

Energy partitioning in dairy cows

**Effects of lipogenic and glucogenic diets on
energy balance, metabolites and reproduction
variables in early lactation**

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Energy partitioning in dairy cows

**Effects of lipogenic and glucogenic diets on
energy balance, metabolites and reproduction
variables in early lactation**

Ariëtte T.M. van Knegsel

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Abstract

Van Knegsel, A.T.M. (2007). Energy partitioning in dairy cows – Effects of lipogenic and glucogenic diets on energy balance, metabolites and reproduction variables in early lactation. PhD thesis Wageningen University, The Netherlands.

Dairy cows experience a negative energy balance (NEB) in early lactation which results from high energy requirements for milk production accompanied by a limited energy intake. Nutrition has been indicated as an important factor in the incidence and severity of NEB and NEB-related metabolic and reproductive disorders, like ketosis and delayed resumption of ovarian activity. A metabolic effect of a NEB in dairy cows is suggested to be an unbalanced availability from glucogenic and lipogenic nutrients. The objective of this thesis was to study the effect of lipogenic and glucogenic diets on the energy balance (EB) and risk of metabolic and reproductive disorders in dairy cows in early lactation. The first study, a literature survey, illustrated that feeding extra glucogenic nutrients relative to lipogenic nutrients, decreased milk fat and seemed to decrease plasma non-esterified fatty acid (NEFA) and β -hydroxybutyrate (BHBA) concentrations. Since studies were scarce and mostly included a confounding effect of dietary energy source with energy intake, it was difficult to draw conclusions on the energy source effects on EB and fertility. Therefore, in the second study, 16 dairy cows were either fed a glucogenic or a lipogenic diet. Diets were isocaloric and equal in intestinal digestible protein. Energy balance was determined in climate-respiration chambers from week 2 until week 9 of lactation. The glucogenic diet decreased milk fat yield and milk energy and tended to decrease body fat mobilisation compared with the lipogenic diet. The objective of the third study was to study the effect of dietary energy source on EB, metabolites and some reproduction variables. Dairy cows (n=111) were fed glucogenic, lipogenic or mixed diet from week -3 until week 9 relative to calving. Multiparous cows fed the glucogenic diet had lower milk fat yield, higher calculated EB, and lower plasma NEFA, BHBA and liver tri-acyl glyceride concentration and tended to have less days postpartum till first ovulation. Additionally, in the third study, the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows was determined. Relations were detected between NAb and EB and plasma metabolites, suggesting that alterations in immune competence peripartum are reflected in the humoral part of the innate immune system.

In conclusion, increasing the glucogenic nutrient availability improved the EB and had potential to reduce the risk of metabolic disorders and to improve reproductive performance in dairy cows.

Keywords: dairy cows; dietary energy source; glucogenic nutrients; lipogenic nutrients; negative energy balance; metabolic disorders; reproduction, immune system

Voorwoord

Na ruim vier en een half jaar ligt er nu eindelijk een proefschrift; daar was het tenslotte allemaal om te doen! Natuurlijk is het super om straks dit project te kunnen afsluiten, maar het betekent ook het afscheid van een erg leerzame periode waarin ik de gelegenheid heb gehad met veel verschillende mensen te kunnen samenwerken aan een project dat meer op mijn lijf geschreven bleek dan menigeen in eerste instantie had kunnen bedenken. Naast het onderwerp en het multidisciplinaire karakter van het onderzoek is ook het contact en de samenwerking met deze mensen een belangrijke motivatie geweest.

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Ariëtte

Contents

Chapter 1	General introduction	11
Chapter 2	Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle – review-	16
Chapter 3	Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition	39
Chapter 4	Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones	57
Chapter 5	Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation	73
Chapter 6	Natural antibodies related to energy balance in early lactation dairy cows	95
Chapter 7	General discussion	111
References		131
Summary/Samenvatting		149
Curriculum vitae		159
Colophon		165

CHAPTER 1

Introduction

Intense genetic selection, improved dairy nutrition and cow management have significantly increased milk yield of dairy cows in the past decades. However, selection on high genetic merit for milk yield is only partially compensated by an increase in feed intake resulting in an ongoing increase in negative energy balance (NEB) for dairy cows in early lactation (Veerkamp *et al.*, 2000). Negative energy balance has been associated with an increase in incidence and severity of metabolic disorders, like fatty liver, ketosis (Grummer, 1993) and ruminal acidosis (Bobe *et al.*, 2004), an increase in incidence in infectious diseases (Collard *et al.*, 2000) and a decrease in reproductive performance, like delayed resumption of ovarian activity (Staples *et al.*, 1990), diminished estrous expression (Lopez *et al.*, 2004), attenuated follicle quality (Lucy *et al.*, 1991a), lower conception rates, and more days open (Reist *et al.*, 2003b; Reksen *et al.*, 2002).

Several nutritional strategies to reduce the severity and incidence of metabolic and reproductive disorders in early lactation have been studied. Most studies aimed at improving the energy balance (EB) by increasing energy intake in the periparturient period, hereby reducing the risk of metabolic and reproductive disorders in early lactation (Drackley *et al.*, 2003; Reist *et al.*, 2003; Hayirli and Grummer, 2004). A common approach, aiming for an increase in energy intake in early lactation, is increasing the energy density of the diet by e.g. decreasing the forage to concentrate ratio (Andersen *et al.*, 2002) or by dietary supplementation of energy dense ingredients like fat (Beam and Butler, 1997) or non-fibre carbohydrates (Patton *et al.*, 2004; Simas *et al.*, 1995). However, increasing the dietary energy density entails a risk of compromising dry matter intake. Feeding rumen fermentable carbohydrates accompanied by low dietary NDF concentration is associated with ruminal acidosis and decreased dry matter intake (DMI) (Owens *et al.*, 1998). Also, dietary fat has been suggested to have the potential to depress DMI (Palmquist and Jenkins, 1980), which may be explained by limited palatability of dietary fat (Grummer *et al.*, 1990) or possible effects of dietary fat on ruminal fermentation and gut motility (Allen, 2000).

An alternative approach is to improve the EB in early lactation by decreasing the caloric demand of milk production. Recently, feeding calcium salts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) has been presented as a dietary regimen to decrease milk energy output and hereby improve the EB in transition cows (Castaneda-Gutierrez *et al.*, 2005; Odens *et al.*, 2007). Alternatively, decreasing the lipogenic to glucogenic nutrient ratio has been suggested to decrease the milk fat content and hereby improve the EB (reviewed in Chapter 2). Milk fat-depressing diets are suggested to lower the priority for milk relative to fat deposition in body reserves (Van Soest, 1963). It can be hypothesised that glucogenic diets, which decrease milk fat yield, decrease the partitioning of energy into milk and consequently increase the partitioning of energy into body reserves. Hereby improving the EB and diminishing body fat mobilisation.

It can be postulated that a dairy cow in NEB experiences a relative shortage of glucogenic nutrients relative to lipogenic nutrients resulting from high lactose production and limited energy intake. It has been suggested that the availability of lipogenic relative to glucogenic nutrients is positively related to the risk of metabolic disorders like fatty liver and ketosis (Adler, 1970; Kronfeld, 1976). Finally, it can be hypothesised that as a status of NEB is related to fertility problems (Butler, 2003) and infectious diseases (Collard *et al.*, 2000), an improvement of the EB by decreasing the lipogenic to glucogenic nutrient ratio could be related to a decreased risk for reproductive disorders and infectious diseases for high-producing dairy cows in early lactation.

Aim and outline of this thesis

The objective of this thesis is to study the effect of lipogenic nutrients and glucogenic nutrients on the EB, metabolic disorders, reproductive performance and risk of infectious diseases of dairy cows in early lactation. The first study (chapter 2), a literature survey, describes the possibilities to modify the availability of lipogenic and glucogenic nutrients and described the relation between dietary energy source and milk production, EB, metabolic and reproductive disorders. In chapter 3, the effect of two diets, differing in lipogenic and glucogenic nutrient availability in the diet, on energy partitioning between milk and body reserves in multiparous dairy cows in NEB, as determined in climate-respiration chambers, is discussed. Chapter 4 discusses the plasma profiles of metabolites and metabolic hormones in the climate-respiration chamber experiment. However we had to conclude that animal numbers were limited in this experiment which prevented to make conclusions on the relation between dietary energy source and the risk of metabolic disorders as indicated by plasma metabolites and metabolic hormones. Therefore, experiment 2 was set up to focus on the second part of the above mentioned objective (chapter 5). This experiment evaluated the effects of a mainly glucogenic or lipogenic diet on calculated EB, plasma metabolites and metabolic hormones and reproductive variables in high-producing dairy cows in early lactation. Further, to test a possible parity-effect both primiparous and multiparous cows were included in this experiment. Additionally, an intermediate diet was added in order to further outline the relation between different glucogenic and lipogenic nutrient availability and energy metabolism. While chapter 2 till 5 focus on the EB, metabolic disorders and some fertility measurements, chapter 6 focuses on the relation between EB, dietary energy source and potential risk of infectious diseases, as indicated by some immune variables. In this chapter, the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows is discussed and relations are described between NAb and EB and indicators of metabolic disorders. Finally chapter 7 discusses feeding of glucogenic nutrients as a tool to reduce milk energy output, improve the EB and health in early lactation dairy cows.

CHAPTER 2

Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle - review -

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Abstract

The pathway for oxidation of energy involves a balanced oxidation of C2 and C3 compounds. During early lactation in dairy cattle this C2/C3 ratio is out of balance, due to a high availability of lipogenic (C2) products and a low availability of glucogenic (C3) products relative to C2 and C3 products required for milk production. This review compares studies which manipulated dietary energy source and shows that dietary energy source can affect the balance in C2/C3 ratio, as indicated by plasma NEFA, β -hydroxybutyrate (BHBA) and glucose levels. It is shown that glucogenic nutrients increase glucose and insulin concentrations and decrease NEFA and BHBA plasma levels. Extra lipogenic nutrients elevate NEFA and BHBA and decrease plasma glucose concentrations. Lipogenic nutrients generally increase milk fat percentage and decrease milk protein percentage, suggesting a surplus of C2 compounds. The inverse is the case for feeding extra glucogenic nutrients, implying reduced deamination and oxidation of glucogenic amino acids. Feeding extra glucogenic nutrients improved the energy balance (EB), in contrast to ambiguous results of lipogenic nutrients on EB. Moreover, glucogenic feed may reduce the severity of ketosis and fatty liver, but increased incidence of (sub)clinical acidosis. As studies are scarce it seems difficult to draw conclusions on the effects of dietary energy source on reproduction. However, lipogenic nutrients decrease glucose and increase NEFA and BHBA plasma levels. High plasma NEFA and BHBA and low plasma glucose levels are associated with decreased reproductive performance, which might imply the C2/C3 compound balance to be important for reproductive function.

Introduction

Negative energy balance and related disorders

Over the last several decades, intense genetic selection, improved dairy nutrition and cow management have significantly increased milk yield of dairy cattle, in particular in the Holstein Friesian cattle breed. It is now well known that these economically favorable developments are accompanied by some negative consequences, such as an increase in incidence of metabolic diseases and a reduction in reproductive performance (Pryce *et al.*, 1999; Rajala-Schultz and Frazer, 2003; Van Arendonk *et al.*, 1989; Westwood *et al.*, 2002), as illustrated for pregnancy rate of dairy cattle in the Netherlands in Figure 1a (adapted from (Nederlands Rundvee Syndicaat, 2003)). However, in the Netherlands pregnancy rate seems to stabilize from 1995 onwards. Dutch dairy farmers were able to consolidate the pregnancy rate by increasing the number of days postpartum (pp.) till first artificial insemination (Figure 1b; adapted from (Nederlands Rundvee Syndicaat, 2003)), resulting in an increase in inter calving interval (from 393 days in 1992 to 417 days in 2002). Veerkamp *et al.* (2000) indicated that the increase in genetic merit for feed intake did not parallel the increase in genetic merit for milk yield. They suggested that selection on high genetic merit for milk yield is only partially compensated by an increase in feed intake resulting in an ongoing increase in negative energy balance (NEB) status during early lactation.

