Impacts of feeding intensity and breed on metabolism, negative energy balance and reproductive efficiency in dairy cows

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Abstract

Despite improved breeding, management, and nutritional strategies, decreased fertility in dairy cows is still widespread. Several studies have highlighted the unfavourable correlation between negative energy balance (NEB) and reproductive performance. However, there is a continuing need for more information regarding the effects of the interaction between different nutritional strategies with animals of different genetic background.

This thesis evaluated the effect of high energy-diet (HE) and low energy-diet (LE) on Holstein and SRB dairy cows. The HE represents what is, in general, common practice among most high milk producing herds. The lower feeding intensity may be representative of e.g. organic dairy producing systems.

In three studies, the metabolic status, milk yield, body condition score (BCS), and NEB were evaluated in cows fed either a HE or a LE diet.

Associations between NEB, plasma adipokines, metabolism, and reproductive parameters were also investigated.

Holstein cows had lower body condition score (BCS) than SRB cows within each energy-diet group. However, diet had no effect on BCS or subcutaneous adipose tissue thickness in Holstein cows irrespective of whether they received a HE or LE diet. The HE group tended to have a less severe energy deficit than the LE group. Holstein cows tended to be in a more severe energy deficit during the first 45 days after calving than SRB cows. Holstein had a lower nadir in energy balance than the SRB.

In conclusion, our results indicate that nutritional strategies might have a stronger association to endocrine and traditional fertility traits than breed. However, breed had a stronger association to the energy balance variables than nutritional strategies. In addition, SRB cows prioritized energy differently when compared to Holstein cows in such a way that the SRB cows maintained homeostasis better than Holsteins who had a deeper energy deficit than SRB cows.

Keywords: residual feed intake, transition period, progesterone, reproductive efficiency, fertility

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Inflytande av utfodringsintensitet och ras på negativ energibalans, metabolism och reproduktionsförmåga hos mjölkkor

Sammanfattning

Nedsatt fruktsamhet är ett stort problem i mjölkkobesättningar och leder till stora ekonomiska förluster för lantbrukare. Flera studier har visat att det finns ett ogynnsamt samband mellan negativ energibalans och fertilitet hos mjölkkor. Dock finns det ett behov av mer information kring samspelet mellan olika utfodringsintensiteter och djur med olika genetisk bakgrund.

Denna avhandling utvärderade effekten av hög (HE) och låg (LE) utfodringsintensitet hos mjölkkor av raserna Holstein och SRB. Den höga utfodringsintensiteten motsvarar den normala utfodringsintensiteten som tillämpas i högproducerande mjölkbesättningar. Den längre utfodringsintensiteten uppnåddes genom en lägre kraftfoderandel och en högre grovfoderandel i foderstaten. En lägre utfodringsintensitet tillämpas exempelvis generellt inom ekologisk mjölkproduktion.

I tre olika studier utvärderades metabolisk status, mjölkavkastning, hull (BCS) och NEB hos kor som hade en HE eller en LE utfodringsintensitet. Därutöver studerades möjliga samband mellan NEB, plasma adipokiner, metabolism och fertilitet.

Holstein-kor hade lägre BCS än SRB-kor. Utfodringsintensiteten hade däremot ingen effekt på BCS eller underhudsfettets tjocklek hos Holstein-kor. Energiförlusten efter kalvning tenderade att vara lägre hos HE-gruppen än hos LE-gruppen. Holstein-kor tenderade att ha ett större energiunderskott under de första 45 dagarna efter kalvning än SRB-kor. Holstein hade en djupare vändpunkt i den negativa energibalansen än SRB.

Sammanfattningsvis tyder våra resultat på att utfodringsintensiteten kan ha ett starkare samband med fertiliteten än ras. Dock hade rasen ett starkare samband med olika mått på energibalans efter kalvning än utfodringsintensitet. SRB-korna kunde behålla homeostas bättre än Holstein, som hade en större energibrist efter kalvningen än SRB-kor.

Nyckelord: energibalans, transitionsperioden, progesteron, nötkreatur, fertilitet

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Dedication

To my boys, Michail and Andreas

När du beträder hemfärden till Ithaka, så önska dig att lång blir denna resa och full av äventyr och full av lärdomar

Σα βγείς στον πηγαιμό για την Ιθακη, να εύχεσαι νάναι μακρύς ο δρόμος, γεμάτος περιπέτειες, γεμάτος γνώσεις.
Konstantinos Kavafis

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I T. Ntallaris*, P. Humblot, R. Båge, Y. Sjunnesson, J. Dupont, B. Berglund (2017). Effect of energy balance profiles on metabolic and reproductive response in Holstein and Swedish Red cows. *Theriogenology*, volume 90, pages 276–283.

II T. Ntallaris*, P. Humblot, R. Båge, Y. Sjunnesson, B. Berglund. Relationships between energy balance and reproductive performance in primiparous Holstein and Swedish Red dairy cows on two energy diets. (manuscript)

III N. Mellouk, C. Rame, J.L. Touzé, E. Briant, L. Ma, D. Guillaume, D. Lomet, A. Caraty, T. Ntallaris, P. Humblot, J. Dupont* (2017). Involvement of plasma adipokines in metabolic and reproductive parameters in Holstein dairy cows fed with different energy diets? *Journal of Dairy Science*, volume 100 (issue 10), pages 8518-8533

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The contribution of Theodoros Ntallaris to the papers included in this thesis was as follows:

I. Contributed to the conception and design of the data collection, organized and performed the collection of the samples, performed the statistical analyses and data management, drafted the manuscript, critically revised the manuscript and interpreted the results together with the co-authors. Corresponded with the journal.

II. Contributed to the conception and design of the data collection, organized and performed the collection of the samples, performed the statistical analyses and data management, drafted the manuscript, and interpreted the results together with the co-authors.

III. Performed the statistical analyses, helped in the interpretation of the results, and critically revised the manuscript together with the co-authors.

Additional publication related to this thesis

J Williams, T Ntallaris, JE Routly, DN Jones, J Cameron, A Holman-Coates, RF Smith, P Humblot, H Dobson. *Association of production diseases with motor activity-sensing methods and milk progesterone concentrations in dairy cows* (submitted manuscript)

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Abbreviations

AMH	Anti-Müllerian hormone
AMS	Automatic milking system
ANOVA	Analysis of variance
AUC 12 or 45	Total energy deficit within Days 5 and 12 or within 5 and 45 from calving
BCS	Body condition scores at different days (-30, -14, 14, 30) relative to calving
BFP – 15	Bone morphogenetic protein
CFI	Calving to first insemination
CL	Corpus luteum
CLA	Commencement of luteal activity after calving
CO_2	Carbon dioxide
DMI	Dry matter intake
dNEB	Total number of days after calving with negative energy balance
EB 12 or 45	Mean energy deficit within Days 5 and 12 or within 5 and 45 from calving
EB nadir	Energy balance at nadir
ECM	Energy corrected milk yield
ECM 12	Total energy corrected milk within Days 5-12 relative to calving
ECM 45	Total energy corrected milk within Days 5 and 45 from calving
ELISA	Enzyme-linked immunosorbent assay
FA	Fatty acid

FFA	Free fatty acid
FLI	First to last insemination interval
FOE	First ovulatory estrus
FSH	Follicle stimulating hormone
GDF – 9	Growth differentiation factor 9
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HE	High energy-diet (35 kg ECM / day)
IGFBP	Insulin growth factor binding protein
IGF-I	Insulin-like growth factor 1
IL - 1 or 6	Interleukin – 1or 6
ILI	Inter-luteal interval
IOI	Inter-ovulatory interval
LCFAs	Long chain fatty acids
LE	Low energy-diet (25 kg ECM / day)
LF	Large size follicles (> 7 mm)
LH	Luteinizing hormone
LHR	Luteinizing hormone receptors
LP	Luteal phase length
LSMeans	Least square means
MF	Medium size follicles (> 5 and ≤ 7 mm)
NEB	Negative energy balance
NEFA’s	Non-esterified fatty acids
NF-EEM	No fertilization or early embryonic mortality
P4	Progesterone
PFI	Pregnancy rate at first insemination
PGE, PGF	Prostaglandin E, prostaglandin F
PLP-LEM	Prolonged luteal phase or late embryonic mortality
SEM	Standard error of mean
SF	Small size follicles (3-5 mm)
SJB	Swedish Jersey breed
SKB	Swedish polled breed
SLU	Swedish University of Agricultural Sciences
SRB	Swedish Red breed
TGs	Triglycerides
TMR	Total mixed ratio

TNF-α	Tumor necrosis factor alpha
VFA	Volatile fatty acid
VLDL	Very low density lipoproteins
VMS	Voluntary milking system

1. Introduction

1.1 Dairy production in Sweden

The structure of the Swedish dairy herd has changed significantly over the past years (*Figure 1*). Even though the dairy sector plays a central role in Swedish agriculture, the overall number of dairy herds continues to decline. There were 44143 dairy herds in 1980 compared to 3872 in 2016, a decline of almost 88 percent (Swedish Board of Agriculture, 2017). During this same period, the dairy milk cow inventory declined from 656 thousand in 1980 to 331 thousand dairy cows in 2016. Despite the decrease in dairy milk herds and inventory, average size of herd increased from 15 dairy milk cows in 1980 to 85 in 2016. Milk production by the four most popular breeds in Sweden (Swedish Red SRB; Holstein; Swedish polled, SKB; and Swedish Jersey, SJB) was also increased from 5528 ECM (Kg of energy corrected milk) in 1980 to 8725 kg ECM in 2016, an increase of 63 percent. Herds with more than 75 dairy milk cows accounted for almost 64 percent of all milk produced in 2016, up from 15 percent in 1980. Conversely, herds with less than 75 dairy cows accounted for 36 percent of total milk production in 2016 compared to 85 percent in 1980.

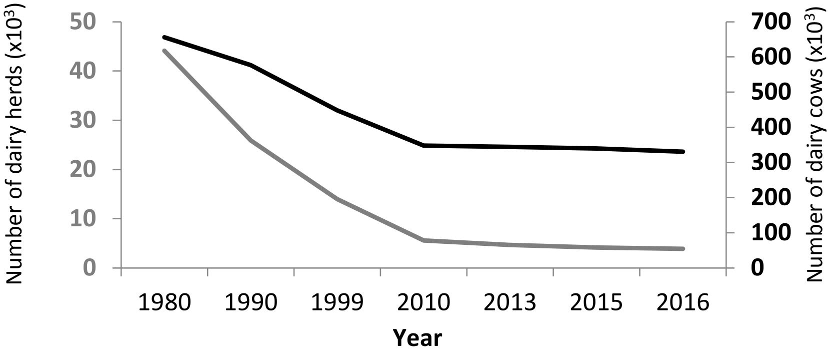


Figure 1. Distribution of herds and dairy cow population in Sweden (Swedish Board of Agriculture, 2017)

In conclusion, the number of small herds (< 75 dairy cows) continues to decline while the larger, more efficient herds continue to increase their share of dairy cow inventory and milk production.

In 2017, approximately 63.2 % of the dairy cattle population was localized in two regions located in the south of Sweden, where the largest herds were also found. The remaining 36.8 % were located in middle and northern Sweden (*Figure 2*).

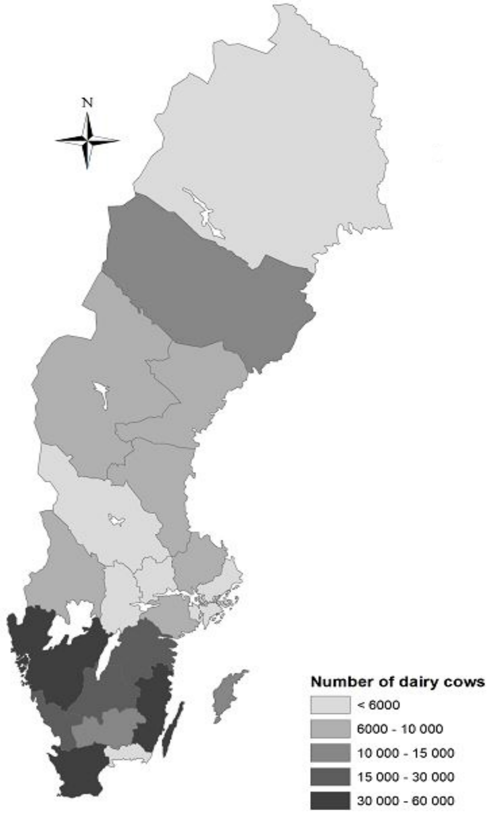


Figure 2. Population distribution of milking dairy cows in Sweden in 2017

In Sweden there are different types of systems (Table 1) used for milk production, as well as different buildings (Swedish Board of Agriculture, 2017). Almost 80% (n = 241 434) of the Swedish cow population is enrolled in the Swedish official milk and health recordings; SOMRS (Cattle statistics, 2017). The characteristics and distribution of the different breeds in Sweden are presented in Table 2. However, different breeds can often be found in the same herd. Holstein (54.8 %) and SRB (36.6 %) are the two most common breeds in Swedish dairy herds. Holstein is preferred due to their high milk production levels and SRB due to their robustness.

