

Drying Off the Dairy Cow

Effects on Metabolism and Udder Health

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Abstract

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Most dairy cows generally have a non-lactating, “dry period” prior to parturition. As the milk production of dairy cows has increased dramatically during the last decades, cows managed conventionally with a 12 to 13 month-calving interval are dried off (DO) while still producing significant quantities of milk. The DO period is probably one of the most physiologically demanding periods for the high-yielding dairy cow, and may negatively affect animal welfare, but few studies have been performed in this area. Therefore, the general aim of the present work was to investigate how different DO procedures and milk yield at DO affect metabolism and udder health of the dairy cow. Furthermore, the effects of different calving intervals and breed were also evaluated.

Three studies are included in this thesis. In the first study cows were randomly assigned to two different feeding treatments. One group was fed straw *ad libitum*, while the other group was fed silage [4 kg dry matter (DM)] daily and straw *ad libitum* to evaluate metabolic changes and health during DO. In the second study, the effects of daily milk yield at DO, different calving intervals (12 and 15 months) and breed on metabolism and udder health were studied when feeding additional silage at DO. The third study was conducted on over 10 000 lactating cows and the association between daily milk yield prior to DO and veterinary-treated clinical mastitis (VTCM) from DO until early lactation was evaluated.

The overall results of this thesis show that extensively restrictive feeding DO protocols may have negative effects on metabolism. The plasma concentration of non-esterified fatty acids (NEFA) was markedly elevated during DO as an effect of restrictive feeding. Furthermore, plasma NEFA was related to the milk yield prior to DO. Feeding straw only during DO affected the rumen environment as shown by increased pH, a reduced concentration of volatile fatty acids, urea, and lower number of protozoa. Restrictive feeding also increased the plasma cortisol, which indicates an elevated metabolic load in this group.

In addition, high milk yield at DO increased the risk for VTCM and the number of open teat canals during the dry period. Swedish Holsteins were at higher risk for VTCM from DO until early lactation compared to Swedish Red and White cows.

In conclusion, this thesis shows that the common DO procedure to feed straw only may give rise to metabolic disturbances. However, this might be avoided without any apparent negative effects on udder health if a limited amount of silage is added during DO. As high daily milk yields at DO increase the risk for mastitis, alternative ways to reduce the milk yield at DO should be considered.

Keywords: acute phase proteins, breed, calving intervals, cortisol, dairy cow, dry-off, metabolic load, metabolism, NEFA, somatic cell count, udder health

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*"We can't solve problems by using the same
kind of thinking we used when we created them."*

Albert Einstein

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II. Odensten, M. O, Holtenius K., & Persson Waller K. 2006. Effects of Two Different Feeding Strategies during Dry-off on Certain Health Aspects of Dairy Cows. *Submitted*.

III. Odensten, M. O., Berglund, B., Persson Waller, K., & Holtenius, K. 2006. Metabolism and Udder Health at Dry-off in Cows of Different Breeds and Production Levels. *Submitted*.

IV. Odensten, M. O., Lindberg, A., & Gustafsson, A. H. 2006. Associations Between Milk Yield at Dry-off and Veterinary-Treated Clinical Mastitis During the Dry Period and Early Lactation. (Manuscript).

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List of abbreviations

APP	acute phase protein
ATP	adenosine triphosphate
BCS	body condition score
BHBA	β -hydroxybutyric acid
CI	calving interval
CI-12	12-month calving interval
CI-15	15-month calving interval
CNS	coagulase-negative staphylococci
DM	dry matter
DO	dry-off
ECM	energy corrected milk
FIL	feedback inhibitor of lactation
HFI	cows selected for high milk fat percentage index
HP	haptoglobin
HY	high-yield group
IMI	intra-mammary infections
LFI	cows selected for low milk fat percentage index
LY	low-yield group
ME	metabolizable energy
MY	medium-yield group
NEFA	non-esterified fatty acids
PD4	4 weeks prior to dry-off
PP1	1 week post-parturition
PP4	4 weeks post-parturition
PRL	prolactin
SAA	serum amyloid A
SCC	somatic cell count
SH	Swedish Holstein breed
SRB	Swedish Red and White breed
VFA	volatile fatty acids
VTCM	veterinary-treated clinical mastitis

Introduction

In the beginning of the 20th century, cows had an average milk production of about 2500 kg 4% fat corrected milk per cow and year. Since then, milk production has increased dramatically. In 2005, the average lactation yield in Sweden was 9022 kg energy corrected milk (ECM¹) for the Swedish Red and White breed (SRB) and 9550 kg ECM for the Swedish Holstein breed (SH) (Larsson, 2006). Milk production has more than doubled since 1960 (Swedish Dairy Association, 2005). With the efficient animal breeding, feeding and management systems used today, this trend seems to be continuing.

Milk production in Sweden is influenced by the payment system for milk. During the last decades, the Swedish system has emphasised milk volume, in contrast to the earlier focus on milk fat production, resulting in high milk production. The economy for dairy farmers has also changed with declining milk prices and higher costs for feed. This is one reason behind the continued trend of fewer numbers of dairy farmers in the country, and increasing numbers of cows per farm, which means that the farmer spends less time per cow. Taking this into account, it is very important that we improve our understanding of ways to develop better management routines, feeding systems, and, by far the most important, how to advance animal welfare and health.

Dry-off

A non-lactating period prior to parturition, commonly referred to as the dry period, is important for optimal milk production during the subsequent lactation. A dry period of less than 40 days results in reduced milk production during the following lactation (Swanson, 1965; Sørensen & Enevoldsen, 1991). If only milk production is considered, the optimal dry-period interval is 40 to 60 days. This duration of the dry period would balance loss of milk production during the current lactation with increased production during the following lactation (Sørensen & Enevoldsen, 1991; Rastani *et al.*, 2005). Eliminating the dry period has been shown to improve metabolic status during the following periparturient period, but the total yield during the following lactation decreased (Andersen *et al.*, 2005; Rastani *et al.*, 2005).

A survey among feed advisors and veterinarians was initially conducted to investigate and retrieve more information about dry-off (DO) practices in Sweden (Odensten, unpublished). The results from the survey concluded that the DO procedure generally starts about 6 to 8 weeks prior to expected parturition. The farmers feed straw when it is available and use different milk-cessation methods depending on the milk yield and udder health at DO. Milk yields of over 30 kg per day at DO are not uncommon. One could conclude that there are as many DO strategies as there are farmers (Odensten, unpublished).

¹ Sjaunja *et al.*, 1990

The DO period is probably, with the exception of the weeks around parturition, the most physiologically demanding period for the high-yielding dairy cow. High-yielding cows managed conventionally with a 12 to 13 month-calving interval (CI) are dried off while still producing large quantities of milk. Because the production economy is thought to benefit from a short CI, a general practice in dairy herds with intensive milk production is to breed cows with the aim of establishing a CI of 12 months (Holmann *et al.*, 1984; Strandberg & Oltenacu, 1989). Österman & Bertilsson (2003) on the other hand, suggested that prolonged CI in high-producing dairy cows has a beneficial effect due to better utilization of the milk producing capacity. Extended CI also improved fertility as the time for resumption of reproductive functions is prolonged (Ratnayake *et al.*, 1998), and Rehn *et al.* (2000) stated that prolonged CI reduces udder health problems due to high milk production.

Reduction of nutrients

A common practice among dairy farmers is to reduce the nutrient supply when drying off the dairy cow. Some farmers have adopted DO routines involving short-term drastic reductions in the feed supply, but concerns have been raised that major changes in the nutrient supply at DO might lead to metabolic problems, especially among high-yielding cows (Skidmore *et al.*, 1997). If farmers offer only straw during DO, the cows will experience a marked negative nutrient balance. In early lactation, such a negative balance gives rise to high non-esterified fatty acid (NEFA) concentrations in blood and is associated with endocrine changes, such as increased cortisol and impairment of the immune system (Preisler *et al.*, 1999; Perkins *et al.*, 2001). This results in the cows being more vulnerable to infections, e.g. in the udder (Rukkwamsuk *et al.*, 1999; Suriyasathaporn *et al.*, 2000). However, this has not been studied at DO.

Feed-deprived cows appear to be able to maintain plasma glucose levels in spite of the fact that glucose precursors are largely lacking (Baird *et al.*, 1972; Reid *et al.*, 1977; McGuire *et al.*, 1995; McGuire *et al.*, 1998; Agenäs *et al.*, 2003). Apparently, it is not reduced plasma glucose that gives rise to reduced mammary lactose synthesis. Among proposed factors contributing to the reduced lactose synthesis during food deprivation are down-regulation of glucose transport proteins, decreased mammary blood flow and prolonged transit time of blood through the mammary gland (Davis & Collier, 1985; Farr *et al.*, 2000; Shennan & Peaker, 2000).

Samuelsson *et al.* (1996) also showed that plasma cortisol increased and prolactin decreased in cows that were feed-deprived for two days. Prolactin may also be involved in the decreased lactose synthesis. Lactose synthesis, as well as milk protein synthesis, is promoted by prolactin, whereas no effect of prolactin has been shown on milk fat synthesis (Knight, 2001). It has also been suggested that the tight junctions between the mammary epithelial cells become leaky when prolactin is reduced (Linzell *et al.*, 1975; Flint & Gardner, 1994).

Cessation of milking

Another common practice during DO is intermittent or abrupt cessation of milking. By prolonging the milking intervals, the secretory epithelium in the udder regresses. This leads to inhibited milk secretion (McFadden *et al.*, 1995). A prolonged milking interval induces re-absorption of milk and regression of mammary secretory epithelial cells (Stefano *et al.*, 2002). In milk, the concentration of a putative protein called Feedback Inhibitor of Lactation, (FIL) increases at late lactation and at DO. FIL acts primarily by blocking constitutive secretion of the alveolar epithelial cells as an effect of milk engorgement in the gland (Peaker & Wilde, 1996; Wilde *et al.*, 1998). Infrequent or ceased milking would decrease milk production by increasing the amplitude of changes in FIL concentration (Wilde *et al.*, 1995; 1998). FIL has also been shown to have a positive effect on apoptosis (programmed cell death) in mammary epithelial cells at the end of lactation and during involution (Stefano *et al.*, 2002). In dairy cows, there is no detectable loss of mammary epithelial cells in contrast to rodents, in which the mass of secretory cells during the non-lactating period is reduced. However, a non-lactating period is nonetheless important for maximizing milk production during the following lactation (Rastani *et al.*, 2005; Andersen *et al.*, 2005). Increased cell turn over during the dry period may promote this effect by removing epithelial cells and renewing the population of mammary secretory cells (Capuco & Akers, 1999).