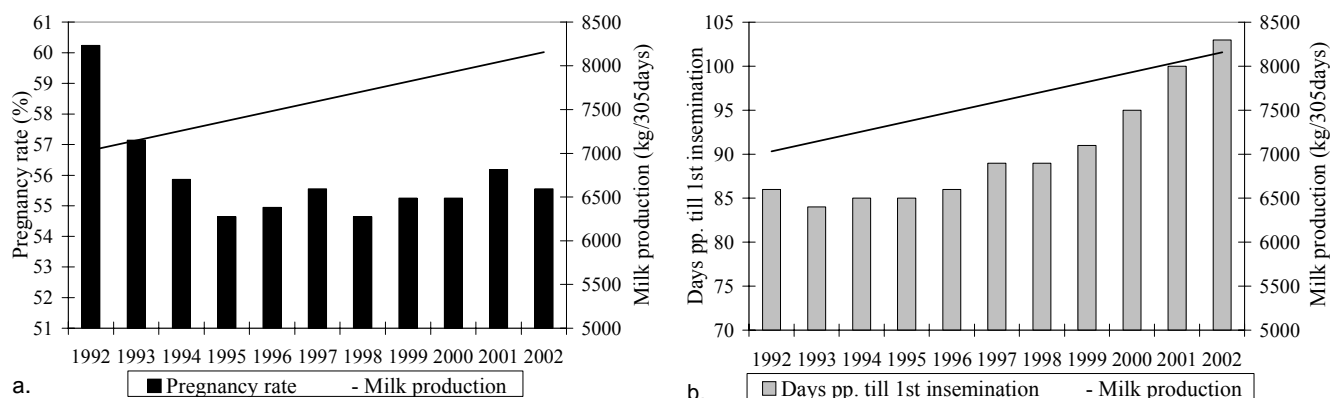


Figure 1. Pregnancy rate and annual milk production of dairy cows in the Netherlands from 1992-2002 (a); Interval pp till 1st insemination and annual milk production of dairy cows in the Netherlands from 1992-2002 (b). Data based on >1 million calvings per year (adapted from (Nederlands Rundvee Syndicaat, 2003)).

Energy balance (EB) can be defined as the difference of net energy intake minus net energy expenditure for maintenance and milk production. If energy expenditure is higher than intake, EB is negative (Heuer *et al.*, 2000) and cows lose body weight. Most studies on the effect of EB on reproductive performance in dairy cattle estimate the EB from estimated dietary net energy intake minus an estimation of energy requirement for maintenance and minus the energy produced in milk (Beam and Butler,

1997; Beam and Butler, 1998; Butler et al., 1981; Canfield and Butler, 1991; Harrison et al., 1990; Lucy et al., 1991a; Reksen et al., 2002; Son et al., 1996; Spicer et al., 1990; Staples et al., 1990; Zurek et al., 1995). Other studies use the change in body weight or body condition score (BCS) as indicators for a cow's energy status (Domecq et al., 1997; Grimard et al., 1995; Wright et al., 1992). Several reviews have been published concerning the effect of EB status in dairy cattle on reproductive efficiency (Beam and Butler, 1999; Boland et al., 2001; Britt, 1993; Butler, 2000; Butler, 2003; Butler and Smith, 1989; Ferguson, 1996; Formigoni and Trevisi, 2003; Friggens, 2003; Gwazdauskas et al., 2000; Jorritsma et al., 2003; Lucy et al., 1992b; Pryce et al., 2004; Wade, 1998). A status of NEB decreases LH pulse frequency, growth rate and diameter of the dominant follicle, weight of the corpus luteum (CL), peri-estrous hormone concentrations like oestradiol (E2) and progesterone (P4) (Beam and Butler, 1999; Butler, 2000; Canfield and Butler, 1991; Mackey et al., 1999; VandeHaar et al., 1995; Villa-Godoy et al., 1988; Yung et al., 1996). In addition, NEB has been related to more days till the first observed estrus postpartum (pp) (Harrison et al., 1990; Liefers et al., 2002; Spicer et al., 1990), more days till first ovulation (Beam and Butler, 1997; Butler and Smith, 1989; Canfield and Butler, 1991; Lucy et al., 1992b), more days open pp (Reist et al., 2003b), decreased conception rates following artificial insemination (Butler, 2000; Domecq et al., 1997; Reist et al., 2003b; Reksen et al., 2002) and lower pregnancy rates (Son et al., 1996).

As recently reviewed (Ingvarlsen et al., 2003), the early lactation period in dairy cattle have been clearly identified with an increased disease incidence. NEB has been indicated as an important factor involved. Epidemiological studies have related NEB directly or indirectly via milk yield to laminitis, leg problems, mastitis and metabolic disorders like ketosis, ruminal acidosis and displaced abomasum (Collard et al., 2000; Grohn et al., 1989; Heuer et al., 1999).

Energy metabolism in high-producing dairy cattle

Metabolism has been recognized to supply the intermediate signals in the relations between NEB and reproduction or health status in dairy cattle. Figure 2a (adapted from (Webster, 1993)) illustrates the pathway of substrates used for energy metabolism in non-lactating dairy cattle. The dietary ingredients fibre, carbohydrates and protein provide substrates for ruminal fermentation and result in the ruminal production of volatile fatty acids (VFA's). The main VFA's produced are acetate and butyrate, which are or can split up into fragments containing two carbon atoms (C2) (lipogenic), and propionate, which is a fragment containing three carbon atoms (C3) (glucogenic). Rumen resistant dietary ingredients and microbial matter can be digested and absorbed in the intestine and provide either C2 or C3 compounds.

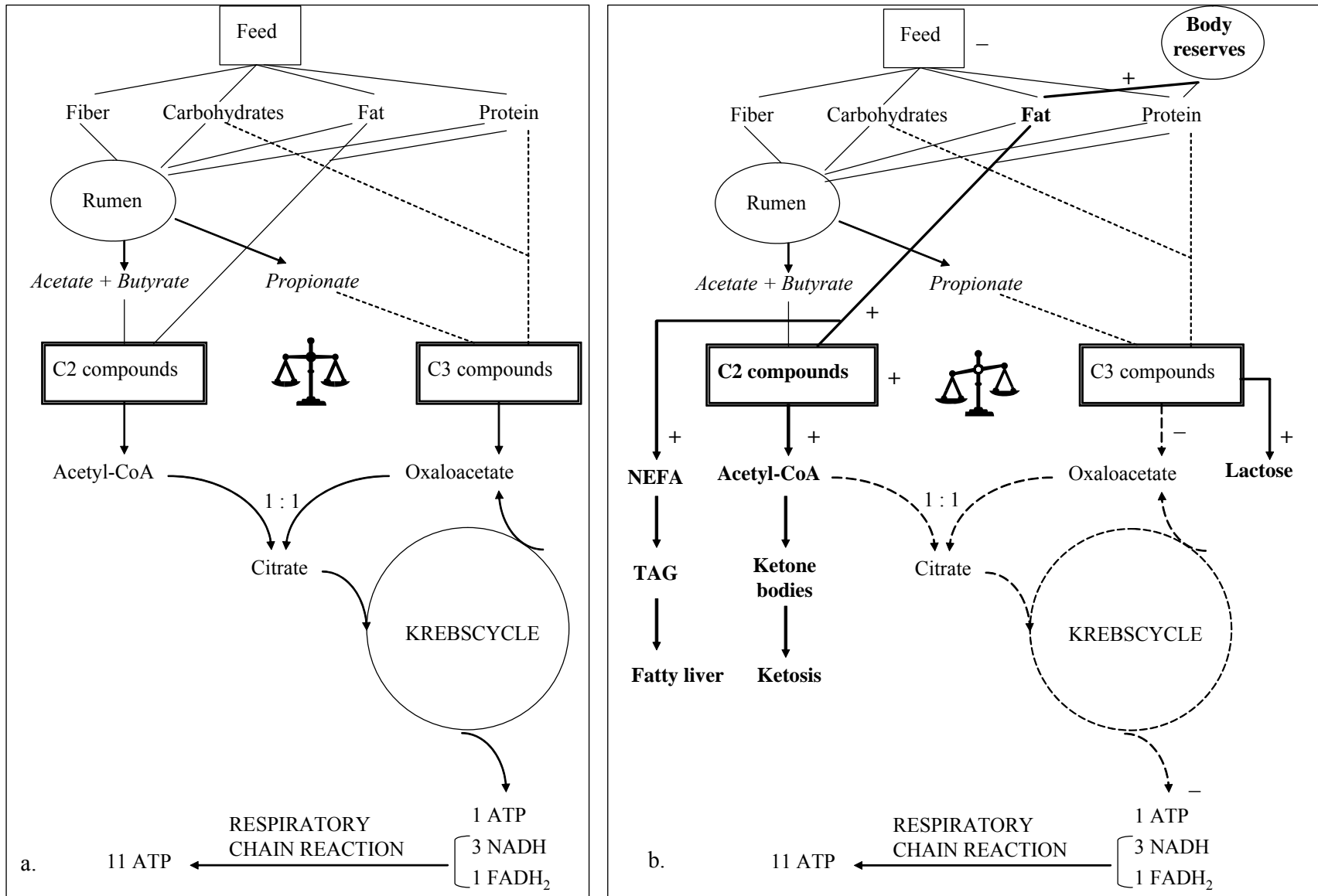


Figure 2. Energy metabolism of non-lactating dairy cattle (a) ; Energy metabolism of lactating dairy cattle in a negative energy balance (b) (adapted from (Webster, 1993)).

The final common pathway for oxidation involves the oxidation of a C2 (Acetyl-Coenzyme-A) and a C3 (Oxaloacetate) fragment to form citrate in a molecular ratio 1:1. Citrate proceeds through a series of intermediate reactions of the Krebs cycle to make available ATP, NADH and FADH₂. NADH and FADH₂ can react with oxygen to produce energy for the body as ATP (respiratory chain reaction). In addition, Figure 2b shows that dairy cattle in early lactation usually have a limited dry matter intake and are therefore in a negative energy balance. This results in the mobilisation of body reserves. Mobilised body reserves are mostly body fat (mainly C2 compounds) and to a lesser extent body protein (partly C2, partly C3 compounds). Mobilisation of body fat results in elevated blood NEFA levels, which can be oxidized to Acetyl-CoA or stored in the liver as tri-acyl glycerol (TAG), possibly causing fatty liver. The high milk production in early lactation requires a high lactose production (from C3 compounds) which results in decreased glucose and insulin levels. The production of Acetyl-CoA from acetate, butyrate and fatty acids from body reserves is high whilst at the same time C3 compounds from glucose and glucogenic precursors, including glucogenic amino acids, are driven towards lactose. Consequently, the ratio of oxaloacetate to acetyl-CoA is out of balance. The availability of citrate to form ATP in the Krebs cycle is decreased. Acetyl-CoA is diverted to the production of ketone bodies, acetone, acetoacetate and β -hydroxybutyrate (BHBA), resulting in a status of ketosis.

In summary, the metabolic effects of a NEB are an imbalance in C2/C3 nutrient ratio and low plasma glucose and insulin concentrations and high concentrations of plasma NEFA, BHBA, acetone, acetoacetate and liver TAG.

Several reviews indicated nutrition to be important in the prevention and treatment of NEB related disorders (Butler, 1998; Butler, 2000; Chilliard, 1993; Diskin et al., 2003; Gong, 2002; Grummer and Carroll, 1988; O'Callaghan and Boland, 1999; Robinson, 1996; Staples et al., 1998). The relation of NEB with protein metabolism has been reviewed (Butler, 1998; Butler, 2000), just as the relation between dietary fat (C2 compounds or lipogenic) and metabolic and reproductive disorders (Chilliard, 1993; Grummer, 1993; Grummer and Carroll, 1988; Grummer and Carroll, 1991; Staples et al., 1998). However, the key problem that occurs in the metabolism of a dairy cow in early lactation seems to be the unbalanced availability of C3 (glucogenic) and C2 (lipogenic) compounds derived from nutrients and body reserves.

The ratio C2/C3 compounds can be manipulated by ingredients in the diet. Lipogenic dietary ingredients, like dietary fat or forages that stimulate the ruminal production of acetate and butyrate, are expected to increase the ratio C2/C3 compounds. Glucogenic nutrients are either ruminal fermented and result in the production of propionate or intestinal digested and absorbed as glucose. Consequently, glucogenic nutrients like grain, nonfibre carbohydrates or propylene glycol are expected to decrease the ratio C2/C3 compounds.

The scope of this review is to find evidence implying the possibilities to modify the C2/C3 ratio by dietary ingredients, measured by altered blood parameters that indicate the EB status. In addition, to find evidence that indicates that a more glucogenic or lipogenic diet affects metabolic disorders, milk production, energy balance and reproductive function.