Table 1. Distribution between types of housing systems for milk production in Sweden (Swedish Board of Agriculture, 2017).

%	Herds	Cows
Conventional	85.8	84.2
Organic	14.2	15.8
Automatic Milking System	22.3	27.8
Loose housing system	36.7	50.6
Tied-up system	41.0	21.6

Table 2. Distribution of milk recorded cows and average yield per breed (Cattle statistics, 2017).

	Distribution (%)	ECM (kg)	Fat (%)	Protein (%)
SRB	36.6	9747	4.4	3.6
Holstein	54.8	10452	4.1	3.4
SKB	0.4	5716	4.4	3.6
SJB	0.8	8984	5.9	4.1
Others	7.3	9879	4.3	3.5

1.2 Reproductive performance of SRB and Holstein dairy cows

The extended calving interval between 1985 and 2016 for the extreme dairy Holstein cow and SRB dual-purpose type cow (*Figure 3*) shows the different rate of decline in their reproductive performance at. However, during the last decade the calving interval tended to become shorter in both breeds. It is also clear that SRB have slightly shorter calving intervals than

Holstein cows over the last three decades. It is known that calving interval can be affected by various parameters such as the voluntary waiting period before the first AI and by the interval between calving and last AI (CLI). However, the first to last insemination interval (FLI), is a less biased indicator of reproductive performance as it is not influenced by management and human decisions. The decrease in CFI interval in both breeds during the last decade is presented in *Figure 4*. The same differences and trends are also noticed for the CLI and FLI intervals (*Figures 4 and 5*, Table 3).

Figure 3. Average calving interval (months) for SRB and Holstein cows in Sweden during milk-recording year 1984/85 to 2015/16 (Cattle statistics, 2017).

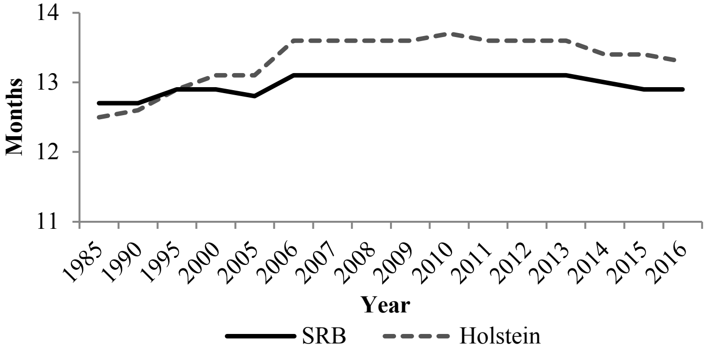


Figure 4. Calving to first insemination interval (CFI) and calving to last insemination interval (CLI) for SRB and Holstein cows in Sweden during milk-recording year 1984/85 to 2015/16 (Cattle statistics, 2017).

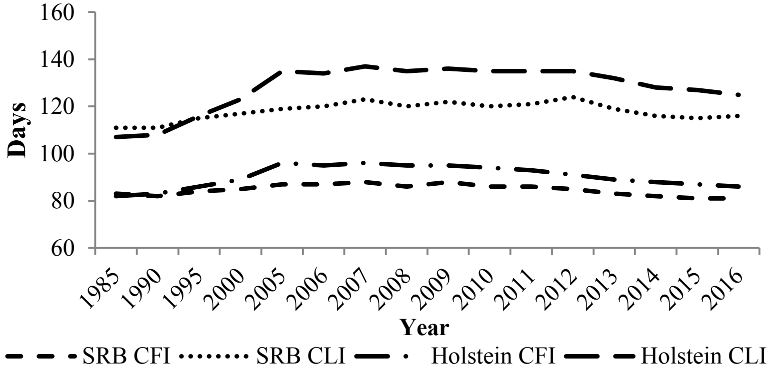


Figure 5. First to last insemination interval (days) for SRB and Holstein cows in Sweden during milk-recording year 1984/85 to 2015/16 (Cattle statistics, 2017).

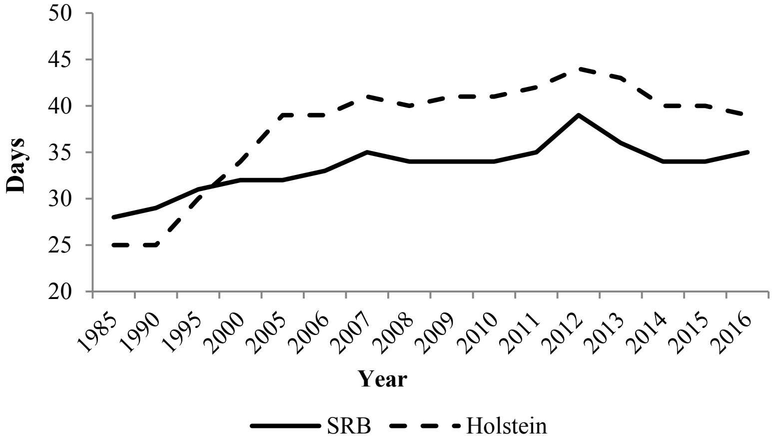


Table 3. Fertility data for the SRB and Holstein cows (Cattle statistics, 2017).

	SRB	Holstein
Herds	2381	2422
Age at calving	27.5	27.3
CFI[1]	81	86
CLI[2]	116	125
Calving interval (months)	12.9	13.3
total AI[3]	1.81	1.83
Culled due to fertility problems, %	6.4	5.8
Difficult calving, %	3.34* / 1.56	3.66* / 1.60
Still birth, %	5.32* / 3.75	8.34* / 4.27
Twins, %	1.03* / 3.47	0.98* / 3.58

[1] interval between calving and first AI (days)

[2] interval between calving and last AI (days)

[3] if we include the AI performed within 6 days after the first AI then percent is increased by 5.2

*first parity cows

1.3 Fatty acids metabolism

The process of lipolysis is controlled by complex mechanisms. In ruminants, the rumen together with the reticulum and cecum are the key actors in the metabolism of fatty acids (FAs) (Loften et al., 2014). Lipolysis, biohydrogenation, and microbial lipid synthesis are the three major processes that are taking place in the rumen and reticulum (Enjalbert et al., 2017). The end-products of lipolysis in the rumen and reticulum are free FAs (FFAs), also known as non-esterified fatty acids (NEFAs). After lipolysis, FAs are no longer connected to the glycerol backbone and they proceed further to cecum where they form triglycerides (TGs) after connecting with glycerol. Cholesterol, together with TG, will form the chylomicrons that are also known as very low density lipoproteins (VLDP). Those proteins can bypass liver metabolism and reach different target tissues such as udder, adipose tissue, or muscle via the bloodstream.

Volatile fatty acids (VFAs) have a central role in a ruminant's metabolism. Acetate and butyrate acids have lipogenic properties compared with propionate that has glycogenic properties (Black et al., 1966). Diet can influence the production and ratio of VFAs produced in the rumen (Van Dung et al., 2014). Forage-rich diets can promote acetate and butyrate production while a grain-rich diet can promote propionate production. There is a positive correlation between increased acetate and butyrate concentrations in the rumen with increased beta hydroxyl butyrate (BHBA) and NEFA concentrations in blood. In contrast, increased propionate concentrations in the rumen will promote gluconeogenic processes increasing glucose and insulin levels in blood (Brzozowska and Oprzadek, 2016). Whereas insulin will increase lipogenesis by inhibiting the above process, glucagon, corticosteroids, ACTH and catecholamines will promote lipolysis.

Overall, tissue mobilization results in the production of NEFAs which can be taken up later by the liver and esterified. In the hepatic mitochondria, fatty acids will undergo β-oxidation producing ketone bodies or they will enter the Kreps cycle producing propionate and carbon dioxide (CO_2). Fatty acids can also be stored in hepatocytes as triglycerides or transformed to very low density lipoproteins (VLDLs) and used by the mammary grand as milk fat. The metabolic balance is preserved by the parallel increase of FFA concentrations which will increase the concentration of glucose in the blood. However, the so called process of “fatty liver” begins, before the appearance of negative energy balance leading to further adaptation processes in an undesirable direction.

1.4 Postpartum ovarian cyclicity

1.4.1 Growth of follicles

Follicle growth on the ovaries is a long process taking place over a period of 3 to 4 months and can be categorized into two stages depending on their gonadotropin dependency (Webb et al., 2004, Crowe, 2008). During the gonadotropin-dependent stage, cohorts of antral follicles (1 to 4 mm) will grow in waves (Rajakoski, 1960, Savio et al., 1988) followed by either atresia or ovulation. During ovarian follicular growth, there is a transfer of dependency from FSH to LH, indicating the presence of a mechanism involved in selection of follicles for continued growth (Webb et al., 2004). Follicles will undergo recruitment and selection followed by a dominance phase. The recruitment phase is characterized by the growth of a cohort of small antral follicles (≥ 5 mm in diameter) and a transient increase in FSH secretion (Adams et al., 1992, Sunderland et al., 1994). During the recruitment phase, the levels of LH in blood and estradiol produced by the small antral follicles are low and inhibin is not being produced yet. During the selection phase some of the recruited follicles will undergo atresia while others will be selected to continue growth. The number of follicles that will be selected depends on the species and occurs in the phase of declining FSH concentrations in blood (Sunderland et al., 1994, Crowe, 2008). During this stage, follicles are producing moderate concentrations of estradiol while FSH, LH, and inhibin concentrations are low. Dominance happens when a large preovulatory follicle exerts a major inhibitory effect on other antral follicles from the recruited and selected cohort (Sunderland et al., 1994). During this stage, follicles are producing high levels of estradiol while inhibin, LH, and FSH concentrations in the blood are low. Dominance is followed by atresia or ovulation depending on the LH pulse frequency. During the luteal phase (in cyclic animals), LH pulse frequency is low, at 1 pulse per 4 hours, while during the follicular phase the LH pulse frequency is up to 4 pulses per hour. The LH pulse frequency has a detrimental effect on the final maturation of the follicle and on the positive feedback on GnRH that will induce LH surge followed by ovulation (Sunderland et al., 1994). At that stage, the fully grown antral follicle is called a pre-ovulatory or Graafian follicle and is ready for ovulation (Kidder and Vanderhyden, 2010). After ovulation, the follicle remnant (granulosa and theca cells) develops into a highly vascularized endocrine organ, the corpus luteum (CL), the primary function of which is to produce progesterone (Kidder and Vanderhyden, 2010).

The number of follicular waves in a cyclic heifer can vary from 1 to 4 although having 3 follicular waves under a 21 day cycle is the most common (Savio et al., 1988, Murphy et al., 1991, Crowe, 2008). Cyclic cows may have 2 to 4 follicle waves during the oestrus cycle (Savio, 1990, Sartori et al., 2004, Crowe, 2008).

At the primary oocyte stage, development of the oocyte within the follicle is halted at prophase 1 of meiosis (Sorensen and Wassarman, 1976). However, under the influence of a gonadotrophin surge associated with the process of ovulation, meiosis resumes within the oocyte (Sorensen and Wassarman, 1976). When ovulation takes place, the oocyte undergoes a meiotic division to form a large secondary oocyte and the first polar body. Meiosis will be halted again in metaphase II until fertilization.

1.4.2 The “two-cell, two-gonadotrophin” model theory

Binding of LH to the theca cell LH receptors (LHR) will cause the production of androgens that exert paracrine effects in neighboring granulosa cells (Orisaka et al., 2006). This process includes the conversion of cholestenone into testosterone by the theca cells, which will be diffused to the neighbouring granulosa cells. In parallel, granulosa cells express FSH receptors and convert the diffused testosterone from the theca cells to estradiol (E2) (Strauss et al., 2014). The latter enters the blood stream through capillaries to reach different tissues such as the brain and reproductive tract. Signals produced by the oocyte, such as growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BFP – 15), will have an impact over the neighbouring granulosa cells, such as cumulus expansion. Granulosa cells will gradually develop LHR that will promote inhibin production despite the FSH dependency during the recruitment phase (Strauss et al., 2014). Inhibin will increase testosterone synthesis and hence oestrogen formation in the preovulatory follicles (Hillier et al., 1994). Granulosa cells are also responsible for the production of Anti-Müllerian hormone (AMH). This AMH regulates follicle development by attenuating the effects of FSH on follicle growth and inhibiting primordial follicle recruitment (Mossa et al., 2017). Absence of AMH will result in faster follicle recruitment, leading to an exhausted pool of primordial follicles at a younger age (Durlinger et al., 1999, Mossa et al., 2017).

