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Energy and protein nutrition of dairy cows during the dry period and early lactation: Production performance and adaptation from pregnancy to lactation

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Academic dissertation

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ABSTRACT

This thesis consists of studies concerning the effects of dry period energy feeding, and early lactation energy and protein feeding on the metabolic and hormonal status, tissue mobilisation and lactation performance in dairy cows. In all studies, restricted amounts of grass silage were fed during the dry period, and lead feeding with concentrate was applied during the last three weeks of pregnancy. After calving, grass silage was fed ad libitum and the daily concentrate allowance was increased to fixed levels according to preplanned schedules.

In experiment I, the effect of two levels of concentrate and glucogenic feed was studied during early lactation. The concentrate levels were 11 and 15 kg/d for multiparous cows and 9 and 12 kg/d for primiparous cows. Glucogenic liquid feed consisted of propylene glycol, polyols, molasses and niacin, and it was fed at 0 or 1 l/d. In multiparous cows, milk production responses to concentrate supplementation differed between the groups with and without glucogenic feed. The high response (1.3 kg ECM/kg concentrate DM) to concentrate supplementation with glucogenic feed was attributed to increased protein intake, since CP contents of silage and glucogenic feed were low. A high substitution rate of concentrate for silage was associated with the lack of milk yield response in multiparous cows without glucogenic feed. Milk yield response of primiparous cows to concentrate supplementation was low. Higher concentrate allowance elevated plasma insulin concentrations and increased live weight gain in primiparous cows after peak yield. Concentrate supplementation and glucogenic feed did not decrease lipid mobilisation, but glucogenic feed decreased concentrations of blood ketone bodies in multiparous cows.

In experiment II, the effect of early lactation protein supplementation was examined. A cereal-based concentrate mixture including 0%, 15% or 30% of rapeseed meal was fed to multiparous cows. High milk yield response between 0% and 15% rapeseed meal was attributed to increased lipid mobilisation and more efficient utilisation of metabolizable energy for milk production. The lack of further milk yield response between 15% and 30% levels was associated with the absence of feed intake and diet digestibility responses, and conversion of extra nitrogen to urea. Protein supplementation did not affect concentrations of blood ketone bodies.

In experiment III, the effects of concentrate proportion in lead feeding and the increase rate of concentrate after calving were investigated. The concentrate proportions of 20%, 40% or 60% of individual energy requirements during lead feeding had no consistent effect on silage intake after calving. High proportions of concentrate increased plasma insulin and low proportions of concentrate slightly increased plasma concentration of non-esterified fatty acids one week before calving. The increase rate of concentrate after calving did not affect silage intake and fast increase of concentrate did not increase the number of off-feed incidences. The highest milk yields during lactation weeks 1 to 5 were observed in the groups with medium or high concentrate proportion before calving and fast increase rate of concentrate after calving. The combination of low concentrate proportion before calving and slower increase rate of concentrate after calving elevated blood concentration of ketone bodies one week after calving.

In experiment IV the effects of body fatness at calving and glucogenic supplementation during the transition period and early lactation were studied. Increased mobilisation of lipid and protein reserves was observed in fatter cows. Increased lipid mobilisation was associated with higher in vitro lipolytic responses to norepinephrine. Glucogenic supplement did not decrease lipid or protein mobilisation, but it prevented the increase of blood ketone body concentration in fatter cows. Plasma leptin started to decrease before calving and the lowest concentrations were measured during the first week of lactation. During early lactation plasma leptin concentrations remained low, but were significantly higher in fatter cows than in

control cows. Plasma leptin was positively related to body fatness and plasma insulin throughout the experiment. No significant differences in feed intake or milk yield were observed. Feed intake did not decrease during the last two weeks of pregnancy, when the cows were fed according to energy recommendations. Mobilisation of tissue reserves commenced before calving and continued for several weeks after calving.

Meta-analyses were conducted to more effectively evaluate the direct reponses to concentrate and protein supplementation during early lactation, and to compare these responses to those obtained in mid-lactation studies. Data analysis indicated that the milk and protein yield responses to concentrate supplementation are higher during early than during mid-lactation, and suggested that the lower responses of mid-lactation cows are associated with a tendency to partition more energy to live weight gain. Linear substitution rate of concentrate for silage was approximately 0.4 kg DM per increased kg DM concentrate in both lactation stages. Protein supplementation increased silage intake and milk production similarly in early- and mid-lactation studies.

Lead feeding by increasing concentrate to approximately 4 kg/d at calving can be recommended in separate feeding of grass silage and concentrate with restricted energy allowance. The importance of avoiding obesity at calving in preventing excessive lipid mobilisation and risk of subclinical ketosis is emphasized. Glucogenic supplement can be used to decrease concentrations of blood ketone bodies. Milk production responses to concentrate supplementation are high during early lactation, but fast increase of concentrate after calving retards the increase of silage intake. Similar responses to soya bean meal supplementation in the meta-analysis of early- and mid-lactation studies suggest that no benefit can be expected by allocating more protein supplement in early than in mid-lactation. However, further early lactation studies using rapeseed feed as the protein supplement are needed.

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Vantaa, October 2005
Tuomo Kokkonen

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications subsequently referred to in the text by their Roman numerals:

I Kokkonen, T., Tesfa, A., Tuori, M., Hissa, K., Jukola, E. & Syrjälä-Qvist, L. 2000. Effects of early lactation concentrate level and glucogenic feed on feed intake, milk production and energy metabolism in dairy cows and heifers. J. Anim. Feed Sci. 9: 563-583.

II Kokkonen, T., Tesfa, A.T., Tuori, M., Yrjänen, S. & Syrjälä-Qvist, L. 2002. Effect of concentrate crude protein level on grass silage intake, milk yield and nutrient utilisation by dairy cows in early lactation. Arch. Anim. Nutr. 56: 213-227.

III Kokkonen, T., Tesfa, A., Tuori, M. & Syrjälä-Qvist, L. 2004. Concentrate feeding strategy of dairy cows during transition period. Livest. Prod. Sci. 86: 239-251.

IV Kokkonen, T., Taponen, J., Anttila, T., Syrjälä-Qvist, L., Delavaud, C., Chilliard, Y., Tuori, M. & Tesfa, A.T. 2005. Effect of body fatness and glucogenic supplement on lipid and protein mobilization and plasma leptin in dairy cows. J. Dairy Sci. 88: 1127-1141.

The experiments were conducted at the University of Helsinki research farms in Suitia (publications I and III) and Viikki (publications II and IV).

The author was responsible for planning and conducting the experiments documented in publications II, III and IV, and participated in planning and conducting the experiment documented in publication I. The author was responsible for calculation of the results, statistical analysis and preparation of the manuscripts documented in publications I to IV.

ABBREVIATIONS

3-MH	3-methylhistidine
AAT	Amino acid absorbed from the intestine
ADF	Acid detergent fibre
AIA	Acid-insoluble ash
BCS	Body condition score
BHB	Beta-hydroxy butyrate
CPT-1	Carnitine palmitoyltransferase-1
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
D-value	Digestible organic matter in the dry matter
ECM	Energy-corrected milk
FM	Fish meal
HMG-CoA synthase	3-hydroxy-3-methylglutaryl-CoA synthase
IGF-1	Insulin-like growth factor 1
LW	Live weight
LWC	Live weight change
ME	Metabolizable energy
N	Nitrogen
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
OM	Organic matter
RMSE	Root mean square error
RSE	Rapeseed expeller
RSM	Rapeseed meal
SBM	Soya bean meal
TMR	Total mixed ration
UFL	Unite Fourragere Lait
VFA	Volatile fatty acid
VLDL	Very low density lipoprotein

CONTENTS

1. INTRODUCTION

Dairy cows are conventionally dried off approximately 60 d before the expected calving date. Traditionally, the dry period has been thought to be necessary for replenishment of body reserves, regeneration of mammary tissue, and for maximal benefits from lactogenic endocrine events near parturition (Annen et al. 2004). Omitting the dry period results in substantial decrease in milk yield during subsequent lactation, which is mostly due to carry-over of "old" mammary cells to the next lactation and concomitant reduction of secretory capacity (Annen et al. 2004).

Current Finnish recommendations for feeding during the dry period (Suvitie 2001) are based on avoiding obesity of cows at calving. During the early dry period Finnish dairy cows are usually fed solely with forage, which can be low quality grass silage (often leftovers from lactating cows) or hay. In addition, straw is often fed ad libitum to dry cows to increase fill and to dilute energy and protein concentration of the diet, if grass silage is of moderate or good quality. Including straw in the dry period diet might enhance the physical ability of the rumen to accommodate higher intakes post-calving, but the evidence for this is scarce (Friggens et al. 2004).

The close-up dry period starts typically 3 to 4 wk before the expected parturition. Lead feeding with cereal concentrates is common practice during this period. The rationale of lead feeding is to prepare the digestive tract for the next lactation. Special interest has been put into enhancing volatile fatty acid (VFA) absorption capacity through rumen wall and adaptation of rumen micro-organisms to a change of feeding around calving (Friggens et al. 2004). In Finnish dairy herds concentrate allowance is usually increased to approximately 3 to 5 kg/d at calving, although there seems to be considerable variation in practices between farms (Nousiainen 2001).

The transition period is often defined as 3 wk prepartum until 3 wk postpartum (Grummer 1995, Drackley 1999). Nevertheless, it is evident that no clear start or end point can be given for the metabolic and endocrinological changes that enable dairy cows to adapt to the increasing demands of the onset of lactation. Feeding or management of cows during previous lactation can have long term effects on nutritional or metabolic status that have repercussions through the dry period through to subsequent lactation. However, it is clear that the majority of metabolic and endocrinological changes involved in adaptation from pregnancy to lactation take place during the last weeks of pregnancy and the first weeks of lactation (Bell 1995).

Transition from pregnancy to lactation is an enormous challenge to the metabolism of dairy cows. Bell (1995) calculated that for a milk yield of 30 kg/d, mammary requirements for glucose, amino acids and fatty acids at 4 d postpartum are approximately 2.7, 2.0 and 4.5 times those of the gravid uterus during late pregnancy. The abrupt increase of nutrient demand at the onset of lactation can not be met by feed intake. Consequently, dairy cows are predestined to mobilise lipids and amino acids from tissue reserves. Non-esterified fatty acids (NEFA) are released from adipose tissue into the bloodstream and are utilized for milk fat synthesis or used as oxidative fuel in the liver or peripheral tissues. Amino acids are mobilised primarily from skeletal muscles and used for gluconeogenesis and increased protein synthesis in the liver and in the splanchnic tissue (Bell et al. 2000). The rate of gluconeogenesis in the liver is increased, and nutrient utilisation of nonmammary tissues is altered to spare glucose for mammary use (Bell and Bauman 1997).

The success of the transition period has a strong influence on the profitability of the cow during lactation. The majority of production diseases occur very early in lactation (Ingvartsen et al. 2003). The peak of disease incidence coincides with the rapid acceleration of milk yield. Metabolic and hormonal changes associated with the acceleration of milk yield during the transition period and early lactation may, directly or indirectly, compromise the immune system and increase susceptibility to bacterial infections (Ingvartsen et al. 2003). The periparturient cow may be particularly susceptible to mastitis, which may adversely affect lactation and reproductive performance (Overton and Waldron 2004). Excessive lipid mobilisation predisposes cow to fatty liver and ketosis (Grummer 1993). Further, an extreme negative energy balance and associated metabolic and endocrinological changes impair reproductive success (Rukkwamsuk et al. 1999). Hyperketonemia appears to have multiple negative effects on immune function (Overton and Waldron 2004) that may be related to the decreased capacity of fat infiltrated liver to clear bacterial endotoxins from circulation (Rukkwamsuk et al. 1999).

During early lactation Finnish dairy cows are usually fed with grass silage ad libitum. Concentrates are often fed separately from grass silage, although a total mixed ration (TMR) feeding system has become more common during the past decade. Feeding to yield is probably the most commonly used concentrate feeding strategy. In this context profitability and lactation responses of concentrate feeding have been discussed in Finland during the past decade. This discussion has been based largely on the extensive body of studies conducted during post-peak lactation.

The overall objective of this thesis was to study the effects of dry period and early lactation energy and protein feeding on lactation performance in dairy cows. A special emphasis was put on studying metabolic and hormonal status, and the mobilisation of tissue reserves. More specifically the objectives were to

- study the effects of concentrate supplementation on milk and protein yields, grass silage intake and lipid mobilisation during early lactation (I)
- assess the effects of protein supplementation on milk and protein yields, grass silage intake, diet digestibility, lipid mobilisation and energy utilisation during early lactation (II)
- investigate the effects of concentrate proportion in lead feeding and increase rate of concentrate after calving on feed intake, milk production and lipid mobilisation (III)
- examine the effects of body fatness on lipid and amino acid mobilisation, lipolytic response of adipose tissue and plasma leptin concentration (IV)
- evaluate the effects of glucogenic supplementation on tissue mobilisation and blood ketone concentration (I and IV)

In addition, to quantify the lactation responses to concentrate and protein supplementation, meta-analyses including our studies and selected literature were conducted. Early lactation responses were compared to the more abundant data of mid-lactation responses, to assess the potential difference of responses between the two lactation stages.

2. MATERIAL AND METHODS

2.1. Experimental animals and feeding

Experiments documented in publications I and III were conducted using Friesian dairy cows housed in a stanchion barn. Experiments II and IV were conducted using Ayrshire dairy cows kept in tie stalls. The cows were milked twice a day and had continuous access to water. Average 305 d milk yields from previous lactation of multiparous cows were 7778 kg, 9103 kg, 8030 kg and 9529 kg respectively in experiments I to IV.

Diets were formulated using feeds typical of those fed in Finnish dairy farms. Grass silage was ensiled with acid-based additives. During the early dry period, all cows (except the test group in publication IV) were fed with restricted amounts of silage, individually according to the energy recommendation for pregnant cows (Rehutaulukot ja ruokintasuositukset 2004). During the close-up dry period (three weeks before calving), concentrates were included in the ration. During lactation, silage was offered ad libitum. The postpartum concentrate allowance was gradually increased and preplanned, fixed levels were achieved 10 to 35 days after calving.

2.2. Experimental procedures and treatments

Experimental procedures are described in detail in individual publications, only a brief summary is presented here (Table 1). Detailed descriptions of analytical procedures can be found in publications I to IV.

The experiment documented in publication I was carried out to study the effect of concentrate level during early lactation on milk and protein yields, ad libitum grass silage intake and lipid mobilisation. In addition, the effect of glucogenic supplementation on blood glucose and ketone concentrations and lipid mobilisation was also assessed. In a 2 x 2 factorial design, 16 primiparous and 16 multiparous cows were divided into four treatment groups using a randomized complete block design. The experimental period started from calving and lasted for 12 weeks. Concentrate levels were 11 kg/d and 15 kg/d for multiparous cows and 9 kg/d and 12 kg/d for primiparous cows. These levels were achieved within 16 days after calving. The dose of liquid glucogenic supplement, containing propylene glycol, polyols, molasses and nicotiamide, was 0 or 1 l/d.

The experiment documented in publication II was conducted to study the effect of postpartum protein supplementation on milk and protein yields, silage intake, energy utilisation and lipid mobilisation. In a randomized complete block design, 21 multiparous cows were divided into three treatment groups receiving 0%, 15% or 30% rapeseed meal (RSM) in cereal-based concentrate, consisting of barley, oats and molassed sugar beet pulp. The experimental period started from calving and lasted for 10 weeks. A fixed daily concentrate allowance, 15 kg/d, was achieved within 16 days of calving.

Table 1. Description of experiments

Exp.	n	Experimental period	Treatments	Groups
I	32	Wk 1 to 12 postpartum	• Concentrate levels: 11 and 15 kg/d in multiparous cows (C11 or C15), 9 and 12 kg/d in primiparous cows (C9 or C12) • Glucogenic supplementation: 0 or 1 l/d (G0 or G1)	C11G0, C11G1, C15G0, C15G1, C9G0, C9G1, C12G0, C12G1
II	21	Wk 1 to 10 postpartum	• Protein supplementation level: 0%, 15% or 30% RSM in concentrate	RSM0, RSM15, RSM30
III	30	Wk 3 prepartum to wk 10 postpartum	• Concentrate proportion in lead feeding: 20% (L), 40% (M) or 60% (H) concentrate of the energy requirement • Increase rate of concentrate after calving: fast (F) (11 – 18 d) or slow (S) (23 – 37 d) increase to fixed level	LS, LF, MS, MF, HS, HF
IV	24	Wk 8 prepartum to wk 8 postpartum	• Energy level between wk -8 and wk -3: according to requirement (Control) or +34 MJ ME/d (Test) • Glucogenic supplementation between wk -2 and wk +8: 0 or 1kg/d (G0 or G1)	Control G0, Control G1, Test G0, Test G1

The experiment documented in publication III was carried out to investigate the effects of concentrate proportion in the diet during lead feeding and concentrate increase rate postpartum on feed intake, milk yield and lipid mobilisation. In a 3 x 2 factorial design, 30 multiparous Friesian cows were divided into six treatment groups using a randomized complete block design. The experimental period started three weeks before parturition and lasted for 10 weeks after calving. Before calving, the proportion of concentrate in the diet was 20%, 40% or 60% of individual energy requirements. The rest of the energy requirement was met with grass silage. After calving, the slow increase of concentrate was 0.5 kg/d during the first 10 lactation days and 0.3 kg/d thereafter. In the fast groups, the daily increase was 2 kg for two days after parturition, followed by 1 kg for the next two days and 0.5 kg thereafter. The daily allowance of 15 kg/d was achieved 11 to 37 days after calving in different treatment groups. The concentrate mixture consisted of grain and peas. RSM was given at 0.8 kg/d as a protein supplement.