Dietary energy source related to blood metabolites, metabolic hormones and metabolic disorders

Effect of lipogenic and glucogenic nutrients on blood metabolites and metabolic hormones

Plasma NEFA and BHBA levels are recognized as indicators for body fat mobilisation and NEB in dairy cattle in early lactation (Butler et al., 2003; Butler and Smith, 1989; Chilliard, 1993; Grummer, 1993). Decreased plasma glucose and insulin levels have been associated with NEB (Butler et al., 2003; Harrison et al., 1990; Lucy et al., 1991a; Veenhuizen et al., 1991) and elevate as cows progress towards a more positive EB (Jorritsma, 2003). Table 1 shows reported effects of feeding either extra glucogenic or lipogenic nutrients on plasma NEFA, BHBA, glucose, insulin and growth hormone (GH) levels. Concerning plasma NEFA levels, 13 (Beam and Butler, 1998; Drackley et al., 2003; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Lucy et al., 1993; Moallem et al., 1997; Oldick et al., 1997; Ruegsegger and Schultz, 1985; Ruppert et al., 2003) out of 15 studies found an increase after feeding extra lipogenic nutrients. In contrast to studies where extra glucogenic nutrients were fed; 13 (Drackley et al., 2003; Grum et al., 1996a; Hurtaud et al., 1998; Knowlton et al., 1998; Leonard and Block, 1997; Minor et al., 1998; Oldick et al., 1997; Ramanzin et al., 1997; Ruppert et al., 2003) out of 14 reported studies found a decrease in plasma NEFA levels. Plasma BHBA levels increased after feeding extra lipogenic nutrients in five out of eight studies (Eastridge et al., 1988; Hurtaud et al., 1998; Jerred et al., 1990; Ruppert et al., 2003). Extra glucogenic nutrients were related to a decrease in plasma BHBA levels in eight out of nine reported studies (Aiello et al., 1984; Eastridge et al., 1988; Grum et al., 1996a; Hurtaud et al., 1998; Knowlton et al., 1998; Minor et al., 1998). Plasma glucose (10 out of 13 studies) and insulin (6 out of 9 studies) concentrations were decreased after feeding extra lipogenic nutrients and in almost all cases increased after feeding extra glucogenic nutrients (glucose 13 out of 14 studies; insulin 7 out of 8 studies) (Aiello et al., 1984; Beam and Butler, 1997; Beam and Butler, 1998; Drackley et al., 2003; Eastridge et al., 1988; Grum et al., 1996a; Knowlton et al., 1998; Leonard and Block, 1997; Lucy et al., 1993; Minor et al., 1998; Miyoshi et al., 2001; Oldick et al., 1997; Ruegsegger and Schultz, 1985; Ruppert et al., 2003). Plasma GH concentration was increased in most studies (4 out of 6 studies) after feeding extra lipogenic nutrients (Beam and Butler, 1997; Beam and Butler, 1998; Grum et al., 1996a; Lucy et al., 1993). In contrast to a decrease after feeding extra glucogenic nutrients in 5 out of 6 studies (Grum et al., 1996a; Knowlton et al., 1998; Leonard and Block, 1997).

In addition, liver triglyceride (Vazquez-Anon et al., 1997), and plasma triglyceride (Oldick et al., 1997) concentrations were elevated after feeding extra lipogenic nutrients. In contrast

to a study who found a decrease in liver triglyceride after feeding more non-fermentable carbohydrates (Minor *et al.*, 1998) and studies who reported a decrease in plasma triglyceride levels after abomasal glucose infusion (Oldick *et al.*, 1997; Ramanzin *et al.*, 1997). Two studies found a positive effect of feeding extra glucogenic nutrients on liver glycogen content (Knowlton *et al.*, 1998; Minor *et al.*, 1998), as a representative of stored carbohydrates.

Table 1. Numerical responses in metabolites and metabolic hormones to either extra lipogenic^a or glucogenic^b nutrients in dairy cattle based on means per treatment group.

Category	Responses (based on means per treatment group)			Total no. of animals compared
	Rise	No response	Decline	
NEFA				
Lipogenic nutrients	12	0	3	394
Glucogenic nutrients	1	0	13	165
BHBA				
Lipogenic nutrients	5	2	1	144
Glucogenic nutrients	0	5	5	106
Glucose				
Lipogenic nutrients	3	0	10	229
Glucogenic nutrients	13	0	1	167
Insulin				
Lipogenic nutrients	3	0	6	194
Glucogenic nutrients	9	0	1	167
GH				
Lipogenic nutrients	4	1	1	156
Glucogenic nutrients	0	1	5	44

^aLipogenic nutrients: prilled fat, CaLCFA, tallow.

^bGlucogenic nutrients: grain, concentrates, starch, nonfibre carbohydrates, propylene glycol, glucose infusion.

Based on: lipogenic nutrients: (Beam and Butler, 1997; Beam and Butler, 1998; Drackley *et al.*, 2003; Eastridge *et al.*, 1988; Ferguson *et al.*, 1990; Garcia-Bojalil *et al.*, 1998b; Grum *et al.*, 1996a; Hurtaud *et al.*, 1998; Jerred *et al.*, 1990; Lucy *et al.*, 1993; Moallem *et al.*, 1997; Oldick *et al.*, 1997; Rueggsegger and Schultz, 1985);

glucogenic nutrients: (Aiello *et al.*, 1984; Drackley *et al.*, 2003; Eastridge *et al.*, 1988; Grum *et al.*, 1996a; Hurtaud *et al.*, 1998; Knowlton *et al.*, 1998; Leonard and Block, 1997; Minor *et al.*, 1998; Miyoshi *et al.*, 2001; Oldick *et al.*, 1997; Ramanzin *et al.*, 1997; Ruppert *et al.*, 2003)

Apart from an increase due to higher dietary fat content (*Eastridge et al., 1988; Hurtaud et al., 1998; Jerred et al., 1990*), plasma BHBA were also elevated with increasing dietary forage level (*Jerred et al., 1990*). Ruppert et al. (2003) found no increase in BHBA, as an indicator of NEB, when fat was added to a corn silage diet, in contrast to an alfalfa silage diet. As corn silage is mainly glucogenic (high proportion of C3 nutrients) and alfalfa silage mainly lipogenic (high proportion of C2 nutrients), it seems logical BHBA levels on a corn silage diet are less increased than BHBA levels in cows on a alfalfa silage diet upon fat addition. In the corn silage diet the extra C2 nutrients of the fat addition are easier to metabolise because of the higher availability of C3 nutrients, compared to the alfalfa silage diet. It seems that the effect of dietary fat addition depends on the nature of other nutrients in the diet, suggesting the C2/C3 balance to be a factor in the concentration of plasma BHBA.

The considerable variation in effects of fat supplementation on metabolites and metabolic hormones may be explained by saturation of the fat source, as indicated by Thomas et al. (1997). In this study a positive effect was found of poly-unsaturated fat with mainly 16- and 18-carbon fatty acids (soybean oil) on insulin and IGF-1 levels compared to control, saturated (animal tallow) and highly polyunsaturated fat with considerable > 20-carbon fatty acids (fish oil), possibly caused by a difference in ruminal fermentation patterns between the fat sources as the poly-unsaturated fat treatment was expected to modify the rumen fermentation pattern in favour of propionic acid production. Moreover, digestibility of fat in the intestine depends on chain length and saturation. In general, fatty acids with more than 18-carbon atoms have a reduced digestibility whilst digestibility appears to be higher for unsaturated than saturated fatty acids (*Doreau and Chilliard, 1997*). Thus, variation in the type of fat source added may increase variation in effects on metabolites because of variation in the amount of extra metabolisable energy obtained.

Santos et al. (2000) reported lower NEFA concentrations and higher glucose and insulin levels in cows fed steam-flaked sorghum compared to steam-rolled corn. This can be explained by a higher ruminal starch digestibility, and resulting higher ruminal propionate secretion, of steam-flaked sorghum compared to steam-rolled corn. This is in line with Simas et al. (1995) who found elevated blood glucose levels in cows fed steam-flaked sorghum, compared to dry-rolled sorghum, which has a lower ruminal starch degradability. A study on starch infusion reported a difference in effect on plasma metabolite levels between infusion sites. Abomasal starch infusion tended to decrease plasma NEFA levels more than ruminal infusion. This is probably caused by the production of the VFAs propionate, butyrate and acetate from starch ruminally infused, while abomasal starch infused is intestinally digested and absorbed as glucose (*Knowlton et al., 1998*).

In addition, several studies presented a diurnal rhythm for glucose, insulin, NEFA and BHBA in ruminants (*Cisse et al., 1991; Lefcourt et al., 1999; Marie et al., 2001*). Especially, plasma

NEFA levels seem to be more sensitive and variable before feeding compared to after feeding. This implies a time-of-day or a time-after-feeding effect when interpreting dietary effects on blood metabolites and metabolic hormones.

In conclusion, feeding extra lipogenic nutrients generally increases NEFA, BHBA and GH levels and decreases plasma glucose and insulin levels. Increased availability of glucogenic nutrients, e.g. dietary corn, starch infusion or propylene glycol increases plasma glucose and insulin levels, and decreases plasma GH, NEFA and BHBA concentrations. This shows that indicators for an imbalance in C2/C3 ratio can be effectively manipulated by dietary energy source. The effect of the manipulation seems to be dependent on the availability of the energy source as metabolic C2 or C3 compound. Secondly, the effect of manipulation also seems to depend on the balance between C2 and C3 compounds, in the rest of the diet.

Effect of lipogenic and glucogenic nutrients on metabolic disorders

As earlier reviewed (Nocek, 1997; Owens et al., 1998), increasing the availability of glucogenic nutrients, in particular of readily fermentable carbohydrates in the rumen, results in an increased incidence of both clinical (pH < 5.0) and subclinical (pH < 5.5) acidosis. This observation is confirmed by later studies on replacing alfalfa silage with corn silage (Ruppert et al., 2003) or replacing a high-fat concentrate with a high-starch concentrate (Keady and Mayne, 2001), where in both cases the ruminal pH decreased with increasing availability of glucogenic nutrients compared to lipogenic nutrients. With subclinical or clinical acidosis, the ruminal tissue wall may become damaged and reduced intake, digestion, laminitis and liver anomalies can occur. Thus, the level as well as the type of glucogenic nutrients (rapidly versus slowly fermentable carbohydrates) influence the occurrence of (sub)clinical acidosis and this contributes to the observed variability in effect of glucogenic supplements on production, energy balance and reproductive characteristics.

Grummer and Carroll (1991) have suggested in their review that long-term fat supplementation might cause fatty liver via chronic elevation of plasma NEFA levels. This theory is supported by firstly other studies that found a positive relationship between elevated NEFA, decreased glucose levels and the incidence of fatty liver (Jorritsma, 2003; Reid, 1980; Veenhuizen et al., 1991); secondly, evidence that indicates the relationship between liver triglyceride content and plasma NEFA concentrations (Jorritsma, 2003); and thirdly, associations between dietary fat supplementation and elevated plasma NEFA levels (Beam and Butler, 1998; Garcia-Bojalil et al., 1998b).

Several studies found a decreasing effect of glucogenic feed on plasma NEFA, BHBA and triglyceride levels (Drackley et al., 2003; Minor et al., 1998; Studer et al., 1993), suggesting also that glucogenic feed may reduce the incidence and severity of ketosis and fatty liver. Grummer et al. (1993) supposed propionate, as a product of rumen degradable

glucogenic feed, to be anti-ketogenic and advised to maximize hepatic glycogen stores to decrease the triglyceride/glycogen ratio in the liver, which has been indicated as a risk factor for fatty liver and ketosis.

In ketosis-induced cattle, by feed restriction plus 1,3-butanediol, Veenhuizen et al. (1991) reported that triglyceride infiltration in the liver increases and liver glycogen content decreases as cows progress towards clinical ketosis. Kronfeld (1982) reviewed that the supply of lipogenic precursors for milk production relative to glucogenic precursors in the diet determines the susceptibility of cows to spontaneous ketosis; liver triglyceride to glycogen ratio indicates the relative supply of these nutrients (Grummer, 1993). In addition, both Grummer (1993) and Drackley (2003) suggested in their reviews the occurrence of fatty liver also may have a direct effect on carbohydrate metabolism by an impaired gluconeogenesis in the liver, resulting in an increased susceptibility to ketosis.

In vitro, high levels of ketone bodies have a negative effect on the chemotactic (Suriyasathaporn et al., 1999) and proliferative (Franklin et al., 1991; Targowski and Klucinski, 1983) capacity of lymphocytes and the secretion of immunoglobulins by lymphocytes (Franklin et al., 1991; Nonnecke et al., 1992; Suriyasathaporn et al., 1999; Targowski and Klucinski, 1983) has been identified. In vivo, BHBA levels have been positively related to the severity of mastitis as indicated by bacterial counts (Kremer et al., 1993). Such data indicate that ketosis may negatively affect some aspects of the immune system.

High plasma NEFA and BHBA levels and low glucose levels have been related to fatty liver and a status of ketosis (Grummer, 1993). Lipogenic and glucogenic nutrients have an effect on ruminal pH and the metabolites NEFA, BHBA, glucose and liver triglyceride content. As a result it can be expected that also the incidence and severity of fatty liver and ketosis as well as acidosis is affected by the lipogenic/glucogenic nutrient ratio. Herewith considering Figure 2, this indicates the C2/C3 nutrient balance to be an important element in the reduction of metabolic disorders in dairy cattle in early lactation

Dietary energy source related to milk production and EB

The selection of the experiments presented in Figures 3, 4 and 5 is based on the criteria: dietary treatment (extra lipogenic and/or glucogenic nutrients fed in the treatment group) and presence of mainly multiparous lactating dairy cattle. Table 2 in the appendix shows the dietary treatment, no of cows, parity and DMI of the experiments presented in Figures 3 to 6.

