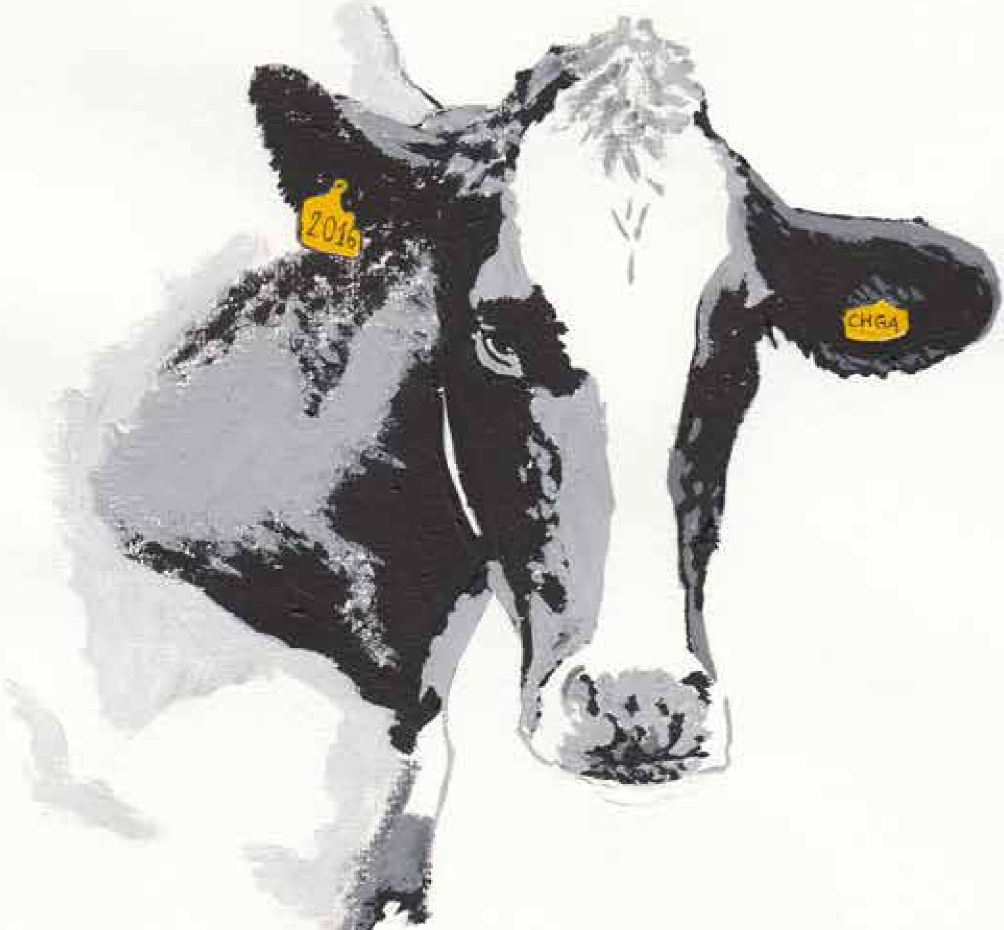
EXTENDED LACTATION AND FEEDING STRATEGIES IN DAIRY COWS

CHARLOTTE GAILLARD

PhD THESIS · SCIENCE AND TECHNOLOGY · 2016

AARHUS UNIVERSITY



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Cover – paintings from Charlotte GAILLARD

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PREFACE

The overall aim of this thesis was to investigate the relationships between lactation duration, feeding strategy and parity, in Holstein cows. This was done by using experimental data from an individual live-weight feeding strategy in early lactation and by using a cow-lifetime prediction model. In both cases, cows' productive and reproductive performances were examined.

Moreover this thesis intends to fulfill the requirements for obtaining a Ph.D. degree in Animal Science from the Faculty of Science and Technology, Aarhus University. My scholarship was funded by the Danish Council for Strategic Research, The Programme Commission on Health, Food and Welfare; and the Department of Animal Science, Faculty of Sciences and Technology, Aarhus University, Denmark. The experimental work was carried out at the experimental facilities of the Department of Animal Science, Aarhus University. The modelling work was carried out at INRA – AgroParisTech, UMR 0791 Modélisation Systémique Appliquée aux Ruminants, Paris, France.

Foulum, February 2016

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LIST OF SCIENTIFIC PAPERS INCLUDED IN THE THESIS

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LIST OF ABBREVIATIONS

AI artificial insemination
AMS automatic milking system
BCS body condition score
BHBA β-hydroxybutyrate
DFS days from shift in diet
DIM days in milk
DM dry matter
DMI dry matter intake
EB energy balance
ECM energy corrected milk
FDA functional data analysis (package on R software)
GARUNS dynamic and stochastic model of Martin and Sauvant (2010) describing the nutrient portioning regarding life priorities (Growth, Ageing, balance of body Reserves, nutrient supply to the Unborn, Newborn and Suckling calf).
HD high energy density diet
HD-LD cows fed the HD diet followed by the LD
IGF-1 Insulin-like Growth Factor-1
LD low energy density diet
LD-LD control strategy, cows fed the LD diet all over the lactation
LPM lactation persistency model
LW live weight
LWg live weight gain
ME metabolisable energy
NE net energy
NE_L net energy for lactation
NEFA non-esterified fatty acids
NDF neutral detergent fiber
NorFor Nordic feed evaluation system
SARA sub-acute ruminal acidosis
VFA volatile fatty acid

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SUMMARY

In the last decades, milk production of Holstein cows has increased while pregnancy rate has decreased. Delaying rebreeding to a stage of positive energy balance (**EB**) is expected to improve cows' pregnancy rates and increase lactation persistency. The overall objective of this thesis was to determine the effect of a live-weight (**LW**) based feeding strategy in early lactation on the production and reproduction of Holstein cows all managed for a 16 months extended lactation.

Paper 1 examines the effects of the individualized feeding strategy on plasma indicators of energy balance. The first hypothesis was that cows receiving a diet enriched in energy (**HD** diet) in early lactation until individual LW nadir would reduce the intensity of negative EB in that period compared with cows fed a standardized energy diet, lower in energy (**LD** diet). The cows fed the HD diet were then shifted to the LD diet at LW nadir (**HD-LD** cows). The second hypothesis was that after the shift in diet, the effect of the HD diet would be carried-over and would extend the negative EB further which could be detected by the concentrations of plasma metabolites. It was concluded that in early lactation, the HD diet reduced the negative EB: the cows fed the HD diet had lower BHBA and NEFA levels than the cows fed the LD diet. Plasma NEFA was higher in HD-LD than LD-LD cows from diet shift until 36 weeks after calving, indicating a carry-over effect of the early lactation HD diet to late lactation metabolism.

Paper 2 examines the effects of using the HD diet in early lactation to reduce the intensity of mobilization and thereby to sustain the mobilization for a longer period on extended lactation. It was hypothesized that the extra energy concentration during early lactation mobilization period would have a positive carry-over effect on milk production during extended lactation. It was concluded that 1) during early lactation the HD diet increased milk production of multiparous cows compared to the cows fed the LD diet but no effect was observed for primiparous cows, and 2) after the shift in diet, the HD diet given in early lactation had a 300 days negative carry-over effect on the lactation persistency for all the cows compared to cows fed the LD diet through extended lactation.

Paper 3 determines the effects of 8 consecutive estruses on milk production and reproduction of Holstein cows, as well as the effect of LW change in early lactation on these parameters. It was hypothesized that milk yield would decrease throughout the 8 estruses and that the 8th estrus would be more expressed than the 2nd estrus. It was also expected that high LW loss in early lactation would decrease milk production and

pregnancy rates compared to cows with lower LW loss. It was concluded that there was a similar loss of 0.56 kg of milk for each estrus day, and that more cows expressed the 8th estrus than the 2nd estrus. The level of LW loss in early lactation had no effect on the reproductive parameters.

Paper 4, presents a comparison of the number of artificial inseminations (AI) per pregnancy and the pregnancy rates between a 16 months lactation and the previous and following 10 months lactations. It also evaluates the effect of a double AI technique around estrus on pregnancy rates compared with a single AI per estrus. It was hypothesized that 1) the pregnancy rates would be higher for the 16 months lactation compared with the previous 10 months lactation, 2) the extended lactation would not have a negative effect on the following 10 months lactation reproductive performance, and 3) the double AI around estrus would increase pregnancy rates compared with a single AI per estrus. It was concluded that pregnancy rates and number of AI per pregnancy were similar for the 16 months lactation and the previous and following 10 months lactations. The double AI technique did not improve pregnancy rates compared with a single AI per estrus.

Paper 5 involves a lifetime performance model (GARUNS) which takes into account the changing priorities of an animal during its life and through repeated reproduction cycles. The aims of this paper were 1) to determine if the GARUNS model of Martin and Sauvant (2010) would be able to fit individual curves of milk yield, live weight, body condition score, dry matter intake, milk fat, milk protein, and milk lactose, of cows managed for 16 months of lactation, 2) to determine if the full model, including the reproductive sub-model of Phuong et al. (2015), would be able to predict the reproductive performance of cows managed for 16 months lactation, 3) to predict which lifetime scenarios would be beneficial, in terms of performance and reproduction, depending on parity and lactation length. It was concluded that the GARUNS model was able to fit individual extended lactation curves. A16 months lactation for the primiparous cows followed by 10 months lactation for the multiparous cows was shown to be the best scenario in terms of productive and reproductive performance.

To conclude, high yielding Holstein cows are able to maintain a 16 months extended lactation. This extended lactation is beneficial in terms of milk production and reproduction, mainly when it is used since the first lactation. The feeding strategy imposed in early lactation did not make major differences in terms of productive and reproductive performance.

SAMMENDRAG (DK)

Mælkeproduktionen hos Holstein-køer er steget de seneste årtier, mens drægtighedsraten er faldet. Udsættelse af reproduktion, til koen er i positiv energibalance (EB), forventes at forbedre drægtighedsraten og at forøge laktationens persistens. Det overordnede formål med denne afhandling var at bestemme effekten af en vægtbaseret (levende vægt = LW) fodringsstrategi tidligt i laktationsperioden på produktionen og reproduktionen hos Holstein-køer over en 16-måneders forlænget laktationsperiode.

Artikel 1 undersøger effekten af individuelle fodringsstrategier på plasmaindikatorer for EB. Den første hypotese var, at køer på en foderration med øget energiindhold (HD ration) tidligt i laktationsperioden og indtil individuel LW minimum (nadir) ville have en reduceret grad af negativ EB i den periode sammenlignet med køer på en foderration med et lavere energiindhold (LD ration). Køerne, som fik HD rationen, skiftede til LD rationen ved LW nadir (HD-LD køer). Den anden hypotese var, at skiftet i foderration vil medføre yderligere forlængelse af den negative EB, indikeret i koncentrationen af plasmametabolitter. Konklusionen var, at HD rationen reducerede den negative EB tidligt i laktationen: Køerne på HD rationen havde lavere BHBA- og NEFA-niveauer end køerne på LD rationen. Plasma NEFA var højere hos HD-LD-køer end hos LD-LD-køer fra skift af foderration og indtil 36 uger efter kælvning, hvilket viser en overslæbningseffekt af HD rationen tidligt i laktationen til koens metabolisme sent i laktationen.

Artikel 2 undersøger effekten på forlænget laktation, når HD-LD fodringsstrategien anvendes til at reducere graden af mobilisering tidligt i laktationen og fastholde mobiliseringen i længere tid. Hypotesen var, at den ekstra energikoncentration tidligt i laktationen ville have en positiv overslæbningseffekt på mælkeproduktionen under den forlængede laktation. Det blev konkluderet, 1) at HD rationen øgede mælkeproduktionen hos ældre køer (anden og senere pariteter) tidligt i laktationen sammenlignet med køer på LD rationen, mens der ingen effekt var hos køer i første paritet, og 2) efter foderskiftet havde HD-foderet, der blev givet tidligt i laktationen, en 300-dages negativ overslæbningseffekt på laktationens persistens hos alle køer sammenlignet med køer, der fik LD-foderet under hele laktationsperioden (LD-LD køer).

Artikel 3 undersøger effekten af 8 brunstperioder i træk på Holstein-køers mælkeproduktion og reproduktion samt effekten af LW ændringen tidligt i laktationen på disse parametre. Hypotesen var, at mælkeydelsen ville falde under hver af de 8

brunstperioder, og at den ottende brunst ville komme tydeligere til udtryk end den anden brunst. Det blev også forventet, at et stort LW tab tidligt i laktationen ville nedsætte mælkeproduktionen og drægtighedsraten sammenlignet med køer med et lavere LW tab. Det blev konkluderet, at der var et ensartet ydelsesfald under alle brunster på 0,56 kg mælk for hver brunstdag, og at flere køer viste brunsttegn under den ottende brunstperiode end den anden brunstperiode. Niveauet af LW-tab i den tidlige laktation havde ingen effekt på de reproduktive parametre.

Artikel 4 viser en sammenligning af antallet af insemineringer (AI) per drægtighed samt drægtighedsraten imellem en 16-måneders laktation og den forrige og efterfølgende 10-måneders laktation. Artiklen evaluerer også effekten af en dobbelt AI-teknik omkring brunsttidspunktet på drægtighedsraten sammenlignet med en enkelt AI per brunstperiode. Hypotesen var, 1) at drægtighedsraten ville være højere for 16-måneders laktationen sammenlignet med den foregående 10-måneders laktation, 2) at den forlængede laktation ikke ville have en negativ effekt på reproduktionen i den følgende 10-måneders laktation, og 3) at den dobbelte AI omkring brunst ville forøge drægtighedsraten sammenlignet med en enkelt AI per brunstperiode. Det blev konkluderet, at drægtighedsraten og antallet af AI per drægtighed var ens for 16-måneders laktationen og den forrige og følgende 10-måneders laktation. Den dobbelte AI-teknik forbedrede ikke drægtighedsraten sammenlignet med en enkelt AI per brunstperiode.

Artikel 5 inddrager en livstidsmodel for mælkeproduktion (GARUNS), der tager hensyn til koens skiftende prioriteter i løbet af dens levetid og gennem gentagne reproduktionscykler. Formålet med denne artikel var 1) at teste, om GARUNS-modellen udviklet af Martin og Sauvant (2010) kunne fitte køer på 16-måneders laktation og deres individuelle kurver for mælkeydelse, kropsvægt, huld, tørstofoptag, mælkefedt, mælkeprotein og mælkelaktose, 2) at afgøre, om den fulde model, inklusive undermodellen for reproduktion af Phuong et al. (2015), kunne prediktere reproduktionen for køer på 16-måneders laktation, og 3) at prediktere, hvilke levetidsscenarier der vil være mest fordelagtige med hensyn til ydelse og reproduktion. Det blev konkluderet, at GARUNS-modellen kan fitte de individuelle kurver hos køer på 16 måneders laktation. En 16-måneders laktation for køer i første paritet fulgt af 10 måneders laktation i senere pariteter viste sig at være det bedste livtstidsscenarie med hensyn til mælkeproduktion og reproduktion.

Højtydende Holstein-køer er i stand til at opretholde 16 måneders forlænget laktation. Den forlængede laktation er især fordelagtig i forhold til mælkeproduktion og reproduktion over koens livstid, når den praktiseres i den første laktation. Den individuelle fodringsstrategi, der blev brugt tidligt i laktationen, gjorde ikke den store forskel i forhold til mælkeproduktion eller reproduktion.

RÉSUMÉ (FR)

Au cours de cette dernière décennie, la production laitière (PL) des vaches Holstein a augmenté alors que le taux de gestation a diminué. Retarder volontairement le moment de l'insémination à une période où la vache a une balance énergétique (BE) positive devrait améliorer le taux de gestation des vaches et augmenter la persistance de la lactation. L'objectif général de cette thèse est de déterminer l'effet d'une stratégie alimentaire individualisée, basée sur le poids en début de lactation, sur la production et la reproduction des vaches Holstein gérées pour 16 mois de lactation.

L'article 1 examine les effets d'une stratégie alimentaire individualisée sur des indicateurs plasmatiques de BE. Les hypothèses suivantes ont été émises : 1) les vaches recevant une ration enrichie en énergie (ration HD), en début de lactation, jusqu'à un certain critère de poids, auraient un BE moins négatif pour cette période comparées à des vaches nourries avec une ration standard, plus basse en énergie (ration LD). Les vaches nourries avec la ration HD en début de lactation recevaient ensuite la ration LD (vaches HD-LD). 2) Après le changement de ration, l'effet de la ration HD serait reporté et étendrait la période où le BE est négatif, ce qui pourrait être détecté par les concentrations des métabolites plasmatiques. Il a été conclu qu'en début de lactation, la ration HD a réduit la période de BE négatif : les vaches recevant la ration HD ont eu des concentrations de BHBA et NEFA inférieures à celles des vaches recevant la ration LD. Du changement de ration jusqu'à la semaine 36 après vêlage, la concentration de NEFA a été plus élevée chez les vaches HD-LD que chez les vaches LD-LD, indiquant un effet reporté de la ration HD du début de lactation sur le métabolisme du milieu de lactation.

L'article 2 examine les effets sur la lactation prolongée de l'utilisation de la ration HD en début de lactation pour réduire l'intensité de la période de mobilisation et de ce fait étendre la durée de cette période. L'hypothèse a été émise que le supplément d'énergie

apporté en début de lactation aurait un effet reporté sur la production laitière d'une vache en lactation prolongée. Il a été conclu que 1) au début de la lactation, la ration HD a augmenté la PL des multipares comparée à celle des multipares recevant la ration LD. Au contraire, la ration HD n'a pas modifié la PL des primipares. 2) Après le changement de ration, la ration HD distribuée en début de lactation a eu un effet négatif reporté de 300 jours sur la persistance de la lactation.

L'article 3 détermine les effets de 8 œstrus consécutifs sur la PL et la reproduction des vaches Holstein, ainsi que l'effet des variations de poids en début de lactation sur ces paramètres. L'hypothèse a été émise que la PL diminuerait pendant les 8 œstrus et que le 8ème œstrus serait plus exprimé que le 2ème. Il était également attendu qu'une importante perte de poids en début de lactation aurait un effet négatif en début de lactation sur la PL et le taux de gestation comparés à ceux des vaches perdant moins de poids. Il a été conclu qu'il y avait une perte similaire de 0.56 kg de lait par jour d'œstrus, et que plus de vaches exprimaient le 8ème œstrus que le 2ème. Le niveau de perte de poids en début de lactation n'a pas influencé les paramètres de reproduction.

L'article 4 présente une comparaison du nombre d'inséminations artificielles (AI) par gestation et des taux de gestation entre des lactations de 16 mois et de 10 mois. Il évalue également l'effet d'une technique de double AI pendant l'œstrus sur les taux de gestation comparés à ceux obtenus avec une seule AI par œstrus. Les hypothèses suivantes ont été émises : 1) les taux de gestation seront plus élevés pour 16 mois de lactation comparés à ceux obtenus pour 10 mois de lactation, 2) la lactation prolongée n'aura pas d'effet négatif sur les paramètres de reproduction de la lactation suivante de 10 mois, et 3) la technique de double AI par œstrus augmentera les taux de gestation comparée à une seule AI par œstrus. Il a été conclu que les taux de gestation et le nombre d'AI par gestation étaient similaires pour 16 et 10 mois de lactation. La technique de double AI n'a pas amélioré les taux de gestation obtenus avec une seule AI par œstrus.

L'article 5 implique un modèle de performance de vie (GARUNS) qui tient compte de l'évolution des priorités d'un animal au cours de sa vie, et des différents cycles de reproduction. Les objectifs de cette étude étaient de déterminer 1) si le modèle GARUNS de Martin et Sauvant (2010) était capable d'ajuster les courbes individuelles de PL, poids, note d'état corporel, ingestion de matière sèche, matière grasse du lait, protéines du lait, et de lactose du lait, des vaches gérées pour 16 mois de lactation, 2) si le modèle incluant le sous-modèle de reproduction de Phuong et al. (2015) pouvait prédire la performance de

production et de reproduction des vaches gérées pour 16 mois de lactation, 3) s'il était possible de prédire quels scenarios de vie optimiseraient les performances de production et de reproduction des vaches, en fonction de leur parité et de la durée de la lactation. Il a été conclu que le modèle GARUNS est capable d'ajuster les courbes individuelles de lactation prolongée. De plus, 16 mois de lactation pour les primipares suivis de lactations de 10 mois le reste de leurs vies apparait comme le meilleur scenario en termes de performance de production et de reproduction.

Les vaches Holstein hautes productrices sont capables de maintenir une lactation de 16 mois. Cette lactation prolongée est bénéfique en termes de PL et de reproduction, principalement lorsqu'elle est utilisée dès la première lactation. La stratégie d'alimentation imposée en début de lactation n'a pas induit de grandes différences en termes de performance de production et de reproduction.

INTRODUCTION

High yielding dairy cows have been selected over generations for increased milk yield but this is associated with a decrease in health and reproductive performance (Butler, 2000, Gilmore *et al.*, 2011). This decrease in pregnancy rate, from around 60% in 1950 to 40% in 2000 (Lucy, 2001), is mainly due to the negative energy balance associated with high yields in early lactation (Walsh *et al.*, 2011), and the relationship between body lipid reserves and the reproductive cycle (Friggens, 2003). Therefore, the traditional 365 days calving interval (10 months lactation) have become more difficult to achieve as pregnancy rates continue to decline. Moreover, the full capacity of the animals is not well exploited as they are often dried off at a time when they are still producing large amounts of milk (Knight, 2005). An extended lactation management, such as delaying rebreeding until 8 months after calving, would potentially extend the cow's lactation duration (Osterman and Bertilsson, 2003), decrease the number of calvings per year, and decrease the number of health risks periods associated with calving (Knight, 2005, Ingvartsen, 2006). With an extended lactation management, the cow is inseminated in a stage of more positive energy balance, and is dried off at a lower milk yield than when managed for a traditional 10 months lactation. However, the individual lactation persistency is varying, and some cows might not be able to sustain a prolonged lactation (Borman *et al.*, 2004, Kolver *et al.*, 2007) but it can be difficult to "spot" these cows. The feeding strategy and energy level used might influence both, yield and lactation persistency (Kolver *et al.*, 2007, Grainger *et al.*, 2009). In early lactation, the energy output in milk is well above the energy intake from the feed ration (Ingvartsen and Andersen, 2000), and this difference influences the duration and intensity of the negative EB period, which in turn affects milk production, health, and reproduction (Butler, 2000). An individualized early lactation feeding can reduce the negative energy balance by reducing live-weight loss (Bossen *et al.*, 2009), which could attenuate the health problems and metabolic stress induced in that period. This finding suggests that an individualized feeding strategy in early lactation can be a tool to avoid problems associated with negative EB and to sustain the lactation.

The overall objective of this thesis was to determine the effect of a live-weight based feeding strategy in early lactation on production and reproduction of Holstein cows managed for a 16 months extended lactation. The thesis first reviews the status of the Holstein dairy cows in terms of milk production and reproduction, the different means used to support lactation, mainly related to milking frequency and feeding strategies, and the energy balance measurements and variation depending on the energy diet. It also reviews how the extended lactation strategy can be used as a tool to modify milk production and reproductive performance. Then, some results are presented in 5 publications to answer the main objective and test the hypotheses, and finally these results are discussed in an overall manner and put into perspectives.

BACKGROUND

1. Status of dairy cow production and reproduction

1.1. Dairy cows in Europe

Europe produces 42% of the world's cow milk (average from 1992 to 2010, FAOstat 2012) with 135 million tonnes of milk per year produced by 23 million dairy cows (Eurostat, 2010). Germany and France are the main milk producers in Europe (Table 1) ranked as the 6th and 7th respectively in the world milk production (FAOstat, 2012).

Table 1 – Key figures of the milk production and number of cows in 3 EU countries in 2010 (Eurostat, 2010, FAOstat, 2012)

	Number of cows (x 1,000)	% EU herd	Tonnes of milk (x 10^6)	% EU milk production	Average L/ cow
Germany	4,181	18.1	29	21.1	6,877
France	3,641	15.8	23	17.2	6,318
Denmark	573	2.5	5	3.6	8,389

In the last decades, the number of dairy farms in Europe has decreased while the number of cows per farm has increased (Barkema *et al.*, 2015). In Denmark, for instance, the number of farms has decreased from 5,938 in 2004 to 3,419 in 2014. Farms had, on average, 90 dairy cows in 2004, and this number has jumped to162 in 2015 (Table 2). Milk production per cow has increased due to three main factors: genetic selection of the dairy cows, improved feeding, especially better-quality forage, and the improvement of management system (Nygaard, 2006). In Denmark, where Holstein is the predominant breed (72%), followed by Jersey (12%), Red Danish (8%), crossbreds (7%) and Red Holstein (1%) (Nygaard, 2006), the average milk recorded yield of all breeds was 8,442 kg in 2004 and 9,663 kg in 2014, which represents an increase of 12.6% in 10 years.

Table 2 - Key figures of dairy cows in Denmark from Dairy Statistics (2014) and Kristensen et al. (2015)

Denmark	2004	2010	2011	2012	2013	2014	2015
Population of dairy cows (/ 1,000 heads)	598	573	579	579	567	547	
Number of dairy farms	5,938	4,138	3,953	3,887	3,618	3,545	3,419
Average cows per farm	90	126	132	138	148	158	162
Milk yield, kg/cow	8,442	9,079	8,919	9,019	9,138	9,663	
Milk fat, %	4.30	4.30	4.27	4.28	4.26	4.21	
Milk protein, %	3.43	3.45	3.46	3.47	3.48	3.49	

In Europe, most of the cows are managed for a 365 day calving internal in loose-housing systems. The number of grazing cows is very variable, from 99% in Ireland to 30% in Denmark, with an overall decline of the pasture system in Europe. The indoor cows are fed roughage (silage made of ryegrass, grass/clover and maize – in Denmark) and concentrates (cereals, soybean meal, rapeseed, byproducts etc.), mainly fed as total mixed ration or partially mixed ration (when some extra concentrate is given for example at milking). The number of farms equipped with automatic milking systems (AMS) in the world started increasing in 1992 (De Koning, 2011), when the first robot was commercialized. In Denmark, 25% of the dairy farms make use of AMS, with an average of two AMS per farm (Beekman and Bodde, 2015). With AMS and all year housing and calvings, the setting is ideal for using an extended lactation strategy.

Milk quota and economy. In 1984, quotas were introduced in Europe to restrain and regulate milk production, which was exceeding demand. Quotas have increased progressively since 2005 and ended “softly” in March 2015. The end of the quotas is due to a considerable increase in consumption of dairy products in the world, which is expected to keep increasing. In this context, the extended lactation management might be a tool to increase milk production, to answer the quota suppression and the increase of dairy products consumption.

Challenges. During the last decades, dairy cows have been selected for increased milk production. This increase induces a need to modify the management of the cows. The milk production capacity of the cows is not fully utilized as most of them still have a high milk yield at drying off (Knight, 2005), which often exceeds 30 kg/d (Madsen *et al.*, 2008). The price of the feed has increased, as more concentrate feed is used to bring more energy to the high productive cow (Kolver and Muller, 1998, García and Fulkerson, 2005). The ability to conceive and the health status of the cows have decreased with increasing milk production (Butler, 2000, Pryce *et al.*, 2004, Gilmore *et al.*, 2011), leading to considerable economic losses. The increase in milk production requires more energy to be directed to the mammary gland, and this energy does not come completely from feed intake, which forces the cows to further use body reserves and to extend the negative energy balance period, bringing negative effects to pregnancy rates and health (Butler and Smith, 1989, Veerkamp *et al.*, 2003, Pryce *et al.*, 2004). These changes lead to challenges, mainly in the early lactation period, when the cow needs to be fed enough to respond to the high demand of peak milk yield, and requires veterinary attention to treat management/production diseases (mastitis, lameness, undernutrition leading to poor BCS or loss of BCS), and some infectious diseases (Dobson *et al.*, 2007).

These challenges show a clear need to better understand the relationships between production, reproduction, energy balance, health, and metabolic status of the dairy cow, in order to propose pro-active feeding and management solutions.

1.2. Hormonal regulation and nutrients used by the mammary gland

Hormonal regulation of milk production. The lactation cycle of a dairy cow is divided into consecutives stages (mammogenesis, lactogenesis, galactopoeisis, and involution) controlled by hormones (Svennersten-Sjaunja and Olsson, 2005). Three categories of hormones are involved: reproductive hormones, which act directly on the mammary gland (oestrogen, progesterone, placental lactogen, prolactin and oxytocin); metabolic hormones, which are responsible for coordinating the body's response to metabolic changes and stress (GH, corticosteroids, thyroid hormones, insulin, IGF-1, GI hormones); and finally the locally produced hormones, including GH, IGF-1, prolactin and leptin (Neville *et al.*, 2002).

The maintenance of lactation (galactopoeisis) is driven by 2 main factors: the galactopoietic hormones and the removal of accumulated milk from the mammary gland. Growth hormone (GH) and prolactin are considered to be the predominant galactopoietic hormones in ruminants (Bauman, 1992, Tucker, 1994, Flint and Knight, 1997). GH is high in early lactation (Ingalls *et al.*, 1973, Convey, 1974, Gill and Hart, 1980), low in late gestation and controls the nutrient partitioning towards the mammary gland (Neville *et al.*, 2002). GH stimulates IGF-1 production (Frystyk, 2004), and also induces insulin resistance in the adipose tissues and muscles (Takano *et al.*, 2001). The sensibility of these tissues to insulin decreases (increase of insulin resistance) as their glucose uptake is reduced, leaving more glucose available for the mammary gland (Bauman and Currie, 1980, Cronjé, 2000). Prolactin receptors found in the mammary gland have been positively correlated with milk production (Akers, 2002). Prolactin maintains the corpus luteum during early pregnancy (Neville *et al.*, 2002). During lactation, prolactin is released at the time of milk removal and helps maintaining the lactation (Tucker, 1994) by inhibiting apoptosis of the mammary gland cells (Flint and Knight, 1997), even though it's role in this process is still unclear. Oxytocin is released from the pituitary gland when suckling. Oxytocin causes the contraction of the myoepithelial cells surrounding the alveolus resulting in milk ejection (Neville *et al.*, 2002). IGF-1 is mainly produced by the liver, in the mammary gland and many other tissues, and plays a role in the cell growth and differentiation, maintenance of cell function and in apoptosis prevention (Frystyk, 2004, Beattie *et al.*, 2006). IGF-1 is higher in late gestation. Prolactin, GH and IGF-1 are involved in the control of milk secretion and apoptosis (Wilde *et al.*, 1998), but further details on this endocrine regulation of mammary gland metabolism is beyond the scope of this thesis.

Progesterone rises in early pregnancy, falls sharply around parturition and early lactation, increasing again in late gestation (Neville *et al.*, 2002). The estrogens, secreted by the corpus luteum during pregnancy, are rising at the end of the pregnancy. They might also play a role at the end of the lactation by stimulating the involution (Athie *et al.*, 1996, Neville *et al.*, 2002) (Figure 1).

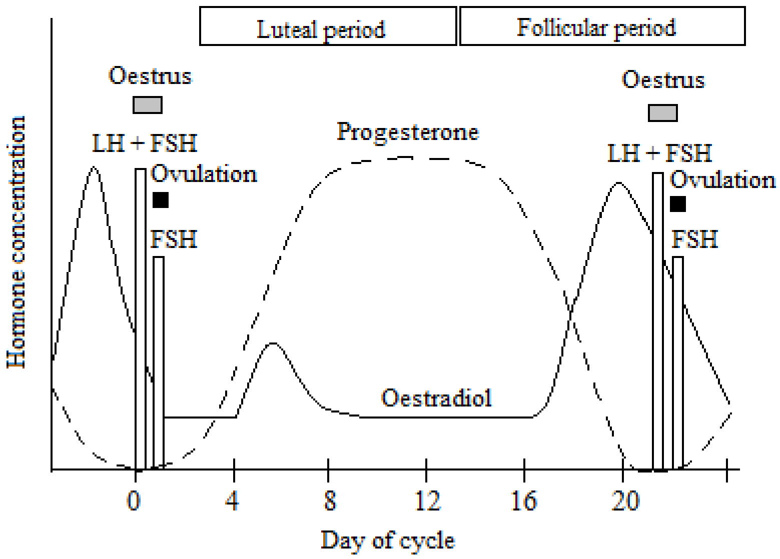

Figure 1 - Changes in hormone concentrations druing the bovine oestrous cycle (Mukasa-Mugerwa (1989)). LH = Luteinising hormone, FSH = Follicle stimulating hormone.

Nutrients from diet to mammary gland. Cow's milk is constituted by 87% water, 4.6% lactose, 3.9% fat, 3.0% protein (casein mainly), minerals and vitamins.

The volatile fatty acids (VFA - acetate, propionate and butyrate) produced by the diet's carbohydrates fermentation in the rumen are the main energy source for the ruminants (60 to 70% of the metabolizable energy supply)(Armentano, 1992, Van Soest, 1994). Acetate is mainly produced by structural carbohydrates present on the diet (fibers like hay and silage), and then used in fatty acid synthesis. Butyrate and a small part of acetate are converted to ketone bodies (BHBA and acetoacetate, respectively). About 17-45% of the fat in milk is built from acetate and 8- 25% from butyrate (McGuire and Bauman, 2003). The rest of milk fatty acids is provided by the lipids in the diet and the lipolysis of adipose tissue triacylglycerol (Parodi, 2004). Propionate is produced by the non-structural carbohydrates (mainly starch and sugar), and is the major precursor of the gluconeogenesis (glucose synthesis from non-carbohydrate sources) in the liver, accounting for 65 to 80% of the net glucose supply (Brockman, 1978, Reynolds *et al.*, 2003). Milk synthesis uses 80% of the total glucose turnover (Stockdale and Roche, 2002): for lactose synthesis (60 to 70%), for pentose phosphates synthesis (20 to 30%), for glycolysis (pyruvate, ATP), and for glycerol synthesis (Figure 2).

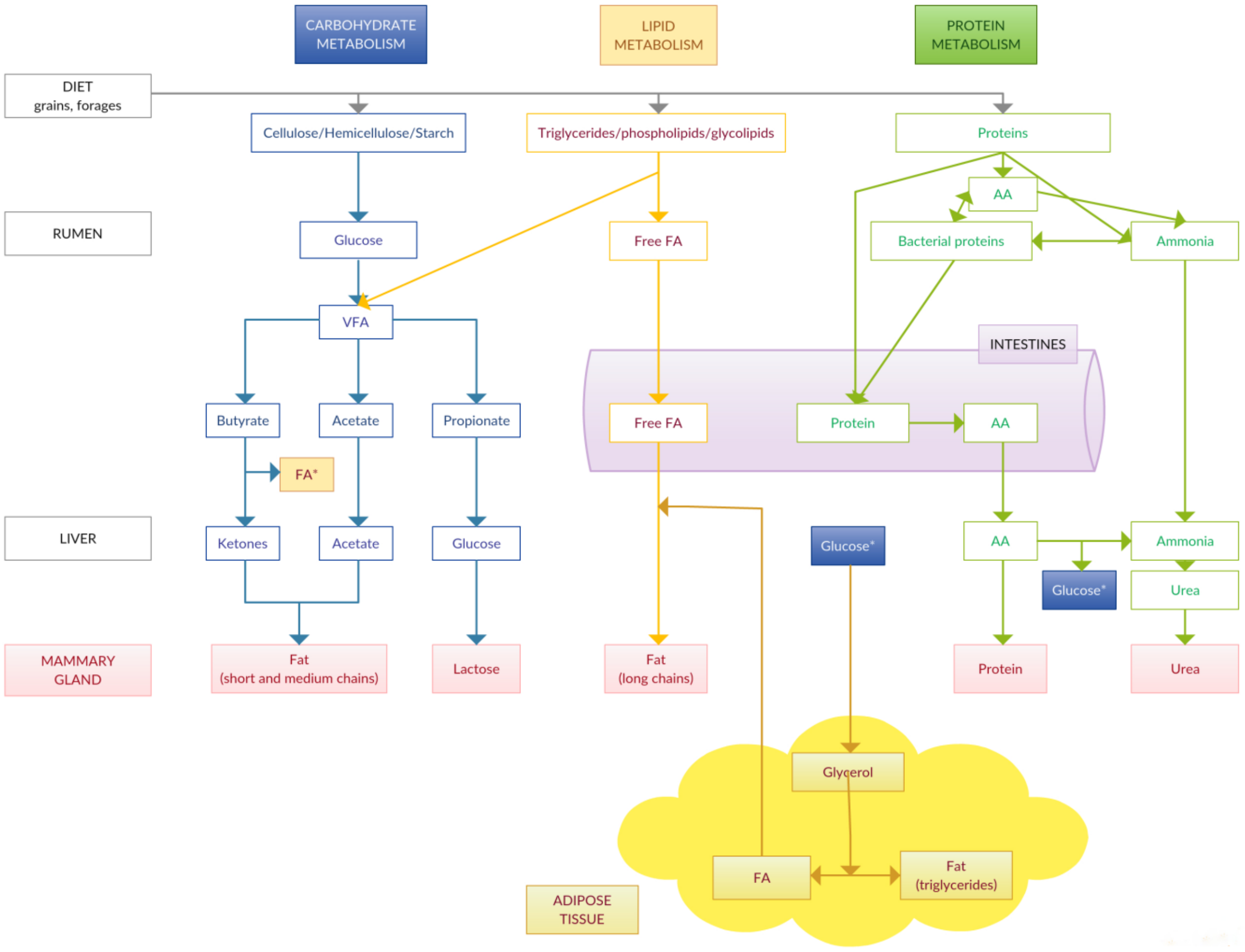

Figure 2 - Carbohydrate, lipid and protein metabolism – from diet to milk components (supported by Walker et al., 2005; Kay, 1969; McGuire and Bauman, 2003; Parodi, 2004; Brockman, 1978; Reynolds et al., 2003). The asterisk (*) indicates that the element produced will then enter the appropriate metabolism way.

Ruminants' main source of available protein is derived from the microbial population (Walker *et al.*, 2005) but some feed protein will escape rumen fermentation and pass to be digested in the stomach (abomasum) and absorbed as amino acids and di- and tri-peptides in the intestines. Ruminants have the ability to utilize other nitrogen sources than amino acids because the microorganisms in their rumen can synthetize amino acids and protein from non-protein nitrogen sources. Urea is excreted through urine, but a part of it is also recycled, returning to the rumen through saliva or the rumen wall to be used by the microbes (Lapierre and Lobley, 2001). Feed proteins are broken down and hydrolyzed by the microorganisms in the rumen into amino acids, and then into ammonia. The ammonia pool is used for bacterial growth and absorbed by the ruminal wall (Walker et al., 2005). After absorption, the ammonia is detoxified in the liver by being converted to urea, which will be excreted in the urine or recycled. Some feed proteins, not degraded in the rumen (40%), and the bacterial proteins (60%), are degraded in the intestines into di- or tri-peptides and amino acids, which are then absorbed by the intestinal wall (Kay, 1969). These amino acids pass the liver, and are then transferred to the mammary gland to produce milk protein, and to the muscles. The amino acids absorbed by the mammary gland are used to synthetize milk proteins (Figure 2). Milk contains around 30 g protein per kg, and about 90% of this protein is casein. The 10% left correspond to the whey proteins (immunoglobulins, α-lactalbumin for lactose synthesis, β-lactoglobulin) and none amino-acids protein like urea (Akers, 2002).

Milk capacity lactation curve. Figure 3 relates milk production, dry matter intake and live weight (LW) during lactation. In high yielding cows, the feed intake peak occurs after the milk yield peak (Coffey *et al.*, 2004). Therefore, cows use their body reserves until the milk yield peak, and regain them in late lactation (Ingvartsen and Andersen, 2000).

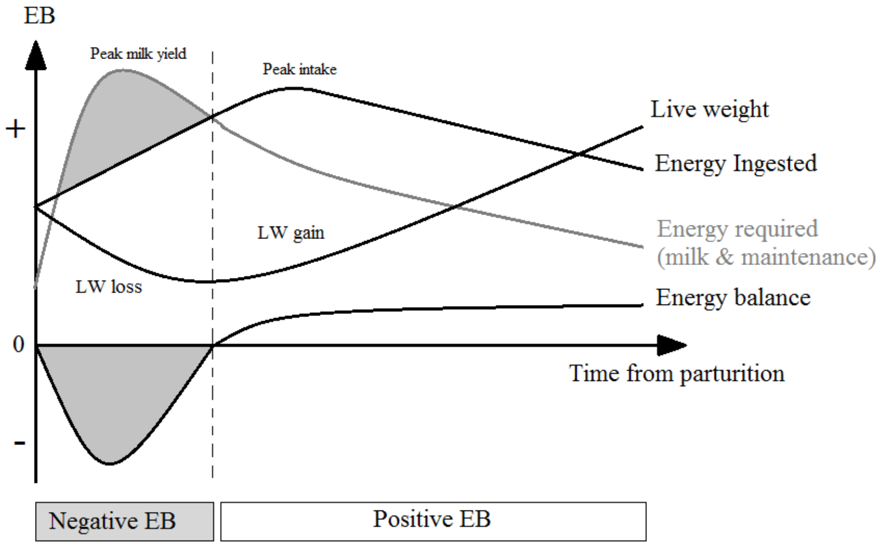

Figure 3 - Relationship between production characteristics during the different phases of the lactation and variation in energy balance (EB)

1.3. Reproduction cycle and conception

Cows are coming in heat throughout the year at regular intervals of around 21 days (range from 18 to 24 days), and this is called estrus cycle (Kerbrat and Disenhaus, 2004). This cycle is divided into two phases, the follicular phase and the luteal phase (Donadeu *et al.*, 2012). The corpus luteum excretes progesterone, and the ovary of the follicle produces estrogens, responsible for the signs of heat (e.g. red swollen vulva, mounting behavior, chin resting) (Esslemont *et al.*, 1980).

A cow is in heat when the progesterone falls and estrogen rises (Figure 1), and is ready to be inseminated during a short period of few hours (14.1 ± 4.5h) (Kerbrat and Disenhaus, 2004), between estrus and ovulation (Donadeu *et al.*, 2012). If the cow becomes pregnant, the progesterone stays high because the corpus luteum is maintained (Zaied *et al.*, 1979). After calving, the cow's cyclicity normally resumes within 40 days but heat signs can be weak on the first estrus.

A nutrition failure can lead to difficulties in conceiving, a lack of nutrients for the growth of the fetus, premature birth, malformations, weak calves, and diseases like milk fever during the delivery of the calf. During gestation, maternal glucose is the main source of energy for the fetus and placenta which provides oxygen and nutrients to the fetus (Thibault and Levasseur, 2001). Around 30 to 40% of the glucose is oxidized in the placenta and the rest is used to produce lactate, which will be partly excreted in fetal blood for ATP production (Bell, 1995, Thibault and Levasseur, 2001). The uterus as well uses amino acids and ketones as energy precursors (Bell, 1995). The placenta is secreting several hormones such as progesterone and estrogens (Klein, 2012). Placental progesterone is positively regulated by IGFs and negatively by estrogens, and helps maintaining the gestation. Placental estrogens participate in the fetal implantation, the development of the uterus and mammary gland, and increase prolactin secretion at calving and at lactogenesis. At the onset of lactation, nutrients requirements for milk production increase by 10-30% (Drackley *et al.*, 2005). In early lactation, the mammary gland is taking up most of the cow's energy and nutrients. For the high yielding cows, selected over generations for increased milk yield, the negative EB is strong (Walsh *et al.*, 2011), and associated with a decrease in reproductive performance (Osterman and Bertilsson, 2003, Gilmore *et al.*, 2011).

Based on progesterone profiles, several studies have also identified major reproductive problems. A high percentage of cows (15 to 25%) have delayed ovulation resumption (Crowe and Mullen, 2013), and around 11-22% of cows have a prolonged luteal phase (Shrestha *et al.*, 2004). These reproductive problems can lead to metritis, abnormal vaginal discharge, retained placenta, and earlier ovulation resumption (Crowe and Mullen, 2013). Other important factors can lead to low pregnancy rates (<50%), such as bad insemination timing due to an imprecise heat detection, poor quality of insemination (semen or technique), infection of the reproductive tract, hormonal disorders, anatomical defects, early embryonic death, or nutritional problems.

2. Energy balance during lactation

2.1. Energy partitioning and negative energy balance

Energy partitioning. Partitioning is the proportionate distribution of absorbed nutrients (or energy) between milk, maintenance, growth, activity, conception, and waste products (Pryce *et al.*, 2004). The energy needed for milk production depends on the yield of the cow, but is around 70% of the energy output, while the energies used for maintenance and activity are around 20 to 25% and 5 to 10%, respectively. The energy needed for growth is small and null for cows above second parity, and the energy for conception is less than 1% (Friggens *et al.*, 2007b).

The energy partitioning varies with the age and the stage of lactation of the cow as proposed by the GARUNS model of Martin and Sauvant (2010). During the first month of the calf's life, the energy is mainly used for the animal's growth and its constitution of body reserves. The energy needed for growth slowly decreases with the animal's age and is almost null around second lactation. At parturition, the energy allocated to body reserves constitution is almost null as it is used for milk production to assure survival of the newborn calf. With the growth of the newborn calf and the decrease of suckling, the energy for milk production decreases while the energy for body reserves increases (Figure 4).

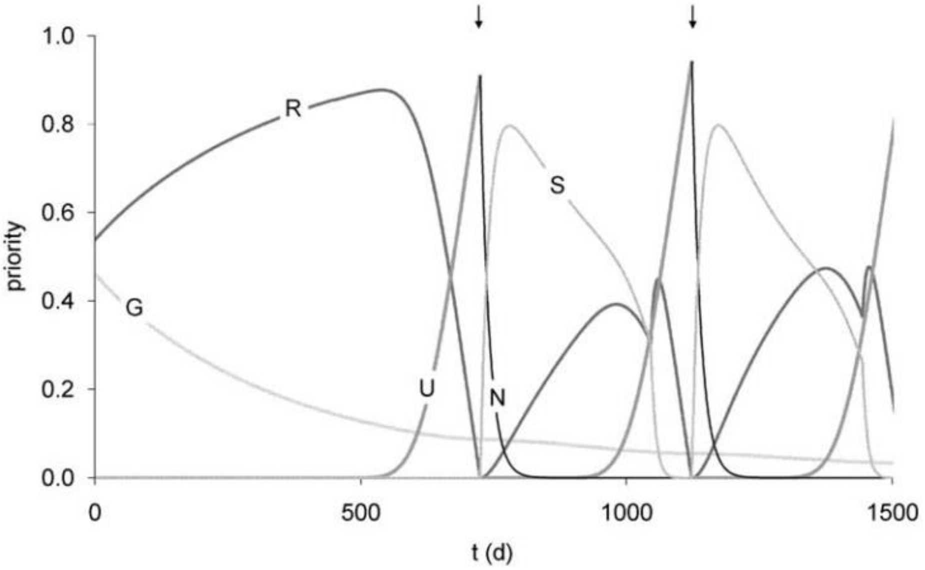

Figure 4 - Trajectories of priorities of the GARUNS model over 1500 days of life. G: growth, R: balance of body reserves, U: ensuring survival of the unborn calf, N: ensuring survival of the newborn calf, S: ensuring survival of the suckling calf. Arrows indicate parturition times of two successive reproductive cycles (from Martin and Sauvant (2010)).

Negative energy balance. Energy balance (EB) is defined as the difference between energy intake from feed and energy required for body maintenance, production and gestation (Heuer *et al.*, 2000), and it constitutes and important parameter for dairy cows. In early lactation, the energy output is higher than the energy input, leading the cow to a negative EB (Bertilsson *et al.*, 1997, Kay *et al.*, 2009). At this time, the cow cannot eat enough to fulfill her requirements and has to draw off her own body reserves to produce milk (Coffey *et al.*, 2004). The EB becomes positive around the feed intake peak (Figure 3).

2.2. Methods for measuring energy balance

Feed intake, milk yield, and live weight.. EB can be calculated by subtracting the energy output from the energy input (Butler and Smith, 1989, McNamara *et al.*, 2003a). The energy input corresponding to the feed energy intake, and the energy output to the sum of the energy requirements for milk, maintenance, growth, conception, and activity (Friggens *et al.*, 2007b). The main difficulty for this calculation is to have all the measurements of input and outputs available.

Body Condition Score (BCS). The BCS reflects the degree of fatness, especially the subcutaneous fatness, and has been used to estimate the EB. Indeed, the larger variation of EB occurs during early lactation, when the cows are mobilizing body reserves, leading to a negative EB. Then the cows enter a deposition phase to recover their body reserves. The BCS is a practical and visual way to have a fast and rough estimation of the energy status of a cow in the farms (Stockdale, 2001). Nevertheless, BCS measurements are subjective, not so precise, and do not describe total fat and protein mobilization/level. New techniques have been developed to give a more accurate estimation of fat mobilization than BCS, by using ultrasound, which gives a measurement of subcutaneous fat on a given point (Domecq *et al.*, 1995), or 3D rear shape analysis (Fisher *et al.*, 2015). Body composition can also be determined using the deuterium oxide dilution technique presented in Bjerre-Harpøth *et al.* (2015).

Live weight gain (LWg). LWg measurement is a more precise alternative to BCS measurements to estimate EB and has the advantage of being done automatically (Maltz, 1997), for example with a platform scale in the AMS. To gain in precision LW measurements can be adjusted for milk and meal-related gut fill (Thorup *et al.*, 2012).