1.4.3 Corpus luteum

The corpus luteum is a transient endocrine gland that produces progesterone (P4) and has a key role in maintaining pregnancy. The development of the local capillary network, and also the growth and development of the steroidogenic cells, will determine CL development. Small and large steroidogenic luteal cells inhabit the major part of the total luteal volume in the cow (Rodgers and O'Shea, 1982, Niswender et al., 1994, Wiltbank et al., 2012). The two cell populations differ in receptors for LH (being greater in small cells), E2 (being greater in large cells), and PGF2α (being greater in large cells) (Wiltbank, 1994). Even though the volume of the CL will significantly increase until Day 7, the production of P4 will continue to increase until day 14 of the estrous cycle (Sartori et al., 2004). Due to the increased size of the ovulatory follicle (Sartori et al., 2004) and to the greater metabolic clearance rate for P4 (Wiltbank et al., 2006), lactating cows have a greater CL volume with a lower circulating P4 than heifers (Wiltbank et al., 2012).

The regression of the CL *(Figure 6)*, starts between days 16 - 17 of the oestus cycle in non-pregnant animals due to multiple pulses of PGF$_2$α released by the endometrium (Niswender et al., 2000). Prostaglandin - F$_2$α will bind to membrane receptors on the large luteal cells of the CL. In parallel, oxytocin is released by the posterior pituitary and binds to oxytocin receptors in the endometrium. This will result in an accelerating effect in which oxytocin release causes more prostaglandin - F2α to be released and vice versa. The two major mechanisms responsible for the regression process are characterized by a functional and structural luteolysis where the capacity to synthesize and number of cells are loss (Shirasuna et al., 2012).

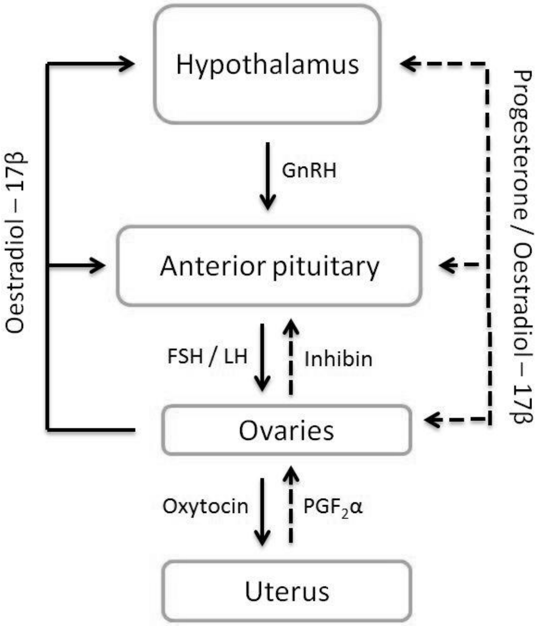


Figure 6. Simplified illustration of the endocrine relationship between the hypothalamus-pituitary ovary-uterus axis. Hormones exert positive (——) or negative effect on the target organs (- - - - -).

1.5 Negative energy balance: A frequent problem impairing fertility

Energy balance is the outcome of the interaction between energy intake and energy demands (*Figure 7*). In dairy cows, negative energy balance is an important cause of infertility (Fenwick et al., 2008). For about 60 years, dairy cows have been very successfully selected to maximise milk yield, but at the same time they have become more sensitive to reproductive disorders (Royal et al., 2000). In early lactation, cows are exposed to negative energy balance as their dry matter intake cannot provide the necessary amount of the energy required for milk production and body maintenances (Berglund and Danell, 1987a, Lucy, 2001, Agenas et al., 2003, Holtenius and Holtenius, 2007, O'Hara et al., 2016). Mobilization of adipose tissue from the body reserves will be followed by the production of leptin, which will contribute additionally in the reduction of appetite, making the energy deficit more severe. Body glucose depots will respond to these high energy demands and it is assumed that more than 60 – 70 % of body glucose will be used for lactose production by the mammary gland (Annison and Linzell, 1964). The missing energy will be provided from body fat reserves and muscles to fill the energy gap (mobilization of body reserves). The extended mobilization, mainly of the adipose tissue, will result in an increased risk of metabolic and reproductive disorders. Studies have shown that several million dairy cows per year in Europe are exposed to reproductive disorders (Grimard et al., 2006, Sheldon et al., 2009).

Homeorhetic adaptation processes will support the basic starting point, maintaining energy balance and high milk production in the early stage of lactation (Heuer et al., 1999, Roche et al., 2009). It will take several months for the cow to adjust and subsequently recover from this new condition of negative energy balance. Adaptation processes will begin at the moment energy balance becomes negative, which usually occurs during the first 2 weeks after calving. Although the animals are in a positive energy balance during the last period of pregnancy, their body will start to adapt to changes in hormonal regulation and mobilization of body fat depots. The critical signal which will trigger adipose mobilization in late gestation comes from the decreasing insulin concentrations and elevated placental lactogenic levels (Bell et al., 1995, Wathes, 2012). Even during this period there are large individual variations in the level of fat mobilization. The mismatch of dietary energy intake and energy demand for maintenance and lactation around calving and mainly during the first 2 months of lactation will lead dairy cows to enter a status of negative energy balance, which is more or less severe (Bauman and Currie, 1980). As a consequence, metabolic status will be altered, affecting the endocrine and somatotropic axis (Mellouk et al., 2017), hence resulting in a decrease in fertility (Figure 8).

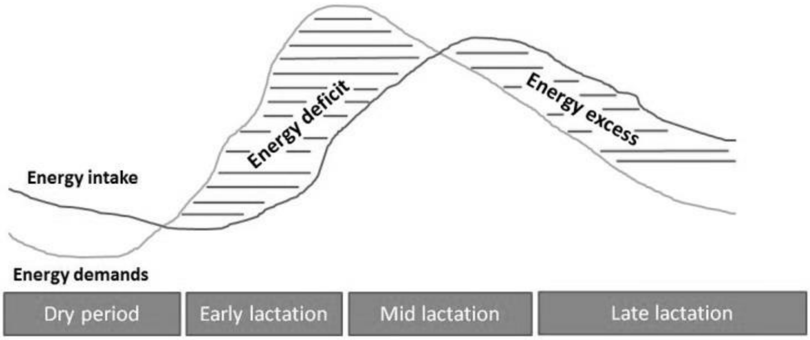


Figure 7. Schematic illustration of the energy balance pattern.

Cows affected by severe negative energy balance have low fertility and extended unproductive periods. They also have a longer period between parturition and first service, and reduced conception rates, resulting in a longer interval from calving to pregnancy (LeBlanc et al., 2002, Runciman et al., 2008, Williams, 2013). Due to the impaired reproductive performance, culling rates are increased and thereby affect herd profitability (Borsberry, 1989, LeBlanc et al., 2002, Kasimanickam, 2004) and hence the sustainability of dairy production systems.

1.5.1 Impact of energy deficit on fatty acids metabolism

Mobilization of fat reserves during negative energy balance is reflected in high concentrations of NEFA in blood and it is associated with peripartum metabolic diseases (Bobe et al., 2004). Insulin, NEFA, and glucose, have been used in different studies as metabolites that can reflect nutrient status in dairy cows (Holtenius et al., 2004, Stengarde et al., 2011). During early lactation, NEFA concentrations in plasma can be used as an evaluation tool of the peripartum energy level in dairy cows (Roche, 2009). Liver is one of the tissues that plays a key role in the absorption and metabolism of NEFA. However, liver has a limited ability to convert TG into VLDL (Hammon et al., 2006). The ability of NEFA to respond quickly to an emerging energy deficit results in a rapid increase and decline in their blood concentration.

Changes in feed intake, lactose synthesis in the mammary gland, and increased circulating glucocorticoids observed during infection are some of the control mechanisms that regulate glucose levels. In high producing dairy cows, metabolic imbalance and glucose deficit may be identified to some extent by characterizing the metabolic profile of the dairy herd (Oltner and Berglund, 1983, Stengarde et al., 2008).

1.5.2 Role of energy deficit on fat tissue

The intensive and prolonged lipolysis during early lactation will induce a remodeling process within the adipose tissue that is characterized by an inflammatory response causing immune cell infiltration and cellular proliferation (Kosteli et al., 2010, Sun et al., 2011). During this period, the amount of adipose tissue will be reduced and the secretion pattern will be altered, favoring the use of FA as an energy source (Bell and Bauman, 1997, Stern et al., 2016). Mobilization of fat tissue during the last months of pregnancy will increase the production of NEFAs which in turn will promote insulin resistance by inhibiting the phosphorylation of insulin receptors (Wathes et al., 2006). The reduced insulin sensitivity of the adipocytes during the transition period will redirect energy in the mammary gland for milk production (De Koster and Opsomer, 2013). The adipocytes′ responsiveness to insulin will increase as days in milk increases, resulting in increased lipogenesis rates (McNamara, 1994). The development of a moderate insulin resistance mechanism during early lactation is generally a beneficial process but which can predispose animals to inflammatory and metabolic diseases by limiting adipocyte capacity for energy buffering (Faulkner and Pollock, 1990, De Koster and Opsomer, 2013).

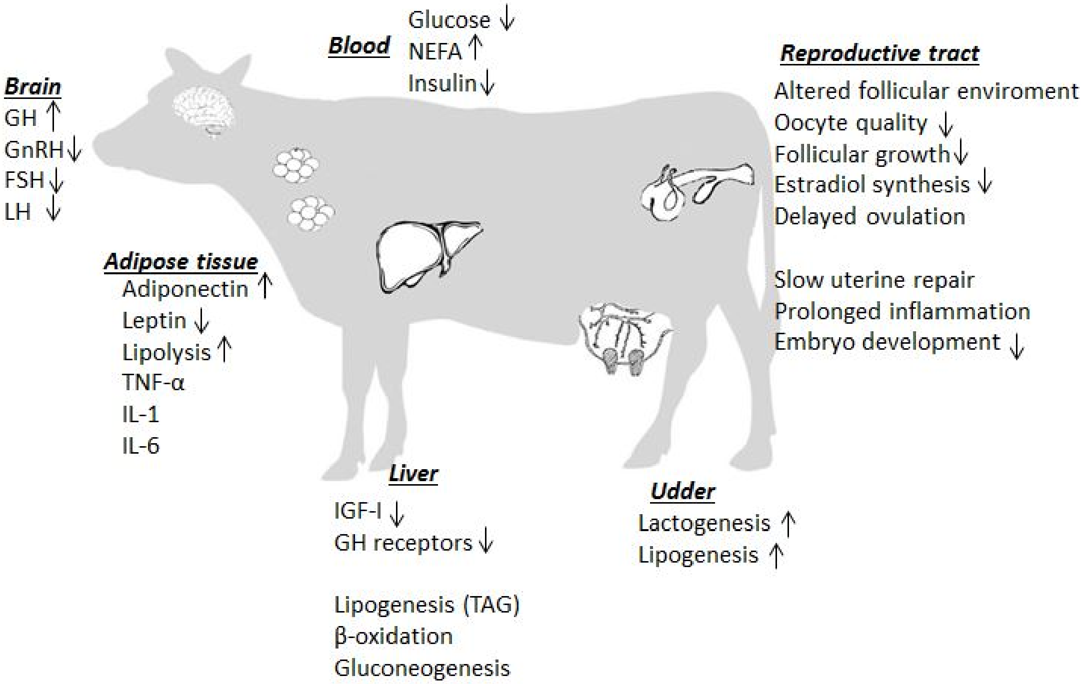


Figure 8 Endocrine and metabolic changes during early lactation and their impact on reproduction

GH = Growth hormone, GnRH = Gonadotropin-releasing hormone, FSH = Follicle stimulating hormone, LH = Luteinizing hormone, NEFA = Non-esterified fatty acid, TNF-α = Tumor necrosis factor alpha, IL - 1 or 6 = Interleukin – 1or 6, IGF-1= Insulin-like growth factor 1, (↓) = low, (↑) = high

1.5.3 Impact of energy deficit on cyclicity and LH surge

Energy deficit has a major effect on the resumption of cyclicity (Bulman and Lamming, 1978, Beam, 1997, Opsomer, 2000) by its ability to reduce LH pulse frequency (Crowe, 2008). The two most common challenges for ovarian function are the prolonged commencement of luteal activity (CLA) and prolonged luteal phase (Crowe, 2008). Energy deficit will alter the metabolic status which will, in combination with the increased tissue mobilization (Overton and Waldron, 2004), cause cows to have a tendency to show anoestrus and to have a low conception rate to AI (Lucy, 2001) . However, prolonged luteal phases are related to uterine problems rather than ovarian problems (Opsomer, 2000, Crowe, 2008).

Correct nutritional management can ensure a moderate, rather than a severe, postpartum energy deficit. Theoretically, nutritional status can be followed by measuring insulin growth factor I (IGF-I) serum concentrations (Zulu et al., 2002).

Energy deficit followed by increased tissue mobilization is negatively correlated with leptin concentrations in serum (Liefers et al., 2003). It is has been shown that leptin can modulate the secretion of LH by its direct actions at the adenohypophyseal level (Amstalden et al., 2003). However, the expression of kisspeptin, GnRH mRNA and the serum concentrations of LH and FSH may also decrease due to energy deficit (Luo et al., 2016).