Effect of lipogenic and glucogenic nutrients on energy intake

As shown in Appendix 1, most studies on altering the glucogenic/ lipogenic nutrient ratio, also alter the energy content of the experimental diets. On the other hand, 22 out of 31 studies (Beam and Butler, 1997; Beam and Butler, 1998; Drackley et al., 2003; Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Harrison et al., 1995; Lucy et al., 1993; Moallem et al., 1999; Moallem et al., 1997; Ruegsegger and Schultz, 1985; Ruppert et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Simas et al., 1998; Simas et al., 1995; Sklan et al., 1994; Sklan et al., 1991; Son et al., 1996; Spicer et al., 1993; Vazquez-Anon et al., 1997; Voigt et al., 2003) reported a decrease in DMI after feeding extra lipogenic nutrients compared to 7 out of 14 studies (Beever et al., 1989; Carroll et al., 1990; Drackley et al., 2003; Gordon et al., 1995; Grum et al., 1996a; Grummer and Davis, 1984; Keady and Mayne, 1998; Minor et al., 1998; Miyoshi et al., 2001; Patton et al., 2004; Pushpakumara et al., 2003; Rigout et al., 2003; Ruppert et al., 2003; Sutton et al., 1991; Vazquez-Anon et al., 1997) on extra glucogenic nutrients which showed a decrease in DMI in the treatment group.

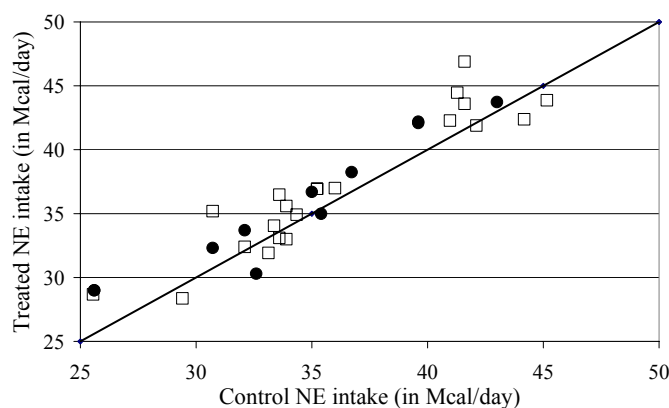


Figure 3. Effect of feeding supplemental lipogenic (□) or glucogenic (●) nutrients on net energy (NE) intake in Mcal per day. Points are based on means per treatment group. Points below the diagonal line represent studies with a negative effect of diet treatment, points above the diagonal line represent studies with a positive effect of diet treatment. Based on (Drackley et al., 2003; Grum et al., 1996a; Harrison et al., 1995; Hurtaud et al., 1998; Jerred et al., 1990; Keady and Mayne, 1998; Knowlton et al., 1998; Leonard and Block, 1997; Lucy et al., 1993; Minor et al., 1998; Moallem et al., 1999; Moallem et al., 1997; Salfer et al., 1995; Simas et al., 1998; Sklan et al., 1994; Son et al., 1996; Spicer et al., 2001; Vazquez-Anon et al., 1997; Voigt et al., 2003).

In general, the negative effect of extra lipogenic sources on dry matter intake is higher when the degree of saturation of fatty acids is lower, probably because of the more

pronounced negative effects of unsaturated fatty acids on rumen carbohydrate fermentation. As a result, 73% of the studies on feeding extra lipogenic nutrients (Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Jerred et al., 1990; Moallem et al., 1999; Moallem et al., 1997; Salfer et al., 1995; Sklan et al., 1994; Son et al., 1996; Spicer et al., 1993) and also 73% of the studies on increasing dietary glucogenic nutrients (Amaral et al., 1990; Grum et al., 1996a; Hurtaud et al., 1998; Knowlton et al., 1998; Leonard and Block, 1997; Minor et al., 1998) obtained a higher net energy (NE) intake in the treatment group compared to the control group, illustrated by Figure 3.

Effect of lipogenic and glucogenic nutrients on milk production

Figure 4 shows an overview of studies who reported milk yield and composition after feeding either more glucogenic nutrients or lipogenic nutrients. Both feeding extra lipogenic nutrients or glucogenic nutrients had similar effects on kg of milk produced per day (Amaral et al., 1990; Beam and Butler, 1997; Drackley et al., 2003; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Gong et al., 2002; Gordon et al., 1995; Grum et al., 1996a; Grummer and Davis, 1984; Harrison et al., 1995; Hoedemaker et al., 2004; Hurtaud et al., 2000; Hurtaud et al., 1998; Jerred et al., 1990; Knowlton et al., 1998; Leonard and Block, 1997; Lucy et al., 1993; McNamara et al., 2003; Minor et al., 1998; Miyoshi et al., 2001; Moallem et al., 1999; Moallem et al., 1997; Oldick et al., 1997; Rigout et al., 2003; Ruegsegger and Schultz, 1985; Ruppert et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Schneider et al., 1988; Simas et al., 1998; Simas et al., 1995; Sklan et al., 1994; Son et al., 1996; Spicer et al., 1993; Sutton et al., 1991; Vazquez-Anon et al., 1997; Voigt et al., 2003). Milk fat percentage was usually elevated after feeding extra lipogenic nutrients (24 out of 32 studies) (Drackley et al., 2003; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Harrison et al., 1995; Jerred et al., 1990; Lucy et al., 1993; McNamara et al., 2003; Moallem et al., 1999; Moallem et al., 1997; Ruegsegger and Schultz, 1985; Ruppert et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Schneider et al., 1988; Simas et al., 1998; Simas et al., 1995; Sklan et al., 1994; Son et al., 1996; Spicer et al., 1993), but decreased after addition of glucogenic nutrients to the diet (23 out of 26 studies) (Amaral et al., 1990; Drackley et al., 2003; Gordon et al., 1995; Grum et al., 1996a; Grummer and Davis, 1984; Hurtaud et al., 2000; Hurtaud et al., 1998; Keady and Mayne, 1998; Knowlton et al., 1998; Leonard and Block, 1997; Minor et al., 1998; Oldick et al., 1997; Patton et al., 2004; Rigout et al., 2003; Ruppert et al., 2003; Sutton et al., 1991; Vazquez-Anon et al., 1997; Voigt et al., 2003). In most cases milk protein percentage decreased after feeding extra lipogenic nutrients (23 out of 28 studies) (Drackley et al., 2003; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Harrison et al., 1995; Jerred et al., 1990; McNamara et al., 2003; Moallem et al., 1999; Moallem et al., 1997; Ruegsegger and Schultz, 1985; Ruppert et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Simas et al., 1998; Simas et al., 1995; Sklan et al., 1994; Son et al., 1996). However, extra glucogenic nutrients increased milk protein percentage in 18 out of 25 studies (Amaral et al., 1990; Drackley et al., 2003; Gordon et al., 1995; Grum et al., 1996a; Hurtaud et al., 2000; Hurtaud et al., 1998; Keady and Mayne, 1998; Knowlton et al., 1998; Leonard and Block, 1997; Minor et al., 1998; Miyoshi et al., 2001; Oldick et al., 1997; Rigout et al., 2003; Ruppert et al., 2003; Sutton et al., 1991; Vazquez-Anon et al., 1997; Voigt et al., 2003). Concerning fat corrected milk (FCM), lipogenic nutrients had a positive effect on FCM in 13 out of 23 studies (Beam and Butler, 1997; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Grum et al., 1996a; Harrison et al., 1995; Jerred et al., 1990; Lucy et al., 1993; Moallem et al., 1999; Moallem et al., 1997; Ruppert et al., 2003; Salfer et al., 1995; Simas et al., 1995; Sklan et al., 1994; Son et al., 1996). After feeding extra glucogenic nutrients FCM decreased in nine of the 14 studies (Grum et al., 1996a; Grummer and Davis, 1984; Hurtaud et al.,

1998; Leonard and Block, 1997; Miyoshi et al., 2001; Oldick et al., 1997; Rigout et al., 2003; Ruppert et al., 2003; Vazquez-Anon et al., 1997).

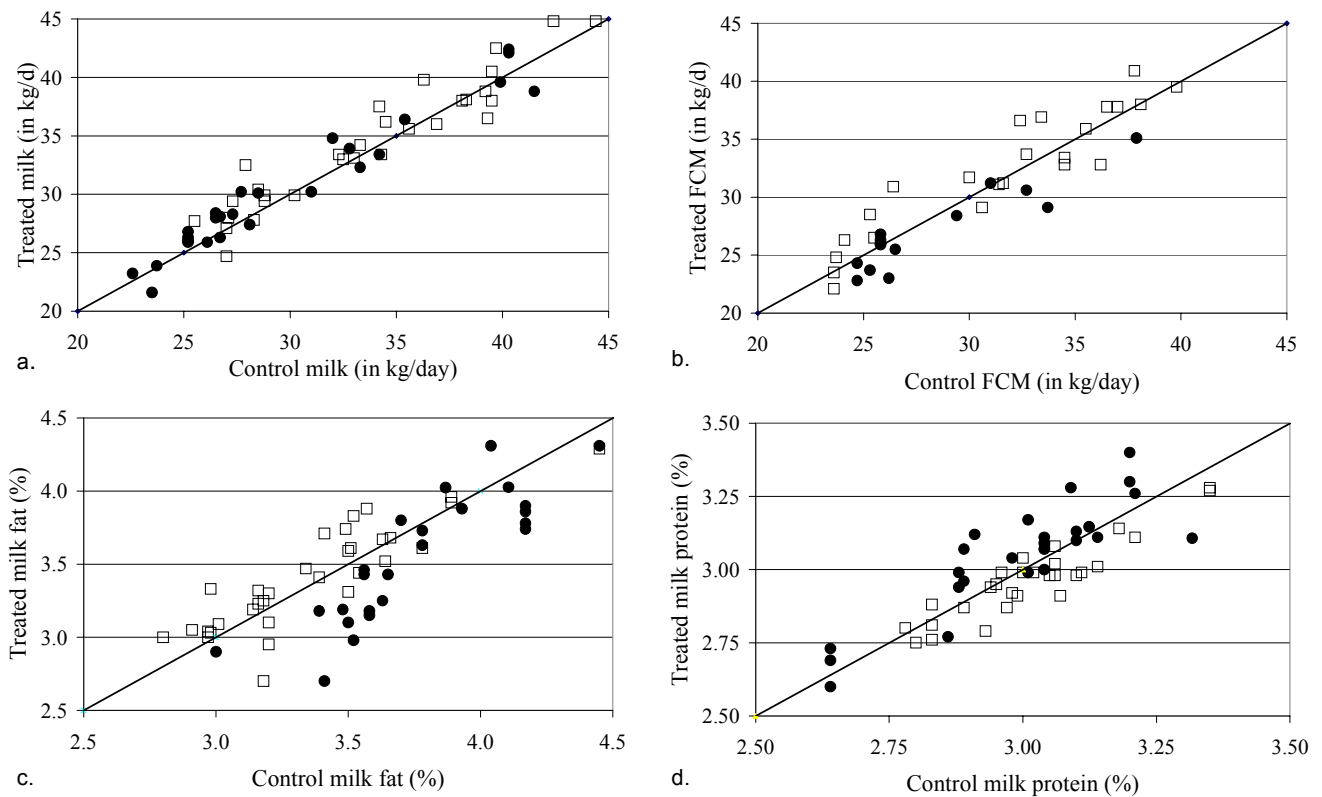


Figure 4. Effect of feeding supplemental lipogenic (□) or glucogenic (●) nutrients on daily milk production in kg (a), daily milk production in fat corrected milk (FCM) (b), milk fat % (c) and milk protein % (d). Points are based on means per treatment group. Points below the diagonal line represent studies with a negative effect of diet treatment, points above the diagonal line represent studies with a positive effect of diet treatment.

Based on (Amaral et al., 1990; Beam and Butler, 1997; Drackley et al., 2003; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Gong et al., 2002; Gordon et al., 1995; Grum et al., 1996a; Grummer and Davis, 1984; Harrison et al., 1995; Hoedemaker et al., 2004; Hurtaud et al., 2000; Hurtaud et al., 1998; Jerred et al., 1990; Knowlton et al., 1998; Leonard and Block, 1997; Lucy et al., 1993; McNamara et al., 2003; Minor et al., 1998; Miyoshi et al., 2001; Moallem et al., 1999; Moallem et al., 1997; Oldick et al., 1997; Rigout et al., 2003; Ruegsegger and Schultz, 1985; Ruppert et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Schneider et al., 1988; Simas et al., 1998; Simas et al., 1995; Sklan et al., 1994; Son et al., 1996; Spicer et al., 1993; Sutton et al., 1991; Vazquez-Anon et al., 1997; Voigt et al., 2003).