Tight junctions between the epithelial cells of the mammary gland form a barrier between the blood and the milk, which prevents paracellular transport. However, milk stasis during prolonged milking intervals causes accumulation of local signals, which makes the tight junctions leaky (Nguyen & Neville, 1998). It has been shown that leaky tight junctions decrease milk production and that lactose seeps into the bloodstream (Stelwagen *et al.*, 1994). Moreover, inhibition of milk production in the udder can be accelerated if hydrolysed casein is injected into the udder (Shamay *et al.*, 2003).

Udder health

At DO, it is of vital importance that the milk synthesis is rapidly inhibited to prevent milk leakage, which may negatively affect udder health (Dingwell *et al.*, 2003). The most common health problem associated with DO is intra-mammary infections (IMI), which most often are unobserved until after calving when clinical mastitis occurs (reviewed by Dingwell *et al.*, 2004). The first line of defence against IMI is the teat end and the formation of the keratin plug. The risk for IMI at DO increases with increasing milk production at DO, occurrence of milk leakage, decreasing teat-end integrity and late formation of the teat-canal keratin plug (Jørstad *et al.*, 1989; Dingwell *et al.*, 2001; Dingwell *et al.*, 2004). The susceptibility to IMI is also influenced by the quality of the immune defence system of individual cows, for example, the antibacterial functions of immune cells. Metabolic disturbances, as well as other stressors, can have negative effects on important immune cell functions, which may increase the susceptibility to infectious diseases such as mastitis (Preisler *et al.*, 1999; Suriyasathaporn *et al.*,

2000). However, the relationship between immune functions and metabolism in dairy cows has been studied mainly in the periparturient and early lactation periods, and not during the end of lactation and at DO.

Aims of the thesis

To ensure economically sustainable dairy farming it is vital to find the best methods to DO high-yielding dairy cows to avoid animal health and welfare problems. Thus, gathering information about the effects of various DO procedures to increase the understanding of this area is essential for both dairy farmers and their advisors.

Therefore, the general aim of this thesis was to increase knowledge about various factors influencing DO in the dairy cow. The studies were performed on cows with different daily milk yields at DO, and various metabolic and health parameters were investigated to clarify cow responses during DO.

The specific aims were:

- To study rumen, metabolic and hormonal changes during DO in cows subjected to two different DO feeding protocols varying in nutrient supply.
- To compare the effects of two DO feeding protocols, varying in nutrient supply, on udder health and certain health related blood parameters.
- To investigate the effects of daily milk yield prior to DO, CI and breed on milk volume reduction, intermediary metabolism and udder health.
- To investigate the association between milk yield at DO, breed, and parity at DO, and the risk of veterinary-treated clinical mastitis (VTCM) from DO until the first 30 days after calving.

Materials and methods

This thesis is based on three separate studies, of which two were experimental. The first experiment was conducted at Kungsängen Research Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden (Kungsängen study) in the fall of 2002 (Papers **I** and **II**). The second experiment was carried out on the research farm Jälla (Jälla study) at the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, from August 2003 to January 2005 (Paper **III**). The third study (Epi-study) was conducted during the fall of 2005, on data from cows enrolled in the official Swedish milk- and health-recording programs (Paper **IV**).

Materials and methods used in this thesis are described in detail in papers **I–IV**. In this chapter, only general comments are made.

Animals and management

In the Kungsängen study (Papers **I** and **II**), 21 SRB cows were used, while 38 SRB and 18 SH cows were used in the Jälla study (Paper **III**). Primi- and multiparous cows were used in both studies. All SRB cows were bred with sires that were indexed for high (HFI) or low (LFI) milk fat percentage, but had the same amount of energy produced in milk (Janson, 1993). The animals in the Jälla study also participated in a long-term trial with two different CI, i.e. 12 months (CI-12) and 15 months (CI-15) (Rehn *et al.*, 2000).

The animals in the Kungsängen and Jälla studies were housed in individual tie stalls with sawdust bedding. Drinking water was always available in automatic water bowls, and the cows had free access to salt licks. During lactation and after DO the cows were fed according to the Swedish requirements for metabolizable energy (ME), protein and minerals, based on actual milk yield and stage of gestation (Spörndly, 2003) (Papers **I–III**).

In the Epi-study (Paper **IV**), 10 741 cows from 488 farms enrolled in the official Swedish milk- and health-recording programs were included. Among these cows, 5090 were SRB, 4896 were SH, and the remaining 755 were other breeds or crossbreeds.

Experimental design

An overview of certain parts of the experimental designs in the Kungsängen study (Papers **I** and **II**) and the Jälla study (Paper **III**) is presented in Fig. 1. The Uppsala Local Ethics Committee approved the experimental designs of these studies.

The Kungsängen study (Papers I and II)

In this experiment, 21 cows were randomly assigned to two different feeding routines at DO, *i.e.* *ad lib* wheat straw (Straw; n=10), or 4 kg of DM silage per day and *ad lib* straw (Silage; n=11) during a DO period of five days. During this period, the cows were milked on day 3 and 5 in the morning.

The Jälla study (Paper III)

Initially, 64 dairy cows of the SRB and SH breeds entered the experiment. Eight of these cows had a milk yield lower than 5 kg milk/day the week prior to DO, and were excluded from further investigations during DO. All the cows received the same feed (4 kg DM silage per day and *ad lib* wheat straw) during the five-day DO period, and were milked on day 2 and 5 in the morning. The cows were grouped according to milk yield prior to DO in a low (LY = 5.0 to 11.4 kg milk per day; n = 19), medium (MY = 11.5 to 17.7 kg milk per day; n = 19), and high (HY = 17.8 to 29.5 kg milk per day; n = 18) yield group.

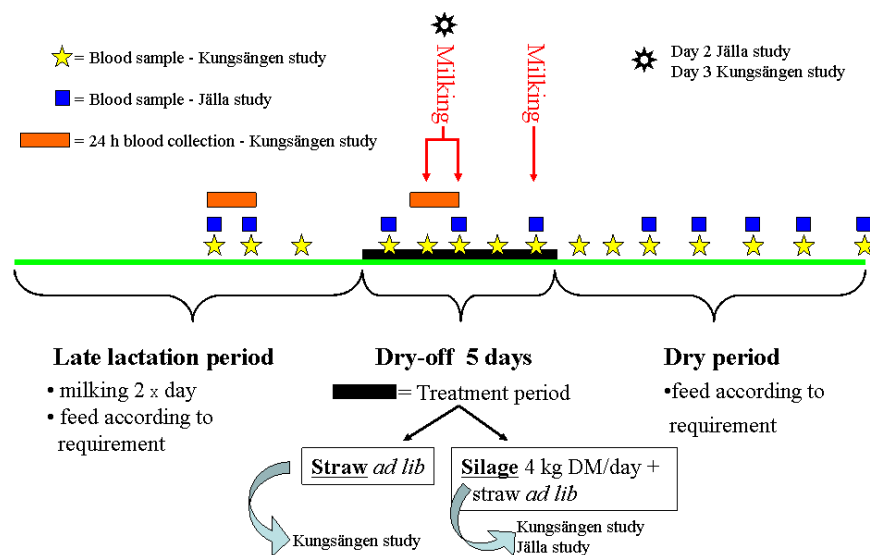


Fig. 1. Schematic drawing showing feeding, milking and blood sampling in the Kungsängen and Jälla studies.

The Epi-study (Paper IV)

This study was conducted on data from cows enrolled in the official Swedish milk- and health-recording programs. Herds having a minimum of 25 lactating cows per herd and in which at least 20% of the cows in the herd had reported DO

date during 2003 were selected. A number of 3240 herds fulfilled these criteria. A random sample of 488 herds with 10 741 cows was selected from these herds.

Data collection and analyses

Daily blood samples were collected (between 1000 and 1100 hours) before, during and after DO, from the jugular or coccygeal vessel into evacuated tubes containing sodium heparin as anti-coagulant (Papers **I–III**). In the Kungsängen study reported in Paper **II**, the diurnal profiles of cortisol were examined using a semi-permanent catheter inserted into the jugular vein. These blood samples were collected every 2 hours for 24 hours.

In the Kungsängen study reported in Paper **I**, plasma insulin was analyzed by means of a commercial radioimmunoassay kit (Coat-A-Count, Diagnostics Products Corporation, Los Angeles, USA). This method has been developed for determination of human insulin and has been evaluated and validated for bovine insulin. In the Jälla study (Paper **III**), an ultrasensitive bovine insulin ELISA kit (Ultrasensitive Bovine Insulin, Mercodia AB, Uppsala, Sweden) was used. In this assay, two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. This assay enables detection of insulin down to a concentration of 0.02 µg/l (0.58 µU/ml). The leptin concentration in blood plasma was determined by means of an ovine-specific radioimmunoassay method validated and evaluated for bovine leptin (Delavaud *et al.*, 2002). The analyses were performed at the Herbivore Research Unit, Adipose Tissue and Milk Lipids Group, INRA-Theix, Saint-Genes-Champanelle, France.

The blood samples collected in the Kungsängen and Jälla studies were also analyzed for plasma glucose, β-hydroxybutyric acid (BHBA), NEFA, and urea. In the Kungsängen study, the blood samples were also analyzed for serum amyloid (SAA), haptoglobin (HP) and Mg.

Milk samples were collected prior to and during DO. Milk yield was registered and milk lactose, protein and fat were analyzed. Bacteriological growth, milk adenosine triphosphate (ATP) and somatic cell content (SCC) were analyzed prior to and during DO, and after parturition (Papers **I–III**). The teat-end condition was scored according to Neijenhuis *et al.* (2000) once weekly after DO, and teat closure was investigated once weekly after DO in two teats (random choice of right or left side) according to Dingwell *et al.* (2004) (Paper **III**). The cows were clinically examined before and during DO (Paper **II**), and disease incidence were registered throughout the studies (Papers **II** and **III**).