1.5.4 The effect of energy deficit on embryo mortality

Energy deficit is a major contributing factor to the low fertility of high milking dairy cows inseminated during the early postpartum (Berglund, 1989). Fertilization failure and / or early embryonic loss (before Day 16 of pregnancy) represent 20 – 45 % of pregnancy failures, whereas later embryonic losses (after Day 16 of pregnancy) account for 8 – 17.5 %, (Humblot, 2001). Studies have shown that there is a correlation between oestrus detection, days to first service and conception rate (Tillard et al., 2008). The low fertility generally associated with energy deficit remains even though the cows are no longer exposed to it (Leroy, 2008a). This could be a lasting effect of energy deficit on the antral follicles that will develop into large dominant follicles 40 - 50 days later (Bage, 2002). In these cases, the concentrations of glucose, insulin, and NEFA in blood serum are reflected in the follicular fluid of the dominant follicle and may influence the ability of the oocyte to be fertilized and promote the development of a healthy and viable embryo (Leroy, 2008a).

In-vitro studies have also shown that short-term exposure of oocytes to low glucose and high NEFA concentrations (Leroy, 2008a), or to altered insulin concentrations (Laskowski et al., 2017), can have a detrimental effect on oocyte quality and embryo development. However, during in vivo conditions, oocytes or early embryo are exposed long-term when cows experience a severe or moderate NEB during early lactation. Hypothetically, the difference in the duration of exposure might enhance the negative effects in in-vivo produced oocytes and embryos.

1.6 The effect of metabolic imbalance on the hypothalamo-hypophyseal-ovarian axis

Decreasing glucose concentrations will down regulate brain functions. The release of FSH and LH from the adenohypophysis will alter as the stimulation by GnRH from hypothalamus will be weak. Estrogen production by the dominant follicle will be restrained by the low levels of blood glucose, insulin, and IGF-I, resulting in a delay of the first ovulatory estrous (Butler, 2000). First ovulatory estrous and conception rate are directly related with the rate of mobilization of body reserves (Butler and Smith, 1989). However, the precise reason for reduced GnRH pulsatility was unknown (Bucholtz et al., 1996, Ohkura et al., 2004) until recently. Recent studiesshowed that kisspeptin drives GnRH release while neurokinin B and dynorphin act as pulse start-andstop signals (Tanco et al., 2016). Increased insulin and IGF-I serum concentrations will increase ovarian estradiol output, although reduced concentrations of both cannot explain the suboptimal GnRH and LH pulse frequency (Butler et al., 2004).

Insulin and IGF-I are metabolic hormones having a multitasking role on the tissue response (Wathes et al., 2006). The liver is the tissue where IGF-I is primarily synthetized under the control of GH produced by the adenohypophysis. However, even though IGF receptors are present in most tissues, IGF availability is controlled by six binding proteins (insulin growth factor binding protein; IGFBP). Under severe NEB due to high milk production, GH concentration is increased but the number of GH receptors in the liver is downregulated (Butler et al., 2003, Garnsworthy et al., 2008). As a result, IGF-I production will decrease, influencing glucose uptake and normal insulin sensitivity (Wathes et al., 2006).

In obesity, leptin sensitivity is decreased causing a reduced feed intake and resulting in a higher susceptibility to NEB. This may partly explain the fact that cows with high BCS before calving will mobilize more fat tissue during early lactation resulting in greater BCS losses than cows with a lower BCS at calving (Garnsworthy et al., 2008). However, BCS losses during early lactation can vary highly between individuals.

1.7 Current situation and strategies for improvement of fertility

Dairy breeds of cattle have been selectively bred over decades for milk-producing traits (Foote, 1970). As a result, today's dairy cows produce more milk than one calf can consume. The side-effect of this intensive selection is that the cow is physiologically incapable of coping with the energy demands during early lactation (Berglund and Danell, 1987b). Today, nutritional disorders are the most cited reason for infertility (Tillard et al., 2007, Tillard et al., 2008, Humblot, 2009). However, new goals for selection and use of genomic selection have been implemented to gain better control of reproduction traits at the genetic level (Barbat, 2010). Reduced reproductive performance due to NEB has also led to the development of management strategies to mitigate the effect of energy deficit (Arbel et al., 2001, O'Hara et al., 2017). However, the current strategies do not take into consideration the high individual variation within the breed.

Current strategies are aiming to maximize dry matter intake in order to obtain a higher milk production. However, results from different studies show that reproductive performance might be improved by modifying feeding and management in such a way that a slightly lower peak milk yield is achieved without compromising the genetic potential of the animal (Bedere et al., 2017a, Bedere et al., 2017b). Reducing the effect of NEB would potentially increase the longevity of the animals and improve welfare, as well as reducing the cost of raising replacement heifers. It this way, herd economy could benefit from the decreased replacement rates.

2 Aims

The overall aim of this project was to study the role of breed on the effects of energy balance and metabolic response on reproductive performance in Holstein and SRB dairy cows. Specific aims were as follows:

- To evaluate the effect of feeding two energy levels and breed during the antepartum and postpartum periods on blood metabolites and reproductive performance (**Papers I, II**).

- To investigate the effect of breed and energy-diet, and to determine any interrelationships between different measures of energy deficit, reproductive performance, or fertility traits in the peripartum period (**Papers I, II**).

- To investigate the association between plasma adipokines concentrations, metabolism, and reproductive parameters in Holstein dairy cows fed diets with different energy levels during the peripartum period (**Paper III**).

3 Overview of Materials and Methods

3.1 Ethical permission

All experimental protocols were approved by the Uppsala Animal Experiment Ethics Board (application C329/12, PROLIFIC) and were carried out in accordance with the terms of the Swedish Animal Welfare Act (studies I, II). An ethics committee (Comité d'Ethique en Expérimentation Animale Val de Loire, CEEA VdL, protocol reference number 2012-10-4) approved all experimental protocols performed in France by INRA, which conformed to the guidelines provided by the French Council for Animal Care (study III).

3.2 Short description and comparison between the studies in Sweden and France

The studies presented in the thesis took place in Sweden (SLU) and France (INRA) and have been performed in the framework of an EU granted project (EU Grant agreement Number 311776). The main project "Prolific" (**P**luridisciplinary study for a **RO**bust and sustainab**L**e **I**mprovement of **F**ertility **I**n **C**ows) was structured in four work packages (WP) with studies being performed in WP2 and especially with partners involved inWP4.

WP2: Molecular approach for fertility markers

WP4: Innovations in farm nutritional management to optimize cow fertility

In both countries, animals were housed indoors and individual feeding was applied. However, in Sweden feed stuffs were fed separately, whereas in France a total mixed ratio (TMR) was used. The Swedish material included two breeds (Holstein and SRB) in order to investigate differences between the extreme dairy type and the dual-purpose type of cow. Milking systems were also different between countries. In Sweden, a voluntary milking system (VMS, DeLaval, Tumba, Sweden) was used, whereas in France cows were milked twice daily in a milking parlour. Overall, the study was also designed to compare a higher and lower intensity of feeding. The higher intensity represents common practice among most high producing Swedish milk producers, whereas the lower feeding intensity may be indicative of e.g. organic dairy producing systems.

3.3 Study design

Three studies were performed:

Study I (Paper I): A study was carried out to characterise the variation between breeds (Holstein, SRB) in terms of nutrient prioritisation and the consequences of this variation on blood metabolites and reproductive performance. This study **focused on metabolism** with little information on reproduction. Plasma glucose, insulin, NEFA, dry matter intake, energy balance, BCS, and embryo mortality were evaluated in Holstein and SRB dairy cows during the ante- and post- partum periods. All biological materials were collected and registrations were conducted at the Swedish University of Agricultural Sciences (SLU). Blood plasma analyses were performed in collaboration with INRA, France. The experimental design is shown in Figure 9.

Study II (Paper II): This study evaluated the effect of two energy diets on the endocrine axis and reproductive performance in Holstein and SRB dairy cows during the peripartum period. This study **focused on the impact of diet and NEB on reproduction**. Possible **interrelationships** between endocrine axis, energy deficit, and reproductive performance were also investigated. Whole-milk P4, activity meter observations for oestrus detection, endocrine and traditional reproductive traits were determined. This work was conducted at SLU. The experimental design is shown in Figure 9.

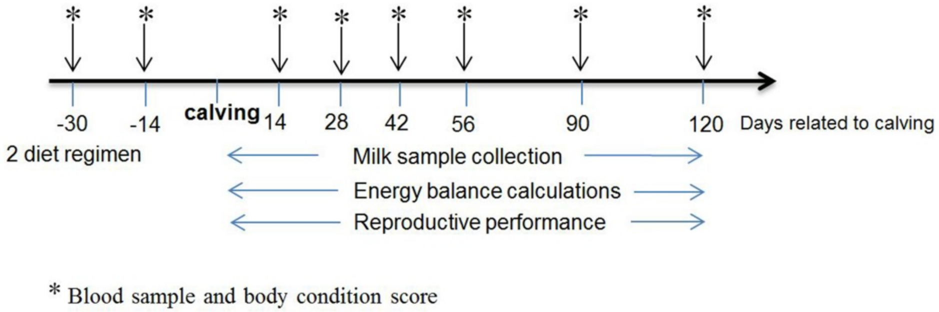


Figure 9. Experimental design (**studies I, II**)

Study III (Paper III): This study investigated the **effect of diet on LH secretion** and the role of adipokines in the **interaction between energy balance and reproductive performance**, in Holstein dairy cows, during the peripartum. Sample collection and measurements were conducted in and by INRA, France. The experimental design is shown in Figure 10.

3.4 Animals and animal management

Study I and II: The experiments were carried out at the Swedish Livestock Research Centre in Uppsala / Lövsta.

In total, 44 heifers were recruited (Table 4) based on the total number of AI (≤ 2). Thirty days before expected calving (Day − 30), the pregnant heifers were moved to a straw-bedded close-up group pen before being moved to an individual calving pen on the day of calving. After calving, the animals were relocated to a loose housing barn where they stayed for 120 days (Day 120) and were milked in a VMS. During the whole study period, the animals were kept indoors and had free access to water.

Pregnancy diagnosis was carried out on all cows by the same investigator using transrectal palpation and ultrasonography with a 7.5 MHz linear probe (DRAMINSKI iScan, Olsztyn, Poland) on Day 35 and once more between 60 and 90 days after AI.

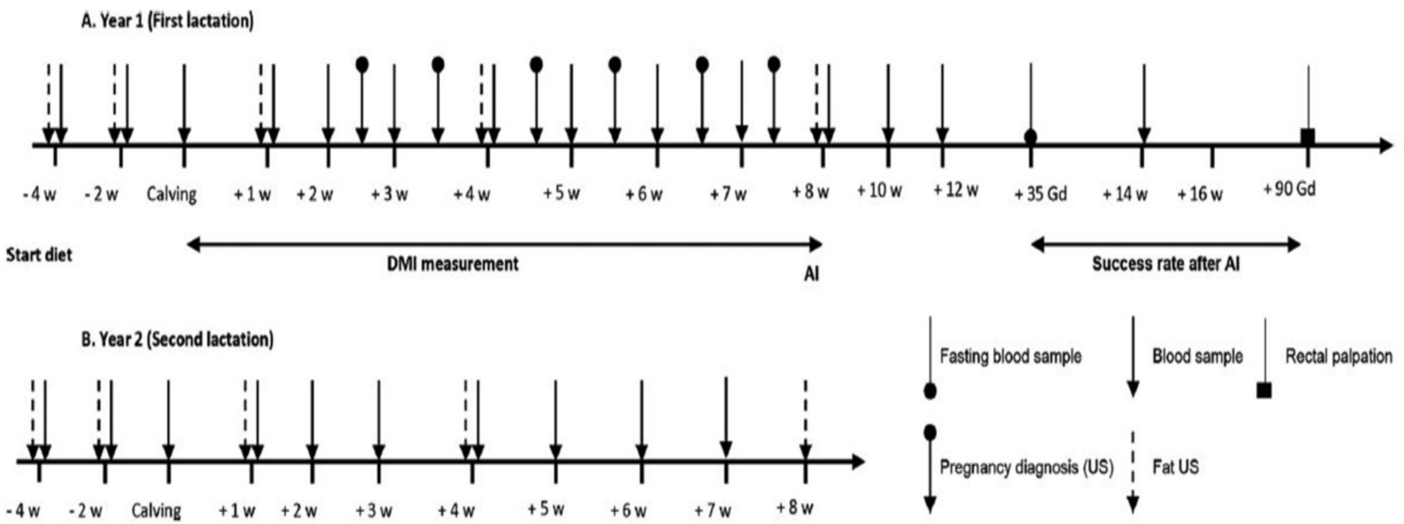


Figure 10. Experimental design (**study III**)

w: week, Gd: gestation day, US: ultrasound

Table 4. Number of pregnant heifers included in the experiment (**studies I and II**).