Effect of lipogenic and glucogenic nutrients on EB

The increased NE intake after feeding extra lipogenic nutrients, accompanied by an increase in milk fat percentage, suggests that the gained NE intake by feeding lipogenic nutrients is probably beneficial to milk fat percentage and not to EB. This observation is supported by studies that found an increased body weight loss (Beam and Butler, 1998; Sklan et al., 1991) and body condition score loss (Sklan et al., 1989) or a more negative EB (Son et al., 1996) after dietary fat supplementation. Son et al., (1996) even reported on a study with increased milk yield, increased milk fat percentage, but lower NE intake after dietary fat supplementation. This suggests more body fat mobilisation in the fat supplemented group than the control group. Concerning milk composition, the inverse effect of feeding extra lipogenic nutrients is observed in a majority of the studies on feeding extra glucogenic nutrients. In a majority of the studies on feeding extra glucogenic nutrients, milk fat percentage is decreased and milk protein percentage increased. This can imply that proteins are saved from use as a glucogenic energy source after feeding extra glucogenic nutrients, indicated by the increased milk protein percentage. Figure 5 further supports these hypotheses, by presenting 18 studies on either feeding more lipogenic or glucogenic nutrients on the calculated EB. Eight of the 12 studies reported an increase in EB after feeding extra lipogenic nutrients (Beam and Butler, 1997; Beam and Butler, 1998; Harrison et al., 1990; Lucy et al., 1993; Oldick et al., 1997; Son et al., 1996; Spicer et al., 1993). In contrast to feeding extra glucogenic nutrients where 12 out of 13 studies were able to improve, not-significantly, the calculated EB (Beever et al., 1989; Gordon et al., 1995; Hurtaud et al., 2000; Hurtaud et al., 1998; Keady and Mayne, 1998; Leonard and Block, 1997; Minor et al., 1998; Miyoshi et al., 2001; Oldick et al., 1997; Sutton et al., 1991; Voigt et al., 2003).

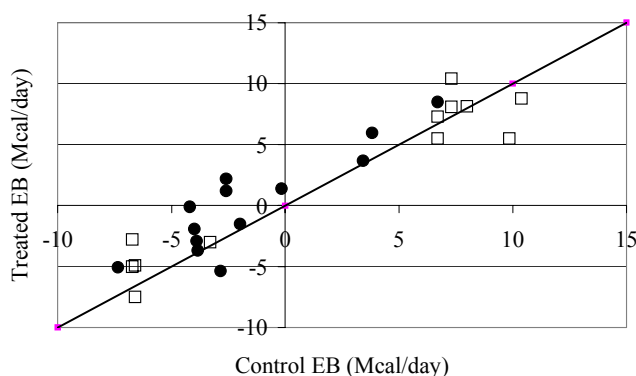


Figure 5. Effect of feeding supplemental lipogenic (□) or glucogenic (●) nutrients on EB. Points are based on means per treatment group. Points below the diagonal line represent studies with a negative effect of diet treatment, points above the diagonal line represent studies with a positive effect of diet treatment. Based on (Beam and Butler, 1997; Beam and Butler, 1998; Beever et al., 1989; Gordon et al., 1995; Harrison et al., 1995; Hurtaud et al., 2000; Hurtaud et al., 1998; Keady and Mayne, 1998; Leonard and Block, 1997; Lucy et al., 1993; Minor et al., 1998; Miyoshi et al., 2001; Oldick et al., 1997; Son et al., 1996; Spicer et al., 1993; Sutton et al., 1991; Voigt et al., 2003).

In conclusion, extra lipogenic nutrients had variable results on EB and increased milk yield and milk fat percentage. This suggests a surplus of C2 compounds in the C2/C3 balance, resulting in elevated milk fat output. In contrast, glucogenic nutrients seem to increase the EB, decrease the milk fat percentage and increase the milk protein percentage, implying a more balanced C2/C3 ratio and therefore a protein-saving effect of glucogenic nutrients.

Dietary energy source related to reproduction

The selection of the experiments presented in Figure 6 is based on the criteria: dietary treatment (extra lipogenic and/or glucogenic nutrients fed in the treatment group) and presence of mainly multiparous lactating dairy cattle. Table 2 in the appendix shows the dietary treatment, no of cows, parity and DMI of the experiments presented in Figure 6.

Effect of lipogenic and glucogenic nutrients on reproductive efficiency

Figure 6 illustrates the effects of feeding supplemental lipogenic or glucogenic nutrients on days to first ovulation, conception rate following first insemination, services per conception and number of days open. Increasing glucogenic nutrients was reached by supplying extra dietary starch (Gong et al., 2002), maize gluten (Armstrong et al., 1990; Carroll et al., 1990), ground shelled corn (Armstrong et al., 1990; Carroll et al., 1990), abomasal glucose infusion (Oldick et al., 1997) or propylene glycol supplementation (Miyoshi et al., 2001). Days postpartum (pp.) till first ovulation (Carroll et al., 1990; Miyoshi et al., 2001; Oldick et al., 1997; Pushpakumara et al., 2003) is reported to be reduced after feeding extra glucogenic nutrients in three out of four studies. Six out of 12 studies (Beam and Butler, 1997; Beam and Butler, 1998; Carroll et al., 1990; Garcia-Bojalil et al., 1998b; Lucy et al., 1992c; Oldick et al., 1997; Sklan et al., 1991; Son et al., 1996; Spicer et al., 1993) on feeding extra lipogenic nutrients reported an increase, four a decrease (Beam and Butler, 1997; Garcia-Bojalil et al., 1998b; Oldick et al., 1997; Son et al., 1996) and two found (Garcia-Bojalil et al., 1998b; Son et al., 1996) no effect on days pp. till first ovulation. Conception rate following first insemination is increased after supplemental glucogenic nutrients in both reported studies (Gong et al., 2002; Miyoshi et al., 2001). Six out of 14 studies showed an increase in conception rate following first insemination by increasing the lipogenic nutrient proportion in the diet (Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Lucy et al., 1992c; McNamara et al., 2003; Moallem et al., 1997; Schneider et al., 1988; Scott et al., 1995; Sklan et al., 1994; Sklan et al., 1991; Son et al., 1996). Number of services per conception is increased in four (Armstrong et al., 1990; Gong et al., 2002) out of five studies on feeding extra glucogenic nutrients. Only Miyoshi et al. (2001) found the number of services per conception to be reduced after propylene glycol supplementation. A majority of the studies (11 out of 15 studies) (Ferguson et al., 1990; Lucy et al., 1992c; McNamara et al., 2003; Moallem et al., 1997; Pushpakumara et al., 2003; Salfer et al., 1995; Schingoethe and Casper, 1991; Schneider et al., 1988; Scott et al., 1995; Sklan et al., 1991) reported a decrease in services per conception after addition of lipogenic nutrients. Number of

days open are reported to be reduced by glucogenic nutrient supplementation in two out of three studies (Gong et al., 2002; Miyoshi et al., 2001). A few studies (3 out of 10 studies) (Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Moallem et al., 1997; Ruegsegger and Schultz, 1985; Salfer et al., 1995; Schingoethe and Casper, 1991; Scott et al., 1995; Sklan et al., 1991) showed a decrease in number of day open after fat addition to the diet.

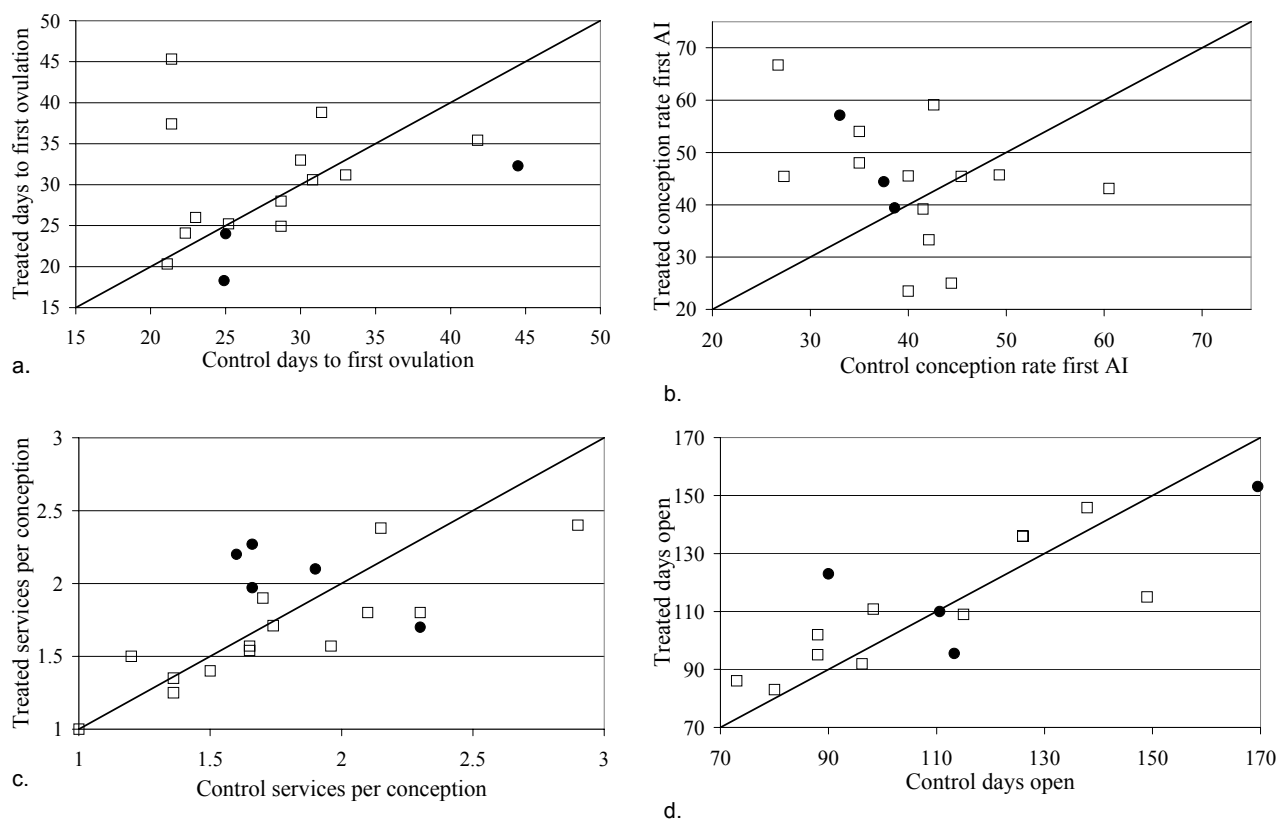


Figure 6. Effect of feeding supplemental lipogenic (\square) or glucogenic (\bullet) nutrients on days to first ovulation (a), conception rate following first artificial insemination (b), services per conception (c) and days open (d). Points are based on means per treatment group. Points below the diagonal line represent studies with a negative effect of diet treatment, points above the diagonal line represent studies with a positive effect of diet treatment. Based on (Armstrong et al., 1990; Beam and Butler, 1997; Beam and Butler, 1998; Carroll et al., 1990; Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Gong et al., 2002; Lucy et al., 1992c; McNamara et al., 2003; Miyoshi et al., 2001; Moallem et al., 1997; Oldick et al., 1997; Pushpakumara et al., 2003; Ruegsegger and Schultz, 1985; Salfer et al., 1995; Santos et al., 2000; Schingoethe and Casper, 1991; Schneider et al., 1988; Scott et al., 1995; Sklan et al., 1994; Sklan et al., 1991; Son et al., 1996; Spicer et al., 1993)

In addition, several studies found increased pregnancy rates (Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Robinson et al., 2002; Schneider et al., 1988; Son et al., 1996), elevated plasma progesterone (P4) level (Ferguson et al., 1990; Garcia-Bojalil et al., 1998b; Sklan et al., 1991; Son et al., 1996), increased diameters of preovulatory follicles (Lucy et al., 1993) and greater follicular populations (Lammoglia et al., 1997; Lucy et al., 1991b; Robinson et al., 2002) after dietary fat supplementation. However, other groups detected a negative relation between dietary

fat addition and follicular development (*Beam and Butler, 1998*), pregnancy rates (*Lucy et al., 1992c; Moallem et al., 1997; Sklan et al., 1994*) or plasma P4 concentration (*Lucy et al., 1993*).

Figure 6 confirms the conclusion of Staples et al. (1998) that effects of dietary prilled fat on reproductive performance are variable. In addition, Figure 6 shows that feeding extra glucogenic nutrients has variable effects on reproductive parameters as well. Several factors contribute to the diversity in effects of supplemental lipogenic and glucogenic nutrients on fertility in dairy cattle. First, as discussed before, the type of lipogenic nutrients (chain length and degree of saturation of long chain fatty acids) as well as the type of glucogenic nutrients (rate of fermentation in the rumen) affects the profile of nutrients absorbed from the gastro-intestinal tract which in turn may impact on reproductive parameters. For supplemental lipogenic nutrients a possible role for fatty acid composition has been suggested and confirmed in several studies (*Petit et al., 2001; Thomas et al., 1997*). Petit et al., (2001) found a difference in conception rate after first insemination and plasma P4 levels between cows fed extracted flaxseed meal (MEGALAC) or whole flaxseed (FLAX). They suggested, next to an increased DM intake, a lower daily milk production and a less negative NEB, also the increased concentration of linolenic acid in FLAX could be beneficial by increasing progesterone levels, decreasing prostaglandin levels and consequently increasing conception rate. They discussed the potential inhibition of prostaglandin secretion by absorbed linolenic acid, as supported by the increased milk progesterone concentration in cows fed FLAX. In addition, another study (*Thomas et al., 1997*) found an enhancing effect of poly-unsaturated fatty acids on plasma insulin level and number of follicles compared to a control group and groups fed saturated or highly poly-unsaturated fatty acids. Similarly, variation in type of glucogenic nutrients contributes to the observed variation in effects on reproduction. Santos et al. (2000) observed a non-significant increase in luteal activity and P4 levels in cows on a diet high in rumen degradable starch (RDS), compared to a rumen resistant starch diet. The authors suggested the increased EB of the RDS group to be an explanation for this observation.