Blood markers. Glucose, BHBA and NEFA are the main blood indicators of energy status and mobilization of adipose tissues.

Several studies (Walsh *et al.*, 2007, Duffield *et al.*, 2009, Ospina *et al.*, 2010, Chapinal *et al.*, 2012, McArt *et al.*, 2012, Roberts *et al.*, 2012, Suthar *et al.*, 2013, Berge and Vertenten, 2014) showed that the level of BHBA and NEFA are high in plasma during early lactation, due to high mobilization of adipose tissue and, consequently, high negative EB (Bell, 1994, Vernon, 2005, Roche *et al.*, 2009). The concentrations are slowly decreasing with the recovery of body reserves and the return to positive EB. Indeed, an increased mobilization means that the lipolysis of the triglycerides stored in the adipose tissue increases and produces NEFA and glycerol. Consequently, the amount of NEFA absorbed by the liver increases. In the liver the NEFA are stored as triglycerides or oxidized in the mitochondria into ketone bodies, e.g. BHBA (Jesse *et al.*, 1986, Bauchart *et al.*, 1996, Vernon, 2005), that can be exported to other organs to serve as an energy source (Drackley and Cu, 2001). So, NEFA concentrations reflect the magnitude of fat mobilization from body reserves, whereas BHBA concentrations reflect the completeness of oxidation of fat in the liver. This explains the positive correlation found between BHBA and NEFA (Harrison *et al.*, 1990) (Figure 5).

The BHBA and NEFA are also good indicators and predictors of the health status of the cow. A concentration above 1.2-2.9 nmol of BHBA per liter of blood (McArt *et al.*, 2012) indicates that the cows are more likely to present health risks (displaced abomasum, metritis, ketosis, culling)(McArt *et al.*, 2012, Suthar *et al.*, 2013), reproduction problems (failure to conceive, retained placenta)(Walsh *et al.*, 2007, Ospina *et al.*, 2010), and decreased milk production (Duffield *et al.*, 2009, Chapinal *et al.*, 2012).

Plasma glucose decreases in early lactation due to its high consumption by the mammary gland (Davis *et al.*, 1979, Bell, 1995), and is consequently negatively correlated with EB (Harrison *et al.*, 1990, Reist *et al.*, 2002, Clark *et al.*, 2005). The mammary gland also takes up acetate, BHBA and glucose to synthetize triglycerides used for the formation of milk fat (Barber *et al.*, 1997, Bauman and Griinari, 2003) (Figure 5).

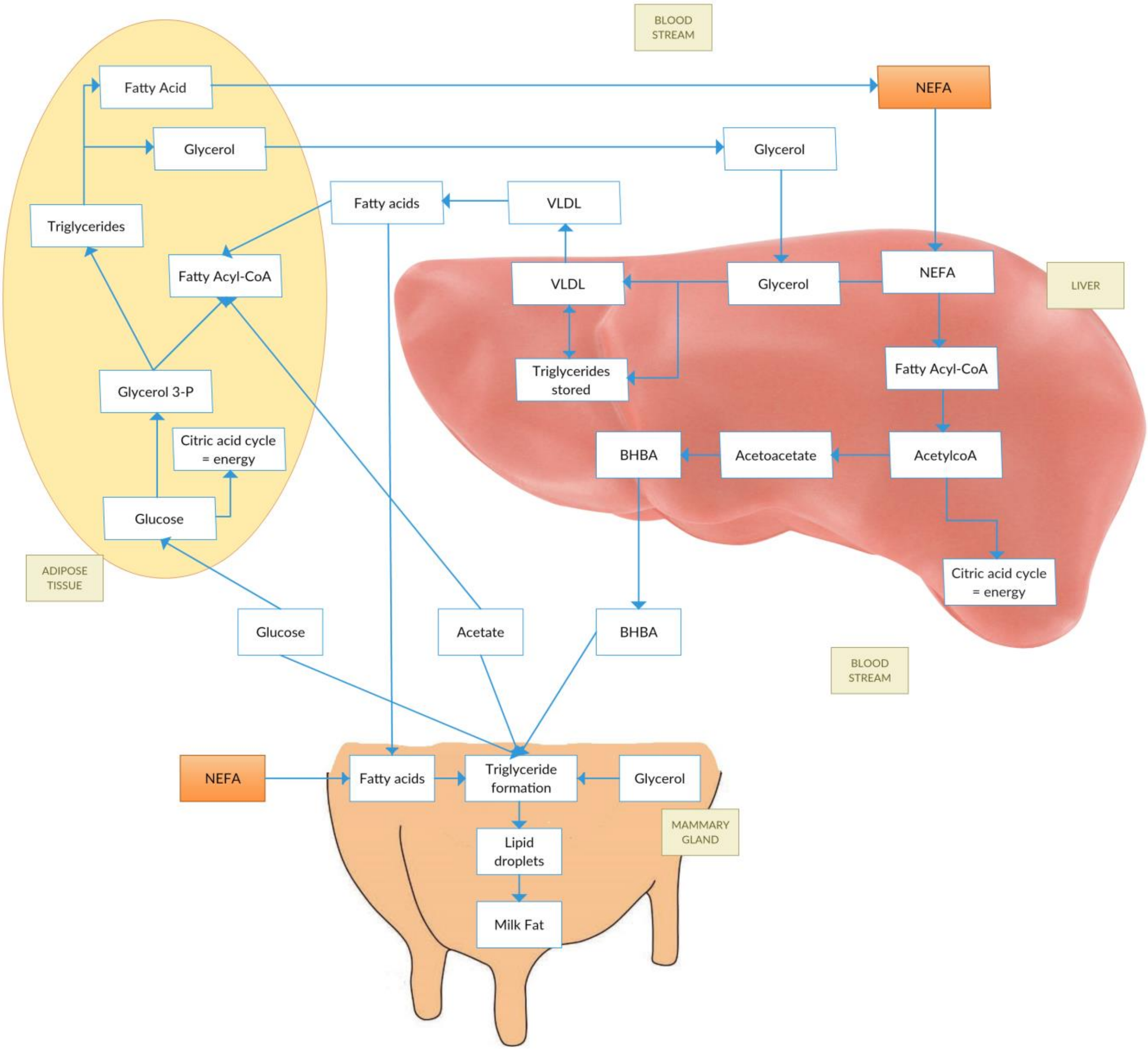

Figure 5 - Overview of the relationships between adipose tissue, liver and mammary gland focusing on the production and utilization of NEFA, BHBA, and glucose (inspired by Bjerre-Harpøth, (2015) and Vernon (1980))

Insulin is a key regulator of adipose tissues as it stimulates the synthesis and storage of adipose tissues (Brockman, 1978) by increasing the glucose uptake and promoting synthesis and activation of acetylCoA carboxylase and lipoprotein lipase (Vernon, 2003). Blood insulin is low in early lactation and increases thereafter. With low concentrations of insulin, the secretion of the hormone sensitive lipase is stimulated, increasing lipolysis with the subsequent release of NEFA to the bloodstream (Melendez *et al.*, 2009). The high NEFA concentration at that time could also have a toxic effect on pancreatic cells (Cnop *et al.*, 2001, Maedler *et al.*, 2001, Bossaert *et al.*, 2007).

IGF-1 is mainly produced in the liver (Sjogren *et al.*, 1999, Yakar *et al.*, 1999) in response to GH (Rose, 2002), insulin and nutritional status (Thissen *et al.*, 1994, Jones and Clemmons, 1995). This relationship between IGF-1 and GH constitutes the somatotropic axis "GH-IGF-1" which is "coupling" when GH stimulates IGF-1 properly. In negative EB, for example in early lactation, even though GH is high (Neville *et al.*, 2002), it can't stimulate the hepatic IGF-1 production (Donaghy and Baxter, 1996) because the insulin concentration is too low to stimulate enough the synthesis of the GH receptors needed for GH action (Kobayashi *et al.*, 1999). Moreover, the low stimulation of the IGF-1 genes expression by insulin contributes to the low IGF-1 production (Butler *et al.*, 2003). These two factors induce a low IGF-1 concentration, and this phenomenon is called "GH resistance" (Chilliard, 1999, Kay *et al.*, 2009) or "uncoupling" of the GH-IGF-1 axis. This resistance is reduced when insulin increases (Butler *et al.*, 2003), when the EB increases (Spicer *et al.*, 1990) (Figure 6). This positive correlation between EB and IGF-1 (Clark *et al.*, 2005) makes IGF-1 a relevant indicator of energy status of the dairy cow.

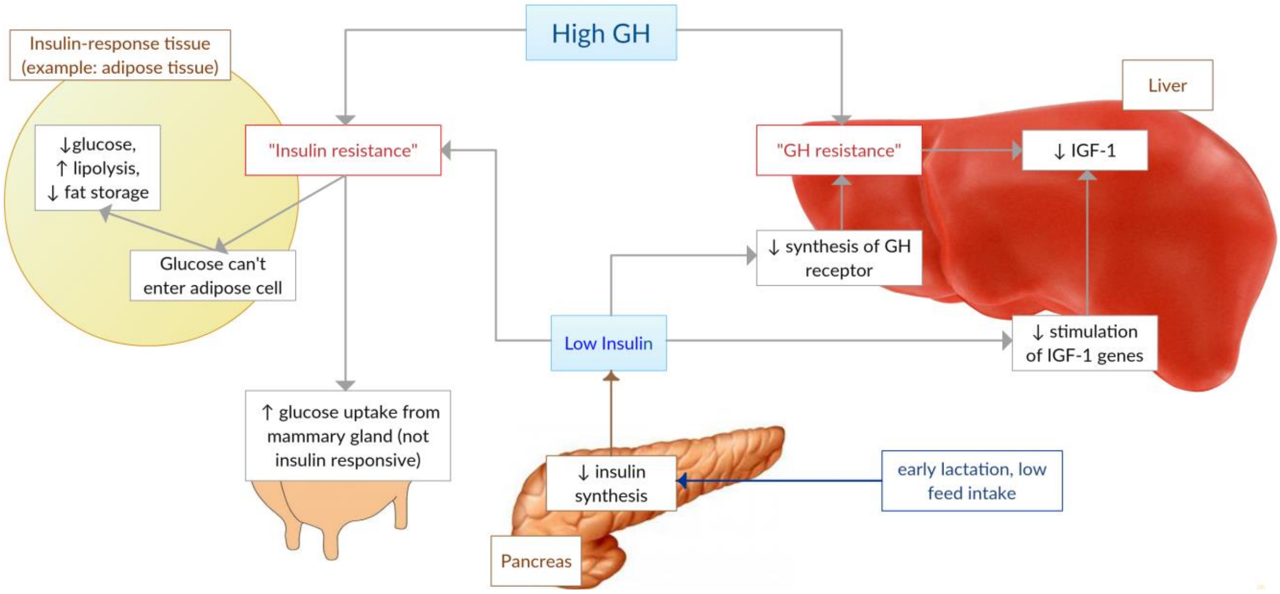

Figure 6 - GH resistance and insulin resistance in early lactation (supported by Neville et al., 2002, Donaghy and Baxter, 1996, Kobayashi et al, 1999, Butler et al., 2003, Chilliard, 1999, Kay et al., 2009, Spicer et al., 1990)

Urea is produced in the liver from the ammonia accumulation and absorption in the rumen initially produced by the dietary amino acid catabolism. Most urea is excreted in the urine, some recycled in saliva, and some secreted in milk. Urea concentration is a good indicator of protein excess, but a weak indicator of EB as only weak correlations have been found (Reist *et al.*, 2002, Clark *et al.*, 2005).

***Milk markers*.** The physiological status of the cow is reflected in the metabolism of the mammary gland (Bjerre-Harpøth, 2015). Looking at the variation of milk composition, e.g. fat, protein, lactose (Grieve *et al.*, 1986, Nielsen *et al.*, 2003, Bjerre-Harpoth *et al.*, 2012), during the different phases of lactation can give a good estimation of the energy status of the cow (Garnsworthy *et al.*, 2006, Stoop *et al.*, 2009, Larsen and Moyes, 2015). For example, the deficit in energy in early lactation will be seen as by higher milk fat, lower or unchanged milk protein, and lower milk lactose. Major components measured in milk can be used to predict the EB (Reist *et al.*, 2002, Friggens *et al.*, 2007a). Using the BHBA in milk has been of interest to detect ketosis and estimate EB (Enjalbert *et al.*, 2001), although it is influenced by feed rations (Murphy, 1999), time and frequency of feeding (Blum *et al.*, 1985, Eicher *et al.*, 1999), so the sampling time has to be fixed and chosen far from feeding time to avoid bias.

2.3. Relationships between energy balance, milk production, and reproduction

Production. A cow producing 35 kg of milk daily requires 3 times more energy for production than for body maintenance (Butler and Smith, 1989). The negative EB, encountered in early lactation, enhances the lipolysis leading to high production of NEFA and ketone bodies i.e. BHBA. When ketone bodies increase, the risk of health issues (ketosis, displaced abomasum, and sub-acute rumen acidosis - SARA, defined by a pH < 5.5 for at least 180 minutes) rises, and the immune cell function drops which can lead to a decrease in DMI and consequently of milk yield, and an increased risk of retained placenta that can lead to metritis (Figure 7).

Reproduction. The negative EB also affects the reproductive performance of the cows in different ways (Butler, 2000). On one side, the negative EB reduces the blood glucose and insulin, which sends a signal to the central nervous system to decrease GnRH (Gonadotropin Releasing Hormone). The GnRH is also decreased by the reduction of leptin secretion induced by the increase of lipolysis (Remppis *et al.*, 2011). This fall of GnRH induces a decrease of FSH (follicle stimulating hormone) and LH leading to a decrease of estradiol secretion. The lack of estradiol causes a prolonged follicular phase and a poor estrus expression, later seen as a delayed ovulation and errors in heat detection (Canfield and Butler, 1991, Jolly *et al.*, 1995). On the other side, the decrease in insulin and IGF-1, induced by the negative EB, slow down the follicular growth and provoke ovarian dysfunctions (Nebel and McGilliard, 1993, Liefers *et al.*, 2003, Veerkamp *et al.*, 2003, Patton *et al.*, 2007). Indeed, with negative EB the concentrations of insulin are lowered (McGuire *et al.*, 1995), which decreases the production of hepatic GH receptors (Pell and Bates, 1990). Even though the GH concentration is unchanged during negative EB (McGuire *et al.*, 1995), the lack of GH receptors induces a decrease of IGF-1 synthesis, needed for the development of the ovarian follicle (Giudice, 1992, Jones and Clemmons, 1995), resulting in a decrease of pregnancy rate. Moreover, during negative EB, the NEFA concentration is high while the glucose is low, so there is less energy available for the ovary. This lack of energy reduces the development of the follicles and the quality of the ovocyte (Jorritsma *et al.*, 2003b). Another effect of excessive negative EB is lowering the concentration of progesterone (Spicer *et al.*, 1990, Reksen *et al.*, 2002) which negatively affects the ability to conceive (Folman *et al.*, 1990) and the embryo survival (Thibault and Levasseur, 2001). The embryo mortality between 30 and 90 days after insemination is one of the causes of low pregnancy rate (Figure 7).

Production and Reproduction. The pregnancy rate and the number of days from calving to first insemination decrease as milk yield increases (Spalding *et al.*, 1975) and with high genetic merit of the cow (Veerkamp *et al.*, 2003).

To summarize, the negative EB has some possible direct negative effects. The release of NEFA is associated with ketone bodies, a useful source of energy for general metabolism and milk fat synthesis, but which also increases the risks of health issues (ketosis, fatty liver, retained placenta), decreasing DMI and consequently, milk yield. The negative EB has some possible negative indirect effects as it decreases the cell immune function, it increases the reproduction problems and the risk of SARA.

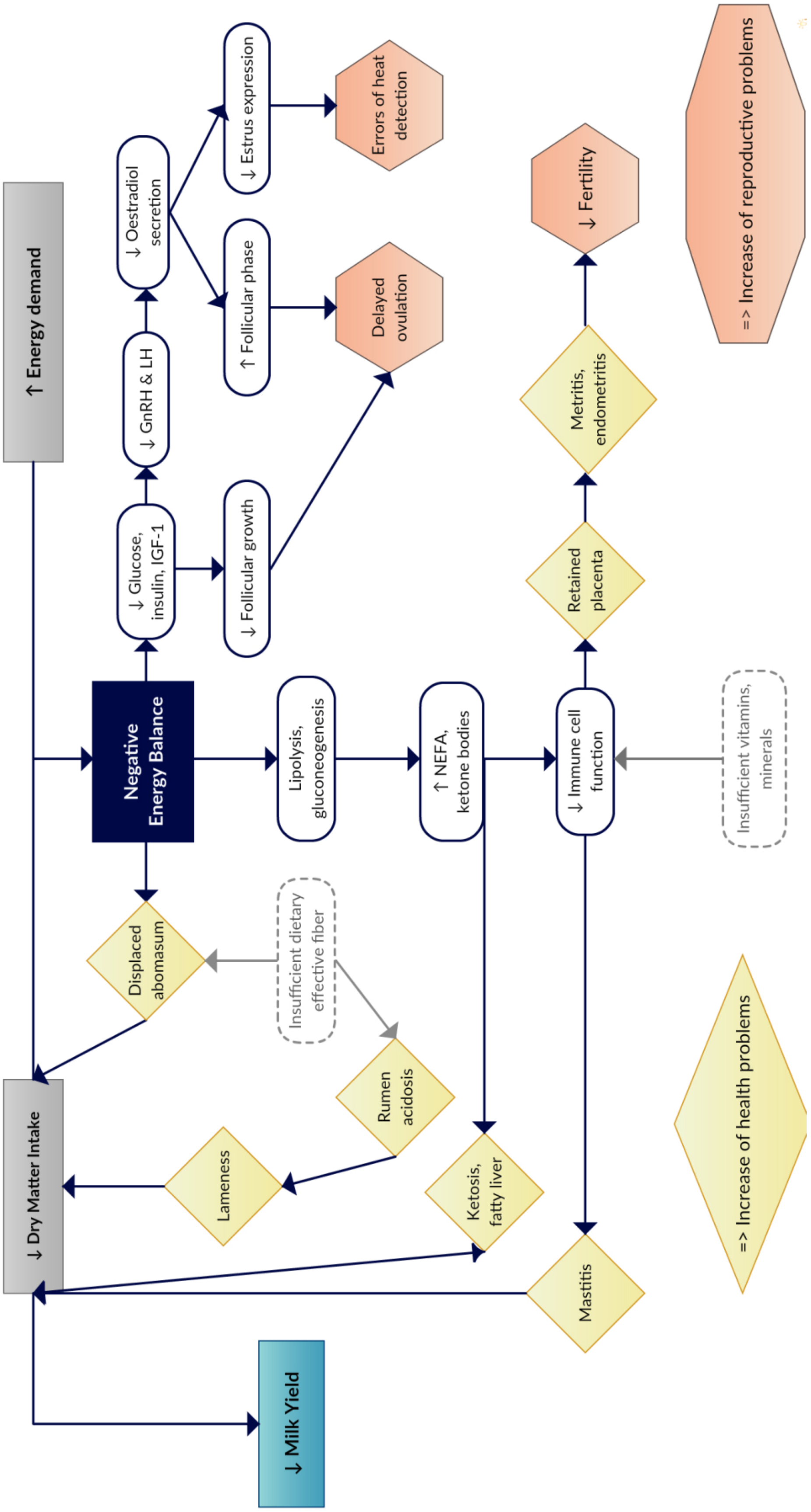

Figure 7 – Some of the possible negative effects of negative EB on health, productive and reproductive performance (adapated from Esposito et al. (2014))

3. Strategies to support milk production and reproduction

3.1. Milking frequency to support milk production

Increasing the milking frequency from twice to thrice daily has been shown to increase milk production during the entire lactation, on average of 3.5 kg of milk per day (Erdman and Varner, 1995) (Table 3).

Table 3 - Summary of selected literature - Effect of milking frequency and parity on milk production of Holstein cows [Adapted from Wall and McFadden (2012)]

Reference	Duration of increased frequency	Change in milk yield 2 vs. 3 times/d
Smith *et al.* (2002)	Full lactation	+16%
Klei *et al.* (1997)	Full lactation	+10.4%
	From 100 DIM	+7.8%
	From 200 DIM	-0.06%
Campos *et al.* (1994)	Full lactation	+17.3% primiparous
Barnes *et al.* (1990)	Full lactation	+6% multiparous
		+14% primiparous
Allen *et al.* (1986)	Full lactation	+13.4% multiparous
		+19.4% primiparous
Gisi *et al.* (1986)	Full lactation	+12% multiparous
		+14% primiparous
Amos *et al.* (1985)	Full lactation	+18.5% multiparous
		+ 25.2% primiparous
Depeters *et al.* (1985)	Full lactation	+17% for multiparous
Poole (1982)	First 20 weeks	+19% multiparous*
		+13% primiparous*
Pearson *et al.* (1979)	Full lactation	+20%

*Effect maintained after 20 wk when cows are then milked 2 times per day. They observed a general milk yield higher during the lactation, and an increase of the duration of the lactation (Poole, 1982)

However, increasing milking frequency required more working hours by the farmers. It became easier in 1992 with the commercialization of the automatic milking systems (AMS) in Europe (Talukder *et al.*, 2015). The number of farms in the world equipped with AMS has increased continuously over the years: more than 100 farms in 1997, 2,000 in 2003 (De

Koning and Rodenburg, 2004), and more than 10,000 farms in 2010 (De Koning, 2011). Automatically milked herds in Europe produce around 5 to 10% more milk than the herds milked with the conventional parlor system (De Koning and Rodenburg, 2004, Bijl *et al.*, 2007). However, large variations in milk production can be seen between herds and within a herd, as some cows are milked less than twice a day with automatic milking therefore producing less milk. In many larger US herds with highly automated conventional parlors, 3 daily milking is a common practice. For these herds already practicing the 3 daily milkings with conventional parlors, adopting the automatic milking system would decrease the milk production of 5 to 10% (De Koning and Rodenburg, 2004).

The AMS has several advantages. It reduces the labor requirements and allows an individual management of the cows (extra concentrate distribution, limitation of milking frequency, real time individual milk production data). Moreover, it avoids the effect of the milker's behavior on milk yield, which has been shown that rough behavior at milking time increases the residual milk by 70%, and consequently decreases the milk production (Seabrook, 1994). With the AMS the cows are autonomous, in a routine with minimal human stressors. Even so, in some farms equipped with AMS, the milk yield didn't increase as expected (Billon and Tournaire, 2002). This has been related to shorter length of lactation due to irregular milking intervals and failure of cup attachments (Bach and Busto, 2005) leading to a decrease of blood flow and extraction of nutrients from the blood (Delamaire and Guinard-Flament, 2006b and 2006a), two main factors for milk synthesis. Indeed, the cow's milking interval is fluctuating between individuals and consequently should be controlled by the manager to obtain the expected milk yield (Figure 8). Moreover, the AMS system only works with well-functioning cow traffic to the machine (Svennersten-Sjaunja and Pettersson, 2008). Feeding concentrates at the AMS helps to motivate the cows to visit it (Prescott *et al.*, 1998), and increases milk flow (Samuelsson *et al.*, 1993) as oxytocin release is increased by concentrate consumption (Svennersten *et al.*, 1995).

One of the main advantages of the AMS is the possibility to control the milking frequency depending on the lactation stage of the cow. It has been shown that increased milking frequency in early lactation increases milk yield and persistency (Poole, 1982, Bar-Peled *et al.*, 1995, Klei *et al.*, 1997), by increasing cells proliferation and decreasing apoptosis. When cows were milked 4 times a day versus 2 times a day in early lactation (3 first weeks), milk yield increased during this period, and even after return to 2 times milking per day (increase of approximately 8% over the entire lactation) (Capuco *et al.*, 2003). After the early lactation, the effect of milking frequency differs depending on the studies. Some have found that it is still possible to improve milk yield and persistency by increasing milking frequency in mid-lactation (Hillerton *et al.*, 1990, Capuco *et al.*, 2003), while others have reported that in mid-lactation it is too late to positively modify the persistency of the lactation and, consequently, milk production (Klei *et al.*, 1997).

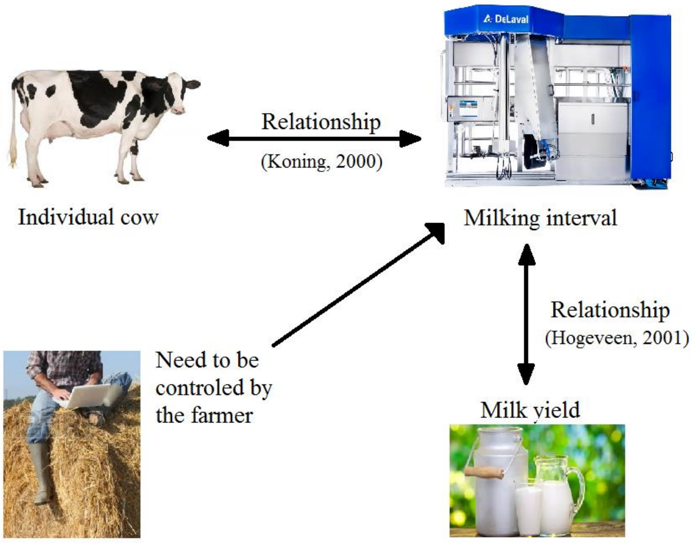

Figure 8- The individual cow's milking interval is controlled by the farmer, informed by the milking robot if he has to pick up the cows for milking, to obtain the expected milk yield

However, increasing milk yield by increasing milking frequency requires some extra energy cost (Bar-Peled *et al.*, 1995) and also reduces the lying and feeding time, major elements for milk production and health of the cow (Gomez and Cook, 2010).

3.2. Feeding strategies to support milk production

Dietary factors affecting voluntary dry matter intake (DMI). The DMI is the first limiting factor to produce milk. Indeed, a high milk yield will always depend on a high DMI. If the cow doesn't eat enough, a decrease in LW and in milk production will be observed. The DMI is limited by several factors, one being the reticulo-rumen volume, which determines the potential physical intake of forages of a cow (Forbes, 1995). This factor is the main limitation in early lactation as the energy in milk exceeds the energy intake (Conrad *et al.*, 1964, Reynolds, 2006), leading to a negative EB state. The diet's energy content is the second most influential factor in feed intake regulation (Mertens, 1987, Forbes, 1995, Allen, 2000). Finally, environmental factors (e.g. the temperature) and characteristics associated with the animal's ability to utilize energy from the diets, such as the health status of the animal, the stage of lactation, the parity, the physiological status (pregnancy), the level of production of the cow etc. (Forbes, 1995), also affect DMI.

Energy and protein. Milk yield is mainly affected by the level of energy and protein ingested (Brun-Lafleur *et al.*, 2010). As seen in the first part, the main energy source for the animal and the rumen microbes are the carbohydrates. The amount of energy influences propionate production in the rumen, which influences the amount of glucose synthetized by the liver. The glucose is then used by the mammary gland to synthetize lactose, and the lactose available influences the amount of milk produced per day.

Milk yield increases when energy intake increases (Broster, 1973, Macleod *et al.*, 1984, Friggens *et al.*, 1995, Jensen, 2014). The energy can be augmented by increasing the amount of DMI, but as said before, the cow has a physical limit for feed intake ingestion. Increasing the concentrate intake or reducing neutral detergent fibers (NDF) increase the dietary energy density and consequently the energy intake and the milk yield (Broderick, 2003) (Table 4). Nevertheless, a too high proportion of concentrates leads to a reduced milk yield as it can result in acidosis and reduced fiber digestibility (Broderick, 2003). Moreover, the proportion of concentrates in the diet has a strong influence on milk fat concentration, since the lack of fibers depresses the acetate formation in the rumen (Wattiaux, 2015).

The changes in milk yield due to an increase of energy concentration in the diet are stronger in early lactation (Kirkland and Gordon, 2001). The answer to an energy increase in the diet will vary depending on parity. Indeed, energy partitioning between milk yield and weight gain for primiparous cows is independent (Jensen, 2014) so the primiparous cows are to a larger extent utilizing the energy for live weight gain instead of milk production while the multiparous cows prioritize milk production to a relatively higher level (Coulon and Remond, 1991, Bossen and Weisbjerg, 2009). Milk yield also increases when dietary protein supply is increased (Macleod *et al.*, 1984, Verite *et al.*, 1997, Metcalf *et al.*, 2008).

It has been shown that a protein-energy interaction might have an effect on milk yield (Rulquin, 1982): milk yield increases when a) increasing dietary protein content above requirement for high energy density diets (Broster, 1973, Cowan *et al.*, 1981), or b) energy density is increased when the protein requirement is met or above (Brun-Lafleur *et al.*, 2010). This is due to the fact that when the protein ingested increases, the DMI is increasing as well so the energy ingested is higher. Milk yield response to changes in energy/protein in the diet are greater for high production potential cows, and different depending on parity (Brun-Lafleur *et al.*, 2010).

Cows in better energy status have a better chance to become pregnant (Butler, 2000, Stockdale, 2001, Jorritsma *et al.*, 2003a). Most of the studies have found that different feeding strategies can be used to improve the energy status of the cows and reduce the risk of low reproduction (Table 4). Nevertheless, high dietary protein diets are correlated with low reproductive performance (Butler, 2000). Indeed, this nutritional strategy alters uterine pH and modifies the concentrations of uterine secretion constituents. These two changes modify the uterine environment and consequently increases embryo mortality (Butler, 1998). Moreover, high protein diets increase the ammonia and urea concentrations in blood and milk (Butler, 1998), and decrease progesterone concentration (Westwood *et al.*, 1998), both highly correlated with decreased ability to conceive (Folman *et al.*, 1990, Butler, 1998, Westwood *et al.*, 1998, Wittwer *et al.*, 1999). The best diet to improve pregnancy rate while not decreasing milk yield should maintain high insulin levels at critical times of lactation, so the number of GH receptors in the liver and elsewhere are sufficient and IGF-1 is secreted sufficiently; and it should also lower the ammonia and urea production during critical periods of follicular development (Royal *et al.*, 2000).

Fat supplementation. Fat supplementation is used to increase energy density which often has a positive effect on milk yield. The main dietary sources of fat are oilseeds and palm oils (Rabiee *et al.*, 2012). The absorbed fatty acids are then utilized directly for milk secretion or tissue deposition, or are oxidized (Palmquist, 1994). So the fat supplementation increases the fatty acids available for absorption and secretion in milk (Garnsworthy, 1997), but it also modifies the fat to propionate ratio (Ikwuegbu and Sutton, 1982) and the fiber digestion in the rumen, which consequently decreases the acetate and butyrate production and de novo milk fat synthesis. A certain level of fat supplementation should not be exceeded to avoid dry matter intake reduction. This negative effect of fat supplementation can vary depending on the stage of lactation, but the results obtained in

Table 4 – Selected litterature indicating the effects of differents feeding strategies on milk production and reproduction.

	Strategy	Effect on production	Effect on reproduction
Butler (2000)	Prot	↑	↓ pregnancy rate
Westwood *et al.* (1998)	Prot	NA	Lower
Folman *et al.* (1990)	Prot	NA	Lower
Brun-Lafleur *et al.* (2010)	E/Prot	↑	NA
Machado *et al.* (2014)	E	↑	NA
Jensen (2014)	E	↑ multiparous	NA
		Ø primiparous	NA
Garcia *et al.* (2007)	E	↑	NA
Andersen *et al.* (2004)	E	↑	NA
Broderick (2003)	E	↑	NA
Reksen *et al.* (2002)	E	↑	Lower
Kirkland and Gordon (2001)	E	↑ early lactation	NA
Murphy (1999)	E	↑	NA
Broster (1973)	E	↑	NA
Weisbjerg *et al.* (2013)	Fat	↓	NA
Onetti and Grummer (2004)	Fat	↑ early lactation	NA
Kokkonen *et al.* (2004)	Fat	↓ early lact	NA
Weiss and Wyatt (2003)	Fat	↑	NA
Ruegsegger and Schultz (1985)	Fat	↓ early lact	NA
Azeemi *et al.* (2014)	P Fat	NA	↓ progesterone
Duque Q *et al.* (2013)	P Fat	None	↓ day to first ovulation
Gowda *et al.* (2013)	P Fat	↑	Improved
Strusinska *et al.* (2006)	P Fat	↑	NA
Sklan *et al.* (1991)	P Fat	↑	Improved

E: energy supplementation, Prot: protein supplementation, Fat: fat supplementation, P Fat: protected fat supplementation previous studies are conflicted on this point (Grummer, 1988, Jerred *et al.*, 1990, Chilliard, 1993, Simas *et al.*, 1995, Drackley *et al.*, 1998, Onetti and Grummer, 2004, Weisbjerg *et al.*, 2008). The milk yield response to fat supplementation is as well complex, varying with the fat type saturation and the stage of lactation, thus difficult to clearly determine. Fat supplementation has been stated to have a positive effect on milk yield only in early lactation (Onetti and Grummer, 2004), no effect in early lactation with a general negative effect over the lactation (Weisbjerg *et al.*, 2013), a negative effect in early lactation (Ruegsegger and Schultz, 1985, Kokkonen *et al.*, 2004) or higher responses in late lactation (Chilliard, 1993).

Protected fats, such as calcium soaps or fat prills, by-pass the rumen and reach the omasum or the abomasum and thereby provide fatty acids for direct absorption in the intestine. This additional energy is gained without help from the rumen, already working at full capacity in early lactation. The use of protected fat results, in general, in higher milk production, and has a positive impact on body condition and subsequently on the ability to conceive (Table 4). Even so, the literature reporting the effect of protected fat supplementation on reproductive parameters is quite limited.

Feeding strategies depending on stage of lactation and energy level. Despite the fact cows can regulate their energy intake to meet their energy requirements (Friggens *et al.*, 1998), feeding all the cows the same way might limit the expression of their milk potential (Bossen and Weisbjerg, 2009). Feeding the cows depending on the stage of lactation (Friggens *et al.*, 1998) or even more precisely, individually, based on their EB should potentially increase milk production.

As described earlier, during the early lactation period the cows are in negative EB and responses to a specific feeding treatment are stronger than later in the lactation. When the cow receives a high-energy diet in early lactation, the feed intake is higher without significant effect on LW or BCS (McNamara *et al.*, 2003a, McNamara *et al.*, 2003b, Rabelo *et al.*, 2003, Andersen *et al.*, 2004, Rabelo *et al.*, 2005, Nielsen *et al.*, 2010), while the milk production and persistency are increased (McNamara *et al.*, 2003b, Andersen *et al.*, 2004) or unaffected (Rabelo *et al.*, 2003, Nielsen *et al.*, 2010).

Bossen *et al.* (2009) proposed an individual feeding strategy based on EB and nutriments requirements of the individual cow. They used automatic LW measurements to detect mobilization and deposition phases, associated with physiological stages of negative and positive EB, respectively (Maltz, 1997). They have shown that cows fed a higher energy diet during the mobilization period had higher milk yield, and that the energy supply to the individual cow can be reduced in the deposition phase without any negative effects on milk production (Bossen and Weisbjerg, 2009, Hymøller *et al.*, 2014). This change to a diet lower in energy also allowed the extension of the mobilization period which contributes to a higher milk production.

3.3. Effect of feeding strategies on metabolic changes

The composition of the feed affects the amount, absorption and allocation of the nutrients to the udder. The metabolic activity regulates the milk synthesis (Nielsen and Jakobsen, 1994), and the changes in milk yield, feed intake, and EB have been found to be associated with characteristic changes in plasma metabolites concentrations (Reist *et al.*, 2003). These changes in plasma metabolite concentrations provide information about the metabolic status of the cow and can be used to determine the pattern of nutrients extracted by the mammary gland (Alstrup *et al.*, 2015).

When increasing the energy in the ration, for example by replacing forage with concentrate, more propionate is produced, leading to lower rumen pH. When high energy diets are fed in early lactation, plasma glucose increases while BHBA and NEFA decrease. When the cows' diets are supplemented with proteins, the plasma urea increases, as well as the blood protein concentration (Law *et al.*, 2009). This protein supplementation does not affect plasma NEFA or glucose (M'Hamed *et al.*, 2001, Law *et al.*, 2009). When the cows' diets are supplemented with fat, the glucose concentration decreases while insulin (Palmquist and Moser, 1981), and NEFA concentrations increases (Grum *et al.*, 1996, Drackley *et al.*, 1998). This fat supplementation also induces insulin resistance which impairs the transport of amino acids to the mammary gland and consequently have a negative effect on milk protein synthesis (Palmquist and Moser, 1981).

Cows with a negative EB have low pregnancy rates regardless of milk producing ability. The IGF-1 is increased when the energy intake in early lactation increases (Obese *et al.*, 2003). Moreover, a positive correlation between IGF-1 and pregnancy rates has been found (Patton *et al.*, 2007), as IGF-1 affects ovarian follicular development and time to first ovulation (Adashi, 1998, Webb *et al.*, 2004).

4. Extended lactation, a potential tool to improve production and reproduction?

4.1. Extended lactation to improve reproductive performance and health

The low pregnancy rate observed in the modern dairy cow has been mainly attributed to the insufficient time to recover from the effects of early lactation stress and calving (Borman *et al.*, 2004). Delaying insemination after the peak yield would increase pregnancy rates (Schindler *et al.*, 1991), decrease the number of inseminations per pregnancy and the frequency of anestrus treatment (Larsson and Berglund, 2000). Moreover, delaying rebreeding can reduce the annual culling rate (reduction of 10% in high producing cows in seasonal system), and improve genetic gain (Borman *et al.*, 2004) as the number of cows involuntarily culled due to conception failures would allow a more selective culling based on performance and functional traits (Beaudeau *et al.*, 2000). Nevertheless, the genetic gain rates would also be slower as the generation interval gets longer.

The cows are genetically selected to produce more milk and consequently to partition nutrients towards the mammary gland (Veerkamp *et al.*, 1995). However, this increase of production also increases energy deficits in early lactation (elevated negative EB), despite of the cows having high energy intake (Borman *et al.*, 2004). The potential for milk production is not only improved by genetics. Milk yield is positively correlated with live weight and age at calving (Markusfeld and Ezra, 1993) due to feeding rather than genetics (Lin *et al.*, 1985, Lee *et al.*, 1997). Feed intake is a relevant indicator of weight at first calving and is highly heritable (Lee *et al.*, 1992). So increasing age and weight at calving might improve milk yield in the first lactation (Borman *et al.*, 2004). Another important factor for increasing milk production is the feeding management as a higher dry matter intake or proportion of concentrates in the ration increase milk production.

The health risks (mastitis, lameness, metabolic problems at peak lactation), from which 60% occur around the calving period (Ingvartsen, 2006), will as well be reduced when delaying rebreeding as the number of calving per cow per year will decrease (Knight, 2005) (Figure 9).

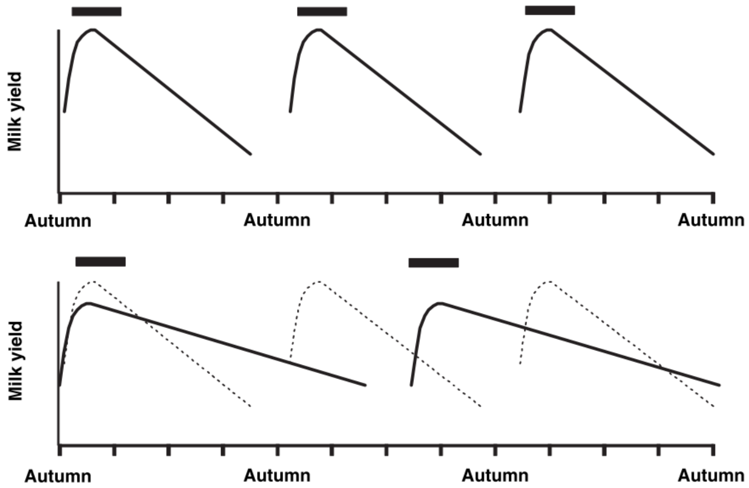

Figure 9 – Comparison of the number of health risks periods (black squares) between a conventional 10 months lactation (above) and an extended (below) (Dobson et al., 2007)

Finally, extended lactation management has been studied in terms of costs, and seems to be a potential management strategy if the cows selected are high producing (Borman *et al.*, 2004). When based only on milk production and feed costs, little is known about the economic benefit of extended lactation (Holmann *et al.*, 1984). However, the costs for rearing heifers represent the second largest annual expenses of a dairy farm, after the feed costs (Bach *et al.*, 2008). On average, the daily cost (including feed, facilities, health, reproduction) for rearing a heifer varies from 2.37 $ (2.18 €, or 16 DKK) to 2.73 $ (2.52 €, or 18 DKK), including the labor cost (Tranel, 2014). Managing the cows for extended lactation should decrease this cost as the number of replacement heifers per year would decrease, as well as the insemination costs.

4.2. Mechanisms of extended lactation to maintain milk production

Several studies have shown that high producing cows are able to extend their milk production beyond 305 days (van Amburgh *et al.*, 1997b, Auldist *et al.*, 2007, Kolver *et al.*, 2007, Butler *et al.*, 2010), up to 670 days (24 month calving interval) for cows in pasture based systems (Auldist *et al.*, 2007, Kolver *et al.*, 2007, Phyn *et al.*, 2008, Grainger *et al.*, 2009, Butler *et al.*, 2010). Some cows also have an unplanned extended lactation due to failure at earlier rebreeding (Cole and Null, 2009, Steri *et al.*, 2009, Jankowska *et al.*, 2012, Steri *et al.*, 2012), like in Australia, where it is common and practiced for pasture based system to have 16 months lactations (Abdelsayed *et al.*, 2015). However, some cows are not able to maintain significant milk production longer and are dried off too early in relation to their conception date. In that case, longer dry periods follow which is not economically beneficial and might also be negative for the cows' health (Sawa *et al.*, 2012).

The persistency and the peak of milk production are defining the total milk production of the lactation. In several studies, persistency is defined as the rate of decline in milk yield from peak yield (Dekkers *et al.*, 1998, Borman *et al.*, 2004, Cole and Null, 2009, Pettersson *et al.*, 2011). So a high persistency involves a low decline of milk yield, while a high peak yield has been negatively correlated with good persistency. Persistency is the key to increase milk yield and thus, make the extended lactations profitable (Sorensen *et al.*, 2008). Persistency has a low heritability (Borman *et al.*, 2004) but can be improved by nutrition and management like milking frequency (Sorensen *et al.*, 2008). If the persistency is only improved by 1% in extended lactations, milk yield in 3 years with two 16 months lactation or three normal 10 months lactations should be the same (Knight, 2005). Extended lactation could improve the production and reproduction of the modern dairy cow by better utilizing the cow's potential, avoiding drying off while it is still producing more than 30 kg of milk per day, and by delaying rebreeding to a period where the cow is in a more positive EB to improve pregnancy rates (Madsen *et al.*, 2008).

A few experiments, mainly with cows in pasture based system, have been performed on extended lactation (Table 5). In general, the milk yield is increased with extended lactation compared with normal 10 months lactation. Extended lactation seems more advantageous, in terms of daily milk yield and economical profitability, for primiparous than multiparous cows (Arbel *et al.*, 2001, Osterman and Bertilsson, 2003) as primiparous cows have higher persistency than multiparous cows so they can be inseminated later (Ratnayake *et al.*, 1998). Regarding the pregnancy rates, results are rare and vary a lot. Despite of pregnancy rates seem to be improved with extended lactation, in most cases no significant difference has been found between the pregnancy rates of extended lactation and normal 10 months lactation cows (Arbel *et al.*, 2001).

Table 5 - Selected references about extended lactation and its effects on production and reproduction

Reference	System	Parity	Effect EL on daily MY	Pregnancy rates at 1st IA (N vs. EL)
Arbel *et al.* (2001)	Indoor, 750 cows	Primi Multi	= ↓	40.3 vs. 43.5% NS 36.6 vs 38.7% NS
Kolver *et al.* (2007)	Pasture, 56 cows	Multi	↑	28.5 vs. 53.5%
Schindler *et al.* (1991)	Indoor, 180 cows	Primi Multi	NA	66.6 vs. 69.2% NS 35.7 vs. 64.4% **
van Amburgh *et al.* (1997a)	Indoor, > 4000 cows	All	↑	35% for both
Osterman and Bertilsson (2003)	Indoor, 52 cows	All	Total ↑, but daily yield =	NA
Larsson and Berglund (2000)	Indoor, 148 cows	All	↓	AI1 49 vs. 56% AI2 46 vs. 58%
Jankowska *et al.* (2012)	Indoor, 132 cows	All	↑	↓
Bertilsson *et al.* (1997)	Indoor, 90 cows	All	↓	41.5 vs. 50%
Grainger *et al.* (2009)	Pasture/Indoor	All	=	NA
Butler *et al.* (2010)	Pasture, 46 cows	All	NA	52%
Auldist *et al.* (2007)	Pasture, 125 cows	All	↑	NA
Sorensen *et al.* (2008)	Pasture, 25 cows	All	↑	NA
Williams *et al.* (2013)	Pasture, 16 cows	All	↑	NA
Phyn *et al.* (2008)	Pasture, 56 cows	Multi	Total ↑, but efficiency ↓	NA
Christiansen *et al.* (2005)	Pasture, 60 cows	All	Total ↑, but daily yield =	NA
Vargas *et al.* (2000)	Database	All	↑	NA
Sawa and Bogucki (2009)	Database	All	↑	↓
Allore and Erb (2000)	Model	All	↑ 15%	↑

4.3. Modelling lactation

Modelling lactation curves brings useful information. A model can predict milk yield and milk components (Dematawewa *et al.*, 2007, Dijkstra *et al.*, 2010), and can define and estimate the lactation characteristics of individual cows for genetic selection (Dekkers *et al.*, 1998, VanRaden *et al.*, 2006).

Mechanistic models are describing biological processes occurring during the lactation, in terms of metabolic and physiological responses (Beever *et al.*, 1991). Some mechanistic models have been used to describe extended lactation curves (Grossman and Koops, 2003, Dematawewa *et al.*, 2007, Hanigan *et al.*, 2007, Dijkstra *et al.*, 2010), but they require a large number of parameters to describe the biological processes, making them difficult to interpret (Abdelsayed *et al.*, 2013).

Empirical models are preferentially used and some appear suitable for the modelling of extended lactation (Abdelsayed *et al.*, 2013). The Wood model is the more commonly used to model lactation curves as it is simply based on 3 parameters, defining the yield at the beginning of the lactation, the rate of increase before peak and the rate of decline after peak (Wood, 1967). However, this model may not be appropriate for extended lactation curves (Vargas *et al.*, 2000, Grossman and Koops, 2003). Vargas *et al.* (2000) tested several models on extended lactation curves, and concluded that the diphasic model (Grossman and Koops, 2003) is the most suitable for extended lactation as it overcomes the problem of the autocorrelation detected in models like Wood based on a gamma function (Wood, 1967). This diphasic model is based on a logistic function, as well as the lactation persistency model (LPM), or the reduced LPM, providing lactation persistency measurements (Grossman *et al.*, 1999). More complex models, based on multiple regressions (Ali and Schaeffer, 1987) can also be used to model extended lactation curves, nevertheless, they require more parameters. Finally, random regression models (Miglior *et al.*, 2007, Stoop *et al.*, 2007), or test-days models, have some advantages over the mechanistic models as they are more flexible on fitting the individual curves but they also require lots of daily recording (Abdelsayed *et al.*, 2015). Like the LPM, they propose an evaluation of persistency (Yazgan *et al.*, 2010), accounting for herd effect, and providing accurate genetic selection (Schaeffer *et al.*, 2000).

GARUNS model. The GARUNS model from Martin and Sauvant (2010) is a dynamic and stochastic model, which takes into account the changing priorities of an animal as it ages, through repeated reproduction cycles and over successive generations. It is composed of a regulating sub-model and an operating sub-model. The regulating sub-model describes the nutrient portioning regarding life priorities (**G**rowth, **A**geing, balance of body **R**eserves, nutrient supply to the **U**nborn, **N**ewborn and **S**uckling calf, GARUNS) while the operating sub-model describes, for example, changes in live weight (LW) and milk yield.

A reproduction sub-model was added by Phuong *et al.* (2015a) using the prediction of milk yield, BCS, and EB from GARUNS, as inputs to stimulate reproductive responses in terms of pregnancy rates and productive lifestime (Figure 10).