	High-energy	Low-energy	Total
Holstein	12	10	22
SRB	11	11	22
Total	23	21	**44**

High-energy: targeted 35 kg ECM / day, Low-energy: targeted 25 kg ECM / day, ECM: energy corrected milk

Study III: This experiment was carried out at the experimental unit UEPAO (Institut National de la Recherche Agronomique, Nouzilly, France). From the 39 animals studied during their first lactation, 35 were studied during their second lactation (2 non-pregnant cows were excluded and 2 cows died for reasons unrelated to the study).

Table 5. Number of pregnant heifers included in the experiment (**study III**).

	High-energy	Low-energy	Total
Holstein	17	22	**39**

High-energy: targeted 35 kg ECM / day, Low-energy: targeted 25 kg ECM / day, ECM: energy corrected milk

3.5 Diet groups

Study I and II: Animals were randomly divided into two diet groups, a high-energy (HE; Holstein n = 12, SRB n = 11) and a low-energy (LE; Holstein n = 10, SRB n = 11) group. The HE group (n = 23) was fed according to Nordic standards (Volden, 2011) for high-producing cows (expected production per day of 35 kg ECM). The low-energy group (n = 21) targeted expected milk production of 25 kg ECM per day, which was achieved by giving 50% less concentrate than in the control group.

Diets were implemented at Day - 30 before expected calving. During the dry period the animals were kept in groups for which the total DMI was estimated from the amount fed. After calving, feed stuffs were fed separately and **the cows were individually fed**. The amount of the concentrate offered in the VMS and in concentrate stations in the feeding area was individually adjusted. Silage was fed in forage mangers placed on weighing cells (CRFI, BioControl, Rakkestad, Norway). Silage and concentrate were fed separately to all cows through the experimental period.

Study III: In total, thirty-nine pregnant Holstein heifers were distributed in two dietary treatment groups according to back fat thickness measurement. Animals were fed either HE energy- diet calculated to yield 35 kg of milk / cow per day (n = 17) or LE energy-diet aiming to yield 25 kg of milk / cow per day (n = 22), as for Study II. The diets started at Day − 30 before expected calving and were then applied during 2 lactations. Diets were calculated according to the INRA French feeding system (Agabriel and Institut national de la recherche agronomique, France, 2007).

3.6 Feed intake

Study I and II: Prior to calving, the pregnant heifers were kept in groups, for which the total dry matter intake (DMI) was estimated from the amount fed for the group. After calving, the feed intake of each animal was individually controlled and documented from calving until the end of the experiment, Day 120 (CRFI, BioControl, Rakkestad, Norway). During this period, the total DMI was individually estimated from the amount fed.

Study III: The DMI was calculated daily for the HE and LE diets, from calving to Day 60. Diet was distributed twice daily, at 09:00 and 15:00 h, and each cow had access to several feeders (Insentec B.V., Markness, The Netherlands). During the dry period, animals were kept in groups in loose housing where the total DMI from feed was estimated from the amount given.

3.7 Sampling and data recording for milk yield and composition

Study I and II: At the entrance to the milking parlour, cows were identified by an electronic collar, and the milk yield of each cow was automatically recorded in the VMS. Once weekly, milk samples (20 ml) pooled from two consecutive milkings (afternoon milking and the following morning milking) were collected from Day 7 after calving until approximately Day 120, and milk composition was analysed. These samples were analysed for fat, protein and lactose content at the laboratory of the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, using MilkoScan FT 120 (FOSS Electric A/S, Hillerød, Denmark).

Study III: At the entrance to the milking parlour, cows were identified by an electronic collar and the milk yield of each cow was automatically recorded (software R-Manufeed 500 pro, vc5 version 2.011.14, 1996, Manus-Delaval, Elancourt, France). After calving, cows were milked twice daily (software RIC version RW 1.7).

3.8 Body condition score and subcutaneous adipose tissue thickness

Study I and II: Body condition score (BCS) was visually scored by one person, starting one month before expected calving and continuing every two weeks during lactation. Body condition was scored using a scale from 1 = very lean to 5 = fat, with .25 unit increments (Edmonson, 1989).

Study III: Adipose tissue mobilization was assessed through subcutaneous fat thickness measurements in the sacral region using ultrasonographic examination with a linear probe (LA 332 3.5 / 10.0 MHz transducer; Mylab-30vet; R-Esaote, Hospimedi, Saint-Crépin-Ibouvillers, France), as described by Schroder and Staufenbiel, (2006).

3.9 Energy balance calculations

Studies I and II: Energy balance (expressed in MJ / day) was calculated as the difference between feed energy input and energy requirements for milk, lean tissue growth, and maintenance from calving to Day 120. These calculations were performed using NorFor, a semi-mechanistic feed evaluation programme for cattle that is the reference system in the Nordic countries (Volden, 2011).

Study III: Energy balance (expressed in Mcal / day) was calculated from calving to Day 60. Calculations were performed using the INRA feeding system (INRA, 2007) as the difference between energy intake and energy requirements for maintenance, milk yield, and pregnancy.

3.10 Progesterone concentrations and determination of luteal activity

Studies I and II: Milk samples were automatically collected in the VMS (DeLaval, Tumba, Sweden) three times per week, starting from Day 7 postpartum until Day 120, and analyzed for milk P4. Approximately 5 ml of milk were taken within 60 min of milking, placed in tubes containing 100 µl preservative (2% Bronopol with 0.05% methylene blue), and stored at +4°C until analysis.

An activity meter system (DeLaval AG, Tumba, Sweden) was used in combination with visual observations that were carried out three times daily for estrous detection. The possible day of oestrus as determined from activity and estrous observations was merged with the corresponding P4 recording to set limits for luteal activity (Leroy, 2008b, Petersson, 2006b, O'Connell, 2011).

Day of ovulation was estimated as the day after detection of oestrus or the day before the onset of metoestrus bleeding. All milk P4 recordings taken within the first 60 days postpartum were used to calculate the percentage of samples above the limit for luteal activity. The threshold for luteal activity (≥ 3 ng / ml) was defined so that 95 % of the possible days of oestrus were below this threshold.

Studies III: Progesterone was measured in plasma. The threshold for luteal activity (> 0.70 ng /ml) was defined from the full data set as the value at which 95 % of the minimum concentrations flanking the supposed day of oestrus were higher than this concentration.

3.11 Ovarian dynamics

Study III: Before AI, ovarian follicular dynamics were monitored three times a week during the cycle by transrectal ultrasonography using a linear probe (LV 513 6.0/8.0-MHz transducer; Mylab30; Esaote). Based on these observations, the growth of each follicle was followed and the number of follicular waves was determined as previously described (Sirois and Fortune, 1988). The length of the cycle was calculated while combining the results from ovarian scans and data from oestrus detection. The beginning of the cycle was defined as the day of oestrus detection and confirmed by ovulation. In case of silent ovulation, the beginning of the cycle was defined as the day when the dominant follicle of the ovulatory wave reached its maximal size before an ovulation was detected. The end of the cycle was the day of AI determined by oestrus detection. After AI, ovulation was confirmed by measuring progesterone three times a week until Day 21 post-AI. Ovarian follicles were classified according to their diameter: small (SF, 3–5 mm), medium (MF, > 5 and ≤ 7 mm) and large (LF, > 7 mm), and counted. The mean number of follicles in each class per cow was calculated from the total number of follicles of a given class from both ovaries (SF, MF, or LF) divided by the number of ultrasonographic examinations.

3.12 LH pulsatility and occurrence of preovulatory LH surges

Study III: Between Days 20 and 60 after first calving, LH profiles were determined from 11 HE and 12 LE primiparous cows to characterize the pulsatility and preovulatory LH surges at time of first ovulation. Only data from animals for which an LH peak was observed were retained for analysis. The synchronization protocol is illustrated in Figure 14. In the morning on Day 10, animals were cannulated with a 2-mm-diameter catheter placed in the jugular vein under local anesthesia with lidocaine at several points (5 mL, Lurocaïne, Vetoquinol, Lure Cedex, France). Twenty hours after the removal of the progestogen implant, LH secretion profiles were determined from samples taken every 10 min for 6 h (to characterize LH pulsatility) and every 2 h for 113 h (to study the preovulatory LH peak). At the end of 113 h, the catheters were removed and the animals were submitted to daily blood sampling from the coccygeal vein until Day 7 to measure circulating progesterone concentrations from the newly formed corpus luteum. Luteinizing hormone pulsatility was characterized from a software version based on use of the DynPeak algorithm (Vidal et al., 2012). The duration of the preovulatory LH surge was defined as the interval from the time that serum LH concentrations exceeded 1 ng / ml (baseline) until serum LH declined to < 1 ng / ml.

3.13 Plasma metabolites

Study I: In total, eight blood samples per cow (about 10 ml per sampling, *Figure 12*) were collected from the coccygeal vein into 10 ml vacuum tubes containing EDTA anticoagulant (BD Vacutainer, 4550 Kremsmünster, Austria). Blood plasma was analysed for **glucose** (GAGO20-1KT, Sigma), **NEFA** (enzymatic colorimetry assay) and **insulin** (RIA) at Unité Physiologie de la Reproduction et des Comportements, INRA, 37380 Nouzilly, France. Samples were collected between 10:00 am and 11:30 am and kept on ice until they were centrifuged at 4000 x g for 10 minutes at + 4 °C. After centrifugation, plasma was harvested, divided into five aliquots and stored at – 20 °C until assays were conducted.

Study III: Blood samples were collected from the coccygeal vein in heparinized Vacutainers (Dutcher, Brumath, France) before diet distribution (*Figure 13*). Blood was immediately centrifuged at 2000 × g for 15 min at + 4 °C. After centrifugation, plasma was separated and stored at – 20 °C until assay. **Plasma fatty acids**, **glucose**, and **insulin** concentrations were recorded from Days – 30 to Day 49 (first and second lactations). Plasma **leptin, resistin, and adiponectin** concentrations were assessed from Day − 30 to Day 49 in first and second lactations.
Bovine **LH** standard preparations were used and plasma LH concentrations were measured using a previously described RIA (Ohtani et al., 2012, Montgomery et al., 1985). Plasma **Anti-Müllerian hormone** (AMH) was measured with a specific bovine AMH ELISA kit from blood samples collected at Day 7 postpartum in lactation 1.

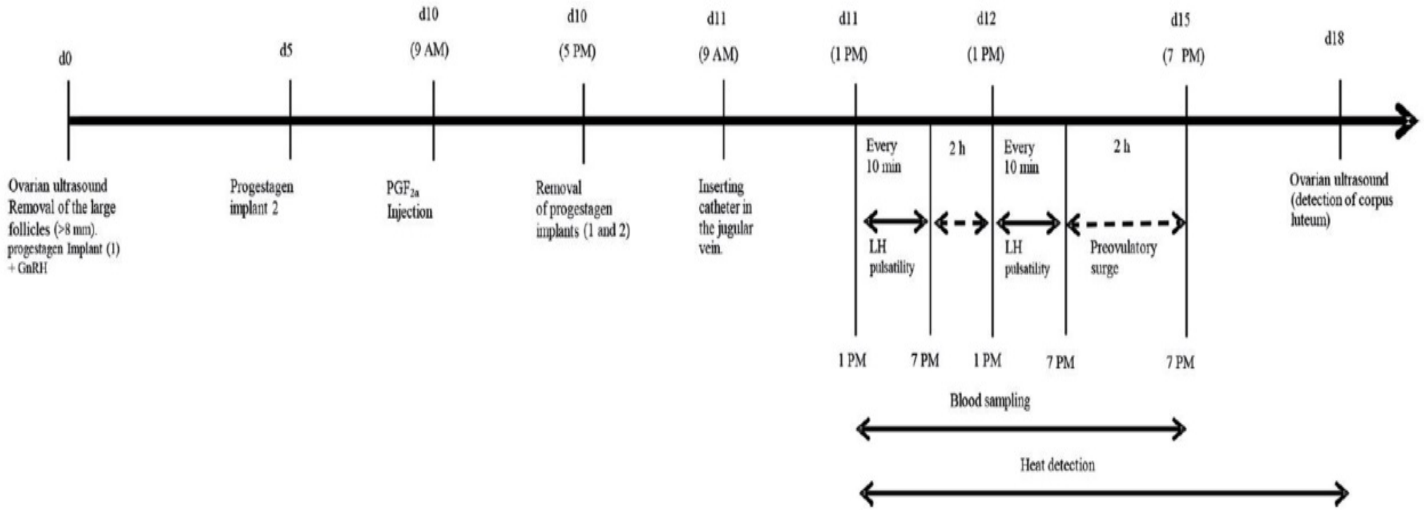


Figure 11. Illustration of the experimental design for LH pulsatility and LH preovulatory surge experiment as adopted by Mellouk et al., 2017 (study III).