The objective of the experiment documented in publication IV was to study the effects of body fatness and glucogenic supplement on early lactation performance of dairy cows. Special emphasis was placed on the plasma leptin concentration, and studying lipid and amino acid mobilisation. Twenty-four multiparous Ayrshire cows were used in a 2 x 2 factorial arrangement. The experimental factors were normal or increased body fatness during the dry period, and glucogenic supplement between 14 d prior to the expected calving date and 56 d after calving. Between days 56 and 21 prepartum half of the cows received restricted amounts of wilted grass silage and oat straw individually according to energy recommendation. To

increase the body fatness, the rest of the cows received an additional energy allowance of 34 MJ ME/d in a diet, which consisted of restricted amounts of wilted grass silage and 1 to 2 kg compound feed. For the final three weeks before calving, all the cows were fed with grass silage and a gradually increased amount of cereal concentrate, according to the energy recommendations for pregnant cows. During the final two weeks of pregnancy, 1 kg/d molassed sugar beet pulp or 1 kg/d glucogenic supplement was added to the cereal mixture. Glucogenic supplement consisted of propylene glycol, molassed sugar beet pulp, lucerne meal, heat-moisture- and expander-treated wheat, wheat bran, polyols (xylitol, arabinitol), nicotinamide and choline chloride. After calving, division into groups with and without glucogenic supplement was maintained. Cows were given a concentrate mixture formulated from barley, oats, molassed sugar beet pulp, and RSM. The fixed daily concentrate allowance of 15 kg/d was achieved within 16 d after calving.

Milk yield was recorded throughout the studies. Milk samples for analyses of milk composition were taken on four consecutive milkings with one, two or four week intervals. Offered feeds were measured daily throughout the studies. Orts from concentrate and silage were collected separately and weighed daily after calving in all studies, and also during the lead feeding period (three weeks before calving) in experiment IV. Live weights (LW) were recorded on two consecutive days with one to four week intervals. All the cows were condition-scored throughout the experiments in conjunction with weighing.

Blood samples for the determination of metabolites and hormones were collected from the jugular vein (publications I to III) or mammary vein (publication IV). Plasma NEFA concentrations were used for assessing lipid mobilisation. In experiment IV, lipid mobilisation was also assessed by measuring subcutaneous fat depth with ultrasonographic scanning, and by monitoring in vitro lipolysis of adipose tissue samples. Plasma creatinine and 3-methylhistidine (3-MH) concentrations, as well as the changes in diameter of the longissimus lumborum muscle were used to estimate amino acid mobilisation.

2.3. Laboratory analysis

The chemical composition of feed (I – IV) and faeces (I and II) were analysed by standard procedures using the AOAC (1995) method 942.05 for ash, method 920.39 for ether extract (after HCl hydrolysis) and method 984.13 for crude protein (CP) analyses. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to Van Soest et al. (1991). NDF was assayed with sodium sulphite (without α-amylase) and is expressed with residual ash. The volatile fatty acids of silage were determined by gas chromatography (Hewlett Packard 5890 Series II) (Huhtanen et al. 1998). Sugar (Somogyi 1945, with modfications of Salo 1965) and ammonium nitrogen (N) (McCullough 1967) contents of silages were measured spectrophotometrically from a water extract of the silage sample. The total N contents of silage and faecal samples were determined from fresh samples by the Kjeldahl method. The lactic acid content of silages was measured with the colorimetric method (Barker and Summerson, 1941). In experiment II, the digestibilities of diets were determined using acid-insoluble ash (AIA) as an internal marker. AIA was assayed by the 2N HCl method (Van Keulen and Young 1977). In vitro organic matter (OM) digestibility was determined using the method of Tilley and Terry (1963) (II) or with cellulase solubility (Friedel 1990) (IV).

Fat, protein and lactose contents of milk were analysed with infrared analysis (MilkoScan 605 (I – III), MilkoScan FT6000 (IV); Foss Electric, Hillerød, Denmark). The urea content of milk

samples was measured with an enzymatic colorimetric method (Rajamäki and Rauramaa 1984) (I – III) or using infrared analysis (MilkoScan FT6000) (IV). Milk acetone content (I – III) was analysed with the methods described by Rajamäki and Rauramaa (1985), and energy-corrected milk (ECM) yields were calculated according to Sjaunja et al. (1991). Separate milk samples were collected for the analysis of casein (II) (Rowland 1938), and progesterone (I and II) (Laitinen 1982).

For the analysis of beta-hydroxy butyrate (BHB) (I – IV; Hansen and Freier 1978) and acetoacetate (I – III; Price et al. 1977), whole blood samples were treated with perchloric acid (Työppönen and Kauppinen 1980). Glucose (Trinder 1969), insulin (RIA, Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA) glucagon (I – III; RIA, Double Antibody Glucagon, Diagnostic Products Corporation, Los Angeles, USA), NEFA (McCutcheon and Bauman 1986), triglycerides (I and III; Wahlefeld 1974), urea (II and III; Gutman and Bergmeyer 1974) and total protein (II; Weichselbaum 1946) were determined from plasma. Plasma creatinine (IV) was analysed with the method of Fabiny and Ertigshausen (1971). Plasma 3-MH (IV) concentrations were analysed with a Biochrom 20 amino acid analyzer (Biochrom Ltd., Cambridge, UK), according to Directive 98/64/EC (European Commission 1998). Plasma leptin (IV) was determined with ovine-specific radioimmunoassay (Delavaud et al. 2000) and validated for bovine plasma (Delavaud et al. 2002).

Lipolysis of adipose tissue samples in vitro (IV) was monitored by following the release of glycerol, assayed with the GPO-Trinder method (McGowan et al. 1983; procedure no. 337; Sigma Diagnostics, Inc., St. Louis, USA).

2.4. Meta-analysis

Two data sets were compiled from our studies (experiments I and II) and earlier studies for meta-analyses to quantify the effect of energy and protein supplementation on early lactation feed intake and milk production responses, and to compare them with responses in mid-lactation and longer-term studies. Data from 36 studies published since 1980 were used for the calculation of responses to concentrate supplementation. Data included 11 studies (20 comparisons) in early lactation, 17 studies (29 comparisons) in mid-lactation and eight studies (14 comparisons) covering both stages. For the calculation of responses to protein supplementation, data from 35 studies published since 1980 were used. Data were included from eight studies (13 comparisons) in early lactation, 21 studies (33 comparisons) in mid-lactation and 6 studies (10 comparisons) covering both stages. Summaries of the studies are presented in Appendices 1 to 4.

The studies that started before the 5^{th} lactation week and ended not later than at the 14^{th} lactation week, were categorized as early lactation studies. Thus, the studies in the early lactation category were mainly conducted before the 10^{th} lactation week. Respectively mid-lactation studies were mainly conducted after the 10^{th} lactation week cut-off point. In addition, a third category for long term studies or change-over studies covering both stages was created. The use of a cut-off point at week 10 is simply an empirically convenient point and does not imply any fundamental change in the biology of intake and lactation. However, most of the tissue mobilisation takes place before this point and the majority of cows are in positive energy balance by this time.

The majority of the studies were conducted with multiparous cows. In some studies primiparous (max. 50% of the animals) were also used but results were not reported separately for different parity groups. In all studies silage and concentrate were fed separately, grass silage was fed ad libitum and silage dry matter intake (DMI) was measured individually. In some studies, a fixed amount of hay (max. 4 kg/d) was fed in addition to grass silage.

To calculate responses to increased metabolizable energy (ME) intake, reported ME intakes were used if available. Digestible energy intake was converted to ME intake with the equation ME = 0.81 x digestible energy (MAFF 1975). UFL intake was converted to ME intake with the equation ME = 11.6 x UFL (Coulon and Rémond 1991). For some of the studies ME content of the concentrate was estimated based on composition and by using feed table values (Rehutaulukot ja ruokintasuositukset 2004). If silage ME content was not reported, it was calculated with the equation ME = 0.16 x D-value (MAFF 1975). If ECM was not reported, it was calculated according to Sjaunja et al. (1991) using the reported average milk yield and milk composition.

2.5. Statistical methods

Detailed description of methods of statistical analysis in individual studies can be found in the respective publications (I to IV). Only a general outline of experimental design is given here. All the studies in this thesis were conducted either through the transition period or started immediately after calving, because a special emphasis was put on studying metabolic and hormonal changes that occur during the transition period. To reduce unexplained variation due to differences between animals, a randomized complete block design was used. Blocks were formed taking into account the expected calving date, LW, milk yield of previous lactation and previous peak yield.

The meta-analyses were conducted with a mixed model methodology as described by St-Pierre (2001), using the PROC MIXED model procedure in the SAS system (2001). Since some studies included several comparisons with different silage quality or type, or with different concentrate composition, a comparison was considered as a random effect. To test homogeneity of the slopes in early- and mid-lactation, a fixed discrete variable 'lactation stage' was included in the model. The homogeneity of the slopes in early- and mid-lactation was tested without the studies covering both stages. Dependent variables were not weighted. Linear models included both fixed and random intercepts and slopes. If the random covariance between intercept and slope, or random slope were not approaching statistical significance, they were removed from the model as described by St-Pierre (2001). In some instances, when the model did not converge or an infinite likelihood was estimated, the estimation of covariance elements was removed using TYPE = VC as an option in the random statement. Regression curves are presented based on predicted values of dependent variables vs. independent variables. Adjusted observations across studies are also plotted. As stated by St-Pierre (2001), when presenting results of mixed model analyses, the Y observations must be adjusted for the additional dimensions represented by individual studies.

Regression relationships between plasma NEFA, insulin and glucagon in the combined data from experiments I to III were calculated using individual observations and the mixed model methodology described above. Treatments nested within experiments were considered as a random effect.

3. GENERAL DISCUSSION

3.1. Feed intake

The voluntary DMI of dairy cows decreases during the last trimester of pregnancy (Ingvartsen and Andersen 2000). The lowest voluntary DMI occurs at calving. Ingvartsen et al. (1992) showed that dairy heifers reduced voluntary DMI from pregnancy wk 26 by 1.5% per week until 3 wk before calving. Grummer (1995) emphasized the importance of maximizing prepartum feed intake in order to reduce fatty acid mobilisation from adipose tissue and hepatic lipid accumulation. Furthermore, he observed a positive relationship between DMI at 1 d before calving and 21 d after calving.

The physical compression of the rumen by the growing uterus and the increasing amount of abdominal fat have been proposed as possible causes of decreased intake (Mäkelä 1956). Results with bulky diets confirm the role of the physical component in intake regulation, since feeding with grass silage and straw has resulted in lower intake than feeding with only grass silage during the prepartum period (Dewhurst et al. 2000, Moorby et al. 2002, McNamara et al. 2003b).

Voluntary DMI also decreases with more energy-dense diets. With high energy diets the initial intake at 3 wk before calving is considerably higher, but the decrease after that (especially during the last week of pregnancy) is more pronounced than with low energy diets. This was observed with concentrate-rich diets vs. low concentrate diets (Coppock et al. 1972, Hernandez-Urdaneta et al. 1976, Johnson and Otterby 1981, Rabelo et al. 2003), and with grass silage vs. grass silage and straw (Dewhurst et al. 2000). On the other hand, in studies by Wu et al. (1997) and Agenäs et al. (2003), cows with ad libitum feeding of moderate or high energy diets maintained DMI even close to parturition. Huyler et al. (1999) observed no decrease in prepartum DMI despite the diets containing 30% straw. Therefore, it is likely that factors other than physical compression of the rumen have the primary influence on feed intake during the periparturient period. A large number of metabolic factors, including nutrients, metabolites, hormones and peptides, are potentially involved (Ingvartsen and Andersen 2000).

An alternative approach to minimize the decline in intake before calving is to restrict feed intake to below or at the level of predicted requirements. As shown in Figure 1a, total DMI of cows fed according to requirements did not decline in experiment IV near calving. Similarly, the absence of a feed-intake dip near calving has been reported in cows fed at the level of requirements (Kunz et al. 1985, Agenäs et al. 2003), or below requirements (Holcomb et al. 2001, Agenäs et al. 2003). Putnam and Varga (1998) reported that restriction of prepartum DMI to 1.5% of the live weight attenuated the decline of DMI over the last 3 wk prepartum.

After calving, voluntary DMI of dairy cows starts to increase. The rate of increase and maximum level of intake vary considerably, depending on the diet fed during lactation (energy density, forage quality) and animal factors (breed, age, production potential, body fatness, diseases) (Ingvartsen and Andersen 2000). Typically maximum intake is reached later than peak milk yield (8 to 22 wk vs. 5 to 7 wk after calving) (Ingvartsen and Andersen 2000).

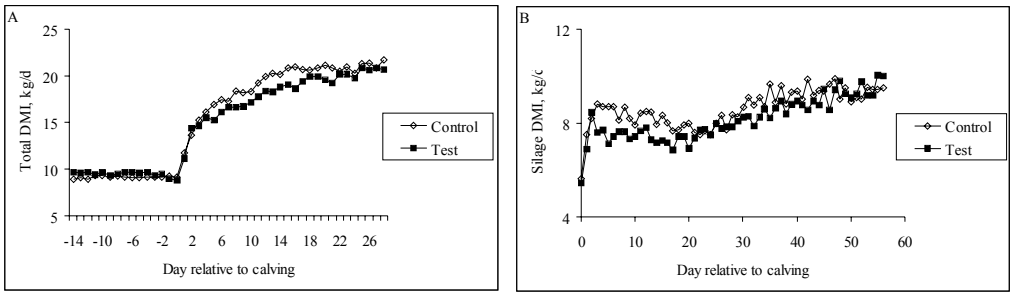

Figure 1. Total DMI (A) and silage DMI (B) of fatter (Test) and normal (Control) cows around calving (experiment IV).

In experiment IV, where concentrate was increased from 4 kg/d at calving to 15 kg/d in 16 days and grass silage was fed ad libitum after calving, the average increase of silage intake between calving day and lactation day 1 was approximately 1.7 kg dry matter (DM) (Figure 1b). Silage intake was further increased 1 kg DM between days 1 and 2, but then it reached a plateau or a slight decrease was seen. Silage DMI started to increase slowly during lactation wk 3 or 4. A similar general pattern was seen in all experiments except in experiment III, where the highest silage DMI was achieved at lactation wk 1. It is apparent that the rapid increase in concentrate after calving resulted in the stagnation of silage DMI increase. Thus, the increase of total DMI during lactation wk 1 to 3 was solely due to increased concentrate allowance.

Voluntary silage DMI stagnated during the period of extensive lipid mobilisation (i.e. for 3 to 4 wk after calving). Therefore, it can not be totally excluded that the stagnation of silage DMI increase was due to physical compression of the rumen volume due to abdominal fat or extensive lipid mobilisation. It has been hypothesized that the increased concentrations of NEFA or ketone bodies would down-regulate voluntary DMI during early lactation (review by Ingvartsen and Andersen 2000). Studies by Garnsworthy and Topps (1982) and Treacher et al. (1986) support the idea of the negative feedback effect of body fat on feed intake, or the idea of a physical limitation imposed by internal fat on reticulorumen fill.

Excluding fatter groups in experiment IV, the cows in experiments I to IV were fed with restricted amounts of feed during the dry period, with the aim of avoiding obesity at calving. Therefore, it is unlikely that physical compression of abdominal fat or extensive lipid mobilisation would have had a major role in limiting the increase of silage DMI, although fatter cows (Test group in Figure 1b) had numerically lower silage DMI than the normal cows during the first 3 wk of lactation. The limited role of physical compression in feed intake regulation is evidenced by the studies of Doreau et al. (1985) and Reynolds et al (2004), which suggest that filling capacity of the rumen (size) is not increased during early lactation and increased DMI is probably associated with greater gut fill.

3.2. Mobilisation of tissue reserves

3.2.1. Lipid mobilisation

Using the slaughter technique Gibb et al. (1992) estimated that cows mobilised on average 40 kg of fat between calving and lactation wk 12, whereas Andrew et al. (1994) estimated mobilisation of 42 kg fat between 2 wk before and 5 wk after calving. Using the D_2O dilution

technique estimates have been 25 to 35 kg (Chilliard et al. 1991) during the first 7 wk of lactation, and 71 kg (Komaragiri and Erdman 1997) by lactation wk 12. Estimates based on respiration chamber studies vary between 30 to 60 kg (as reviewed by Chilliard 1987). The majority of fat mobilisation occurs by peak lactation, although mobilisation may often continue longer, up to approximately lactation wk 12 (Gibb et al. 1992, Komaragiri and Erdman 1997). The duration of the mobilisation period and the extent of mobilisation are affected by factors like breed, milk yield potential, feeding level and balance of nutrients (Chilliard 1987).

Concentration of NEFA in plasma is a good indicator of mobilisation of body fat (Bell 1995). The decrease of reesterification of fatty acids released by lipolysis is probably the main factor contributing to the massive increase of plasma NEFA in periparturient cows (Chilliard 1999). In addition, adipose tissue lipogenesis is suppressed (McNamara and Hillers 1986). Based on in vitro studies basal lipolytic rate, indicated by glycerol release, is either increased (Metz and van den Bergh 1977), remains relatively unchanged (McNamara and Hillers 1986) or may even temporarily decrease at the onset of lactation (Rukkwamsuk et al. 1998, IV).

Bell (1995) estimated that approximately half of the NEFA released into the bloodstream is either oxidized or incorporated into milk triglycerides. The mobilised fatty acids accounts for 40 to 50% of fatty acids in milk triglycerides during the first month of lactation (Bell 1995, Chilliard 1999). The contribution of mobilised energy to milk production during the first 10 weeks of lactation is also sizable. Chilliard (1999) estimated that mobilisation of 55 kg of lipids would contribute energy for 550 kg of milk. Based on indirect calorimetry Sutter and Beever (2000) calculated, that 520 kg (19%) of milk produced during the first 8 lactation weeks was derived from mobilised tissue. During lactation wk 1 tissue energy contributed 38% of milk energy (84 kg milk / wk).