In the Kungsängen study, rumen fluid was collected from each cow 1 week prior to DO, during DO, and 2 weeks post DO, using a technique developed at the Department of Agricultural Research for Northern Sweden, Forage Research Centre, SLU, Umeå. A full description of the technique is given by Hetta (2004). Rumen fluid was collected into pre-warmed thermos bottles using an esophageal tube (Fig. 2).



Fig. 2. Esophageal tube used for collecting rumen fluid.

Within 2 min after collection, pH was registered (MP125, Mettler-Toledo, GH-8603, Schwerzenbach, Switzerland). A test was conducted before the Kungsängen study started on a rumen-fistulated cow where rumen samples were collected through the fistulae and compared to a rumen sample collected by the esophageal tube. Virtually no differences in pH were detected between fluid collected by means of esophageal tube or through the fistulae, indicating that saliva contamination was negligible (data not presented). The rumen fluid was also analyzed for volatile fatty acids (VFA), ammonium nitrogen ($\text{NH}_3\text{-N}$) and protozoa counts.

In the Kungsängen study, 117 faeces samples were collected before, during and after DO. Analysis of faecal cortisol metabolites (Palme & Mostl, 1997) by enzyme immunoassay was performed at the Institute of Biochemistry, Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria. The faecal cortisol metabolites have been shown to correlate with plasma cortisol and furthermore, elevated levels of cortisol metabolites have been observed in cows subjected to stressful treatments (Mostl *et al.*, 2002).

The data extracted in Paper IV included information on cow ID number, milk recording data (test-day milk yields, milk fat, milk protein content, and SCC, DO date (in 2003), calving date (after DO date), parity, breed, veterinary treatment dates, and reasons for treatment. The cows were divided into five groups (0–10, >10–15, >15–20, >20–25, and >25 kg/day) according to estimated milk yield at DO.

Statistical analysis

Statistical analyses are described in detail in each paper. In papers I–III, procedure mixed models with repeated measurements (Littell *et al.*, 1998) were used for all analyses using SAS software (version 8.02). Chi-square and Fischer's exact tests were used in papers II and III for analyzing IML, bacteriological findings and disease incidence. In the Epi-study (Paper IV), the statistical software STATA was

used. Generalized estimation equations with a logit link and binomial error distribution were used to model the probability of VTCM between DO and calving. The differences presented in results are significant ($P < 0.05$), if nothing else is stated.

Results

In this section, the results are summarized. A more detailed description is presented in each of Papers **I-IV**.

Feed consumption (Papers **I-III**)

In the Kungsängen study (Papers **I** and **II**), all cows in the Silage group consumed their silage ration, 4 kg DM/day, during DO in addition to 4.8 ± 0.3 kg DM straw per day. The Straw cows consumed 5.6 ± 0.7 kg DM straw per day. In the Jälla study (Paper **III**), all cows consumed a silage ration of 4 kg DM/day. Straw consumption was not determined.

Milk yield and milk composition (Papers **I-IV**)

In the Kungsängen study (Papers **I** and **II**), the milk yield prior to DO was 16.8 ± 0.8 kg/day in the Straw cows and 17.3 ± 0.8 kg/day in the Silage cows. All cows reduced their milk production during DO, and there was no difference in milk production between DO treatments. On day 3, the milk yield of the Straw and Silage cows was 9.0 ± 0.6 and 9.2 ± 0.6 kg, respectively, while the milk yield on day 5 was 2.3 ± 0.3 and 4.2 ± 0.6 kg, respectively. In the Jälla study (Paper **III**), the daily milk yield the week prior to DO was 8.6 ± 0.5 kg/day for LY cows, 14.7 ± 0.5 kg/day for MY cows, and 21.2 ± 0.5 kg/day for HY cows.

The extrapolated milk yield at DO in the Epi-study (Paper **IV**) was 13.9 ± 5.5 kg for SRB cows, 15.4 ± 5.8 kg for SH cows, and 12.9 ± 5.9 kg for other breeds/crossbreeds.

The milk lactose content decreased, while the milk fat and protein content increased during DO in all cows investigated (Papers **I-III**). The milk fat percentage increased in both the Straw and Silage treatment groups of the Kungsängen study, but the increase was most pronounced among the Straw cows (Paper **I**). In the Jälla study, the rise in milk fat percentage at day 5 was higher in the MY and HY groups than in the LY group (Paper **III**).

Intermediary metabolism (Papers **I-III**)

Plasma NEFA increased markedly during DO in all cows, peaked at day 3, and declined thereafter reaching pre-DO values at day 10 (Paper **I**) and day 12 (Paper **III**). The rise was more pronounced in cows fed straw only during DO, and

primiparous cows had higher NEFA concentrations throughout the study (Paper I). Plasma NEFA was related to milk yield prior to DO, with higher levels in the HY group than in the LY group (Paper III).

The plasma BHBA concentration decreased during DO, and remained lower than in late lactation (Papers I and III). In the Jälla study, the LY cows generally had lower plasma BHBA concentrations compared to the MY and HY cows. Breed affected the BHBA concentrations with a higher overall concentration in SRB than in SH cows, and primiparous cows had lower mean plasma BHBA levels compared to multiparous cows (Paper III).

A drop in plasma insulin during DO compared to late lactation was observed in all cows in the Kungsängen (Paper I), and Jälla studies (Paper III). In the Kungsängen study, the drop was most pronounced in the cows subjected to straw treatment. There were no yield effects on plasma insulin concentration (Paper III). After introduction of the dry-period feed ration, the insulin level returned to the level observed prior to DO (Papers I and III).

The plasma leptin concentration was reduced during the latter part of DO (Fig. 3), increased slowly during the dry period, and was slightly higher day 19 than in late lactation (Paper I).

The plasma glucose concentration did not change during DO compared to late lactation (Papers I and III), with the exception of the Silage group (Paper I), which responded with a slight increase during DO. An overall effect was seen in HFI cows, which had a lower glucose level than LFI cows (Paper I). This effect was not observed in the Jälla study. The CI-15 cows had higher plasma glucose concentrations compared to CI-12 (Paper III).

A reduction in the plasma urea concentration was induced by DO, and it remained low during the sampling performed in the dry period (Papers I and III). In both studies, there was an effect of parity where primiparous cows had lower urea values compared to multiparous cows (Papers I and III).

The plasma Mg concentration decreased in all cows during DO and remained low during the remaining part of the study (Paper II). The levels tended to be lower in the Straw group than in the Silage group. In addition, parity had an effect on Mg, with lower concentrations in primiparous cows than in multiparous cows (Paper II).

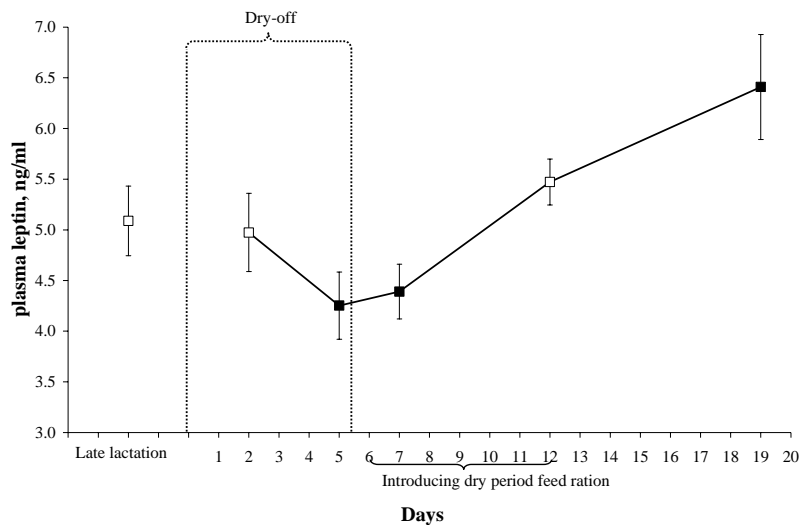


Fig. 3. Mean concentrations of plasma leptin before, during and after DO in cows fed straw only or straw plus 4 kg DM silage per day. The symbols represent the mean of all 21 cows (Paper I). Values that differ significantly ($P < 0.05$) from prior to DO are indicated by filled symbols.

Rumen metabolism (Paper I)

The rumen concentration of total VFA, as well as the individual acids propionate, butyrate, and acetate, decreased during DO. The reduction was more pronounced among cows fed straw only during DO (Paper I). Two weeks after DO there was no difference between treatment groups. The pH was higher at the sampling performed during DO than during the other two samplings, especially among the straw cows. Protozoa counts and $\text{NH}_3\text{-N}$ decreased markedly during DO in both treatment groups. The protozoa count was still low two weeks after DO despite feeding the dry-period feed ration.

Clinical investigations (Papers II–III)

The heart rate decreased during DO in all cows in the Kungsängen study, and the rate was numerically lower in the Straw group compared to the Silage group. The rectal temperature was not affected by DO. There was a decrease in the number of rumen contractions at DO, with numerically lower numbers in the Straw group, but the difference between groups was not significant. One cow in the Straw group and two cows in the Silage group developed clinical mastitis within 10 days post-parturition, but no other clinical diseases were recorded during the experiment (Paper II).

The disease incidence did not differ among yield groups, breeds or CI in the Jälla study. From DO to 2 months after calving, 23% of the cows suffered from clinical mastitis, and 30% suffered from some other clinical disease (mainly puerperal paresis) (Paper III).

Cortisol and acute phase proteins (Papers II and III)

Straw cows had higher plasma cortisol concentrations during DO than Silage cows, and the Straw group had higher cortisol levels during DO than in late lactation (Paper II). Milk yield prior to DO had a direct effect on plasma cortisol with increased numerical values in all groups, but the increase was only significant in the MY and HY groups (Paper III). In both studies, primiparous cows had lower plasma cortisol values than multiparous cows. The concentration returned to pre-DO values when the cows were introduced to the dry-period feed (Papers II and III). In the Kungsängen study, the diurnal cortisol profile revealed a treatment effect with higher cortisol values in Straw cows compared to Silage cows. Prior to DO, there were no differences between the two groups (Paper II). The concentration of cortisol metabolites in faeces showed a large variation and did not correlate to plasma cortisol, probably mainly due to uneven faecal production during DO (data not shown).