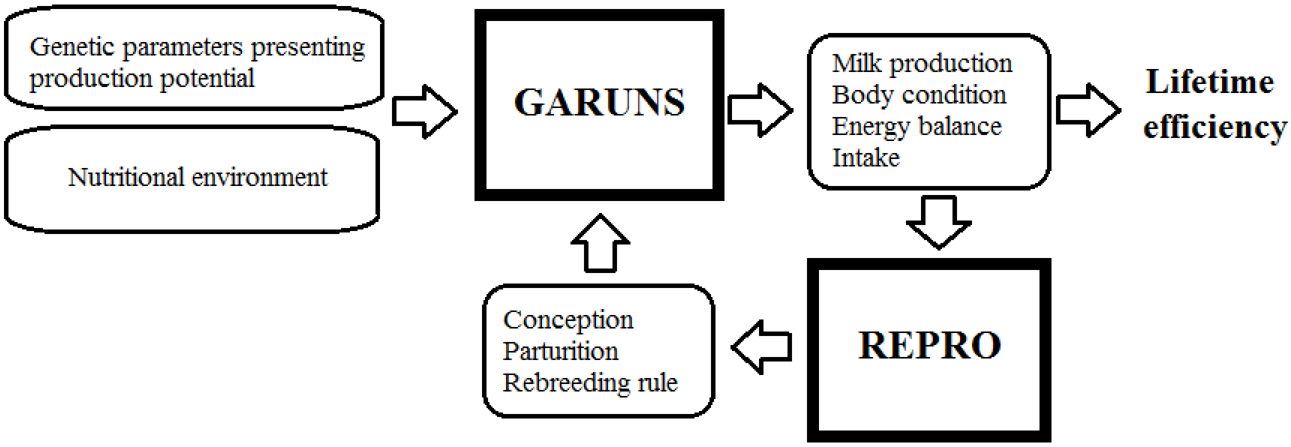

Figure 10 – Schematic representation of the model for predicting lifetime efficiency of individual cows that includes the GARUNS model (Phuong et al., 2015a)

The GRAUNS model, with and without the reproductive model, has been tested on cows managed for 10 months lactation (Phuong *et al.*, 2015a, Phuong *et al.*, 2015b). The next step would be to test this model for cows managed for longer lactation to widen its utilization.

OBJECTIVES AND HYPOTHESES

Objective 1: To investigate the interaction between a feeding strategy based on live-weight changes in early lactation and an extended lactation in dairy cows with focus on metabolic and milk production adaptations.

We hypothesized that:

- An energy enriched diet from calving until live-weight nadir will reduce the severity of the negative energy balance, measured in terms of production, live weight, and plasma metabolites.
- The reduction in diet energy concentration from LW nadir will extend the negative energy balance further compared to cows not changing energy concentration
- This feeding strategy will have a positive carry-over effect on milk production in mid and late lactation.

This objective was studied in papers 1 and 2.

Objective 2: to investigate the effects of the feeding strategy based on live-weight changes in early lactation and of the lactation length on the productive and reproductive performance.

We hypothesized that:

- The feeding strategy will have no effect on the pregnancy rates and number of inseminations per pregnancy (paper 3)
- Delaying rebreeding increases mounting behavior and insemination success (papers 3 and 4).
- The consecutive estruses in extended lactation influence negatively the milk yield and milking frequency (paper 3).
- The length of the lactation needs to be adjusted depending on the age of the cow to obtain the highest productive and reproductive performance (paper 5).

APPLIED METHODOLOGY

1. Experimental set up

Animals and Housing. The experiment, approved by The Animal Experiments Inspectorate under the Danish Veterinary and Food Administration, was carried out at the Danish Cattle Research Centre (DCR) at Aarhus University, AU-Foulum, Denmark. Sixty-two Holstein pregnant cows and heifers entered the experiment at drying off 8 weeks before expected calving, from October 2012 to September 2013. Dry cows and heifers received the same dry cow standard diet and were housed on slatted floor with cubicles for the first 5 weeks of the dry period, on straw-bedded litter for the next 3 weeks and in a calving pen for the last 6 to 24 h before calving. After calving, they spent some days in a care group pen before entering the experimental group section. Cows and heifers were blocked according to parity, expected calving date and previous heifer treatment (if any), and allocated to one of the two feeding strategies. It was not possible to also balance the heifers (30%) for their potential genetic yield as we already had 3 blocking criteria and multiple sires were used.

All lactating cows were housed in a common loose housing system with slatted floor, cubicles with mattresses and sawdust bedding, and with free access to water. They had access to one automatic milking system (AMS, DeLaval AB, Tumba, Sweden), equipped with a platform scale (Danvaegt, Hinnerup, Denmark), where 3 kg of concentrate was offered daily. The cows were fed different rations through automatic feeders (Insentec, Marknesse, the Netherlands). Records of the amount of feed eaten per cow were taken. Two partially mixed rations with different level of energy were distributed ad libitum in the feeders. This housing and milking system is a common farming system in Denmark which settles our experiment in a situation close to reality and is well-suited for an extended lactation experiment.

The insemination of the 62 cows started after 220 DIM in the aim to complete a16 months lactation, as it has been shown that high yielding dairy cows are able to maintain this duration of lactation (van Amburgh *et al.*, 1997b, Osterman and Bertilsson, 2003, Auldist *et al.*, 2007). A 16 months lactation seems to be a good trade-off as some cows might not be able to complete longer lactations, and shorter lactation (> 10 months but < 16 months) might bring up indistinct results compared with a 10 months lactation. In the present experiment, all the cows where managed for 16 months lactation so there was not 10 months lactating cows to compare our results with, because this has been done previously (Osterman and Bertilsson, 2003), and the main objective was to compare diets effects on productive and reproductive performance of cows managed for 16 months lactation. During the experiment, among the 62 cows, 6 multiparous cows were culled from E-Coli infection in mid-lactation, and 3 multiparous cows were dried off earlier than the planned 16 months due to a low milk yield (<12 kg/d) or mastitis. At the end of the lactation, the milking rules were changed 70 days before planned calving (Table 6). At the same time, the concentrates allowed at the AMS were reduced by 0.5 kg/d for these cows, so after 6 days they didn't receive more concentrates at the AMS. On the Friday after, the cows were transferred to another pen for the weekend where they received 6 kg TMR. On Monday they were milked one last time.

Table 6 - AMS settings and procedures

Days	Either Time from latest milking, h	Or Exp. milk yield, kg	Manual pick up [1] for milking if time since latest milking reach, h
0 to 150 DIM	5 [2]	7	15
150 DIM to 70 before expected calving	8 [4]	8	15
70 before exp. calving to drying off[3]	12	10	20

[1] To be picked up during daytime working hours

[2] When milking is incomplete (less milk than estimated) in the period 0-150 DIM the setting of time from latest milking is increased to 8 hours

[3] Concentrate feeding is reduced from 9 weeks before expected calving

[4] Reduced to 6.7 hours if a cow appears on the 'observation list' in order to have it milked 2 times within working hours

Barn technology. The Insentec RIC system (Insentec, Marknesse, the Netherlands) allow the daily measurement of individual feed intake, as well as the use of different feeding strategies for cows housed together. The feeding stations identify the cow, thanks to the chip in their necklace, and open to the cows registered for each station, with its associated ration. Each cow was offered a maximum of 3 kg of concentrate per day in the AMU. If a cow ate less than the daily 3 kg concentrate allowed, the amount not eaten (up to 1.5 kg) was allowed on top of the 3 kg allowance for the next day. Individual daily DMI was calculated as the sum of the partially mixed ration ingested at the Insentec and the amount of concentrate ingested at the AMU within a day.

The AMS allows the automated recording of individual milk yield. Access to the milking unit was restricted for the cows depending on their last milk yield. The cows had to be milked at least twice a day, and if not, an automatic alert informed the farmer who then manually picked up the cow for milking. One inconvenience of the AMS is that the number of milkings per cow per day is not fixed; just a minimum number of milking (twice daily) is required. The milking frequency possible effects on our measurements should therefore be studied to understand its possible effects.

The AMS is equipped with a platform scale (Danvaegt, Hinnerup, Denmark) allowing the recording of each cow's live-weight 6 times per second during milking time. The measurements had then to be cleaned up, removing the first and last 200 measurements associated to when the cow enters and leaves the platform (Bossen *et al.*, 2009), due to a large variability during milking, between milkings, and between days.

Every second week, the BCS of each cow was evaluated visually on a grading scale from 1 to 5 (thin to fat) (Ferguson *et al.*, 1994), by one of the same two trained persons to avoid bias as it is a subjective evaluation.

2. Feeding strategies and live-weight changes

The cows were allocated to two feeding strategies at calving. Two diets were used; one with a high energy density (HD) and a 50:50 forage to concentrate ratio, and one with a lower energy density (LD, control diet) and a 60:40 forage to concentrate ratio. A 10%-units energy density difference was planned and expected between the LD and HD diets; however, the obtained difference was smaller (4%-units). For both strategies, a maximum of 3 kg of concentrate was offered daily in the milking robot, meaning that the difference between the feeding strategies was only obtained by different partially mixed rations fed in the Insentec. Half of the cows were fed with the HD diet until they reached at least 42 days in milk and a LW gain ≥ 0 kg/day based on a 5-days LW average, and were then shifted to the LD diet (strategy HD-LD). The other half of the cows were fed the LD diet throughout the lactation (control strategy LD-LD). Both diets, LD and HD, were offered ad libitum (Figure 11). The rations were optimized using the NORFOR model and standards (Volden, 2011).

The change of diet for the LD-HD cows was based on LW changes as done previously by Bossen *et al.* (2009). LW changes correspond to a physiological stage of the lactation, when cows are losing weight right after calving because they mobilize body reserves to produce milk (mobilization phase), and then gaining weight as they start to reconstitute their body reserves (deposition phase). The shift was done after 42 days to give enough time to the LW to stabilize in its change of direction (increase or decrease), in order to no misinterpret an early and maybe brief increase of LW as the end of the mobilization period.

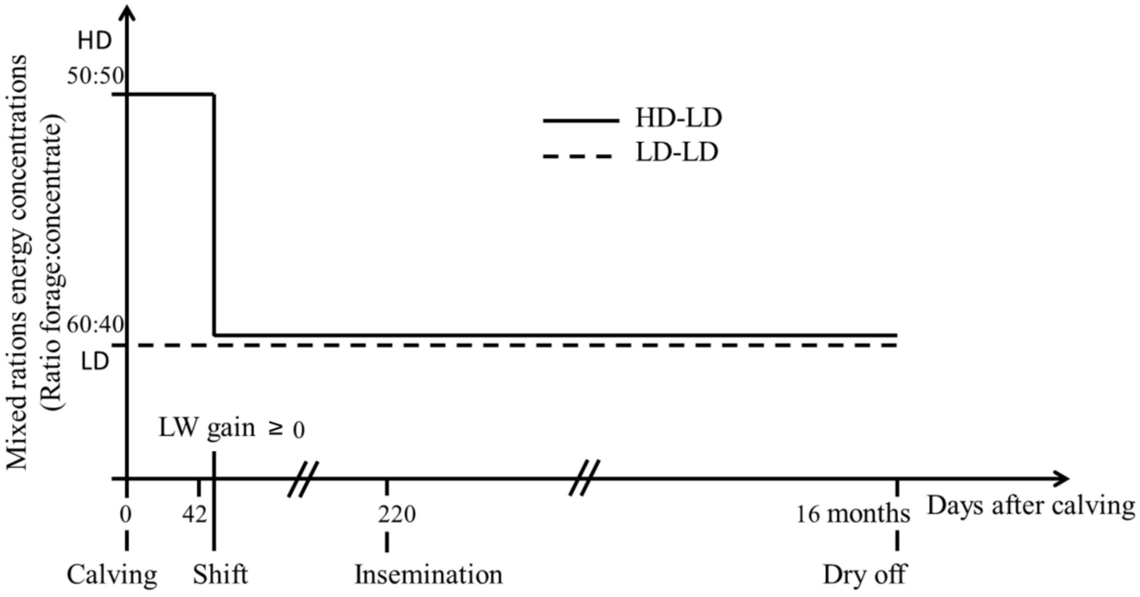

Figure 11 - Feeding strategies HD-LD and LD-LD used in the experiment

3. Samples, calculations and modelling

Blood samples. Blood samples were taken every week from calving to 12 weeks in milk, and every second week from 12 to 36 weeks in milk. The samples were always taken at the same time, at 10.00 am before the fresh feed delivery at noon, to minimize the day time effect on the measurements, which can affect mainly BHBA concentration (Mansion *et al.*, 1981). Analyses were performed on all the plasma samples for glucose, NEFA. BHBA, as they are metabolites giving information on the energy status of the cow. The concentrations of IGF-1 and insulin were measured at 4 time points only (at week 3, 5, 12 and 24) because the main variations occur in the first week after calving and the analysis technique is expensive. No blood samples were taken after week 36 as the early lactation is the period where the main physiological and EB variations occur (Marett *et al.*, 2011, Bjerre-Harpoth *et al.*, 2012).

Calculations. The energy corrected milk (ECM, 3.140 MJ/kg) was calculated according to Sjaunja's equation (Sjaunja *et al.*, 1991):

$$\text{ECM} = \text{milk yield} \times ((38.3 \times \text{fat} + 24.2 \times \text{protein} + 15.71 \times \text{lactose} + 20.7) / 3.140)$$

with ECM and milk yield in kg; fat, protein, and lactose in g/kg. The daily energy balance (EB, MJ of NE_L/d) was calculated using the following basic equation subtracting the energy output (lactation, maintenance, activity) to the net energy inputs (intake). The net energy intake was calculated with the Scandinavian feed units system (SFU), where 1 SFU = 7.89 MJ of NE_L/kg. The energy used for growth (only for the first and second parity), and the energy used from conception were not included in the energy output, as they were shown to be less than 1% of the energy output (Friggens *et al.*, 2007b).

Smoothing. The Functional Data Analysis (fda) package of R (Ramsay *et al.*, 2010) was chosen to smooth our daily production data (ECM, LW, BCS, DMI, net energy intake, fat in milk, protein in milk, lactose in milk, SCS, milking frequency), as it can be applied for all our production data whatever the shape of their curves. A single common equation such as the Wood function (Wood, 1967), which is often used to describe lactation curves, would not fit our extended lactation data, as the shapes of the curves were longer than the traditional 10 months lactation and varied according to parity and the treatment of our cows. Moreover, it could not be applied to all the other performance data. Polynomials could have been a solution to fit all our data the same way, but in this case, all the points of the smoothed curves were correlated, leading to a decrease in the quality of information. With the fda method, there are only local correlations between points because of the knots which provide independence of fitting for data that are separated by 3 knots - in this case 3 DIM. Moreover, the smoothed data allowed us to calculate the slopes of the curves as the derivative of the smoothed data, at any day, even without raw data at this precise time. This allowed easy comparison of the shape of the curves between groups, one of our main interests. Indeed, the slopes are key indicators to study milk production in dairy cows. For the ECM curve, the slope represents the persistency of the lactation, as it has been defined as the rate of decline in milk yield from the peak yield by several previous studies (Dekkers *et al.*, 1998, Cole and Null, 2009, Pettersson *et al.*, 2011). For the other variables studied, the slopes represent the rate of decay of an initial difference.

Modelling dairy cow's lifespan. The GARUNS model of Martin and Sauvant (2010) was chosen to simulate the lifetime efficiency of the cows managed for different lactation lengths during their life.

Previous studies have shown that mathematical models can be used to explore concepts and hypotheses regarding nutrient partitioning in dairy cows (Baldwin *et al.*, 1987a, Baldwin *et al.*, 1987b, Oldham and Emmans, 1989), and they have potential to predict long term efficiency (Dumas *et al.*, 2008, Friggens *et al.*, 2013). Prediction of nutrient partitioning should also consider genetic variability between cows and differences in expression of their genotypes through time, as it has been shown that these differences exist and are important (Kirkland and Gordon, 2001, Yan *et al.*, 2006). The GARUNS model allows genetic variation to be incorporated in the prediction of the lifetime efficiency. Moreover, it has been successfully tested on Holstein cows managed for 10 months lactation (Phuong *et al.*, 2015b).

RESULTS

1. Paper 1 – Effects of individualized feeding strategy in early lactation on indicators of energy balance in Holstein cows managed for extended lactation

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Effects of live weight adjusted feeding strategy on plasma indicators of energy balance in Holstein cows managed for extended lactation

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In early lactation, most of the dairy cows are in negative energy balance; the extent and duration depend in part on the feeding strategy. Previous studies showed an increased lactation milk yield by use of a live weight (LW) adjusted feeding strategy with a high energy diet before and a reduced energy diet after LW nadir compared with a standard diet throughout lactation. The objective of the present study was to examine how such an individualized feeding strategy affects plasma indicators of energy status. It was hypothesized that an energy-enriched diet until LW nadir will reduce the severity of the negative energy balance, and that the reduction in diet energy concentration from LW nadir will extend the negative energy balance period further. Sixty-two Holstein cows (30% first parity) were managed for 16 months extended lactation and randomly allocated to one of two feeding strategies at calving. Two partially mixed rations were used, one with a high energy density (HD) and a 50 : 50 forage : concentrate ratio, and one with a lower energy density (LD, control diet) and a 60 : 40 forage : concentrate ratio. Half of the cows were offered the HD diet until they reached at least 42 days in milk and a LW gain ⩾ 0 kg/day based on a 5-days LW average, and were then shifted to the LD diet (strategy HD-LD). The other half of the cows were offered the LD diet throughout lactation (control strategy LD-LD). Weekly blood samples were drawn for analysis of plasma metabolites and hormones. Before the shift in diet, the HD-LD cows had higher glucose and lower beta-hydroxybutyrate and non-esterified fatty acids (NEFA) concentrations than the LD-LD cows. After the shift until 36 weeks after calving, plasma NEFA was higher in HD-LD than LD-LD cows. Insulin and insulin-like growth factor-1 were not affected by the feeding strategy. To conclude, in early lactation, the energy-enriched diet reduced the negative energy balance. Plasma NEFA was higher in HD-LD than LD-LD cows from diet shift until 36 weeks after calving, indicating a carry-over effect of the early lactation HD diet to late lactation metabolism.

Keywords: Holstein cows, feeding, energy balance, metabolites, hormones

Implications

In early lactation, automatic live weight recordings can be used to adjust feeding of individual cows managed for 16 months extended lactation. An energy-enriched diet in early lactation reduced the magnitude of the negative energy balance as indicated by the plasma NEFA concentration. A carry-over effect from the early lactation high energy density diet to the remaining lactation period was detected as an increased plasma NEFA.

Introduction

Some intensively managed European dairy farms have started to use an extended lactation strategy to avoid unsuccessful early rebreeding (Osterman and Bertilsson, 2003). Indeed in the traditional 12 months lactation cycle systems, insemination occurs during peak milk yield when most of the cows are in negative energy balance and mobilize body reserves leading to high reproduction failure (Santos *et al*., 2009). However, Holstein cows respond individually to the extended lactation management regarding their propensity to gain weight and in their level of feed intake (Kolver *et al*., 2007; Grainger *et al*., 2009). This means that for use in high yielding dairy herds, the extended lactation strategy needs to be individually modified in order to obtain any potential advantages in improving peak milk yield using high energy diets and keep the absolute milk yield higher for a longer period. Some automatic milking systems are generating data, such as the live weight (LW), that are rarely used by the farmers. One way to utilize these data would be to provide automated individual feeding strategies

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for cows, especially during early lactation. It has been shown that an individualized and LW adjusted feeding strategy imposed in early lactation increased the lactation milk yield in early and mid-lactation, and also increased persistency of lactation in cows on a 12 months lactation cycle without changing the feed efficiency and with reduced LW loses (Bossen and Weisbjerg, 2009; Bossen *et al.*, 2009). Nevertheless, the effect of this individualized feeding strategy on metabolism and energy balance has not been studied in an extended lactation scenario. Additional knowledge on the interaction between the physiology of the dairy cow and the feeding strategy is needed to optimize the diet composition. Previous studies showed a strong relationship between the diet composition, the energy status of the cow, and the concentration of plasma metabolites and hormones (Sorensen and Knight, 2002; Delany *et al.*, 2010). Plasma concentrations of beta-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), glucose, urea, uric acid, insulin, and insulin growth factor 1 (IGF-1) are considered to be relevant indicators of negative energy balance in dairy cows (Aeberhard *et al.*, 2001; Kessel *et al.*, 2008).

The objective of this study was to determine the impact of an individualized feeding strategy on indicators of energy balance (glucose, BHBA, NEFA, insulin and IGF-1) in Holstein cows. An energy-enriched diet was distributed to half of the cows until each cow reached a constant or positive LW gain based on a 5-days average. First, it was hypothesized that an energy enriched feeding strategy in early lactation would reduce the intensity of the mobilization, increase plasma glucose, and decrease plasma NEFA and BHBA. Second, it was hypothesized that after the shift, the effects would be carried-over, prolong energy mobilization, and decrease energy deposition.

Table 1 *Planned composition of dry period ration, LD and HD rations including concentrates in the milking robot (% of DM), and associated chemical composition (g/kg DM)*

	Diet dry period	$LD + C^1$	$HD + C^1$
Ingredients (% of DM)			
Barley	–	4.3	4.0
Wheat NaOH treated[2]	–	0	15.8
Sodium bicarbonate	–	0.5	–
Rapeseed cake, 10.5% fat	–	17.2	15.8
Rapeseed meal, 4% fat	6.4	–	–
Sugar beet pulp, dried	1.5	8.6	7.9
Grass/clover silage	27.8	31.1	23.7
Corn silage	43.5	27.9	22.2
Spring barley straw	19.9	–	–
Urea	–	0.1	0.1
Minerals[3]	0.9	0.6	0.6
Concentrates in milking robot[4]	–	10.7	9.9
Forage : Concentrate	–	60 : 40	50 : 50
Energy density (MJ NEL/kg DM)	–	7.49	7.81
Chemical composition (g/kg DM)			
Ash	52	78	67
CP	119	171	165
Crude fat	27	45	44
Sugar	–	47	45
Starch	112	144	228
NDF	481	337	305

LD = lower energy density; HD = high energy density; DM = dry matter.

[1] LD + C/HD + C: partially mixed ration/partially mixed ration enriched in energy + concentrates (C) in the milking robot.

[2] The NaOH treated wheat was used to increase the proportion of by-pass starch, and was prepared by adding 30 kg of NaOH and 100 l water to 1000 kg of whole wheat kernels and mixing for 10 min.

[3] Minerals (per kg): Ca 203.5 mg, Mg 78.2 mg, K 0.5 mg, Na 0.1 mg, Cl 0.1 mg, S 12.6 mg, I 184.2 mg, Se 36.8 mg, Mn 3,684 g, Zn 5,263 g, Co 26.3 mg, Cu 1,052 mg. Vitamins (per kg): A 526 1000 IU, D3 116 1000 IU, alfa-tocopherol 2874 mg, E 3158 IU.

[4] Composition of the concentrates in milking robot (%DM): 16.9% sugar beet pulp, dried; 16.8% rapeseed meal, 14.6% barley, 14.6% wheat, 9.0% soybean meal, dehulled; 7.0% sunflower meal, dehulled; 7.0% citrus pulp, dried; 5.0% wheat bran, 5.0% alfalfa meal, 3.0% molasses, cane; 0.7% sodium chloride, 0.3% mineral premix, 0.1% magnesium oxide.

Minerals (kg): Fe 59 mg, Zn 46 mg, Mn 38 mg, Cu 8 mg, J 0.7 mg, Se 0.30 mg, Co 0.11 mg. Vitamins (per kg): A 1000 IU, D3 1000 IU, alfa-tocopherol 45 mg, E 50 mg.

Material and methods

Facilities and animals

The experiment, approved by The Animal Experiments Inspectorate under the Danish Veterinary and Food Administration, was carried out at the Danish Cattle Research Centre at Aarhus University, AU-Foulum, Denmark. Sixty-two Holstein pregnant cows and heifers entered the experiment 8 weeks before expected calving, from October 2012 to September 2013. Cows and heifers received the same dry cow standard diet (Table 1) through the last 8 weeks of pregnancy, which corresponded to the dry period. Dry cows and heifers were housed on slatted floor with cubicles the first 5 weeks of dry-off, on straw-bedded litter the next 3 weeks and in a calving pen for the last 6 to 24 h before calving. After calving, they entered the experimental group section. All cows were fed the same dry cow ration during this pre-experimental period. At calving, they were blocked according to parity (primiparous or multiparous) (Table 2) and expected calving time, and allocated to one of two feeding strategies. All cows were housed in a common loose-housing system with slatted floor, cubicles with mattresses and bedding, and access to water. The cows had access to one automatic milking unit (DeLaval AB, Tumba, Sweden). The milking frequency was not influenced by the feeding strategy ($P = 0.34$), and was on average 2.77 ± 0.18 per day for the period studied (from calving to 36 weeks). The milking robot was equipped with a device that delivered a restricted amount of concentrate per cow per milking per day and recorded the concentrate refusals. The milking robot was also equipped with a platform scale (Danvaegt, Hinnerup, Denmark) to record the live weight of the animal at each milking. Feed intake of the partially mixed ration was recorded by the Insentec RIC system (Insentec, Marknesse, the Netherlands).

Experimental design and feeding

The cows were allocated to one of two feeding strategies at calving with different diets. Two partially mixed rations were used; one with a high energy density (HD) and a

Table 2 *Live weight (± standard error) characteristics of Holstein cows for each strategy (LD-LD or HD-LD) and parity (primiparous or multiparous)*

Parity	Primiparous		Multiparous	
Strategy	LD-LD	HD-LD	LD-LD	HD-LD
Number of cows	9	8	22	23
LW at calving (kg)	613 ±11.7	601 ± 17.0	675 ± 18.5	682 ± 9.4
LW at nadir (kg)	569 ± 10.6	571 ± 18.0	639 ± 15.8	667 ± 12.6
DIM at nadir (days)	53 ± 4.2	51 ± 2.8	47 ± 1.1	52 ± 1.3

LD = lower energy density; HD = high energy density.
(LD-LD): strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio).
(HD-LD): strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio).

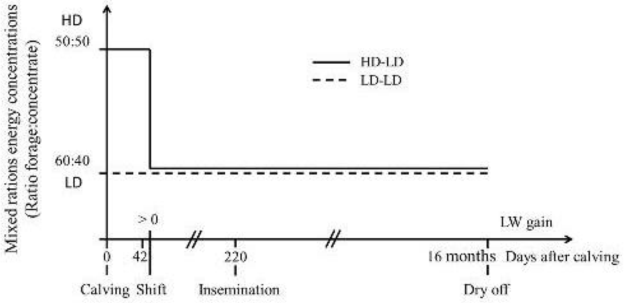

Figure 1 Mixed ration energy concentration during lactation on the two feeding strategies. The graph for the HD-LD Holstein cows represents an example of a cow shifted at 42 days after calving. LD-LD: strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio). HD-LD: strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio). HD = high energy density; LD = lower energy density.

50 : 50 forage : concentrate ratio, and one with a lower energy density (LD, control diet) and a 60 : 40 forage : concentrate ratio. The composition of the diets is shown in Table 1. For both strategies, a maximum of 3 kg concentrate daily was offered in the milking robot. Half of the cows were fed with the HD diet until they reached at least 42 days in milk and a LW gain ⩾ 0 kg/day based on a 5-days LW average, and were then shifted to the LD diet (strategy HD-LD). The other half of the cows were fed the LD diet throughout lactation (control strategy LD-LD) (Figure 1). Both diets, LD and HD, were offered *ad libitum*. The rations were optimized using the NORFOR model and standards (Volden, 2011). Weekly analyses of dry matter content in forages were performed for adjustment of ration composition.

At the first heat detected after 220 DIM, the insemination started. The cows were dried off 8 weeks before expected calving, or if the milk production dropped below 12 kg per day in two subsequent weeks.

Data recording

Blood samples. Blood was sampled by venipuncture in the tail at 4 ± 1 DIM weekly from calving to 12 weeks in milk (WIM) on Thursday mornings between 10:00 and 12:00. From 14 to 36 WIM, blood samples were taken every second week. The samples were harvested in vacutainers (9–10 ml Sodium-Heparin-vacutainer tube, Becton Dickinson Vacutainer Systems, Plymouth, UK) and put on ice before being centrifuged at 2000 × ***g*** at 4°C for 10 min. Plasma was harvested to polypropylene tubes and stored at –20°C until analyzed.

Recording. LW was recorded when the cows were visiting the milking robot. Weight recordings (10 times per second) were 'cleaned up' following the procedure described in Bossen *et al.* (2009) to obtain one daily LW per cow. Each time the cow visited the Insentec feeder, feed intake was recorded and daily dry matter intake (DMI R) was calculated. The daily concentrate intake in the milking robot (a maximum of 3 kg/day) was also recorded and daily concentrate dry matter intake calculated (DMI C). Body condition score (BCS) of all cows was scored manually every second week by the same two trained persons. A 1 to 5 points scale with 0.25 points intervals was used (Ferguson *et al.*, 1994). The milk yield was recorded at each individual milking in the milking robot and the milk components (fat, protein, lactose, cells) were measured every week.

Chemical analyses

Metabolites. All the plasma samples (24 per cow) were analyzed for the concentrations of glucose (mmol/l), NEFA (mmol/l), BHBA (mmol/l), uric acid (μmol/l), and urea (mmol/l) by use of an autoanalyzer (ADVIA 1650 Chemistry System; Siemens Medical Solution, Tarrytown, NY, USA). For the quantitative determination of NEFA, the Wako NEFA-HR(2) reagent (Wako Chemicals GmbH, Neuss, Germany), an enzymatic colorimetric method assay, was used. The ADVIA urea nitrogen method is based on the Roch-Ramel enzymatic reaction, using urease and glutamate dehydrogenase. The inverse reaction was used for measuring BHBA. The uric acid method is based on the Fossati enzyme reaction using uricase with a Trinder colorimetric procedure (Fossati *et al.*, 1980).

Hormones. Plasma samples of 3, 5, 12 and 24 WIM were also analyzed for insulin and IGF-1 (ng/ml). The Mercodia Bovine Insulin ELISA, a solid phase two sites enzyme immunoassay, was used to determine insulin concentrations. The IGF-1 concentration was measured in extracted samples after removal of IGF-binding proteins by use of an in-house double-sandwich FIA (Time-Resolved Fluorescence Immunoassay) validated and described earlier (Frystyk *et al.*, 1995).

Data analysis

Calculation of live weight (LW) gain and energy corrected milk (ECM). The Wilmink model (Wilmink, 1987), equation (1) below, was used to fit the daily individual LW measurements, where LW is the live weight (kg); DIM are the days in milk; the factor −0.05 and the parameters *a*, *b* and *c* are associated with the general LW during the lactation, the increase of LW after LW nadir, the decrease of LW toward the LW nadir; and the moment of LW nadir around 50 days after calving calculated from Table 2. The parameters were calculated for each cow by linear regression (lm function in R). Equations for LW gain (2) were found as the derivative of the LW equations for individual cows at 24 time points representative of the 24 blood samples taken per cow. The energy corrected milk (3.14 MJ/kg) was calculated according to the equation (3) (Sjaunja *et al.*, 1991), with fat, protein and lactose in g/kg; ECM and yield in kg.

$$\mathrm{LW} = a + b \times \mathrm{DIM} + c \times \exp(-0.05 \times \mathrm{DIM}) \qquad (1)$$

$$\mathrm{LW\ gain} = b - 0.05 \times c \times \exp(-0.05 \times \mathrm{DIM}) \qquad (2)$$

$$\mathrm{ECM} = \mathrm{yield} \times (38.3 \times \mathrm{fat} + 24.2 \times \mathrm{protein} + 15.71 \times \mathrm{lactose} + 20.7)\ /\ 3.14 \qquad (3)$$

Calculation of energy balance. Daily energy balance (EB, in MJ NEL/day) was calculated using the following basic equation:

$$\mathrm{EB} = \mathrm{E_{intake}} - (\mathrm{E_{lact}} + \mathrm{E_{maint}} + \mathrm{E_{act}})$$

$$\mathrm{E_{lact}} = \mathrm{ECM} \times 3.14,\ \mathrm{E_{maint}} = \mathrm{LW}^{0.75} \times 0.08 \times a,$$
$$\mathrm{E_{act}} = \mathrm{LW} \times 0.01 \times b$$

The $\mathrm{E_{intake}}$ is the total energy intake (MJ NEL/day, with 1 Scandinavian Feed Units = 7.89 MJ NEL/kg, Strudsholm *et al.*, 1997). The $\mathrm{E_{lact}}$ is the daily energy required for the milk production (MJ NEL/day) with ECM the energy corrected milk (kg ECM/day), and 3.14 the energy needed to produce 1 kg ECM (MJ NEL/kg ECM) (Sjaunja *et al.*, 1991). The $\mathrm{E_{maint}}$ is the daily energy required for maintenance (MJ NEL/day), where the maintenance requirement for NEL is set at 0.08 Mcal/kg $\mathrm{LW}^{0.75}$ (NRC, 2001), '*a*' is the coefficient of conversion from Mcal to MJ, equal to 4.184, and LW is the live weight (kg/day). The $\mathrm{E_{act}}$ is the energy required for activity of loose housed cows (MJ NEL/day), 0.01 corresponds to the 10% activity for voluntary activity (MJ ME/kg) (NRC, 2001), and '*b*' is the coefficient of conversion from ME to NEL, equal to 0.65. The energy used for growth was not included as it has been shown to be <1% of the energy output and only for the first and second parity (Friggens *et al.*, 2007). The cows were not inseminated before 8 months of lactation so the energy for conception was not included in the calculation as the period studied was from calving to insemination. The EB calculated per period is shown in Table 3.

Calculation of RQUICKI values. RQUICKI values were calculated to indicate insulin sensitivity:

$$\mathrm{RQUICKI} = 1\ /\ (\log(\mathrm{Gl}) + \log(\mathrm{In}) + \log(\mathrm{NEFA}))$$

where Gl represents the plasma concentration of glucose (mg/dl), In the plasma concentration of insulin (μU/ml), and NEFA the plasma concentration of NEFA (mmol/l) (Holtenius and Holtenius, 2007).

Statistical analyses

As the cows shifted diet individually, the data were divided and analyzed separately for three different periods of the lactation, reflecting the shift in diet. The first period was from calving to 5 WIM before the shift in diet. The second period was right after the shift from 10 to 12 WIM. The third period was further after the shift from 14 to 36 WIM. The cows were randomly allocated to a given feeding strategy and sampled for blood several times during the lactation. The influence of the feeding strategy on the metabolites, hormones and production data (daily averages per cow), was analyzed applying a linear mixed-effects model before and after the shift:

$$Y_{ijkl} = \mu + S_i + P_j + W_k + (SP)_{ij} + C_{ijl} + \epsilon_{ijkl}$$

where μ is the overall mean within cows, the effects of the i^{th} feeding strategy S (i = LD-LD, HD-LD), the j^{th} parity P (j = primiparous, multiparous), and W is the k^{th} WIM (k = 1 to 36). The $(SP)_{ij}$, denotes the two-factors interactions, C_{ijl} is the random effect of the l^{th} cow within i strategy and j parity, and ε_{ijkl} is the random residual error. With R version 3.0.0, the LME function, from the NLME package (Pinheiro *et al.*, 2015), was used to fit the linear mixed-effects model (Laird and Ware, 1982). The correlation over weeks within each cow was calculated with the temporal corAR1 function, representing an autocorrelation structure of order 1 (Pinheiro and Bates, 2000). For the three periods before and after the shift, the results are presented as LS means values for concentrations of metabolites, hormones, EB, and production data for each strategy and parity, indicating if these two factors had an effect on the variables (Table 3). An example of the NEFA graph is presented in Figure 2.

Simple correlations between EB, LW gain, NEFA, BHBA, glucose, urea, uric acid, insulin and IGF-1, were calculated at 3, 12 and 24 WIM, using the Pearson's method.

Results

Metabolites in blood plasma

From 1 to 5 WIM. Before the shift in diet, the strategy had an effect on plasma glucose, BHBA, NEFA, and urea concentrations. The HD-LD cows had higher concentrations of glucose than the LD-LD cows. From 1 to 2 WIM, glucose decreased and then slightly increased for both strategies until WIM 5. The BHBA and NEFA concentrations were lower for the HD-LD cows. From 1 to 5 WIM, NEFA decreased. For BHBA, the concentrations were not influenced by the week. Urea was lower for the HD-LD multiparous cows but there was no effect of strategy on primiparous cows. Concentrations of urea were higher and stable for the LD-LD cows compared with the HD-LD cows, in which a decrease of the urea concentration occurred from 1 to 2 WIM followed by an increase until 4 WIM. Uric acid was not influenced by the strategy, but

Table 3 *Plasma concentrations of glucose (mmol/l), BHBA (mmol/l), NEFA (mmol/l), urea (mmol/l), uric acid (μmol/l), insulin (ng/ml), IGF-1 (ng/ml), LW gain (kg/day) and energy balance (EB, MJ NEL/day) of Holstein cows in three periods; (a) before the shift, (b) after the shift, (c) further after the shift, according to strategy (LD-LD or HD-LD) and parity (primiparous or multiparous)*[1]

Parity	Primiparous		Multiparous			*P*-values		
Strategy	LD-LD	HD-LD	LD-LD	HD-LD	SE	S	P	S × P
(a) From 1 to 5 WIM								
Glucose	3.8	4.0	3.4	3.6	0.05	0.02	<0.001	0.64
BHBA	0.74	0.57	0.86	0.68	0.06	0.04	0.24	0.99
NEFA	0.52	0.42	0.45	0.35	0.03	0.01	0.10	0.72
Urea	2.8	2.2	3.0	2.5	0.08	<0.001	0.02	0.007
Uric acid	29	31	31	32	1.4	0.34	0.51	0.57
Insulin	0.15	0.20	0.21	0.14	0.08	0.59	0.94	0.79
IGF-1	89	112	69	83	6	0.03	0.004	0.59
EB	−25	−21	−11	−8	5	0.001	0.39	0.94
LW gain	−0.46	−0.47	−0.41	−0.26	0.2	0.62	0.59	0.75
(b) From 10 to 12 WIM								
Glucose	3.9	3.9	3.7	3.7	0.04	0.74	0.04	0.65
BHBA	0.5	0.6	0.7	0.8	0.04	0.11	0.01	0.37
NEFA	0.13	0.15	0.14	0.15	0.01	0.23	0.78	0.35
Urea	3.3	3.5	3.5	3.8	0.09	0.03	0.07	0.41
Uric acid	33	30	29	25	1.5	0.07	0.19	0.55
Insulin	0.34	0.24	0.25	0.25	0.07	0.62	0.93	0.46
IGF-1	124	116	90	103	6	0.34	0.002	0.17
EB	7	7	3	8	3	0.48	0.05	0.06
LW gain	0.31	0.27	0.22	0.26	0.05	0.72	0.24	0.41
(c) From 14 to 36 WIM								
Glucose	3.8	3.8	3.7	3.8	0.03	0.18	0.31	0.17
BHBA	0.6	0.6	0.6	0.7	0.02	0.11	0.003	0.75
NEFA	0.09	0.11	0.09	0.10	0.004	0.006	0.39	0.52
Urea	3.8	3.8	4.0	4.1	0.07	0.38	0.06	0.72
Uric acid	33	28	30	30	1.1	0.29	0.70	0.14
Insulin	0.41	0.26	0.32	0.39	0.07	0.88	0.81	0.10
IGF-1	111	113	135	145	6	0.43	<0.001	0.59
EB	15	19	12	20	4	0.86	0.08	0.52
LW gain	0.39	0.32	0.26	0.28	0.05	0.91	0.08	0.35

BHBA = beta-hydroxybutyrate; NEFA = non-esterified fatty acids; LD = lower energy density; HD = high energy density; LW = live weight; S = strategy; P = parity; WIM = weeks in milk.

LD-LD: strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio).

HD-LD: strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio).

[1] Least squares means (LS Means) values are presented.

changes occurred over weeks of lactation: the concentration dropped from 2 to 3 WIM from around 34 to 29 μM. Regarding parity, the primiparous had a lower concentration of urea but a higher concentration of glucose than the multiparous. No other effect of parity was found (Table 3).

From 10 to 12 WIM. After the shift in diet for the HD-LD cows, the concentrations of glucose, BHBA, and urea were not different between strategies (Table 3). The NEFA concentrations decreased over WIM and were numerically higher for the HD-LD cows than the LD-LD cows, but the difference was not significant. The HD-LD compared with LD-LD cows tended to have a higher concentration of uric acid. Parity influenced the concentrations of glucose and tended to affect urea; the primiparous having numerically higher concentrations of glucose and lower concentrations of urea than the multiparous.

From 14 to 36 WIM. A few weeks after the shift in diet, some changes appeared in metabolite concentrations. The glucose concentration was numerically higher for the HD-LD cows compared with the LD-LD cows. The glucose concentration increased with DIM for both strategies. The concentrations of BHBA were only influenced by parity; the multiparous cows had higher concentrations than the primiparous cows. Numerically, the NEFA concentrations were similar to the previous period, but in this period, the strategy had an effect on NEFA concentrations, as the HD-LD cows had a higher concentration of NEFA than the LD-LD cows. Moreover, NEFA concentrations decreased for both strategies until week 22, and were then low (<0.1 mmol/l) and stable until 36 weeks. Urea tended to be higher for the multiparous compared with the primiparous, and the concentration increased with DIM. Finally, uric acid was not affected by parity, week, or strategy (Table 3).

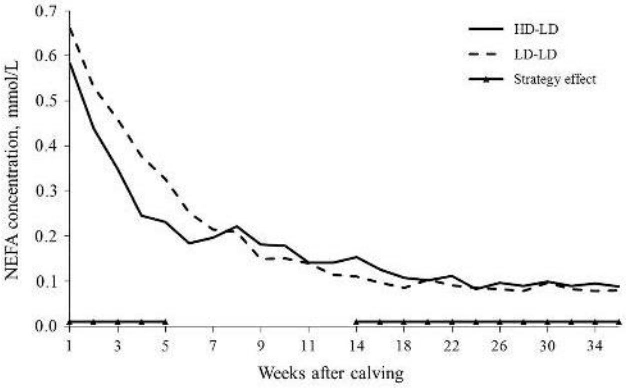

Figure 2 Evolution of the NEFA plasma concentration of Holstein cows, from week 1 to 36 after calving, for both feeding strategies (LD-LD dash line, HD-LD full line). Weeks in which the effect of the strategy is significant are indicated by the horizontal line on the graph (Strategy effect). LD-LD: strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio). HD-LD: strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio). NEFA = non-esterified fatty acids; LD = lower energy density; HD = high energy density.

Hormones

Insulin and IGF-1. The insulin concentration increased with time, from 5 to 24 WIM ($P < 0.002$) but was not affected by strategy or parity. Nevertheless, at 3 WIM, the multiparous LD-LD cows had a numerically higher insulin concentration than the multiparous HD-LD cows or the primiparous from both strategies (0.5 ng/ml for the multiparous LD-LD *v.* <0.15 ng/ml for the other). The same observation was made for week 24, where the primiparous LD-LD cows also had a higher concentration than the primiparous HD-LD cows. The IGF-1 increased over time until week 24 ($P < 0.001$). Before the shift from 1 to 3 WIM, the HD-LD cows had a higher concentration of IGF-1 than the LD-LD cows, but after the shift from 12 to 24 WIM, the strategy had no effect. Parity also influenced IGF-1. The primiparous had higher concentrations of IGF-1 than the multiparous.

RQUICKI. Table 4 shows the means values of glucose, insulin, NEFA and RQUICKI. The period had an effect on the plasma concentrations of glucose, insulin and NEFA, but had no effect on RQUICKI values. The strategy ($P = 0.23$) and parity ($P = 0.63$) had no effect on RQUICKI. The Pearson correlation between BCS and RQUICKI was negative ($r = -0.23$, $P < 0.002$), showing that RQUICKI increased with decreased BCS.

Table 4 *RQUICKI values for the three periods studied; (a) before the shift, (b) after the shift, and (c) further after the shift; and* ***P****-values showing the effects of lactation period (Means ± SE)*

	Lactation period			
	(a) 3 and 5 WIM	(b) 12 WIM	(c) 24 WIM	*P*-value
Glucose (mg/dl)	66.44 ± 0.65	68.19 ± 0.82	67.79 ± 0.83	<0.001
Insulin (μU/ml)	4.39 ± 0.85	6.78 ± 1.05	8.63 ± 1.08	0.002
NEFA (mmol/l)	0.35 ± 0.02	0.13 ± 0.02	0.09 ± 0.02	<0.001
RQUICKI	0.54 ± 0.18	0.58 ± 0.18	0.56 ± 0.18	0.89

NEFA = non-esterified fatty acids; WIM = weeks in milk.

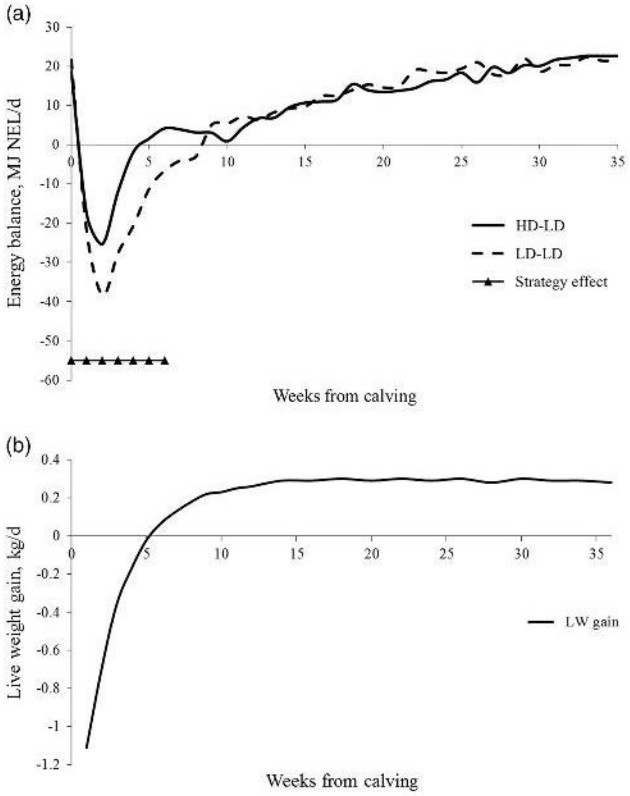

Figure 3 (a) Energy balance (MJ NEL/day) of Hostein cows regarding feeding strategies (LD-LD dash line, HD-LD full line). Weeks in which the effect of the strategy is significant are indicated by the horizontal line on the graph (Strategy effect). (b) Average live weight gain (kg/day) of Holstein cows regarding the week after calving. There was no effect of strategy or parity on live weight and live weight gain. LD-LD: strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio). HD-LD: strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio). LD = lower energy density; HD = high energy density.

Energy balance and indicators of energy balance

The EB was not influenced by parity during the periods studied (Table 3). From calving to 5 WIM, the EB was negative, and was more negative for the LD-LD cows than for the HD-LD cows ($P = 0.001$). From 10 to 12 WIM, the EB was not influenced by the feeding strategy anymore, and was positive. From 14 to 36 WIM the EB kept being positive for all the cows (Table 3, Figure 3a). The LW characteristics of the cows regarding the parity are shown in Table 2. The LW gain, calculated by deriving the LW equations of the Wilmink model, was not influenced by strategy ($P = 0.71$) or the parity ($P = 0.42$) (Table 3). It increased with time, starting to be negative from calving until around WIM 5, which defines the mobilization period. Then it was kept positive and stable, while the cows entered the deposition period (Figure 3b).

At week 3, NEFA and BHBA were negatively correlated with the LW gain ($r = -0.38$, $P < 0.002$ and $r = -0.34$, $P < 0.006$, respectively), while the EB was correlated with glucose ($r = 0.40$, $P < 0.001$), NEFA ($r = -0.53$, $P < 0.001$), BHBA ($r = -0.32$, $P = 0.01$), insulin ($r = 0.39$, $P = 0.02$), and IGF-1 ($r = 0.25$, $P = 0.05$). At week 3, the EB and LW gain were positively correlated ($r = 0.40$, $P = 0.001$). At week 12, the LW gain was positively correlated with glucose and IGF-1 ($r = 0.36$, $P = 0.003$, and $r = 0.35$, $P = 0.004$, respectively) while EB was correlated with urea and insulin ($r = 0.45$, $P < 0.001$, and $r = 0.33$, $P = 0.01$, respectively). At week 12, there was no correlation between the EB and LWG ($r = -0.08$, $P = 0.52$). At week 24, the LW gain was positively correlated with IGF-1 and insulin ($r = 0.23$, $P = 0.06$, and $r = 0.32$, $P = 0.01$, respectively) while the EB was correlated with the BHBA ($r = -0.29$, $P = 0.03$). The EB and LW gain were positively correlated at week 24 ($r = 0.31$, $P = 0.02$).

Sum milk yield, sum feed intake and mean BCS

The feeding strategy had no effect on the averages ECM, and DMI of concentrates (DMI C) in milking robot, for the three periods studied from 0 to 36 WIM (Table 3). The DMI R intake (partially mixed ration without the concentrates at the milking robot) of LD or HD was different from 0 to 42 days between LD-LD and HD-LD cows (15.6 *v.* 16.0 kg DM/day, respectively) but this difference was quite small; 0.38 ± 0.49 kg DM/day higher for the HD-LD cows. The primiparous had lower LD or HD intake and ECM than the multiparous ($P < 0.001$), but similar DMI of concentrates and BCS (Table 5).

Discussion

Early lactation

During early lactation, plasma glucose was higher, and NEFA and BHBA were lower for the HD-LD cows compared with the LD-LD cows, when the HD-LD cows were fed a high energy diet. Moreover, correlations were found between LW gain and NEFA as well as BHBA. These results confirmed our first hypothesis indicating that the HD diet reduced the magnitude of negative energy balance in early lactation. Results from previous studies on the effect of increasing the amount of concentrate in early lactation are in accordance with ours. Cows fed a high energy diet by increasing the amount of concentrates in the ration had a higher concentration of plasma glucose (Jenny and Polan, 1975; Sutton *et al.*, 1986) and a lower concentration of plasma BHBA (Andersen *et al.*, 2004) and NEFA (Dhiman *et al.*, 1991; Nachtomi *et al.*, 1991) than cows fed a diet lower in energy. Increased energy intake by supplementing diets with glycerol or propylene glycol increased plasma glucose and decreased NEFA and BHBA as well (Lomander *et al.*, 2012). These results confirm that the energy balance can be improved by feeding an energy-enriched diet in early lactation. In addition, our results indicated that HD-LD cows had lower plasma concentrations of urea compared with LD-LD cows. One explanation is that this result suggests a smaller protein mobilization and oxidation as previously found (Cucunubo *et al.*, 2013). The multiparous had higher urea concentrations than the primiparous. Another study found no effect of parity on urea, but that urea was low at the beginning of the lactation and then increased (Dhiman *et al.*, 1991). Finally, in early lactation, we found that the primiparous had higher concentrations of glucose than the multiparous in accordance with Dhiman *et al.* (1991).