Live weight is still widely used as an index of tissue mobilisation, although it is well known that live weight change (LWC) is a poor indicator of tissue mobilisation during early lactation. This is due to simultaneous increase of feed intake and hence gut content, and water repletion of body tissues, that mask the real magnitude of tissue mobilisation (Sutter and Beever 2000). In addition, the increase of milk yield confounds LW measurements during the first weeks of lactation. Sutter and Beever (2000) observed that LW loss was only 50% of the estimated body tissue loss based on indirect calorimetry. Calculation of corrected LWC by subtracting feed intake and milk yield (II) may give a more realistic estimate of tissue mobilisation.

Body condition score (BCS) may give a more realistic view of the lipid reserves than LW. Estimates of BCS are significantly correlated to subcutaneous fat (Garnsworthy and Topps 1982, Domecq et al. 1995, IV). However, BCS is insensitive to changes in reserves measured in early lactation, the scale of observations may be subjective (IV) and BCS determined by different persons and in different studies are not directly comparable. Finally, ultrasound measurements can be used to assess subcutaneous fat depth and its changes. Subcutaneous fat correlates strongly with total body fat (Faulkner et al. 1990).

3.2.2. Protein mobilisation

A major source of mobilised amino acids is protein breakdown in skeletal muscles, although skin, uterine involution and myometrial protein degradation may have some contribution (Blum et al. 1985, Bell et al. 2000). Botts et al. (1979) determined mobilisable protein

reserves in a depletion-repletion experiment by N balance and found that protein reserves range from 25 to 27% of total body protein, which is over 20 kg of protein in a 600 kg cow. Direct slaughter experiments have yielded smaller estimates from zero loss between 7 d prepartum and 63 d postpartum (Andrew et al. 1994) to 6.6% over the first 8 wk of lactation (Gibb et al. 1992). Using the D_2O dilution technique Chilliard et al. (1991) observed body protein loss of 0.5 and 3.3 kg during the first 7 wk of lactation, when cows were fed low and high energy diets and milk yield was 30 kg/d. With the same technique Komaragiri and Erdman (1997) estimated that body protein loss was 21 kg between wk 2 prepartum and wk 5 postpartum. The average milk yield in that study was 41 kg/d over lactation wk 1 to 16. Phillips et al. (2003) estimated body protein loss of 8 kg during the first 60 d of lactation in cows producing over 40 kg milk/d.

Part of the variation of the estimates of mobilised protein can be explained by differences in methodology. Protein mobilisation also seems to increase with increased milk yield potential. The level of protein and energy nutrition probably also affect the estimates (Komaragiri and Erdman 1997). In practice dairy cows are not normally called upon to lose LW at rates greater than 1 to 2 kg/d, which gives an estimate of 150 to 300 g protein/d, assuming that body tissue lost contained 15% protein. This is sufficient to provide amino acids for 5 to 10 kg/d of milk (Bauman and Elliott 1983). Tamminga et al. (1997) calculated based on the data from five production trials that protein mobilisation reached a maximum of 4.6 kg in the 4^{th} week of lactation. Mobilised protein was sufficient for milk yield of 3.9 kg/d for the first 4 wk of lactation.

Nitrogen balance studies are probably the most reliable method to measure N retention in the body, but they are quite costly and laborious, and not easily repeatable. Alternative methods are semi-quantitative or indicative, but are more repeatable. Ultrasound measurements of longissimus lumborum or longissimus dorsi muscle diameter can be used to indicate mobilisation or accretion of muscle protein (Bruckmaier et al. 1998). Plasma and urine concentrations of 3-MH have been widely used as an indicator of muscle protein catabolism in several species, including cattle. 3-MH is liberated to plasma as a consequence of actin and myosin breakdown, and it can not be reincorporated into protein (Blum et al. 1985, Rathmacher 2000). Plasma creatinine can be taken as a measure of lean muscle mass, and the decrease of creatinine reflects skeletal muscle breakdown (Bruckmaier et al. 1998). Creatinine is formed in muscle from creatine. Urinary creatinine excretion can also be used as an index of body protein mass, if kidney function is normal (Bruckmaier et al. 1998). Finally, a positive correlation between BCS and muscle depth (Reid et al. 1986, Moorby et al. 2002, IV) indicates that at least to some extent changes of BCS also represent changes in protein reserves.

In periparturient cows plasma concentrations of 3-MH are increased around calving (Blum et al. 1985, Burhans et al. 1997, IV). Blum et al. (1985) observed maximal concentrations 1 wk after calving, and a rapid increase between 1 wk before and 1 wk after calving. Burhans et al. (1997) reported that plasma 3-MH peaked at d 2 postpartum. In the study by Overton (1998) the ratio of 3-MH to creatinine in urine peaked at d 3 postpartum.

The rapid increase of 3-MH concentration and the decrease of muscle diameter between d 7 prepartum and d 1 postpartum also in experiment IV suggest that protein mobilisation commences before calving, even with moderate CP concentration of prepartum diet (approximately 150 g/kg DM). The data presented by Bell and Bauman (1997) also shows that the rapid increase of plasma 3-MH concentration starts a few days before calving. In

accordance with this Overton (1998) reported no increase in the ratio of 3-MH to creatinine in urine before d 3 prepartum and a rapid increase between d 3 prepartum and d 1 postpartum.

In experiment IV, 3-MH concentrations were below the prepartum level at 28 d postpartum. Similarly, Zurek et al. (1995) and Burhans et al. (1997) detected a decline in plasma 3-MH concentrations between d 1 or d 2 and d 22 postpartum. After a decline from peak values, plasma 3-MH or the ratio of 3-MH to creatinine in urine seem to reach a plateau 3 to 5 wk after calving (Blum et al. 1985, Zurek et al. 1995, Burhans et al. 1997, Overton 1998), indicating that the most intensive period of muscle protein mobilisation is over by that time.

Plasma creatinine and muscle diameter decreased through the period from wk 1 before calving to wk 4 after calving in experiment IV. The decrease of muscle diameter may continue up to wk 8 after calving (Bruckmaier et al. 1998, Kokkonen et al unpublished), and there seems to be considerable variation between animals. Moorby et al. (2002) also reported that longissimus dorsi muscle diameter starts to decrease before calving and reaches a minimum between 4 to 7 wk of lactation.

3.2.3. Regulation of tissue mobilisation

Lipid mobilisation during the periparturient period is facilitated by the increased responsiveness of adipose tissue to adrenergic stimulation, as shown with in vitro glycerol release (Metz and van den Bergh 1977, Jaster and Wegner 1981, McNamara and Hillers 1986, Reid et al. 1986). In experiment IV and in the study by Rukkwamsuk et al. (1998) the increased responsiveness to adrenergic stimulation was seen relative to basal lipolytic rate rather than in absolute terms. Jaster and Wegner (1981) observed that the number of β-adrenergic receptors nearly doubled in adipose tissue of cows between 30 d prepartum and 30 d postpartum.

The suppression of de novo lipogenesis and esterification in adipose tissue after the onset of lactation (McNamara and Hillers 1986, Rukkwamsuk et al. 1999) is associated with low levels of plasma insulin (Chilliard 1999) and decreased responsiveness of adipose tissue to insulin (Bell 1995). It has also been shown that the insulin secretion response in lactating cows to glucose or propionate infusion is much lower than in dry cows (Chilliard 1999). In experiments I to IV, a transient decrease of plasma insulin was also seen near calving and during the first weeks of lactation. However, the response of plasma NEFA to insulin is not totally abolished (Chilliard 1999). A hyperinsulinemic-euglycemic clamp study by Mashek et al. (2001) showed that insulin effectively decreased plasma NEFA concentration at lactation wk 4. This was also illustrated in experiment II by the higher plasma concentration of insulin and lower concentration of NEFA in the RSM0 group than in other groups at lactation wk 1. There was a negative linear relationship between plasma NEFA and insulin in the combined data from experiments I to III (Figure 2a), but the individual variation was large.

The increased responsiveness of adipose tissue to adrenergic stimulation and decreased responsiveness to insulin is orchestrated by growth hormone (Bell 1995). Plasma growth hormone concentrations rise during late pregnancy, peak at parturition and remain elevated through early lactation. As reviewed by Bell (1995) and Chilliard (1999) growth hormone probably has no direct lipolytic effect on adipose tissue. It enhances lipolytic responsiveness to adrenergic stimulation, and decreases the rate of lipogenesis and activities of lipogenic enzymes.

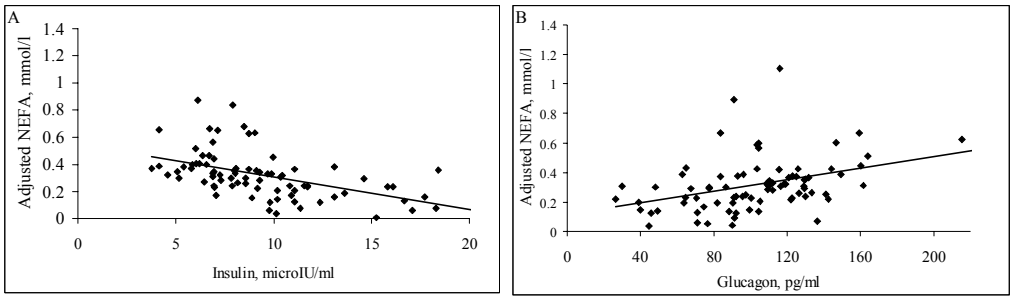

Figure 2. Relationships between plasma (A) insulin and adjusted NEFA (lactation wk 4 in experiments I to III). NEFA = -0.0241Ins + 0.5456 (n = 82, linear P = 0.02, R^2 = 0.28); (B) glucagon and adjusted NEFA (lactation wk 4 in experiments I to III). NEFA = -0.002Glu + 0.112 (n = 82, linear P = 0.01, R^2 = 0.16).

Glucagon is only weakly lipolytic in ruminants (Brockman and Laarveld 1986). Hippen et al. (1999a, 1999b) observed no stimulatory effects of glucagon infusion on lipolysis on the basis of plasma NEFA concentrations in early lactation dairy cows. In fact, glucagon infusion stimulated insulin secretion, which may counteract the possible lipolytic effect of glucagon (Hippen et al. 1999a, Hippen et al. 1999b), because insulin probably has a stronger regulatory effect on peripheral metabolism than glucagon (Brockman and Laarveld 1986). In the combined data from experiments I to III, there was a significant positive relationship between plasma NEFA and glucagon (Figure 2b), but the individual variation was substantial. On the other hand, plasma glucagon decreased transiently near calving in experiments II and III, i.e. at the time when plasma NEFA concentrations peaked. After calving plasma glucagon increased in experiments I to III, and especially in II and III.

In parallel with increased response to β-adrenergic stimulation, antilipolytic responses to α-2-adrenergic stimulation are also increased (Chilliard 1999, Vernon and Houseknecht 2000). In addition, adipose tissue produces prostaglandin E2 and adenosine, which both inhibit catecholamine-stimulated lipolysis (Vernon and Houseknecht 2000). Therefore, lipolytic capacity may represent the balance between opposing actions of lipolytic and antilipolytic effectors (Bell 1995). Yet another factor which may inhibit high rates of lipolysis is albumin supply to adipose tissue, since fatty acids released by adipocytes are transported in blood bound to albumin (Vernon and Houseknecht 2000).

Both the decrease of protein synthesis and the increase of protein degradation contribute to the net mobilisation of amino acids from skeletal muscles and skin. These changes are consistent with decreased concentration of insulin and thereby decreased inhibition of catabolism. In parallel with insulin, plasma concentration of insulin-like growth factor 1 (IGF-1) decreases, and stimulation of protein synthesis by both of these factors is thus reduced during the periparturient period (Bell et al. 2000). The increased mobilisation of amino acids is also facilitated by growth hormone-induced insulin resistance of skeletal muscle (Bell and Bauman 1997). On the other hand, there is some evidence that a high concentration of ketone bodies may limit protein mobilisation to protect the body from hazardous protein degradation (Holtenius and Holtenius 1996).

3.3. Adaptation of glucose metabolism

A sudden increase in glucose demand occurs without any increase of feed intake before parturition (Bell and Bauman 1997). Glucose metabolism adapts to the increased demands of lactation by decreasing oxidation of glucose in peripheral tissues (Bennink et al. 1972) and by increasing hepatic gluconeogenesis. Reynolds et al. (2003) reported that liver glucose release doubles from 9 d prepartum to 11 d postpartum. Furthermore, Reynolds et al. (2003) observed that during the transition period measured glucose output was more than adequate to meet glucose requirements for maintenance, gestation and milk lactose synthesis. During the transition period and especially during the early postpartum period, the relative proportions of amino acids (especially alanine), lactate and glycerol as precursors of gluconeogenesis increase (Overton 1998, Reynolds et al. 2003).

Glucagon stimulates hepatic gluconeogenesis, while insulin has an inhibitory effect. Change of the insulin to glucagon ratio may therefore be a major factor affecting gluconeogenesis (Chilliard 1999, Drackley et al. 2001). Glucagon also has a glycogenolytic effect (Hippen et al. 1999b), which can contribute to maintaining glucose homeostasis in the short term. The increased concentrations of glucagon in early lactation cows in experiments I to III are consistent with the studies of De Boer et al. (1985) and Smith et al. (1997). Due to steeper and more extended decrease of plasma insulin near calving relative to glucagon, the insulin to glucagon ratio decreased during the transition from pregnancy to lactation in experiments II and III. In experiment II the average insulin to glucagon ratio (mol / mol) decreased from 2.1 at 1 wk prepartum to 1.3 at 1 wk postpartum and remained low thereafter. In experiment III the average insulin to glucagon ratio decreased from 3.9 to 1.8 between wk 1 prepartum and wk 1 postpartum, but it was slightly increased after that.

3.4. Ketogenesis

Hepatic conversion of NEFA to ketone bodies can be seen as a strategy to spare glucose during times of deficit. In ruminant animals, heart, kidney, skeletal muscle, mammary gland, and gastrointestinal tract can oxidize ketone bodies (Drackley et al. 2001). A prerequisite for ketogenesis is increased supply of long-chain fatty acids in the liver. Fatty acid uptake to the liver is related to NEFA concentration in blood (Grummer 1993). In the liver fatty acids can be oxidized either via the citric acid cycle or to ketone bodies, or they are esterified to triglycerides (Drackley et al. 2001).

Earlier, the prevailing theory on the development of ketosis was a lack of oxaloacetic acid to combine with acetyl-CoA. Instead of combustion in the citric acid cycle, excess acetyl-CoA is converted to ketone bodies (Holtenius and Holtenius 1996, Drackley et al. 2001). According to more recent theories hepatic ketogenesis is regulated by the activity of carnitine palmitoyltransferase-1 (CPT-1) and the intramitochondrial activity of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase). CPT-1 promotes entry of fatty acyl-CoA into mitochondria for acetyl-CoA production, and HMG-CoA synthase activity is the regulatory step in the conversion of acetyl-CoA to ketone bodies (Drackley et al. 2001).

De Boer et al. (1985) suggested that glucagon deficiency resulting from impaired synthesis or secretion may predispose cows to ketosis during early lactation. Concentrations of glucagon decrease during ketonemia (De Boer et al. 1985) and glucagon concentrations are lower in the

blood of obese cows (Smith et al. 1997). Although apparently contradictory to earlier results, weak positive relationships between plasma glucagon and NEFA (Figure 2b) and between plasma glucagon and BHB (data not shown, R^2 0.15 and 0.09, $P < 0.05$, lactation wk 1 and 4) in the combined data from experiments I to III probably only reflect a slight increase in lipomobilisation and ketonemia in high-yielding cows. Intravenous infusions of glucagon for 48 h or 14 d did not increase blood BHB in early lactation cows (Hippen et al. 1999a, Hippen et al. 1999b). Therefore, there is no substantial evidence that glucagon stimulates ketogenesis in dairy cows.

NEFA mobilisation results in lipid infiltration of the liver. Some degree of lipid infiltration is normal during the periparturient period (Grummer 1993, Overton and Waldron 2004). The low rate of synthesis and secretion of very low density lipoprotein (VLDL) in ruminant liver is a major factor leading to lipid infiltration of the liver (Grummer 1993). If lipid infiltration becomes severe, resulting in fatty liver syndrome, it may increase susceptibility to ketosis (Grummer 1995) and impair gluconeogenic and ureagenic activity of the liver (Overton and Waldron 2004). Ketotic cows have a higher percent of fat infiltration in the liver than healthy cows (Gröhn et al. 1983).

Using a threshold of 1.5 mmol/l BHB in blood, the proportion of cows having at least one episode of hyperketonemia during the first two months of lactation was high in experiments I and III: 31% (multiparous cows) and 37%, respectively. In experiments II and IV the corresponding proportions were 10% and 17%. The prevalence of hyperketonemia in earlier studies has ranged from 8.9 to 34%, as reported in the review by Duffield (2000). The potential effect of breed on the difference in hyperketonemia between experiments can not be assessed, since breed and experiment were confounded (i.e. Friesian (Black and White) cows were used in experiments I and III and Ayrshire cows in experiments II and IV). In a large retrospective cohort study, Schnier et al. (2004) observed a 1.44 times greater predicted incidence risk of ketosis in Finnish Black and White cows than in Ayrshire cows in tie stalls. Differences in management and/or production potential of cows may have contributed to the observed differences in hyperketonemia. A similar fixed concentrate allowance was used in experiments II to IV, and for half of the multiparous cows in experiment I. Yet, based on previous milk yields the production potential of cows in experiments I and III may have been lower than that of cows in experiments II and IV.