The SAA concentration increased during DO in both Straw and Silage cows (Paper II). However, at days 10 and 15, the SAA values were lower than prior to DO. The HP concentration was below the detection range throughout the study, and was excluded from the statistical analysis.

Udder health (Papers II-IV)

Milk SCC and milk ATP (Papers II and III)

The cow composite log SCC increased during the two milkings at DO compared to prior to DO in all cows in the Kungsängen and Jälla studies (Papers II and III). The SCC was similar in the Straw and Silage groups (Paper II), but LY cows had higher SCC compared to the MY and the HY cows (paper III). The SRB cows had lower log SCC compared to SH cows both prior to DO, and after parturition (Paper III). The log SCC decreased again in the samples post-parturition in all cows (Papers II and III).

The udder quarter milk ATP was not affected by treatment, but changed over time in all quarters, with higher ATP values during DO than in late lactation (Paper II). At days 4 to 8 after parturition, the ATP values were higher than, or similar to, those during DO. At the first sampling after parturition, the ATP was numerically higher in the Silage group than in the Straw group in all udder quarters. However, such a trend was not observed at 6 to 8 days after parturition (Paper II).

Bacteriology (Papers II and III)

The proportion of cows with new IMI (all udder pathogens) during DO and post-parturition was 33% in both Straw and Silage groups (Paper II). Overall, the proportions of cows with IMI at 4 weeks prior to DO (PD4), during DO, 1 week post-parturition (PP1) and 4 weeks post-parturition (PP4) were 26.8, 42.8, 39.3

and 23.2%, respectively. In Paper **III**, the proportion of cows with IMI at PP1 was lower in LY than in MY and HY. At DO, new IMI were more common in LY than in HY, but at PP1, new IMI were less common in LY than in MY and HY.

Coagulase negative staphylococci was the most common finding in both studies (Papers **II** and **III**). Other bacteria detected were *Staphylococcus aureus*, *Streptococcus uberis*, *Arcanobacterium pyogenes*, *Escherichia coli*, and *Enterococcus* spp. (Paper **II**).

Milk leakage, teat-end condition and teat-end closure (Paper III)

Milk leaking from the teats between or just before milkings was observed during DO on 1 to 3 occasions in each of 5 cows. Among the 37 cows examined for teat-end condition and closure, 16, 13 and 8 cows belonged to the LY, MY, and HY group, respectively. Teat-end callosity did not differ between production groups, but the proportion of cows scored as having open teat ends was lower in the LY than in the HY group. Cows with teats that were scored as closed on all occasions had fewer IMI at DO than cows that were scored open, but the groups did not differ at PP1 and PP4.

Mastitis incidence (Paper IV)

The results showed that milk yield at DO was significantly associated with the risk for VTCM during the period from DO to 30 days after calving. For cows with daily milk yield over 25 kg at DO, the risk for VTCM increased by 72% compared to cows with milk yields less than 10 kg at DO. Cows with a daily milk yield of 10 to 15 kg/day at DO did not run a higher risk for VTCM compared to cows milking 0 to 10 kg of milk. For cows with a milk yield of over 15 kg at DO there was an increasing risk for VTCM. Also, SCC higher than 200 000 cells/ml at DO were associated with a higher risk for VTCM, and SH cows were 43% more likely to have VTCM compared to SRB cows.

General discussion

Does DO cause stress?

During the peripartum period, it is generally accepted that high-yielding dairy cows may experience metabolic stress due to negative nutrient balance, which alters their homeostasis and exposes the cows to illness (Colitti & Stefanon, 2006). However, concerns have been raised that DO might also lead to problems similar to those that periparturient cows may experience (Skidmore *et al.*, 1997). In general, inadequate nutritional intake alters the endocrine regulation towards tissue mobilisation, and gives rise to elevated NEFA concentrations in blood plasma of monogastric species as well as in ruminants (Grummer *et al.*, 1994; Barb *et al.*, 2001; Nielsen *et al.*, 2003). In the present thesis (Papers **I** and **III**), the cows responded with an increased plasma level of NEFA during DO probably reflecting

mobilization of adipose tissue (Pullen *et al.*, 1989; Dunshea *et al.*, 1990). Metabolic stress in early lactation related to negative nutrient balance and its correlation to health disorders is well documented (Emanuelson *et al.*, 1988; Pryce *et al.*, 1998). However, there is a lack of information about metabolic stress during the DO period. The present results indicate that cows subjected to harsh nutritional cutbacks may experience metabolic stress. The cows in our trials that were fed straw only during DO were in a deeper negative nutrient balance as shown by higher plasma levels of NEFA than cows also fed silage. The results also showed that primiparous cows had higher overall NEFA concentrations during DO compared to multiparous cows. The results are in line with previous findings showing higher plasma NEFA concentrations postpartum in primiparous cows indicating that these animals mobilized more of their adipose tissue reserves than multiparous cows (Belyea *et al.*, 1975; Drackley *et al.*, 2003). Thus, primiparous cows may be more sensitive to harsh reductions in nutrients during DO procedures.

Furthermore, plasma insulin and leptin were both reduced during DO. These results indicate that the reduced nutrient supply during DO gave rise to endocrine regulation towards tissue mobilization. Increased plasma BHBA has frequently been used as a marker for identifying animals in a negative energy balance (Kelly, 1977; Duffield, 2000). However, data presented in the present thesis show that the BHBA concentration in plasma was reduced during DO despite the fact that the cows were in a negative energy balance. It has also been shown previously that cows, which were deprived of feed in lactation week 10, did not respond with increased BHBA in plasma (Baird *et al.*, 1972; Agenäs *et al.*, 2003). The drop reported in papers **I** and **III** probably reflected a reduced ruminal production of butyrate, resulting in reduced conversion of butyrate to BHBA in the rumen epithelium. This shows that BHBA is not a useful marker for detection of negative energy balance in late lactation.

The concentration of cortisol in blood has proved to be a useful indicator of stress (Broom & Johnson, 1993; Mason, 2000). However, several factors may induce elevated plasma cortisol. Therefore, caution when interpreting cortisol profiles is advised. It is interesting that the cows fed only straw during the DO period showed a significant increase in plasma cortisol whereas the cows that were subjected to a limited reduction in nutrient supply did not respond with increased cortisol levels (Paper **I**). It is reasonable to assume that the elevated cortisol level observed during DO in the cows fed straw only was an effect due to feed restriction. The results may suggest that these cows were stressed only when subjected to a marked drop in nutrient supply during DO. This is in agreement with Agenäs *et al.* (2003) and Barb *et al.* (1997), who showed that feed deprivation gave rise to an increased plasma level of cortisol in cattle and swine, respectively.

In papers **I** and **II**, the mean milk production prior to DO was moderate (about 17 kg milk per day). In the Jälla study (Paper **III**), all cows were fed silage, 4 kg DM/day, and *ad lib* straw during DO thus undergoing a limited reduction in nutrient supply. The cows were divided into three groups according to their milk

production prior to DO. Only the two groups with the highest milk production responded with a significant increase in plasma cortisol during DO. In early lactation cows, the degree of negative energy balance is not related to the plasma level of cortisol (Beerda *et al.*, 2004). It is possible that the elevated cortisol levels were related to a higher intra-mammary pressure in cows with a higher milk production at DO. In HY cows, the cortisol level was elevated also at 2 days after the end of the DO period. At that time, the differences in energy balance should have been negligible, but the intra-mammary pressure may still have been significant. However, a relationship between cortisol and energy balance cannot be ruled out since the HY cows were assumed to have a more pronounced negative energy balance during the DO period. It would have been interesting to combine these results with a behavioural study. If the increase in cortisol was due to elevated mammary pressure, the cows might have experienced discomfort and therefore avoided lying down. Österman & Redbo (2001) indicated that low milking frequency reflects discomfort which is displayed in the lying-down behaviour.

Cortisol can also be released if the cows perceive danger or become excited. The blood sampling procedure may have caused a cortisol release that affected the results. Venipuncture affects the hypothalamic-pituitary-adrenal-cortical axis, and cortisol thus peaked approximately 15 min after the puncture (Alam & Dobson, 1986; Hopster *et al.*, 1999). In the present studies, the samplings were performed rapidly after approaching the cow, and during the 24-hour collection for the diurnal plasma cortisol profiles (Paper II), a permanent catheter was used to avoid the risk of disturbing the animals. Thus, it is not likely that the increased plasma cortisol was due to the blood sampling procedure.

Acute phase proteins (APP) in plasma may be used as indicators for clinical and sub-clinical inflammatory processes in animals. In the present study, the concentration of SAA, but not of HP, increased in plasma during DO, thereafter returning to values lower than prior to DO. However, there were no differences between the Straw and the Silage groups (Paper II). Asemgeest *et al.* (1995) have shown that in calves, stressful situations did not induce any increase in plasma cortisol or HP, but elevated the level of serum SAA. These authors also suggested that SAA might be a more sensitive variable to assess physical welfare than cortisol. However, the results from this thesis indicate that the rise in SAA was not caused by stress since both feeding groups had an increased level of SAA, but only the Straw group had elevated cortisol levels. In the present study, no differences in APP or udder health were observed between DO treatments. The involution of the udder is a form of inflammatory reaction. Thus, such a reaction is likely to induce an acute phase reaction and release of APP. An increase in both SAA and HP has been observed after calving, probably due to inflammatory reactions in the reproductive organs associated with involution of the uterus (Uchida *et al.*, 1993; Meglia *et al.*, 2005). Therefore, it is likely that the involution of the udder would result in an increase in both SAA and HP, but this was not observed in our studies. High SAA and HP levels have also been found in cows with a modest increase of triglyceride infiltration in the liver (Ametaj *et al.*, 2005). Kobayashi *et al.* (2002) have shown that cows in late lactation fed a restrictive

diet, responded with an elevated level of NEFA in plasma concomitant with an accumulation of triglycerides in the liver. In early lactating dairy cows, there is a correlation between the plasma level of NEFA and the degree of triglycerides in the liver (Laarveld *et al.*, 1981). The cows investigated in the studies reported here responded with increased NEFA in plasma during DO. It is thus possible that there was some degree of triglyceride accumulation in the liver during DO. It could thus not be excluded that an elevated level of liver triglycerides contributed to the increased SAA (Paper II).