After the shift

After the shift from 10 to 36 WIM, it was expected that the concentrations of BHBA and NEFA for the HD-LD cows would still be similar or higher than those of the LD-LD cows the first week after the shift and then similar, as the cows recovered from the shift. However, from 10 to 12 WIM, there were no statistical differences between the strategies, while from 14 WIM, NEFA concentrations were higher for the HD-LD cows compared with the LD-LD cows. Even so, by looking closely at the differences in NEFA concentrations between the LD-LD cows and the HD-LD cows we found that they were numerically similar from 10 to 12 WIM and from 14 to 36 WIM (the difference between LD-LD and HD-LD cows equals 0.015 mmol/l for both intervals). However, for both LD-LD and HD-LD cows, the variation among the cows at 10 to 12 WIM was larger compared with 14 to 36 WIM (SEM = 0.01 and 0.004 mmol/l, respectively) explaining the non-significance of the difference between the LD-LD and HD-LD cows from 10 to 12 WIM (Table 3). The HD-LD cows were not shifted from HD to LD on the same day and, consequently, the adaptation to the new feeding strategy and the duration of the mobilization period of each cow were different, which induced a metabolic variation among the cows. These results are in accordance with Kessel *et al.* (2008), where it was indicated that the changes of metabolites and hormones concentrations differ among animals depending on their ability to cope with their metabolic stress.

Our results from 10 to 12 WIM are in accordance with other results. In a previous study, some cows were restricted for 4 days, and the metabolic changes occurred during these restriction days. Then the cows were shifted back to the standard TMR diet *ad libitum*, and 1 week after, there were no metabolic differences between the groups (Bjerre-Harpoth *et al.*, 2012). This result is similar to our result, where metabolic changes were detected during the shift period, but not 1 week after the last cow had been shifted to the control diet (from 10 to 12 WIM). This could indicate that the cows needed at least one week to adjust to the change of diet. The general decline in NEFA during the lactation period in our experiment can be related to a decrease in milk synthesis and rates of lipolysis, as well as an increase of re-esterification of fatty acids (Ronge *et al.*, 1988). This decrease in plasma NEFA is in accordance with some previous work (Marett *et al.*, 2011).

From 14 to 36 WIM, the HD-LD cows had higher NEFA concentration compared to the LD-LD cows. The same pattern was found for cows fed *ad libitum* before parturition and then fed restrictively according to requirements after calving (Kunz *et al.*, 1985). These cows had higher concentrations of NEFA after parturition than cows fed the same restricted diet before and after parturition and indicated an increase in ketone bodies (Kunz *et al.*, 1985). This could

Table 5 *Milk yield (kg/day), fat in milk (%), protein in milk (%), lactose in milk (%), cells in milk (log(cells)), ECM (kg ECM/day), milking frequency (Milkings/day), BCS, and DMI (kg DM/day) of Holstein cows for three periods (WIM) (a) before the shift, (b) after the shift, (c) further after the shift, regarding strategy (LD-LD or HD-LD) and parity (primiparous or multiparous)*

Parity	Primiparous		Multiparous			*P*-values		
Strategy	(LD-LD)	(HD-LD)	(LD-LD)	(HD-LD)	SE	S	P	S × P
(a) From 1 to 5 WIM								
Milk	30.2	26.9	38.6	40.5	1.8	0.52	<0.001	0.06
ECM	32.5	29.0	40.5	41.3	1.9	0.99	<0.001	0.19
Fat	4.57	4.58	4.49	4.18	0.1	0.04	0.06	0.17
Protein	3.34	3.44	3.45	3.48	0.05	0.25	0.109	0.48
Lactose	4.87	4.94	4.78	4.84	0.03	0.02	0.001	0.90
SCC	1.89	2.05	2.19	2.11	0.07	0.87	0.04	0.17
Milkings	2.4	2.6	3.0	3.2	0.2	0.19	<0.001	0.92
BCS	3.1	3.2	3.1	3.2	0.04	0.11	0.91	0.61
DMI R^1	13.4	12.9	17.9	19.1	0.6	0.04	<0.001	0.10
DMI C^2	2.1	2.1	2.2	2.1	0.07	0.27	0.84	0.31
(b) From 10 to 12 WIM								
Milk	33.1	30.5	41.3	42.0	1.7	0.95	<0.001	0.26
ECM	32.4	30.4	40.1	40.0	1.4	0.79	<0.001	0.46
Fat	3.87	4.07	3.86	3.81	0.11	0.88	0.19	0.25
Protein	3.23	3.19	3.13	3.17	0.06	0.64	0.56	0.82
Lactose	4.96	4.89	4.96	4.87	0.04	0.48	0.02	0.71
SCC	1.84	1.85	2.00	1.98	0.15	0.95	0.25	0.90
Milkings	2.39	2.83	2.87	3.13	0.26	0.09	0.06	0.67
BCS	3.2	3.2	3.1	3.2	0.07	0.19	0.71	0.79
DMI R^1	17.8	15.8	21.7	21.8	0.7	0.49	<0.001	0.06
DMI C^2	2.4	2.4	2.4	2.4	0.06	0.43	0.69	0.59
(c) From 14 to 36 WIM								
Milk	32.2	29.8	37.3	37.3	1.6	0.70	<0.001	0.42
ECM	32.0	30.9	36.9	36.8	1.5	0.88	<0.001	0.69
Fat	3.92	4.28	3.98	3.98	0.14	0.40	0.35	0.14
Protein	3.45	3.45	3.42	3.41	0.07	0.96	0.53	0.97
Lactose	4.89	4.92	4.84	4.79	0.03	0.23	0.01	0.18
SCC	1.77	1.95	1.95	1.98	0.14	0.47	0.33	0.51
Milkings	2.43	2.50	2.71	2.73	0.19	0.75	0.11	0.89
BCS	3.2	3.3	3.2	3.2	0.06	0.06	0.82	0.76
DMI R^1	18.8	17.7	21.7	21.8	0.6	0.69	<0.001	0.26
DMI C^2	2.4	2.4	2.3	2.4	0.07	0.23	0.99	0.60

LD = lower energy density; HD = high energy density; S = strategy; P = parity; DMI R^1 = DMI of the partially mixed rations (LD and HD); DMI C^2 = DMI of the concentrates at the milking robot; WIM = weeks in milk; SCC = somatic cell counts.
LD-LD: strategy where Holstein cows were fed LD diet (60 : 40 forage : concentrate ratio).
HD-LD: strategy where Holstein cows were fed HD diet (50 : 50 ratio) followed by LD diet (60 : 40 ratio).

indicate a difference of ability between the HD-LD cows and the LD-LD cows to maintain extended lactation. It has previously been shown that the concentrations of growth hormone, IGF-1 and NEFA in the late stage of extended lactation can give information about the persistency of the lactation of a cow and indicate its ability to maintain lactation (Marett *et al.*, 2011). Plasma NEFA have previously been used as an indication of increased adipose tissue mobilization through lipolysis (McNamara and Hillers, 1986) and cows with high concentrations of NEFA could have a better persistency than cows with lower NEFA concentrations (Kay *et al.*, 2009). However, Marett *et al.* (2011) found no evidence of this, maybe because all the cows in their study were in a positive energy balance or because the differences were too small to be detected.

Hormones

Insulin was not affected by feeding strategy but increased over the time in accordance with previous results. Similarly, Sorensen and Knight (2002) found that insulin was unaffected by nutrition (one group fed 3 kg extra concentrates compared to another group). A low plasma insulin concentration reduces glucose uptake by the muscles and the adipose tissue and consequently saves glucose for uptake by the mammary gland (van Knegsel *et al.*, 2007). With low concentrations of insulin, the secretion of the hormone-sensitive lipase is stimulated, increasing lipolysis with the subsequent release of NEFA to the bloodstream (Melendez *et al.*, 2009). According to Staples *et al.* (1998), plasma insulin concentrations reflect the energy intake. In our study, insulin is not reflecting energy intake in early lactation, but this may be the case later in lactation. This finding in early lactation could indicate a resistance to insulin indicated by a reduced insulin responsiveness during this period (Bell and Bauman, 1997; Sinclair, 2010). As previously found by Holtenius and Holtenius (2007), plasma components vary over the periods but not the RQUICKI index, so it seems a good indicator to identify changes in insulin sensitivity for these cows. Moreover, we found a correlation between BCS and RQUICKI, in accordance with Holtenius *et al.* (2003) suggesting that cows with high BCS should have a lower RQUICKI and be more insulin resistant than the thin cows (Holtenius *et al.*, 2003). Our RQUICKI values were not affected by the period of the lactation. However, the RQUICKI was lower in the first weeks of lactation than later around 36 weeks (0.54 and 0.56 ± 0.18, respectively) which supports our hypothesis of insulin resistance.

As lactation progressed IGF-1 tended to increase, which has also been found in many other studies (Sorensen and Knight, 2002; Marett *et al.*, 2011). In early lactation, it has been shown that cows fed a high energy diet had higher plasma IGF-1 concentrations than cows fed a low energy diet (Andersen *et al.*, 2002; Andersen *et al.*, 2004). This is in accordance with our results as IGF-1 for the HD-LD cows was higher than for the LD-LD cows at least until five WIM. Finally, we found that the primiparous cows had higher concentrations of IGF-1 than the multiparous cows while another study found no effect of parity on IGF-1 (Kang *et al.*, 2007).

LW gain, NEFA, and BHBA, relevant indicators of energy balance

In early lactation, the LW gain is positively correlated with the EB. Both are correlated with NEFA and BHBA, which points out that the negative energy balance, or the loss of weight, is related with an increase in NEFA and BHBA concentrations indicating an increase of mobilization. Thus, the LW gain, NEFA and BHBA are good indicators of EB in early lactation. This result has been established previously like in Kessel *et al.* (2008), where the concentrations of BHBA were chosen as the single best indicator of energy balance. The inexistent correlation between LW gain and EB right after the shift period may indicate that the model used to describe the LW data may not be the most accurate tool to characterize the adaptive capability of the cows, as the variation in LW measures within and between individuals is high during this period. Moreover, the lack of correlations between LW gain and the metabolites could also indicate a delay between the metabolic response and the LW gain response, as the change in diet induced a change in metabolite concentrations, which was later seen as LW gain changes. Further after the shift, from 14 to 36 WIM, the positive correlation between LW gain and EB is restaured. Even so we found an effect of the feeding strategy on the NEFA concentration, there was no correlation between this metabolite and EB or LW gain. The differences in NEFA concentrations between the groups might be too small to traduce an effect on the LW gain or EB.

Conclusions

The aim was to study the effects of an individualized live weight adjusted feeding strategy on plasma indicators of energy balance in Holstein cows managed for extended lactation. From calving to 5 weeks after calving, the energy-enriched diet reduced the negative energy balance of HD-LD cows indicating a reduced mobilization of HD-LD cows compared with LD-LD cows. The energy-enriched diet in early lactation had some carry-over effects in later lactation metabolism, from 14 to 36 WIM. The LW gain is highly related to energy balance in early lactation and in mid-lactation. Nevertheless, the LW gain was unable to detect small changes in energy induced by diets. In this case, metabolites such as BHBA and NEFA are better indicators of energy balance. To conclude, these results indicate that the HD-LD strategy with an increased dietary energy concentration in early lactation followed by an individually managed reduction in dietary energy concentration at weight nadir reduces the magnitude of the mobilization during early lactation, slightly sustain the mobilization for a longer period of time, and induces metabolic changes in later lactational stages. Moreover, the individual feeding adjustment in early lactation managed using the live weight data can be improved by measuring weekly plasma BHBA, NEFA, and glucose.

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2. Paper 2 – Effects of an individual weight-adjusted feeding strategy in early lactation on milk production of Holstein cows during extended lactation

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Effects of an individual weight-adjusted feeding strategy in early lactation on milk production of Holstein cows during extended lactation

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ABSTRACT

Extending lactation by voluntarily delaying rebreeding aims to improve fertility and milk production in the modern dairy cow. Previous studies have shown that increased energy concentrations in the ration induced greater total milk yield and lactation persistency defined by the duration and the shape of the lactation curve. In this paper, we hypothesized that increasing the supply of energy during the early lactation mobilization period would have a positive carryover effect on milk production during extended lactation. A total of 53 Holstein cows completed a 16-mo lactation, including 30% primiparous cows. The cows were divided into 2 feeding strategies: half of the cows received a high-energy density diet (HD) in early lactation followed by a lower-energy density diet (LD; strategy HD-LD). The change in diet was defined individually after 42 d of lactation, and when the live weight (LW) gain of the cow was ≥ 0 based on a 5-d average. The other half of the cows were fed the LD diet during the entire lactation (strategy LD-LD). Both groups received 3 kg of concentrates per day during milking. Weekly milk composition (fat, protein, lactose, and somatic cells), daily milk production, daily feed intake, daily LW, and body condition score every second week were recorded. The fda package of R was used to model the curves of these different variables for the 53 cows that had a lactation over 400 d. The fitted values of these curves and the associated slopes were then compared for parity and treatment effects using a linear mixed-effects model. The HD-LD and LD-LD cows had a similar length of lactation (461 ± 7 d). The HD diet reduced the intensity of the mobilization period and increased the milk production of the multiparous cows in early lactation compared with the cows fed the LD diet. The primiparous cows used the extra energy to grow and gain weight, but not to produce more milk. After the shift in diet, the treatment had little short-term carryover effect on milk yield or LW, but it affected the slopes of some curves. From 0 to 50 d from shift, milk fat content of the LD-LD cows decreased faster than that of the HD-LD cows whereas milk lactose increased. From 250 to 350 d from shift, the energy-corrected milk decreased faster for the HD-LD cows than for the LD-LD cows. The lactose content in milk decreased faster for the LD-LD cows than for the HD-LD cows, and the fat content in milk was significantly higher for the primiparous HD-LD than for the primiparous LD-LD cows. In conclusion, the supply of extra energy during the mobilization period had a 300-d negative carryover effect on lactation persistency.

Key words: extended lactation, energy balance, persistency, milk production

INTRODUCTION

The fertility of dairy cows is decreasing due to an intense in genetic selection for milk production, and the cows are often dried-off while they are still producing a high amount of milk per day (Knight, 2005). When rebreeding is voluntarily delayed, the lactation of the cows is extended beyond the traditional 10 mo, allowing a better exploitation of the capacity of the cow's production (Knight, 2005). This management also avoids the insemination occurring at the same time as the peak of production and the reconstitution of body reserves, which could improve pregnancy rates (Borman et al., 2004). The plasticity of the lactation curve offers the possibility of prolonging the lactation and increasing the persistency of the lactation (Grossman and Koops, 2003). Lactation persistency is defined as the slope of the decline in milk yield from peak lactation (Sorensen et al., 2008) and is affected by

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genetics, parity, stage of pregnancy (Nørgaard et al., 2005), milking frequency, and nutrition (Sorensen et al., 2006). Indeed, the energy density of the ration has to be adapted for each stage of the lactation, especially in early lactation, where diets with a high energy concentration can reduce the intensity of the mobilization period and lead to a higher milk production (Bossen et al., 2009). However, as cows respond individually to feeding strategies, an individual diet adjustment would improve milk production (Bossen and Weisbjerg, 2009). Even so, this individual adjustment requires a precise definition of when to adjust the diet for each cow. Our objectives in the present study were to examine the effects of an individually live weight-adjusted feeding strategy in early lactation, based on 5 consecutive daily weightings, aiming to reduce the intensity of the mobilization in early lactation, and thereby to sustain the mobilization for a longer period. It was hypothesized that an increased supply of energy during the mobilization period will have a positive carryover effect on milk production and consequently will support a planned 16-mo extended lactation.

MATERIALS AND METHODS

Experimental Facilities and Animals

The experiment was approved by The Animal Experiments Inspectorate under the Danish Veterinary and Food Administration and was carried out at the Danish Cattle Research Centre at Aarhus University, Foulum, Denmark. A total of 53 Danish Holstein cows (17 primiparous and 36 multiparous) entered the experiment 2 mo before calving from December 2012 to September 2013, and completed a 16-mo lactation and an 18-mo calving interval. Cows were housed in a loose housing system with slatted floors and cubicles with mattresses and sawdust as bedding. Cows had free access to water, automatic feed bins (RIC system, Insentec, Marknesse, the Netherlands), and an automatic milking unit (**AMU**; DeLaval AB, Tumba, Sweden). The AMU was equipped with a weighing platform (Danvaegt, Hinnerup, Denmark) to record the live weight (**LW**) of the animal at each milking and a device delivering and recording the amounts of concentrates and refusals.

Experimental Design

Before entering the experiment, cows with previous lactations were dried off 8 wk before expected calving date. Dry cows and heifers were housed on slatted floors with cubicles the first 5 wk of dry-off, on deep litter bedded with straw the next 3 wk, in a calving pen for the last 6 to 24 h before calving, and entered the experimental group pen immediately after calving. All cows were fed the same dry cow ration during this pre-experimental period (Table 1).

The experimental animals were blocked according to expected calving date and parity and randomly allocated to 1 of 2 feeding strategies (Table 2). In the high-low energy diet strategy (**HD-LD**) the cows received a partially mixed ration with a high energy density (**HD**), 50:50 forage:concentrate ratio, until they reached at least 42 d of lactation and a LW gain ≥0 kg/d over 5 d, on average. When these criteria were fulfilled, the cows were individually shifted to a diet with lower energy density (**LD**), with a 60:40 forage:concentrate ratio. The cows in the low-low energy diet strategy (**LD-LD**) were fed the LD diet during the whole lactation. Diets were formulated using the NORFOR model and standards (Volden, 2011). The composition of the diets is shown in Table 1. Both groups were fed the mixed part of the ration ad libitum and each cow was further offered 3 kg of concentrates per day in the milking robot. Weekly analyses of the dry matter content of forages were performed for adjustment of ration composition.

The cows were inseminated at the first heat observed after 220 DIM to achieve an 18-mo calving interval. Finally, the cows were dried off 8 wk before expected calving, or if the average milk production dropped below 12 kg of milk per cow per day in 2 subsequent weeks.

Data Recording

Feed Intake. Feed intake (kg) was recorded for each cow at each visit to the Insentec feeder. All cows were offered a maximum of 3 kg of concentrate per day in the AMU. If a cow ate less than the daily 3 kg concentrate allowed, the amount not eaten (up to 1.5 kg) was allowed on top of the 3-kg allowance for the next day. Individual daily DMI was calculated as the sum of the partially mixed ration ingested at the Insentec and the amount of concentrate ingested at the AMU within a day.

Daily Milk Yield and Milking Frequency. The milk yield was individually recorded at each visit to the AMU. The milk yields recorded at the first and last milking of a 24-h day were divided proportionally according to time from midnight, and allocated to the days, −1 or +1, to obtain the daily milk yield. The same adjustment was made to calculate the daily milking frequency. Individual milk samples were collected weekly by the AMU using a modified automatic sampler (XMS, DeLaval; Løvendahl and Bjerring, 2006). The milk samples were taken over a 48-h period starting on Sunday at noon and finishing on Tuesday at the same time. The milk samples were preserved with bronopol and kept cold (2°C) until they were analyzed.

LW. Individual LW was recorded 10 times per second at each AMU milking. Aberrant weight records were excluded using the procedure described in Bossen et al. (2009). The daily LW was calculated as the average of the multiple recordings.

BCS

Starting at calving, the BCS was individually scored every second week by 1 of the same 2 trained persons. A 1- to 5-point scale with 0.25-point intervals was used where 1 represents a very lean cow and 5 a very fat cow (Ferguson et al., 1994).

Chemical Analyses

Feed. Samples of all feeds were collected weekly. Corn silage samples were composited every 6 wk, whereas barley and concentrate feed samples were composited every 3 mo. Samples were milled through a 1-mm screen before chemical analysis. Dry matter content was determined by drying in a forced-air oven at 60°C for 48 h. Ash was analyzed by weighing after combustion at 525°C (AOAC International, 2000). Crude protein was calculated based on analysis of total N according to the Dumas principle (Hansen, 1989) using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany). Crude fat was analyzed by Soxhlet extraction with petroleum ether after hydrolyzing with HCl. Sugar was analyzed by the Luff-Schoorl method (European Community, 2012; 71/250/EEC). Starch was analyzed

Table 1. Ingredient composition (% of DM) of the dry period ration, the partially mixed rations with a high-energy density (HD) and lower-energy density (LD), including the concentrates received at the automatic milking unit (AMU), and the associated chemical compositions (g/kg of DM)

Item	Diet dry period	LD + C[1]	HD + C[1]
Ingredients, % of DM			
Barley	—	4.3	4.0
Wheat NaOH treated[2]	—	0	15.8
Sodium bicarbonate	—	0.5	—
Rapeseed cake, 10.5% fat	—	17.2	15.8
Rapeseed meal, 4% fat	6.4	—	—
Sugar beet pulp, dried	1.5	8.6	7.9
Grass/clover silage	27.8	31.1	23.7
Corn silage	43.5	27.9	22.2
Straw (spring barley)	19.9	—	—
Urea	—	0.1	0.1
Minerals and vitamins[3]	0.9	0.6	0.6
Concentrates in AMU[4]	—	10.7	9.9
Forage:concentrate ratio	—	60:40	50:50
Energy density, MJ of NE_L/kg of DM	—	7.49	7.81
Chemical composition, g/kg of DM			
Ash	52	78	67
CP	119	171	165
Crude fat	27	45	44
Sugar	—	47	45
Starch	112	144	228
NDF	481	337	305
AAT[5]	—	145	160
PBV[5]	—	16	8

[1] LD + C and HD + C = partially mixed low- or high-energy density ration + concentrates (C) received at the AMU.

[2] The NaOH-treated wheat was prepared by adding 30 kg of NaOH and 100 L of water to 1,000 kg of whole wheat kernels and mixing for 10 min.

[3] Minerals (per kg): Ca = 203.5 mg, Mg = 78.2 mg, K = 0.5 mg, Na = 0.1 mg, Cl = 0.1 mg, S = 12.6 mg, I = 184.2 mg, Se = 36.8 mg, Mn = 3,684 g, Zn = 5,263 g, Co = 26.3 mg, Cu = 1,052 mg. Vitamins (per kg): A = 526 kIU, D_3 = 116 kIU, α-tocopherol = 2,874 mg, E = 3,158 IU.

[4] Composition of the concentrates in milking robot (% DM): 16.9% sugar beet pulp, dried; 16.8% rapeseed meal; 14.6% barley; 14.6% wheat; 9.0% soybean meal, dehulled; 7.0% sunflower meal, dehulled; 7.0% citrus pulp, dried; 5.0% wheat bran; 5.0% alfalfa meal; 3.0% molasses, cane; 0.7% sodium chloride; 0.3% mineral premix; 0.1% magnesium oxide. Minerals (per kg): Fe = 59 mg, Zn = 46 mg, Mn = 38 mg, Cu = 8 mg, I = 0.7 mg, Se = 0.30 mg, Co = 0.11 mg. Vitamins (per kg): A = 1,000 IU, D_3 1,000 IU, α-tocopherol = 45 mg, E = 50 mg.

[5] AAT/PBV = protein evaluation system for ruminants used in the Nordic countries. AAT represents the amino acids absorbed in the small intestine and PBV the protein balance in the rumen (Volden, 2011).

Table 2. Cow characteristics and effects of feeding treatment and parity on milking frequency, ECM, milk, fat, protein, lactose, SCS, live weight (LW), BCS, DMI, net energy intake, and energy balance (EB) (means ± SEM)

	Primiparous		Multiparous			*P*-value[1]		
Item	LD-LD[2]	HD-LD[3]	LD-LD[2]	HD-LD[3]	SEM	T	P	T × P
Characteristics								
Number of animals	9	8	18	18	—	—	—	—
Duration of lactation, d	464	463	462	454	12	0.55	0.60	0.74
Pregnancy time, DIM	242	252	242	228	15	0.27	0.58	0.21
Total milk yield, kg	14,565	12,928	15,671	15,928	901	0.70	0.02	0.25
Means before the shift (0 to 42 DIM)								
Milking frequency	2.4	2.6	3.1	3.4	0.2	0.11	<0.01	0.80
ECM, kg/d	32.5	28.9	40.1	42.9	1.5	0.41	<0.01	0.02
Milk 0–7 DIM, kg/d	21.1	17.9	28.0	30.2	1.4	0.71	<0.01	0.02
Milk 35–42 DIM, kg/d	34.3	30.7	42.6	46.8	1.8	0.18	<0.01	0.02
Fat, %	4.59	4.53	4.49	4.11	0.10	0.02	0.06	0.21
Protein, %	3.34	3.37	3.42	3.38	0.07	0.72	0.47	0.51
Lactose, %	4.89	4.95	4.81	4.84	0.03	0.18	<0.01	0.74
SCS, log(cells)	1.87	1.98	1.98	2.09	0.07	0.17	0.21	0.96
LW at calving, kg	613	602	658	692	13	0.54	<0.01	0.13
LW at 42 DIM, kg	559	574	629	678	11	0.05	<0.01	0.21
BCS	3.08	3.19	3.11	3.20	0.03	0.01	0.68	0.85
DMI, kg of DM/d	15.5	15.1	20.0	21.1	0.6	0.13	<0.01	0.16
Energy intake, MJ of NE_L/d	116	118	149	164	5	<0.01	<0.01	0.09
EB, MJ of NE_L/d	−25.1	−11.3	−19.7	−13.8	4.2	0.01	0.66	0.27
Means after the shift (0 DFS to the end of the lactation)								
Milking frequency	2.3	2.4	2.5	2.6	0.1	0.59	0.19	0.80
ECM, kg/d	31.8	29.7	32.9	33.3	1.4	0.76	0.08	0.33
Milk, kg/d	31.4	27.8	32.6	32.9	1.5	0.48	0.02	0.13
Fat, %	4.07	4.48	4.18	4.12	0.12	0.45	0.37	0.08
Protein, %	3.61	3.64	3.56	3.56	0.04	0.94	0.27	0.78
Lactose, %	4.87	4.87	4.82	4.73	0.02	0.05	0.01	0.19
SCS, log(cells)	1.87	1.88	1.97	2.12	0.11	0.21	0.09	0.48
LW at 0 DFS,[4] kg	567	575	627	663	12	0.16	<0.001	0.37
LW at 300 DFS, kg	689	675	726	757	19	0.49	0.01	0.34
BCS	3.24	3.35	3.30	3.37	0.04	0.11	0.42	0.72
DMI, kg of DM/d	21.1	19.9	23.8	24.0	0.6	0.78	<0.001	0.13
Energy intake, MJ of NE_L/d	164	153	179	181	4.7	0.64	<0.001	0.14
EB, MJ of NE_L/d	16.6	13.4	20.1	18.9	2.8	0.43	0.06	0.68

[1] T = treatment; P = parity; T × P = interaction.

[2] LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation.

[3] HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when LW gain ≥0 and at least after 42 DIM.

[4] DFS = days from shift.

by an enzymatic colorimetric technique (Knudsen et al., 1987). The NDF content was analyzed by neutral detergent extraction (Mertens et al., 2002) using a Fibertec M6 System (Foss Analytical, Hillerød, Denmark). The chemical composition of mixed rations is presented in Table 1.

Milk Components. Milk samples were analyzed weekly using mid-infrared spectroscopy (Eurofins, Aarhus, Denmark) for the percentages of fat, protein, lactose, and SCC; the SCC data were log-transformed to give the SCS.

Data Analysis

Calculation of ECM. Energy-corrected milk (3.140 MJ/kg) was calculated according to Sjaunja's equation (Sjaunja et al., 1991):

$$\mathrm{ECM} = \text{milk yield} \times [(38.3 \times \text{fat} + 24.2 \times \text{protein} + 15.71 \times \text{lactose} + 20.7)/3.140],$$

with ECM and milk yield in kilograms, and fat, protein, and lactose in grams per kilogram.

Calculation of Energy Balance. Daily energy balance (**EB**; MJ of NE_L/d) was calculated using the following basic equation:

$$\mathrm{EB} = \mathrm{E_{intake}} - (\mathrm{E_{lact}} + \mathrm{E_{maint}} + \mathrm{E_{act}}),$$

where $E_{lact} = ECM \times 3.14$, $E_{maint} = LW^{0.75} \times 0.08 \times a$, and $E_{act} = LW \times 0.01 \times b$. The E_{intake} is the total energy intake (MJ of NE_L/d). The E_{lact} is the energy output with milk (MJ of NE_L/d) calculated by multiplying the ECM (kg/d) by 3.140 MJ/kg (Sjaunja et al., 1991).

The E_{maint} is the energy required for maintenance (MJ of NE_L/d), calculated by multiplying the LW (kg) by 0.08 Mcal/kg/d (NRC, 2001), and a is the coefficient of conversion from megacalories to megajoules, equal to 4.184. The E_{act} is the energy required for the activity of loose housed cows (MJ of NE_L/d) which is considered to be 10% of the LW (0.01 MJ of ME/kg; NRC, 2001), and b is the coefficient of conversion from ME to NE_L, equal to 0.65. The energy used for growth (only for the first and second parity), and the energy used for conception were not included, as they have been shown to be less than 1% of the energy output (Friggens et al., 2007).

Smoothing with the 'fda' Package. The functional data analysis (fda) package of R (Ramsay et al., 2010) was chosen to smooth our daily production data, as it can be applied for all our production data whatever the shape of their curves. A single common equation, such as the Wood function (Wood, 1967), which is often used to describe lactation curves, would not fit our extended lactation data because the shapes of the curves were longer than the traditional 10-mo lactation and varied according to parity and the treatment of our cows. Moreover, it could not be applied to all the other performance data. Polynomials could have been a solution to fit all our data the same way, but in this case all the points of the smoothed curves should have been correlated leading to a decrease in quality of the information. With the fda method, only local correlations exist between points because of the knots which provide independence of fitting for data that are separated by 3 knots, in this case 3 DIM. Moreover, the smoothed data allowed us to calculate the slopes of the curves as the derivative of the smoothed data, at any day, even without raw data at this precise time. This allowed easy comparison of the shape of the curves

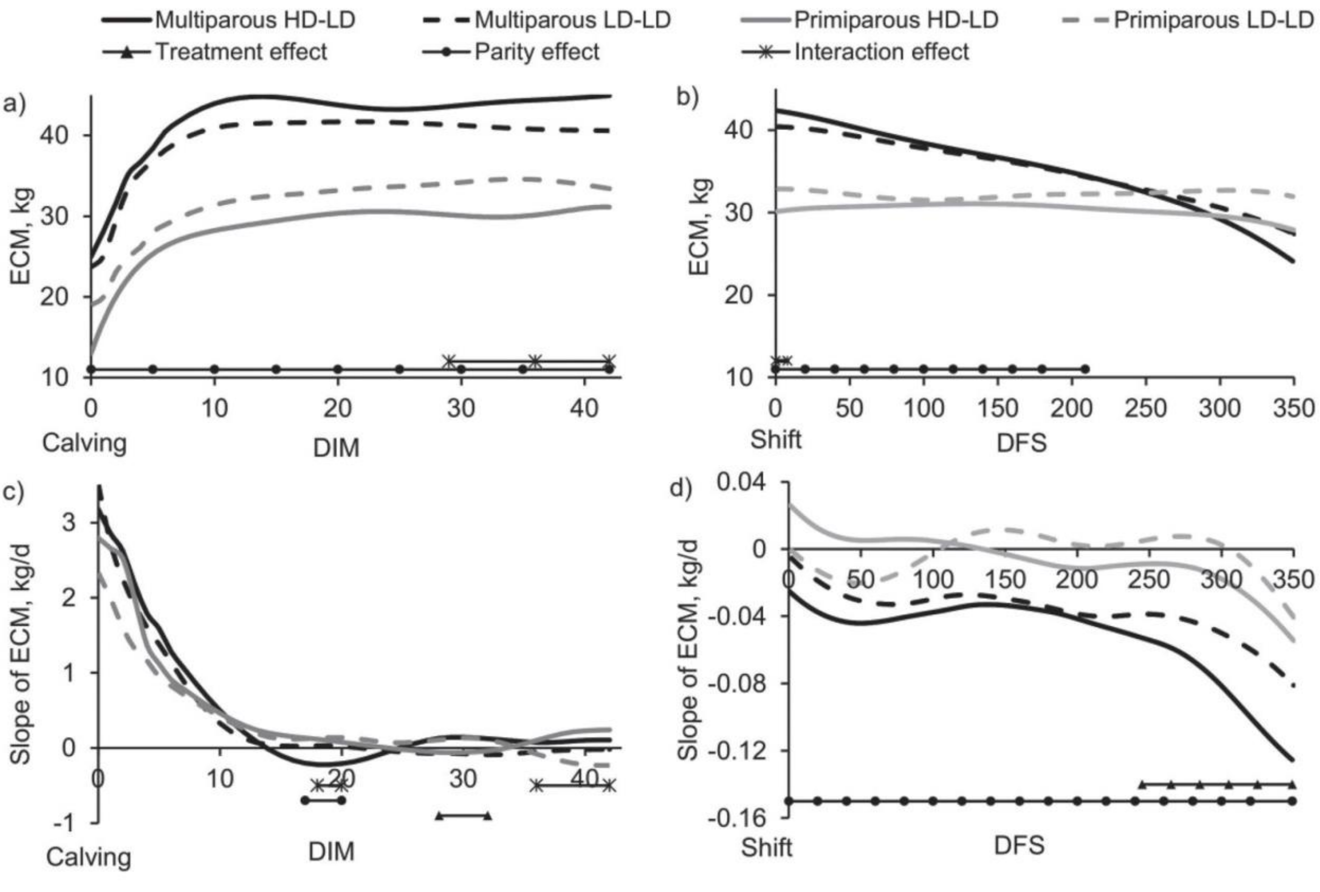

Figure 1. Smoothed ECM (kg/d) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dashed line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

between groups, and it was one of the main interests of the current study. Indeed, the slopes are key indicators to study milk production in dairy cows. For the ECM curve, the slope represents the persistency of the lactation, as it has been defined as the rate of decline in milk yield from the peak yield by several previous studies (Pettersson et al., 2011). For the other variables studied, the slopes represent the rate of decay of an initial difference.

The smoothing with the fda package uses a B-spline basis function and does not require assumptions about the shape of the lactation curve. This last point was an important advantage, as we worked with longer lactations and changes in energy supply. The spline functions of the fda package are constructed by joining polynomials end-to-end at argument values called knots (in our case, the knots are the DIM). These spline functions are smoothing on the integral of the squared deviations from an arbitrary differential operator. The degree of smoothing can be adjusted by applying a penalty on the derivative(s) of the functions. In the present paper, a penalty of $1e^{+6}$ was applied to the second derivatives of polynomials of order 4. With this penalty, the required level of smoothing was achieved and fitted the lactation peak and the following part of the lactation. However, the first week after calving, milk production was overestimated for most cows; therefore, a penalty of $1e^{+2}$ was also used to fit the individual curves and to fit the first weeks of the lactation properly. The smoothed data and associated sloped obtained with the penalty of $1e^{+2}$ were kept to study the period from calving to 42 DIM, whereas the one obtained with the penalty of $1e^{+6}$ were kept to study the postshift period from the day from shift (**DFS**) to late lactation (350 DFS).

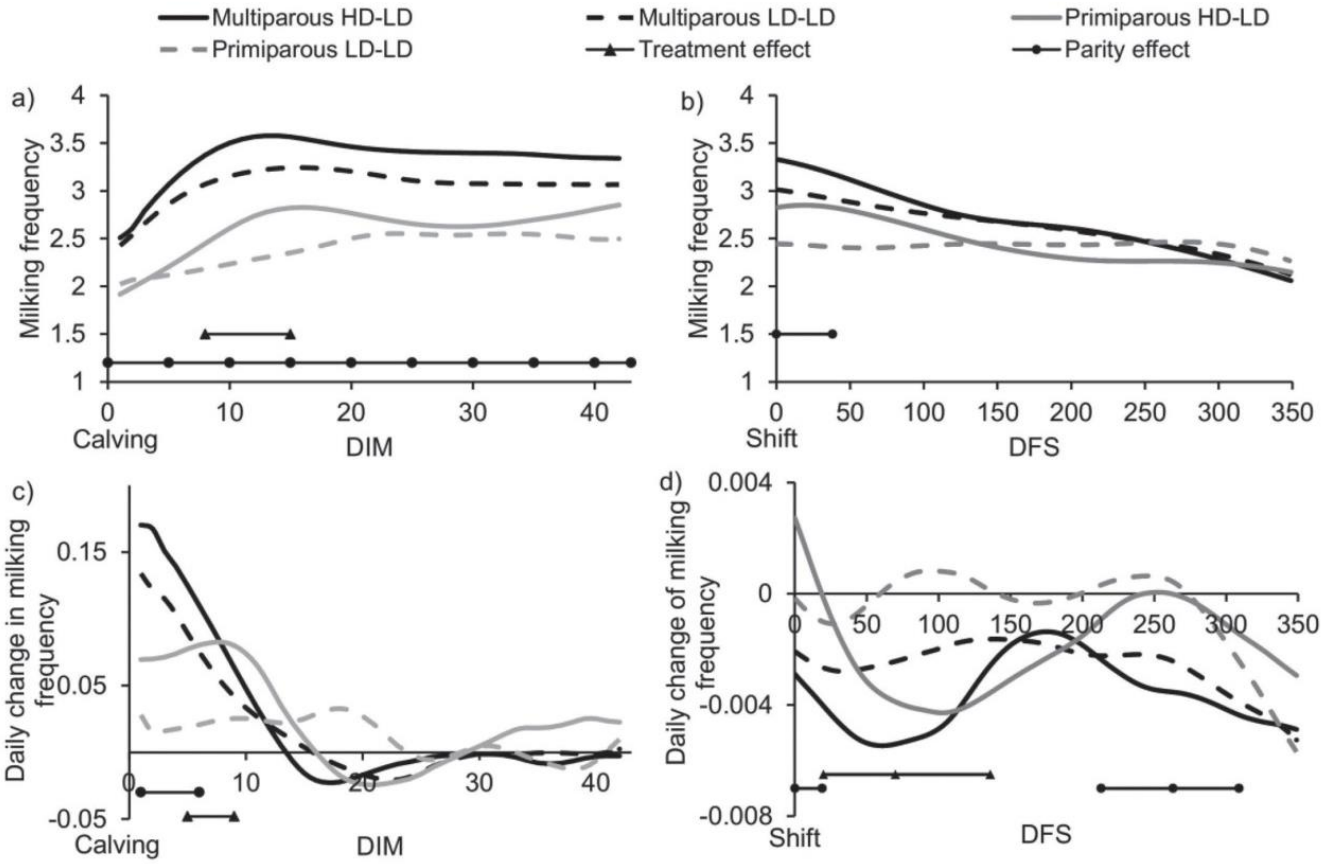

Figure 2. Smoothed milking frequency and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

Statistical Analysis

The effects of treatment and parity on the smoothed daily ECM (kg/d), LW (kg), BCS (point), DMI (kg of DM/d), net energy intake (MJ of ME/d), fat (%), protein (%), lactose (%), SCS, milking frequency, and associated slopes were analyzed with R software version 3.0.0 (R Development Core Team, 2015). The lme function from the nlme package (Pinheiro et al., 2015) was used to fit the linear mixed-effects model (Laird and Ware, 1982) described below:

$$Y_{ijl} = \mu + T_i + P_j + (TP)_{ij} + C_{ijl} + \varepsilon_{ijl},$$

where μ is the overall mean, and the model includes the effects of the ith feeding treatment T (i = LD-LD, HD-LD), the jth parity P (j = primiparous, multiparous), $(TP)_{ij}$ denotes the 2-way interaction, C_{ijl} is the random effect of the lth cow within i treatment and j parity, and ε_{ijl} is the residual error.

The model was applied to each day of the 2 periods studied from 0 to 42 DIM and from 0 to 350 DFS. The effect of the day was not included directly in the model because of the highly correlated nature of smoothed data. The effects of parity and treatment were determined for each day to understand the possible carryover and duration of the HD diet on the production variables during mid and late lactation. Figures 1 to 10 present the results for the 2 periods studied, indicating when the effects of parity, treatment, and interaction were significant. Averages of ECM (kg/d), LW (kg), BCS (point), DMI (kg DM/d), net energy intake (MJ of ME/d), fat (%), protein (%), lactose (%), SCS, and milking frequency were also calculated using raw data, and are given in Table 2 for the 2 periods studied.

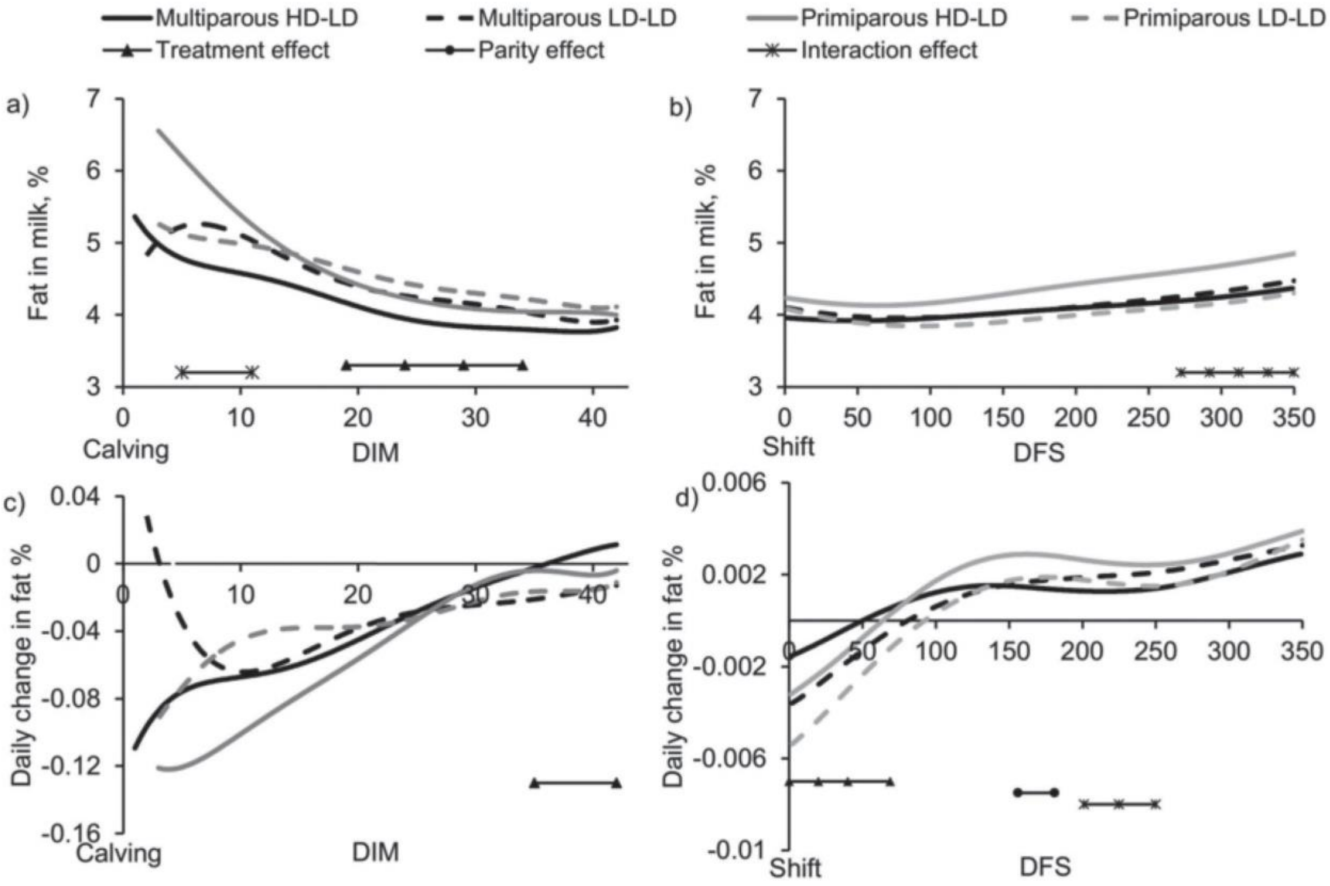

Figure 3. Smoothed fat in milk (%) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 :concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

RESULTS

Lactation Duration and Total Milk Yield

The 53 Holstein cows had an average (±SEM) lactation length of 461 ± 7 d regardless of the feeding treatment ($P = 0.60$) and the parity ($P = 0.55$). Total milk yield was not influenced by treatment ($P = 0.70$), and primiparous cows had a lower total milk yield than multiparous ($P = 0.02$, 13,746 and 15,799 kg, respectively; Table 2).

Before the Diet Shift

Table 2 shows the means of the production variables before the shift in diet from calving to 42 DIM. Figures 1 to 10 present the smoothed data (a) and associated curves (c) for the production variables from calving to 42 DIM.

The DMI was similar for the LD-LD and HD-LD cows ($P = 0.13$) and higher for multiparous cows than primiparous cows ($P < 0.001$). The net energy intake was greater for the HD-LD than the LD-LD cows ($P = 0.002$) and greater for multiparous than primiparous ($P < 0.001$). The EB was less negative for the HD-LD cows than for the LD-LD cows (−12 vs. −22 MJ of NE_L/d respectively, $P = 0.01$). The BCS was higher for the HD-LD than the LD-LD cows ($P = 0.01$), but similar regarding parity. The LW at calving was similar for both treatments ($P = 0.54$), but higher for the HD-LD cows at 42 DIM ($P = 0.05$). The LW was always higher for multiparous compared with primiparous ($P < 0.001$). From 0 to 42 DIM, an interaction between treatment and parity for ECM was noted ($P = 0.02$), showing that the primiparous LD-LD had a higher ECM than the primiparous HD-LD whereas multiparous LD-LD had a lower ECM than multiparous HD-LD. However,

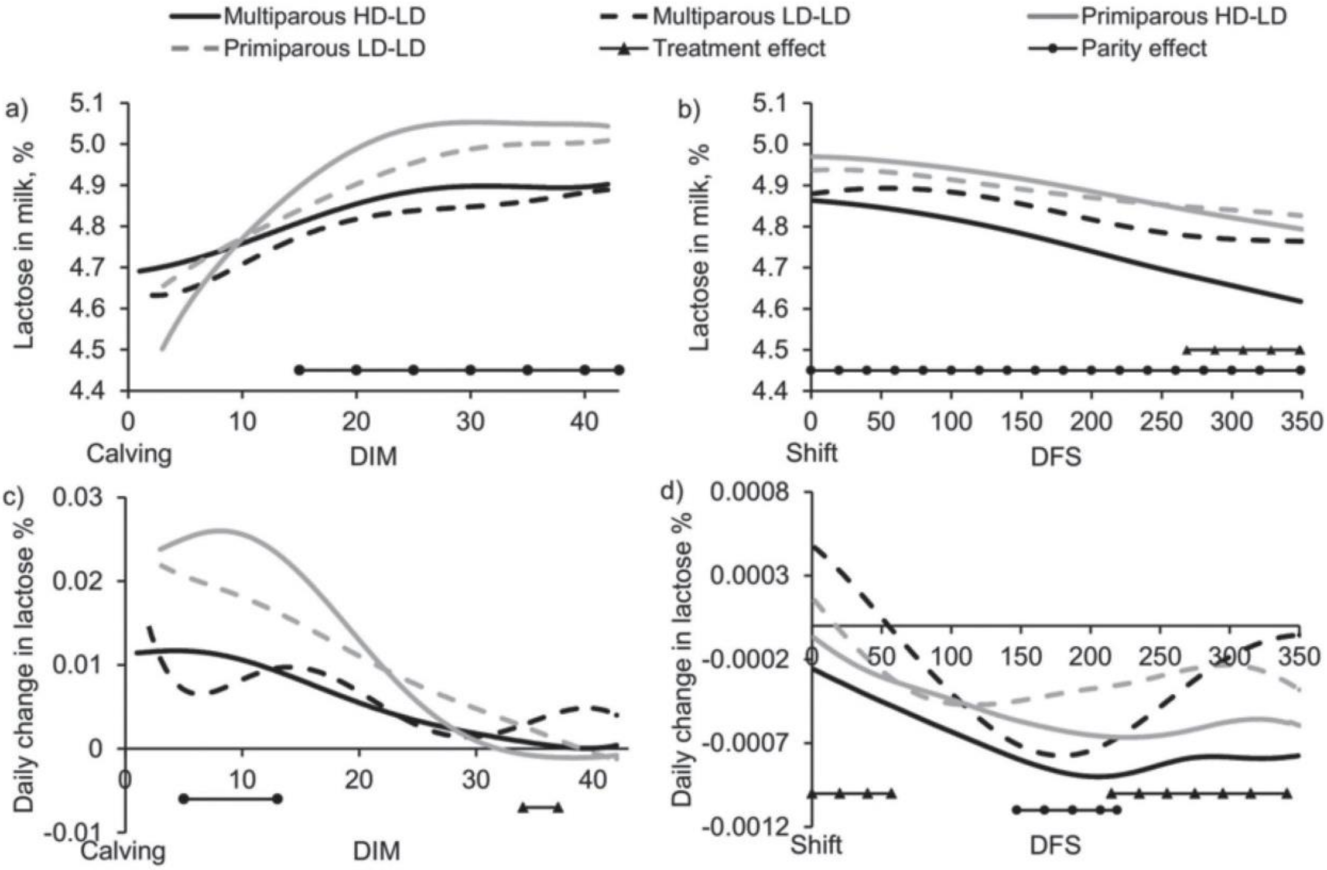

Figure 4. Smoothed lactose in milk (%) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

this interaction was present from the start of the trial, as looking at the milk yield during the first week after calving (from 0 to 7 DIM) showed the same interaction ($P = 0.02$). There was no effect of treatment on the milking frequency ($P = 0.11$). The multiparous cows had more daily milkings than the primiparous (3.2 vs. 2.5 ± 0.1 respectively, $P < 0.001$). For the same period, the milk fat was lower for the HD-LD cows than for the LD-LD cows ($P = 0.02$), and no significant difference between treatments was found for the other milk components. An effect of parity was found only on milk lactose ($P = 0.008$), where primiparous cows had a higher lactose than multiparous cows. Regarding the slopes, the HD-LD cows had a faster increase of DMI from calving to 10 DIM compared with the LD-LD cows. No effect of the feeding treatment was observed on the EB, LW, BCS, ECM, milking frequency, and milk components slopes.