3.5. Leptin

Leptin is primarily produced in white adipose tissue, and is involved in the regulation of energy homeostasis, feed intake and immunity. Administration of leptin has increased energy expenditure in rodents (Ahima and Flier 2000), and decreased feed intake in rodents, pigs and sheep (Ingvartsen and Boisclair 2001). In rodents, the circulating leptin concentration decreases quite rapidly during food restriction, and the fall of leptin decreases basal metabolic rate by affecting thyroid hormone secretion (Ahima and Flier 2000). Effects of leptin on energy homeostasis and feed intake are central and mediated by orexigenic and anorexigenic neuropeptides in the hypothalamus (Ingvartsen and Boisclair 2001, Vernon et al. 2002). Leptin may have a role in activation of lipid oxidation (Ahima and Flier 2000), and may protect nonadipose tissue from excessive fat accumulation (Unger 2000). The development of insulin resistance is associated with the accumulation of triglycerides in skeletal muscles and low leptin concentration may contribute to this development (Vernon et al. 2002).

In the long term, circulating leptin concentrations in adult cattle are positively associated with adiposity and the plane of nutrition (Delavaud et al. 2002). In the short term, severe feed restriction or feed deprivation decreases plasma leptin concentrations in dairy cows (Block et al. 2003, Chelikani et al. 2004). Leptin probably has no role as a short-term satiety factor in dairy cattle (Delavaud et al. 2002, Chelikani et al. 2004).

Experiment IV showed, in agreement with earlier studies (Block et al. 2001, Holtenius et al. 2003, Liefers et al. 2003, Reist et al. 2003), that plasma leptin concentration decreases during periparturient period. This decrease is most likely due to decreased synthesis in adipose tissue (Block et al. 2001, Leury et al. 2003). Leptin starts to decrease before calving (Liefers et al. 2003, IV) and reaches nadir at calving (Liefers et al. 2003) or during the first week of lactation (Reist et al. 2003, IV). The rise of plasma leptin concentration is very slow or non-existent after calving (Block et al. 2001, Holtenius et al. 2003, Liefers et al. 2003, IV), although Liefers et al. (2003) observed a temporary rise soon after calving. Contrasting with other studies, Reist et al. (2003) observed a steady increase in plasma leptin between lactation wk 1 to 20.

Block et al. (2001) attributed the reduction of plasma leptin to the negative energy balance caused by the initiation of copious milk secretion. Temporal changes of plasma leptin and energy balance near calving are parallel, and plasma leptin and negative energy balance nadirs seem to coincide. However, despite the increase of energy balance during advancing lactation, the increase of plasma leptin concentration can be slow (Block et al. 2001, IV). In the study by Holtenius et al. (2003) plasma leptin levels remained low even in cows which had reached positive energy balance at lactation wk 12. In experiment IV, fatter cows were in more negative energy balance, but had significantly higher plasma leptin concentrations than the control group. On the other hand, Liefers et al. (2003) and Reist et al. (2003) reported positive relationships between plasma leptin and energy balance during early lactation.

The uncoupling of plasma leptin concentration and energy balance may be due to mobilisation of adipose tissue and therefore decreased secretory capacity (Block et al. 2001). The significant positive correlations between BCS, fat depth and plasma leptin during early lactation give some support to this theory (IV). Higher plasma leptin concentrations in fatter cows than in the control group (IV) may thus reflect preservation of greater body lipid reserves, despite the more extensive lipid mobilisation in fatter cows. Reist et al. (2003) also observed a positive relationship between BCS and plasma leptin in post parturient cows, whereas Holtenius et al. (2003) did not.

Based on the significant correlations between the plasma concentrations of leptin, insulin, growth hormone and NEFA, Block et al. (2001) suggested that these factors could represent co-regulation by energy balance, and perhaps mediate the effect of energy balance on leptin synthesis. In agreement with this, significant correlation was found between plasma leptin and insulin in experiment IV. Plasma glucose also tended to be positively correlated with leptin, although not statistically significant at all time points.

Studies with a hyperinsulinemic-euglycemic clamp (Block et al. 2003, Leury et al. 2003) and sustained jugular infusion of glucose (Chelikani et al. 2003) showed that insulin or insulin-mediated glucose metabolism (possibly IGF-1) stimulates secretion of leptin during late lactation and late pregnancy, but not in early lactation. Inhibitory effects of growth hormone on leptin expression and release, and reduced adipose tissue sensitivity to insulin, may explain the lack of responses during early lactation (Leury et al. 2003, Chelikani et al. 2003). Block et

al. (2001) and Leury et al. (2003) also speculated that β-adrenergic signals could be associated with the reduction of plasma leptin around parturition.

The decrease of plasma leptin during the periparturient period increases metabolic efficiency by decreasing basal metabolic rate, and may contribute to glucose sparing by affecting the development of insulin resistance in muscles. It may also have a long-term effect to facilitate the increase of feed intake at the onset of lactation (Block et al. 2001, Vernon et al. 2002). However, the lack of association between feed intake depression and plasma leptin in hyperinsulinemic-euglycemic clamp studies suggest that a decrease of plasma leptin around parturition may not drive the increase of feed intake at the onset of lactation (Leury et al. 2003). Finally, the depression of leptin may contribute to the immunosuppression around calving and during early lactation (Ingvartsen and Boisclair 2001).

3.6. Dry period overfeeding and body fatness

3.6.1. Feed intake after calving

Research shows that cows fed with restricted feeding (75 to 100% of requirements) for 6 to 10 wk before calving increase their feed intake faster after calving than cows with a higher prepartum energy allowance (ad libitum feeding or 125% of requirements) (Kunz et al. 1985, Tesfa et al. 1999, Agenäs et al. 2003). In addition, cows fed with restricted diet don't decrease their feed intake before calving (Kunz et al. 1985, Agenäs et al. 2003, IV), whereas this is often observed in ad libitum fed cows (Grummer 1995, Overton and Waldron 2004).

Several studies have also shown that as BCS at calving increases, the rate of increase in feed intake after calving decreases (Garnsworthy and Topps 1982, Treacher et al. 1986, Garnsworthy and Jones 1987). Differences in average BCS between categories of fatter and thinner cows have been large in these studies (approximately 1 to 2 BCS units on 4 to 6 point scales). In addition to differences in BCS, differences in the size of cows in the different body condition groups appear to explain some of the differences in DMI (Ingvartsen et al. 1996). Furthermore, energy content of mobilised tissue per BCS unit change may be different at the lower and higher ends of the scale, since thin cows have no subcutaneous fat depots and BCS changes at the lower end of the scale may reflect changes in tissue water in addition to changes in protein and lipids (Otto et al. 1991). When the differences between condition scores at calving have been smaller (0.8 BCS units or less), average feed intake of fatter and thinner cows has not differed significantly during early lactation (Fronk et al. 1980, Boisclair et al. 1986, Holter et al. 1990, IV). Some of the studies described dry period feeding inadequately, but large differences of BCS at calving were most likely achieved by moderate to severe restriction of nutrient intake or substantial overfeeding during the dry period (ad libitum or very high energy feeding) and/or by overfeeding or feed restriction during late lactation (Garnsworthy and Topps 1982, Treacher et al. 1986, Garnsworthy and Jones 1987, Agenäs et al. 2003).

3.6.2. Lipid mobilisation

Cows with a restricted energy allowance during the prepartum period have lower lipid mobilisation after calving than do overfed cows (Kunz et al. 1985, Holtenius et al. 2003), and this can be associated with faster increase in feed intake in early lactation. Similarly, studies with large differences in BCS at calving have shown that fatter cows mobilise more lipids during early lactation (Garnsworthy and Topps 1982, Reid et al. 1986, Treacher et al. 1986,

Garnsworthy and Jones 1987, Pedron et al. 1993). In some studies larger lipid mobilisation of fatter cows was observed without significant differences in feed intake between fatter and thinner cows (Boisclair et al. 1986, Holter et al. 1990, IV). Fronk et al. (1980) reported increased lipid mobilisation with dry period overfeeding in one experiment, but observed no differences in another. In a study by Tesfa et al. (1999), 25% under- or overfeeding for 6 wk before calving had no effect on BCS at calving or lipid mobilisation during early lactation. The lack of increase in mobilisation in some studies may be explained by small differences in prepartum LW gain. If the difference in mobilisation between fatter and thinner cows is less than 30-40 kg LW, feed intake appears not to be depressed (Ingvartsen et al. 1996).

Restricted feed intake or decreased energy density of diet during the dry period is essential to avoid extensive lipid mobilisation after calving. However, it must be noted that severe restriction of nutrient intake during the dry period to reduce body fatness of cows will cause mobilisation of maternal tissues before calving, which may compromise subsequent lactation performance, particularly if nutrient supply from the lactation diet is inadequate (Agenäs et al. 2003). Severe feed restriction may also impede mammary gland and foetal development (Friggens et al. 2004).

The results from experiment IV and from Rukkwamsuk et al. (1998) suggest that more extensive lipid mobilisation of fatter cows is facilitated by increased responsiveness to adrenergic stimulation. Contrasting these studies, Reid et al. (1986) observed no differences in the adrenalin-stimulated glycerol release in adipocytes of fatter and thinner cows.

3.6.3. Ketogenesis

Increased body fatness is a well known risk factor for ketosis (Rukkwamsuk et al. 1999, Duffield 2000). Fat cows have lower feed intake and higher lipid mobilisation than normal or thin cows during early lactation. Lowered feed intake can decrease propionate supply, which in turn may decrease insulin secretion. Since propionate is antiketogenic, the combination of decreased propionate and increased NEFA supply favours ketogenesis in the liver (Drackley 1999).

Although overfeeding has increased plasma insulin during the dry period in several studies, after calving the difference in insulin levels between fatter and thinner cows disappears (Fronk et al. 1980, Kunz et al. 1985, Reid et al. 1986, Rukkwamsuk et al. 1998, IV). Kunz et al. (1985) and Rukkwamsuk et al. (1998) observed higher blood ketone concentrations and Holter et al. (1990) higher urine ketone levels in fatter cows, whereas Fronk et al. (1980) and Reid et al. (1986) observed no differences in blood ketone concentrations. Duffield (2000) reported that incidence of subclinical ketosis (serum BHB $\geq$ 1.4 mmol/l) in a field trial was increased from 27 to 40%, with higher BCS precalving ($\leq$ 3 vs. BCS between 3.25 and 3.75). When BCS precalving was $\geq$ 4, the incidence of subclinical ketosis was 78%.

In experiment IV fatter cows had higher plasma NEFA concentrations during the immediate periparturient period, although differences in plasma insulin concentrations were not detected at that time. Blood BHB was increased only in fatter cows without glucogenic supplementation, which implies that glucogenic supplementation can effectively decrease ketogenesis during extensive lipid mobilisation. Experiment I also showed the efficacy of glucogenic supplement. Similarly, Miettinen (1995) observed that feeding 200 ml glucogenic substance (50% propylene glycol, 23% sugar alcohols, 1% nicotinic acid) twice a day decreased milk acetone levels. In experiments I and IV the positive effect was not achieved by

reducing lipid mobilisation, and therefore glucogenic supplementation possibly affected regulatory enzymes of ketogenesis in the liver. A possible mechanism is via increased propionate supply, since the glucogenic supplement used in I and IV included propylene glycol, which is, at least partly, absorbed as propionate in the rumen (Nielsen and Ingvartsen 2004). The glucogenic supplement used in experiment IV also contained xylitol, which is antiketogenic (Sakai et al. 1996), and other sugar alcohols. Niacin has shown a tendency to reduce plasma concentrations of BHB, but statistically significant differences were found only in few studies (review by Nielsen and Ingvartsen 2000).

3.6.4. Effects on milk yield

Several studies have shown that dry period overfeeding and/or increased body fatness at calving do not affect the subsequent milk yield during early lactation (Fronk et al. 1980, Garnsworthy and Topps 1982, Kunz et al. 1985, Garnsworthy and Jones 1987, Boisclair et al. 1986, Holter et al. 1990, Agenäs et al. 2003, IV). Contrasting these studies Treacher et al. (1986) observed lower milk yield in fatter cows, and Tesfa et al. (1999) reported that feeding 125% of energy requirements during the last 6 wk of gestation resulted in lower milk yields during the first 8 wk of lactation.

Fatter and thinner cows maintain their milk yield differently. Fatter cows increase lipid mobilisation, whereas thinner cows rely more on increased feed intake (Garnsworthy and Topps 1982, Garnsworthy and Jones 1987). Thus, when feed consumption during the dry period is taken into account, overfeeding during the dry period is an inefficient way to produce milk. Garnsworthy (1988) illustrated this using energetic efficiencies presented by MAFF (1975). In this system the efficiency of conversion of metabolizable dietary energy directly to milk energy is 0.62. During the dry period efficiency of ME utilisation for LW gain is lower (depends on the diet, but 0.435 can be used as an example), and when the body reserves are utilized for milk production with an efficiency of 0.82, the overall efficiency is 0.82 x 0.435 = 0.36.

3.7. Lead feeding with concentrate

Lead feeding is a practice of increasing concentrates during the last 2 to 4 wk prior to calving. This can be done by changing the composition of TMR, by supplementing ad libitum fed forage with concentrates or by replacing part of the forage with concentrates in restricted feeding. Lead feeding is widely used in Finnish dairy farms and also in other countries.

3.7.1. Adaptation of the rumen and effects on feed intake

In theory, prepartum concentrate feeding may have positive effects on postpartum feed intake by increasing VFA absorption capacity, as a study by Dirksen et al. (1985) seemed to indicate that energy-rich feeding during the dry period stimulated the growth of rumen papillae. However, only a few cows were used in that study. Dirksen et al. (1985) speculated that the development of rumen papillae was essential to reduce VFA accumulation in the rumen and to prevent the decrease of rumen pH during early lactation, when starch-rich rations are fed. They suggested that, to ensure full development of rumen papillae, energy-rich feeding should commence 4 wk before parturition. They also noted that this may induce excessive fat deposition.

Some subsequent studies seem to support the above-mentioned theory. Doepel et al. (2002) observed that cows fed a hay- and silage-based diet including approximately 30% oat hulls and 6 to 12% barley for 3 wk prepartum had lower DMI postpartum than the cows, which were fed a hay- and silage-based diet with 19 to 28% barley prepartum. McNamara et al. (2003b) reported that supplementation of a grass silage-based diet with concentrate during the last 4 wk of pregnancy had a positive effect on DMI postpartum in comparison to a diet consisting solely of a mixture of grass silage and straw. However, it must be noted that average energy intake of the group fed with grass silage and straw was approximately 30% below recommendations, which may have negatively affected postpartum performance. Furthermore, initial total DMI at calving in that group was 1.5 kg/d lower than in the group with prepartum concentrate feeding. The rate of increase in DMI postpartum was similar in both groups. Thus, the difference in average total DMI after calving was due to different initial levels at calving.

The primary factor stimulating rumen epithelium growth is the concentration of short chain fatty acids, in particular propionate and butyrate, in the rumen liquid (Kauffold et al. 1977), which can be altered by increasing concentrate feeding. Andersen et al. (1999) hypothesized that larger fluctuations of rumen VFA concentration would enhance stimulation of rumen epithelial growth. To test this theory, they used a special feeding strategy called 'acid load'. Approximately 4 kg DM/d starch-rich concentrate was fed at the morning feeding and silage at the afternoon feeding for the last 4 wk of pregnancy. However, they found no differences in ruminal epithelium (increased rumen papillae area or other signs of increased growth of the rumen epithelium) between concentrate-rich feeding ('acid load') and control groups. Sehested et al. (2000) observed that rumen epithelia of 'acid load' cows had increased capacity for butyrate absorption, which was not attributed to changes in epithelial surface area and structure but rather to changes in transport processes of epithelial cells. Rabelo et al. (2001) found no effect of increased concentrate proportion (8%, 38% or 52% of DM) on rumen papillae width during the dry period, but rumen papillae tended to be shorter with low concentrate proportion at one of the two measurement sites. Reynolds et al. (2004) observed that 0.8 kg DM/d supplemental barley during the dry period did not increase rumen papillae mass and length, whereas the number of papillae tended to increase, and the width and surface area of papillae decreased. Finally, Holtenius et al. (1994) detected that long-term (6 wk) intraruminal loads of propionic acid, butyric acid and acetic acid had no effect on rumen epithelial morphology in sheep fed with hay. It seems that a major effect on rumen epithelial growth can not be achieved in practical feeding, but it remains possible that the VFA absorption capacity of the rumen wall can be increased with concentrate feeding.

The negative effect of body fatness induced by increased prepartum energy levels may mask the potential positive effect of increased VFA absorption capacity on feed intake, and fatness may induce health problems. To test the effect of prepartum concentrate feeding without the confounding effect of energy level, concentrate proportions of 20%, 40% or 60% of the recommended dietary energy (Rehutaulukot ja ruokintasuositukset 2004) were fed in experiment III. No consistent effect of prepartum concentrate feeding on postpartum silage intake was observed. Comparably, Holcomb et al. (2001) and Keady et al. (2005) studied two different ratios of forage:concentrate at restricted energy levels during the last 4 wk of pregnancy and found no effect on feed intake at subsequent lactation. In the 'acid load' studies, Aaes et al. (1994) observed that increased prepartum concentrate feeding caused the average silage DMI to be 13% higher during the first 5 wk of lactation, whereas no differences were found by Ingvartsen et al. (2001).

Also in the studies with ad libitum feeding during dry period, increased proportion of concentrate in the prepartum diet during the last 3 to 4 wk of pregnancy has not affected postpartum feed intake (VandeHaar et al. 1999, Holcomb et al. 2001, Keady et al. 2001). Concentrate-rich feeding did not affect BCS in these studies (Holcomb et al. 2001) or the increase of BCS was modest (VandeHaar et al. 1999, Keady et al. 2001). Increased prepartum energy intake did not induce greater lipid mobilisation postpartum in the studies by VandeHaar et al. (1999) and Holcomb et al. (2001), which would have masked the potential positive effect of prepartum concentrate feeding on the development of feed intake capacity. In the study by Keady et al. (2001), BCS loss was greater for animals supplemented with concentrate prior to calving.