Does feed restriction or cessation of milking during DO matter?

It is well known that complete feed withdrawal leads to a marked reduction in milk production and changes in milk composition in dairy cows (Agenäs *et al.*, 2003; Chelikani *et al.*, 2004). However, a more limited reduction in feed intake leads to a less prominent reduction in milk production and virtually no alterations in milk composition (Friggens *et al.*, 1998; Lacy-Hulbert *et al.*, 1999; Andersen *et al.*, 2003). Presumably, a small reduction in nutrient intake would not be sufficient to create any significant changes in milk production. Thus, it is reasonable to assume that the changes in milk production and composition observed at the two milkings performed during DO in cows receiving 4 kg DM silage and straw *ad lib* were not primarily effects of the reduced feed intake. However, both the milk production and the lactose concentration were numerically lower in cows receiving only straw as compared to in cows that also received 4 kg DM silage (Paper I), suggesting that the more marked reduction in feed supply contributed to rapid inhibition in milk production during DO to some extent. It was also observed that the cows were able to maintain the glucose concentration in blood in spite of the reduced nutrient intake. Several factors may have contributed to this. The most important factors are a decrease in blood flow due to increased pressure on the capillaries and prolonged transit time through the mammary gland (Chaiyabutr *et al.*, 1980; Farr *et al.*, 2000) resulting in reduced mammary glucose uptake and lactose synthesis. Another contributing factor is the reduction in insulin, which, in turn, reduces insulin-dependent glucose uptake and utilization in peripheral tissues.

The rumen environment was affected due to the restrictive feeding (Paper I). The production of VFA was lowered as expected, and the decrease was more pronounced in the straw *ad lib*-fed cows, as was the increase in ruminal pH. Both DO procedures induced disturbances of the rumen environment, since both rumen nitrogen and the number of protozoa decreased. A reduction of the protozoa number of the same magnitude has been observed in fasting steers (Galyean *et al.*, 1981) and in buffaloes fed wheat straw (Rajvir *et al.*, 1996). In these studies, however, the number of protozoa increased after resuming feeding, but this was not seen in the Kungsängen study (Paper I).

Cessation of milking efficiently inhibits milk synthesis. It causes down regulation of mammary secretory epithelial cells, which results both in reduced milk lactose concentration and milk production (Capuco *et al.*, 1997). Already

within 18 hours after cessation of milking there are changes in the mammary tissue that affect and down regulate milk production and after 36 hours the milk secretion is completely inhibited (Hamann & Dodd, 1992; Stelwagen & Lacy-Hulbert, 1996). These reports agree well with the results of the present thesis showing a drop in milk lactose concentration concomitant with a reduced milk production during DO. The marked drop in lactose in milk suggests that the content of sodium and chloride increased in order to maintain the osmolality of milk, although this was not verified. Stelwagen *et al.* (1994) suggested that an elevated intra-mammary pressure due to cessation of milking might increase the permeability of tight junctions of the mammary epithelium, causing paracellular leakage of lactose to blood plasma and that this may contribute to the decreased lactose concentration in milk. With the intervals between milkings used in the present studies (61 and 48 hours in the Kungsängen study and 39 and 72 hours in the Jälla study), it is reasonable to assume that these feedback mechanisms blocking constitutive secretion of the alveolar epithelial cells were activated and thus efficiently inhibited milk synthesis. However, it is interesting that the cows in the Kungsängen study, which were subjected to a longer initial milking interval during DO than the Jälla cows responded with a more pronounced reduction in milk production. It is possible that the longer initial milking interval facilitated a rapid inhibition of milk synthesis during DO.

Corticosteroids appear to have both stimulating and inhibiting effects on milk synthesis. It has been shown that corticosteroids may inhibit milk production in ruminants (Shamay *et al.*, 2000). On the other hand, in early-lactating cows, elevated plasma cortisol reduced the permeability of mammary tight junctions when ACTH was induced, which would facilitate milk synthesis (Stelwagen *et al.*, 1998). The results of the present thesis show that cortisol increased during DO especially in cows receiving straw only (Paper I) and in cows with a high-milk yield prior to DO (Paper III). Cows that did not respond with increased cortisol during DO, however, were as efficient in reducing milk production. Thus, increased cortisol levels in blood plasma probably did not contribute significantly to the cessation of milk production.

The design of the studies in this thesis made it impossible to clearly distinguish between feed restriction and prolonged milking interval as a means to inhibit milk production. The present results and published literature together, however, suggest that reduced milking frequency during DO is more efficient than reduced nutrient supply in order to inhibit milk production at DO.

Does the milk yield at DO matter?

In the Epi-study, the highest estimated milk production at DO was as high as 38 kg per day, which must be considered as very high. Data presented (Paper IV), clearly indicate that a daily milk yield above 15 kg milk per day at DO significantly increases the risk for VTCM. In line with the results presented in this thesis, high milk production at DO has been associated with an increased risk of udder infections and clinical mastitis in other studies (Jørstad *et al.*, 1989;

Dingwell *et al.*, 2004; Rajala-Schultz *et al.*, 2005). As our results only included VTCM and not IMI, it is likely that the actual risk for udder health disturbances was underestimated in the present study.

The teat canal of the udder has two important defence mechanisms: the sphincter muscle and the formation of the teat keratin plug that prevent potential udder pathogens from entering the udder (Paulrud, 2005). The udder is most resistant to dry-period infections when it has become fully involuted and has a natural teat canal keratin plug acting as a physical barrier in each teat (Capuco *et al.*, 1992; Williamson *et al.*, 1995).

The function of keratin in the teat is not just to keep fluid inside the body and keeping xenobiotics out, but also assumes the role of sealing the teat canal during DO and between milkings. The formation of keratin in the teat canal is a continuous process. At each milking, the keratin plug is partly flushed out by the milking machine and after each milking the formation of a new keratin plug begins (Paulrud, 2005). The teat canal keratin physically blocks the teat canal and thereby prevents bacteria from entering the udder. The risk for milk leakage during DO increases if the keratin plug is not formed.

In paper **III**, five animals were observed having milk leakage during DO and the leakage was evenly distributed between the two highest milk yield groups. There were only 56 cows in that experiment, but the results indicate that higher milk production, *i.e.* higher intra-mammary pressure, increases the risk of milk leakage. Moreover, the proportion of open udder quarters was lower in the LY than in the HY group from DO to 5 weeks into the dry period. When observing each weekly observation (week 1 to 5 after DO), differences were found at week 2 and 3 when fewer cows and udder quarters were found open in LY compared to the MY groups. Dingwell *et al.* (2004) also showed that cows producing more than 21 kg milk per day at DO had over 50% open teats 5 weeks after DO but cows milking less than 21 kg per day had only about 20% open teats. Furthermore, Dingwell *et al.* (2004) showed that quarters forming a keratin plug early in the dry period had a lower probability of IMI than those that did not close. Increased intra-mammary pressure may cause milk leakage from the teat end, which allows bacteria to penetrate the teat canal and colonize the gland (Cousins *et al.*, 1980). In addition, the increased volume of milk in the gland results in lower concentrations of natural protective factors such as lactoferrin, immunoglobulins and phagocytic cells (Bushe & Oliver, 1987; Paape *et al.*, 1992). Thus, reducing the level of production at drying-off might directly increase the resistance of the udder in the early dry period by minimizing both of these effects. In support of this, cows that leaked milk following drying-off were four-fold more likely to develop clinical mastitis during the dry period (Schukken *et al.*, 1993).

In agreement with Emanuelson *et al.* (1988), the SCC increased significantly at the end of lactation (Papers **II** and **III**). During the DO procedure, the SCC increased even more. In healthy udders, this is mainly a concentration effect, as the milk production decreases, and an effect of cell migration during involution of the mammary gland. However, there is also an increased risk of IMI during this

phase, which results in an increase in SCC (Dingwell *et al.*, 2004). In Paper IV, high SCC (> 200,000 cells/ml) prior to DO increased the risk for VTCM from DO to early lactation by 46%. This could be due to a persisting chronic infection in the udder, which may develop into a case of clinical mastitis at calving. This indicates that the control of the SCC in the milk prior to DO is of great importance.

Practical implications of the results

The results presented in this thesis clearly show that high milk yield at DO should be avoided as both the risk for VTCM and the metabolic load is strongly associated with high milk yield at DO. With the trend towards increasing milk production, there is an obvious risk that these problems will be exaggerated if the present management routines are not changed. Prolonging CI might be one way to reduce milk production at DO especially among primiparous cows that have a flat lactating curve. This latter group appears to be more sensitive to harsh DO routines, and would thus benefit the most from management systems that reduce milk yield prior to DO. Based on previous knowledge of the cows' pedigree, the farmer could delay the first service by a month or two and thereby decrease the milk yield at DO. A Swedish study has shown that total milk yield increases by 15 to 16 % with 15 months CI, and that those primiparous cows were able to maintain their production (Rehn *et al.*, 2000). In addition, SH cows with high peak yield also maintained their production longer than SRB cows (Rehn *et al.*, 2000). A possible recommendation could be to extend the CI for primiparous SH cows to reduce the problems with high yields at DO.

This thesis clearly indicates that feeding straw only to a high-producing dairy cow is not an appropriate routine as it increases the metabolic load of the animal. This may in turn have negative effects on the immune response and increase the risk for mastitis. Instead, a combination of a moderate restrictive feeding (around 4 kg DM silage per day) and cessation of milking are keys to a successful DO. On many farms, the cows are also moved to another location in the barn at DO, which is thought to facilitate DO. In agreement with this, it has been shown that stress caused by isolating cows created leaking tight junctions and reduced milk yield (Stelwagen *et al.*, 2000).