GnRH = Gonadotropin-releasing hormone, $PGF_2\alpha$ = Prostaglandin $F_2\alpha$

3.14 Reproductive performance

Study I: Non - fertilisation rate or early embryonic mortality (NF-EEM) was estimated as the number of cows that were not pregnant at 21 days after AI (cows with milk progesterone < 5ng / ml) divided by the total number of inseminated cows. Prolonged luteal phase or late embryonic mortality (PLP-LEM) rate was estimated as the number of cows that had a high milk progesterone level by day 35 after the first AI (cows with milk progesterone ≥ 5 ng / ml), but not at later stages, , divided by the total number of inseminated cows (Humblot, 2001). Cows were inseminated 12 h after the onset of standing oestrus and milk progesterone was checked retrospectively, at the time point of AI, to confirm if cows were in heat at AI. Cows that were found to have high milk progesterone on the day of AI were treated as NF-EEM. Postpartum CLA was defined as the first day that the milk progesterone concentration was ≥ 5 ng / ml.

Study II: Milk P4 concentrations were used to describe and characterize the first postpartum estrous cycle (Royal et al., 2000, Horan, 2005, Petersson, 2006a). The first two postpartum P4 values above the limit for luteal activity (≥ 3 ng / ml) preceded by a low P4 value and not earlier than Day 10 after calving were used to define CLA (*Figure 12)*.

- **Early CLA** was defined as the first two consecutive measurements of milk P4 concentration ≥ 3 ng / ml occurring within the first 30 days after calving.
- The length of the **luteal phase (LP)** was measured as the time of the first consecutive milk sample of ≥ 3 ng / ml to the final consecutive milk sample of ≥ 3 ng / ml.
- The **interluteal interval (ILI)** was measured as the time interval from the first milk sample of < 3 ng / ml following luteolysis to the last consecutive milk sample of < 3 ng / ml.
- The **interovulatory interval (IOI)** was defined as the period of time from the commencement of one LP to the commencement of the next LP.
- **First ovulatory estrous (FOE)** was estimated based on both low P4 (< 3 ng/ml) and visual heat.

The traditional fertility traits used in this study included calving to first insemination interval (CFI), first to last insemination interval (FLI), and pregnancy rate at first insemination (PFI). If an insemination was followed by a new insemination within 6 days, the first insemination was omitted from the analysis.

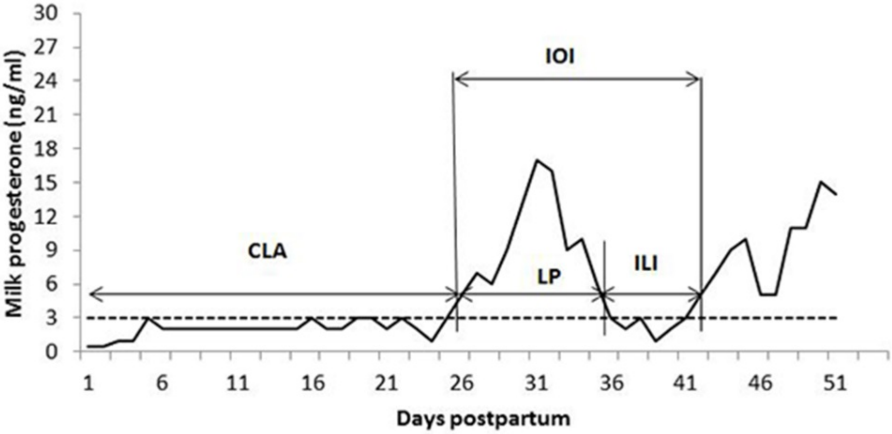


Figure 12. Reproductive parameters monitored using milk progesterone profiling (study II).

CLA = Commencement of luteal activity, LP = length of the luteal phase, ILI = interluteal interval, IOI = interovulatory interval,

Two luteal activity profiles were used to categorize the pattern of P4 levels as “normal” or “atypical” (including delayed ovarian activity, prolonged luteal phase, and cessation of luteal activity (Opsomer, 2000, Horan, 2005, Nyman, 2014).

- **“Normal” luteal activity** was defined as a P4 rise starting before Day 45 postpartum followed by regular cyclicity.
- **“Delayed” luteal activity** was defined as a P4 rise starting after Day 45 postpartum.

- **“Prolonged” luteal activity** was defined as a normal rise in P4 before Day 45 postpartum, but with the P4 concentration remaining ≥ 3 ng / ml for at least 19 days.
- **“Cessation” of luteal activity** was indicated as starting a P4 rise normally before Day 45 postpartum, but interrupted with a period of low P4 concentration for 12 or more days.

Study III: Postpartum CLA was defined as the first day that the plasma progesterone concentration was > 0.70 ng / ml. This threshold was defined from our full data set as the value at which 95 % of the minimum concentrations flanking the supposed day of oestrus were higher than this concentration. The pregnancy rate was determined twice after AI: on 35 d by ultrasonographic examination and on 90 d by transrectal palpation. Pregnancy rate was calculated as the number of pregnant females divided by the total number of inseminated females.

3.15 Statistical analyses

Study I, II, and III: All statistical analyses were performed with SAS® software version 9.3 (SAS Institute., 2011).

Continuous variables were analyzed using the MIXED procedure for linear mixed models. Backward elimination was used to build the models, excluding non-significant effects ($p > 0.20$) at each step.

The area under the curve (AUC) was calculated for the total area using the trapezoidal rule.

Models included a repeated effect of time (week or day ante- / postpartum) within animals. The correlations between test days were accounted for by specifying a correlation structure (spatial power) among residuals to consider that time intervals between samplings were not exactly the same.

All variables were analyzed first for normality of distribution. When needed, variables which deviated from a normal distribution were log-transformed. However, to improve clarity and in order to facilitate interpretation of the data, non-transformed values are presented in the tables. Sampling times were treated as actual days postpartum in the analysis of the material and presentation of the results.

Least square means (LSMeans ± standard error of the mean, sem) estimated by the models were compared. Hypothesis for subclasses of main effects were investigated using the contrast option and Scheffé's adjustment was used for multiple-post ANOVA comparisons.

As a complement, the multivariate ANOVA (MANOVA) option was used in the multivariate GLM model to estimate the strength and direction of associations between variables measured.

For binary variables, generalized lineal models in the GENMOD procedure in SAS 9.3 were used. The GLM parameterization of CLASS variables and the logit option statement were used. Scheffé's adjustment was used for multiple-post ANOVA comparisons.

4 Summary of results

4.1 Dry matter intake and milk yield

During the postpartum period, DMI (kg / day) and milk yield (kg ECM / day) increased over time (**studies I and III**). Holstein cows in the HE energy-group had significantly higher DMI (**studies I and III, pages 279 and 8523 respectively**) and ECM (**study I**) values throughout the observation period than the other groups. However, no difference was found within the SRB energy-diet groups (**study I**).

4.2 Body condition score and subcutaneous adipose tissue thickness

Body condition score and subcutaneous adipose tissue thickness decreased significantly during the early postpartum (**studies I and III, pages 280 and 8523, respectively**). Holstein cows had lower BCS than SRB cows within each energy-diet group (**study I**). However, within the Holstein cows, no difference was found in BCS or subcutaneous adipose tissue thickness between the HE or LE groups (**studies I and III**). In addition, subcutaneous adipose tissue thickness was greater in year 1 than in year 2 (**study III**).

4.3 Energy balance

The magnitude and severity of energy deficit (Figure 13, panels 1 and 2) was greater during early postpartum (**studies I, II, and III**). The lowest value for energy balance (EB nadir) was lower in Holstein cows than in SRB cows (**study II**). Overall, Holstein cows had a strong tendency to a more severe energy deficit than SRB cows (**studies I and II**).However, the HE diet group tended to have a less severe energy deficit than the LE diet group (**studies I, II, and III**). The mean energy deficit within Days 5 and 12 from calving was greater in Holstein cows than in SRB cows (**study II**). In addition, Holstein cows tended to be in a more severe energy deficit during the first two weeks and during the first 45 days after calving than SRB cows (**study II**). Overall, HE diet group had a less severe energy deficit than LE in lactation 1 and in lactation 2 (**study III**). However, the severity and magnitude of energy deficit in Holstein cows tended to be lower in lactation 1 than in lactation 2 (**study III**).

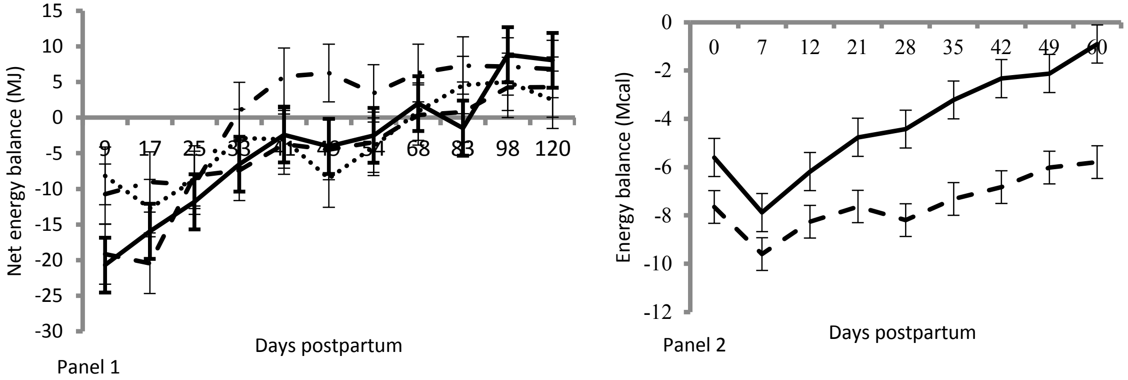


Figure 13. Net energy balance. *Panel 1:* Primiparous Holstein and Swedish Red (SRB) dairy cows in two energy diet groups (HE: high energy; 35 kg ECM/day, LE: low energy diet; 25 kg ECM/day). Holstein HE (solid line, n=12), Holstein LE (dashed line, n=10), SRB HE (dashed-dotted line, n=11), SRB LE (squared dotted line, n=11), (**studies I and II**). *Panel 2:* In Holstein dairy cows in two energy diet groups during the first 2 years of lactation (HE: high energy; 35 kg ECM/day, LE: low energy diet; 25 kg ECM/day). Holstein HE (solid line, n=17), Holstein LE (dashed line, n=22), (**study III**).

4.4 Plasma concentrations of glucose, fatty acid, and insulin

Overall, Holstein cows had lower glucose plasma concentrations on average than SRB cows (**study I**). The difference between the two breeds in terms of glucose plasma concentration was highest in early postpartum (Day 14); with lower values in Holstein cows than in SRB cows (**study I**). However, diet and time of sampling had no effect on glucose plasma concentrations (**studies I and III, pages 280 and 8523 respectively**). In addition, glucose concentrations were lower in lactation 1 than in lactation 2 (**study III**).

The NEFA plasma concentration increased after calving and then decreased gradually during lactation in both energy-diet groups (**studies I and III**) and breeds (**study I**). Plasma NEFA concentrations tended to be lower in Holstein cows at Day -14 than in SRB cows (**study I**). In contrast, NEFA plasma concentrations were higher in Holstein cows at Day 14 postpartum than in SRB cows (**study I**). Overall, NEFA plasma concentrations tended to be higher in the LE than in the HE diet group in study I. However, NEFA concentrations were not affected by diet in study III. In addition, NEFA plasma concentrations were higher in lactation 1 than in lactation 2 (**study III**).

Plasma insulin concentrations did not differ between breeds (**study I**). However, at Days 14 and 28 postpartum, insulin plasma concentration tended to be lower in Holstein cows than in SRB cows. Overall, plasma insulin concentrations were higher in the HE diet group than in the LE diet group in study I. However, plasma insulin concentrations were not affected by diet in study III. In addition, concentrations were higher in lactation 1 than in lactation 2 (**study III**).

4.6 Plasma concentrations of adipokines

Overall, plasma concentrations of leptin, adiponectin, and resistin were affected by diet, year (except for plasma leptin concentration), and week (**study III, page 8525**). Plasma leptin and adiponectin concentrations decreased before calving and then increased progressively throughout lactation. Higher concentrations of these 2 adipokines were found in HE cows than in LE cows during first and second lactations. In contrast, plasma resistin concentrations increased before calving and decreased after calving. Lower concentrations of resistin were found in HE cows than in LE cows during the first 2 lactations.

4.6 Reproductive performance

Breed and energy-diet group had no effect on NF-EEM and PLP–LEM rates after the first AI (**studies I and III, pages 280 and 8526**). However, the total number of cows with NF-EEM and PLP–LEM tended to decrease when BCS was ≤3.5 at the time of first AI or as CFI increased (**study I**).

Energy-diet groups tended to have a stronger correlation to the endocrine and traditional fertility traits than breed (table 6, **study II**). Breed had no effect on CLA (**study II**). The CLA after first calving occurred earlier in HE than in LE animals in study III but later in study II. However, after second calving, CLA did not differ between energy-diet groups (**study III**). The CLA was positively correlated with both the severity and the magnitude of energy deficit (**study II**).