Taken together, if cows have received grass silage during the dry period, lead feeding with concentrate has usually not increased postpartum silage intake. It is possible that small amounts of concentrate or even good quality grass silage as sole feed induces sufficient stimulus for rumen papillae growth. It is noteworthy that in the majority of the above mentioned studies (excluding Keady et al. 2001, McNamara et al. 2003b and Keady et al. 2005), there was no control group with sole forage diet. As noted by Sehested et al. (2000), the positive effects on epithelial surface area suggested by Dirksen et al. (1985) were primarily obtained by shifting animals from a pure straw diet to a high concentrate diet. Inclusion of straw in the precalving diet may attenuate adaptation of the rumen epithelium for a high intake of concentrate postpartum.

The studies by Hernandez-Urdaneta et al. (1976), McNamara et al. (2003b) and Keady et al. (2005) have shown that abrupt changes from sole grass silage or low concentrate diet to a lactation diet with a moderate concentrate level can be done at calving or within a few days of calving without long term disturbances in feed intake. Furthermore, in experiment III, the daily concentrate allowance in one treatment group was increased from an average of 1.8 kg to 15 kg within 18 days without any off-feed incidences during that period. In fast increase groups, concentrate allowance increased 6 kg from the initial level within 4 d of calving without off-feed incidences.

Abrupt changes from sole forage or very low concentrate diet to a high concentrate diet increases lactate accumulation in the rumen, since lactate producers (*Streptococcus bovis* and lactobacilli) are more tolerant to low pH than lactate utilizers (e.g. *Megasphaera elsdenii*) (Russell and Hespell 1981). The nutritional pathogenesis of laminitis involves rumen acidosis (Nocek 1997). Therefore, to prevent laminitis, abrupt changes in concentrate feeding are not advisable.

3.7.2. Effects on lipid mobilisation and blood ketone concentrations

Grummer (1995) emphasized the importance of maximizing feed intake during the close-up dry period and suggested that increasing the energy density of a close-up diet with concentrate would reduce lipid mobilisation before calving and would attenuate the risk of fat infiltration of liver and susceptibility to ketosis.

Increasing the energy density of ad libitum-fed TMR diets with concentrate has decreased plasma NEFA in several studies during 1 to 2 wk prepartum (Minor et al. 1998, VandeHaar et al. 1999, Holcomb et al. 2001) or at calving (Doepel et al. 2002), but plasma NEFA was not affected in the study of Mashek and Beede (2000). Supplementation of ad libitum-fed grass silage with concentrate decreased plasma NEFA before calving or at calving in one study

(Keady et al. 2005), but not in another (Keady et al. 2001). In most of these studies concentrate supplementation increased voluntary total DMI during 3 to 4 weeks prepartum, but did not prevent a DMI dip at calving (Minor et al. 1998, VandeHaar et al. 1999, Keady et al. 2001, Keady et al. 2005). Doepel et al. (2002) also observed the dip in voluntary DMI, but found no differences in average DMI between prepartum energy levels.

Olsson et al. (1998b) observed that increasing energy intake by a high level of concentrate supplementation (7.9 kg DM/d at calving) combined with restricted grass silage feeding decreased plasma NEFA at calving, but not before calving. Increased concentrate proportions at restricted feeding levels decreased plasma NEFA prepartum in experiment III, but not in the study by Olsson et al. (1997).

Consistent with decreased plasma NEFA, an increase in plasma insulin before calving was observed in III, as well as in other studies (Olsson et al. 1998b, Holcomb et al. 2001). Plasma insulin also tended to increase in the study of Mashek and Beede (2000). In the study of Doepel et al. (2002) plasma insulin increased when high energy was combined with high protein, but not when combined with low protein.

Holtenius et al. (1996), Olsson et al. (1997) and Holcomb et al. (2001) observed large variations in serum insulin concentrations with high concentrate feeding prepartum. This was also seen in III, where the standard deviation of plasma insulin was much larger in groups with a concentrate proportion of 60% at one week before calving. It is also noteworthy that the cows fed 60% concentrate had substantially higher average plasma insulin concentrations than the cows fed 40% concentrate. Yet the cows fed 60% concentrate showed no further decrease in plasma NEFA prepartum in comparison with the cows fed 40% concentrate. Holtenius et al. (1996) suggested that heterogenous changes in insulin concentrations can be a sign of a disturbance in the periparturient adaptation of metabolism. They noted that suppression of lipolysis between 1 wk prepartum and 1 - 3 d postpartum was followed by a prolonged period of lipolysis. Olsson et al. (1997) found no evidence that the high insulin level prepartum would have affected subsequent lactation performance. Also in experiment III lactation performance was not impaired, but the HF group (60% concentrate prepartum and fast increase of concentrate postpartum) failed to show any increase of plasma insulin at lactation wk 4 and 8, unlike other groups. Simultaneously, this group also had elevated blood ketone concentrations.

Most studies with ad libitum feeding have shown no effect of increased concentrate prepartum on plasma NEFA postpartum (Mashek and Beede 2000, Keady et al. 2001, Doepel et al. 2002, Keady et al. 2005). The studies with different concentrate proportions and restricted feed intake prepartum (Olsson et al. 1997, III) are in line with this, although in experiment III a slight tendency towards higher plasma NEFA was observed at lactation wk 8 with increased concentrate proportion in the prepartum diet. No effects on plasma insulin postpartum have been observed (Olsson et al. 1997, Mashek and Beede 2000, III).

In line with the absence of differences in NEFA concentrations, enhancing the carbohydrate status of cows during the close-up dry period has not affected BHB concentrations in blood after calving in most studies (Olsson et al. 1997, Keady et al. 2001, Doepel et al. 2002), although in experiment III blood BHB was lower 4 wk after calving in the low concentrate prepartum group. Increased concentrate supplementation has decreased blood BHB prepartum in some studies (Mashek and Beede 2000, III).

3.7.3. Milk yield and composition

There are not many studies comparable to III, where a proportion of concentrate has been studied with a restricted energy allowance during the last weeks of pregnancy. Aaes at al. (1994) and Ingvartsen et al. (2001) found no effect on subsequent milk yield or milk composition, when cows were fed with a restricted energy allowance and with or without an 'acid load' diet (same energy level). Olsson et al. (1997) and Keady et al. (2005) also found no effect of different prepartum concentrate/forage ratios on subsequent milk production. Holcomb et al. (2001) observed no differences in milk yield or composition between restricted feeding of high or low forage diets during the prepartum period.

Furthermore, there have been several studies in which the energy density of ad libitum-fed TMR diets has been increased with concentrates during the last 3 to 4 wk of pregnancy (Johnson and Otterby 1981, VandeHaar et al. 1999, Mashek and Beede 2000, Holcomb et al. 2001, Doepel et al. 2002, Keady et al. 2005). In other studies, restrictively or ad libitum-fed forage has been supplemented with different levels of concentrates and hence with different energy levels (Nocek et al. 1986, Olsson et al. 1998b, Keady et al. 2001). All these studies have shown very little or no effect of enhancing prepartum energy status with concentrates on subsequent milk yield and/or milk composition.

The most marked exception is the increased milk yield during the first weeks of lactation observed with high concentrate allowance at calving (Olsson et al. 1998b, III). Due to the very different initial levels at calving between high and low prepartum concentrate groups in these studies, the difference in concentrate allowances extended until lactation wk 3. Therefore, the increased milk yield in these studies (Olsson et al. 1998b, III) during the first weeks of lactation was not a true residual effect of prepartum concentrate feeding. Keady et al. (2001, 2005) observed increased milk fat content with increased energy density in a prepartum diet, which was associated with higher BCS at calving and concomitant increased lipid mobilisation after calving. Finally, the study by McNamara et al. (2003b) showed that concentrate supplementation of a sole forage diet (grass silage or mixture of grass silage and straw) during the last 4 wk of pregnancy may increase the subsequent milk yield, when BCS is below 3 before calving.

3.8. Postpartum concentrate supplementation

3.8.1. Substitution of concentrate for silage

Substitution rate can be influenced by several factors (e.g. digestibility of forage, conservation method, chemical composition of forage, type and level of concentrate) as reviewed by Broster and Thomas (1981). Illustrating this, the random slope in the meta-analysis model was statistically significant indicating that individual regressions within the comparison were not parallel. The average linear substitution rate was 0.41 (Table 2), which is the same as that calculated from eight experiments by Rook et al. (1991). It is close to the mean substitution rate of 0.45 in a series of experiments in early- and mid-lactation by Kristensen and Skovborg (1990), and 0.39 in a review by Ryhänen et al. (1996) and slightly lower than those reported in the reviews by Thomas (1987) (0.52) and Huhtanen (1998) (0.53). Faverdin et al. (1991) reported a higher mean substitution rate (0.61) for grass silage-based diets in a series of experiments in mid-lactation.

Table 2. Mixed model regression relationships between concentrate DMI (independent variable, kg/d), silage DMI (dependent variable, kg/d) and live weight change (dependent variable, kg/d)

Stage	n	Intercept	SE	Linear	SE	P	Quadratic	SE	P	R^2	RMSE
Silage DMI:											
All data	163	13.33	0.33	-0.41	0.02	<0.001				0.93	0.30
Early	45	12.20	0.48	-0.37	0.04	<0.001				0.98	0.14
Mid	79	14.52	0.52	-0.43	0.03	<0.001				0.90	0.37
Both stages	39	12.45	0.53	-0.42	0.04	<0.001				0.97	0.17
Live weight change:											
All data	75	-0.56	0.12	0.08	0.01	<0.001				0.90	0.07
All data	75	-0.77	0.17	0.14	0.04	0.001	-0.004	0.002	0.08	0.89	0.07
Early	31	-0.78	0.14	0.09	0.01	<0.001				0.82	0.12
Early	31	-1.00	0.16	0.17	0.04	0.001	-0.005	0.002	0.06	0.85	0.12
Mid	23	-0.62	0.30	0.09	0.03	0.012				0.99	0.03
Both stages	21	-0.25	0.10	0.06	0.01	0.002				0.87	0.05
Both stages	21	-0.81	0.21	0.23	0.04	<0.001	-0.014	0.002	<0.001	0.91	0.05

The quadratic fixed effect of concentrate intake on silage DMI was not statistically significant. In line with this, Thomas (1987) observed no evidence for increased substitution rate with increased concentrate between 2.5 and 8 kg/d in a review of 27 experiments. Additionally, Rook et al. (1991) found no curvilinearity in substitution rate in the data from eight experiments over lactation wk 4 to 13 with a concentrate DMI range 2.9 to 11 kg/d. Similarly, Kristensen and Skovborg (1990) found no evidence of curvilinearity in substitution rate between three concentrate levels (approximately 3.5, 6.5 and 9 to 9.5 kg DM / d). In contrast, Østergaard (1979), Faverdin et al. (1991) and Huhtanen (1998) suggested that substitution rate increases with a high amount of concentrate. However, as there are few observations with daily concentrate intake above 10 kg DM/d, particularly in the mid-lactation category, it is unlikely to prove a curvilinear substitution rate in this study.

These data suggest that there are no differences (P = 0.28) in substitution rate between early- and mid-lactation. The predicted responses were more variable in mid-lactation studies, as indicated by the larger root mean square error (RMSE). In addition, Bertilsson (1987) and Kristensen and Skovborg (1990) observed no significant effect of lactation stage on substitution rate with concentrate levels below 10 kg DM/d. In contrast, the studies by Ekern (1972) and Taylor and Leaver (1986) suggest that substitution rate in mid-lactation is only approximately 50 to 60% of that in early lactation. In experiment I the substitution rate decreased in multiparous cows (0.88 vs. 0.45) during the periods of lactation weeks 1 to 6 and 7 to 12, when concentrate was increased without glucogenic feed. Thus, one factor which probably contributed to the high early lactation substitution rates reported by Ekern (1972) and Taylor and Leaver (1986) was that substitution rates were calculated for shorter periods (i.e. 4 or 5 wk) than the values used in the meta-analysis. In addition, higher concentrate levels in the studies by Ekern (1972) and Taylor and Leaver (1986) were rather generous relative to milk yield potential of the animals. Furthermore, in the study by Taylor and Leaver (1986), the high substitution rate observed during early lactation was largely due to one high concentrate feeding treatment.

3.8.2. Tissue mobilisation

In general, increased concentrate allocation during early lactation increases the energy balance (McNamara et al. 2003a, III) and decreases LW loss or increases LW gain (Thomas et al. 1986, Phipps et al. 1988, Kristensen and Skovborg 1990, Aston et al. 1995, McNamara et al. 2003b, III). In contrast, LW loss tended to be greater with higher concentrate level in multiparous cows in experiment I. This was possibly due to higher initial mobilisable fat depots at calving in the cows allotted to high concentrate groups, as indicated by greater BCS 3 wk before calving. Plasma NEFA concentrations gave no indication of increased fat mobilisation after calving, but this does not exclude possible differences in mobilisation before the first blood sampling at 2 wk after calving. Alternatively, differences in gut-fill may have contributed to observed differences in LWC. Ingvartsen et al. (2001) observed a larger LW loss during the first 3 wk after calving with faster increase of daily concentrate allowance. They attributed this, at least partially, to differences in gut-fill. In experiment III, the effect of increased concentrate allowance on LWC was also variable but during lactation weeks 1 to 5, LW loss was smaller with high than with medium concentrate allowance.

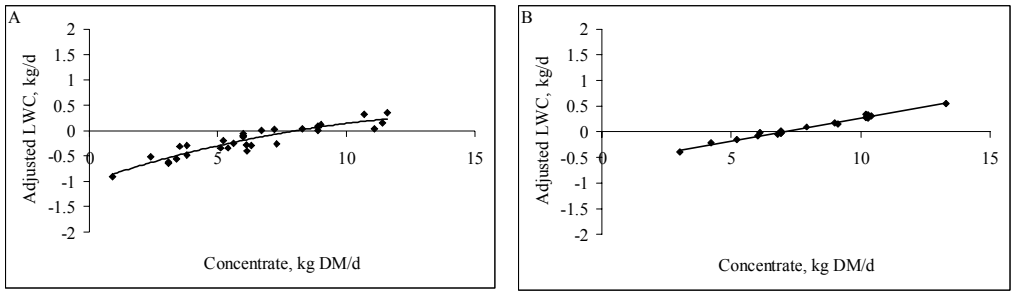

Figure 3. Relationships between concentrate DMI and adjusted live weight change in early- and mid-lactation studies. (A) Early lactation. LWC = -0.005ConcDMI^2 + 0.17ConcDMI - 1.00 (n = 31, quadratic P = 0.06, R^2 = 0.85). (B) Mid-lactation. LWC = 0.09ConcDMI - 0.62 (n = 23, linear P = 0.012, R^2 = 0.99).

The meta-analysis of concentrate supplementation data shows an average linear increase of 0.08 kg/d in LW per increased concentrate kg DM without significant differences between early- and mid-lactation studies. However, the quadratic effect of concentrate intake on LW gain was statistically significant in the studies covering both stages and approached significance in early lactation studies. This suggests that early lactation cows tend to partition a greater proportion of additional energy to milk production at high concentrate levels than mid-lactation cows (Table 2, Figure 3). The difference between lactation stages is probably related to differences in circulating insulin concentrations and tissue responsiveness to insulin. In line with this, McNamara and Hillers (1986) observed that rates of de novo lipogenesis and esterification of palmitate increased dramatically in adipose tissue between 1 and 2 months postpartum and the increase continued through mid-lactation. Using indirect calorimetry, Kirkland and Gordon (2001) observed that early lactation cows partitioned more of the change in ME intake to milk energy output than cows in later stages of lactation. Conversely, mid-lactation cows tended to partition a greater proportion of the change in ME intake to tissue gain. However, it must be noted that in their study mean initial days in milk of early- and mid-lactation cows were 77 and 225, and therefore these categories of lactation stages are not directly comparable to the categories of the present meta-analysis.

3.8.3. Effect on ketogenesis

The elevated blood glucose and decreased BHB 1 wk after calving in the high concentrate prepartum group in experiment III are a consequence of high concentrate allocation of these groups during the first lactation week. Comparably, Vik-Mo and Refsdal (1984) stated that improved intakes of liberal amounts of concentrated feeds and silage ad libitum depressed ketonemia during early lactation. Similarly, Minor et al. (1998) observed that increased proportions of nonfibre carbohydrates in the diet decreased plasma NEFA and BHB concentrations at lactation days 28 and 60. However, the increased concentrate level had no effect on concentrations of blood ketone bodies in experiment I and in the study by Olsson et al. (1997).

3.8.4. Milk yield response

In early lactation energy requirements are almost never met (Coulon and Rémond 1991). Therefore, early lactation cows are in negative energy balance for several weeks after calving. As discussed above, concentrate supplementation increases energy balance and decreases LW loss. However, at high concentrate levels the effect of concentrate supplementation on LW gain seemed to be lower in early lactation than in mid-lactation. This can be attributed to decreased responsiveness of adipose tissue to insulin and lowered insulin secretion response to increased glucose or propionate supply in early lactation cows. Thus, it could be expected that lower partitioning of energy to tissue gain would favour higher milk yield responses in early lactation than in mid-lactation cows. In line with this, the linear response of milk yield was 0.98 kg/kg concentrate DM in early lactation studies, and 0.68 kg/kg concentrate DM in mid-lactation studies (Table 3). The interaction between the fixed effect of concentrate DMI and lactation stage was statistically significant (P = 0.05). In studies covering both stages, the average linear response was 0.78 kg/kg concentrate DM. The average linear response (0.76 kg/kg concentrate DM) in the whole data is similar to the estimates reported in the reviews by Thomas (1980) and Ryhänen et al. (1996). ECM responses to concentrate supplementation followed milk yield responses (Table 3). However, the interaction between the fixed effect of concentrate DMI and lactation stage was not statistically significant (P = 0.12). Milk yield and ECM responses were similar in mid-lactation, whereas in early lactation milk yield response was larger than ECM response. This indicates that the higher milk yield response in early lactation is derived largely from increased lactose production.