The importance of a cow having a healthy udder prior to DO must be emphasized as there is an increased risk for VTCM if the cow has elevated SCC on the last milk-test day and this knowledge should form the basis for relevant control regimes.

From the results of the present thesis, it was not possible to clearly distinguish between feed restriction and prolonged milking interval as a mean to inhibit milk production. To find optimal milking intervals at DO, further investigations are needed to combine moderate restrictive feeding protocols with different milking intervals. Such studies should evaluate the routines that give the best inhibition of milk production at DO.

Main conclusions

- Cows fed only straw at DO experienced a more pronounced metabolic load shown as higher NEFA and lower plasma insulin values during DO compared to in those cows fed additional silage.
- Addition of 4 kg DM silage daily to the DO diet did not have any negative effects on reduction of milk production or health.
- Primiparous cows may be more sensitive to harsh nutrient cutback DO procedures and are therefore more sensitive to DO diets based on straw only.
- As daily milk yield at DO increases, the metabolic load is elevated and the risk for VTCM from DO until 30 days after calving is increased. The latter is associated with an increased incidence of open teat canals.
- The risk for VTCM from DO until 30 days after calving is significantly higher in cows having a composite milk SCC over 200 000 cells/ml at DO compared to in cows having a SCC below 200 000 cells/ml. The risk is significantly higher in SH cows than in SRB cows.
- A long initial milking interval at DO appears to facilitate a rapid inhibition of milk synthesis.
- The results indicate that cows with prolonged CI have a better nutrient balance in late pregnancy.

Populärvetenskaplig sammanfattning

Under de senaste 40 åren har svenska mjölkors avkastning mer än fördubblats och det är rimligt att anta att ökningen kommer att fortsätta. Under kontrollåret 2005 hade Svensk röd och vit boskap (SRB) en årlig avkastning per ko på 9022 kg energi korrigerad mjölk (ECM) och Svensk lågland (SLB) hade en avkastning på 9550 kg ECM (Larsson, 2006). Denna mjölmängd har uppnåtts inte bara genom avelsarbete utan även genom bättre utfodring och skötsel. Den ökade avkastningen innebär att dagens mjölkko utsätts för betydande metaboliska och hälsomässiga påfrestningar.

I dagens svenska mjölkproduktion eftersträvas ett kalvningsintervall på cirka 12 månader med en rekommenderad sintidslängd på ca 2 månader. Den ökade mjölkavkastningen medför att kon ofta har en hög avkastning även sent i laktationen vid tiden för sinläggning. Sinläggningen är sannolikt, förutom perioden runt kalvningen, det tillfälle då de mest omfattande förändringarna sker vad gäller fysiologiska anpassningar hos en mjölkko. Sinläggningen måste ske på snabbast möjliga sätt, dels för att minimera risken för mastit och för att det är oekonomiskt för lantbrukaren med utdragen sinläggning. Snabb sinläggning är svårt att uppnå vid hög avkastning.

En i Sverige vanlig rutin vid sinläggning av kor som producerar mycket mjölk är att utfodra med enbart halm, vitaminer och mineraler under några dagar samtidigt som man snabbt minskar urmjölkningensgraden. Näringsinnehållet i halmen inskränker sig till en liten mängd omsättbar energi och inget smältbart råprotein. Syftet med strategin är att skapa näringsbrist hos kon vilket påskyndar minskningen i mjölkproduktionen. Men vid näringsbrist förändras kons metabolism och hon måste mobilisera sina vävnadsreserver, inte minst för att kunna förse det växande fostret med näring. Det saknas dokumentation om hur utfodring med enbart halm påverkar kons ämnesomsättning.

Det finns en omfattande dokumentation som beskriver den metabola stress, och därtill kopplade hälsostörningar, som mjölkkor utsätts för under perioden runt kalvning. Under tiden efter kalvning ökar mjölkavkastningen snabbare än foderkonsumtionen. Kon måste därför mobilisera kroppsreserver främst i form av fett och protein för att täcka behovet. Detta leder i sin tur till att kon hamnar i en kraftigt negativ näringsbalans. Detta medför på nytt stora påfrestningar på kons ämnesomsättning och därmed ökad risk för ämnesomsättningssjukdomar. Idag saknas emellertid kunskap om den metabola stress som särskilt högvastande mjölkkor utsätts för vid sinläggning.

Hur påverkas en ko metabolisk under en ”normal” sinläggning med utfodring med halm och skiljer sig detta gentemot kor som utfodras med tillskott av ensilage? Spelar avkastningsnivån någon roll vid sinläggningen? Finns det skillnader mellan Sveriges två vanligaste raser, SRB och SLB i dessa frågor? Finns det några samband mellan avkastningsnivån och incidensen av behandlade mastit fall runt nästa kalvning?

Detta var bakgrunden till ett fyraårigt forskningsprojekt som har genomförts i mjölkbesättningen på Kungsängens Forskningscentrum, SLU och Jälla Naturbruksgymnasium samt en omfattande studie på data från kodatabasen, Svensk Mjölk.

I det första försöket undersöktes skillnaderna mellan två olika sinläggningsrutiner på 21 SRB kor. Korna hade en medelavkastning på 17 kg/dygn mjölk vid sinläggningen. Deras våmfunktioner, ämnesomsättning, vissa hälsoparametrar samt hur väl de svarade på sinläggningsrutinerna studerades. Korna delades in i två grupper där ena gruppen utfodrades med fri tillgång på halm och den andra gruppen utfodrades med 4 kg torrsbstans (ts) ensilage och fri tillgång på halm. Denna behandling fortgick under 5 dagar och alla kor mjölkades på morgonen dag 3 och dag 5. Efter detta skedde en stegvis upptrappning av foder för båda grupperna till sintidsfoderstat.

I det andra försöket var syftet att studera olika avkastningsnivåer och även undersöka skillnader avseende metabolism och hälsa mellan raser (SRB och SLB) och olika kalvningsintervall (12 respektive 15 månader). I denna studie ingick 56 kor och alla kor fick samma sintidbehandling, 4 kg ts ensilage och fri tillgång på halm under 5 dagar och de mjölkades på morgonen dag 2 och dag 5.

I den tredje studien undersöktes sambandet mellan avkastningsnivån vid sinläggning och risken för mastit runt nästföljande kalvning. I denna studie ingick 10741 kor från 488 olika besättningar runt om i Sverige, informationen hämtades från Svensk Mjölks kokontroll.

Alla kor i de två första försöken sinlades inom 5 dagar. I den grupp där korna bara utfodrades med halm visade resultaten att dessa kor hamnar i en kraftig negativ energibalans med mobilisering av kroppsreserver som följd. Resultaten tyder också på att förstakalvare har svårare att återhämta sig efter att ha utsatts en negativ energibalans. Den negativa energibalansen har visat sig kunna sänka immunförsvaret vilket kan leda till ämnesomsättningssjukdomar och att korna är mindre motståndskraftiga mot mastit.

Kortisol är ett hormon som är kopplat till kroppens stressreaktioner. De kor bara utfodrades med halm under sinläggning hade höga kortisolhalter i blodet jämfört med de kor som fin tillskott av ensilage vis sinläggning. De högre kortisolhalterna betyder att korna var utsatta för någon form av stress. Kor med en hög mjölkavkastning vid sinläggning visade sig också ha höga halter kortisol, trots att de utfodrades med tillskott av ensilage. Detta kan tyda på att det ökade trycket från mjölk i juvret också kan vara stressande för kon.

Kor som läcker mjölk under sinläggningen har en högre risk för utveckla mastit och i våra studier fanns det tecken på att hög avkastning vid sinläggningen försämrar spenens förmåga att hålla tätt. Resultaten från tredje studien visar ett starkt samband mellan avkastningen vid sinläggningen och risken för att kon skall få mastit under sinperioden och runt kalvningen. Antalet celler i mjölken vid sista

provmjölknigen hade också ett samband med risken för mastit. Om korna har mer än 200 000 celler/ml mjölk, är det dubbelt så stor risk att de drabbas av mastit under sintiden eller runt nästföljande kalvning än för kor med lägre än 200 000 celler/ml mjölk. Vidare fann vi tydliga skillnader mellan raser för risken att få mastit i samband med sinläggning, och att risken var högre hos SLB kor än hos SRB kor.

Resultaten visar att hög avkastning vid sinläggning ökar risken för mastiter och ämnesomsättningsproblem under sintiden och runt kalvning. I och med att mjölkavkastningen hos dagens mjölkkor verkar fortsätta öka, så kommer sinläggningen att bli ett allvarligt problem om man inte finner nya sinläggningsrutiner. Det går att undvika sinläggning vid höga avkastningsnivåer genom att förlänga kalvningsintervallet. Detta är särskilt lämpligt för förstakalvare, som har en uthålligare laktation och dessutom visade sig vara extra känsliga för dagens sinläggningsrutiner.

För att undvika onödig belastning på korna vid sinläggning är det viktigt att tänka på att:

- ✓ Förlänga första mjölkningsintervallet till mer än 40 timmar. Detta för att juvret i detta stadium av laktationen tillbakabildas om mjölken stannar kvar i juvret.
- ✓ Ge tillskott av ensilage (runt 4 kg ts per dag) under sinläggningen. I studierna var det inte några problem att sinlägga korna på denna foderstat och utfodring med enbart halm leder till onödiga risker för kon.
- ✓ Flytta kon inför sinläggningen. Praktisk erfarenhet visar att detta påskyndar sinläggningen vilket bland annat kan förklaras med att stressen av att byta miljö påskyndar minskningen av mjölkproduktionen. En annan faktor som säkert är viktig är att kon kommer ifrån den invanda miljön där mjölkning brukar ske och att man därigenom undviker att hon får mjölknedsläpp när de andra korna skall mjölkas.
- ✓ Korna bör få lika mycket tillsyn under sinläggningen som efter kalvning.