Holstein cows tended to have longer LP intervals than the SRB cows (**study II**). However, the HE had longer LP intervals than the LE diet group (**study II**).

The FOE did not differ between breeds (**study II**). However, the HE diet group tended to have a later FOE than the LE group (**study II**).

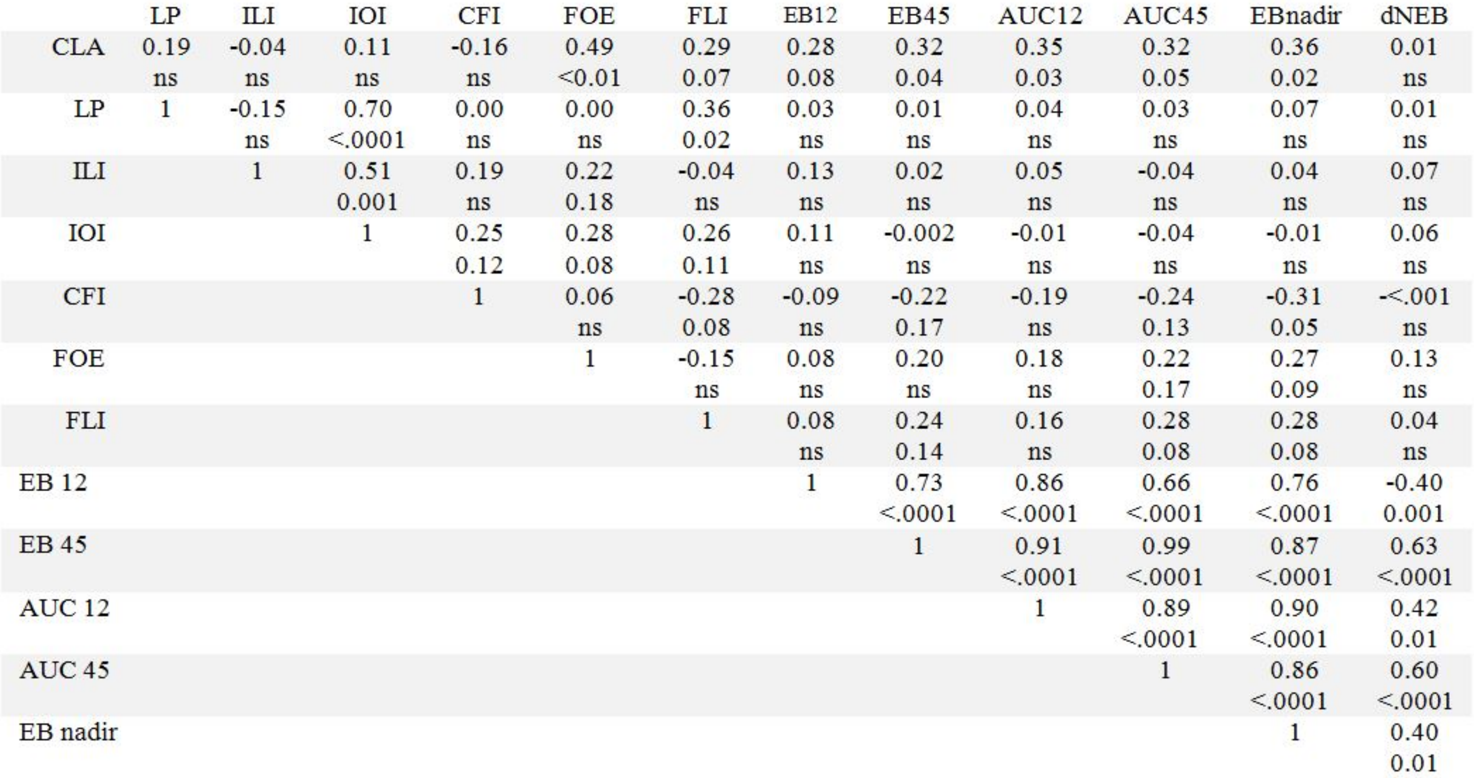
Energy-diet groups had no effect on the length of the oestrus cycle or on the number of small, medium, and large sized follicles (**study III**). However, the HE -diet group had more frequent 3-wave cycles than the LE group (**study III**). In addition, no difference in AMH concentration was found between the two energy-diets (**study III**).

No difference was found between energy-diet groups or breeds on the distribution of normal and atypical P4 profiles (**study II**). However, the proportion of atypical P4 profiles for the Holstein HE group (58.3%) tended to be higher ($p = 0.08$) compared with the other three groups (Holstein LE, 33.3%; SRB HE, 27.3%; and SRB LE, 27.3%). Cows with an atypical P4 profile had longer CLA (29.0 ± 3.2 days) than cows with a normal P4 profile (20.4 ± 2.4 days). Moreover, cows with an atypical P4 profile had longer LP intervals (21.2 ± 2.2 days) than cows with a normal P4 profile (7.0 ± 1.7 days).

The interval between first and second calving tended to increase in LE cows compared with HE cows (**study III**). No significant difference between diet groups was found for the interval between calving and first AI (**studies I and III**).

Basal LH concentrations did not differ between LE and HE cows (**study III**). However, the mean pulse frequency was higher in HE cows than in LE cows. No effect of the diet was observed on the magnitude or duration of the LH surge.

Table 6. Partial Correlation Coefficients from the Error Matrix (r and p values) for the first luteal activity after correction for breed (Holstein and Swedish red primiparous dairy cows) and energy-diet (high; 35kg ECM/day, low; 25kg ECM/day).



	LP	ILI	IOI	CFI	FOE	FLI	EB12	EB45	AUC12	AUC45	EBnadir	dNEB
CLA	0.19 ns	-0.04 ns	0.11 ns	-0.16 ns	0.49 <0.01	0.29 0.07	0.28 0.08	0.32 0.04	0.35 0.03	0.32 0.05	0.36 0.02	0.01 ns
LP	1	-0.15 ns	0.70 <.0001	0.00 ns	0.00 ns	0.36 0.02	0.03 ns	0.01 ns	0.04 ns	0.03 ns	0.07 ns	0.01 ns
ILI		1	0.51 0.001	0.19 ns	0.22 0.18	-0.04 ns	0.13 ns	0.02 ns	0.05 ns	-0.04 ns	0.04 ns	0.07 ns
IOI			1	0.25 0.12	0.28 0.08	0.26 0.11	0.11 ns	-0.002 ns	-0.01 ns	-0.04 ns	-0.01 ns	0.06 ns
CFI				1	0.06 ns	-0.28 0.08	-0.09 ns	-0.22 0.17	-0.19 ns	-0.24 0.13	-0.31 0.05	-<.001 ns
FOE					1	-0.15 ns	0.08 ns	0.20 ns	0.18 ns	0.22 0.17	0.27 0.09	0.13 ns
FLI						1	0.08 ns	0.24 0.14	0.16 ns	0.28 0.08	0.28 0.08	0.04 ns
EB 12							1	0.73 <.0001	0.86 <.0001	0.66 <.0001	0.76 <.0001	-0.40 0.001
EB 45								1	0.91 <.0001	0.99 <.0001	0.87 <.0001	0.63 <.0001
AUC 12									1	0.89 <.0001	0.90 <.0001	0.42 0.01
AUC 45										1	0.86 <.0001	0.60 <.0001
EB nadir											1	0.40 0.01

ns = p ≥ 0.20 denoted as ns, ECM = energy corrected milk, CLA = commencement of luteal activity after calving, LP = luteal phase length, ILI = interluteal interval, IOI = interovulatory interval, CFI = calving to first insemination, FOE = first ovulatory estrous, FLI = first to last insemination interval, EB 12 or 45 = mean energy balance within Days 5 and 12 or within 5 and 45 from calving, EB nadir = energy balance at nadir, AUC 12 or 45 = total energy balance within Days 5 and 12 or within 5 and 45 from calving, dNEB = total number of days after calving with negative energy balance.

5 Discussion

5.1 The novelty of the project

This project evaluated the effects of breed and nutritional strategies on reproductive performance, postpartum commencement of luteal activity, and oestrous cycle characteristics. Infertility is a multi-factorial problem influenced by individual animals, their environment, and management factors. In this project, we integrated information from genetics (breed) through collection of a large amount of phenotypic data. Our results highlighted the interaction between nutritional strategies and breed as a key factor influencing fertility and reproductive performance in dairy cows. Genetics (breed), animal physiology, and animal nutrition were studied, with a particular focus on the cow's reproductive tract, adipose tissue, and metabolic status. We showed that breed might have different adaptive metabolic responses to different feeding intensities and management strategies.

Our results also indicate that individual metabolic response to nutritional strategies might have a stronger effect on the endocrine and traditional fertility traits than breed. However, breed was more strongly associated with the energy balance variables than nutritional strategies. These differences might be of use to help herd health advisors, nutritionist, and farmers to design the most efficient systems to meet the nutritional requirements of the cows and especially avoid overconsumption of energy. In addition, we found that SRB cows prioritized energy differently when compared to Holstein cows: SRB cows maintained homeostasis better than

Holsteins, which had a deeper energy deficit than SRBs. Moreover, most of the endocrine (CLA, LP, IOI, and FOE) and traditional (CFI and FLI) traits were more favourable in the LE -diet group when compared with the HE -diet group, indicating faster activation (better adaptation) of the endocrine axis in these cows.

5.2 Dry matter intake and energy corrected milk

The severity and the magnitude of NEB in our cows were positively correlated with both DMI and ECM, as previously described by others (Gross et al., 2011). However, diet had a significant effect on DMI in Holstein cows but not in SRB cows. In contrast, the effect of diet on ECM was not consistent between studies I and III. Thus these patterns appear to have a genetic basis, suggesting that the energy balance in early lactation may mainly be driven by genetic factors (Friggens, 2004).

5.3 Body condition score and subcutaneous adipose tissue thickness

Diet was not associated with back fat thickness or BCS suggesting that adipose tissue mobilization is independent of nutrition and depends more on genetics. However, the risk of severe negative energy balance after calving was shown to increase as the difference in BCS before and after calving increased. The results from studies I and III, obtained in different environmental conditions, were very consistent. Our findings are in agreement with studies made by Garnsworthy and Torps (1982), showing that BCS is affected in cows with NEB. High BCS at AI was also correlated negatively with NP-EEM frequency, showing the practical use of measurement for BCS in reproductive management.

Our results demonstrate that animals from different breeds mobilize fat reserves differently in order to cover energy needs, indicating that breed may play a role in the interaction between energy level and BCS (Hjertén, 2006). These results are in agreement with the concept that breed difference, in coping with energy deficit might help to reduce the impact of NEB on endocrine and metabolic function and reduce embryonic mortality (Bilodeau-Goeseels, 2003). A possible advantage of the energy metabolism in SRB compared to Holstein cows may be related to a better adjustment of their metabolic response to energy demands, providing them the possibility to relocate or save energy for reproductive processes. However, as reported before, results show that this advantage might cause some SRB individual cows to become obese, resulting in decreased fertility (Awasthi, 2010).

5.4 Energy balance

The development of NEB is a result of a mismatch between energy intake and energy requirements (Bauman and Currie, 1980, Butler, 2000). The relationships between NEB and the endocrine axis were studied in SRBs and Holstein cows in the early ´80s (Berglund and Danell, 1989). However, due to genetic selection for milk production, improved management, and nutrition, these breeds and production levels are very different today, which is why other relationships may have developed.

Our results show that the effect of diet on NEB was not associated with differences in NEFA concentrations, BCS, or back fat thickness. This suggests that no difference in fat mobilization existed between the two energy-diet groups.

In the present study, endocrine and traditional fertility traits were positively unfavourable correlated with the severity and magnitude of NEB during early postpartum. This relationship might be explained by the high metabolic and nutritional requirements due to the rapid increase in milk production. The two breeds studied differ in terms of energy balance in early lactation. The energy balance in Holstein cows reached a lower nadir combined with a higher total energy deficit compared to SRB cows, which experienced less severe NEB during early lactation. In addition, this study could not support the hypothesis that high-energy diets can reduce NEB. Several Swedish farmers keep mixed herds, combining purebred Holstein and SRB cows and feeding them as if they were one breed. Our results might aid the development of nutritional strategies that are relevant to dairy farmers to improve the energy status of dairy cows during early lactation. High energy-diets may be adapted and favourable to Holsteins. In contrast, these high energy-diets may not be optimal for some SRB cows.

The difference in the severity and magnitude of NEB in favour of SRB cows might be explained by a difference in the way SRB and Holstein cows prioritize energy. A possible difference in energy prioritization might indicate than Holstein cows promote homeorhesis rather than homeostasis (Bauman et al., 1989), which is the case in SRB cows. Despite Holstein cows having a more pronounced NEB, no direct unfavourable effect could be found on endocrine or traditional fertility traits when compared with SRB. Some limitations may be due to the relatively low number of animals under study. However, the negative impact of high BCS / very moderate NEB on the fertility of SRB cows should also be taken into consideration.