Quadratic relationships between concentrate DMI and milk yield can be seen in Figures 4a and 4b. These relationships reveal that the predicted response in mid-lactation studies was close to zero when the concentrate level was above 10 kg DM/d. In early lactation studies and in studies covering both stages, the decrease of responses were much smaller. Therefore, using only mid-lactation studies in a curvilinear model for predicting milk production responses to concentrate supplementation underestimates early lactation responses.

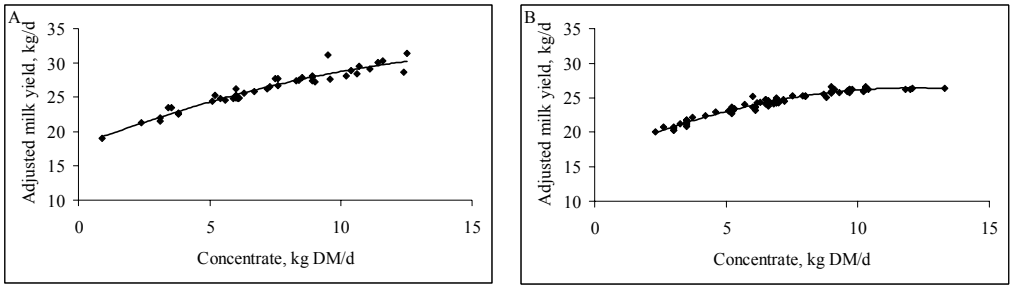

Figure 4. Quadratic relationships between concentrate DMI and adjusted milk yield in early- and mid-lactation studies. (A) Early lactation. Milk yield = $-0.039\text{ConcDMI}^2 + 1.47\text{ConcDMI} - 17.91$ (n = 45, quadratic $P < 0.001$, $R^2 = 0.95$). (B) Mid-lactation. Milk yield = $-0.068\text{ConcDMI}^2 + 1.64\text{ConcDMI} - 16.52$ (n = 79, quadratic $P < 0.001$, $R^2 = 0.96$).

Table 3. Mixed model regression relationships between concentrate DMI (independent variable, kg/d), milk yield (dependent variable, kg/d) and ECM yield (dependent variable, kg/d)

Stage	n	Intercept	SE	Linear	SE	P	Quadratic	SE	P	R^2	RMSE
Milk yield:											
All data	163	19.39	0.49	0.76	0.04	<0.001				0.92	0.58
All data	163	17.25	0.60	1.43	0.15	<0.001	-0.048	0.010	<0.001	0.94	0.52
Early	45	19.17	0.50	0.98	0.07	<0.001				0.92	0.84
Early	45	17.91	0.82	1.47	0.26	<0.001	-0.039	0.019	0.05	0.93	0.72
Mid	79	19.72	0.79	0.68	0.06	<0.001				0.91	0.55
Mid	79	16.52	0.81	1.64	0.18	<0.001	-0.068	0.012	<0.001	0.97	0.34
Both stages	39	17.71	0.89	0.78	0.10	<0.001				0.86	0.72
ECM yield:											
All data	161	19.58	0.56	0.75	0.05	<0.001				0.92	0.57
All data	161	16.48	0.61	1.72	0.14	<0.001	-0.07	0.01	<0.001	0.96	0.42
Early	45	19.57	0.76	0.88	0.08	<0.001				0.94	0.61
Early	45	16.76	0.65	1.79	0.18	<0.001	-0.07	0.01	<0.001	0.95	0.58
Mid	79	20.00	0.89	0.68	0.06	<0.001				0.88	0.65
Mid	79	16.22	0.94	1.85	0.19	<0.001	-0.08	0.01	<0.001	0.96	0.36
Both stages	37	17.62	0.96	0.82	0.09	<0.001				0.92	0.56
Both stages	37	15.91	1.32	1.45	0.36	0.002	-0.05	0.03	0.10	0.92	0.54

In general, the response of milk yield to concentrate input follows the law of diminishing returns (Gordon 1981, Ferris et al. 2001). This is partly due to increased partitioning of additional energy and nutrients to LW gain and partly due to negative associative effects on digestion (Ferris et al. 1998, Huhtanen 1998). High concentrate ration induces low rumen pH, which decreases cellulolysis. In addition, silage NDF is more digestible than concentrate NDF and thus NDF digestibility tends to decrease when concentrate allowance is added (Huhtanen 1998). Although the effect of increased concentrate on silage DMI was linear in the present meta-analysis, some earlier reviews have suggested that substitution rates increase with a high amount of concentrate (Faverdin et al. 1991, Huhtanen 1998).

Table 4. Mixed model regression relationships between ME intake (independent variable, MJ/d) and milk yield (dependent variable, kg/d).

Stage	n	Intercept	SE	Linear	SE	P	R^2	RMSE
All data	157	7.29	0.84	0.09	0.005	<0.001	0.97	0.56
Early	39	6.10	1.49	0.11	0.01	<0.001	0.98	0.52
Mid	79	6.96	1.26	0.08	0.01	<0.001	0.97	0.54
Both stages	39	7.85	1.44	0.08	0.01	<0.001	0.91	0.78

Due to the differences in substitution rates and silage quality between experiments, the responses were also calculated relative to ME intakes. The relative differences between lactation stages remained similar to those calculated per increased concentrate intake. Linear response (kg milk/MJ ME increase in intake) was higher in early lactation studies than in mid-lactation studies (0.11 vs. 0.08; interaction P = 0.05) (Table 4). The tendency for higher responses in early than in mid-lactation studies is in agreement with reviews by Broster and Broster (1984) and Coulon and Rémond (1991). In line with this, Kirkland and Gordon (2001) observed that milk yield response to changes in ME intake was negatively correlated with days in milk. Average milk yield responses in that study for early-, mid- and late lactation cows were 0.149, 0.078 and 0.025 kg/MJ change in ME intake, respectively.

In contrast to evidence shown here for higher milk yield response in early lactation, studies comparing flat-rate versus feeding to yield systems or high/low feeding (Østergaard 1979, Taylor and Leaver 1984a, Taylor and Leaver 1984b, Taylor and Leaver 1986, Aston et al. 1995, Nousiainen 2002) with fixed total amounts of concentrate have failed to show any benefit of allocating more concentrate at early than at mid-lactation. In these studies, the total milk yield with different concentrate allocation systems was not different, and they indicate that an enhanced persistency can compensate for a reduction in peak yield, when silage is fed ad libitum (Broster and Thomas 1981). Greater LW losses during early lactation in the low concentrate groups (Taylor and Leaver 1984a, Taylor and Leaver 1986, Aston et al. 1995 exp. 1) show that with sufficient amino acid supply from concentrate (approximately 190 to 200 g CP/kg DM), cows with a low concentrate allowance could compensate for the energy deficit by tissue mobilisation. Furthermore, in the study by Taylor and Leaver (1986) and in one of the two experiments by Aston et al. (1995), substitution rate declined as lactation progressed, whereas in the current data set, average substitution rates were similar in early- and mid-lactation studies.

The above mentioned concentrate allocation studies were conducted with cows having peak milk yields of approximately 30 kg/d. Modern dairy cows have considerably higher peak

yields than that and they tend to mobilise more fat than cows with moderate genetic merit (Ferris et al. 1998). Moreover, Ferris et al. (1998) suggested that medium merit cows tend to partition more nutrients to tissue gain and less to milk energy than high merit cows. The applicability of flat-rate feeding in high yielding cows remains to be studied. The overall performance of cows in experiments I to IV shows that relatively high (15 kg/d) fixed concentrate levels can be used in feeding high yielding cows during early lactation.

Finally, it must be noted that concentrate supplementation increases both energy and protein supply. In a review of the effects of energy supplementation, Coulon and Rémond (1991) stated that the CP content of diets used in some early lactation trials may have been insufficient. The response to energy supplementation was higher if protein requirements were not met at lower levels of energy supply and protein supply increased with energy supply. In the majority of studies included in the meta-analysis, concentrate was increased without changing its composition. Furthermore, metabolizable protein intake is closely correlated to energy intake. Thus, the response attained is derived from an increase in both energy and protein supply (Kuoppala et al. 2004b). This is illustrated by the high response to increased concentrate between groups of multiparous cows with glucogenic supplementation in experiment I. Although the low milk yield of the group with lower concentrate level (C11G1) was probably partly due to random animal factors, low protein contents in glucogenic supplement and silage may have contributed to the low milk yield by decreasing AAT supply. Similarly, a very high ECM yield response to fast increase of concentrate was observed in experiment III, when CP content of silage was low.

3.8.5. Milk composition responses

Increased concentrate allowance decreased milk fat content during the early weeks of lactation in experiment III, but not in experiment I. Other early lactation studies have shown variable responses. Ingvartsen et al. (2001) observed a temporary decrease of milk fat content with a faster rather than slower increase rate of daily concentrate allowance. Increased concentrate proportion in the diet decreased milk fat content in studies with fixed energy levels (Olsson et al. 1997, Olsson et al. 1998a). When grass silage was fed ad libitum, moderate levels of concentrate supplementation did not affect milk fat content (Steen and Gordon 1980b, Mayne and Gordon 1984, Bertilsson 1987, McNamara et al. 2003b). Aston et al. (1995) observed that high and low levels of concentrate (12 kg DM/d and 3 kg DM/d) decreased milk fat content relative to intermediate amounts, when silage was fed ad libitum.

In the meta-analysis, the average linear decrease of milk fat content in the whole data was 0.12 g/kg per increased kg concentrate DM (Table 5). The interaction between the fixed effect of concentrate intake and lactation stage was not statistically significant ($P = 0.95$). Responses were very variable in different studies, which is partly due to large variation in concentrate compositions (e.g. starch content) (Sutton 1989). In line with the studies by Ferris et al. (1999) and Ferris et al. (2001), a significant quadratic effect predicts larger decreases of milk fat content with high concentrate allowance in early- and mid-lactation studies. Furthermore, the current model based on the whole data predicts that milk fat content is fairly stable across concentrate ranges 2 to 10 kg DM/d. Within these ranges milk fat content does not vary more than approximately 1 g/kg with increased concentrate supplementation (Figure 5a).

Table 5. Mixed model regression relationships between concentrate DMI (independent variable, kg/d), milk fat and protein contents (dependent variables, g/kg), and fat and protein yields (dependent variables, g/d)

Stage	n	Intercept	SE	Linear	SE	P	Quadratic	SE	P	R^2	RMSE
Fat content:											
All data	163	41.81	0.62	-0.12	0.06	0.04				0.12	0.83
All data	163	39.24	0.83	0.72	0.20	<0.001	-0.060	0.014	<0.001	0.34	0.79
Fat yield:											
All data	163	826.4	26.3	26.6	2.11	<0.001				0.85	28.6
All data	163	662.4	27.5	78.2	6.26	<0.001	-3.71	0.44	<0.001	0.93	20.1
Protein content:											
All data	161	30.22	0.30	0.22	0.03	<0.001				0.75	0.33
All data	161	29.61	0.42	0.42	0.10	<0.001	-0.014	0.007	0.04	0.76	0.33
Protein yield:											
All data	161	581.6	16.9	29.2	1.54	<0.001				0.96	14.7
All data	161	507.4	20.3	52.1	4.59	<0.001	-1.66	0.31	<0.001	0.98	11.4
Early	45	592.7	29.9	33.3	2.79	<0.001				1.0	1.36
Mid	79	593.9	25.7	27.0	2.08	<0.001				0.95	16.3
Mid	79	497.3	27.6	56.8	5.58	<0.001	-2.12	0.36	<0.001	0.98	9.74
Both stages	37	542.9	34.6	28.0	3.50	<0.001				0.92	18.9

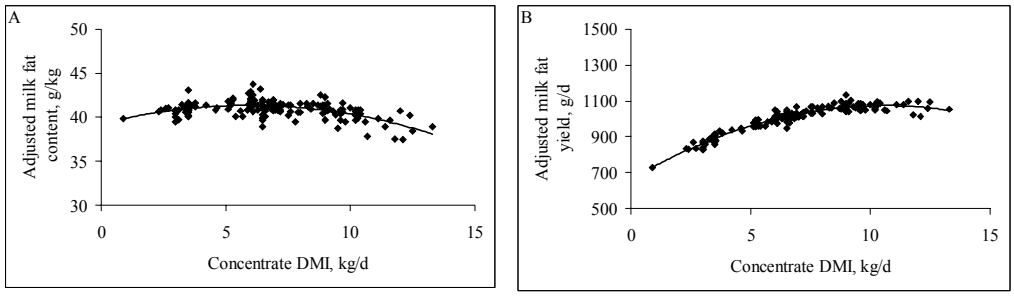

Figure 5. Quadratic relationships between concentrate DMI and adjusted milk fat content and yield. (A) Fat content = -0.060ConcDMI^2 + 0.72ConcDMI - 39.24 (n = 163, quadratic P < 0.001, R^2 = 0.34). (B) Fat yield = -3.71ConcDMI^2 + 78.2ConcDMI - 662.4 (n = 163, quadratic P < 0.001, R^2 = 0.93).

Decrease of milk fat content in III was probably mainly a dilution effect since milk and fat yield tended to increase with increased concentrate allowance during the first weeks of lactation. Yet another factor, which may have contributed to lower milk fat content in III, is decreased lipid mobilisation, since mobilised fat reserves represent a major source of fatty acids for milk fat during negative energy balance (Bell 1995). In experiment III, ME balance was higher with increased concentrate supply, and average ME balance in H groups was slightly positive, and LW loss was smaller in these than in other groups during days 1 to 35.

Linear increase of milk fat yield in the whole data was on average 26.6 g/kg per increased kg concentrate DM (Table 5). No interaction (P = 0.26) between the the fixed effect of concentrate intake and lactation stage was detected. The quadratic model of the whole data predict diminished responses in fat yield with high concentrate allowance, and suggest an inclination point at 11 kg DM/d (Figure 5b).

Higher concentrate levels have increased milk protein content in some early lactation studies (Mayne and Gordon 1984, Aston et al. 1995, McNamara et al. 2003b), but not in others (Steen and Gordon 1980b, Bertilsson 1987). In experiment III, milk protein content tended to increase with fast increase of concentrate and higher initial concentrate level at calving (treatments MF and HF), but concentrate level did not significantly affect milk protein content in experiment I.

In the meta-analysis the average linear increase of milk protein content in the whole data was 0.22 g/kg per increased kg concentrate DM (Table 5). The interaction between the fixed effect of concentrate intake and lactation stage was not statistically significant (P = 0.33). The quadratic model predicts diminished responses in milk protein content with higher concentrate allowances in early- and mid-lactation studies.

In general, increases in milk protein contents reflect increased ME intakes. The positive response of milk protein content on increased energy supply has been shown in several studies as reviewed by Spörndly (1989), Sutton (1989) and Coulon and Rémond (1991). In the whole data set the average linear increase (0.030 g/kg per increased MJ ME intake) was similar to that reported by Spörndly (1989). Coulon and Rémond (1991) observed that the protein content response to increased energy supply was twice as low in early lactation as in mid-lactation. They suggested that when energy supply is lower than requirements, milk production increases more than protein content. In the present data set, no statistically

significant interaction (P = 0.33) between the linear effect of lactation stage and ME intake was detected. In agreement with the studies by Spörndly (1989) and Coulon and Rémond (1991) no curvilinearity between energy intake and milk protein content was observed.

The linear increase of milk protein yield in early lactation studies was on average 33.3 g/kg per increased kg concentrate DM, which was significantly higher than in the mid-lactation category (interaction P = 0.03) (Table 5). Furthermore, the effect of concentrate supplementation on milk protein yield was linear in early lactation studies, whereas a curvilinear relationship was observed in mid-lactation studies (Figures 6a and 6b). It is also noteworthy that the predicted responses were surprisingly uniform in early lactation studies, as indicated by the very small RMSE.

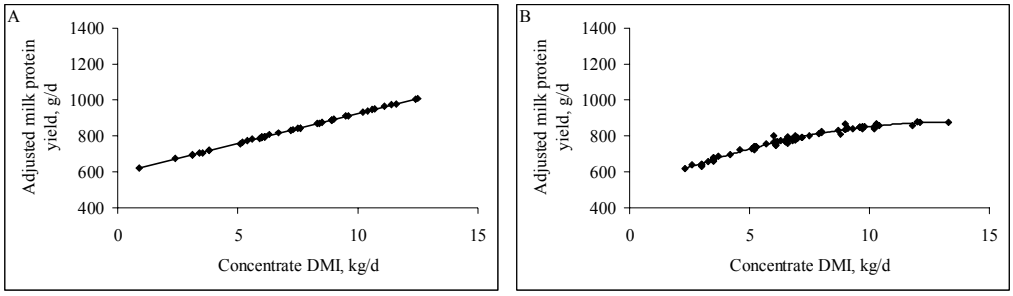

Figure 6. Relationships between concentrate DMI and adjusted milk protein yield in early- and mid-lactation studies. (A) Early lactation. Protein yield = 33.3ConcDMI - 592.7 (n = 45, linear P < 0.001, R^2 = 1.0). (B) Mid-lactation. Protein yield = -2.12ConcDMI^2 + 56.8ConcDMI - 497.3 (n = 79, quadratic P < 0.001, R^2 = 0.98).