References

- Agenäs, S., Dahlborn, K., & Holtenius, K. 2003. Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. *Livestock Production Science* 83:153-164.
- Alam, M. G., & Dobson, H. 1986. Effect of various veterinary procedures on plasma concentrations of cortisol, luteinising hormone and prostaglandin F2 alpha metabolite in the cow. *Veterinary Records* 118:7-10.
- Alsemgeest, S. P., Lambooy, I. E., Wierenga, H. K., Dieleman, S. J., Meerkerk, B., van Ederen, A. M., & Niewold, T. A. 1995. Influence of physical stress on the plasma concentration of serum amyloid-A (SAA) and haptoglobin (Hp) in calves. *The Veterinary Quarterly* 17:9-12.
- Ametaj, B. N., Bradford, B. J., Bobe, G., Nafikov, R. A., Lu, Y., Young, J. W., & Beitz, D. C. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Canadian Journal of Animal Science* 85: 165-175.
- Andersen, J. B., Friggens, N. C., Sejrsen, K., Sørensen, M. T., Munksgaard, L., & Ingvarsen, K. L. 2003. The effects of low vs. high concentrate level in the diet on performance in cows milked two or three times daily in early lactation. *Livestock Production Science* 81:119-128.
- Andersen, J. B., Madsen, T. G., Larsen, T., Ingvarsen, K. L., & Nielsen, M. O. 2005. The effects of dry period versus continuous lactation on metabolic status and performance in periparturient Cows. *Journal of Dairy Science* 88:3530-3541.
- Baird, G. D., R. J. Heitzman, & K. G. Hibbitt. 1972. Effects of starvation on intermediary metabolism in the lactating dairy cow. A comparison with metabolic changes occurring during bovine ketosis. *Biochemical Journal* 128:1311-1318.
- Barb, C. R., Barrett, J. B., Kraeling, R. R., & Rampacek, G. B. 2001. Serum leptin concentrations, luteinizing hormone and growth hormone secretion during feed and metabolic fuel restriction in the prepuberal gilt. *Domestic Animal Endocrinology* 20:47-63.
- Barb, C. R., Kraeling, R. R., Rampacek, G. B., & Dove, C. R. 1997. Metabolic changes during the transition from the fed to the acute feed-deprived state in prepuberal and mature gilts. *Journal of Animal Science*. 75:781-9.
- Beerda B., Kornalijnslijper, J. E., van der Werf, J. T. N., Noordhuizen-Stassen, E. N., & Hopster, H. 2004. Effects of milk production capacity and metabolic status on HPA function in early postpartum dairy cows. *Journal of Dairy Science* 87:2094–2102.
- Belyea, R. L., Coppock, C. E., & Lake, G. B. 1975. Effects of silage diets on health, reproduction, and blood metabolites of dairy cattle. *Journal of Dairy Science* 58:1336-46.
- Broom, D. M., & Johnson, K. G. 1993. *Stress and Animal Welfare*, (Chapman & Hall, London). 87-144.
- Bushe, T. & Oliver, S. P. 1987. Natural protective factors in bovine mammary secretions following different methods of milk cessation. *Journal of Dairy Science* 70:696-704.
- Capuco, A. V., & Akers, R. M. 1999. Mammary involution in dairy animals. *Journal of Mammary Gland Biology and Neoplasia* 2:137 - 144
- Capuco, A. V., Akers, R. M., & Smith, J. J. 1997. Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. *Journal of Dairy Science* 80:477-487.
- Capuco, A. V., Bright, S. A., Pankey, J. W., Wood, D. L., Miller, R. H., & Bitman, J. 1992. Increased Susceptibility to Intramammary Infection Following Removal of Teat Canal Keratin. *Journal of Dairy Science* 75: 2126-2130.
- Chaiyabutr, N., Faulkner, A., & Peaker, M. 1980. Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. *Research in Veterinary Science* 28:291-295.
- Chelikani, P.K., Ambrose, J. D., Keisler, D. H., & Kennelly, J. J. 2004. Effect of short-term fasting on plasma concentrations of leptin and other hormones and metabolites in dairy cattle. *Domestic Animal Endocrinology* 26:33-48.

- Colitti, M., & Stefanon, B. 2006. Effect of natural antioxidants on superoxide dismutase and glutathione peroxidase mRNA expression in leukocytes from periparturient dairy cows. *Veterinary Research Communications* 30:19-27.
- Cousins, C.L., Higgs, T.M., Jackson, E. P., Neave, F. K., & Dodd, F. H., 1980. Susceptibility of the bovine udder to bacterial infection in the dry period. *Journal Dairy Research* 47:11–18.
- Davis, S.R., & Collier, R.J. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. *Journal of Dairy Science* 68:1041-58.
- Delavaud, C., Ferlay, A., Faulconnier, Y., Bocquier, F., Kann, G., & Chilliard, Y. 2002. Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. *Journal of Animal Science* 80:1317-1328.
- Dingwell, R. T., Kelton, D. F., & Leslie, K. E. 2003. Management of the dry cow in control of peripartum disease and mastitis. *The veterinary clinics of North America Food animal practice* 19:235–265.
- Dingwell, R. T., Leslie, K. E., Schukken, Y. H., Sargeant, J. M., Timms, L. L., Duffield, T. F., Keefe, G. P., Kelton, D. F., Lissemore, K. D., & Conklin, J. 2004. Association of cow and quarter-level factors at drying-off with new intra-mammary infections during the dry period. *Preventive Veterinary Medicine* 63:75–89.
- Dingwell, R., D. Kelton, K. Leslie, & V. Edge. 2001. Deciding to dry-off: does level of production matter? Pages 69-79. *National Mastitis Council Annual Meeting Proceedings.*, Ontario, Canada. Natl. Mastitis Council, Inc., Arlington, VA.
- Drackley, J. K., Cicela, T. M., & LaCount D. W. 2003. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. *Journal of Dairy Science* 86:1306-1314.
- Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. *Veterinary Clinics of North American- Food animal practice* 16:231–253 review.
- Dunshea, F. R., Bell, A. W., & Trigg, T. E. 1990. Non-esterified fatty acid and glycerol kinetics and fatty acid re-esterification in goats during early lactation. *British Journal of Nutrition* 64:133-145.
- Emanuelson, U., Olsson, T., Mattila, T., Astrom, G., & Holmberg, O. 1988. Effects of parity and stage of lactation on adenosine triphosphate, somatic cell count and antitrypsin content in cows' milk. *Journal of Dairy Research* 55:49-55.
- Farr V.C., Prosser, C. G., & Davis, S. R. 2000. Effects of mammary engorgement and feed withdrawal on microvascular function in lactating goat mammary glands. *American Journal of Physiology-Heart Circulatory Physiology* 279:813–818.
- Flint, D. J., & Gardner, M. 1994. Evidence that growth hormone stimulates milk synthesis by direct action on the mammary gland and that prolactin exerts effects on milk secretion by maintenance of mammary deoxyribonucleic acid content and tight junction status. *Endocrinology* 135:1119-1124.
- Friggens, N. C., Emmans, G. C., Kyriazakis, I., Oldham, J. D., & Lewis, M. 1998. Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. *Journal of Dairy Science* 81:2228-2239.
- Galyean, M. L., Lee, R. W., & Hubert, M. E. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. *Journal of Animal Science* 53:7-18.
- Grummer, R. R., Winkler, J. C., Bertics, S. J., & Studer, V. A. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein heifers. *Journal of Dairy Science* 77:3618-23.
- Hamann, J., & Dodd, F. 1992. Milking routines. Pages 69-96. In: *Machine Milking and Lactation*. Bramley et al. ed. Insight books, Berkshire, England.
- Hetta, M. 2004. Timothy and Red clover as Forage for Dairy Production-In vitro Degradation Characteristics and Chemical Composition. Doctoral Thesis, Dept. of Agricultural Research for Northern Sweden, SLU. *Acta Universitatis Agriculturae Suecia Agraria* vol. 460. pp. 42.
- Holmann, F. J., Shumway, C. R., Blake, R. W., Schwart, R. B., & Sudweeks, E. M. 1984. Economic value of days open for Holstein cows of alternative milk yields with varying calving intervals. *Journal of Animal Science* 67:636-643.

- Hopster, H., van der Werf, J. T., Erkens, J. H., & Blokhuis, H. J. 1999. Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. *Journal of Animal Science* 77:708-14.
- Janson, L. 1993. Vad händer vid urval för låg respektive hög mjölkfetthalt? *SLU info rapporter. Allmänt 181pp. N22. Swedish Univ. of Agric. Sci., Uppsala, Sweden.* In Swedish.
- Jørstad, A., Farver, T. B., & Riemann, H. 1989. Teat canal diameter and other cow factors with possible influence on somatic cell counts in cow milk. *Acta Veterinaria Scandinavica* 30:239-245.
- Kelly, J. M. 1977. Changes in serum β -hydroxybutyrate concentrations in dairy cows kept under commercial farm conditions. *Veterinary Records* 101:499-502.
- Knight, C. H. 2001. Overview of prolactin's role in farm animal lactation. *Livestock Production Science* 70:87-93.
- Kobayashi, Y., Boyd, C. K., McCormack, B. L., & Lucy, M. C. 2002. Reduced insulin-like growth factor-I after acute feed restriction in lactating dairy cows is independent of changes in growth hormone receptor 1A mRNA. *Journal of Dairy Science* 85:748-754.
- Laarveld, B., Christiansen, D. A. & Brockman, R. P. 1981. The effect of insulin on net metabolism of glucose and amino acids by the mammary gland. *Endocrinology* 108:2217-2221.
- Lacy-Hulbert, S. J., Woolford, M. W., Nicholas, G. D., Prosser, C. G., & Stelwagen, K. 1999. Effect of milking frequency and pasture intake on milk yield and composition of late lactation cows. *Journal of Dairy Science* 82:1232-1232.
- Linzell, J. L., Peaker, M., & Taylor, J. C. 1975. The effects of prolactin och oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. *Journal of Physiology* 253: 547-563.
- Littell, R. C., Henry, P. R. & Ammerman, C. B. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal Animal Science* 76:1216-1231.
- Mason, W. A. 2000. Early developmental influences of experience on behavior, temperament and stress. Pages 269-290. *The biology of animal stress; Basic principles and implications for animal welfare.* Edited by G. P. Moberg and J. A. Mench. CABI Publishing, New York, USA.
- McFadden, T. B., Callagan, M. R., & Davis, S. R. 1995. Regulation of lactose production by ovine mammary acini in culture. *Proceedings of the New Zealand society of animal production* 55:17-20.
- McGuire, M. A., Bauman, D. E., Dwyer, D. A., & Cohick, W. S. 1995. Nutritional modulation of the somatotropin/insulin-like growth factor system: response to feed deprivation in lactating cows. *Journal of Nutrition* 125:493-502.
- McGuire, M. A., Dwyer, D. A., Bauman, D. E., & Smith, D. F. 1998. Insulin-like growth factors in plasma and afferent mammary lymph of lactating cows deprived of feed or treated with bovine somatotropin. *Journal of Dairy Science* 81:950-957.
- Meglia, G. E., Johannisson, A., Agenas, S., Holtenius, K., & Persson Waller, K. 2005. Effects of feeding intensity during the dry period on leukocyte and lymphocyte subpopulations, neutrophil function and health in periparturient dairy cows. *The Veterinary Journal* 169:376-384.
- Mostl E., Maggs J. L., Schrotter G., Besenfelder U., & Palme R. 2002. Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* 26:127-39.
- Neijenhuis, F., Barkema, H., W., Hogeveen, H., & Noordhuizen, J. P. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. *Journal of Dairy Science* 83:2795-2804.
- Nguyen, D. A., & Neville, M. C. 1998. Tight Junction Regulation in the Mammary Gland. *Journal of Mammary Gland Biology and Neoplasia* 3:233-46.
- Nielsen, N. I., Ingvarsen, K. L., & Larsen, T. 2003. Diurnal variation and the effect of feed restriction on plasma and milk metabolites in TMR-fed dairy cows *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine* 50:88-97.