Additionally, also the reported increase in insulin concentrations with the RDS treatment, probably resulting from an increased production of ruminal propionate, can be beneficial to ovarian function. Secondly, it is important to differentiate between isocaloric and non-isocaloric diets, as dietary energy density has been reported to have significant effects on reproductive performance (*Gwazdauskas et al., 2000; Sanz et al., 2004*). As illustrated in Figure 4, most studies increased the NE intake by adding more lipogenic or glucogenic nutrients to the diet. To study the effect of dietary energy source it is highly favorable to offer isocaloric diets to prevent this interaction with dietary energy density. Thirdly, as shown in Figure 5, several authors found an effect of glucogenic or lipogenic nutrients on the (calculated) EB (*Minor et al., 1998; Miyoshi et al., 2001*) which might interact with the effect of energy source on reproduction.

Concerning above mentioned explanations, they all have, besides an effect on reproductive performance, also an effect on EB. This suggests that the effect of dietary energy source is not necessarily a direct effect of energy source availability, but might be an indirect effect via alterations of EB status by dietary energy source. This implies EB to be an intermediary in the effect of dietary energy source on reproductive performance.

A fourth consideration, concerning the interpretation of the effects of dietary energy source on reproductive parameters, is that parameters as the number of services per conception or conception rate at first AI could largely depend on the protocol of the experiment. The minimum number of days until first AI seems particularly important in this aspect. A part of the presented studies in figure 4b do not specify the determination of timing of AI (*Gong et al., 2002; Hoedemaker et al., 2004; McNamara et al., 2003; Moallem et al., 1997*). Most studies applied a waiting period till first AI ranging from 39 to 90 DIM (*Ferguson et al., 1990; Scott et al., 1995; Sklan et al., 1991; Son et al., 1996*), other studies inseminated the cows after synchronization with a prostaglandin analogue (*Garcia-Bojalil et al., 1998a; Sklan et al., 1994*).

A fifth explanation can be that several authors suggested that optimum nutritional conditions for follicle growth are not necessarily recognized as optimum conditions for embryo-survival (*O'Callaghan and Boland, 1999*). Exact knowledge about this hypothesis is lacking, however it has been indicated that impaired body condition and prolonged low energy intake are detrimental to fertility. In contrast, short-term restrictions in dietary intake have been shown to increase subsequent pregnancy rates in heifers (*Nolan et al., 1998*). Explanations can probably be found in intermediate metabolic signals. Increased energy status is related to increased plasma insulin concentration (*Miyoshi et al., 2001; Oldick et al., 1997*) which is beneficial to follicular development (*Gong, 2002*). On the other hand body fat mobilisation, resulting from feed restrictions, is associated with increased plasma P4 levels (*Nolan et al., 1998; Rabie et al., 2001*), probably caused by the steroid-storage function of fat tissues, which can be beneficial to pregnancy establishment and fertility.

Summarizing, it is difficult to draw conclusions on the effects of feeding either extra lipogenic or glucogenic nutrients on reproductive parameters. Firstly, reported effects seem to be variable due to type of lipogenic or glucogenic nutrients and NE intake level effects on EB. Secondly, research on the relation between glucogenic nutrient addition and EB and reproductive parameters is desirable as studies on this subject are still scarce. Some suggestions can be made as glucose and insulin are increased after feeding extra glucogenic nutrients and are suggested as positive metabolic signals to the reproductive axis (*Butler et al., 2003; Butler, 1993; Canfield and Butler, 1991; Gong et al., 2002; Grimard et al., 1995; Lucy et al., 1991a*). Plasma NEFA and BHBA levels are increased after feeding extra lipogenic nutrients and associated with decreased reproductive performance and anestrus (*Beam and Butler, 1998; Lucy et al., 1992a; Moallem et al., 1997; Villa-Godoy et al., 1990; Walters et al.,*

2002). Both observations might imply that the C2/C3 compound balance is important for reproductive performance.

Conclusion

Dietary energy source affects the balance in C2 and C3 compound availability, as supplied by dietary ingredients and adipose tissue mobilisation. Alterations in C2 and C3 compound availability result in modifications in blood metabolic profiles and production performance in lactating dairy cattle. These observations, together with the described effects of C2/C3 compound ratio on energy balance and reproductive performance in dairy cattle, suggest a relationship between the availability of C2 and C3 compounds and EB and reproduction. However, as the described effects on reproduction are rather incoherent and studies on feeding extra C3 compounds and reproductive performance are scarce, further research could validate these suggestions.

Appendix

Table 2. Studies presented in Figures 3 to 6. Dietary treatment, no of cows, parity and DMI.

Reference	Dietary treatment	Isocaloric ^a Y/N	Extra nutrients (% DM)	Extra nutrients (kg/d)	forage : concentrate (DM basis)	Cows (no)	Treatment period (DIM)	Parity	DMI (kg DM/d)
<i>Lipogenic studies</i>									
Beam and Butler, 1997	Control				50:50	45	0-84	≥2	23.1
	tallow + yellow grease	N	8.7	2.09					24.0
	tallow + yellow grease	N	17.4	3.74					21.5
Beam and Butler, 1998	Control				45:55	42	0-100	≥2	17.3
	Prilled LCFA	N	2.5	0.39					15.5
Ferguson et al., 1990	Control, herd 1				52:48	95	0-150	≥2	
	LCFA, herd 1	N		0.50					
	Control, herd 2					47			
	LCFA, herd 2	N		0.50					
	Control, herd 3					47			
Garcia-Bojalil et al., 1998a,b	Control, low protein				48:52	45	1-120	≥2	19.6
	Megalac ^b , low protein	N	2.2	0.42					19.0
	Control, high protein				47:53				19.4
	Megalac ^b , high protein	N	2.2	0.44					19.8
Harrison et al., 1999	Control				46:54	48	21-119		23.1
	Whole cotton seed (WCS)	N							23.9
	WCS + Megalac ^b	N	2.7	0.58					21.6
Lucy et al., 1992	Control				45:55	90	0-75	≥1	
	Ca salts of LCFA	N	3						
Aiello et al., 1984	Control				52:48	18	80	≥1	20.0
	Ca salts of LCFA	Y	2.2		65:35				19.7
	Ca salts of LCFA	N	2.2		52:48				20.5
McNamara et al., 2003	Control				84:16	201	0-70	≥1	
	Megalac plus ^d	N	3	0.40					
	Megapro gold ^e	N	3	1.50					
Moallem et al., 1997	Control				37:63	48	0-150	≥2	24.1
	Adolac ^c	N	2.2	0.50					24.3
Moallem et al., 1999	Control				34:66	36	0-150	≥2	24.0
	Adolac ^c	N		0.55					23.3
Rueggsegger and Schultz, 1985	Soybean meal					58	0-105	≥2	22.7
	Heat treated soybeans	N							22.4
Salfner et al., 1995	Control				45:55	47	-14- 35	≥1	15.3
	Ttallow	N	2	0.40					15.5
Schingoethe and Casper, 1991	Control				50:50	153	0-28		20.8
	Oilseeds	N							20.4
Schneider et al., 1988	Control				44:56	108	14-135	≥1	
	Megalac ^b	N		0.50					
	Control					48	14-63		
Scott et al., 1995	Adolac ^c	N		0.45					
	Control				45:55	220	0-200	≥1	
Simas et al., 1995	Megalac ^b	N		0.45					
	Dry-rolled sorghum (DRS)				34:66	36	5-96	≥1	21.6
Simas et al., 1998	DRS + Megalac ^b	Y	2.5						17.8
	Steam-flaked sorghum (SFS)								23.1
	SFS + Megalac ^b	Y	2.5						19.5
Sklan et al., 1991	Dry-rolled sorghum				38:62	40	85 ± 50	≥1	28.9
	DRS + Megalac ^b	N	2.5		37:63				30.8
	Steam-flaked sorghum	Y			38:62				26.7
	SFS + Megalac ^b	N	2.5		37:63				26.1
	SFS + Megalac ^b	N	5		36:64				26.8
Sklan et al., 1994	Control				25:75	126	0-120	≥2	20.3
	Ca soaps of fatty acids	N	2.6	0.53					20.2
Sklan et al., 1994	Control				36:64	66	0-120	≥2	20.6
	Adolac ^c	N	2.5	0.51					20.3

Table 2, continued. Studies presented in Figures 3 to 6. Dietary treatment, no of cows, parity and DMI.

Reference	Dietary treatment	Isocaloric ^a Y/N	Extra nutrients (% DM)	Extra nutrients (kg/d)	forage : concentrate (DM basis)	Cows (no)	Treatment period (DIM)	Parity	DMI (kg DM/d)
Son et al., 1996	Control, low protein				49:51	34	14-84		24.2
	Tallow, low protein	Y	3	0.72	65:35				22.8
Spicer et al., 1993	Control, high protein				49:51	34			25.1
	Tallow, high protein	N	3	0.68	65:35				24.1
	Control				39:61	14	0-84	≥2	25.9
	Megalac ^b	N	1.8	0.36					24.4
<i>Glucogenic/lipogenic studies</i>									
Drackley et al., 2003	control				60:40	18	161	≥1	21.0
	high fat (white grease)	N	3						20.1
	high concentrate level (corn)	Y			40:60				21.3
Grum et al., 1996	low ground shelled corn level				45:55	8	52	≥2	19.2
	LC + prilled LCFA	N	3	0.621					20.7
	high ground shelled corn level	N			70:30				20.2
	HC + prilled LCFA	N	3	0.582					19.4
Hurtaud et al., 1998	control				70:30	4	60		16.6
	VFA infusion (rumen)	N							16.4
	propionate infusion (rumen)	N							16.6
	glucose infusion (duodenum)	N							16.5
Oldick et al., 1997	control				26:74	4	111 ± 14	≥2	22.5
	glucose infusion (abomasal)	N		1.00					22.4
	tallow infusion (abomasal)	N		0.45					22.7
	yellow grease inf. (abomasal)	N		0.45					21.5
Ruppert et al., 2003	High alfalfa silage (HA)				50:50	6	31	≥2	24.8
	HA + tallow	N	4						22.9
	High corn silage (HC)	Y			50:50				22.6
	HC + tallow	N	4						21.4
Vazquez-Anon et al., 1997	high fat diet (tallow)				65:35	43	150 ± 3	≥2	21.6
	high grain diet (ground corn)	Y			50:50				22.5
Voigt et al., 2003	Tapioca			2.00		32	0-100		20.9
	Ca salts of palm and soya oil	Y							20.2
<i>Glucogenic studies</i>									
Amaral et al., 1990	control				86:14	4	60-90	≥2	18.5
	glucose infusion (intravenous)	N		0.34					18.8
	glucose infusion (intravenous)	N		0.74					19.7
Armstrong et al., 1990	low concentrate level					90	0-350	≥1	
	medium concentrate level	N		4					
	high concentrate level	N		7.2					
Beever et al., 1989	molassed sugar beet feed				30:70		21	≥2	16.3
	barley	N							15.9
Carrol et al., 1990	high forage:concentrate ratio				84:16	46	5-100	≥2	20.9
Jerred et al., 1990	low forage:concentrate ratio	N			45:55				24.8
Gong et al., 2002	control							2.8 ±	
	extra starch	Y	16						
Gordon et al., 1995	fibre concentrate (sugarbeetpulp,citruspulp,cottonseed)					6	32 ± 7	≥2	17.4
	starch concentrate (barley, wheat, maize)	N							17.0
Grummer et al., 1984	control				50:50	6	49-133	≥2	18.8
	high concentrate (74%groundshelled corn, 21%soyabean meal)				17:83				17.3
Hoedemaker et al., 2004	control					234	-13-12	≥1	
	propylene glycol	N		1					
Hurtaud et al., 2000	control				48:52	4			19.9
	glucose infusion (duodenum)	Y		2.25					16.5
Keady and Mayne, 1998	low starch (0.5%)				52:48	50	46 ± 17		16.6
	high starch (38.4%)	Y							16.4
Knowlton et al., 1998	control				50:50	4	24 ± 2.1	≥2	22.5
	starch infusion (abomasum)	N		1.5					21.8
	starch infusion (rumen)	N		1.5					21.7

Table 2, continued. Studies presented in Figures 3 to 6. Dietary treatment, no of cows, parity and DMI.