After the Diet Shift

Table 2 shows the means of the production variables after the shift in diet from the shift (0 DFS) to the end of the lactation. During this period, the feeding treatment had no effect on our production variables. The primiparous had a lower daily milk yield, LW, DMI, energy intake, and higher lactose in milk than the multiparous cows. Figures 1 to 10 present the smoothed data (b) and associated curves (d) for the production variables from 0 to 350 DFS.

ECM. From 0 to 200 DFS, the primiparous cows produced less ECM than the multiparous cows (Figure 1). The first 8 d following the shift, an interaction between parity and treatment was noted where the multiparous HD-LD produced more milk than the multiparous LD-LD, whereas the primiparous HD-LD produced less milk than the primiparous LD-LD.

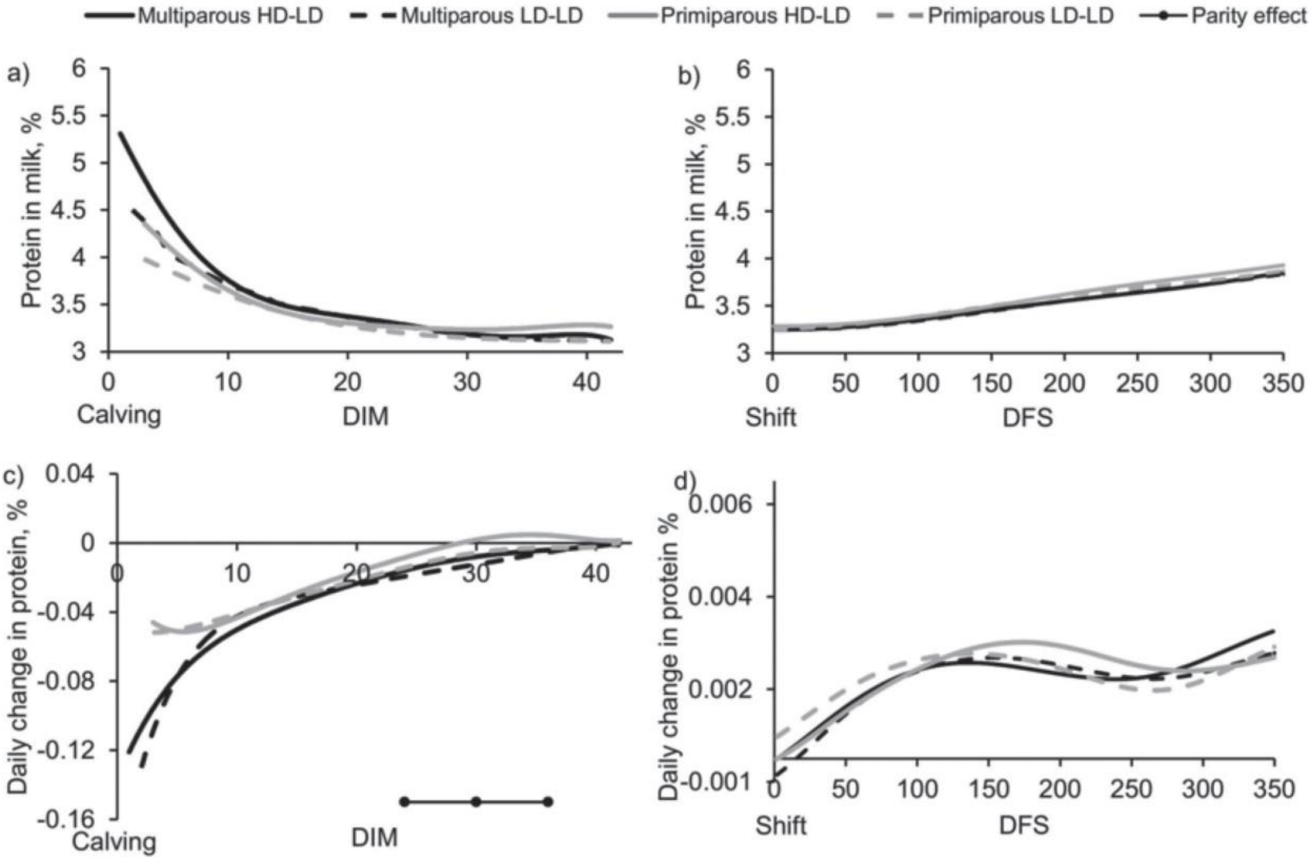

Figure 5. Smoothed protein in milk (%) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

The shapes of the ECM curves differed between parities from 0 to 350 DFS. The primiparous cows had a flat curve with slopes around zero, whereas the multiparous had a decreasing curve and, thus, an increasingly negative slope. Moreover, the treatment had a late effect on the slopes of ECM from 250 to 300 DFS. The ECM of the HD-LD cows decreased faster than the ECM of the LD-LD cows regardless the parity.

Daily Milking Frequency. The treatment had no effect on the milking frequency (Figure 2), but the slopes of the groups were different from 20 to 140 DFS where the milking frequency decreased faster over days for the HD-LD cows than for the LD-LD cows. From 0 to 40 DFS, the primiparous cows had fewer milkings than multiparous cows (2.6 vs. 3.1 ± 0.2 milkings per day, respectively, at 20 DFS, $P = 0.10$). Parity also had an effect on the slopes of the curves of number of milkings from 0 to 20 DFS and from 200 to 300 DFS, the milking frequency decreased faster for the primiparous than for the multiparous cows.

Milk Components. After 270 DFS, the primiparous HD-LD cows had a higher fat in milk than the primiparous LD-LD cows, whereas the multiparous HD-LD cows had lower fat in milk than the multiparous LD-LD cows. Treatment had an effect on the shape of the curves from 0 to 60 DFS, where the fat in milk decreased faster for the LD-LD cows than for the HD-LD cows (Figure 3). From 0 to 350 DFS, the primiparous cows had higher lactose in milk than the multiparous cows. From 240 to 350 DFS, the HD-LD cows had less lactose in milk than the LD-LD cows. Moreover, from 0 to 50 and from 200 to 350 DFS, the lactose in milk of the HD-LD cows decreased faster than that of the LD-LD cows (Figure 4). No effect of treatment or parity was noted on the protein in milk and on the associated slopes (Figure 5). From 270 DFS

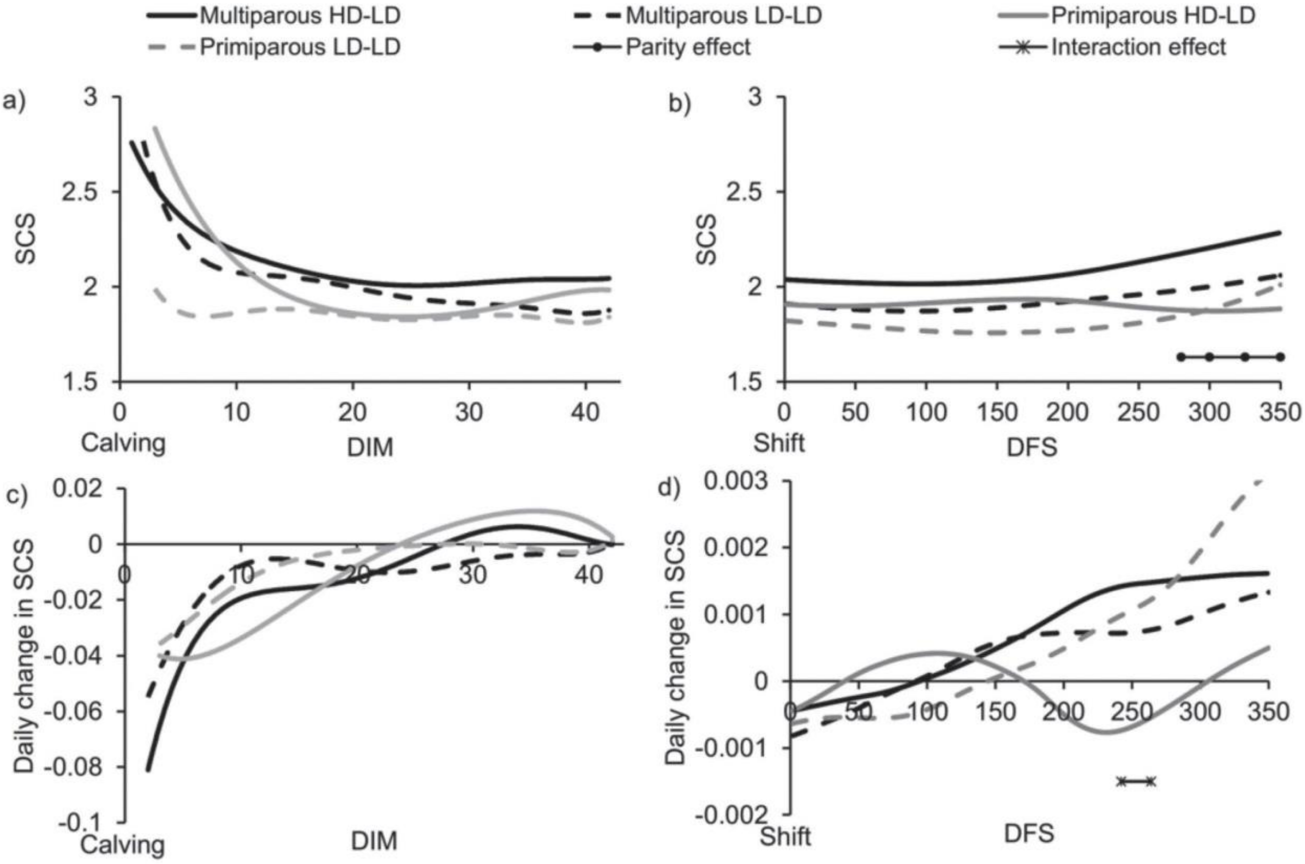

Figure 6. Smoothed SCS and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

to the end of the lactation, the SCS was higher for the multiparous cows compared with the primiparous cows. There was no effect of treatment on the SCS for this period and no differences on the slopes between groups or parities (Figure 6).

BCS and LW. For the whole postshift period, both parities had the same BCS (Figure 7), and the primiparous cows were lighter than multiparous cows regardless of the treatment (Figure 8). The shapes of the LW and BCS curves were similar between the LD-LD and HD-LD cows. From 150 to 350 DFS, the primiparous cows had a slower increase of BCS than the multiparous cows.

Feed and Energy Intake. The DMI of the primiparous cows was lower than the DMI of the multiparous cows. The interaction effect with DMI found for the first 50 DFS indicated that the multiparous HD-LD cows ate more than the multiparous LD-LD cows, whereas the primiparous HD-LD cows ate less than the primiparous LD-LD cows. From 20 DFS to the end of the lactation, only the parity affected the shape of the DMI curves. The results for the net energy intake followed the DMI results (Figure 9).

Energy Balance. From 0 to 350 DFS, the EB increased for both groups (Figure 10); no effect of treatment on EB was observed. From 0 to 50 DFS the EB of the LD-LD cows increased faster than the EB of the HD-LD cows, and from 230 to 300 DFS the EB of the HD-LD cows increased faster than the EB of the LD-LD cows.

Complementary Results

Reproduction. More cows expressed mounting behavior at 8 mo than at 2 mo after calving, the feeding

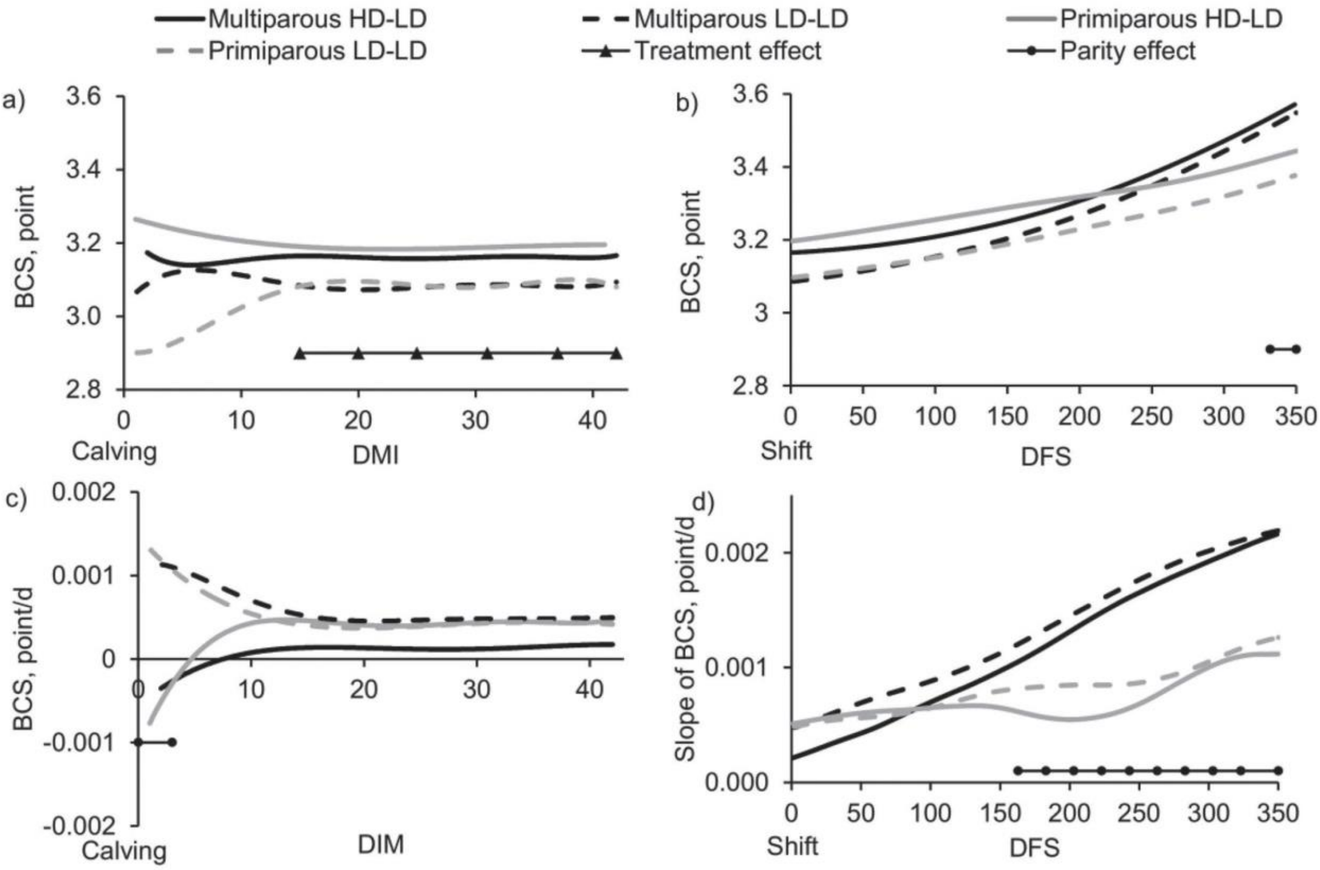

Figure 7. Smoothed BCS (points) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

treatment had no effect on pregnancy rates (40% at first insemination; Gaillard et al., 2016), and the conception rates and number of inseminations were similar for the 16-mo lactation and the previous and following 10-mo lactations (C. Gaillard, J. Sehested, and M. Vestergaard, AU Foulum, Tjele, Denmark, unpublished data).

Duration of the Mobilization Period. After the shift in diet (shift day on average at 50 DIM) and until 36 wk after calving, the plasma nonesterified fatty acids were higher for the HD-LD cows than for the LD-LD cows, indicating that the HD-LD cows sustain the mobilization for a longer period of time than the LD-LD cows (Gaillard et al., 2015).

DISCUSSION

Mobilization Period (From Calving Until Day of Diet Shift)

Group Characteristics. The treatment had no effect on the milk yield or ECM of primiparous cows before the shift in diet. The results indicate that the groups LD-LD and HD-LD of primiparous cows were not well balanced with respect to yield potential. Indeed, the milk production of the LD-LD primiparous was higher than that of the HD-LD primiparous the first days following calving, and the difference was the same at 42 DIM (i.e., around 4 kg milk more per day

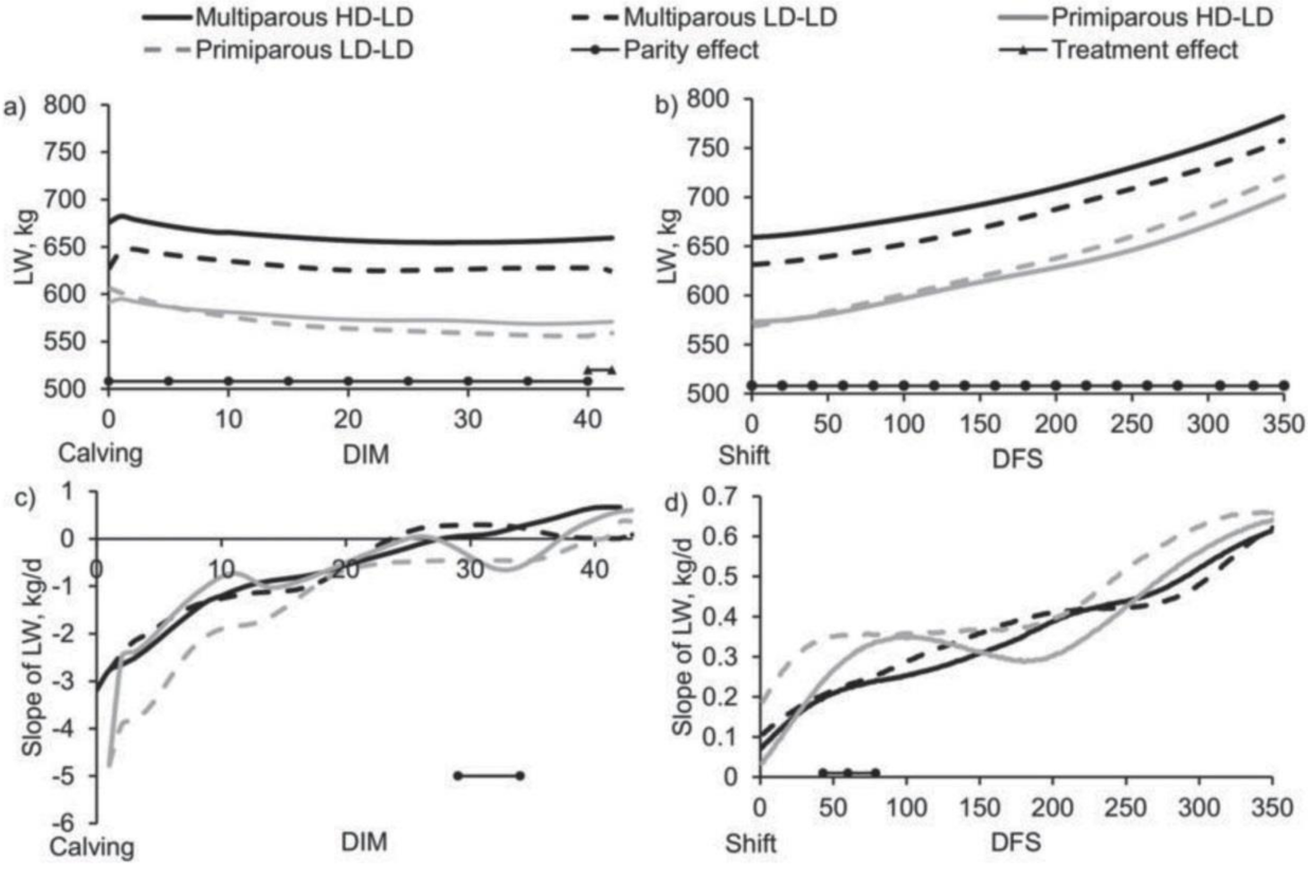

Figure 8. Smoothed live weight (LW; kg/d) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when LW gain ≥0 and at least after 42 DIM.

for LD-LD). However, blocking according genetic potential was not possible due to multiple sires. For the multiparous cows, the groups LD-LD and HD-LD were well balanced regarding milk potential as indicated by similar milk production right after calving. At around 42 DIM, the HD-LD multiparous cows produced around 4 kg milk more per day than the LD-LD multiparous indicating the effect of the feeding treatment.

Use of Extra Feed Energy. Before 42 DIM, the energy intake of the HD-LD cows was higher than that of the LD-LD cows, whereas DMI was unaffected by treatment. For the multiparous cows, this extra energy was used for both milk and body reserves. The HD-LD cows lost less weight during the mobilization period than the LD-LD cows and, consequently, had a higher LW at the end of the period than the LD-LD cows; this is in accordance with previous studies (Andersen et al., 2004; Bossen and Weisbjerg, 2009; Machado et al., 2014). For the primiparous cows, the extra energy was only used for growth and body reserves, but not to increase the milk yield. This is in accordance with Bossen et al. (2009), one of the rare studies presenting result on primiparous cows, where feeding a higher energy density had no significant effect on the primiparous milk yield but a positive effect on the multiparous milk yield.

Carryover Effect of Energy Feeding in Early Lactation

After the mobilization period, all cows received the same diets. The treatment had few small carryover

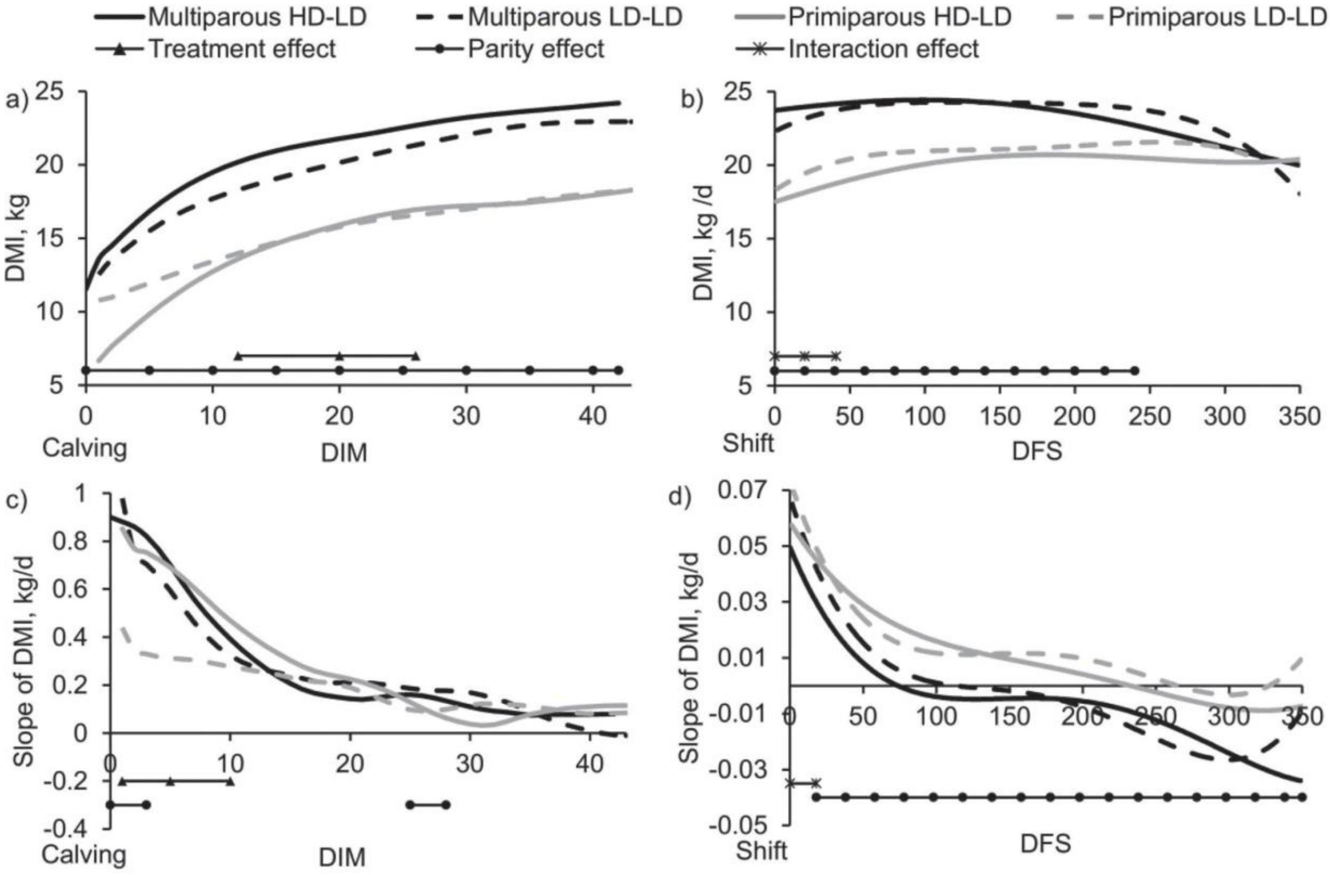

Figure 9. Smoothed DMI (kg/d) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

effects after the shift, whereas the shapes of several curves were different right after the shift and at the end of the lactation.

Short-Term Carryover. During the 50 d following the diet shift, DMI, and consequently energy intake, of HD-LD was higher than LD-LD for multiparous cows. This indicates a short-term carryover effect of energy feeding in early lactation. The higher intake immediately postshift explains the small increase of milk production for the multiparous HD-LD compared with the multiparous LD-LD observed for the 8 first days following the shift. The slopes of fat and lactose also reflect this difference of energy intake between LD-LD and HD-LD for the 50 d following the shift. These short-term carryover effects were expected as part of the adaptation period for cows changing diet. Bossen et al. (2009) also found a reduced milk yield right after the change from an energy enriched diet to a lower-energy diet. They also indicated that this short-term effect was stabilized after 2 wk, as in the present experiment. In general, milk reduction is affected by the stage of the lactation: the earlier the change to a lower-energy diet is introduced, the smaller is the reduction (Bossen et al., 2009). However, it should be noted that this effect was not seen in primiparous cows, which is probably due to their pretrial differences in milk production levels.

Long-Term Carryover. As can be seen from the significant effects of treatment (and treatment interactions) in late lactation, there were long-term carry-over effects of energy feeding in early lactation on milk composition (Figure 2). Postshift lactose in milk for the HD-LD cows was lower and decreased faster than for the LD-LD cows. The fat in milk of the HD-LD cows was lower for the multiparous and higher for the primiparous than for the LD-LD cows. These results are in accordance with Dessauge et al. (2011), who found a

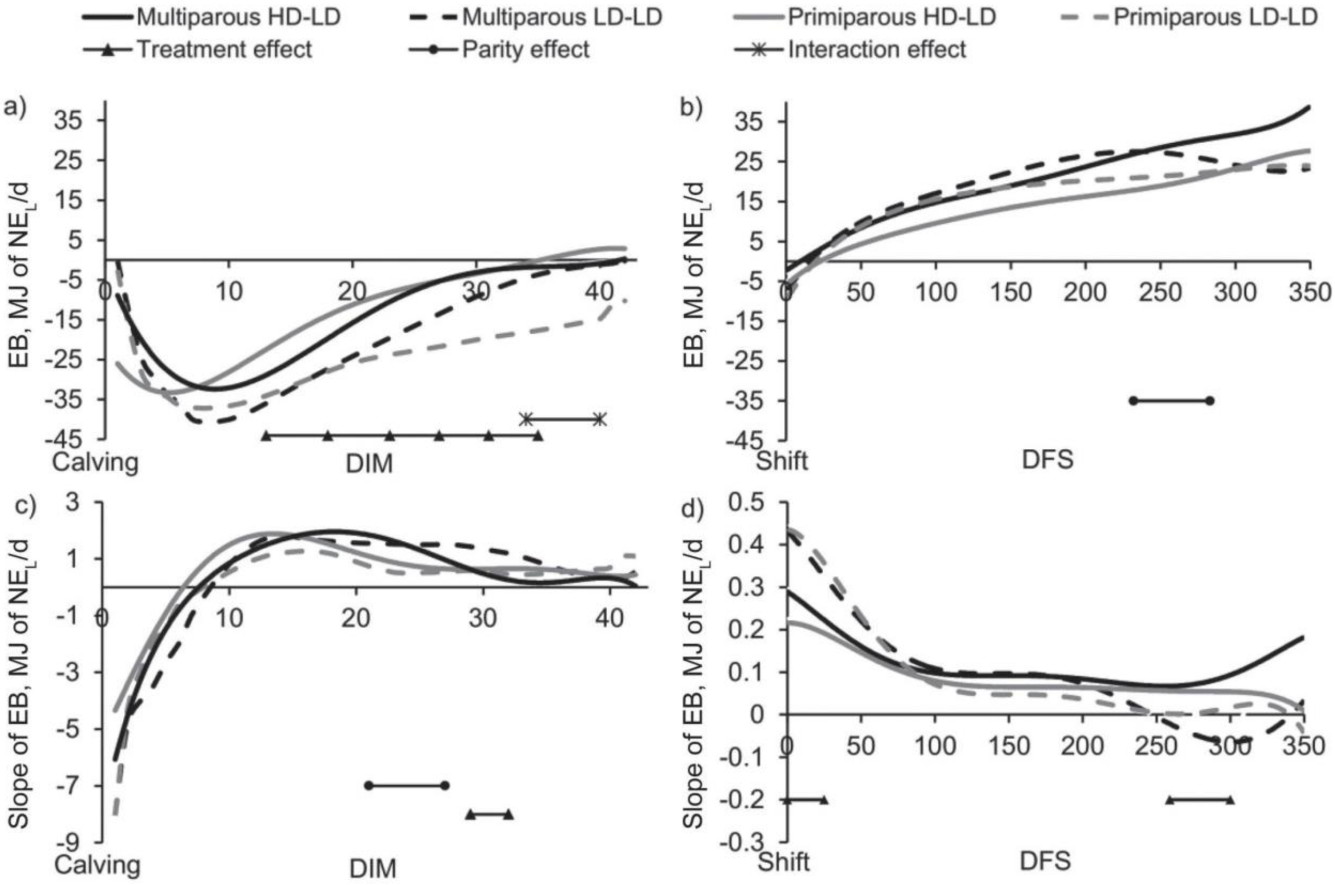

Figure 10. Smoothed energy balance (EB; MJ of NE$_L$/d) and associated slopes from calving to 42 DIM (a and c, respectively), and after the shift back to a common diet for all cows [days from shift (DFS) = 0] until 350 DFS (b and d, respectively), according to treatment (full line HD-LD, dash line LD-LD) and parity (black multiparous, gray primiparous). The days on which the effects of treatment, parity, and their interaction are significant ($P < 0.05$) are indicated by the horizontal bars on the graphs. LD-LD = group with 1 partially mixed ration 60:40 forage:concentrate during the entire lactation. HD-LD = group with 2 partially mixed rations 50:50 followed by 60:40 forage:concentrate when live weight gain ≥0 and at least after 42 DIM.

decline of lactose and fat in milk when the cows where fed restrictively.

At the end of the period, from 250 to 350 DFS, the ECM dropped faster for the HD-LD cows than for the LD-LD cows. This late carryover effect was unexpected given that carryover effects on ECM appear to diminish rapidly during the first 50 d postshift. Days in milk at which pregnancy started were compared between the groups to make sure that this late decrease of persistency was not due to an earlier pregnancy for the HD-LD cows. The results indicated that the time of pregnancy was similar between the groups LD-LD and HD-LD (246 and 240 ± 8.8 DIM, respectively; $P = 0.27$; Table 2). Moreover, at insemination time, cows were fed the same diet and had similar DMI. Thus, the late difference observed on persistency between the LD-LD and HD-LD cows can be considered as a negative long-term carryover of the early treatment.

Feeding an energy-enriched diet in early lactation has been found to increase the mid-lactation milk yield for cows managed for 10 mo of lactation (Bossen et al., 2009); thus, we were expecting a similar long-term effect, including an increase in persistency, after returning to a lower-energy diet. However, studies have also shown that the positive effect of a high-energy diet in early lactation is often counterbalanced by a reduced milk yield persistency when the diet energy is reduced (Everson et al. 1976; Cassel et al., 1984). In the present experiment with extended lactation, no mid-lactation increase in milk production was noted, but there was a decrease in persistency at the end of lactation. This negative long-term effect on persistency might be due to an increase of apoptosis of the epithelial cells of the mammary glands as well as a decrease of cell proliferation, as it has been found that an energy restriction during lactation negatively affects mammary cell proliferation (Dessauge et al., 2011). In this context, our shift to a lower-energy diet can be considered as a restriction in energy that may lead to a decrease in cell proliferation of the mammary gland and, thus, could explain the decrease of persistency observed in late lactation.

CONCLUSIONS

Feeding an energy-enriched diet in early lactation reduced the intensity of the mobilization period independently of parity, as it reduced weight loss. The energy-enriched diet also increased the milk production of multiparous cows. This dietary treatment had a little short-term carryover on the production data when cows returned to the control diet, and had a negative long-term carryover effect on lactation persistency, which was significant from 250 d from diet shift.

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3. Paper 3 – Milk yield and estrus behavior during eight consecutive estruses in Holstein cows fed standardized or high energy diets and grouped according to live weight changes in early lactation

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Milk yield and estrous behavior during eight consecutive estruses in Holstein cows fed standardized or high energy diets and grouped according to live weight changes in early lactation

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ABSTRACT

Cows managed for extended lactation go through several estruses before rebreeding. The aims of this study were (1) to quantify the effect of the first 8 estruses after calving on milk yield, milking frequency, and estrous behavioral activity, and (2) to determine the effects of early lactation live weight gain (LWG) as an indication of energy balance on milk yield, plasma insulin-like growth factor 1 (IGF-1) concentration, estrous behavioral activity, interval from calving to first estrus, between-estrus intervals, and pregnancy risk. Milk yield, live weight, and estrous behavioral activity were measured daily in 62 Holstein cows, 17 primiparous and 45 multiparous, managed for an 18-mo calving interval. Blood plasma obtained at wk 3, 5, 12, and 24 after calving was analyzed for IGF-1. Estrus was detected by use of milk progesterone profiles combined with visual observations (i.e., mounting behavior and other). The cows were divided into 2 groups: the cows having a negative LWG in each of the first 5 wk postpartum and the cows having a positive LWG in at least 1 of the first 5 wk after calving. The results indicate a similar decrease of 0.56 kg of milk per day of estrus during each of the 8 consecutive estruses. The activity level was 17 ± 1 movements per hour higher during the 8 estruses compared with the basic activity level. More cows expressed mounting behavior at estrus 8 than at estrus 2 (63.3 and 45.9%, respectively). The negative LWG cows had lower IGF-1 and higher milk production than the positive LWG cows. Both LWG groups had similar interval from calving to first estrus, on average 55 d. To conclude, the decrease in milk yield during estrus is marginal and similar in consecutive estruses. Moreover, estrous behavior is more highly expressed in the later estruses compared with the earlier estruses. Reproductive parameters (frequency of mounting, pregnancy risk, interval to first estrus, and between-estrus intervals) were not influenced by the live weight change during early lactation.

Key words: extended lactation, estrus, pregnancy risk, energy balance

INTRODUCTION

High-yielding dairy cows have been selected over generations for increased milk yield, but this is associated with a decrease in reproductive performance (Gilmore et al., 2011). This decrease is mainly due to the negative energy balance (**EB**) associated with high yields in early lactation (Walsh et al., 2011) and the relationship between body lipid reserves and the reproductive cycle (Friggens, 2003). With an 18-mo calving interval, as compared with a traditional 12-mo interval, rebreeding is postponed (Sorensen et al., 2008) to take place during a period in which most if not all cows are in a positive EB. Indeed, the EB is negative in early lactation (Lucy, 2001), but it returns to positive starting around 6 wk after calving (Butler et al., 1981; Gilmore et al., 2011); therefore, the first ovulations typically occur while the high-yielding cow is still in negative EB. Postponing rebreeding to later estruses seems to be a good management option in some situations (Sorensen and Knight, 2002); however, it will also result in more consecutive estruses, which might have a negative effect on milk production and will likely increase mounting activity. Increased activity and expression of mounting behavior during estrus might, however, have a positive influence on the ease of estrus detection (Nielsen et al., 2010). Nevertheless, increased activity could also negatively affect short-term milk production (Steensels et al., 2012). It has been shown that the milk production decreases, on average, 2.25 kg during the day of estrus with a traditional 12-mo calving interval (Schofield et al., 1991). A cow is in estrus when it is sexually receptive; usually this occurs every 21 d (range = 18–24 d) and the estrus lasts for some hours (14.1 ± 4.5 h in Kerbrat and Disenhaus, 2004). This

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period was previously detected by observing specific mounting behavior (the cow stands still while mounted by another cow), but these days fewer cows are expressing mounting behavior (Kerbrat and Disenhaus, 2004). Measurements of milk progesterone can be used (Kerbrat and Disenhaus, 2004), associated with visual observations (i.e., chin resting, color and consistency of the mucus in the vulva; Esslemont et al., 1980; Foote, 1975), and measurements of activity (i.e., pedometers, collars; Hurnik et al., 1975) to precisely detect the day of estrus (Kerbrat and Disenhaus, 2004). The type of housing affects the number of cows expressing estrus. Indeed, more cows housed in stanchion barns expressed mounting than the cows housed in freestalls or pasture when driven twice daily to an observation area for 30 min (De Silva et al. 1981), and more mountings were observed on dirt than on concrete floors (Britt et al. 1986). A decline in estrous behavior expression is often associated with a decrease in reproductive performance and an increase in milk production (Spalding et al., 1975). Spicer et al. (1990) showed that cows expressing estrous behavior during the first and second estrous cycle, as detected by blood progesterone levels, had less negative EB than cows that did not express estrous behavior. Finally, IGF-1 has been identified as a mediator of EB-related effects on luteal function as it stimulates progesterone production (McArdle and Holtore, 1989).

We intended to quantify the decrease in milk production due to estrus and the mounting behavior over several consecutive estruses in cows managed for extended lactation (18-mo calving interval) in a loose housing system with milking robots. Thus, the objectives of the present study were to determine (1) the effect of consecutive estruses on milk yield, milking frequency, and estrus-related activity in dairy cows during extended lactation, and (2) the effect of live weight changes in early lactation on estrous behavioral activity, return to estrus, pregnancy risk, and plasma IGF-1 of cows in extended lactation.

MATERIALS AND METHODS

Animals, Housing, and Feeding Strategies

The experiment was carried out at the Danish Cattle Research Centre at Aarhus University, Foulum. It involved 62 Holstein cows, 17 primiparous and 45 multiparous, managed for an 18-mo calving interval. The number of cows was optimized for the measurement of production parameters of this feeding trial. All the cows were housed in one group pen on slatted floor with freestalls equipped with mattresses and sawdust bedding. The cows had access to water and an automatic milking system (**AMS**; DeLaval AB, Tumba, Sweden) and were voluntarily milked (mean ± SD) 2.77 ± 0.18 times per day during the period studied.

The cows were fed according to 1 of 2 strategies using 2 partially mixed rations, one with a high energy density (**HD**; 7.81 MJ of NE_L/kg of DM) and 50:50 forage-to-concentrate ratio; and one with a lower standardized energy density (**LD**; control diet, 7.49 MJ of NE_L/kg of DM) and 60:40 forage-to-concentrate ratio, but still sufficient to meet the daily energy demands of a high-yielding lactating cow. Half of the cows were fed ad libitum the LD diet during the entire lactation (control strategy **LD-LD**, with 9 primiparous and 22 multiparous cows). The other half of the cows was fed the HD diet until they reached at least 42 DIM and a live weight gain (**LWG**) ≥ 0 kg/d based on a 5-d live weight (**LW**) average, and were then shifted to the LD diet (strategy **HD-LD**, with 8 primiparous and 23 multiparous cows). Insemination was initiated at first estrus after 220 DIM (e.g., at estrus 7.8 ± 1.4 d postpartum). The herd-average milk production for a 305-d lactation period was around 11,000 kg in 2014.

Measurements and Calculations

Estrus Detection, Behavior, and Determination of the Day of Estrus. Estruses were detected and recorded for each cow until estrus number 8. The day of estrus was defined based on milk progesterone profiles in combination with the visual observations. Progesterone (ng/mL) was automatically measured in individual cow milk, based on an immunoassay in the Herd Navigator (Lattec I/S, Hillerød, Denmark) system connected to the AMS. Visual observations [mounting or being mounted (i.e., estrous behavior), vulva swollen red, mucus discharge (i.e., viscous, clear elastic strands of mucus hanging from the vulva), presence of blood in the genital area] were recorded by the farm personnel (2 experienced persons) daily for the cows at 21 ± 3 d after the latest estrus and for those having high activity. The activity of the cows was recorded by a collar activity meter system (DelPro, DeLaval, Tumba, Sweden) mounted around the neck of each cow. The collar recorded the activity of each animal continuously and it was then expressed as number of movements per hour. The activity at the day of estrus was compared with the activity at d 5 relative to the day of estrus. To determine the day of estrus, the following procedure was followed. First, the period in which an estrus could occur was defined by a low level of progesterone (<3 ng/mL; Lamming and Bulman, 1976). Within this period, the day of estrus was defined based on the visual observations in the following anticipated order of importance: (1) the day where the cow is mounting another cow or being mounted itself. If these observa-

Table 1. Visual criteria for definition of day of estrus

Visual observation	Defined day of estrus relative to day of observation
Mounting, mounted	Day of observation
Vulva swollen red, mucus discharge	Day of observation
Presence of blood in the genital area	Day 2 before the day of observation

tions were not recorded during the estrus period, but (2) "swollen red, mucus discharge" was recorded, then this day was defined as the day of estrus. Finally, if neither (1) or (2) were recorded but (3) the presence of blood in the genital area was observed, then the day of estrus was defined as 2 d before this blood was observed (Table 1). One of these 3 criteria were recorded for each estrus period.

Insemination Recordings and Pregnancy Risk. The result of insemination (pregnancy or not) and the number of inseminations per pregnancy were recorded throughout the lactation. Pregnancy was detected by high level of progesterone for at least 30 d after insemination, and confirmed by rectal palpation. The average number of inseminations per pregnancy and average pregnancy risk after first and second inseminations were calculated.

LW and LWG Calculation. Live weight was recorded (Danvaegt, Hinnerup, Denmark) at each milking in the AMS. Weight recordings (10 times per second) were cleaned up, following the procedure described in Bossen and Weisbjerg (2009), and a daily average was calculated. To attenuate daily variations, the Wilmink model (Wilmink, 1987; equation [1]) was used to fit the daily individual LW measurements:

$$\text{LW} = \text{a} + \text{b} \times \text{DIM} + \text{c} \times \exp(-0.05 \times \text{DIM}), \quad [1]$$

where LW is in kilograms; the factor −0.05 is the average time from calving at LW nadir (50 d); a is the level of LW; b is the increase of LW after LW nadir; and c is the decrease of LW toward the LW nadir. The parameters a, b, and c were calculated for each cow by linear regression (lm function in R). The equation for LWG [2] was the derivative of the LW equation for individual cows at each 7 DIM, representing the average weekly LWG:

$$\text{LWG} = \text{b} - 0.05 \times \text{c} \times \exp(-0.05 \times \text{DIM}). \quad [2]$$

Average weekly LWG was then used as an indicator of energy balance. Based on their weekly LWG, cows were divided into 2 groups. One group comprised the cows having a negative LWG in each week during the first 5 wk after calving (**NLWG**) and the other group comprised the cows having a positive LWG in at least 1 of the first 5 wk after calving (**PLWG**). The characteristics and mean LWG of these groups, as well as the distribution of PLWG and NLWG cows on the feeding strategies, are presented in Table 2.

Milk Yield and Milking Frequency. Milk yield was recorded by the AMS at each milking to calculate a daily yield for each cow. The data set used in the present study contains the daily yield (kg/d) for the day of estrus and baseline yield, defined as the average daily yield of d −6, −5, and −4 relative to the day of estrus, expressing milk yield in the nonestrus period. Average of the daily milk yield was also calculated for wk 3, 5, 12, and 24. Milking frequency was based on individual cow visits to the AMS per day. The cows had free access to the AMS, but if the last visit occurred within 5 h the cows were denied access. If the cow spent 15 h not visiting the AMS, it was manually picked up and lead to the AMS.

Blood Samples. Blood was sampled during wk 3, 5, 12, and 24 after calving from the tail vessel on Thurs-

Table 2. Characteristics of the live weight gain (LWG) groups[1]

Item[2]	NLWG	PLWG
Total of cows	29	33
Number of primiparous	8	9
Number of HD-LD cows	13	18
Number of LD-LD cows	16	15
Average LWG when wk ≤5, kg/d ± SEM	−0.66 ± 0.07	0.15 ± 0.07
Average LWG when wk >5, kg/d ± SEM	0.28 ± 0.03	0.33 ± 0.03

[1] PLWG = cows having a positive LWG in 1 of the first 5 wk after calving; NLWG = cows having a negative LWG in each of the first 5 wk after calving.

[2] HD-LD = cows fed 2 partially mixed rations, a 50:50 forage-to-concentrate diet during the mobilization period and the control diet after (60:40 forage-to-concentrate); LD-LD = cows fed the same partially mixed ration, a 60:40 forage-to-concentrate diet during all the lactation.

day mornings between 1000 and 1200 h. Plasma was harvested and kept frozen at −20°C until analyzed for IGF-1 using a noncompetitive time-resolved immunofluorometric assay of the sandwich type, as described by Frystyk et al. (1995).

Statistical Analysis

Linear Mixed-Effects Models. Three different linear mixed-effects models were used as 3 different time frames (DIM, days relative to the day of estrus, or weekly average at wk 3, 5, 12, and 24) were studied. Model [1] tests the effects of parity, estrus number, feeding strategy, and event (estrus, nonestrus) on daily milk yield, daily activity, or daily milking frequency. Model [2] tests the effect of days from estrus, parity, and feeding strategy on milking frequency and daily milk yield around estrus. Model [3] tests the effects of parity, feeding strategy, and LWG group on daily milk yield, IGF-1, LWG, interval from calving to first estrus, intervals between estruses, DIM at first insemination, and number of estruses at first insemination.

$$\text{Model [1]: } Y_{ijklm} = \mu + E_i \times P_j \times S_k \times N_m + C_{jkl} + \varepsilon_{ijklm},$$

$$\text{model [2]: } Y_{ijkl} = \mu + D_i \times P_j \times S_k + C_{jkl} + \varepsilon_{ijkl}, \text{ and}$$

$$\text{model [3]: } Y_{ijkl} = \mu + G_i \times P_j \times S_k + C_{jkl} + \varepsilon_{ijkl}.$$

In model [1], Y is the variable studied (daily milk yield, daily activity or daily milking frequency), μ is the overall mean, E is the effects of the ith estrus event (estrus, nonestrus), P is the effect of the jth parity (primiparous, multiparous), S is the effect of the kth feeding strategy (LD-LD or HD-LD), N is the effect of the mth estrus number ($m = 1$ to 8), with the respective 2- and 3-factor interactions, C_{jkl} is the random effect of the lth cow within j parity and k strategy, and ε_{ijklm} is the random residual error. The feeding strategy, and its interactions, was excluded from the final model after it was tested not to have effect on the variables analyzed.

In model [2], Y is the variable studied (daily milking frequency or daily milk yield), μ is the overall mean, D is the effects of the ith days from estrus (−6 to 6), P is the effect of the jth parity (primiparous, multiparous), S is the effect of the kth feeding strategy (LD-LD or HD-LD), with the respective 2-factor interactions, C_{jkl} is the random effect of the lth cow within j parity from estrus and k strategy, and ε_{ijkl} is the random residual error. The feeding strategy, and its interactions, was excluded from the final model after it was tested not to have effect on the variables analyzed.