5.5 Plasma concentrations of glucose, fatty acid, and insulin

In this study, diet had no effect on glucose and NEFA plasma concentrations. This is consistent with findings made by other groups indicating a potential interaction between genotype and diet in the control of lipolysis (Roche, 2006, Andersen, 2004). However, the lack of difference between energy-diet groups in terms of blood metabolites may imply that another mechanism is involved in tissue mobilization during early lactation. Regulation of lipolysis is largely genetically controlled, whereas lipogenesis is primarily regulated by the environment (McNamara, 1986a, McNamara, 1986b, Herdt, 2013). The use of different nutritional strategies had no effect on body lipid mobilization during early postpartum, which is in agreement with other studies (Delaby, 2009). The absence of an effect of energy-diets on metabolic variables might be due to high individual variation in the results and the small number of animals studied. However, differences with previous studies (Leroy, 2014) may also be due to the magnitude of the difference between feeding intensities, levels of feeding and periods studied, as has been found in other studies (Berglund and Danell, 1987).

5.6 Plasma concentrations of adipokines

Our results are in agreement with other studies showing that the concentrations of plasma leptin, adiponectin, and resistin are correlated to NEB (Sadri et al., 2011, Giesy et al., 2012, Reverchon et al., 2014). Our results are also in agreement with other studies showing that the period around calving is characterized by the most prominent changes in plasma adipokines (Giesy et al., 2012, De Koster et al., 2017). In our study, leptin concentrations declined to a nadir at parturition and decreased rapidly during periods of moderate undernutrition. Reduced leptin concentrations are correlated with delayed commencement of luteal activity (Kadokawa et al., 2000, Liefers et al., 2003).

The profiles of the three adipokines studied in this project were affected by the energy content of the diet. This result might imply that the effect of energy-diet on plasma concentration of adipokines is correlated with mechanisms that regulate NEB and maintain fat reserves.

The correlation between plasma adipokines and reproductive performance was weak. However, we believe that these correlations deserve further investigations, because of the low number of recruited animals and high individual variation.

The correlation between resistin with NEFA suggests that resistin may contribute to the regulation of lipolysis during the peripartum period. This correlation might be explained by previous studies showing that recombinant bovine resistin increases the release of glycerol and mRNA levels for adipose triglyceride lipase and hormone-sensitive lipase in adipose tissue explants (Reverchon et al., 2014).

However, our results on plasma adipokines and their correlation with NEB and reproductive performance are limited only to Holstein cows, highlighting the need for further investigations in cows with different genetic backgrounds.

5.7 Reproductive performance

Several authors have reported unfavourable effects of NEB on reproductive performance in dairy cows (Berglund and Danell, 1987, Butler and Smith, 1989, Lucy, 2001, Kanyima, 2013, Valour, 2013). No direct effect of breed or diet on reproductive performance was found in this study. High milk production and metabolic imbalance can affect fertility (Grimard et al., 2006) but this was relationship not be observed in the present study. A possible explanation for this discrepancy might be that despite significant differences in the magnitude and severity of NEB between the two breeds, the differences were not large enough to affect reproductive performance.

A lack of power to detect differences in reproductive performance could also be noted. However, as discussed before, the negative impact on fertility of animals with a high BCS at time of AI may have contributed to this lack of difference.

The synthesis and secretion of GnRH and LH can be affected by the severity and magnitude of NEB resulting in altered endocrine and fertility traits (Peter, 2009, Humblot, 2009). Poor follicular growth and delayed LH peak might lead to poor signs of oestrous expression and poor oocyte quality, thus negatively affecting the fertility traits, especially those based on visual oestrus observation (Leroy, 2008a). Considering the above information a reduction in reproductive performance in LE and Holstein cows was expected.

Despite the fact that no major effect of diet was found on NEB, endocrine traits seemed to be more favourable in the LE than in the HE -diet group. The effect of diet on CLA was not consistent between the studies performed under different environmental conditions. A possible explanation might be the differences in response in NEFA plasma concentrations between the studies, nutritional management (TMR vs. separate feed stuffs) and / or in differences of the effect of breed and diet interaction in milk production between the studies (kg ECM per day). However, our results showing a tendency for better conception rates (first 3 AIs) in the LE group than in the HE group are in agreement and consistent with those of Cutullic et al. (2011), showing that low-level feeding strategies do not disturb reproductive function in such a way that fertility is affected.

6 Conclusions

- The interaction between nutritional strategies and breed is a key factor influencing fertility and reproductive performance in dairy cows.
- Breed has different adaptive metabolic responses to different feeding intensities and management strategies.
- Individual metabolic response to nutritional strategies has a stronger effect on endocrine and traditional fertility traits than breed.
- Breed is more strongly associated with energy balance than the nutritional strategies.
- SRB cows can maintain homeostasis better than Holsteins, which have a deeper energy deficit than SRBs.
- Low energy-diet groups have a faster activation (better adaptation) of the endocrine axis when compared with high energy-diet groups.
- The interaction between high BCS at calving and incorrect individual management routines can increase the risk for developing a severe energy deficit after calving.
- Limited negative energy balance in early lactation is one prerequisite for high milk production and does not affect necessarily reproductive performance.
- Continued studies of possible breed differences in the relationship between energy balance and nutritional management while considering the continuously increasing milk yield.

7 Future perspectives

The results of this thesis highlighted the impact of the interaction between cows of different genetic background and nutrition. However, despite clear differences in energy balance between the breeds, the impact of NEB on reproductive performance was very limited. Reproductive performance was related more to other individual factors such as BCS and management. A limitation of this study may be related to the experimental design.

Due to high individual variation within breeds, it would be interesting to investigate the effect of different nutritional strategies further, based on a more individualised management for a bigger sample size. Currently, cows are fed based on their milk production despite their genetic background to maximize the dry matter intake. However, as found in our study, this results in some cows having too high BCS, compromising their reproductive potential.

Modifying the nutritional management routines to induce slightly lower peak milk yield without compromising the genetic potential of the animal would probably be beneficial for reproductive performance. It would also be interesting to include the Swedish results from the second lactation and investigate possible differences on the metabolism, NEB, and reproductive performance between parities, breeds and energy-diets.

Such studies could be the source of innovative solutions for robust and sustainable improvement of fertility in dairy cows. However, molecular approaches are needed to unravel the underlining molecular mechanisms involved in a cow’s production and fertility. In this respect, it would be interesting to investigate the impact of fat mobilization and / or accumulation on the production of pro-inflammatory cytokines and their relationships with fertility on a larger scale.

Popular science summary

This thesis describes the impact of **breed and feeding intensity on reproductive performance** in dairy cows. Despite the improved breeding, management and nutritional strategies, decreased fertility in dairy cows is still widespread. Most of the dairy cows are in negative energy balance during the period after calving as the amount of consumed feed is not enough to cover the energy demands for milk production and maintenance. **It is natural to mobilize body reserves** but a too rapid mobilization makes the cow susceptible to metabolic disorders and impaired fertility.

The studies presented in this thesis were conducted in the framework of the **EU project "Prolific"** (Grant; 311776) and took place in Sweden (SLU; experimental station "Lövsta") and France (INRA). For the project around 85 cows were recruited (44 in Sweden and 39 in France) that were followed for two consecutive years. Animals were submitted either to a high energy diet (High energy; HE) or to a restricted diet (Lower energy; LE).

From the studies in Sweden, metabolic patterns in response to diet were expressed differently between the SRB and Holstein cows. **Diet had no effect on glucose and free fatty acid plasma concentrations** but **differences** were found **between breeds**, indicating a potential interaction between breed and diet in the control of lipolysis. Our results were indicating that the regulation of lipolysis is largely controlled by breed, whereas lipogenesis is primarily regulated by the diet. Mobilization of fat tissue was not affected by the different nutritional strategies during early postpartum.

Holstein cows had more pronounced energy deficit than SRB cows. When exposed to a high energy diet, Holstein cows prioritised milk production whereas SRB cows fed this regime had a tendency to build up excessive body reserves.

Nutrition had no effect on BCS but this variable was influenced by breed, suggesting that adipose tissue mobilization depends more on genetics and not so much on diet. The results from studies made in Sweden and France demonstrated that the risk of developing a severe negative energy balance after calving increased as the BCS losses before and after calving increased. Our findings are showing that **BCS is affected in cows with NEB**. High BCS at AI tended to be unfavourable correlated with **embryo mortality frequency**, showing the practical use of measurement for BCS in reproductive management.

Our results demonstrated that animals of **different breeds mobilize fat reserves differently** in order to cover energy needs. A possible advantage of the energy metabolism in SRB compared to Holstein can provide them the possibility to relocate or save energy for reproductive processes. However, this advantage might also cause some SRB individual cows to become obese, resulting in decreased fertility. **Limited negative energy balance in early lactation does not affect necessarily reproductive performance;** however, maybe is a prerequisite for high milk production.

Today's nutritional strategies aim increasing the dry matter intake but due to high individual variation within breed, it would be interesting to further investigate the effect of different management strategies (shorten dry period, prolonged lactation) based on a more **individualized management.**

Populärvetenskaplig sammanfattning

I denna avhandling studerades **rasens och utfodringsintensitetens inverkan på reproduktionen** hos mjölkkor. Trots förbättrade avels-, utfodrings- och skötselstrategier är dålig fruktsamhet hos korna fortfarande en realitet. De flesta kor befinner sig i en negativ energibalans i en period närmast efter kalvningen eftersom deras konsumtionsförmåga inte kan täcka energibehovet för mjölkproduktion och underhåll. Kon mobiliserar då energi från sitt kroppshull, men en alltför snabb och kraftig sådan gör kon mer mottaglig för metaboliska störningar och nedsatt fruktsamhet.

Studiena i avhandlingen utfördes inom ramen för EU-projektet ”Prolific” (Bidragskontrakt nr 311776), och genomfördes i Sverige (SLU, Forskningscentrum Lövsta) och i Frankrike (INRA). Cirka 85 kor rekryterades till projektet (44 i Sverige och 39 i Frankrike) och de följdes under två år. Korna blev fördelade på två utfodringsintensiteter, hälften på en hög energiintensitet (Hög energi; HE) och den andra hälfen på en lägre energiintensitet (Låg energi; LE).

Baserat på resultaten i de svenska studierna så var det **metaboliska mönstret för de två utfodringsintensiteterna** olika hos SRB och holsteinkor. Utfodringsintensiteterna hade ingen effekt på glukos eller plasmakoncentration av NEFA (fria fettsyror), men de rasskillnader som existerade kan tyda på ett möjligt samspel mellan ras och utfodringsintensitet i regleringen av lipolysen (fettnedbrytningen). Våra resultat tyder på att **regleringen av lipolysen** till stor del styrs av ras, medan **lipogenesen** (upbyggnaden av fett) främst styrs av utfodringen.

Mobiliseringen av fettvävnad under de första veckorna efter kalvning påverkades inte av de två olika utfodringsintensiteterna.

Holstein hade en större energibrist efter kalvningen än SRB-kor. På den högre utfodringsintensiteten prioriterade Holstein energin till mjölkavkastning medan SRB tenderade att i högre utsträckning fylla på sina fettreserver.

Utfodringsintensitet hade ingen effekt på kroppshullet (BCS), däremot var hullet påverkat av ras vilket kan tyda på att mobiliseringen av underhudsfett i större utstäckning är styrd av genetisk bakgrund än utfodring. Resultaten från såväl de svenska som franska studierna visade att risken för att utveckla en allvarlig negativ energibalans efter kalvningen ökade när BCS-förlusterna före och efter kalvningen ökade. Våra resultat visar att **BCS påverkas hos kor i negativ energibalans (NEB).** Ett kraftigt kroppshull vid insemineringstidpunkten hade också ett ogynnsamt samband med **embryodödlighet**, vilket visar den praktiska nyttan av att registrera hullet för fruktsamhetsresultatet.

Våra resultat visar att **kor av olika ras mobiliserar på olika sätt från sina fettreserver** för att täcka sina energibehov. En möjlig fördel för SRB jämfört med holstein är att de kan använda en del av sina inlagrade fettreserver till reproduktionsprocessen. Denna fördel kan dock också leda till att några individuella SRB-kor blir för feta, vilket i sin tur kan leda till försämrad fruktsamhet. **En begränsad negativ energibalans i tidig laktation behöver inte påverka reproduktionsresultatet**, men det kan vara en nödvändig förutsättning för en hög mjölkavkastning.

Dagens utfodringsstrategier syftar till att öka foderintaget av torrsubstans, men då detta har en stor individuell variation inom ras, skulle det vara intressant att vidare undersöka effekten av olika skötselstrategier (kortare sintidsperiod, förlängd laktation) baserat på mera **individualiserad skötsel.**

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