Reference	Dietary treatment	Isocaloric ^a Y/N	Extra nutrients (% DM)	Extra nutrients (kg/d)	forage : concentrate (DM basis)	Cows (no)	Treatment period (DIM)	Parity	DMI (kg DM/d)
Leonard and Block, 1997	control glucose infusion (intravenous)	N			46:54	10	15-41	≥2	19.2 17.8
Minor et al., 1998	control high non-fibre carbohydrate	N			40:60	75	-19-280	≥1	21.2 21.3
Miyoshi et al., 2001	control propylene glycol	N		0.50	50:50	35	0-42	≥1	15.7 15.4
Patton et al., 2004	Megalac ^b + barley Megalac ^b + propionate + propylene glycol			0.41 0.41	61:39	24	0-350	≥2	13.6 16.1
Pushpakumara et al., 2003	control extra barley	N		0.80	50:50	20	-42 - 0	≥2	15.7 16.0
Rigout et al., 2003	control Glucose infusion (duodenum) Glucose infusion (duodenum) Propionate infusion (rumen) Propionate infusion (rumen)	Y N Y N		1.72 ^d 3.45 ^d 1.72 ^d 3.45 ^d	55:45	5	53 ± 12		15.6 16.6 16.7 16.0 16.3
Sutton et al., 1991	Molassed sugar beet feed + fishmeal Barley + fishmeal Molassed sugar beet feed + soyabean meal Barley + soyabean meal	N N N N			50:50 48:52	16	0-126	≥2	16.0 15.5 15.5 16.0

^a Treatment diet is isocaloric compared to control diet: Y(es)/N(o)

^b Ca salts of palm fatty acids

^c Ca salts of long chain fatty acids (LCFA)

^d in Mcal/d

^e Ca salts of palm fatty acids, extracted rapeseed meal and whey permeate

CHAPTER 3

Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition

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Abstract

Nutrition has been indicated to be important in limiting the severity of a negative energy balance (NEB) in dairy cows in early lactation. NEB related metabolic problems suggest a role for the balance in supply of lipogenic and glucogenic nutrients. To test the effect of lipogenic and glucogenic nutrients on energy partitioning, energy and nitrogen balance of 16 lactating dairy cows were determined by indirect calorimetry in climate-respiration chambers from week 2 to 9 postpartum (pp). Cows were either fed a diet high in lipogenic nutrients or a diet high in glucogenic nutrients from week 3 prepartum till week 9 pp. Diets were isocaloric (net energy basis) and equal in intestinal digestible protein. There was no effect of diet on metabolisable energy intake and heat production. Cows fed the lipogenic diet partitioned more energy to milk than cows fed the glucogenic diet (1175 ± 18 vs. 1073 ± 12 kJ/(kg^{0.75}·d)) and had a higher milk fat yield (1.89 ± 0.02 vs. 1.67 ± 0.03 kg/d). The increase in milk fat production was caused by an increase in C16:0, C18:0 and C18:1 in milk fat. No difference was found in energy retained as body protein, but energy mobilised from body fat tended to be higher in cows fed the lipogenic diet than in cows fed the glucogenic diet (190 ± 23 vs. 113 ± 26 kJ/(kg^{0.75}·d)). Overall, results demonstrate that energy partitioning between milk and body tissue can be altered by feeding isocaloric diets differing in lipogenic and glucogenic nutrient content.

Introduction

Late gestation and early lactation can be considered as the most critical period for high producing dairy cows. This period is often characterised by metabolic disorders like fatty liver, ketosis (Grummer, 1993) and ruminal acidosis (Owens et al., 1998), and a reduced fertility (Butler, 2003). Metabolic and reproductive disorders in dairy cows in early lactation have been allocated to a negative energy status, resulting from a genetic potential for high milk production accompanied by a delay in feed intake peripartum (Veerkamp et al., 2003).

Several reviews indicated nutrition to be an important factor in the prevention and severity of negative energy balance (NEB) and related metabolic disorders (e.g. Gong, 2002; Grummer and Carroll, 1988)). The characteristics of NEB related metabolic problems suggest a role for the balance in availability of lipogenic and glucogenic nutrients (chapter 2) (Adler, 1970). In ruminants, lipogenic nutrients either originate from fibre that stimulates the ruminal production of acetate and butyrate or originate from dietary fat or are derived from body reserves. Glucogenic nutrients originate from starch escaped from rumen degradation or gluconeogenesis. In descending order of importance, propionic acid, glucogenic amino acids and lactic acid are the main contributors to gluconeogenesis in ruminants (Brockman, 2005). The contribution of intestinally digested starch towards glucose is highly variable, depending on factors including dietary source of rumen resistant starch, technological treatment and intake level (Mills et al., 1999).

Over 40 years ago, it was already suggested that milk-fat depressing diets lower the priority for milk fat production relative to fat deposition in body reserves (Van Soest, 1963). This implies that lipogenic nutrients, which increase milk fat yield (Jerred et al., 1990), increase the partitioning of metabolisable energy (ME) into milk (Baldwin et al., 1985) and consequently decrease the partitioning of ME into body reserves. However, the effect of lipogenic nutrients on milk fat also depends on the degree and type of saturation of dietary fat. Poly-unsaturated fatty acids and specific intermediates of their biohydrogenation in the rumen, notably C18:2, trans10, cis12, depress milk fat (Bauman et al., 2006) in contrast to saturated fat sources (Baumgard et al., 2001; Schroeder et al., 2003). On the other hand, glucogenic nutrients decrease milk fat content (e.g. Grum et al., 1996a; Ruppert et al., 2003)) and increase plasma insulin (Miyoshi et al., 2001). These observations suggest that glucogenic nutrients stimulate body fat deposition and the partitioning of ME into body tissue. This indicates possibilities to improve the energy balance of dairy cows in early lactation by increasing the availability of glucogenic nutrients at the expense of lipogenic nutrients.

In addition, not only milk fat yield, also milk fatty acid composition is affected by lactation stage (Garnsworthy et al., 2006; Palmquist et al., 1993) and diet composition (Chilliard and Ferlay, 2004). Both dietary fat addition and body fat mobilisation increase the bioavailability of C18 fatty acids (Ward et al., 2002). Furthermore, the

early lactation period is usually characterised by a higher concentrate intake compared with the mid and late lactation or dry period, which results in a lower ruminal acetate/propionate ratio (Bannink et al., 2006). Accordingly, the availability of precursors for *de novo* lipogenesis is reduced (Bauman and Griinari, 2003), resulting in a decrease in medium and short-chain fatty acids in milk fat. Thus dietary fat, body fat and the reduced acetate/propionate ratio may increase the proportion of long chain fatty acids in milk fat. Hence a relationship between dietary energy source and a NEB with milk fat composition can be expected. However, very few comparisons on the interaction between body fat mobilisation and diet composition on milk fat composition have been made (Smith et al., 1978).

The objective of this study was to compare on an isocaloric basis the effects of a mainly glucogenic or a mainly lipogenic diet on energy partitioning and milk fat composition in early lactation dairy cows in a NEB. The companion paper (chapter 4) focuses on the effects of dietary energy source on blood metabolites and metabolic hormones and their relationship with the determined energy balance by indirect calorimetry.

Materials and methods

Experimental design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. Energy balance of 16 lactating dairy cows was determined as energy retention in body mass (ER) using climate-respiration chambers (Verstegen et al., 1987) over 8 successive balance periods (1 week) from week 2 to week 9 postpartum (pp). The experiment was divided in 4 groups. Within each group 2 pairs of 2 cows were assigned to 2 experimental diets 3 weeks prepartum. Experimental diets were either mainly lipogenic or mainly glucogenic.

Animals and housing

Sixteen Holstein-Friesian dairy cows, 4 cows per group, with comparable milk production (> 9500 kg fat and protein corrected milk per 305 d), were selected from a group of 44 cows which were inseminated at synchronised ovulation (the Crestar+ method, Intervet, Boxmeer, The Netherlands). Ovulation was synchronised to obtain natural calving cows with at maximum 4 d variation in calving date. Selection of the 16 experimental cows was based on calving date. Post calving, parity of the selected cows ranged from 2 to 4. Within 1 week pp 4 cows were transported and housed, (2 cows per chamber) in 2 identical, large, open-circuit, indirect climate-respiration chambers (Verstegen et al., 1987). Per group dietary treatment was assigned alternately to one of the 2 chambers. The cows were housed in the chamber in a tie stall. Chamber temperature was maintained at 16°C and relative humidity was set at

70%. Air velocity was < 0.2 m/s. Cows were exposed to 16 h of light (0600h to 2200h) and 8 h of darkness. Body condition was scored (1-5 scale) every 3 weeks from week 3 prepartum till week 9 pp. Body weight was recorded at start and end of each one-week balance period. Cows were milked twice daily (0600h and 1700h) in the climate respiration chamber with a mobile milking system.

Diets

Three weeks prepartum cows were fed 1 kg/d of the experimental concentrates, succeeded by 2 kg/d in the last week prepartum. Roughage did not differ between diets and was supplied ad libitum and consisted of grassilage, cornsilage and wheat straw in a ratio 45:45:10 (DM basis). Postpartum, wheat straw was replaced by 1.5 kg/d of chopped alfalfa hay. Concentrate supply was increased stepwise by 0.5 kg/d pp, until concentrate intake reached 10.0 kg/d with a concentrate to forage ratio of 40:60 (DM basis). Ingredient and chemical composition of both concentrates and diets are presented in Table 1 and 2. Calculated fatty acid composition of the concentrates is presented in Table 3. Diets were fed as total mixed ration (TMR), were isocaloric according to the Dutch NE for lactation system (Van Es, 1975) and were equal in intestinal digestible protein and degraded protein balance (DVE/OEB system) (Tamminga et al., 1994). Diets were fed twice a day in equal proportions prior to milking.

Energy and nitrogen balance

Faeces with urine were collected quantitatively per chamber, pooled per balance period, sampled for energy and nitrogen analysis and stored at -20°C until analysis. Feed intake was monitored per cow per balance period and feed samples were collected per day and pooled per balance period, sampled for energy and nitrogen analysis and stored at 4°C until analysis. Milk was sampled each milking, 3 g/kg milk, pooled per cow per balance period and stored at 4°C during the balance period and at -20°C after each balance period. Gross energy (GE) values of feed, energy in faeces with urine and energy in milk were measured using bomb calorimetry (IKA-C700, Janke & Kunkel, Heitersheim, Germany) and N content by Kjeldahl analysis. Intake of ME per chamber was calculated from the GE content of the feed, faeces with urine and produced methane. Heat production (HP) was determined indirectly at 9-min intervals by measuring the exchange of oxygen, carbon dioxide, and methane as described earlier (Verstegen et al., 1987). Energy retention in body mass was calculated by subtracting the HP and energy in milk from the ME. The total N retention (NR) (g/d) was estimated from N in feed, faeces, urine, milk, dust, water that condensed on the heat exchanger and in acidified liquid samples through which outflowing air from the chambers was led to trap aerial ammonia. Energy retention as body protein (ER_p) was derived from the protein gain ($N \times 6.25$; exception N in milk $\times 6.38$) multiplied by 23.6 kJ/g (energetic value of body protein (ARC, 1980)). The energy retention as fat (ER_f) was calculated from the difference between ER and ER_p. Energy retention data

are expressed per $\text{kg}^{0.75}$, where the mean body weight per cow per balance period was used to calculate the metabolic body weight.

Table 1. Ingredient and chemical composition of glucogenic and lipogenic concentrate.

Item	Concentrate	
	Glucogenic	Lipogenic
Ingredient, g/kg		
Rapeseed meal	127.2	98.5
Lupins		166.2
Maize	265.6	
Maizeglutenfeed		63.3
Milocorn	250.0	
Palmkernel, expeller	31.6	125.0
Sugar beet pulp		294.5
Peas	131.5	
Soyabean meal, formaldehyde treated	124.0	
Rapeseed meal, formaldehyde treated		148.3
MEGALAC ¹		25.0
Molasses-cane	40.0	40.0
Palmoil		25.0
Calcium carbonate	13.6	
Magnesium oxide	0.7	
Sodium chloride	8.3	6.6
Mineral-vitamin mixture ²	7.5	7.5
Chemical composition		
DM, g/kg	873.2	895.8
Crude protein, g/kg DM	201.8	212.7
Crude fat	31.0	85.9
NDF	152.2	350.6
Starch	448.0	3.8
Ash	60.9	98.5
DVE ³	137	134
OEB ⁴	13	22
NE _L ⁵ , MJ/kg	6.69	

¹ Ca salts of palm fatty acids (Volac, Hertfordshire, United Kingdom).

² Contained per kg mix: 115 g Ca (CaCO₃); 385 mg Mg (MgO); 1.67 mg Cu (CuSO₄·5H₂O); 1.34 mg Mn (MnO₂); 2.67 mg Zn (ZnSO₄·7H₂O); 0.04 mg Co (CoSO₄·7H₂O); 0.13 mg I (KI); 0.03 mg Se (Na₂SeO₃); 667000 IU Vitamin A; 160000 IU cholecalciferol (Premix 2031, PreMervo, Utrecht, The Netherlands).