In model [3], Y is the variable studied (average daily milk yield at wk 3, 6, 12, and 24; IGF-1, LWG, interval from calving to first estrus, intervals between estruses, DIM at first insemination, and number of estruses at first insemination), μ is the overall mean, G is the effects of the ith LWG group (NLWG or PLWG), P is the effect of the jth parity (primiparous, multiparous), S is the effect of the kth feeding strategy (LD-LD or HD-LD), with the respective 2- and 3-factor interactions, C_{jkl} is the random effect of the lth cow within j parity and k feeding strategy, and ε_{ijkl} is the random residual error. For the IGF-1 analysis, the week factor was added to the model as well as its 2- and 3-factor interactions.

With R version 3.0.0 (R Development Core Team, 2014), the lme function from the nlme package (Pinheiro et al., 2015) was used to fit the models. This generic function fits a linear mixed-effects model in the formulation described by Laird and Ware (1982). The correlation between repeated measurements within each cow was calculated with the corAR1 function, a temporal autocorrelation structure of order 1 that uses the order of the observations in the data set as covariate (Pinheiro and Bates, 2000).

Chi-Square Tests and Pearson Correlations. The effects of LWG group, feeding strategy, parity, and estrus number on the number of cows exhibiting estrus (mounting or being mounted), pregnancy risks, and number of inseminations per pregnancy were tested using a chi-square test on R software (chisq.test function; Agresti, 2007). In addition, the chi-square test was used to compare the length of intervals between estrus 1 to 2 and estrus 7 to 8. To do so, the length of intervals was categorized as regular (18–26 d) or irregular (31–57 d). Correlations between daily milk yield and milking frequency, between LWG and plasma IGF-1, and between estrus number and number of cows expressing mounting behavior were quantified by a simple Pearson correlation with R software (cor.test function; Hollander and Wolfe, 1973).

RESULTS

Effect of Estrus on Milk Yield and Milking Frequency

The feeding strategy was found not to have an effect on the daily milk yield ($P = 0.92$) and milking frequency ($P = 0.55$). Daily milk yield was 0.56 ± 0.19 kg lower ($P < 0.001$) on the day of estrus compared with the average of d −4, −5, and −6 relative to estrus (Figure 1). This difference in milk yield was not affected by parity ($P = 0.51$) or estrus number ($P = 0.25$). The milking frequency was positively correlated with the daily milk yield ($r = 0.49$, $P < 0.001$). Even so,

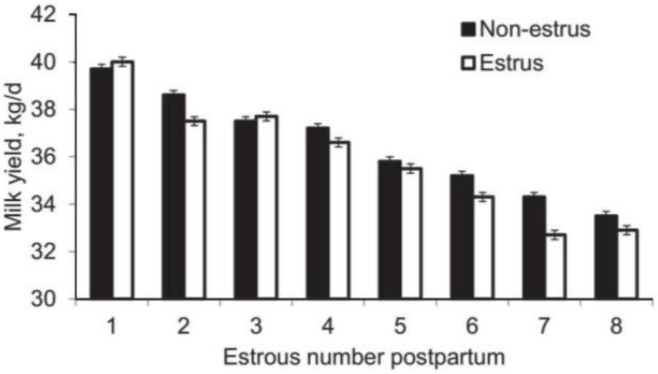

Figure 1. Milk yield (kg/d; mean ± SEM) during estrus and nonestrus (-6 to -4 d relative to estrus).

no difference in milking frequency was noted between the day of estrus and the d -6, -5, and -4 relative to estrus ($P = 0.23$, average 2.7 ± 0.09 milking per day). An increase in milking frequency was observed at d -3, -2, and -1 relative to estrus ($P < 0.001$), as well as an increase in milk yield at d -2 relative to estrus compared with d -3 relative to estrus (36.8 vs 36.1 ± 0.02 kg, $P = 0.03$; Figure 2).

Variation in Daily Activity During Estrus

The feeding strategy was tested not to have effect on the daily activity ($P = 0.31$). Activity was generally higher during the 3 first estruses and then decreased until estrus 8 ($P < 0.001$; Figure 3). Activity was, on average, 17 ± 1 movements per hour higher during estrus compared with 5 d before estrus ($P < 0.001$). There was an interaction between parity and estrus number, indicating that during nonestrus the multiparous cows were more active than the primiparous cows from estrus number 2 to 6, whereas during estrus the primiparous were more active than the multiparous in estruses 1, 2, 3, and 6 ($P < 0.001$) and less active than the multiparous in estruses 5 and 7 ($P < 0.001$). A positive correlation was noted between estrus number and the number of cows expressing mounting behavior (r = 0.79, $P = 0.02$) (Figure 4). More cows tended to express mounting behavior at estrus 8 than 2 (63.3 vs. 45. 9%, $P = 0.06$).

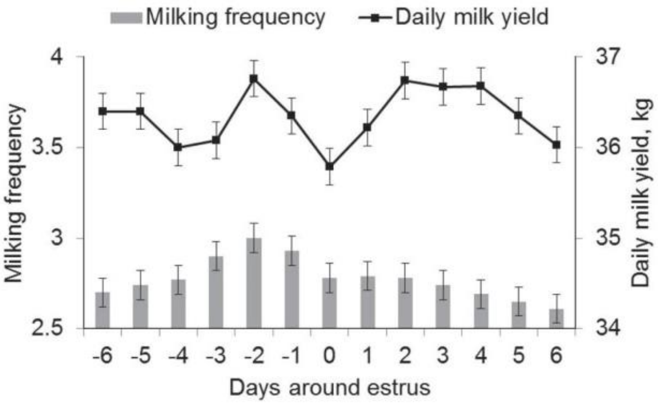

Figure 2. Daily milk yield (kg/d; mean ± SEM) and daily milking frequency around estrus (d = 0).

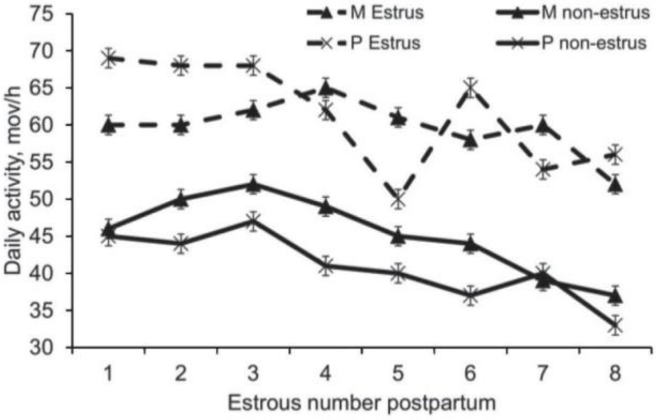

Figure 3. Activity [number of movements per hour (mov/h); mean ± SEM] during estrus and nonestrus for multiparous (M) and primiparous (P) cows.

Relationships Between LWG Grouping and IGF-1, Milk Yield, Estrous Behavior, and Estrus Intervals

LWG Groups. During the first 5 wk after parturition LWG was -0.66 ± 0.07 kg/d for the NLWG group and 0.15 ± 0.07 kg/d for the PLWG group ($P < 0.001$). During the period from 6 to 24 wk after parturition, the LWG was 0.28 ± 0.03 kg/d for NLWG and 0.33 ± 0.03 kg/d for PLWG ($P = 0.22$; Figure 5). Parity and feeding strategy had no effect on the LWG ($P = 0.78$

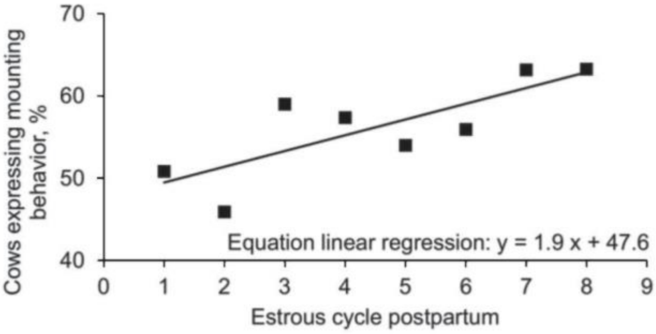

Figure 4. Percentage of cows exhibiting estrous behavior (mounting, being mounted) at each of the 8 estruses postpartum, and regression line from the correlation analysis.

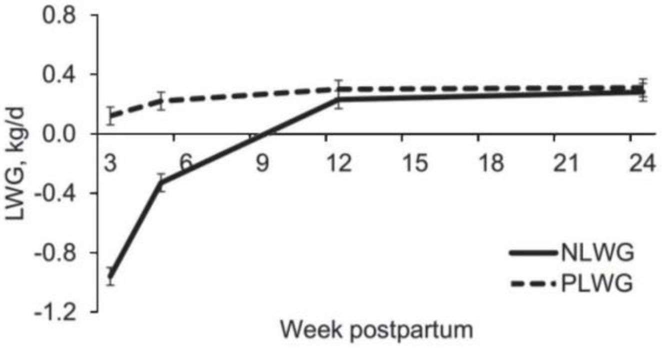

Figure 5. Live weight gain (LWG, kg/d; mean ± SEM) for each group (NLWG and PLWG) in wk 3, 5, 12, and 24 postpartum. PLWG = cows having a positive LWG in 1 of the first 5 wk after calving; NLWG = cows having a negative LWG in each of the first 5 wk after calving.

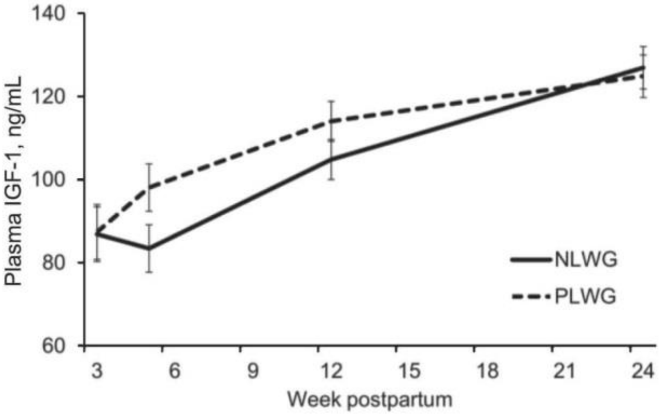

Figure 6. Plasma IGF-1 concentration (ng/mL; mean ± SEM) for each live weight gain (LWG) group (NLWG and PLWG) in wk 3, 5, 12, and 24 postpartum. PLWG = cows having a positive LWG in 1 of the first 5 wk after calving; NLWG = cows having a negative LWG in each of the first 5 wk after calving.

and P = 0.98, respectively). There was no effect of LWG groups or feeding strategy on the LW at calving, indicating that the groups were homogenous in LW at calving. The NLWG cows lost more weight than the PLWG cows from calving to nadir (day of shift of diet for the HD-LD cows; $P = 0.005$). Moreover, from calving to first insemination, the NLWG cows lost weight whereas the PLWG gained weight ($P < 0.001$). There was no effect of feeding strategy on LWG from calving to nadir or from calving to insemination (Table 3).

IGF-1 Concentration and LWG. Plasma IGF-1 concentrations were different between the LWG groups at 5 wk postpartum, where the PLWG cows had higher levels of IGF-1 than the NLWG cows (98 vs. 83 ± 5.6 ng/mL; $P = 0.05$). Plasma IGF-1 increased for both groups from 3 to 24 wk postpartum (Figure 6). The IGF-1 was higher for primiparous cows than for multiparous cows ($P < 0.001$), and was higher for the (HD-LD) cows than for the (LD-LD) cows at 3 wk ($P = 0.03$). In addition, weekly plasma IGF-1 was positively correlated with the LWG (r = 0.35, $P < 0.001$).

Milk Yield and LWG. Average daily milk yield across the 4 time points studied (wk 3, 5, 12, and 24) was consistently higher for the NLWG cows than for the PLWG cows (on average 37.5 and 34.7 ± 0.9 kg, respectively; $P = 0.03$). There was no interaction between milk yield at the 4 time points and the LWG groups.

Estrus Intervals and LWG. Interval from calving to first estrus was similar for PLWG and NLWG cows, but higher for the primiparous compared with the multiparous and higher for the HD-LD cows compared with the LD-LD cows (Table 4). The interval

Table 3. Effects of feeding strategy and live weight gain (LWG) group on LWG, daily milk yield, DIM at first insemination, number of estrus at first insemination, number of inseminations per pregnancy, and pregnancy risks

	LWG group[1]				Feeding strategy[2]			
Item	NLWG	PLWG	SEM	*P*-value	LD-LD	HD-LD	SEM	*P*-value
Number of cows	29	33	—	—	31	31	—	—
Daily milk yield, kg/d	37.5	34.7	0.9	0.03	36.1	36.1	0.9	0.85
Live weight at calving, kg	666	639	13	0.13	647	656	13	0.62
LWG from calving to nadir[3]	−34	−15	5	0.005	−25	−18	5	0.25
LWG from calving to first insemination	−9	31	9	<0.001	11	15	9	0.74
DIM at first insemination	227	224	3	0.46	226	221	3	0.27
Estrus number at first insemination	8.1	7.9	0.2	0.55	8.4	7.4	0.2	0.003
Number of inseminations per pregnancy	2.59	2.48	0.25	0.61	2.35	2.71	0.25	0.21
Pregnancy risk, first insemination, %	34.5	33.3	—	0.92	45.2	22.6	—	0.06
Pregnancy risk, second insemination, %	50.0	54.5	—	0.77	68.7	41.7	—	0.09

[1] PLWG = cows having a positive LWG in 1 of the first 5 wk after calving; NLWG: cows having a negative LWG in each of the first 5 wk after calving.

[2] HD-LD = cows fed 2 partially mixed rations, a 50:50 forage-to-concentrate diet during the mobilization period and the control diet after (60:40 forage-to-concentrate); LD-LD = cows fed the same partially mixed ration, a 60:40 forage-to-concentrate diet during all the lactation.

[3] Nadir: day of shift of diet for the HD-LD cows.

from calving to first estrus was higher than the following intervals ($P < 0.001$), and the interval from first to second estrus was higher than the interval between estruses 4 and 5, as well as for the following intervals ($P < 0.05$). There was no effect of LWG groups, parity, or feeding strategy on the interval from first to second estrus, this interval was, on average, 26.4 ± 1.0 d for all the cows. Similarly, no effect of these factors was noted on the following intervals, which, on average, were 24.6 ± 0.4 d. On average, for the interval between estrus 1 and 2, 85.5% of the cows had an interval between 18 and 26 d, and 14.5% between 31 and 57 d. For the interval between estrus 7 and 8, 96% of the cows had an interval between 18 and 26 d, and 4% between 31 and 43 d. The interval between estrus 7 and 8 tended to be more regular (between 18 and 26 d) than the interval between estrus 1 and 2 ($P = 0.06$).

Reproduction and LWG. The frequency of mounting behavior was similar for both LWG groups ($P = 0.71$). The feeding strategy, parity, and LWG group had no effect on the total number of inseminations per pregnancy (average 2.53 ± 0.25 inseminations per cow). The pregnancy risk was not affected by parity group. Pregnancy risk was also similar for both LWG groups (Table 3). The pregnancy risk tended to be higher for the LD-LD cows than for the HD-LD cows (45.2 and 22.6% or 14 and 7 cows, respectively, for the first insemination, $P = 0.06$; and 68.7 and 41.7% or 12 and 10 cows, respectively, for the second insemination, $P = 0.09$). The day of first insemination was similar for LWG groups (225.5 ± 2.6 d) and for the 2 feeding strategies (223.4 ± 3.0 d). The estrus number at first insemination was similar for the LWG groups but was higher for the LD-LD cows compared with the HD-LD cows (8.4 and 7.4 ± 0.2, respectively; $P = 0.003$).

DISCUSSION

Effect of Delayed Rebreeding on Production

After calving, the EB of the cow is often negative (Butler et al., 1981) and returns to positive approximately 6 wk after calving (Gilmore et al., 2011). The several estruses occurring during an 18-mo calving interval take place in cows of different metabolic conditions, so the reduction in milk yield due to an estrus could be expected to be different at estrus 1 compared with estrus 8. Our results indicate that the first 8 estruses after calving decrease milk yield to the same extent on the day of estrus (average loss of 0.56 ± 0.19 kg of milk per estrus). No other comparable results were found in the literature, as few studies have been conducted on this topic with cows on extended lactation; however, Schofield et al. (1991) found a similar decrease in milk

Table 4. Mean interval (d ± SEM) between consecutive estruses of the live weight gain (LWG), parity, and feeding strategy groups

	LWG group[1]				Parity				Feeding strategy[2]			
Intervals between estrus, d	NLWG	PLWG	SEM	*P*-value	Multiparous	Primiparous	SEM	*P*-value	LD-LD	HD-LD	SEM	*P*-value
Calving to estrus 1	52.5	57.4	4.2	0.41	51.4	64.6	5.4	0.04	49.5	60.9	3.9	0.05
Estrus 1 to 2	26.8	26.1	1.5	0.74	26.1	27.3	1.2	0.62	26.6	26.3	1.5	0.88
Average estrus [2, 8]	24.9	24.8	0.8	0.93	25.3	23.7	0.7	0.22	23.8	25.8	0.8	0.13
Estrus 7 to 8	23.9	23.2	0.7	0.46	23.8	22.8	0.6	0.38	23.1	24.1	0.8	0.33

[1] PLWG = cows having a positive LWG in 1 of the first 5 wk after calving; NLWG = cows having a negative LWG in each of the first 5 wk after calving.

[2] HD-LD = cows fed 2 partially mixed rations, a 50:50 forage-to-concentrate diet during the mobilization period and the control diet after (60:40 forage-to-concentrate); LD-LD = cows fed the same partially mixed ration, a 60:40 forage-to-concentrate diet during all the lactation.

yield at first and second estrus for cows managed for a 12-mo calving interval. In our study, the decrease in milk yield at estrus was not due to a decrease in milking frequency. The loss of milk might be due to the fact that, during estrus, cows spend more time on activity and expressing estrus and probably also less time eating, leading to a reduction in the energy available for milk production (Hurnik et al., 1975; Kerbrat and Disenhaus, 2004). Moreover, no difference in milking frequency was observed between the day of estrus and the days preceding or following it. It has not been possible to find other studies that described variations of milking frequency around estrus with AMS and in an extended lactation setting.

Effect of Estrus on Activity and Estrous Behavior

The increase in activity during estrus is in accordance with previous studies on cows managed for a 12-mo calving interval, where the activity of the cows as well as the restlessness increased during estrus (Farris, 1954; Van Eerdenburg et al., 1996). In a more recent study, an algorithm using pedometer data was developed to identify the changes in behavior to detect estrus (Løvendahl and Chagunda, 2010). Until now the activity of cows during an extended lactation has not been studied. Our results showed a decrease in daily activity from estrus 4 to 8. Even so, the difference in activity between day of estrus and 5 d before estrus is similar for all the estruses (±17 movements per hour). This can be explained by an increase of mounting behavior found with estrus number. This may indicate that, during extended lactation, the nature of the cows' activity is changing toward spending more time walking and less time mounting in early estruses, whereas the opposite is true in later estruses (Figure 7).

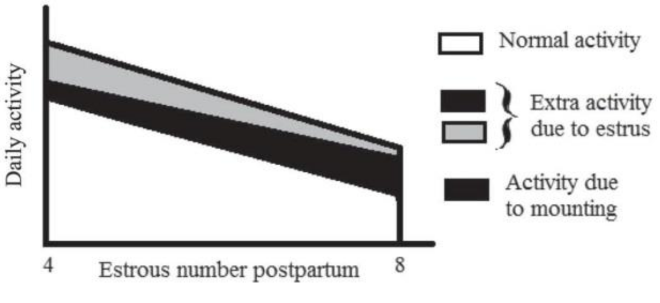

Figure 7. Diagram describing the changes in activity types and level over the estrus 4 to 8. Daily activity is decreasing from estrus number 4 to 8 (in white), whereas mounting behavior activity is increasing (in black) during estrus. The difference of activity (in gray and black) between estrus and 5 d before estrus (normal activity) is the same for each estrus number postpartum.

Grouping the Cows According to Their LWG in Early Lactation

Grouping the cows according to their LWG in early lactation was based on previous studies showing that the LWG is a good indicator of EB, and that the EB in early lactation is important for determining the onset of ovarian activity (Butler et al., 1981). In general, the more the cows are losing weight in early lactation, the greater their energy deficit, leading to a longer delay of return to estrus (Friggens, 2003). Unfortunately, a lack of knowledge exists about the importance of the effect of early lactation management on late insemination (i.e., around 8 mo), because most of these studies were conducted with cows managed for 12-mo calving interval; consequently, the effect of the early lactation was only tested on short-term insemination success, as this occurred around 2 mo of lactation. In our study, during the first 5 wk of lactation the NLWG cows were still below their calving weight at first insemination, whereas the PLWG cows were above their calving weight at their first insemination. The HD-LD cows, receiving more energy during early lactation, were expected to be over-represented in the PLWG, but the HD-LD cows were almost equally distributed between the 2 LWG groups. It seems that the LWG calculations are not accurate enough to detect small changes in energy diet. This might be due to the large daily LW variation or the small difference of energy density in the diets.

Relationships Between Estrus and Other Parameters

The NLWG cows produced more milk than the PLWG cows. As these groups were balanced according to the feeding strategy, the difference in EB between the groups suggests an individual variation and adaptation to the lactation. The NLWG cows mobilized more body tissues and for a longer time than the PLWG cows. Plasma IGF-1 level was positively correlated with LWG, and the NLWG cows had lower plasma IGF-1 than PLWG cows at 5 wk. This result is in accordance with Spicer et al. (1990), who found that serum IGF-1 concentration decreases during negative EB. Moreover, negative EB has previously been linked to reduced ovarian activity during early lactation (Butler et al., 1981; Spicer et al., 1990), and it has been shown that reproductive performance is affected by the mobilization of body energy reserves (Friggens, 2003), with thin cows being delayed in their return to estrus. In our study, the NLWG cows lost more LW than the PLWG cows until the first insemination, but the pregnancy risks between the LWG groups were similar. This last result might be due to the fact that our sample size was limited for evaluating fertility outcomes. Larger trials, such as the ones conducted by Santos et al. (2009), have shown negative effects of BCS and LW loss on return to estrus, conception rates, and pregnancy loss.

Factors Influencing Pregnancy Risks

It has been shown that the duration of the interval from calving to first estrus is related to the LW of the cow during early lactation (Haresign and Lewis, 1979); thin cows, or cows losing more weight in early lactation, need more time to return to estrus resulting in a longer interval to first estrus (Friggens, 2003). When more energy is available in early lactation, more glucose and insulin should increase the LH secretion and induce ovulation at regular intervals with stronger estrous behavior (Butler and Smith, 1989). Our results are not completely in accordance with these results; the HD-LD cows fed a more energy dense diet had the same weight loss in early lactation as the LD-LD cows and a longer interval from calving to first estrus. Regarding the LWG groups, the NLWG cows lost more weight in early lactation than the PLWG cows, but had the same interval from calving to first estrus. Moreover, a high BW loss in early lactation leads to a decrease in reproduction efficiency due to delay in insemination and lower pregnancy risks (Haresign and Lewis, 1979; Gilmore et al. 2011); but, again, our results differed from these findings, maybe due to our smaller sample size. There was no difference in pregnancy risks between our LWG groups, maybe because the difference in LWG between groups was too small and short in time to have an effect. The LD-LD cows tended to have higher pregnancy risks than the HD-LD cows. Moreover, the LD-LD cows had a shorter interval from calving to first estrus than the HD-LD cows, and, consequently, more estruses from calving to first insemination could explain the tendency for higher pregnancy risks. Indeed, it has previously been shown that an increase in the number of estrous cycles reduced the services per conception and increased the pregnancy risk at first insemination (Thatcher and Wilcox, 1973). The tendency of HD-LD cows to have lower pregnancy risks than LD-LD cows might also be due to the effect of the change to a lower energy density diet in early lactation, which prolonged mobilization and slightly decreased energy balance until the cows adapt to the new diet.

CONCLUSIONS

Milk yield decreased to 0.56 kg of milk on the day of estrus and was not affected by estrus number 1 to 8 in cows managed for an 18-mo calving interval. The decrease in milk yield induced by estrus was independent of milking frequency. The activity of the cow increased at the day of estrus compared with nonestrus days. More cows expressed mounting behavior at estrus 8 compared with estrus 2. Reproductive parameters (frequency of mounting, pregnancy risk, interval to first estrus, and between-estrus intervals) were not influenced by the LW change during early lactation.

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4. Paper 4 - Effects of delayed insemination and double insemination technique on the reproductive performance of Holstein cows

C. Gaillard, J. Sehested and M. Vestergaard Effects of extended lactation and double insemination technique on the reproductive performance of Holstein cows.
Submitted in December 2015

Highlights

- Similar reproductive outcomes of an insemination in early or late lactation.
- Comparison of the effect of a double versus single insemination per estrus.
- The double insemination did not improve pregnancy rates.

Short Communication:

Effects of delayed insemination and double insemination technique on the reproductive performance of Holstein cows

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ABSTRACT

An earlier study showed that deliberately delaying rebreeding until after peak lactation improved pregnancy rates compared with the traditional rebreeding in early lactation. The first objective was to compare the number of artificial inseminations (AI) per pregnancy, and the pregnancy rates at first and second AI between a 16 mo lactation (rebreeding at around 8 mo after calving) and the previous and following 10 mo lactations (rebreeding at around 2 mo after calving) of each of 62 Holstein cows with lifetime recordings. The second objective was to evaluate the effect of a double AI technique around estrus, in which cows received 2 AI with 2 days apart compared with a single AI per estrus. The results showed that the pregnancy rates and number of AI per pregnancy were similar for the 16 mo lactation and the previous and following 10 mo lactations. The double AI technique did not improve pregnancy rates compared with a single AI per estrus, and induced confusion on how to report the herd reproductive performance.

Key Words: extended lactation, pregnancy rate, number of inseminations, double insemination

INTRODUCTION

During the recent decades, the milk production per cow has increased and fertility has decreased in Holstein cows which can be seen as a negative correlation between yield and fertility (Butler, 2000, Gilmore et al., 2011). Indeed, as the cows are usually inseminated around peak yield, more artificial inseminations (AI) in succeeding estruses are often needed to attain pregnancy which is then involuntarily delayed (Borman et al., 2004). The cows have been selected for high milk yield which is negatively correlated with estrus duration; probably due to a decrease in circulating estrogens (Lopez et al., 2004). The decrease of estrus behavior expression does not facilitate the AI at the right time during estrus (Kerbrat and Disenhaus, 2004), and contributes to the reproductive failure (Spalding et al., 1975). Thus, attempts to overcome the problems with estrus detection have included the use of a double AI technique, with which cows are inseminated 1 or 2 days apart (Stevenson et al., 1990). Schindler et al. (1991) found that deliberately delaying rebreeding until after peak lactation improved pregnancy rates compared with rebreeding in early lactation. In that case, the first AI occurs in a period of more positive or less negative energy balance compared with the early lactation period (Butler et al., 1981, Walsh et al., 2011), and furthermore the return to estrus has become regular compared to the early lactation period (Butler et al., 1981) which facilitates estrus detection. The use of delayed AI increased the number of cows expressing estrus behavior at time of AI (Gaillard et al., 2015). Delayed AI fits with extended lactation and increased calving interval which would reduce the number of calvings per cow per year and consequently reduce the health risks associated with calving time (Ingvartsen, 2006, Knight, 2005). Nevertheless, the effect of delayed AI and extended lactation on the reproductive performance has not been studied up to now. The objectives of the present study were firstly to compare the number of AI per pregnancy and the pregnancy rates between a 16 mo lactation (delayed AI at around 8 mo after calving) and the previous and following 10 mo lactations (early AI at around 2 mo after calving), and secondly to evaluate the effect of a double AI technique around estrus on pregnancy rates compared with a single AI per estrus. We hypothesized that 1) the pregnancy rates will be higher for the 16 mo lactation compared with the previous 10 mo lactation, 2) the extended lactation will not have a negative effect on the following 10 mo lactation reproductive performance, and 3) that the double AI around estrus will increase pregnancy rates compared with a single AI per estrus.

MATERIALS AND METHODS

Animals, Housing and Feeding Strategies

The experiment was performed at the Danish Cattle Research Centre at Aarhus University, Foulum. The number of AI per pregnancy and the pregnancy rates at first and second AI were recorded for the lifetime of 62 Holstein cows having one extended lactation period. For each cow, the lactations where categorized into 3 lactation groups: the 16 mo extended lactation (EL), the previous 10 mo lactation (PL), and the following 10 mo lactation (FL). Lactation type represents either the EL, or the 10 mo lactations (Normal, NL) grouping the PL and FL. The number of primiparous and multiparous cows for each lactation group is detailed in Table 1. All the cows were housed in one group pen on slatted concrete floors with cubicles equipped with mattresses and sawdust bedding. The cows had access to water and an automatic milking system (AMS; DeLaval AB, Tumba, Sweden) and were fed partially mixed rations with complementary concentrates supplied during visits in the AMS. For the 10 and 16 mo lactations the AI was initiated at the first estrus after 60 and 220 days in milk, respectively.

Insemination technique

In this life time data set, some heifers (16 among 62 observations) and cows (80 among 173 observations) were inseminated 2 times around a given estrus day, with on average 2 days between these 2 inseminations. This double AI technique was used with the objective to increase the chances of pregnancy. The herdsmen decision to inseminate a cow twice was based upon if the cow stayed in heat the days following the first AI. There were no other criteria used to decide when to apply this double AI technique. The number of cows assigned to single or double AI per lactation group is given in table 1. In order to be able to analyze the effect of the AI technique, we defined 2 variables to calculate the number of AI per pregnancy. The ‘basic number of AI per pregnancy’ represented the number of AI excluding the double AI for the same estrus cycle, while the ‘real number of AI per pregnancy’ included them. For example, if a cow received 2 AI for the same cycle and get pregnant, the real number of AI per pregnancy was 2, and the basic number of AI per pregnancy was 1. The same two ways of calculation was applied to the pregnancy rates.

Statistical analysis

The statistical analysis was made using R version 3.0.0 (R Development Core Team, 2014). As there was no interaction effect between parity and the lactation group, or between parity and lactation type, a simple linear mixed-effects model was used to test the effects of one factor (parity, lactation group, or lactation type) at a time on the number of AI per pregnancy (basic or real), and on the length of the calving interval. The effect of the AI technique (single or double) on the number of AI per pregnancy was tested with the same model. The factors and their levels are presented in table 2.

A chi-square test was used to determine the effect of parity, lactation group, lactation type, and AI technique, on the pregnancy rate. This test was also used to determine the effect of parity on the percentage of cows receiving the double AI technique. Finally a t-test was used to compare the number of basic and real number of AI per pregnancy.

RESULTS AND DISCUSSION

The parity had no significant effect on the number of AI per pregnancy (Basic AI: $P = 0.19$, Real AI: $P = 0.09$) or on the pregnancy rates (Basic first AI: $P = 0.54$, basic second AI: $P = 0.56$) (Table 3). The number of AI per pregnancy was higher for NL, the normal 10 mo lactations (PL and FL combined) compared with EL, the extended 16 mo lactations (basic AI: $P = 0.02$, real AI: $P = 0.01$). Previous studies also found that delaying AI until after the peak yield decreased the number of AI per pregnancy (Larsson and Berglund, 2000). Despite this finding, there was no significant difference in the number of AI per pregnancy between the 3 lactation groups PL, EL, and FL (basic AI: $P = 0.09$, real AI: $P = 0.12$) (Figure 1). This is in accordance with Christiansen et al. (2005), where the cows managed for 10 mo lactation had 2.0 ± 0.3 AI per pregnancy and the cows managed for 16 mo lactation had 1.5 ± 0.3 AI per pregnancy, with no significant difference between the 2 groups. These results indicate that the extended lactation had no negative effect on the planned timing of AI for the following 10 mo lactation.

The lactation groups had similar pregnancy rates ($P > 0.05$) whatever the way of calculating it ("basic" or "real" values). This result is in accordance with Bertilsson et al. (1997) where no differences in pregnancy rates were found between 16 mo and 10 mo lactating cows. Nevertheless, other studies found an improved pregnancy rate with extended lactation (Schindler et al., 1991). Despite not significant, our "basic" results were numerically more similar to the results of Schindler et al. (1991) than to the results of

Bertilsson et al. (1997): for the “basic” pregnancy rates to first AI our extended lactation group had around 12%-units higher pregnancy rate compared with their previous lactation. The “basic” pregnancy rate at first AI of the FL was 10%-units higher compared with the previous 10 mo lactation indicating that the extended lactation had a positive effect on the pregnancy rates of the following lactation. In all cases, the relatively small number of animals might explain the absence of significant differences in pregnancy rates. Finally, the basic number of AI was lower than the real number of AI (1.9 versus 2.5, $P = 0.001$), and the pregnancy rates were higher for the cows that did not receive the double AI in the same estrus cycle (first AI: $P = 0.006$, second AI: $P < 0.01$) (Figure 2). It indicated that the double AI technique did not have the expected role as pregnancy rates were not improved by using 2 AI per estrus. This is in accordance with Stevenson et al. (1990) who found no benefit of the double AI technique (pregnancy rates of 33.5% with double AI and 32.1% with single AI, $n = 723$) applied to cows failing to be pregnant for at least 2 services. Our results suggest that the pregnancy failure does not arise from an estrus detection problem per se but from another unidentified fertility problem which might be a failure to ovulate or to inappropriate patterns of ovarian cyclicity (Royal et al., 2000).

CONCLUSIONS

During a 16 mo extended lactation, the number of AI per pregnancy and the pregnancy rates after first and second AI were not improved compared with the previous and following 10 mo lactations. Applying the double AI technique around estrus did not improve the pregnancy rates compared with the use of one AI per estrus, and induced confusion on how to report the herd reproductive performance. To conclude, the reproductive performance was not influenced by delayed rebreeding, and the extended lactation had no negative effect on the reproductive performance in the following lactation. Nevertheless, further investigations with larger herds should be carried out to better assess the possible improvement of pregnancy rates with the use of extended lactation.

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Figures

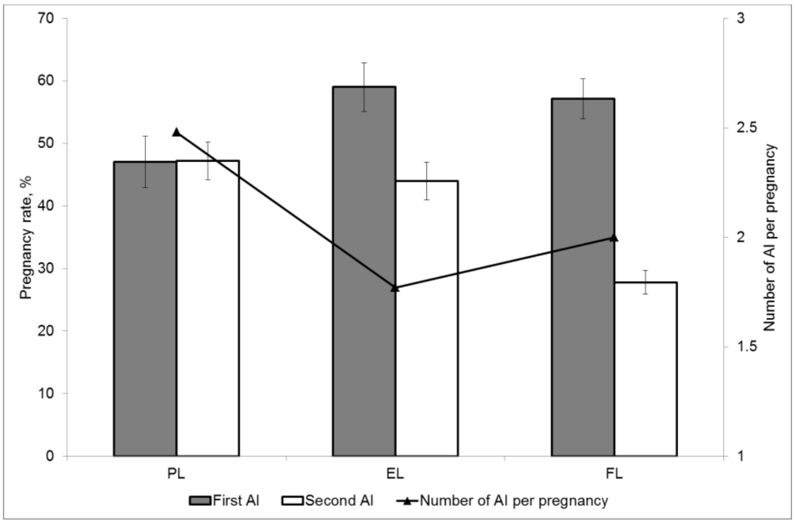

Figure 1 – Basic pregnancy rates (% ± SD) and basic number of AI per pregnancy for the 3 lactation groups (PL, EL, FL), at first and second AI.

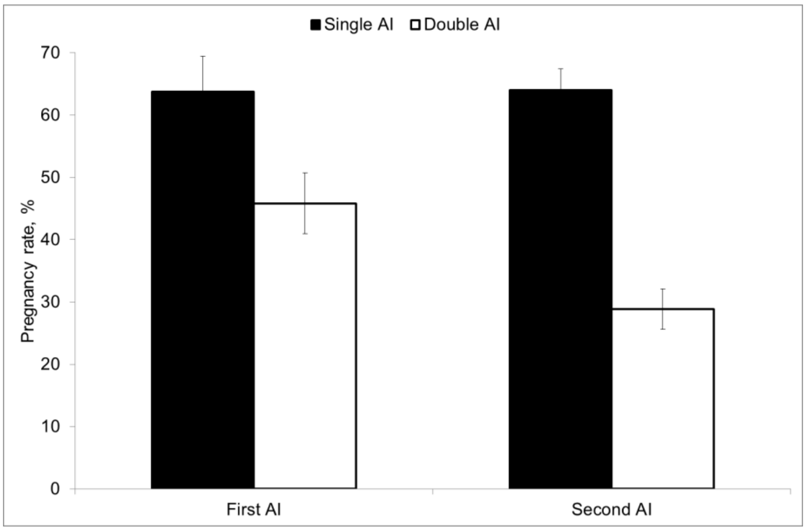

Figure 2 – Basic pregnancy rates (% ± SD) for cows receiving 1 (single) or 2 (double) AI per estrus (Insemination technique), at first and second AI.

Table 1. Effect of the delayed artificial insemination (AI, around 8 mo) compared with AI around 2 mo on the number of AI per pregnancy and pregnancy rate (%) for Holstein cows. Comparison between 3 lactation groups: previous 10 mo lactation (PL), 16 mo extended (EL), and 10 mo following lactation (FL).

	Lactation group				
	PL	EL	FL	SEM	P value
Number of observations	131	62	42	-	-
Number of animals	68*	62	42	-	-
Primiparous	0	17	0	-	-
Multiparous	68	45	42	-	-
Calving interval[1], d	372^b	541^a	368^b	11	< 0.001
Number of observations with double AI technique	46	29	21	-	-
Basic number of AI per pregnancy	2.0	1.8	2.5	0.2	0.09
Real number of AI per pregnancy	2.6	2.3	3.2	0.2	0.12
Basic pregnancy rate at first AI, %	47.1	59.0	57.1	-	0.27
Real pregnancy rates at first AI, %	38.7	40.0	23.8	-	0.18
Basic pregnancy rate at second AI, %	47.2	44.0	27.8	-	0.14
Real pregnancy rates at second AI, %	52.8	53.0	56.2	-	0.28

*Some animals had several lactations before EL, some had none (i.e., the 17 primiparous cows)

[1] Calving interval: lactation period+ dry period.

EL: extended lactation, PL: lactations preceding extended lactation, FL: lactation following extended lactation, AI: Artificial Insemination

Table 2. Factors and levels studied

Factor	Levels
Parity*	0, 1, 2, 3+
Lactation group	Extended lactation (EL), lactations preceding extended lactation (PL), lactation following extended lactation (FL)
Lactation type	Normal 10 mo (NL) [includes PL and FL], 16 mo extended lactation (EL)
AI technique	Single, Double

0 = as replacement heifers, 3+ = cows of third parity or more

Table 3. Parity effect on the percentage of cows receiving double AI, the number of AI per pregnancy (basic or real), and the basic pregnancy rates at first and second AI

Parity group	Number of animals	% cows with double AI	Basic number of AI ± SEM	Real number of AI ± SEM	Basic pregnancy rate at first AI, %	Basic pregnancy rate at second AI, %
0	62	25.8^{a}	1.6 ± 0.2	1.9 ± 0.3	62.9	56.5
1	62	35.5^{b}	1.9 ± 0.2	2.4 ± 0.3	52.4	46.7
2	62	45.2^{c}	2.3 ± 0.2	2.8 ± 0.3	51.7	37.9
3+	49	61.2^{d}	1.9 ± 0.2	2.7 ± 0.3	59.2	40.0
Parity effect (P-value)		< 0.001	0.19	0.09	0.54	0.56

AI: artificial insemination

0 = as replacement heifers, 3+ = 34 cows of 3rd, 11 cows of 4th, and 3 cows of 5th parity

5. Paper 5 – Prediction of the productive and reproductive lifetime performance of Holstein cows managed for different lactation durations, using a model of lifetime nutrient partitioning

C. Gaillard, O. Martin, P. Blavy, N.C. Friggens, J. Sehested, H. N. Phuong. Prediction of the productive and reproductive lifetime performance of Holstein cows managed for different lactation durations, using a model of lifetime nutrient partitioning.
Submitted in February 2016

Summary

Prediction of the lifetime performance of Holstein cows managed for different lactation durations. Gaillard et al. 2016. A lifetime performance model was used to predict the efficiency (milk/intake) and the pregnancy rates of different lifetime scenarios. These two output criteria were used to determine the optimum lifetime scenario based on lactation duration. A 16 mo lactation for the primiparous cows followed by 10 mo lactation for the multiparous, appeared to be the best lifetime scenario in terms of production and reproduction performance.

RUNNING TITLE: Productive and reproductive lifetime performance

Prediction of the productive and reproductive lifetime performance of Holstein cows managed for different lactation durations, using a model of lifetime nutrient partitioning

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ABSTRACT

The GARUNS model is a lifetime performance model taking into account the changing physiological priorities of an animal during its life, and through repeated reproduction cycles. This dynamic and stochastic model has been previously used to predict the productive and reproductive performance of various genotypes of cows across feeding systems. In the present paper, we used this model to predict the productive and reproductive lifetime performance of Holstein cows for different lactation durations, with the aim of determining the lifetime scenario that optimizes the cow's performance. To evaluate the model, data from a 16 mo extended lactation experiment on Holstein cows were used. Generally, the model could consistently fit the body weight, milk yield, and milk components of these cows, while the reproductive performance was over-estimated. Simulation of cows managed for repeated16 mo lactation all their life (scenario EL-EL) had the highest lifetime efficiency (ratio of energy in milk to energy intake), and the longest lifetime compared with shorter (repeated 10, 12, or 14 mo lactations) or longer lactations (repeated 18, 20, or 22 mo lactations). Their pregnancy rates were not significantly improved compared with a 10 mo lactation (scenario N-N). Simulations of cows managed for a 16 mo lactation during their first lactation, followed by 10 mo lactations for the rest of their life (scenario EL-N), had a lifetime efficiency similar to the one of the scenario EL.-EL Simulations of cows managed for a 10 mo lactation during their first lactation, followed by 16 mo lactations for the rest of their life (scenario N-EL), had a lifetime efficiency similar to the one of the N-N scenario. The pregnancy rates of these 4 scenarios (N-N, EL-EL, N-EL, and EL-N) were similar. To conclude, the GARUNS model was able to fit and simulate extended lactation. Those simulations indicated that managing the primiparous cows with a 16 mo extended lactation, followed by 10 mo lactations, allows the lifetime efficiency of these cows to increase being similar to cows managed for 16 mo lactation all their life. Further work should include health incidents (i.e. diseases) to the prediction model to have more accurate and realistic predictions on the lifetime efficiency.

Key words. Lifetime efficiency, nutrient partitioning, extended lactation, modelling

INTRODUCTION

The progressive increase in milk yields over the last decades has led to an increased interest in extended lactation among European farmers (Knight, 2005). In extended lactation management systems, a cow is voluntarily rebred later in the lactation (Sorensen et al., 2008) to extend the lactation duration above the traditional 10 mo (Osterman and Bertilsson, 2003) while keeping a decent milk yield during the lactation, and a lower milk yield at the time of drying off. This management also avoids the insemination to occur at the same time as peak milk yield and the reconstitution of the body reserves, which could improve pregnancy rates (Borman et al., 2004). The annual health risks periods (mastitis, lameness, metabolic problems at peak lactation), of which 65% occur around the calving period (Ingvartsen, 2006), should as well be reduced when delaying rebreeding as the number of calving per cow per year will decrease (Knight, 2005). Extended lactation provides a tradeoff between a longer productive life, partly due to less health risk periods per year, and a lower number of calves per year (Borman et al., 2004). However, there is not enough information concerning the efficiency of extended lactation systems, particularly the long term consequences. This is partly because experiments on extended lactation are time consuming and the number of animals restricted. In this context, prediction models can be useful tools to predict the consequences of different management strategies in terms of production and reproduction. The simulation of nutrient partitioning across physiological functions and according to genotypes has been the subject of a number of models (Dumas et al., 2008, Friggens et al., 2013) with the aim to predict the performance of an animal and help the farmers to take the best management decisions. A dynamic and stochastic model, referred to here as GARUNS, which takes into account the changing priorities of an animal during its life, and through repeated reproduction cycles, has been previously developed (Martin and Sauvant, 2010) and tested for cows managed for 10 mo lactation, for different breeds and parities (Phuong et al., 2015a).

Accordingly, the objectives of the present study were 1) to determine if the GARUNS model of Martin and Sauvant (2010) was able to fit individual curves of milk yield (**MY**), body weight (**BW**), body condition score (**BCS**), dry matter intake (**DMI**), milk fat (**MCF**), milk protein (**MCP**), and milk lactose (**MCL**), of cows managed for 16 mo of lactation, 2) to determine if the full model, including the reproductive sub-model of Phuong et al. (2015), can predict the reproductive performance of cows managed for 16 mo lactation, 3) to predict which lifetime scenarios, based on lactations duration, will be beneficial, in terms of performance and reproduction.

MATERIALS AND METHODS

Facilities and animal

The data were obtained from the REPROLAC experiment described in Gaillard et al. (2015). This experiment was conducted at the Danish Cattle Research Centre at Aarhus University, AU-Foulum, Denmark, from November 2012 to January 2015, and approved by The Animal Experiments Inspectorate under the Danish Veterinary and Food Administration. A total of 62 Holstein cows including 17 first parity cows were managed for

16 mo of lactation by delaying rebreeding to 220 days in milk (**DIM**). All the cows were housed in a single pen with slatted floor and cubicles with mattress. They had a free access to water and were fed ad libitum though automatic feeders (Insentec RIC system, Marknesse, The Netherlands). They had an access to an automatic milking unit (DeLaval AB, Tumba, Sweden), where they received 3 kg extra concentrate per day, and had to visit it at least twice a day. In the REPROLAC experiment half of the cows were fed a diet enriched in energy in early lactation (on average the first 7 weeks of lactation) compared to the standard diet. To simplify the present analysis, and because the difference of energy between the diets was observed to have very few effects on the production variables (Gaillard et al., 2016), only the standard diet was considered. This diet had an average energy density of 11.5 MJ of ME/kg of DM.

Data recording

MY was recorded at each individual milking in the milking robot and the milk components (fat, protein, lactose, and cells) were measured every week. Daily BW was recorded through a platform scale (Danvaegt, Hinnerup, Denmark) in the milking robot. BCS of all cows was scored manually every second week by the same two trained persons. A 1 to 5 points scale with 0.25 points intervals was used (Ferguson et al., 1994). Daily DMI was calculated by adding the concentrates eaten at the milking robot and the dry matter intake registered by the automatic feeders.

GARUNS Model

The GARUNS model is a dynamic and stochastic model, which takes into account the changing physiological priorities of an animal during its life, and through repeated reproduction cycles (Martin and Sauvant, 2010). In this model, an individual cow is the unit modelled. The model is composed of a regulating sub-model and an operating sub-model. The regulating sub-model describes the priorities in terms of **G**rowth, **A**ging, body **R**eserves, energy supply to the **U**nborn calf, to the **N**ew born calf, and to the calf through **S**ucking (GARUNS); while the operating sub-model distributes the energy between the physiological functions according to the priorities and to genetic parameters. To simulate the differences between animals in genetic potential for milk production level, milk components, body weight, and body reserves the model uses the following genetic scaling parameters: peak MY potential ($\boldsymbol{\mu_Y}$), milk fat secretion ($\boldsymbol{\mu_F}$), milk protein secretion ($\boldsymbol{\mu_P}$), and milk lactose secretion ($\boldsymbol{\mu_L}$), nonlabile body mass at maturity ($\mathbf{w_M}$), labile body mass mobilization index (**μ_X**). Moreover, to adapt the original model with the situation of extended lactation, the following parameters that regulate the lactation length and priority for milk production were also used: priority between the suckling calf and body reserves from calving to peak milk yield (**y_N**) and after the peak milk yield (**λ_0**), and priority between the suckling calf and a new born calf (**γ** and **Uwea**). The unit of w_M is kilograms, whereas the remaining parameters are dimensionless energy ratios (MJ/MJ). Moreover, the inputs of GARUNS are the energy density of the ration (**eD**), the genetic scaling parameters to describe innate variability between animals in performance, and the reproductive timing parameters (age from birth to first pregnancy and intervals between calving and pregnancy). A reproduction sub-model was then added by Phuong et al. (2015b) to the GARUNS model using the prediction of MY, BCS, and energy balance from the GARUNS model as inputs to stimulate reproductive responses in terms of pregnancy rate at first insemination and thereby productive lifetime (Figure 1). The culling decisions for this new reproductive GARUNS model are described in Phuong et al. (2015b). In short, a cow was culled if it was not pregnant after 4 inseminations and if MY was below 25 kg/d. If MY was above 25 kg/d after 4 inseminations without being pregnant, a last insemination was allowed. Moreover, a cow managed for a 10 mo lactation was culled if it was not pregnant after 160 d after calving, and after 340 d after calving for a cow managed for 16 mo lactation. The last culling rule for the different lactation lengths used in this paper is presented in Table 1. The effects of disease on the animal's survivability are not currently incorporated in the model.

Model fitting to individual data

In order to use GARUNS for simulating different extended lactations strategies it was first necessary to evaluate its ability to fit extended lactation data, and to estimate the relevant genetic scaling parameters for extended lactation cows. These were then used to define the population of cows used in the simulation. Thus using the observed data of the REPROLAC cows, the model was adjusted individually for each production measurements (MY, BW, DMI, BCS, MCF, MCP, and MCL), and the genetic-scaling parameters fitting the best the observed values were recorded. The software Modelmaker version 3.0 (Cherwell Scientific Ltd., 2000) was used for the optimization using a least square procedure (Phuong, 2015a). A total of 11 genetic scaling parameters (presented in Table 2) were adjusted.