3.9. Postpartum protein supplementation

3.9.1. Feed intake and digestibility of diet

Most early or early to mid-lactation studies (Gordon 1979, Gordon 1980, Thomas et al. 1981, Mayne and Gordon 1984, Sutton et al. 1996, Schei et al. 2005, II) reported only statistically non-significant increases of silage DMI or no effect. In some studies, at low level of concentrate allowance (3 to 6 kg DM/d), protein supplementation increased silage DMI significantly (Murphy et al. 1985, Sutton et al. 1994, Aston et al. 1998).

Preliminary analysis showed that the interaction between the fixed effect of diet CP content and lactation stage was statistically significant (P = 0.01) on silage DMI and milk yield. However, further analysis revealed that this was due to the higher proportion of studies using soya bean meal (SBM) alone or with fish meal (FM) in early than in mid-lactation (see discussion in Chapter 3.9.4). To eliminate the confounding factor of protein source, the final analysis was conducted with studies where SBM alone or with FM was used. There were only three rapeseed feed studies in the early lactation data set, and therefore rapeseed feed studies could not be used to test the effect of lactation stage.

The average linear increase of silage DMI was 0.013 kg DM per increased 1 g/kg CP in diet DM, using only studies with SBM alone or with FM (Table 6). The interaction between the fixed effect of diet CP content and lactation stage was not statistically significant (P = 0.23). The quadratic effect of the increase of diet CP content on silage DMI was close to statistical

significance in all categories except early lactation, indicating diminishing silage DMI response as the CP content of the diet increased.

The average silage DMI response of the whole data with SBM alone or with FM (0.013 kg DM per increased 1 g/kg CP in diet) was lower than that reported in earlier reviews. Oldham (1984) reported an average response of 0.019 kg DM per increased g/kg CP in diet DM. Tuori (1992) and Huhtanen (1998) have reported greater increases (0.027 and 0.029 kg DM per increased 1 g/kg CP in diet DM) in studies which were mainly conducted in mid-lactation and using RSM. Shingfield et al. (2003) reported similar silage DMI responses for rapeseed expeller (RSE) and SBM in a grass silage-based diet (0.0225 and 0.0238 kg DM per increased 1 g/kg CP in diet DM). In the present data set high (183 g/kg DM) average diet CP content in SBM plus FM studies may have reduced silage DMI response, as suggested also by the significance of the quadratic effect. Besides this and the numerically low response in early lactation studies, no explanation is readily found for the low average response in the present data with SBM alone or with FM. In comparison, Aston et al. (1998) observed a tendency for greater potential for silage intake responses between lactation weeks 13 to 21 than between lactation weeks 4 to 12.

Protein supplementation may result in more efficient cell wall digestion in the rumen (Oldham 1984). In the review of Gordon et al. (1981), supplementation with SBM increased DM digestibility of the diet by 0.25 g/kg per 1 g increase in concentrate CP content. However, Huhtanen (1998) reviewed 13 comparisons with RSM and found no effect of protein supplementation on OM and a marginal effect on NDF digestibility. In the present data set, using mid-lactation data, the average linear increases of OM and NDF digestibility of the diet with SBM alone or with FM were 0.16 g/kg and 0.44 g/kg per 1 g increase in diet CP content. However, the linear effects were not statistically significant (P = 0.21). In the studies with rapeseed feeds the corresponding responses were 0.15 g/kg and 0.59 g/kg per 1 g increase in concentrate CP content. Linear NDF digestibility responses of rapeseed feeds vs. SBM alone or with FM were not significantly different (P = 1.0). However, the quadratic effect was statistically significant in rapeseed feed studies (Figure 7). Thus, in line with the review by Huhtanen (1998), average linear increases of OM and NDF digestibility in response to protein supplementation are modest. Further, the quadratic model predicts that rapeseed feed supplementation increases NDF digestibility of the diet only up to 170 g CP/kg DM. This reflects increasing concentrate indigestible NDF content at higher levels of rapeseed feed supplementation (Shingfield et al. 2003).

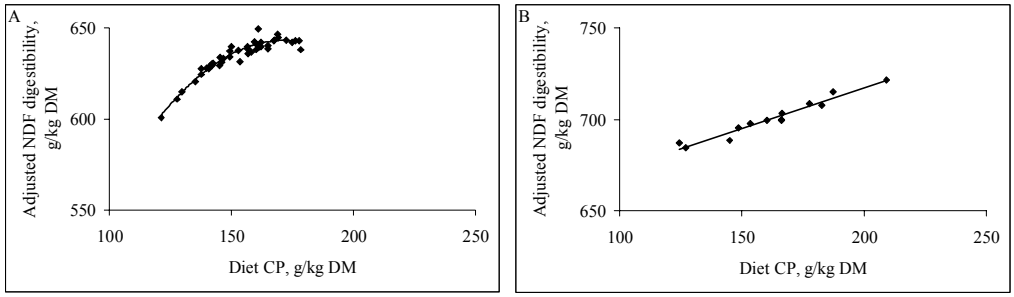

Figure 7. Relationship diet CP content and adjusted NDF digestibility of the diet in the studies with (A) rapeseed feed as protein supplement. NDF dig. = -0.0016DietCP^2 + 5.59DietCP + 163.7 (n = 44, quadratic P = 0.03, R^2 = 0.92). (B) soya bean meal and fish meal as protein supplement. NDF dig. = 0.44DietCP + 628.3 (n = 13, linear P = 0.21, R^2 = 0.95)

Table 6. Mixed model regression relationships between diet CP content (independent variable, g/kg DM), silage DMI (dependent variable, kg/d) and live weight change (dependent variable, kg/d) in the studies with soya bean meal alone or with fish meal.

Stage	n	Intercept	SE	Linear	SE	P	Quadratic	SE	P	R^2	RMSE
Silage DMI:											
All data	63	7.64	0.40	0.013	0.002	<0.001				0.80	0.20
All data	63	4.75	1.45	0.044	0.015	0.008	-0.00008	0.00004	0.05	0.81	0.19
Early	21	7.86	0.65	0.009	0.004	0.04				0.46	0.25
Mid	27	7.56	0.63	0.016	0.003	<0.001				0.91	0.18
Mid	27	3.91	1.77	0.056	0.019	0.014	-0.0001	0.000049	0.06	0.91	0.18
Both stages	15	8.10	0.74	0.010	0.004	0.06				0.73	0.17
Both stages	15	2.10	2.59	0.074	0.027	0.04	-0.00017	0.00007	0.05	0.79	0.16
Live weight change:											
All data	36	-0.45	0.30	0.0022	0.0011	0.07				0.10	0.17
Early	13	-1.21	0.51	0.0042	0.0019	0.08				0.35	0.13
Mid	14	-0.17	0.29	0.0017	0.0016	0.33				0.05	0.17
Both stages	9	0.64	0.30	-0.0022	0.0015	0.23				0.37	0.07

Due to a lack of data in early lactation studies, it was not possible to test the interaction between lactation stage and effect of protein supplementation on digestibility. The modest overall increases of OM and NDF digestibility in response to protein supplementation suggest that if silage DMI is limited by fill in early lactation, protein supplementation does not alleviate this problem. Furthermore, it is most likely that fill is not the prime factor to limit feed intake in dairy cows which are fed with high-energy rations (Ketelaars and Tolkamp 1992, Huhtanen 2002, Huhtanen et al. 2002). In grass silage-based diets with moderate concentrate proportions, both ruminal distension signals and negative feedback from metabolic signals may regulate intake simultaneously (Huhtanen 2002).

3.9.2. Tissue mobilisation

Studies by Ørskov et al. (1981, 1987) and Phipps et al. (1988) showed that increased amino acid supply with limited energy intake during early lactation can induce increased mobilisation of fatty acids from adipose tissue. In these studies forages were of poor quality and results showed a severe negative energy balance. In the study by Schei et al. (2005) BCS loss and plasma NEFA increased with protein supplementation and restricted energy allowance, indicating increased lipid mobilisation during early weeks of lactation. In experiment II, an increased lipid mobilisation was also observed between the unsupplemented group (RSM0) and groups with RSM supplementation as indicated by LWC and plasma NEFA concentrations. In experiment II, silage digestibility was low (silage D-value in vitro was 62), which may have contributed to the increase of lipid mobilisation. On the other hand, rapid increase of daily concentrate allowance and a high level of concentrate feeding probably alleviated severe negative energy balance. It must be noted that the RSM15 cows in experiment II were heavier than the cows in the other groups. Thus, these cows probably had larger mobilisable fat depots.

Other early lactation studies with higher CP levels and adequate energy supplies give no support to theory about increased lipid mobilisation induced by protein supplementation (Roffler and Thacker 1983, Wohlt et al. 1991, Komaragiri and Erdman 1997). Komaragiri and Erdman (1997) stated that the basal diet in the studies by Ørskov et al. (1981, 1987) was very low in ruminally undegraded protein, and therefore any further postruminal addition of protein resulted in higher milk production and greater energy deficit. Under adequate feeding conditions, tissue mobilisation in early lactation is not limited by dietary protein and is not affected by changes in dietary rumen undegradable protein (Komaragiri and Erdman 1997).

In the meta-analysis data the average effect of protein supplementation on LWC was 0.0022 kg/d per increased 1 g/kg CP in diet DM (Table 6). LWC responses were variable in mid-lactation studies and studies covering both stages, and no significant relationship between CP content of the diet and LWC was found. Thus, supporting the above-mentioned studies, the meta-analysis results do not support the theory of enhanced lipid mobilisation with increased protein supplementation. However, it must be noted that LWC in response to protein supplementation are possibly masked by the increase of silage intake and hence the greater gut-fill.

3.9.3. Ketogenesis

Ørskov et al. (1987) showed that protein supplementation combined with severe energy restriction predisposes cows to ketosis during early lactation. Despite tendencies towards

increased plasma NEFA concentrations in RSM-supplemented groups in experiment II, blood BHB concentrations were not increased and plasma glucose did not decrease significantly, which was due to the high concentrate level. In a study by Schei et al. (2005) the combined effect of low energy and high protein supplementation increased blood ketones and decreased glucose. However, the changes were not dramatic and no cases of clinical ketosis were detected. In these two studies (II, Schei et al. 2005) grass silage was fed ad libitum.

Ørskov et al. (1987) observed that the risk of ketosis increased when cows produced 15 to 20 kg fat-corrected milk more than their calculated energy intake. This corresponds to approximately a 75 to 100 MJ ME/d negative energy balance. In the RSM15 group (II) the minimum energy balances were -46 and -42 MJ/d at lactation wk 1 and 2. Based on experiment II and the study by Schei et al. (2005), the risk that protein supplementation increases incidences of ketosis is relatively low, if grass silage is fed ad libitum and a moderate or high amount of concentrate is given.

3.9.4. Milk yield and composition

As discussed earlier, mobilisable protein reserves of dairy cows are small compared to mobilisable fat reserves. Based on these estimates and taking into account that a considerable proportion of absorbed amino acids end up in gluconeogenesis, it could be expected that protein supplementation would be highly beneficial to dairy cows during the early weeks of lactation.

The database included studies with rapeseed feeds (RSM, rapeseed cake and RSE), SBM or FM as protein supplements. Shingfield et al. (2003) showed that inclusion of RSE elicited considerably higher milk and milk protein yield responses than SBM in a grass silage-based diet. Therefore, the possible effect of protein source was tested using mid-lactation data (i.e. without the potential confounding factor of lactation stage). In the present dataset, average milk yield response was twice as high in studies with rapeseed feeds than in studies with SBM alone or with FM (0.091 vs. 0.046 kg per 1 g increase in diet CP content, interaction $P = 0.002$). The average response in the studies with rapeseed feeds is similar to that reported by Huhtanen (1998) for RSM. The large difference in responses between protein supplements may be partially due to higher average diet CP content in SBM plus FM data than in rapeseed feed data (183 vs. 155 g/kg DM). Nevertheless, Shingfield et al. (2003) also reported that milk yield response to increased CP intake was approximately 1.7 times higher with RSE than with SBM.

The effects of lactation stage on milk, ECM and protein yields were analysed with a subset of data with SBM alone or with FM. Interactions between lactation stage and the effect of CP content of the diet were not statistically significant ($P = 0.49$, $P = 0.83$ and $P = 0.96$ for milk, ECM and protein yields). Average linear milk and ECM responses were 0.045 and 0.041 kg per 1 g increase in diet CP content in the whole data with SBM alone or with FM (Table 7). Average linear milk protein yield response was 1.99 g per g/kg DM increase in diet CP content. Quadratic effects were not statistically significant in any of the categories or in the whole subset of studies with SBM alone or with FM.

A difference in the relative proportions of SBM and FM between studies is a potential source of bias in the present comparison of lactation stages. Milk and protein yield responses to FM supplementation have been 20 to 25% higher than to SBM supplementation (Chamberlain et

al. 1989). Amino acid profile and rumen undegradable protein content of the supplements may account for the difference in responses (Chamberlain et al. 1989).

Since the lowest CP contents in many of the studies in the early lactation dataset were above 150 g/kg DM and several studies began 4 wk after calving, the response between RSM0 and RSM15 in experiment II was also compared with results from studies based on maize silage feeding. Roffler et al. (1986) fitted an exponential model to data from 17 early lactation studies with SBM, corn silage and lucerne feeding. The model predicted large milk yields and DMI responses to protein supplementation at low levels of CP in the diet (1.9 kg milk/d between 13% and 15% CP in the diet) and diminishing returns with higher CP contents. Journet and Remond (1981) reported 2.9 to 4.2 kg/d higher milk yields in three protein supplementation trials with SBM and RSM during lactation wk 1 to 8. Crude protein contents of control diets varied between 105 to 138 g/kg DM and CP contents in the supplemented diets were 153 to 170 g/kg DM. Taken together, these studies give some support to the observation in experiment II that large responses can be expected when a low protein diet (RSM0 in experiment II) is supplemented with a moderate amount of protein concentrate during early lactation.

Table 7. Mixed model regression relationships between postpartum diet CP content (independent variable, g/kg DM), milk and ECM yield (dependent variables, kg/d) and protein yield (dependent variable, g/d) in the studies with soya bean meal alone or with fish meal.

Stage	n	Intercept	SE	Linear	SE	P	R^2	RMSE
Milk yield:								
All data	63	15.48	1.04	0.045	0.005	<0.001	0.93	0.36
Early	21	18.01	1.51	0.036	0.008	0.001	0.80	0.47
Mid	27	15.23	1.56	0.046	0.008	<0.001	0.97	0.30
Both stages	15	12.03	2.09	0.057	0.010	0.001	0.89	0.54
ECM:								
All data	63	15.90	1.30	0.041	0.006	<0.001	0.94	0.30
Early	21	18.07	1.61	0.031	0.008	0.005	0.72	0.50
Mid	27	16.72	2.22	0.039	0.011	0.006	0.97	0.22
Both stages	15	12.36	2.06	0.055	0.010	0.001	0.88	0.55
Protein yield:								
All data	63	378.9	51.9	1.99	0.24	<0.001	0.98	8.50
Early	21	418.1	84.8	1.75	0.37	0.001	0.96	9.65
Mid	27	436.9	73.2	1.73	0.34	<0.001	0.97	10.13
Both stages	15	244.3	119.2	2.65	0.55	0.003	1.0	1.42

Nevertheless, it is evident that in experiment II, the very high milk and protein yield responses between RSM0 (grain mixture without protein supplement) and RSM15 (15% RSM in the concentrate) were partly due to larger lipid mobilisation in RSM15 than in the other groups. Average LW of the cows in the RSM15 group was higher after calving and they may have had larger mobilisable fat reserves than cows in the other groups. Therefore, differences in lipid mobilisation and milk production performance may have been partly due to direct and indirect effects of increased amino acid supply and partly due to random animal factors. Similarly to experiment II, Tuori (1992) observed a lack of milk yield response between two

RSM supplementation levels (20% and 33% RSM in concentrate) and numerically lower milk yields at a higher RSM level during lactation weeks 4 to 14.

Increased ME intake in protein supplementation studies is derived from increased silage DMI. The average responses to increased ME intake in early- and mid-lactation protein supplementation studies were similar (0.11 vs. 0.13 kg milk per increased MJ, interaction P = 0.67). In studies covering both stages the average response was 0.13 kg milk per increased MJ. The milk yield response to increased ME intake in protein supplementation studies was higher than the corresponding response in concentrate supplementation studies (0.09 kg milk per increased MJ). However, the difference in responses between concentrate and protein supplementation studies was much smaller than that reported by Huhtanen (1998). Huhtanen (1998) noted that the marginal response to additional ME derived from silage intake exceeded the theoretical value of 0.194, which is predicted on the basis of ME requirements for milk production (Rehutaulukot ja ruokintasuositukset 2004). Shingfield et al. (2003) reported slightly higher responses using RSE than SBM (0.242 vs. 0.206). In the current data set, using mid-lactation studies, the response to increased ME intake also tended to be higher with rapeseed feeds than with SBM alone or with FM (0.17 vs. 0.13, interaction P = 0.09), but both responses remained below the 0.194 theoretical value.

The similar responses in early- and mid-lactation protein supplementation studies suggests that there are no differences in energy partitioning between milk and body tissues, unlike concentrate supplementation studies. It can be speculated that easily digestible carbohydrates in the concentrate increase propionate concentration in the rumen, which elicits an increase in insulin secretion. As discussed above, increased plasma insulin favours tissue gain in mid-lactation. Contrasting this, a moderate increase in silage intake probably has no effect on rumen propionate and plasma insulin.

