-123.

Sorensen, A., et al. (2008). "Extended lactation in dairy cows: effects of milking frequency, calving season and nutrition on lactation persistency and milk quality." Journal of Dairy Research **75**(1): 90-97.

Stastny, L. and V. Pasek (1985). "Dynamics of udder development during the first three lactations in the same population of Czech Pied cows." Sbornik Vysoke Skoly Zemedelske v Praze, Fakulta Agronomicka, B (Zivocisna Vyroba) **42**: 157-170.

Togashi, K. and C. Y. Lin (2009). "Economic weights for genetic improvement of lactation persistency and milk yield." Journal of Dairy Science **92**(6): 2915-2921.

Van Vliet, J. H. and F. J. C. M. Van Eerdenburg (1996). "Sexual activities and oestrus detection in lactating Holstein cows." Applied Animal Behaviour Science **50**(1): 57-69.

Washburn, S. P., et al. (2002). "Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds." Journal of Dairy Science **85**(1): 244-251.

Yániz, J. L., et al. (2006). "Factors affecting walking activity at estrus during postpartum period and subsequent fertility in dairy cows." Theriogenology **66**(8): 1943-1950.

Yoshioka, H., et al. (2007). "Relationship between duration of estrus behavior determined by radiotelemetory system and three factors (milk production, steroid hormone, and ovulation time) in dairy cows." Reproduction Fertility and Development **19**(1): 129-129.

Zaied, A. A., et al. (1979). "Concentrations of progesterone in milk as a monitor of early pregnancy diagnosis in dairy cows." Theriogenology **12**(1): 3-11.

Internet source:

Aarhus University 2014. REPROLAC. Available on: http://agro.au.dk/en/research/projects/reprolac/ Consulted the 30th of April

Delaval 2013. Herd navigator and reproductive management. Available on: http://www.delaval.com/PageFiles/171203/53571175_HN_Reproduktion%20managementLOWRES.pdf Consulted the 2nd of May

Books

Volden H. (2011). Norfor “The Nordic feed evaluation system”. EAAP Publication No 130. Edited by Harald Volden.