Model fitting evaluation

Production variables. We calculated the relative prediction error (**RPE**) to evaluate the ability of the GARUNS model to fit the observed data. As described in Phuong et al. (2015a), the root mean square error (**RMSE**) was first calculated for each DIM across the N cows:

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(O_i - P_i)^2}$$

where O_i and P_i are the i^{th} observed and predicted values of MY, DMI, BW, BCS, MCF, MCP, or MCL, and N is the number of observations. Then the RPE was computed as 100 × RMSE/(mean of the observed data). As done in the Fuentes-Pila et al. (1996) paper, it was assumed that a RPE < 10%, between 10 and 20%, and > 20% indicate a good, moderate, and poor prediction, respectively.

Reproduction model. To evaluate the reproduction model, the observed values of the number of days from calving to first service, the number of days from calving to pregnancy, the duration of the calving interval, and the percentage of pregnant cows at first insemination, were compared with the simulated values obtained with the GARUNS model using a t-test.

Scenarios and statistical analysis

The simulation of different lifetime scenarios regarding the parity and the lactation length were implemented in C++ programing language on the RECORD platform (Bergez et al., 2013). The lifetime of a cow is considered from birth to culling and is only influenced by reproductive performance and culling policy. For each scenario, a total of 300 cows were randomly generated with the genetic parameters drawn in a normal law according to the mean and the standard deviation of the REPROLAC cows' parameters defined after fitting (Table 2).

Firstly, several durations of lactation, presented in Table 1, were tested to define which duration of lactation could be better than the 10 mo lactation in terms of milk production and reproductive parameters. A t-test was used to compare the pregnancy rate, the lifetime efficiency (energy output in milk in MJ, divided by the energy intake in MJ), the milk yield per feeding day (total milk yield in kg divided by total number of days of life), the age at first calving, the life duration (in days), the total days in milk during the life, the number of lactations in life, between the groups to find the "optimum" extended lactation duration regarding these criteria. Secondly, 4 lifetime scenarios were defined taking into account the parity (primiparous or multiparous) and the lactation duration. These scenarios are presented in Table 3. The previous parameters were compared between these 4 scenarios to define which scenario optimizes the reproductive and productive lifetime performance.

RESULTS

Step 1 - GARUNS fitting REPROLAC data

The GARUNS model was able to adequately fit 16 mo extended lactation performance data as shown in Figure 2, where the observed values of MY, DMI, milk components and BW of one cow are plotted (symbols) with the fitted curves of the model simulation (lines). Table 4 gives the means (± sd) of the observed values for each variable, as well as the associated relative prediction errors (RPE in %). BW, MCF, MCP, and MCL, were accurately predicted (RPE ≤ 10%), and MY was acceptably predicted (RPE for all the cows = 13.8%), which in both cases indicate good fits of the model to the actual observations following Fuentes-Pila et al. (1996) assumptions. However, the BCS was poorly predicted (RPE > 20%), and consequently resulted in a poor DMI fit (RPE > 20%). The model underestimated the BCS while it overestimated the DMI. No major changes of prediction accuracy have been observed between parities (Table 4). Table 2 gives the description, the means and standard deviations, of the parameters of GARUNS used to fit the REPROLAC data.

Step 2 – GARUNS reproductive model

Table 5 presents the simulated reproductive performance of the REPROLAC Holstein cows. It shows that the model over-predicted the reproductive performance of these cows as the simulated cows had higher first service to pregnancy (+ 6%, $P = 0.02$), number of days from calving to first service (+ 23 d, $P < 0.01$), number of days from calving to pregnancy (+ 30 d, $P < 0.01$), and longer calving interval (+ 9 d, $P < 0.01$) than the observed values.

Step 3 - Production and reproduction performance for different planned lactation durations

Figure 3 shows that the pregnancy rate was significantly increased from an 18 mo lactation compared with a 10 mo lactation ($P = 0.05$). For shorter lactations (12, 14, 16 mo), the pregnancy rates were higher than for a 10 mo but the difference was not significant (*P*

= 0.34, *P* = 0.47, *P* = 0.73 respectively). For longer lactations (> 18 mo), the pregnancy rate at first insemination was similar to the pregnancy rate at first insemination of an 18 mo lactation. The number of inseminations per pregnancy was similar for the different lactation durations (2.1 ± 0.1 inseminations, *P* > 0.5) The herd average lifetime efficiency and the production per feeding day, increased from a 10 mo lactation to a 12 mo lactation (*P* < 0.001), were similar for a 12, 14, and 16 mo lactations (*P* > 0.05), and started to decrease when the lactation length was ≥ 18 mo. For a 22 mo lactation, the efficiency and the milk production per feeding day were similar to those of a 10 mo lactation (*P* = 0.94). The herd average life duration, the number of lactations per life, and the total number of days in milk increased from a 10 mo to a 16 mo lactation (*P* < 0.01), and were similar for a 16 and 18 mo lactation (*P* = 0.08, *P* = 0.28, *P* = 0.25, respectively). For longer lactations (20 and 22 mo), compared to a 16 mo lactation, the number of lactations per lifetime decreased (*P* < 0.01), the life duration increased (*P* < 0.01), while the total number of days in milk stayed stable (*P* = 0.70). Regarding these 3 criteria (reproduction, production, and life duration) the 16 mo lactation was kept as the optimum extended lactation duration for the step 4.

Step 4 - Best life scenario

The 4 life scenarios presented in Table 3 (N-N, EL-N, N-EL, and EL-EL) had similar pregnancy rates (i.e. between N-N and EL-EL, *P* = 0.73) (Figure 5) and number of inseminations per pregnancy (2.1 ± 0.1 inseminations, *P* > 0.5). The EL-EL and EL-N scenarios had a higher efficiency than the scenarios of N-N and N-EL (*P* < 0.01). The N-N scenario had the lowest milk yield per feeding day and the EL-EL scenario the highest. The milk yield per feeding day was lower for the scenario N-N than EL-N (*P* < 0.001), for the scenario EL-N than N-EL (*P* = 0.025) and for the scenario N-EL than EL-EL (*P* = 0.015). The EL-EL scenario had higher life duration and number of lactations (Figure 6). The number of lactations per life and the life durations were similar for the N-N and EL-N scenarios (*P* = 0.61, and *P* = 0.24, respectively), and were lower than those of the N-EL and EL-EL scenarios. The number of lactations per life and the life durations were lower for the N-EL scenario compared with the EL-EL scenario (*P* = 0.04, and *P* = 0.02, respectively).

DISCUSSION

Model fitting.

Genetic parameters. The genetic scaling parameters presented in Table 2 can be compared to those of the Danish Holstein cows of Phuong et al. (2015b) managed for 10 mo of lactation. The production level (μ_Y) of the REPROLAC cows, managed for 16 mo of lactation, was 12%-units higher than the one of Phuong et al. (2015b), due to the fact that these Holstein cows were coming from different herds fed with different energy density diets (11.5 MJ ME versus 11.1 MJ ME respectively), and the results were recorded at different years so the genetic progress of the last decade might be a reason for the increased milk potential (Phuong et al. (2015b) data sets were recorded in 2011 and 2014). The μ_L and μ_F parameters (lactose and fat concentrations in milk) were also 1%-unit and 9%-units, respectively, higher for Phuong et al. (2015b) compared with the REPROLAC parameters, which was expected as these parameters are negatively correlated with the level of production. The persistency and level of production were independent parameters. The other parameters were not significantly different indicating that the reproductive timing can be considered as the main effect on the lactation shape. These results suggest that the same genetic parameters can be used to build scenarios of different lactation durations.

Production and reproduction performance. The GARUNS model was able to fit the production data of Holstein cows managed for extended lactation. Our results are in accordance with Phuong et al. (2015): the model accurately predicted the individual BW and milk components, acceptably predicted the MY. The model underestimated BCS and consequently overestimated DMI (because predicted DMI is an outcome of the predicted energy requirement, including body reserves). The observed DMI was actually lower than the predicted one, and consequently the observed efficiency (MY/DMI) should be slightly higher than the predicted efficiency. The model over-estimated the reproductive performance of the REPROLAC cows (+10% for all parameters, apart from calving interval where it is only +2%). The simulated outputs of pregnancy rate at first insemination (46 ± 5 %) are comparable with those reported in the literature of approximately 48% for Holstein cows (Kolver et al., 2007). Cows managed for a longer lactation than 10 mo are often observed to have an improved pregnancy rate at first service although the difference is not always statistically significant: 46 vs. 64.4% (*P* > 0.05) (Schindler et al., 1991), 49 vs. 56% (Larsson and Berglund, 2000), 19 vs. 48% (*P* > 0.05) (Kolver et al., 2007), 35% for both (van

Amburgh et al., 1997), and 40.3 vs. 43.5% (P > 0.05) (Arbel et al., 2001). In general, postponing rebreeding to a period of more positive energy balance affects positively the pregnancy rates. Thus, in this study the simulated gain in pregnancy rate for 16 mo of lactation seemed appropriate.

Model limitations. The health problems often encountered in the life of a cow (i.e. mastitis, lameness, ketosis) were not taken into account in this model. These health problems would influence negatively the production variables, as well as the pregnancy rates and lifetime (Esposito et al., 2014). Consequently, in the current model the production and reproduction values were predicted for animals that would never get sick, and should be lower in reality. To take into account the health risks in GARUNS, a "health sub-model" could be built and integrated to the actual model. This sub-model should include the effect of general diseases on survivability as it has been shown that diseases negatively affect pregnancy rates, directly (metritis, retained placenta) by decreasing fertility (Esposito et al., 2014), or indirectly (mastitis, ketosis, acidosis, lameness) by decreasing DMI and consequently increasing negative energy balance which affects negatively the ability to conceive (Butler, 2000, Reksen et al., 2002, Liefers et al., 2003, Patton et al., 2007). The milk yield at drying off, and the drying off technique could also be incorporated in the "health sub-model" as the stagnant milk contained in the mammary gland at drying-off is lower in natural protective factor like immunoglobulins, so a high milk yield at drying-off would increase the risk of infections (Bushe and Oliver, 1987, Paape et al., 1992). The use of an intermittent cessation of milking might help reducing the infections compared with an abrupt cessation of milking (Olivier et al., 1990).

Model predictions.

Realistic outputs. The increase of pregnancy rate with the lactation duration is in accordance with the literature (Schindler et al., 1991, Bertilsson et al., 1997, Kay et al., 2007). The absence of significant difference in predicted pregnancy rates, or number of inseminations per pregnancy, between 10 and 16 mo of lactations is in accordance with other results of the REPROLAC experiment comparing the reproductive outcomes between 10 mo lactations and 16 mo lactations (Gaillard et al., Submitted to Livestock Science). From 18 mo lactation, lifetime efficiency and milk yield per feeding day decreased compared with shorter lactations which is in accordance with previous studies. Milk yield is a driver of efficiency (MY/DMI), and thus for long lactations (>10 mo) there are more days with lower than average milk yield, which might explain the decrease of efficiency (Osterman and Bertilsson, 2003, Kolver et al., 2007). This also highlights the value of using a model like GARUNS to define the optimum duration of a lactation found to be 16 mo in terms of efficiency and pregnancy rates. This is in accordance with Auldist et al. (2007) and Kolver et al. (2007) who found that 16 mo lactation was the more profitable way to extend lactation for pasture-based system. This decrease in lifetime efficiency for long lactation might also come from the fact that, as in real life, the GARUNS model has an in-built aging function (the A in GARUNS) which gradually increases with age, and represents a limit to longevity (around 10 years, as shown by the figure 2 in Martin and Sauvant (2010)). It also places a natural limit on the benefits of very long lactations because cows can't manage enough lactation to compensate the reduced number of peak lactations. Another possibility is that the benefits to late rebreeding get progressively less as lactation duration increases.

For the 10 mo lactations scenario, the simulated value of lifetime (1,969 ± 1,095 d) is in accordance with other studies where it varies from 1,762 to 2,200 d (Pritchard et al., 2013, CRV, 2014, Phuong et al., 2015a). The average number of lactations for the 10 mo lactations scenario was 2.92, sd = 3.03, with is in accordance also with literature (3.3 lactations in Knaus (2009) and 3.0 lactations in Hare et al. (2006)). The high values of lifetime predicted for the extended lactation scenarios (i.e. 8 lactations for a 16 mo scenario) might be attributed to the fact that in the model the lifetime is only defined by the reproductive performance and the culling rules, as the effects of diseases are not incorporated yet in the model (Phuong et al., 2015a). Old literature reported significant numbers of cows with 8 lactations (Lush and Shrode, 1950), but no actual studies can be referred to evaluate these numbers which indicate the lack of knowledge on extended lactation systems in a lifetime perspective.

Environment and economy. From an environmental point of view, the extended lactation scenarios proposed in this paper should reduce the methane emissions. The model of Garnsworthy (2004) shows that herd with improved longevity has a lower environmental impact as by lowering the number of heifers replacements, producing 27% of the methane on farms, the methane emissions would be mitigated. As proposed by

Phuong et al. (2015a), the GARUNS model could also include a calculation of the economic impact of having longer lactations. This would provide a more precise value of the efficiency of each scenario proposed in this paper. For example, the model of Jones (2005) calculates the final impacts of reducing annual milk production to extend the productive life of dairy cows, and concludes that the net financial effect of cutting milk production for a longer productive life of dairy cows was generally a loss.

Optimum lifetime scenario. The 2 best scenarios in terms of production and reproduction performance were the EL-N and EL-EL scenarios. To manage primiparous cows for 16 mo lactation seems to be beneficial for the lifetime efficiency. This is in accordance with previous results where extended lactation seems more advantageous for primiparous than multiparous cows (Arbel et al., 2001, Osterman and Bertilsson, 2003) as primiparous cows have a higher persistency than multiparous cows so they can be inseminated later (Ratnayake et al., 1998). In our case, this management shows that a first lactation of 16 mo will have an effect on the efficiency of the cow for the rest of its life. It seems to be the key element here, as the benefit of extended lactation was only seen for scenarios EL-N and EL-EL, so for primiparous cows with 16 mo of lactation. Bringing the extended lactation later in the cow's life, like for the N-EL scenario didn't give any value in terms of productive and reproductive performance. Regarding the EL-EL scenario, even though, the life duration should be improved compared with the N-N scenario because of the higher duration of the lactation in the EL-EL scenario, the obtained outputs for the life duration and number of lactation were high.

CONCLUSIONS

The GARUNS model was able to fit and simulate extended lactations. Further work should include health incidents to the prediction model to have more accurate predictions on the lifetime efficiency. Regarding our results and the need of improvements of the model, the "safer-best" lifetime scenario seems to be the EL-N scenario (primiparous with 16 mo lactation only), where the efficiency is improved without changing the other parameters.

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Table 1. Waiting period duration and culling rules of the GARUNS model, regarding the lactation duration simulated

Lactation duration*, mo	Voluntary waiting period **, d	Culling rule***, d
10	60	160
12	120	220
14	180	280
16	240	340
18	300	400
20	360	460
22	420	520

*Lactation duration is from calving to drying off

**Waiting period: days from calving to first insemination

***Culling: maximum number of days allowed for a cow to get pregnancy, otherwise the cow gets culled. Calculation: 5 cycles of 21 days + days from calving to first insemination

Table 2. Description, mean and standard deviation of the genetic parameters[1] of the REPROLAC Holstein cows given by the GARUNS model

Parameter	Description	Mean	sd
w_M (kg)	Maturity weight	548	57
μ_Y	Production level	1.4	0.2
λ_0	Persistency	0.001	0.0006
y_N	Start of the lactation	0.68	0.27
Uwea	End of the lactation	3.7	1.2
γ	End of the lactation	0.79	0.38
μ_L	Milk lactose secretion	0.99	0.01
μ_F	Milk fat secretion	1.1	0.11
μ_P	Milk protein secretion	1.04	0.07
μ_X	Ability of mobilizing	1.4	0.8
φ	Weight of uterus/fetus	0.58	0.02

[1] The unit of the parameters is mentioned in the parentheses, otherwise unitless

Table 3. The 4 lifetime scenarios simulated with the GARUNS model taking into account the parity and the duration of the lactation.

Scenario	Primiparous	Multiparous
N-N	N	N
EL-EL	EL	EL
N-EL	N	EL
EL-N	EL	N

EL:16 mo extended lactation, N:10 mo traditional lactation, and eD = 11.5 MJ ME/kgDM

Table 4. GARUNS model accuracy for predicting MY, DMI, BW, BCS, MCF, MCP, and MCL of Danish Holstein managed for a 16 mo lactation.

Variable	Parity	Mean observed ± sd	RPE, %
MY, kg/d	All cows	32.30 ± 7.27	13.84
	Primiparous	29.73 ± 4.22	13.00
	Multiparous	33.49 ± 8.67	13.77
DMI, kg/d	All cows	21.41 ± 3.09	19.56
	Primiparous	18.26 ± 2.89	22.65
	Multiparous	18.23 ± 2.89	22.62
BW, kg	All cows	684 ± 54	3.00
	Primiparous	633 ± 56	3.28
	Multiparous	708 ± 53	2.87
BCS, point	All cows	3.31 ± 0.22	27.37
	Primiparous	3.25 ± 0.17	20.30
	Multiparous	3.33 ± 0.25	30.81
MCF, kg/kg	All cows	0.042 ± 0.004	10.67
	Primiparous	0.04 ±0.005	10.81
	Multiparous	0.0423 ± 0.004	10.59
MCP, kg/kg	All cows	0.035 ± 0.003	10.19
	Primiparous	0.036 ± 0.003	12.17
	Multiparous	0.035 ± 0.004	9.26
MCL, kg/kg	All cows	0.048 ± 0.001	3.93
	Primiparous	0.049 ± 0.001	2.92
	Multiparous	0.048 ± 0.001	4.41

MY: milk yield, DMI: dry matter intake, BW: body weight, BCS: body condition score, MCF: milk fat, MCP: milk protein, MCL: milk lactose, RPE: relative prediction error

Table 5. Mean (± sd) of observed and simulated reproductive data from the REPROLAC Holstein cows managed for 16 mo lactation

	Observed	Simulated	P-value
First service pregnancy, %	40 ± 5	46 ± 5	0.02
Days from calving to first service, d	227 ± 13	250 ± 16	< 0.01
Days from calving to pregnancy, d	251 ± 37	281 ± 6	< 0.01
Calving interval, d	541 ± 20	550 ± 29	< 0.01

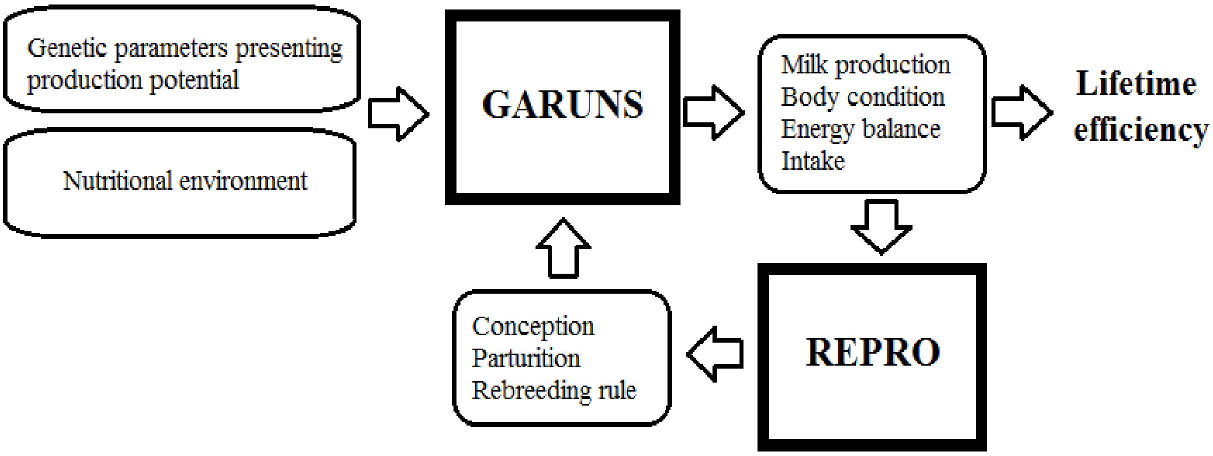

Figure 1. Schematic presentation of the model for predicting lifetime efficiency of individual cows that includes the GARUNS model (from Phuong et al. 2015a).

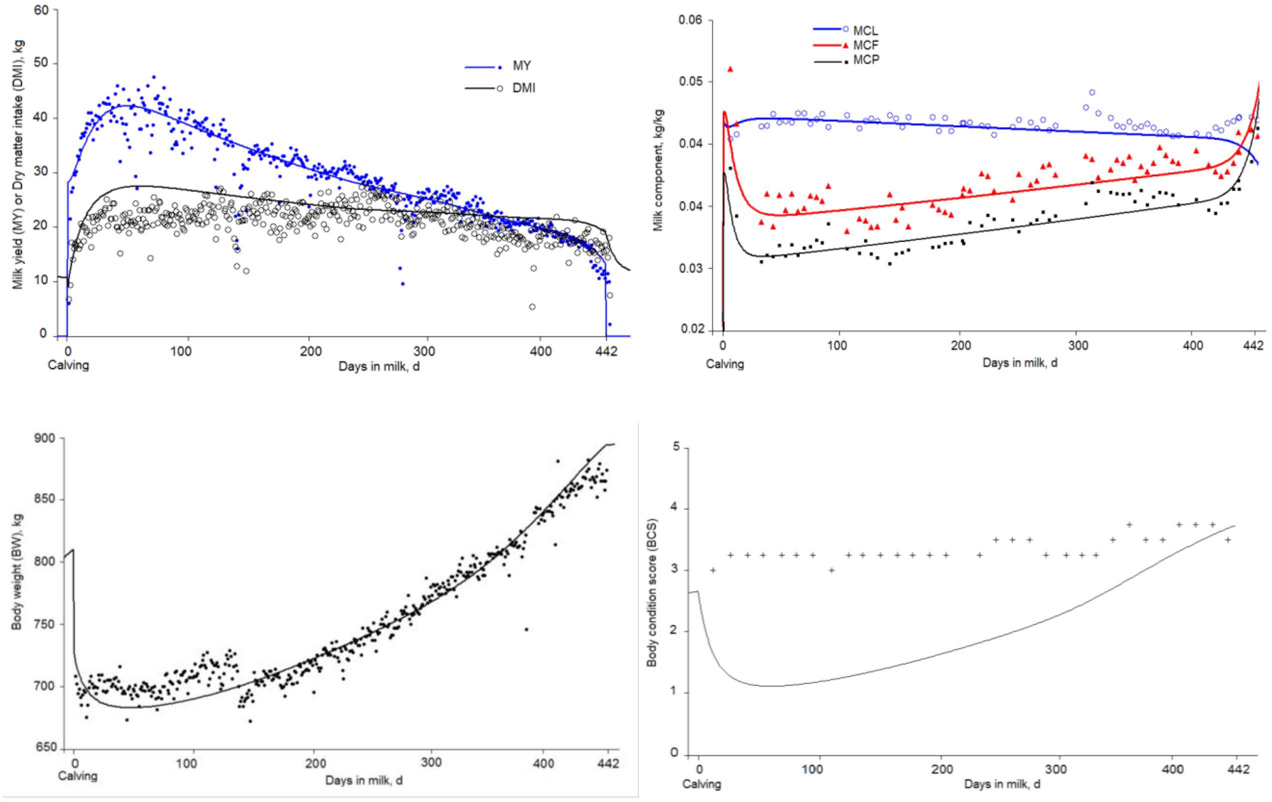

Figure 2. Observations (symbols) and associated fitted curves from the model simulation (solid lines) of milk yield (MY, kg/d), dry matter intake (DMI, kg/d), body weight (BW, kg), milk fat concentration (MCF, kg/kg), milk protein concentration (MCP, kg/kg), and milk lactose concentration (MCL, kg/kg) of a selected cow.

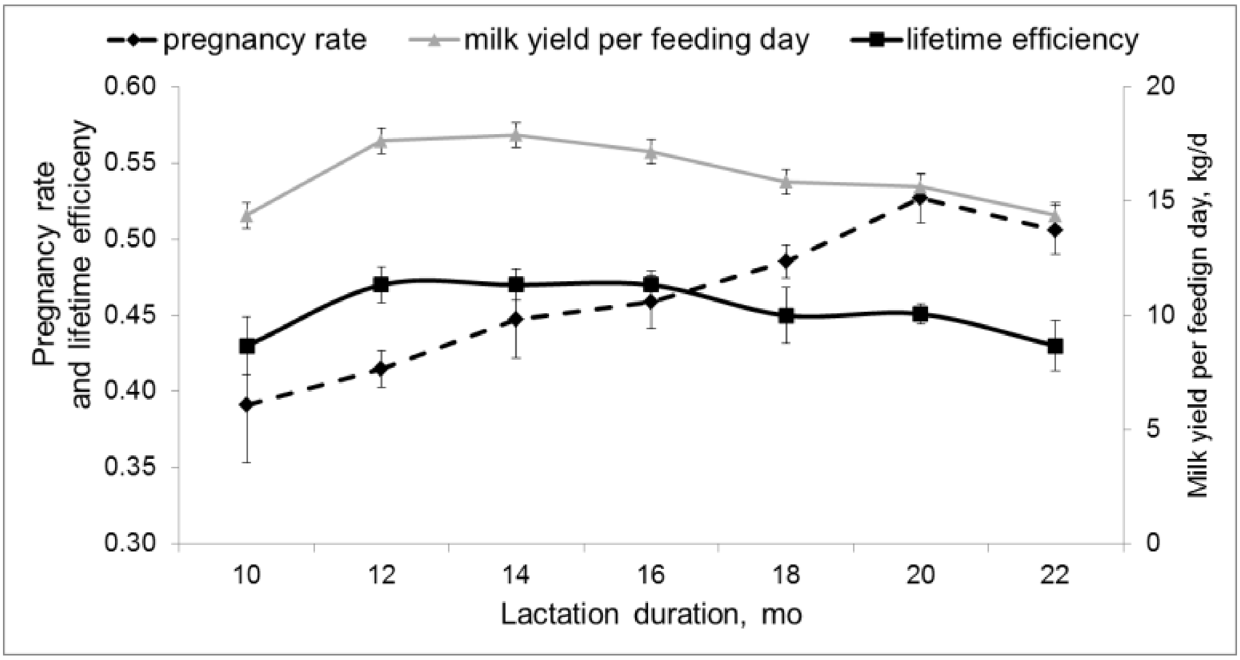

Figure 3. Pregnancy rate (%), lifetime efficiency (energy in milk to energy intake over lifetime, MJ/MJ) and milk production per feeding day (total milk yield total / duration of life, kg/d) of Holstein cows obtained for lactations of different durations (mo)

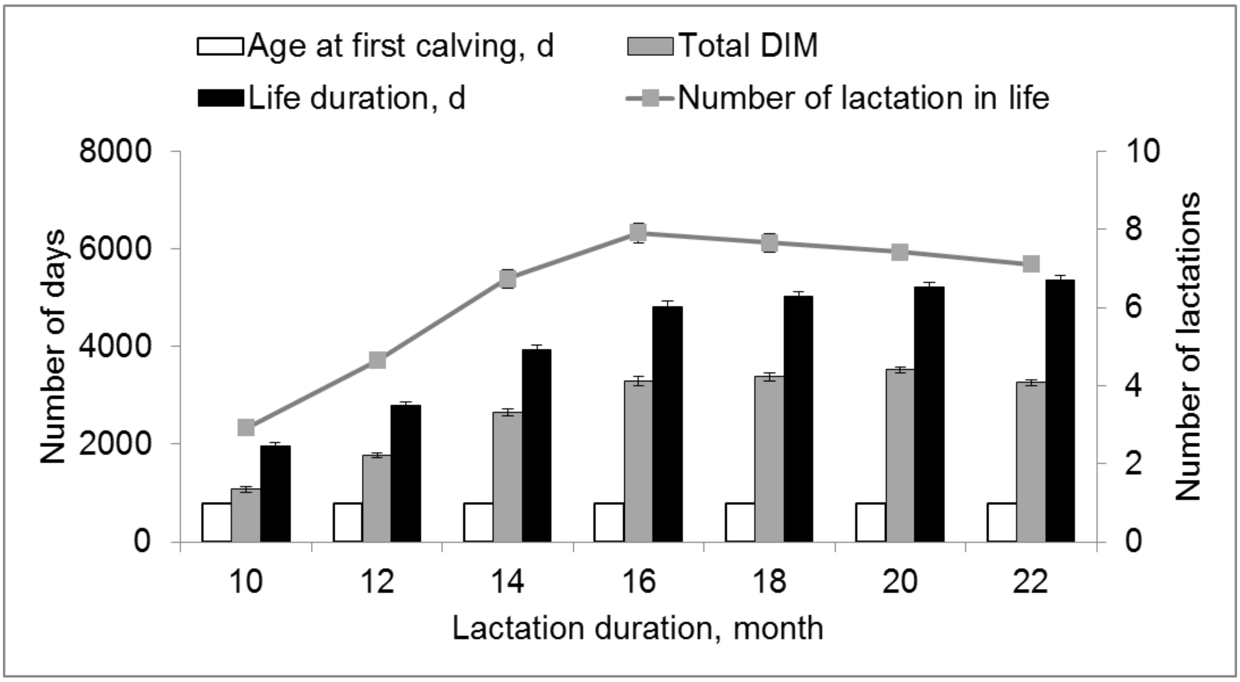

Figure 4. Life duration (d), number of lactations in life, and age at first calving (d) of Holstein cows for different lactation durations (mo)

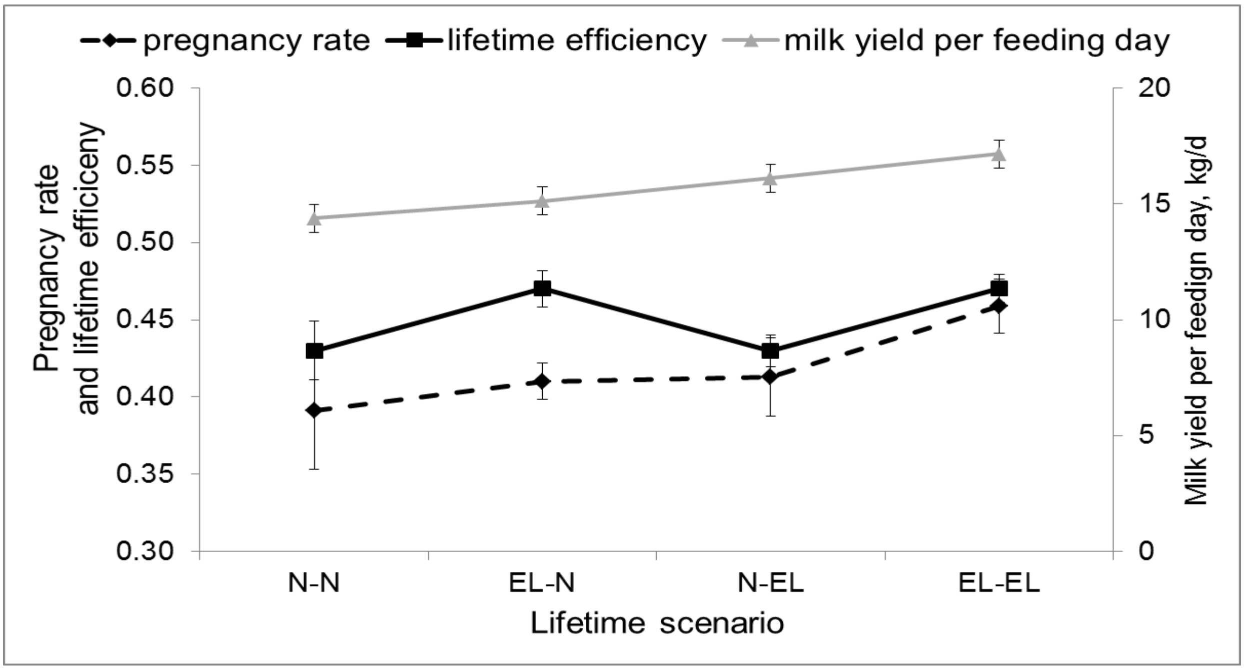

Figure 5. Pregnancy rate (%), lifetime efficiency (energy in milk to energy intake over lifetime, MJ/MJ) and milk production per feeding day (total milk yield total / duration of life, kg/d) of Holstein cows for different lifetime scenarios

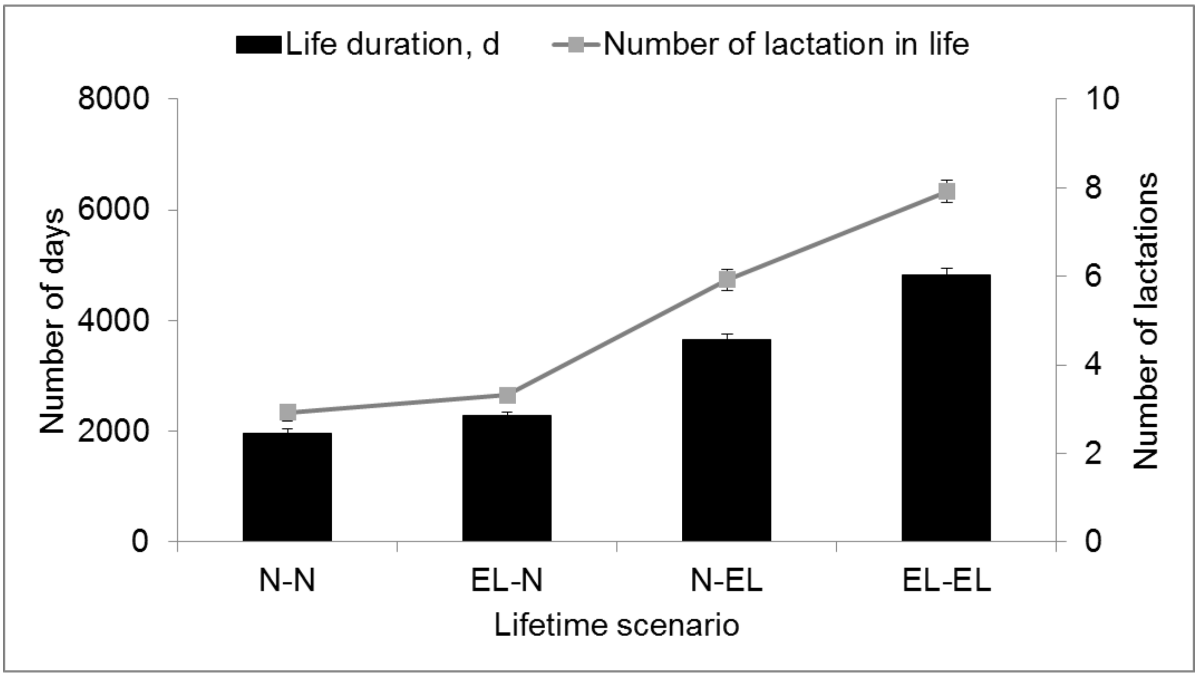

Figure 6. Life duration (d), number of lactations in life, and age at first calving (d) of Holstein cows for different lifetime scenarios

GENERAL DISCUSSION

In this thesis, the effect of a live-weight based feeding strategy in early lactation on the production and reproduction of Holstein cows, all managed for an extended lactation of 16 months, have been studied. The results presented previously to answer the objectives and hypotheses need further discussion. Firstly, the interactions found between the feeding strategy and the metabolic and production parameters will be discussed as well as the pro and cons of such a feeding strategy. Secondly, the relationships between the EB calculation and the different measurements taken during the experiment (physiological and production variables) will be discussed in order to hierarchize the possible indicators of EB. Thirdly, the effects of the feeding strategy and the duration of the lactation on the capacity to conceive will be discussed. The ideal lifetime management for a high yielding dairy cow should combine a high productive and reproductive performance.

1. Effects of the individualized feeding strategy on metabolites and milk production

1.1. Experimental set-up

***Obtained feeding strategy relative to planned*.** A 10%-units energy difference was planned and expected between the LD and HD diets; however, the obtained difference was smaller (4%-units), which can explain the few and little differences observed between the 2 feeding strategies for some of the variables measured.

Three main factors might explain this unplanned smaller difference between the two rations. Firstly, when planning the rations, the two basics rules were to keep the same ingredients and the same forage to concentrate (F:C) ratio during the experiment, as this ratio was considered to be the driver of the energy difference between the rations (60:40 versus 50:50) (Figure 12). From November 2012 to January 2015, several forages were used, which induced some variation in roughage quality and, consequently, might have impacted the energy density of the rations even so the F:C ratio was kept the same. Secondly, what is planned is not always what is realized. A chemical analysis of the ration was performed at the end of the experiment to have precise information on what the cows ate. Thirdly, two different feed evaluation systems were used to plan and evaluate the data. The rations were planned using the NorFor system, while the SFU system was used to evaluate the data, as the used of the NorFor system is challenging for evaluating experimental data. The Nordic feed evaluation system (NorFor) is a dynamic and non-additive net-energy system developed in 2007 in the Nordic countries (Volden, 2011). In this system, the energy value of a feed stuff is not constant and varies according to the other feed stuffs in the ration. In the classical additive energy evaluation system, like the Scandinavian feed unit (1 SFU = 7.89 MJ NE_L) system (Strudsholm *et al.*, 1997), only one energy value for each feed stuff is given. The energy level of forages is evaluated similarly for both systems while the energy concentration of other feed stuffs (concentrates, feed additives etc.) are estimated lower in energy in the NorFor system compared to the SFU system. The non-additive approach results in a decrease in NE_L per kg DM with increased DMI for a given feed ration (Volden, 2011).

However, this difference of 4%-units energy (equivalent to 0.32 MJ NE_L /kg DM) is not negligible as Bossen et al. (2009) found several differences in production data between their diets with a 0.4 MJ NE_L /kg DM difference. One difference between their rations and the HD/LD rations is that: 1) the density energy of the LD diet (standard diet of the present experiment) is not far from the highest density energy diet of Bossen et al. (2009), so consequently the HD diet has a quite high energy density, 2) the jump from Bossen et al. (2009) from a high to a low energy diet is quite high (twice higher than the one of the present experiment), and, consequently, some more extreme effects have been observed in the mobilization period and later on in the study of Bossen et al. (2009) (Figure12).

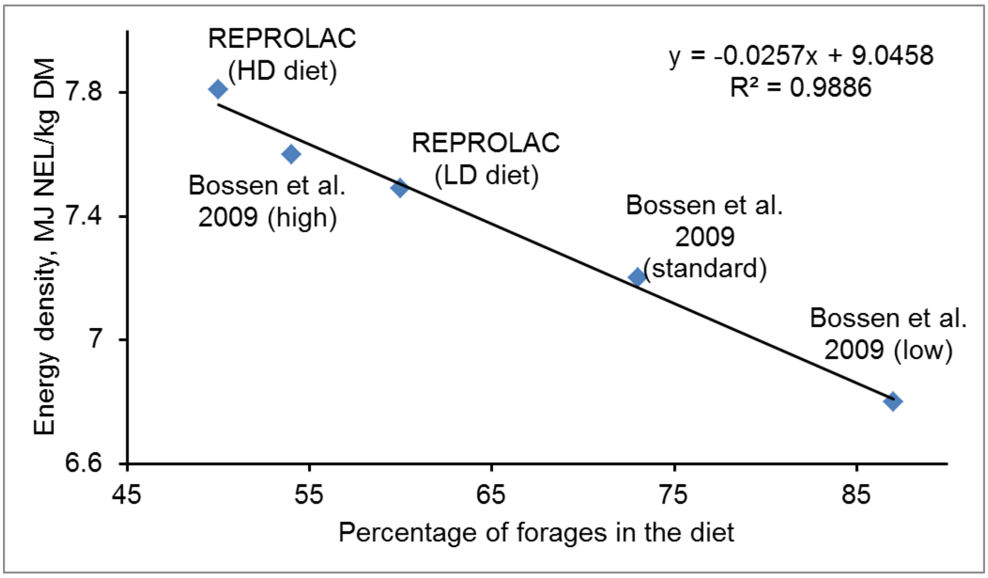

Figure 12 - Energy density regarding the percentage of forages in the diet. Combining data from REPROLAC and Bossen et al. (2009)

Friggens et al. (2003) describes that the cow has a target level of body fatness, which was maybe reached already with our standard LD diet and, consequently, the HD diet was not able to improve it further.

Individual shift criteria. The LD-HD cows were individually shifted after 42 DIM but not before they reached a LWg ≥ 0 on a 5 days average. A day of shift was also calculated for the LD-LD cows using the same criteria; even so they were fed the same diet for all the lactation. The results show that the average day of shift for all cows is equal to 49.7 d with a standard error of 7.1 d without difference between feeding strategies. Most of the cows (84%) had a day of shift between 42 and 55 d after calving (Figure 13). This standard deviation of 15 days indicates that the duration of the mobilization period vary between cows as also found by Bossen and Weisbjerg (2005). This individual variation concerning the end of the mobilization period confirms the interest of shifting the diet of the cows individually instead of doing it at a fixed common day.

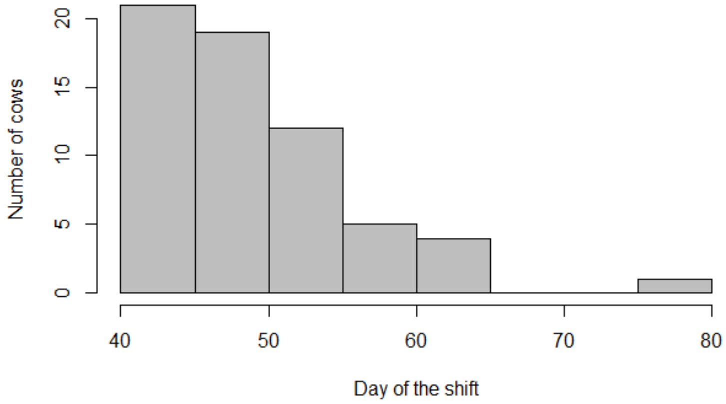

Figure 13 - Histogram of the number of cows per day of shift

Overall performance. In the REPROLAC herd, 85% of the Holstein cows (i.e. 53 cows among the 62) succeed to maintain a lactation longer than 440 d (16 months lactation), on average of 461 ± 12 d. Among the 9 cows with a lactation lower than 440 d, 6 multiparous cows were culled from E Coli infection in mid-lactation (summer period), and 3 multiparous cows were dried-off before 440 d due to low milk yield or mastitis. These 9 cows were equally distributed between the two feeding treatments. The 53 cows that succeeded to maintain 16 months lactation produced on average 14,773 ± 901 kg of milk during their lactation (15,799 kg for the multiparous cows and 13,746 kg for the primiparous cows). These production values are slightly lower than those of Lehmann *et al.* (2016) recorded at a private farm (Herd 1, Holstein cows, conventional system, 16 months lactations, average of 16,000 kg of milk per cow's lactation), and of Jankowska *et al.* (2012) with unplanned extended lactations longer than 420 d (average 16,497 kg of milk per cow's lactation). Lactation above 16 months are feasible by high yielding Holstein cows, and might as well increase the lifetime duration of the cow as suggested in paper 5. An improved longevity has been reported to lower environmental impact as 27% of the methane produced from a dairy farm is due to the herd replacement heifers (Garnsworthy, 2004). Extended lactation management would reduce the number of replacement heifers and, consequently, the methane emissions. Additionally, predictions model of Knapp *et al.* (2014) shows that the enteric methane emissions per unit of ECM (g/kg) are reduced with increased productivity (kg milk per cow per year) and feed efficiency. In the present experiment, the productivity and the efficiency of the cows managed for 16 months lactation were higher than those of the cows managed for 10 months lactations based on model calculations (0.47 vs. 0.43%, respectively, for the lifetime efficiency, and 0.46 vs. 0.39%, respectively, for the pregnancy rate)(paper 5).

1.2. Effects of the individualized weight-based feeding strategy on the intensity of the mobilization period

Mobilization intensity reduced. As presented previously, the shift from mobilization to deposition occurred at around 49.7 ± 7.1 d for all the cows and was as such independent on the feeding strategy in early lactation. The results presented in papers 1 and 2 also indicate that the HD diet reduced the intensity of negative EB in early lactation compared with the LD diet. This result is in accordance with Bossen *et al.* (2009) who found that, in early lactation, the cows fed a high energy density diet mobilized less than the cows fed a standard energy density diet. In Bossen et al. (2009), the level of mobilization, indicated by the LW loss, was higher for the cows fed a high energy diet than for the cows fed the standard diet (39 and 66 kg, respectively). This is in accordance with the results of the present experiment. In this thesis, blood markers also indicated a reduction of the intensity of mobilization for the HD-LD cows compared with that of the LD-LD cows. So, the cows fed the HD diet had lower BHBA and NEFA levels, higher glucose concentration, and lost less weight during the mobilization period than the cows fed the LD diet. These results are in accordance with other studies where an high energy diet in early lactation increases plasma glucose (Jenny and Polan, 1975, Sutton *et al.*, 1986), and decreases plasma BHBA (Andersen *et al.*, 2004) and NEFA (Dhiman *et al.*, 1991, Nachtomi *et al.*, 1991), compared with a standard diet. Kessel *et al.* (2008) also established that the BHBA is the best single blood marker to indicate EB.

Effect of parity on the use of the extra energy. The multiparous cows fed the HD diet also had higher milk yield than the multiparous cows fed the LD diet from calving to 42 DIM. Concerning the primiparous cows, a difference in milk yield already existed between the 2 groups in the first week after calving, indicating that the heifers were not well-balanced regarding their initial potential milk production. At 42 DIM the difference in milk yield between the 2 primiparous groups was the same as the one found in the first week of lactation which indicates that the extra energy offered by the HD diet was not used to produce more milk. In early lactation, the HD-LD primiparous cows gained weight compared with the LD-LD primparous cows but they had a constant BCS which can indicate that they used the extra-energy provided by the HD diet for growing and not for depositing subcutaneous fat. This is in accordance with previous studies showing that the primiparous cows have a partitioning of energy between milk and gain, which is different from the multiparous cows, as primiparous cows prioritize body growth (Spahr *et al.*, 1993, Bossen and Weisbjerg, 2009, Jensen *et al.*, 2015).

Effect of parity on the hormonal concentrations. It has been shown that the cows with reduced blood IGF-1 after calving had a stronger uncoupled somatotropic axis and, consequently, they produce more milk than the cows with higher IGF-1 concentration postpartum (Pereira *et al.*, 2010). After calving, the GH becomes uncoupled with the IGF-1 production by the liver because of down-regulation of liver GH receptors (Lucy, 2001). The somatotropic axis will then recover around 7 weeks postpartum, so that GH is again able to stimulate liver IGF-I synthesis (Butler *et al.*, 2003). For multiparous cows, after calving, IGF-1 concentration was quite low compared with the rest of the lactation, while for primiparous cows IGF-1 concentration did not vary a lot during the lactation. This is in accordance with Wathes *et al.* (2007a) and Taylor *et al.* (2004) who found that IGF-1 was greater in young animals on account of IGF-1s role in regulating growth.

1.3. Effects of the individualized weight-based feeding strategy on the duration of the mobilization period

Production data. In paper 2, the production data (LW, BCS, EB calculations) were used to define when the end of the mobilization period. LW and BCS increased right after the shift so these parameters indicated that the mobilization period ended at the day of the shift. The EB calculation indicated that all the cows were still slightly in negative EB 15 days after the day of the shift defined by the LWg. So, based on the EB calculations the day of shift seems a little too early (15 days too early). One explanation is that the LW registrations were not corrected for milk or rumen/gut fill (Thorup et al, 2012) so the minimum LW might have been overestimated and the LW loss underestimated during the mobilization (up to 5-10 kg; Bossen (2008)). Modification in the shift criteria might improve the precision of the day of shift based on LW measurements, by simply adding 15 days to the decision criteria. With this adjusted and, still, individualized shift criteria, all our cows would have been in positive EB right after the shift. Another solution to improve the precision of the day of shift would be to look at another measurement, for example blood metabolites, to complete and adjust the information given by the LW on the energy status of the cows.

Metabolite data. During the first week following the shift in diet, the HD-LD cows needed an adaptation time to the new diet lower in energy. To compensate for the decrease of energy in the diet, the HD-LD cows increased their mobilization as indicated by higher concentrations of plasma BHBA and NEFA after the shift compared with those before the shift (+ 0.18 ± 0.05 mmol/L and + 0.04 ± 0.004 mmol/L, respectively) and lower concentration of plasma glucose (- 0.05 ± 0.005 mmol/L), which reached the same levels as the LD-LD cows' concentrations (Figure 14). Accorsi *et al.* (2005) also reported high NEFA levels (around 0.3-05 mmol/L) as sign of mobilization from the adipose tissue.

Similar results have been found in experiments with restrictively-fed cows. Nielson et al. (2003) fed cows ad libitum followed by restricted feeding. Feed restriction increased the plasma concentrations of NEFA and BHBA by 121 and 90%, respectively, while glucose concentration decreased by 19%. Bjerre-Harpoth et al. (2012) also fed cows restrictively in early lactation for 4 days (at around 7.0 ± 3.1 weeks in milk), and observed a decrease in plasma glucose and an increase of BHBA the days following the restriction. The first weeks following the shift in diet, no significant differences of plasma glucose, BHBA or NEFA were found between the HD-LD cows and the LD-LD cows. Moreover, the end of the mobilization period was similar for both feeding strategies (49.7 ± 7.1 d, P = 0.14). This result is not in accordance with Bossen et al. (2009) who found that feeding according to LW can be used to manipulate both the intensity and the duration of the mobilization period especially within multiparous cows.