The milk production responses to protein supplementation are mediated only partly through direct effects of increased amino acid supply, and partly through indirect effects of increased silage intake and ration digestibility (Gordon et al. 1981, Oldham 1984). Gordon et al. (1981) estimated that approximately 50% of the response obtained can be accounted for through indirect effects. From the meta-analysis data with SBM alone or with FM, it can be calculated that average silage DMI response (0.013 kg DM per increased 1 g/kg CP in diet DM) corresponds to 1.43 MJ per 10 g/kg CP in diet DM, assuming silage ME content of 11 MJ/kg DM. Using the average response of 0.13 kg milk per increased MJ, 1.43 MJ would yield 0.19 kg ECM. Thus, increased silage DMI accounts for approximately 45% (0.19/0.41) of the milk production response in the present data set.

3.9.5. Protein utilisation

Nitrogen balance studies during early lactation have shown a consistent and substantial increase in urinary excretion of N in response to protein supplementation (Gordon 1980, Mayne and Gordon 1984, Gordon and Small 1990), which is also reflected by increased plasma urea-N concentrations (Gordon 1980, Mayne and Gordon 1984, Gordon and Small 1990, II). As milk protein yield is slightly increased in response to protein supplementation and N retention remains unchanged (Gordon 1980, Gordon and Small 1990), protein utilisation is linearly decreased when CP content of the diet is increased (Mayne and Gordon 1984, II)

4. CONCLUSIONS AND PERSPECTIVES

1. Restricted feeding according to requirements or below requirements prevented the decrease of feed intake during the last two weeks of pregnancy. The simultaneous moderate increase of plasma NEFA was probably hormonally induced. Amino acid mobilisation most likely commenced during the last few days of pregnancy, thus coinciding with the onset of copious milk secretion.

2. Increased body fatness at calving resulted in greater lipid and amino acid mobilisation, which was enhanced during the last week of pregnancy, peaked at the time of calving and continued extensively for several weeks after calving. Increased lipid mobilisation in fatter cows seemed to be facilitated by increased responsiveness of adipose tissue to adrenergic stimulation.

3. Despite the more negative energy balance and greater lipid mobilisation, fatter cows had continuously higher plasma leptin concentrations than cows in normal condition after calving. This might have been due to preservation of larger body fat reserves, as indicated by larger subcutaneous fat depth.

4. Glucogenic supplementation effectively prevented the increase of blood ketone concentration. Glucogenic supplement did not decrease mobilisation of tissue reserves, but can be used to reduce the negative effects of excessive lipid mobilisation in fat cows.

5. The proportion of concentrate or concentrate level in lead feeding during the close-up dry period had only minor effects on postpartum feed intake or milk production. Low concentrate level (2 kg/d or lower) at calving predisposed high yielding cows to subclinical ketosis during very early lactation, when daily concentrate allowance was increased slowly after calving. Fast increase of concentrate retarded the increase of silage intake.

6. The decrease of plasma insulin around calving is part of the adaptation mechanism which allows cows to mobilise body fat and protein. Attempts to block the decrease of insulin by feeding large amounts of concentrate during the close-up dry period are unnecessary and may disturb adaptation from pregnancy to lactation.

7. In practice, lead feeding by increasing concentrate to approximately 4 kg/d at calving can be recommended in separate feeding of grass silage and concentrate with restricted energy allowance. Further, this study emphasizes the importance of avoiding obesity at calving in preventing excessive lipid mobilisation and the concomitant increased risk of subclinical ketosis. The optimal increase rate of concentrate after calving may be related to concentrate composition and requires further studies directed towards rumen acidosis.

8. Direct responses of milk and milk protein yields to concentrate supplementation were greater during early lactation than during mid-lactation. This was due to greater partitioning of increased energy intake towards tissue gain in mid-lactation than in early lactation. In practice, cows can compensate for moderate underfeeding of concentrate during early lactation by increasing tissue mobilisation and through

enhanced persistency after peak lactation. Substitution rates of concentrate for silage were not different in early- and mid-lactation studies.

9. Milk and protein yield responses to protein supplementation on grass silage-based diets were not different in early- and mid-lactation studies. Protein supplementation seemed to have only minor effects on lipid mobilisation and blood ketone concentrations if energy feeding was adequate and protein supply in the control level was not very low (below 130 g CP/kg DM in the diet).

10. Protein supplementation slightly increased silage dry matter intake. Increased ME intake derived from grass silage resulted in greater milk yield response than the increase of ME intake from concentrate.

11. Direct responses of milk and milk protein yields to concentrate supplementation in early lactation are underestimated, if only mid-lactation data is used for prediction. There are abundant data on mid-lactation responses to concentrate and protein supplementation with grass silage-based diets. Further studies should be directed at examining direct and residual effects of early lactation supplementation. In particular, there is a lack of early lactation studies evaluating responses to supplementation with rapeseed feeds.

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Appendix 1. Summary of studies included in the postpartum protein and concentrate supplementation databases.

Protein supplementation studies			**Concentrate supplementation studies**		
Study [1]	Reference	CP content of diet, g/kg DM	Study	Reference	Concentrate, kg DM/d
	Early lactation:			**Early lactation:**	
1S	Aston et al. 1998	160 – 204	1	Aston et al. 1995	3.1 – 11.4
2S	Gordon & Small 1990	166 – 198	2	Aston et al. 1995	6.0 – 11.6
3R	Experiment II	134 – 175	3	Bertilsson 1987	5.2 – 8.2
4S	Mayne & Gordon 1984	144 – 185	4	Coulon et al. 1987	2.4 – 6.3
5R	Murphy et al. 1985	127 – 161	5	Gordon & Small 1990	0.9 – 6.3
6S	Schei et al. 2005	151 – 202	6	Experiment I	9.5 – 12.5
7S	Thomas et al. 1981	175 – 241	7	Mayne & Gordon 1984	5.9 – 8.5
8R	Tuori 1992	141 – 170	8	McNamara et al. 2003b	3.4 – 6.7
	Mid–lactation:		9	Steen & Gordon 1980a	3.8 – 7.3
9S	Aston et al. 1994a	125 – 187	10	Steen & Gordon 1980b	7.5 – 10.6
10S	Aston et al. 1995	194 – 262	11	Thomas et al. 1986	6.0 – 11.1
11S	Aston et al. 1998	160 – 209		**Mid–lactation:**	
12S	Cody et al. 1990	142 – 183	12	Aston et al. 1994b	3.0 – 12.1
13R	Heikkilä 1997	142 – 161	13	Aston et al. 1995	3.0 – 9.1
14R	Huhtanen et al. 1991	136 – 150	14	Bertilsson & Bursted 1983	2.6 – 8.0
15R	Huhtanen et al. 1995	145 – 175	15	Bertilsson 1987	4.2 – 6.8
16R	Huhtanen & Heikkilä 1996	128 – 142	16	Faverdin et al. 1991	3.5 – 7.0
17R	Jaakkola et al. 1996	141 – 160	17	Faverdin et al. 1991	5.2 – 9.3
18R	Khalili et al. 1998	138 – 157	18	Faverdin et al. 1991	5.7 – 10.2
19R	Khalili et al. 2002	138 – 161	19	Faverdin et al. 1991	2.3 – 6.8
20R	Kokkonen et al. 2000	142 – 168	20	Ferris et al. 2003	3.0 – 12.0
21R	Rinne et al. 1999a	135 – 149	21	Heikkilä 1997	5.2 – 10.2
22R	Shingfield et al. 2001	121 – 157	22	Heikkilä et al. 1998	7.9 – 13.3
23R/S	Shingfield et al. 2003	140 – 178	23	Kassem et al. 1987	3.3 – 7.2
24S	Thomas et al. 1981	162 – 233	24	Kristensen & Skovborg 1990	3.5 – 9.6
25R	Tuori 1992	158 – 178	25	Kristensen & Skovborg 1990	3.5 – 9.7
26R	Tuori 1992	145 – 169	26	Kuoppala et al. 2004a	6.9 – 10.4
27R/S	Tuori 1992	160 – 184	27	Rinne et al. 1999a	6.2 – 8.7
28R	Tuori & Syrjälä–Qvist 1995	123 – 159	28	Shingfield et al. 2002	6.3 – 9.0
29R	Vanhatalo et al. 2004	147 – 167		**Both stages:**	
	Both stages:		29	Fitzgerald & Murphy 1999	3.5 – 7.1
30S	Gordon 1980	139 – 213	30	Gordon 1984	3.3 – 8.3
31R	Khalili et al. 2005	169 – 203	31	Kristensen & Skovborg 1990	3.5 – 9.1
32S	Phipps et al. 1988	178 – 192	32	Kristensen & Skovborg 1990	3.5 – 9.2
33R	Rinne et al. 1999b	139 – 169	33	Kuoppala et al. 2004b	6.6 – 10.2
34S	Sutton et al. 1994	163 – 229	34	Phipps et al. 1988	2.7 – 7.8
35S	Sutton et al. 1996	181 – 242	35	Poole 1987	7.2 – 9.4
			36	Sutton et al. 1994	3.0 – 6.0

1) Letter R or S refers to primary protein supplement(s) in the study. R = rapeseed feed. S = soya bean meal alone or with fish meal

Appendix 2. Mean and range of selected variables in the postpartum concentrate supplementation database.

	n	Mean	Std Dev	Minimum	Maximum
Early lactation:					
Concentrate DMI, kg/d	45	7.21	2.83	0.90	12.50
Silage DMI, kg/d	45	9.36	1.52	5.70	13.20
ME intake, MJ/d	39	187	36	115	268
CP intake, g/d	45	2751	511	1724	3857
Milk yield, kg/d	45	26.3	4.3	19.0	38.8
ECM, kg/d	45	25.7	4.3	17.2	36.3
Fat content, g/kg	45	39.5	2.8	34.5	47.3
Protein content, g/kg	45	31.1	1.7	27.3	35.1
Fat yield, g/d	45	1031	167	629	1384
Protein yield, g/d	45	818	162	472	1245
Concentrate CP, g/kg DM	45	187	17	158	240
Silage CP, g/kg DM	45	151	25	112	194
Diet CP, g/kg DM	45	166	17	138	196
Silage D-value, g/kg DM	24	683	31	643	750
LWC, kg/d	31	-0.24	0.43	-1.45	0.38
Mid–lactation:					
Concentrate DMI, kg/d	79	7.00	2.63	2.30	13.30
Silage DMI, kg/d	79	11.03	2.25	6.20	17.40
ME intake, MJ/d	79	200	38	129	302
CP intake, g/d	79	3088	550	1339	4226
Milk yield, kg/d	79	23.9	4.3	15.5	33.8
ECM, kg/d	79	24.1	5.1	15.6	36.6
Fat content, g/kg	79	41.0	3.8	34.6	50.5
Protein content, g/kg	79	31.8	1.9	29.0	36.1
Fat yield, g/d	79	984	221	620	1524
Protein yield, g/d	79	762	162	478	1179
Concentrate CP, g/kg DM	79	194	28	94	234
Silage CP, g/kg DM	79	159	22	108	194
Diet CP, g/kg DM	79	172	22	102	212
Silage D-value, g/kg DM	65	677	44	609	756
LWC, kg/d	23	0.09	0.37	-0.68	0.92
Both stages:					
Concentrate DMI, kg/d	39	6.23	2.27	2.70	10.20
Silage DMI, kg/d	39	9.71	1.57	6.90	14.10
ME intake, MJ/d	39	177	30	114	234
CP intake, g/d	39	2828	475	1829	3729
Milk yield, kg/d	39	22.5	2.6	16.7	27.7
ECM, kg/d	37	22.5	2.9	15.3	27.6
Fat content, g/kg	39	41.1	2.6	35.1	47.0
Protein content, g/kg	37	31.7	1.5	27.9	35.4
Fat yield, g/d	39	923	120	629	1152
Protein yield, g/d	37	711	96	464	887
Concentrate CP, g/kg DM	39	215	49	106	383
Silage CP, g/kg DM	39	157	19	131	194
Diet CP, g/kg DM	39	178	23	136	229
Silage D-value, g/kg DM	35	668	47	601	756
LWC, kg/d	21	0.08	0.19	-0.40	0.38

Appendix 3. Mean and range of selected variables in the postpartum protein supplementation database: studies with soya bean meal alone or with fish meal as protein supplements

	n	Mean	Std Dev	Minimum	Maximum
Early lactation:					
Concentrate DMI, kg/d	21	5.5	2.2	0.7	8.5
Silage DMI, kg/d	21	9.7	1.8	7.9	13.9
ME intake, MJ/d	21	178	26	115	217
CP intake, g/d	21	2766	580	1724	3954
Milk yield, kg/d	21	24.7	2.7	19.0	29.7
ECM, kg/d	21	23.8	2.7	17.5	27.6
Protein content, g/kg	21	30.6	1.5	27.9	32.6
Protein yield, g/d	21	736	107	472	866
Concentrate CP, g/kg DM	21	225	71	104	372
Silage CP, g/kg DM	21	162	32	132	225
Diet CP, g/kg DM	21	182	26	144	241
Silage D-value, g/kg DM	21	715	28	682	750
LWC, kg/d	13	-0.4	0.6	-1.5	0.3
OM digestibility, g/kg DM	6	770	8	757	779
NDF digestibility, g/kg DM	0				
Mid-lactation:					
Concentrate DMI, kg/d	27	6.9	1.9	3.1	9.1
Silage DMI, kg/d	27	10.3	2.1	7.4	14.2
ME intake, MJ/d	27	197	17	160	224
CP intake, g/d	27	3100	659	2030	4626
Milk yield, kg/d	27	24.0	2.5	18.3	28.5
ECM, kg/d	27	24.1	3.4	19.6	32.1
Protein content, g/kg	27	31.9	2.3	26.3	37.2
Protein yield, g/d	27	768	96	606	954
Concentrate CP, g/kg DM	27	212	101	104	575
Silage CP, g/kg DM	27	167	23	142	212
Diet CP, g/kg DM	27	180	35	125	262
Silage D-value, g/kg DM	17	664	41	609	720
LWC, kg/d	14	0.2	0.2	-0.1	0.6
OM digestibility, g/kg DM	13	761	38	687	801
NDF digestibility, g/kg DM	13	708	75	566	764
Both stages:					
Concentrate DMI, kg/d	15	6.6	2.1	3.0	8.8
Silage DMI, kg/d	15	10.2	1.8	7.6	13.3
ME intake, MJ/d	12	193	23	145	214
CP intake, g/d	15	3210	611	2310	4354
Milk yield, kg/d	15	23.2	3.4	18.2	30.9
ECM, kg/d	15	23.1	3.1	18.8	30.1
Protein content, g/kg	15	33.0	1.9	29.6	35.7
Protein yield, g/d	15	761	117	594	1010
Concentrate CP, g/kg DM	15	223	80	106	386
Silage CP, g/kg DM	15	173	24	119	194
Diet CP, g/kg DM	15	191	27	136	242
Silage D-value, g/kg DM	8	664	44	620	707
LWC, kg/d	9	0.2	0.2	-0.1	0.5
OM digestibility, g/kg DM	10	780	19	740	805
NDF digestibility, g/kg DM	4	789	33	743	820

Appendix 4. Mean and range of selected variables in the postpartum protein supplementation database: studies with rapeseed feeds as protein supplements

	n	Mean	Std Dev	Minimum	Maximum
Early lactation:					
Concentrate DMI, kg/d	8	8.5	3.6	5.6	12.9
Silage DMI, kg/d	8	9.1	1.0	7.5	10.2
ME intake, MJ/d	8	193	47	136	253
CP intake, g/d	8	2712	689	1739	3813
Milk yield, kg/d	8	27.8	8.6	17.3	40.2
ECM, kg/d	8	28.4	9.6	16.0	41.0
Protein content, g/kg	8	31.6	1.5	29.7	33.8
Protein yield, g/d	8	882	310	506	1310
Concentrate CP, g/kg DM	8	158	36	111	204
Silage CP, g/kg DM	8	155	17	127	164
Diet CP, g/kg DM	8	153	17	127	176
Silage D-value, g/kg DM	3	619		619	619
LWC, kg/d	8	-0.1	0.1	-0.3	0.1
OM digestibility, g/kg DM	5	708	20	684	724
NDF digestibility, g/kg DM	3	561	4	557	564
Mid-lactation:					
Concentrate DMI, kg/d	53	7.7	0.9	5.9	8.9
Silage DMI, kg/d	53	10.7	1.6	7.1	13.4
ME intake, MJ/d	53	204	18	163	237
CP intake, g/d	53	2803	370	1986	3664
Milk yield, kg/d	53	25.8	3.2	17.3	32.0
ECM, kg/d	53	27.1	3.5	18.4	32.8
Protein content, g/kg	53	32.3	1.1	28.8	33.9
Protein yield, g/d	53	828	106	563	993
Concentrate CP, g/kg DM	53	154	26	116	221
Silage CP, g/kg DM	53	151	17	120	185
Diet CP, g/kg DM	53	153	14	121	178
Silage D-value, g/kg DM	33	672	27	611	726
LWC, kg/d	28	0.2	0.2	-0.2	0.5
OM digestibility, g/kg DM	44	732	28	682	772
NDF digestibility, g/kg DM	44	634	46	531	710
Both stages:					
Concentrate DMI, kg/d	11	9.1	0.3	8.8	9.6
Silage DMI, kg/d	11	11.2	1.0	9.4	12.2
ME intake, MJ/d	11	222	9	205	233
CP intake, g/d	11	3367	376	2798	4130
Milk yield, kg/d	11	30.2	2.4	26.6	33.9
ECM, kg/d	11	31.4	1.2	29.4	33.1
Protein content, g/kg	11	32.0	1.2	30.3	33.3
Protein yield, g/d	11	961	50	870	1030
Concentrate CP, g/kg DM	11	176	23	138	211
Silage CP, g/kg DM	11	150	36	124	195
Diet CP, g/kg DM	11	166	19	139	203
Silage D-value, g/kg DM	11	660	6	656	668
LWC, kg/d	0				
OM digestibility, g/kg DM	11	723	7	713	736
NDF digestibility, g/kg DM	11	614	25	566	642