- Österman, S., & Bertilsson, J. 2003. Extended calving intervals in combination with milking two or three times per day: effects on milk production and milk composition. *Livestock Production Science* 82:139–149.
- Österman, S., & Redbo, I. 2001. Effects of milking frequency on lying down and getting up behaviour in dairy cows *Applied Animal Behaviour Science* 70:167-176.
- Paape, M. J., Miller, R. H., Young, M. D., & Peters, R. R. 1992. Influence of involution on intramammary phagocytic defense mechanisms. *Journal of Dairy Science* 75:1849-1856.
- Palme, R., & Mostl, E. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *International Journal of Mammalian Biology* 62:192-197
- Paulrud, C. O. 2005. Basic concepts of the bovine teat canal. *Veterinary Research Communications* 29:215-245.
- Peaker, M., & Wilde, C. J. 1996. Feedback control of milk secretion from milk. *Journal of Mammary Gland Biology and Neoplasia* 1:307-15.
- Perkins, K. H., VandeHaar, M. J., Tempelman, R. J., & Burton, J. L. 2001. Negative energy balance does not decrease expression of leukocyte adhesion or antigen-presenting molecules in cattle. *Journal of Dairy Science* 84:421-428.
- Preisler, M. T., Weber, P. S. D., Tempelman, R. J., Erskine, R. J., Hunt, H., & Burton, J. L. 1999. Glucocorticoid receptor down-regulation in neutrophils of periparturient cows. *Am. Journal of Veterinary Research* 61:14-19.
- Pryce, J., Veerkamp, R. R., Thompson, R., Hill, W., & Simm, G. 1998. Genetic parameters of common health disorders and measures of fertility in Holstein Friesian dairy cattle. *Animal Science* 65:353-360.
- Pullen, D. L., Palmquist, D. L., & Emery, R. S. 1989. Effect of days of lactation and methionine hydroxy analog on incorporation of plasma free fatty acids into plasma triglycerides. *Journal of Dairy Science* 72:49–58.[
- Rajala-Schultz, P. J., Hogan, J. S., & Smith, K. L. 2005. Association between milk yield at dry-off and probability of intra-mammary infections at calving. *Journal of Dairy Science* 88:577–579.
- Rajvir, S., Salim, I., Singh, D. V., Setia, M. S., Singh, R., & Iqbal, S. 1996. Effect of exclusive feeding of wheat straw on rumen microbes and metabolites in buffalo calves (*Bubalus bubalis*). *Buffalo Journal*. 12 :1-9.
- Rastani, R. R., Grummer, R. R., Bertics, S. J., Gümen, A., Wiltbank, M. C., Mashek, D. G., & Schwab, M. C. 2005. Reducing Dry Period Length to Simplify Feeding Transition Cows: Milk Production, Energy Balance, and Metabolic Profiles. *Journal of Dairy Science*. 88:1004-1014.
- Ratnayake, D. R. T. G., Berglund, B., Bertilsson, J., Forsberg, M., & Gustafsson, H. 1998. Fertility in dairy cows managed for calving intervals of 12, 15 or 18 months *Acta Vet. Scand.*39: 215-228.
- Rehn, H., Berglund, B., Emanuelson, U., Tengroth, G., & Philipsson, J. 2000. Milk production in Swedish dairy cows for calving intervals of 12 and 15 months. *Acta Veterinaria Scandinavica* 50: 263–271.
- Reid, I. M., Stark, A. J., & Isenor, R. N. 1977. Fasting and refeeding in the lactating dairy cow. 1. The recovery of milk yield and blood chemistry following a six-day fast. *Journal of Comparative Pathology* 87:241-51
- Rukkwamsuk, T., Kruip, T. A., & Wensing, T. 1999. Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. *The Veterinary Quarterly* 21:71-77.
- Samuelsson, B., Uvnäs-Moberg, K., Gorewit, R. C., & Svennersten-Sjaunja, K. 1996. Profiles of the hormones somatostatin, gastrin, CCK, prolactin, growth hormone, oxytocin and cortisol .2. In dairy cows that are milked during food deprivation. *Livestock Production Science* 46: 57-64.
- Schukken, Y. H., Vanvliet, J., Vandegheer, D., & Grommers, F. J. 1993. A randomized blind trial in dry cow antibiotic infusion in a low somatic cell herd. *Journal of Dairy Science* 76:2925–2930.

- Shamay, A., Shapiro, F., Barash, H., Bruckental, I., & Silanikove, N. 2000. Effect of dexamethasone on milk yield and composition in dairy cows. *Annales de zootechnie* 49:343-352.
- Shamay, A., Shapiro, F., Leitner, G., & Silanikove, N. 2003. Infusions of casein hydrolyzates into the mammary gland disrupt tight junction integrity and induce involution in cows. *Journal of Dairy Science* 86:1250-1258.
- Shennan, D. B., & Peaker, M. 2000. Transport of milk constituents by the mammary gland. *Physiological reviews* 80:925-951.
- Sjaunja, L. O., Baevre, L., Junkarinen, L., Pedersen, J., & Setälä, J. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. *ICEPMA, 27th session*, July 2-6, Paris, France.
- Skidmore, A., Peeters, K., Sniffen, C., & Brand, A. 1997. Monitoring dry period management. Page 173 In: *Herd Health and Production Management in Dairy Practice*. A. Brant, J. Noordhuizen, & Y. Schukken, ed. Wageningen Pers. Wageningen, The Netherlands.
- Sørensen, J. T., & Enevoldsen, C. 1991. Effect of Dry Period Length on Milk Production in Subsequent Lactation. *Journal of Dairy Science* 74: 1277-1283.
- Spörndly, R., (ed). 2003. *Feed Tables for Ruminants (Fodertabell för idisslare)*. Rapport 257, Department of Animal Nutrition and Management, Swedish University of Agricultural Science, Uppsala, Sweden (in Swedish).
- Stefano, B., Colitti, M., Gabai, G., Knight, C. H., & Wilde, C. J. 2002. Mammary apoptosis and lactation persistency in dairy animals. *Journal of Dairy Research* 69:37-52.
- Stelwagen, K. & Lacy-Hulbert, S. J. 1996. Effect of milking frequency on milk somatic cell count characteristics and mammary secretory cell damage in cows. *American Journal of Veterinary Research* 57:902-905
- Stelwagen, K., Hopster, H., Van Der Werf, J. T., & Blokhuis, H. J. 2000 Short communication: effects of isolation stress on mammary tight junctions in lactating dairy cows. *Journal of Dairy Science* 83:48-51.
- Stelwagen, K., Davis, S. R., Farr, V. C., Prosser, C. G., & Sherlock, R. A. 1994. Mammary epithelial cell tight junction integrity and mammary blood flow during an extended milking interval in goats. *Journal of Dairy Science* 77:426-432.
- Stelwagen, K., van Espen, D. C., Verkerk, G. A., McFadden, H. A., & Farr, V. C. 1998. Elevated plasma cortisol reduces permeability of mammary tight junctions in the lactating bovine mammary epithelium. *Journal of Endocrinology* 159:173-178.
- Strandberg, E.; & Oltenacu, P. A. 1989. Economic consequences of different calving intervals. *Acta Agriculturae Scandinavica* 39:407-420.
- Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, & Schukken, Y. H. 2000. Hyperketonemia and the impairment of udder defense: a review. *Vet. Res.* 31:397-412.
- Swanson, E. W. 1965. Comparing continuous milking with sixty-day dry periods in successive lactations. *Journal of Dairy Science* 48:1205-1209.
- Swedish Dairy Association. 2005. *Cattle statistics*. Swedish Dairy Association, Hållsta, Eskilstuna, Sweden.
- Uchida, E., Katoh, N., & Takahashi, K. 1993. Appearance of haptoglobin in serum from cows at parturition. *Journal of Veterinary Medical Science*. 55:893-894.
- Wilde, C. J., Addey, C. V., Boddy, L. M., & Peaker, M. 1995. Autocrine regulation of milk secretion by a protein in milk. *Biochemical Journal* 305:51-8.
- Wilde, C. J., Addey, C. V., Bryson, J. M., Finch, L. M., Knight, C. H., & Peaker, M. 1998. Autocrine regulation of milk secretion. *Biochemical Society symposia* 63: 81-90.
- Williamson, J.H., Woolford, M.W., & Day, A.M., 1995. The prophylactic effect of a dry-cow antibiotic against *Streptococcus uberis*. *New Zealand Veterinary Journal* 43: 228-234.

Personal communication

- Larsson, N-E. 2006. Swedish Dairy Association, Dairy Statistics. 2006. Personal communication.

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