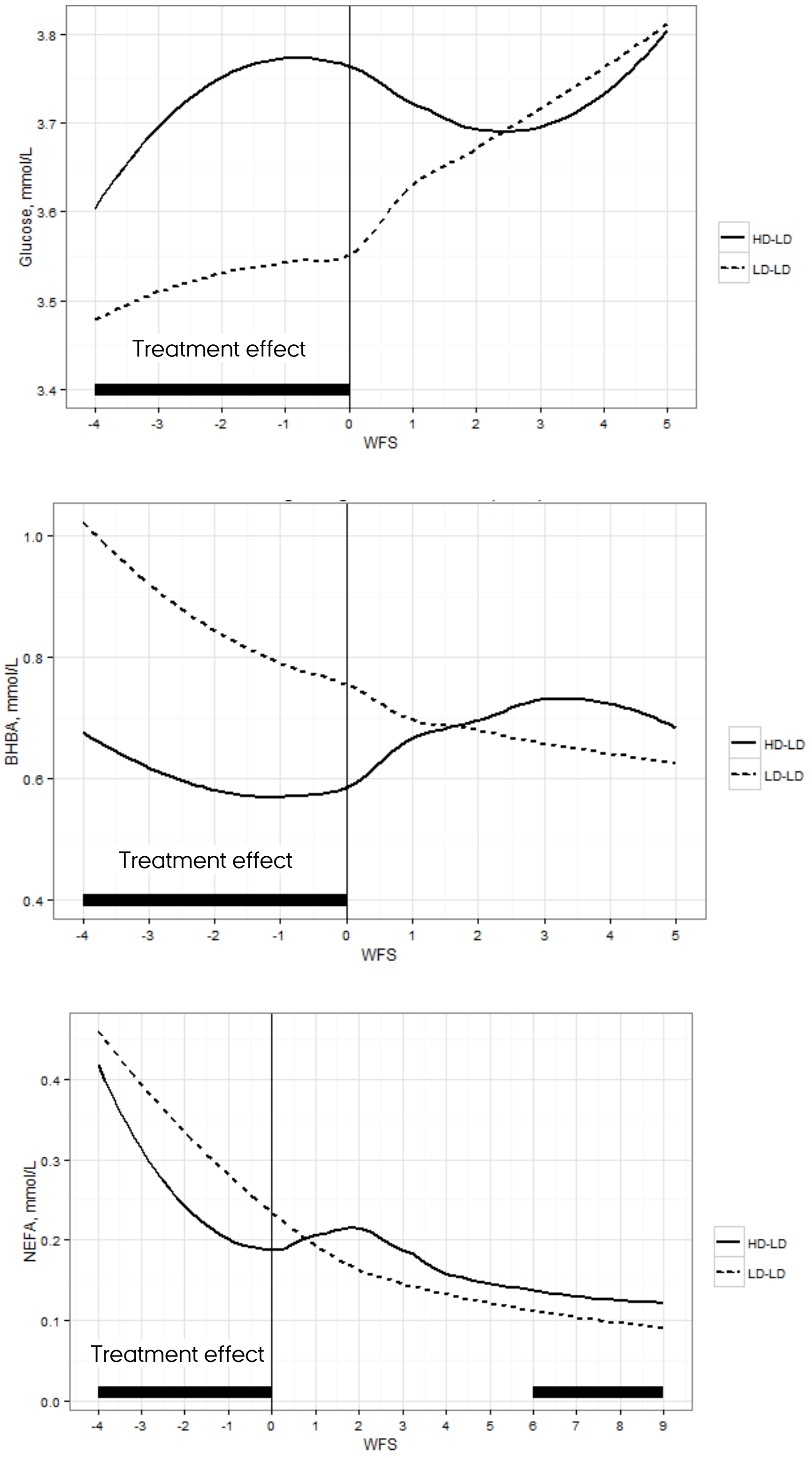

Figure 14 - Glucose, BHBA, and NEFA concentrations regarding week from shift (WFS) and the feeding strategy (HD-LD or LD-LD). The significant effect ($P < 0.05$) of the feeding strategy (treatment) is indicated by the horizontal bars on the graphs.

Effect of the feeding strategy on NEFA concentration. Figure 14 shows that the NEFA concentration of the HD-LD cows was significantly higher from 6 WFS to 9 WFS, and the Figure 2 of the paper 1 indicates that this difference was maintained until week 36 from calving. After the shift, the difference observed between the feeding strategies in plasma NEFA from 6 WFS actually existed from 2 WFS, even so it was not significantly different from 2 to 6 WFS. This absence of statistical significance before 6 WFS is probably due the high individual variation during that period as the cows adapt individually to the new diet. This late difference in NEFA concentration between the diets is also bringing up the question of the correlation between the NEFA concentration and the mobilization period. Indeed, NEFA is considered to be a good indicator of EB and, consequently, of mobilization (Accorsi *et al.*, 2005). But in the present experiment, in mid-lactation the EB was positive and didn't turn negative at any moment for the rest of the lactation. Moreover, the plasma NEFA concentration is connected with the fat mobilization and also with the milk production, but there was no difference observed in the milk production between the groups when the NEFA difference was observed in mid-lactation. As shown by Chelikani *et al.* (2004), NEFA concentration varies in the opposite way as IGF-1 and insulin concentrations. These two hormones are regulating the physiological adaptation mechanism which prioritizes milk production (Pereira *et al.*, 2010). In the REPROLAC experiment only 4 measurements of these 2 hormones were taken (at weeks 3, 5, 12, 24), with a feeding strategy effect only observed on IGF-1 at week 3 after calving (P = 0.03, HD-LD > LD-LD). To find a physiological reason for this observed NEFA difference between the two groups in mid-lactation would require more measurements of IGF-I and NEFA.

Another point to notice is the absence of a difference in BHBA levels between the HD-LD and LD-LD cows after the shift in diet. BHBA and NEFA were established as good indicators of mobilization reacting the same way (Dhiman *et al.*, 1991, Nachtomi *et al.*, 1991, Lomander *et al.*, 2012), but in the present experiment it is not entirely the case. After the shift in diet, BHBA concentrations were not different between the LD-LD cows and the HD-LD cows while a difference was observed between NEFA concentrations. This is in accordance with the results of McCarthy *et al.* (2015), showing that there is only a weak correlation between NEFA and BHBA.

1.4. Effects of the individualized weight-based feeding strategy on the lactation persistency

Negative carry-over effect on late persistency. The results presented in paper 2 show that the HD diet given in early lactation had no positive carry-over effect on the milk production in late lactation, but had a negative carry-over effect on the persistency of the lactation. These results are surprising as the HD-LD cows were expected to produce more milk than the LD-LD cows at least until mid-lactation as previously found in Bossen and Weisbjerg (2009), but also because the lactation persistency of the HD-LD cows was expected to be higher or at least similar than the one of the HD-LD cows. Moreover, the HD-LD and LD-LD cows had the same lactation duration (461 ± 12 d), and the same numbers of days from calving to conception (243 ± 9 DIM), which makes this difference in persistency difficult to interpret. Everson *et al.* (1976) and Cassel *et al.* (1984) found that the positive effect of a high energy diet in early lactation is often counterbalanced by a reduced milk yield persistency when the diet energy is reduced. This might be an explanation for the negative carry-over effect of the HD diet on the late persistency of the HD-LD cows.

Persistency in late lactation. A lower persistency was observed for the HD-LD cows in late lactation compared with the LD-LD cows and explained by a "negative" carry-over effect from the HD diet. But this might not be a "negative" event. For example, considering a cow successfully inseminated at the planned time (i.e. 8 months for a 16 months lactation), if the persistency is still high in late lactation, the milk yield at the planned drying-off time might be too high again, or the dry period might be shortened to avoid drying off at high milk yield. Several studies have reported the effect of the dry period length on the milk yield of the next lactation. They all agree that the mammary tissue benefits from a dry period and, consequently, the milk yield of the following lactation, but they are not in accordance concerning the optimal dry period length. Some studies reported that a dry period of 60 d is the optimal one in terms of milk production for the next lactation (Makuza and McDaniel, 1996, Mantovani *et al.*, 2010, Atashi *et al.*, 2013), while others support a dry period between 30 and 40 d (Bachman and Schairer, 2003, Gulay *et al.*, 2003, Pezeshki *et al.*, 2007). If too long lactation (> 16 months) are planned to avoid drying-off at high milk yields, other variables might change and this management might become less advantageous at the end. For example, as presented in paper 5, cows managed for lactations longer than 16 months would have lower lifetime efficiency (milk yield/dry matter intake) than if they were managed for 16 months. Longer lactations also induce more compromises for individual cows as not all the cows are able to manage a very long lactation. Moreover, longer lactations can lead to high BCS and fat cows at drying-off which have been shown to create problems in the next lactation (Bjerre-Harpøth, 2015).

Feeding strategy and milk yield at drying-off. The HD-LD cows had lower persistency in late lactation and consequently lower milk yield at drying-off than the LD-LD cows. The effect of milk yield level at drying-off on the health of the cow, and on the milk production in the next lactation, need to be studied. During drying-off time, the epidemiology of new intermammary infections and the persistence of these infections up to the next lactation are well recognized (Natzke *et al.*, 1975, Eberhart, 1986). Antibiotics are the main solution to cure the animals at this stage, and several techniques have been developed to enhance the efficacy of these antibiotic treatments (Dohoo and A.H., 1982, Eberhart, 1986, Hogan and Smith, 1994). Drying-off technique (abrupt or intermittent) and milk yield at drying off also impact the health of the animal. Indeed, if the cow is still producing high amounts of milk at drying-off, the intra-mammary pressure is high resulting from a gland that is not being evacuated which may cause leakage from quarters, thus allowing the bacteria to penetrate the teat canal and colonize the gland. Moreover the stagnant milk contained in the mammary gland at drying-off is low in immunoglobulins, so a high milk yield at drying-off would increase the risk of infections (Bushe and Oliver, 1987, Paape *et al.*, 1992). Using an intermittent cessation of milking might help reducing the infections (Oliver *et al.*, 1990). Until now, the 'optimal' yield at drying-off has not been defined even though, considering non-infected cows at drying-off, no associations between the yield at drying-off and the milk production in the next lactation have been found.

2. Relationships between EB, and production/physiological variables

2.1. EB calculation and end of mobilization period

In the first 3 papers, the daily EB (MJ NE_L/d) has been defined as the energy input minus the energy outputs (Butler and Smith, 1989, Heuer *et al.*, 2000, McNamara *et al.*, 2003a):

$$EB = E_{intake} – (E_{lact} + E_{maint} + E_{act}),$$

$$E_{lact} = ECM \text{ x } 3.14,\ E_{maint} = LW^{0.75} \text{ x } 0.08 \text{ x } a,\ E_{act} = LW \text{ x } 0.01 \text{ x } b$$

The E_{intake} is the total energy intake (MJ NE_L/d, with 1 Scandinavian Feed Units = 7.89 MJ NE_L/kg, Strudsholm *et al.* (1997)). The E_{lact} is the daily energy required for the milk production (MJ NE_L/d) with ECM the energy corrected milk (kg ECM/d), and 3.14 the energy needed to produce 1 kg ECM (MJ NE_L/kg ECM) (Sjaunja et al., 1991). The E_{maint} is the daily energy required for maintenance (MJ NE_L/d), where the maintenance requirement for NE_L is set at 0.08 Mcal/kg LW0.75 (NRC, 2001), "a" is the coefficient of conversion from Mcal to MJ, equal to 4.184, and LW is the live weight (kg/d). The E_{act} is the energy required for activity of loose housed cows (MJ NE_L/d), 0.01 corresponds to the 10% activity for voluntary activity (MJ ME/kg) (NRC, 2001), and "b" is the coefficient of conversion from ME to NEL, equal to 0.65.

In the following sections, it has been chosen to consider this calculation as the "reference" to evaluate the EB of dairy cows, and to define the end of the mobilization period (when EB = 0). This choice is debatable for several reasons. First, the energy intake is calculated according to one feed evaluation system (Feed Units system) so the result might be slightly different with another system. Then, the LW data are used to calculate the energy for maintenance and activity, but these data were not corrected for rumen-gut fill and milk produced. Secondly, this equation excludes the energy needed for growth (in the first and second parity) and pregnancy as both have been shown to represent less than 1% of the energy output (Friggens *et al.*, 2007b). However, this calculation seems to be one of the more precise tool to evaluate EB as described in the background part of this thesis (2.2.), and it has been used is several studies (Friggens *et al.*, 2007b, Liu *et al.*, 2009, Moyes *et al.*, 2013, van Knegsel *et al.*, 2014). Researchers are also working on improving the EB equation, mainly by adjusting the variables for example for gut-fill for the LW (Thorup *et al.*, 2012).

In practice, it is not easy to do such a calculation as it requires all the input and outputs measurements. It is often that the LWg alone is used to evaluate EB on real time and take management decisions (Maltz, 1997, Bossen *et al.*, 2009). Other approaches to estimate the EB are presented in the background of this thesis (2.2.) mainly showing that the blood or milk markers can give indication about the energy status of the cows (Roche *et al.*, 2009, McArt *et al.*, 2012, Suthar *et al.*, 2013, Berge and Vertenten, 2014, Larsen and Moyes, 2015). In the present thesis the LWg was used in practice to determine the day where the mobilization period ends (DEM) nearly equivalent to the day of diet shift of the REPROLAC experiment defined when LWg ≥ 0 on a 5 days average and when DIM ≥ 42. It might also be possible to estimate a DEM based on changes in some other measurements for example by utilizing the inflexion point or the point where two tangents are crossing. As a first approach, the Table 7 presents the DEM found with the different measurements based on the average curves of the 62 cows from the experiment. For some measurements it was not possible to find a DEM with this manual technique based on an average curve. An individual analysis might help finding out a DEM of these measurements. For the measurements where a DEM was found, there was no difference of DEM between the feeding strategies which is in accordance with experimental results showing no difference in the day of shift between the feeding strategies.

Table 7 - Day where the mobilization period ends (DEM) defined with different measurements

Measurement	DEM Multiparous	DEM Primiparous
EB calculation	**54**	**70**
LWg	50	52
DMI ECM	Not visible on the average curve	
Fat in milk	56	66
Protein in milk	51	49
Lactose in milk	51	38
Cells in milk Ratio Fat/Protein BCS	Not visible on the average curve	
NEFA	55	55
BHBA	87	111
Glucose	78	78
Urea	Not visible on the average curve	
Uric acid	66	66

Insulin and IGF-1 measurements are not presented in Table 7 as only 3 measurements were taken for these two hormones due to resources constraints. Even if they might bring interesting information about EB, they don’t offer a practical and financial alternative to the actual EB estimations. The milk composition should as well be automatized to obtain daily measurements if it is used as EB indicator. Otherwise, weekly milk composition might be combined with another parameter to define the DEM. The plasma measurements might as well be not easy to use as indicator of EB in practice, but the combination of daily plasma measurements and LWg might improve the detection of the end of the mobilization. For the multiparous cows, five of the measurements (LWg, fat, protein, lactose, NEFA) gave a DEM close to the DEM of EB calculation, while for the primiparous only the fat in milk and uric acid gave a DEM close to the DEM of EB calculation. This might indicate that more physiological changes occur in early lactation for the multiparous cows than for the primiparous cows, and consequently feeding an enriched diet in early lactation with a shift at the end of the mobilization might be of less importance for the primiparous cows than for the multiparous cows. The Table 7 also shows the difficulties to find the DEM for the primiparous cows as those obtained with the different measurements are quite varying. This might be due to the flatter milk curve of the primiparous cows compared with the multiparous.

2.2. Classification of the indicators of EB

Is LW the most appropriate to define EB, mobilization and deposition periods? It has been shown that LW recordings not corrected for rumen/gut fill overestimate minimum LW and underestimate LW loss during the mobilization (5-10 kg, Bossen (2008)). Corrections of LW improve greatly the precision of LW and EB calculations but these corrections are not automated yet so in practice they can’t be done in “real time” (Thorup *et al.*, 2012). Several studies found that acetone in milk, measured several times per week, might be a practical and accurate tool to estimate EB as it reflects the degree of mobilization (Clark *et al.*, 2005, Pedernera, 2008). Bjerre-Harpøth (2012) found that free glucose and isocitrate in milk can be used as indictors of EB. In the same order of idea, plasma measurements could be used to estimate EB. The combination of plasma measurements with production measurements could also be used to estimate where the energy is balanced although Reist *et al.* (2002) found that using blood measurements in addition to milk traits did not improve the precision of EB estimation.

Correlation between LWg and EB calculation was taken as a reference, to compare correlations between EB and other measurements (Table 8), and consequently classify the potential of the measurements as indicator of EB. Coefficient of correlation between EB and each measurement was calculated at 3 different weeks (3, 12, 24) representative of different phases in the lactation as explained in paper 1. The primiparous and multiparous cows were separated for the analysis, as the values for their production and physiological measurements are quite different (papers 1 and 2).

Table 8 - Correlation coefficients between EB and several measurements, at different time point of the lactation (early lactation at 3 weeks, mid-lactation at 12 weeks, and later in the lactation at 24 weeks)

	Multiparous			Primiparous		
Correlation	Week 3	Week 12	Week 24	Week 3	Week 12	Week 24
EB-LWg	**0.39**	**0.23**	**0.50**	**0.59**	**0.26**	**0.73**
EB-fat	-0.39	-0.31	0.52	-0.25	-0.39	-0.77
EB-protein	-0.09	-0.11	0.40	0.57	-0.38	0.26
EB-(fat/prot)	-0.39	-0.31	0.35	-0.58	-0.39	-0.71
EB-lactose	0.44	0.14	0.17	0.53	0.25	-0.60
EB-NEFA	-0.46	-0.34	0.01	-0.69	-0.04	0.34
EB-BHBA	-0.48	-0.47	-0.27	-0.77	-0.06	-0.42
EB-glucose	0.29	0.21	0.17	0.87	0.54	0.56
EB-uric acid	0.11	0.12	0.39	0.71	0.46	0.22
EB-BCS	0.39	0.09	0.47	0.71	0.46	0.22

LWg was strongly correlated with EB at weeks 3 and 24, but less at week 12. Fat in milk was also strongly correlated with EB, with even higher correlation coefficients than those of LWg-EB correlations, unless for primiparous cows at week 3. Fat to protein in milk ratio was as well strongly correlated with EB, a bit less at week 24. Reist *et al.* (2000) also found a correlation between EB and fat to protein in milk ratio. In early lactation, BHBA and NEFA were highly correlated with EB, but poorly later in lactation (week 24). Plasma glucose was positively correlated with EB during all the lactation, as also found by Clark *et al.* (2005) and Parker and Lewis (1977), however in the present study, the correlation was stronger mainly for the primiparous cows, not for the multiparous cows. For BCS, the correlation with EB was positive and strong at week 3 and 24 for multiparous cows, and at weeks 3 and 12 for the primiparous cows. Nevertheless, BCS measurements are subjective, not done often, and among several studies, Senatore *et al.* (1996) and Clark *et al.* (2005) found a poor relationship between BCS and EB which is not in accordance with the present finding. The work of Fischer *et al.* (2015) might help to add precision to the traditional BCS measurements by analyzing the rear shape of dairy cows in 3D. Other indicators not measured in the present experiment might be considered as well. For example, Clark *et al.*

(2005) found that acetone in milk might be a good predictor of EB, and the peak of acetone coincides with the decline in milk yield. However, daily variability in acetone level is high (Winterbach *et al.*, 1993).

To summarize, for the multiparous cows, LWg and fat in milk can be used together to estimate EB during all the lactation. This is in accordance with de Vries and Veerkamp (2000) and Heuer *et al.* (2000) who also found a strong positive correlation between fat in milk and EB in the first 4 weeks after calving. Then, added to fat in milk, the present results show that the NEFA and BHBA levels might as well be used to indicate the EB until week 12 after calving. These results are in accordance with Reist *et al.* (2000) and Clark *et al.* (2005) who found a negative correlation between NEFA levels and EB, and with Verkerk and Guiney (1999) and Kessel *et al.* (2008) who found a positive correlation between BHBA levels and EB. For the primiparous cows, LWg and fat in milk might as well be used together to estimate more precisely EB. However, in early lactation, EB-LWg and EB-fat correlation are weaker than later in lactation. In this period, EB estimation might be more accurate by looking at plasma glucose, plasma uric acid and BCS, highly correlated with EB. In both cases, these results show a possibility to define an index including several measurements to estimate EB, as it has been done by Moyes *et al.* (2013) combining NEFA, BHBA and glucose to define an index of physiological imbalance.

In general, fat in milk seems to estimate EB more accurately than LWg for both parities. In practice, it's easy to measure and it seems possible to increase the number of measurements per week. The end of the mobilization period can be defined by finding the change is slope (i.e. inflexion point) and the DEM obtained is similar to the one of EB calculation (Table 7). This work requires further analysis to define more precisely DEM, mainly by analyzing the individual curves and not the average curve of a measurement. This technique should as well be tested and developed to be used in real time and in practice.

3. Reproduction and life management

3.1. Effects of the feeding strategy on pregnancy rates

Feeding strategy. The results presented in paper 3 indicate that there was no effect of feeding strategy on cows' reproductive performance as hypothesized. However tendencies for higher pregnancy rates for the LD-LD cows were observed. This absence of feeding strategy effect on pregnancy rates might be due to the small energy density difference between the diets, the small number of animals, the fact that the first insemination occurs in mid-lactation while the different diets are fed the first weeks of lactation only, and the fact that both groups had similar BCS near insemination (> 3.10) (paper 2, Figure 7). On this last point, Carvalho *et al.* (2014) found that cows with high BCS (≥ 2.75) at insemination had more chances of becoming pregnant than cows with low BCS (≤ 2.50) (49.2% vs. 40.4% respectively). Only a few studies performed mainly in pasture based systems have investigated the effect of different levels of energy intake on cows' reproductive performance (Table 9). Most of them show no improvement in pregnancy rate. Even though these studies have been done on cows managed for 10 months lactation and not 16 months, it already indicates the difficulty to affect positively pregnancy rate with higher energy intake in early lactation.

Table 9 – Selected litterature about the effect of higher level of energy intake on reproductive performance of dairy cows managed for 10 monhts lactation

Reference	Effect of higher level of energy intake on reproductive performance
Dillon *et al.* (1997)	Improved
Fulkerson *et al.* (2001)	Improved
Logue *et al.* (1999)	No effect
Buckley *et al.* (2000)	No effect
Snijders *et al.* (2001)	No effect
Kennedy *et al.* (2003)	No effect
Pedernera *et al.* (2008)	No effect
McDougall *et al.* (1995)	Reduced

IGF-1 and reproduction. Several hormones and metabolic parameters have been proved to play a role in the relationship between EB and reproductive performance, mainly leptin, NEFA and IGF family, involved in the re-initiation of ovarian activity in post-partum dairy cows (Konigsson *et al.*, 2008). NEFA has already been discussed in the present thesis and recognized as a good indicator of EB (discussion part 2.). The relationship between plasma leptin, body fatness and feed intake is well established, the post-partum period of negative EB such as the delayed resumption of cyclicity is linked to reduced leptin levels (Konigsson *et al.*, 2008). However, leptin was not measured in the present experiment and will not be discussed further.

As the feeding strategy did not have major effects on cows' production and reproduction performance, in paper 3, an attempt to identify other factors being responsible for the results was made. Actual LWg for a cow was considered to be important. Thus, the cows were grouped according to their LWg in the first weeks of lactation (PLWG = the cows having a positive LWg in 1 of the first 5 weeks after calving, NLWG = the cows having a negative LWg in each of the first 5 weeks after calving). This grouping reveals that the individual effect is much more important than the feeding strategy effect as the HD-LD cows were almost equally split in the two LWg groups. The NLWG cows had lower EB in early lactation as they mobilized more body reserves than the PLWG cows; they also had lower IGF-1 concentration in the 12 first weeks after calving. This result in in accordance with Spicer *et al.* (1990) who found that serum IGF-1 concentration decreases with negative EB. Patton *et al.* (2007) and Pedernera *et al.* (2008) also found that cows with high concentrations of IGF-1 in plasma in early lactation are more likely to become pregnant than cows with lower IGF-1 concentration. In the present study, there was no difference in pregnancy rates between the LWg groups, even so this result might be due to the small number of animals which was limiting for evaluating parameters like pregnancy rates. IGF-1 measurements in early lactation (at week 3 and 5) might also be too few to get a strong estimate of IGF-1. On this last point, Falkenberg *et al.* (2008) shows that few measurements of IGF1 concentration in the postpartum period (5 measurements in that experiment) have very limited value to predict individual fertility in dairy cows (some fertility parameters: days open, calving interval, days to first service, and services per conception, the postpartum interval to commencement of luteal activity) (Darwash *et al.*, 1997). Using IGF-1 as a practical tool to predict the reproductive performance of a cow is quite controversial, although many studies consider it has potential (Pushpakumara *et al.*, 2003, Taylor *et al.*, 2004, Patton *et al.*, 2007).

Low pregnancy rates. Papers 3 and 5 reported a low pregnancy rate at first conception (40%) compared with other studies on Holstein cows managed for extended lactation (Table 5) where it averages 48%, or with the simulated pregnancy rate for 16 months lactation in paper 5 (46%).

REPROLAC Holstein cows were high productive cows. It has been shown that high genetic merit cows have lower pregnancy rates (Berry *et al.*, 2003, Veerkamp *et al.*, 2003). BCS at conception day was not correlated with interval from calving to conception (r = 0.23, P = 0.12) which is in accordance with Sorensen (2000) and indicates that EB was not the reason for low pregnancy rates at first insemination. The magnitude and severity of loss in BCS in early lactation has been shown to affect the reproductive performance (Domecq *et al.*, 1997). In the present experiment, BCS was only measured every 2 weeks, so there was not enough measurements in the first 2 weeks after calving to conclude whether or not a correlation existed between BCS loss during early lactation and reproductive performance. However, in paper 3, the LWg groups reflect the LW change during the first 5 weeks of the lactation. The pregnancy rates for the LWg groups were similar, and there was no correlation between LW change in early lactation and pregnancy rates, which is not in accordance with Domecq *et al.* (1997).

Paper 4 also indicates the difficulty in calculating reproductive performance (number of insemination to pregnancy, pregnancy rate at first and second inseminations) as some cows received a double insemination during the same estrus cycle. The way of recording this double insemination created difficulties to have the appropriate outputs. In paper 4, 'basic' calculations are introduced, representing the number of AI per pregnancy and the pregnancy rate excluding the double AI for the same estrus cycle. Compared with 'real' calculations, where all inseminations and results are recorded, the 'basic' calculations overestimate the pregnancy rates and underestimate the number of inseminations per pregnancy. These differences between the basic and real values did not affect our results comparing feeding strategies or LWg groups as the cows receiving the double inseminations were fortunately well balanced between the groups. However, for the farm statistics, the way they are recording the results will change drastically their conclusion on the reproductive performance of the cows having for example a pregnancy rate at first AI of 40% (real) or 60% (basic)! In papers 3 and 5 it was decided to keep the 'real' values of the reproductive outputs to avoid giving 'free benefice' to the extended lactation. As discussed before, the 'real' values were also closer to values reported in the literature than the 'basic' values.

3.2. Effects of delaying rebreeding on activity

Measurements and detection of the day of estrus. Cows' activity was recorded by a collar activity meter system mounted around the neck of each cow. The collar recorded the activity of each animal continuously and it was expressed as number of movements per hour. Lovendahl and Chagunda (2010) proposed to use these measurements, through a complex algorithm, to detect the day of estrus related with an increase of activity. They found that the activity measurements can bring some information about duration and intensity of estrus, but it is preferable to use milk progesterone measurement alone or in combination with activity measurements to detect estrus days. This is due to the high variability of the activity measurements, variability which can also be induced by external factor (visitors stressing the cows, new cow entering the pen etc.). These results are in accordance with the results of paper 3 showing high variability and difficulties to determine days of estrus based on activity measurements alone. In the present study, progesterone measurements in milk were used to determine the period of estrus, i.e. days where progesterone dropped below 3 ng/L (Lamming and Bulman, 1976), as it is recognized to be a precise method (Kerbrat and Disenhaus, 2004, Lovendahl and Chagunda, 2010). However, estrus has been shown to last only few hours (14.1 ± 4.5 hours in Kerbrat and Disenhaus (2004), 13.7 ± 6.5 hours in VanEerdenburg *et al.* (1996)) so the visual observations presented in paper 3 (mounting or being mounted, vulva swollen red or mucus discharge, presence of blood in the genital area) were used to select the precise day of estrus within the period defined by progesterone measurements. These observations were recorded twice a day. Mounting or being mounted is a well-known characteristic criteria to detect estrus (Desilva *et al.*, 1981, Helmer and Britt, 1985) but it seems very unprecise to use only visual observations to detect estrus. Even when using video recording of the cows, the proportion of cows expressing mounting behavior is quite low, i.e. less than 50% (Senger, 1994), with 53% standing estrus (Lyimo *et al.*, 2000), or 37% of estruses detected (Van Vliet and Van Eerdenburg, 1996). Kerbrat and Disenhaus (2004) also proposed to record other behavior only expressed on the day of estrus: chin resting, sniffing and licking ano-genital region, chin rubbing; but this require hours of video analysis which in practice is not realizable. Combination of progesterone measurements and selected visual observations (twice daily) seems to be a good and precise compromise. In the present case, the technique to detect the estrus days of each cow took some time as it was done manually, so for larger herds it might be a bit challenging. Indeed, it seems that no software or algorithm able to combine visual observations and progesterone measurements has been built yet. Homer *et al.* (2013) proposed a novel approach to detect estrus based on the idea that 'standing to be mounted' is the most definitive indicator of estrus. They detected estrus by measuring the relative position of cows in 3D with advanced technology (UWB technology). They showed that UWB technology detected 9 cows out of 10 in estrus, and was also able to indicate all the cows that were not in estrus.

Increase of activity. Compared with non-estrus days, the activity during estrus day was increased by17 movements per hour for all the 8 estruses studied. The number of cows expressing mounting behavior was higher at estrus 8 than at estrus 2 (63.3 vs. 45.9%). These results are in accordance with previous studies showing that activity and restlessness of the cows increased during estrus (Farris, 1954, VanEerdenburg *et al.*, 1996). As shown in the paper 3 (Figure 7), the activity nature of cows managed for extended lactation is changing over estruses toward spending less time walking and more time mounting. The fact that the number of cows expressing mounting behavior increased with estrus number is due to the relationship between body lipid reserves and reproductive cycle (Friggens, 2003). Spicer *et al.* (1990) found that cows expressing mounting behavior during the first and second estrous cycle had less negative EB than cows that did not express estrous behavior. Moreover, IGF-1 was identified as a mediator of EB related effects on luteal function as it stimulates progesterone production during diestrus (Schams *et al.*, 1988, McArdle and Holtore, 1989, Spicer *et al.*, 1990, Diskin *et al.*, 2003) and estradiol during estrus (Bauman and Currie, 1980, Adashi *et al.*, 1985, Spicer *et al.*, 1990, Adashi, 1998, Diskin *et al.*, 2003, Wathes *et al.*, 2007c). These findings are in accordance with ours as the IGF-1 level was higher for the PLWG cows than the NPLG cows, increasing over time and consequently was also higher at estrus cycle 8 than at estrus cycle 2. Vailes *et al.* (1992) showed that estrogen concentration has an effect on the expression of sexual behavior (i.e. mounting) (Figure 15). Mounting activity is negatively correlated to concentrations of progesterone (Alexander *et al.*, 1984) and positively with the estrogen concentration (Hafez, 1987, Roche, 2006). This is logical because ovulation occurs 25 to 32 hours after the onset of mounting/standing heat, so during heat, progesterone concentration is low (no corpus luteum yet), and estrogen concertation is high (produced by the follicle).

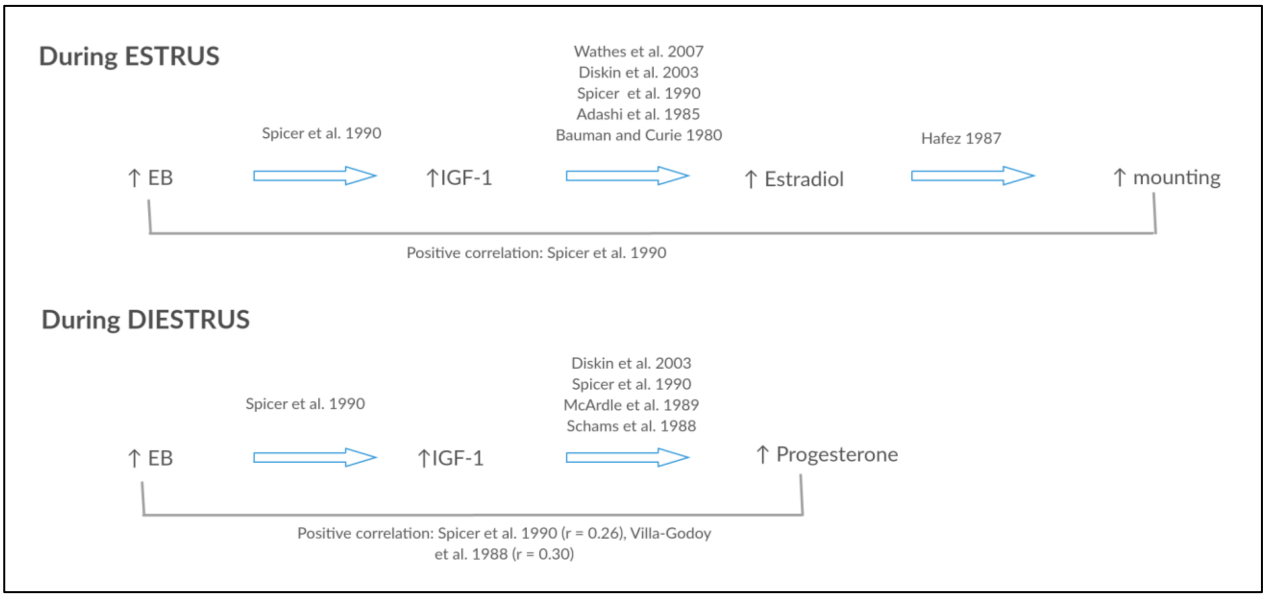

Figure 15 - Effect of EB and IGF-1 on estradiol, progesterone and mounting behavior, during estrus and diestrus

Although mounting behavior increased with estrus number (paper 3), pregnancy rates were not improved for a 16 months lactation compared with a 10 months lactation (paper 4). This result might be due to the small number of animals, or the variation between years as the comparison was done on the same cows but at different lactations and years. This result is not in accordance with Schindler *et al.* (1991) who found improved pregnancy rates with extended lactation, but is in accordance with Bertilsson *et al.* (1997). The present result also corresponds to the predictions presented in paper 5, where only lactations above 16 months had significantly higher pregnancy rates than the one obtained for a10 months lactation. However, the model used in paper 5 to predict the pregnancy rates doesn't take into account the possible health problems (i.e. disease) which have been shown to influence pregnancy rate (Esposito *et al.*, 2014).

3.3. Lifetime managements

According to the results presented in paper 5, the lifetime scenario optimizing the productive and reproductive performance for high yielding Holstein cows would be to have an extended lactation of 16 months at first parity, followed by 10 months lactations (EL-N scenario). The scenario where the cows were managed for 16 months lactation all their life also gave a similar performance to the EL-N scenario, but the outputs concerning life duration and number of lactations during lifetime, were quite high. The high values of lifetime predicted for extended lactation scenarios (i.e. 8 lactations for a 16 mo scenario) might be attributed to the fact that in the model lifetime is only defined by reproductive performance and culling rules, as the effects of diseases are not incorporated yet in the model (Phuong *et al.*, 2015a). However, it is not uncommon to observed cows with 8 lactations in old literature (Lush and Shrode, 1950). No other studies can be referred to evaluate these numbers which indicate the lack of knowledge on extended lactation systems in a lifetime perspective.

Primiparous cows have a lower peak yield than multiparous cows but their persistency is higher (Bar-Anan and Genizi, 1981) so they maintain long lactation with a decent daily milk yield in late lactation (Ratnayake *et al.*, 1998). As described previously, we also observed differences between parities in LW loss, metabolites and hormones concentrations. These differences indicate that primiparous and multiparous cows have a different nutrient partitionning, which may promote nutrient partitioning into growth as well as milk during the first lactation (Wathes *et al.*, 2007b). Extending the duration of the lactation of primiparous cows seems to be advantageous for the following lactations. Compared with cows managed for 10 months of lactation all their life (scenario N-N), or with primiparous managed for 10 months followed by 16 months lactation (scenario N-EL), the lifetime efficiency of the EL-N cows is significantly higher than the one of these 2 other scenarios. The N-N and EL-N scenarios have similar conception rates, lifetime duration and number of lactations (average of 3 lactations). The extended lactation seems more profitable for primiparous cows than multiparous cows as primiparous cows will benefit from it, in terms of efficiency, for all their life. There is no comparable previous studies on lifetime scale, but other studies at the scale of 1 lactation showed that extended lactation was more advantageous for primiparous cows than multiparous cows in terms of daily milk yield and economical profitability (Arbel *et al.*, 2001, Osterman and Bertilsson, 2003) (Figure 16). For multiparous cows, at the scale of one lactation, a 16 months lactation appears to be less advantageous in terms of daily milk production than a 10 months lactation, due to the fact that there are more days with low milk yield at the end of the lactation. However at the lifetime scale, the multiparous cows will benefit from longer lactation by increasing their efficiency and conception rates (paper 5).

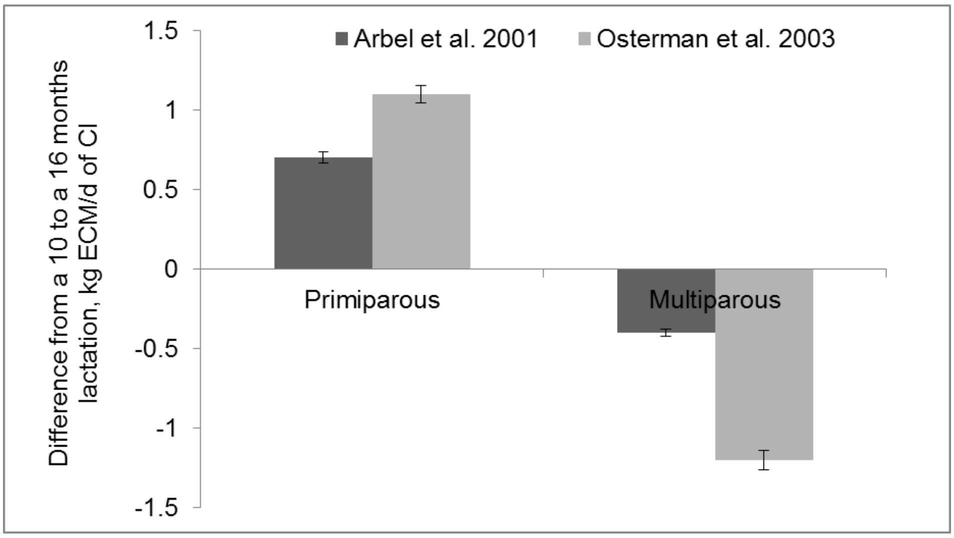

Figure 16 - Difference of milk production (in kg ECM/d of calving interval) from a 10 to a 16 months lactation

The positive carry-over effect of the extended lactation for primiparous cows on the following lactations is difficult to explain. Primiparous cows have a smaller population of secretory cells in early lactation than multiparous cows which is responsible of the lower milk production in early lactation and the flatter lactation curve (Miller *et al.*, 2006). However, due to higher mitogenic (i.e. IGF-1, Weber *et al.* (2000)) or survival factors (Flint and Knight, 1997) for primparous cows than multiparous cows, the mammary gland epithelial cells' rate of proliferation of primiparous cows is higher than the one of the multiparous cows (Miller *et al.*, 2006). So by extending the first lactation with high proliferation rate, it might give more time to the mammary gland to keep the memory of it for the following lactations compared to cows with shorter first lactation.

Fertility improvement. In the EL-N or EL-EL scenario, the extended lactation did not compromise the future reproductive success whether the following lactation is an extended or a 10 months lactation, in the opposite, it seemed to have a positive effect on pregnancy rates (paper 5). This result is in accordance with Sorensen (2000). Which factors could then explain an improved fertility for cows managed for 16 months lactation?

Firstly, the somatotropic axis plays an important role to define the level of fertility. A low postpartum insulin and glucose concentrations suppress the hypothalamic GnRH secretion and subsequent pituitary LH release (Diskin *et al.*, 2003, Ohkura *et al.*, 2004). The insulin stimulates follicular growth and maturation, so when it is reduced, like in early postpartum, it is linked with an ovarian dysfunction (Butler *et al.*, 2004, Vanholder *et al.*, 2005, Kawashima *et al.*, 2007). Consequently, low levels of insulin, IGF-1, glucose, and high levels of NEFA, urea, GH, and NEB in early lactation reduce fertility (Leroy *et al.*, 2008). For cows managed for 16 months lactation, rebreeding occurs at a positive EB (paper 3) with a 'coupled' somatotropic axis so the fertility should be increased compared with a cow rebred at 2 months after calving. However, it has been shown that there is a carry-over effect of the adverse metabolic conditions during early postpartum on the next follicles (Britt, 1992), less capable of producing adequate amount of estrogens and progesterone (following ovulation) which might lead to an oocyte of inferior quality (Britt, 1992, Roth *et al.*, 2001) and, consequently, a decrease in fertility (Carvalho *et al.*, 2014).

Secondly, metabolic and infectious diseases (Grohn and Rajala-Schultz, 2000, Santos *et al.*, 2004), and poor management conditions (housing, herd size, heat detection) also have negative effects on fertility (Leroy *et al.*, 2008). The parturition and postpartum period needs to be planned and managed carefully: a strict follow-up of the cow's health status is needed to prevent a drop in animal appetite and to limit the LW loss. Automatic and precise tools have to be used to follow the changes in LW or BCS, and an optimal feeding strategy is required. A reduced energy intake during the first weeks of the dry period, followed by an increase of energy supply (carbohydrates) after parturition reduce the LW loss and improve the metabolic status of the cow (Overton and Waldron, 2004). Additionally, the source of energy supply in the demanding early postpartum period might affect health and fertility (Grummer, 2007). For example, compared to a lipogenic diet, a glucogenic diet stimulates the energy partitioning towards body reserves in early lactation. In that case, the cows have a lower NEB, a reduced fat mobilization, and they resume ovarian activity early due to higher insulin levels (van Knegsel *et al.*, 2007a, van Knegsel *et al.*, 2007b). However, the cows fed the glucogenic diet also have a milk fat depression and less energy partitioned to milk (van Knegsel *et al.*, 2007a). This decrease in ECM yield in early lactation might not be a problem for cows maintained for 16 months lactation as persistency is negatively correlated with peak milk yield (Sorensen and Knight, 2002), so a lower daily ECM in early lactation might help achieving the extended lactation. However, although the use of diet energy is a potential tool to improve fertility (Grummer, 2007), it is not an easy factor to handle as shown by the results in paper 3 where no differences in pregnancy rates were found between our two feeding strategies.

Finally, the selection for milk traits has a negative effect on fertility, because milk traits are negatively correlated with fertility traits (Lucy, 2007). However, considering other traits for selection additionally to milk traits might improve fertility (Royal *et al.*, 2000). As it has been shown in paper 3, and in the previous paragraph, the PLWG cows had a tendency for higher pregnancy rates than the NLWG cows, so changes in BCS or LW in early lactation have a major impact on reproductive outputs. This loss of BCS depends on the available mass of adipose tissue but also on a genetically determined set-point of BCS which is correlated with reproductive outcome (Lucy, 2007). So, selecting for traits comprising changes in BCS early postpartum should be included in the selection criteria (Royal *et al.*, 2000, Leroy *et al.*, 2008).

CONCLUSION

The severity of the negative energy balance of the cows fed an energy enriched diet from calving until live-weight nadir (cows HD-LD) was reduced. This reduction was followed by lower concentrations of NEFA and BHBA, higher concentration of glucose, and higher milk production and live weight in early lactation than for cows fed the standard diet lower in energy (cows LD-LD). However, the mobilization period of the HD-LD cows was not extended beyond the shift of diet as indicated by similar metabolites concentrations between the HD-LD and LD-LD cows. Nevertheless, the plasma NEFA was higher for the HD-LD cows than the LD-LD cows from the time of diet shift until 36 weeks after calving indicating a carry-over effect of the early lactation HD diet to the late lactation metabolism for NEFA. The HD diet had no carry-over effect on milk production in mid or late lactation though the persistency of the milk production was lower in late lactation for the HD-LD cows than for the LD-LD cows.

The feeding strategy had no effect on pregnancy rates and number of inseminations per pregnancy. The pregnancy rates of a 16 months lactation were not improved compared to those of a 10 month lactation, although more cows expressed mounting behavior when rebreed at around 8 months compared with earlier rebreeding. The decrease in milk yield during estrus was marginal and similar in the consecutive estruses observed for 16 months lactation.

Based on model calculations, higher pregnancy rates were found for lactation above or equal to 18 months compared with 10 months lactation, but lifetime efficiency and milk yield per feeding day were lower. When cows were managed for 16 months lactation for the first lactation, followed by 10 months lactations, the lifetime efficiency was higher than the one observed for cows managed for 10 months lactations all their life, and similar to the one observed for cows managed for 16 months lactations all their life.

To conclude, the higher energy supply in early lactation did not improve productive or reproductive performance of high yielding Holstein cows. The GARUNS model was able to predict lifetime performance of Holstein cows managed for lactations of different durations. Based on model calculations, primiparous cows kept a life benefit in terms of efficiency from the extended lactation of 16 months, which was not the case for multiparous cows. These predictions are based on data from one lactation only and need to be confirmed by fitting lifetime data. Moreover, health factors need to be added to the model to give more precise outputs.

PERSPECTIVES

Which cows for extended lactation? Until now no precise criteria have been defined to determine which cows can be managed for longer lactation than the usual 10 months. In most of the studies of indoor Holstein cows, mainly high producing dairy cows were able to maintain longer lactations. Paper 5 showed that primiparous cows might benefit more from extended lactation than multiparous cows. LW or BCS changes in early lactation might also be interesting criteria to take into account to represent the individual variations between cows. The relationships between production and physiological variables with lactation duration should be studied to identify eventual criteria that can be used in the first 2 months of lactation, to decide if the cow would benefit from an extended lactation.

Diet and milking frequency. The feeding strategy proposed in this thesis can be improved. The daily concentrates allowed per cow can be adjusted every day, as proposed by the LELY milking robot, to optimize the individual energy requirement. Moreover, the milking frequency could be increased (i.e. to 3 times daily) in early lactation in addition to the energy enriched diet, as it has been shown that for 10 months lactation the effects of this combined strategy has a positive carryover effect on milk yield even when the number of milking is reduced in mid-lactation (Norgaard *et al.*, 2005).

Seasonal effect. In the present experiment, the seasonal effect on production and reproduction performances was not studied as the set-up was built up in a way that season would not affect our comparison between feeding strategies. It has been shown, that winter calving is more beneficial in terms of productive and reproductive performance, at least for cows managed for 10 months lactation. The effect of season on these parameters have not been studied yet on indoor dairy cows managed for extended lactations.

Social rank. The feeding strategy used in the present thesis took into account the individual LW changes. To go further into the understanding of the individual cow effects, the social rank and the behavior of each cow should be taken into account. A cow with high social rank will have an easier access to the feed bins, so she will get more fresh food than a low social ranking cow. Moreover, cows might be able to sort the feed while eating as they have a tendency to prefer the concentrate. Despite generally well-mixed TMR is fed, the feed eaten by a lower and higher ranking cow might not be the same in a nutrient point of view. Feed sampling of the leftovers could be analyzed to test this hypothesis.

Farm level. By using extended lactation management, some trade-off will be made and need to be studied to evaluate the feasibility and profitability of this management. Less concentrates will be needed to feed the cows (heifers and early lactation) but they will have more days with lower milk yield at the end of the lactation. More forages will be needed which will require changes in the land used in the farm. Fewer calves will be born per year which might require the use of sexed semen to make sure the born calves will be female which will be later used to renew the herd. Moreover, the longevity of the cows managed for extended lactation should increase otherwise it will be difficult to keep the number of animals per herd. The diminution of the number of bull calf per year will also induce a decrease in the calf meat production which will have to be counterbalanced in some ways.

Environment. More information is needed about the effect of extended lactation on the environment. Extended lactation should reduce the methane emissions thanks to the reduced number of replacement heifers, which are responsible of 27% of methane emissions on farms.

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Interest in extended lactation has increased this last decade to avoid rebreeding in a negative energy balance period and to exploit the full milk yield capacity of dairy cows. This PhD thesis focused on the effects of an early lactation feeding strategy on the productive and reproductive performance of Holstein cows managed for 16 months lactation. A lifetime prediction model was also used to determine the effect of lactation duration and parity on cows' lifetime performance. The new research findings contribute to the understanding of how to manage Holstein cows during their life taking into account individual energy needs, age and lactation duration.