³ Intestinal digestible protein (Tamminga et al., 1994).

⁴ Degraded protein balance (Tamminga et al., 1994).

⁵ Net energy for lactation calculated with VEM system (Van Es, 1975).

Table 2. Ingredient and chemical composition of glucogenic and lipogenic diet¹

Item	Diet	
	Glucogenic	Lipogenic
Ingredient, g/kg		
Corn silage ²	388.3	388.4
Grass silage ³	388.3	388.4
Chopped alfalfa hay ⁴	29.1	29.1
Concentrate	194.3	194.3
Chemical composition		
DM, g/kg	472	482
Crude protein, g/kg DM	162	169
Crude fat	34	54
NDF	324	396
ADF	214	260
Starch	267	95
Ash	78	84
DVE ⁵	88	86
OEB ⁶	14	18
NE _L ⁷ , MJ/kg DM	6.67	6.59

¹ Based on 40:60 concentrate to forage ratio (dry matter basis).

² Corn silage, g/kg: 316 DM, 291 OM, 24 crude protein, 9 crude fat, 134 NDF, 101 starch and sugars, 16 DVE, -10 OEB (Blgg, Oosterbeek, The Netherlands).

³ Grass silage, g/kg: 326 DM, 290 OM, 64 crude protein, 13 crude fat, 170 NDF, 8 sugars, 21 DVE, 21 OEB (Blgg, Oosterbeek, The Netherlands).

⁴ Chopped alfalfa hay, g/kg: 891 DM, 840 OM, 187 crude protein, 28 crude fat, 338 NDF, 42 starch and sugars (Blgg, Oosterbeek, The Netherlands).

⁵ Intestinal digestible protein (Tamminga et al., 1994).

⁶ Degraded protein balance (Tamminga et al., 1994).

⁷ Net energy for lactation calculated with VEM system (Van Es, 1975).

Milk yield and composition

Milk yield was recorded daily. Milk samples for fat, protein and lactose analysis (ISO 9622, Melkcontrolestation, Zutphen, The Netherlands) were collected 4 times per balance period (two a.m. and two p.m. milkings). An individual morning sample per balance period was stored at -20°C until analysis for milk fatty acid composition.

For milk fatty acid analysis, the stored individual milk samples were heated to 50°C and directly centrifuged (20 min at 1600g) at 4°C. The upper layer, fat and cream, was collected and filtered on folded filter paper. The fat-and-cream mixture was collected from the filter and stored overnight at -20°C. The mixture was heated for 10 min at 50°C. The oily substance was centrifuged at room temperature (5 min at 1130g) and the fat fraction was transferred to a tube containing a small amount of anhydrous

sodium sulphate. The milk fats were stored at -20°C until analysis. Before analysis, samples were heated to 50°C . A portion of $50\ \mu\text{l}$ was taken, weighed and added to 5 ml of hexane, containing 0.02% C23 (Alltech, Breda, The Netherlands) as an internal standard. The glycerol-bound fatty acids were transesterified to methyl esters by vortexing 1 min with $200\ \mu\text{l}$ sodium methanolate in methanol (30%). The solution was neutralised with 1 g sodium hydrogen sulphate and dried with anhydrous sodium sulphate. Fatty acid methyl esters (FAME) were injected into a gas chromatograph (Carlo Erba HRGC Mega 2), equipped with a flame ionisation detector, detector temperature 260°C . The carrier gas was helium, inlet pressure 330 kPa. Samples were injected by split injection (split ratio 1:20), injection temperature was 260°C . Separation of FAME was performed with a Supelco column (SP 2560, Sigma-Aldrich, Zwijndrecht, The Netherlands) ($100\ \text{m} \times 0.25\ \text{mm} \times 0.2\ \mu\text{m}$). The oven temperature was programmed from 140°C for 4 min, followed by an increase of $4^{\circ}\text{C}/\text{min}$ to 240°C and held for 30 min. FAME concentrations were determined with Chrom Card program (Thermo Finnigan, Milan, Italy) using Supelco FAME mix (Sigma-Aldrich, Zwijndrecht, The Netherlands) as a standard.

Table 3. Fatty acid composition of the of glucogenic and lipogenic concentrate¹.

Item	Concentrate	
	Glucogenic	Lipogenic
Fatty acid, g/kg DM		
≤10:0	0.14	0.55
12:0	0.97	3.86
14:0	0.37	1.37
16:0	3.18	12.16
16:1	0.10	0.06
18:0	0.50	1.71
18:1	8.07	29.24
18:2	11.31	11.86
18:3	0.89	0.97
≥20:0	0.34	1.67
Total	25.89	63.44

¹Calculated according to Centraal Veevoederbureau (CentraalVeevoederbureau, 2005).

Statistical analysis

In group 2 and 3, in total 3 cows were excluded from the experiment because of a left displaced abomasum, 1 cow fed a lipogenic diet, 2 cows fed a glucogenic diet. Therefore, values are based on 13 cows (glucogenic diet: $n = 6$; lipogenic diet: $n = 7$) for analysis of milk yield, energy in milk and milk fat, protein and lactose (model 1) or 6 climate-respiration chambers ($n = 3$ per dietary treatment per lactation week) for analysis of GE, ME, methane production, HP, ER, ER_p and ER_f (model 2). Whereas multiple measurements per animal cannot be regarded as independent units of observation, repeated measures analysis of variance (PROC MIXED (Littell et al., 2006)

of SAS[®] VERSION 9.1) was performed. Cow (model 1) or chamber (model 2) was included as the repeated subject. Diet (glucogenic or lipogenic), week (2 till 9 pp) and their interaction were included in the model as fixed effects. In case of lack of significance ($P > 0.05$), the diet×week interaction was excluded from the model. A first-order autoregressive structure (AR(1)) was the best fit and was used to account for within-cow variation (model 1) or within-chamber variation (model 2). Model assumptions were evaluated by examining the distribution of residuals. Values are presented as Least Square Means with their SEM.

Results

Animal performance

Body weight (595 ± 14 kg) and BCS (2.7 ± 0.1) at entry of the climate-respiration chambers one week pp were not different between dietary treatments. At the end of the experiment, week 9 pp, body weight (548 ± 13 kg) and body condition score (2.3 ± 0.1) also did not differ between dietary treatments. Dry matter intake and milk yield did not differ between diets (Table 4), but both increased linearly with time pp ($P < 0.05$). Dry matter intake increased from 16.5 kg/d (± 0.6 kg/d) in week 2 to 22.7 kg/d (± 0.2 kg/d) in week 9 pp. Milk yield increased from 34.2 kg/d (± 0.7 kg) in week 2 to 41.5 kg/d (± 0.7 kg/d) in week 9 pp. Milk fat content and daily milk fat yield were higher ($P < 0.05$) in cows fed the lipogenic diet compared with cows fed the glucogenic diet.

Table 4. Dry matter intake, milk production and milk composition of dairy cows fed a mainly glucogenic or lipogenic diet during week 2 till week 9 of lactation (LSM).

Variable	Diet			<i>P</i> -values ¹		
	Glucogenic	Lipogenic	SEM	Diet	Week	D×W ¹
Dry matter intake,	20.8	20.7	0.5	0.94	<0.01	ns
Milk yield, kg/d	39.8	39.8	0.7	0.88	<0.01	ns
Milk lactose						
%	4.44	4.56	0.05	0.08	0.16	ns
kg/d	1.76	1.82	0.04	0.32	<0.01	ns
Milk fat						
%	4.27	4.81	0.15	0.02	<0.01	ns
kg/d	1.68	1.90	0.06	0.03	0.02	0.05
Milk protein						
%	3.11	3.13	0.06	0.64	<0.01	ns
kg/d	1.23	1.24	0.03	0.66	0.11	0.02

¹ Diet×week interaction was excluded from the model if not significant ($P > 0.05$).

Protein content, protein yield, lactose content and lactose yield did not differ between dietary treatments. Milk fat, milk protein and milk lactose content were affected by time pp ($P < 0.01$) (Figure 1). Daily milk fat yield and milk protein yield were affected by the diet×week interaction ($P < 0.05$), but effects of these interactions were small.

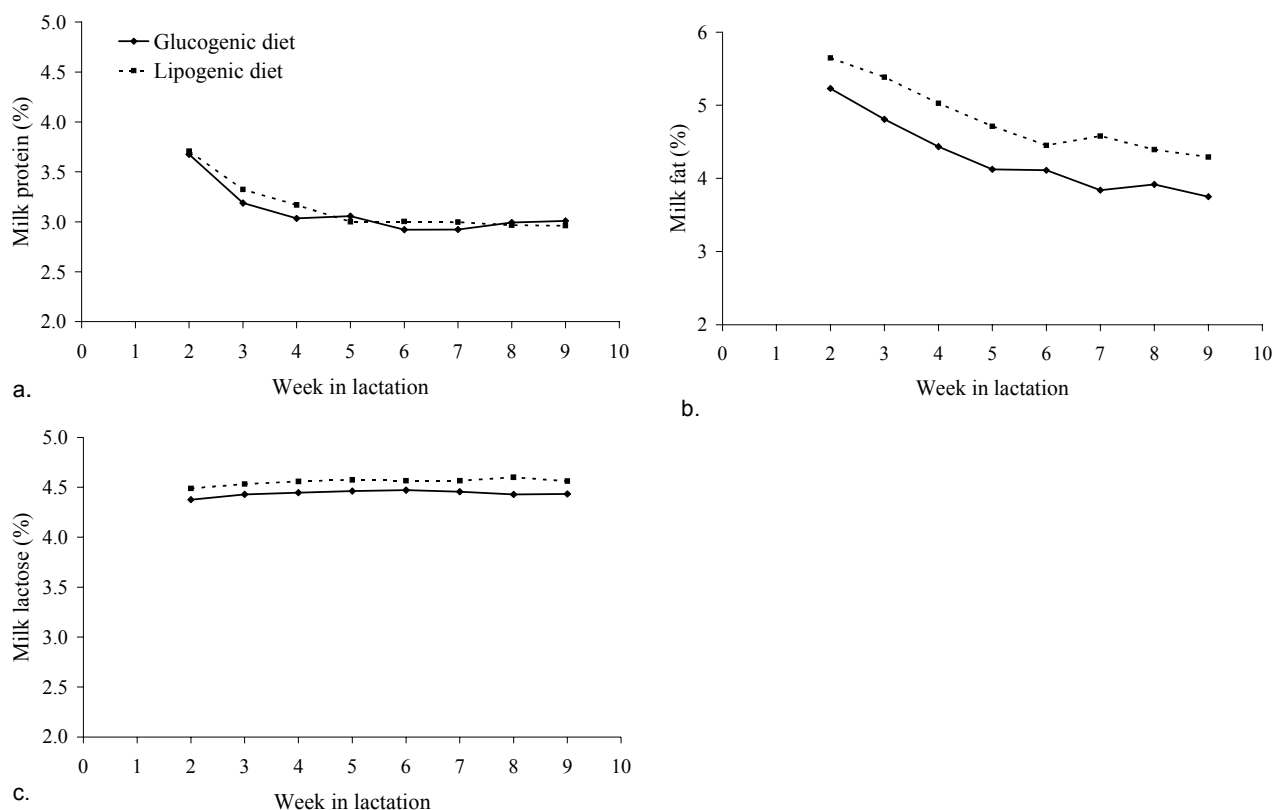


Figure 1. a. Milk protein content; b. Milk fat content; c. Milk lactose content of dairy cows fed a mainly glucogenic or lipogenic diet during week 2 till week 9 of lactation. Values represent means during 8 balance periods of 1 week. Overall SEM: milk protein, 0.03; milk fat, 0.07; milk lactose, 0.01.

Energy partitioning

GE intake, ME intake, methane production and heat production were not different between diets (Table 5). ME intake increased ($P < 0.01$) from week 2 till week 9 pp (Figure 2). The ME:GE ratio was not different between diets, but increased ($P < 0.01$) from week 2 till week 9 pp. Energy in milk was higher ($P < 0.05$) for cows fed the lipogenic diet, compared with cows fed the glucogenic diet. ER tended to be lower ($P = 0.12$) for cows fed the lipogenic diet, compared with cows fed the glucogenic diet. In more detail, ER_p did not differ between diets, but diets tended ($P = 0.09$) to be different for ER_f . For both diets, ER_p was positive from week 3 pp. ER_f was positive from week 8 pp for cows fed the glucogenic diet, whereas ER_f was still negative ($-11 \pm 39 \text{ kJ}/(\text{kg}^{0.75} \cdot \text{d})$) in week 9 pp for cows fed the lipogenic diet.

Table 5. Gross energy intake, metabolisable energy intake, methane production, heat production and energy retention in body mass of dairy cows fed a mainly glucogenic or lipogenic diet during week 2 till week 9 of lactation (LSM).

Variable	Diet		SEM	<i>P</i> -values ¹	
	Glucogenic	Lipogenic		Diet	Week
	kJ/(kg ^{0.75} ·d)				
Gross energy intake (GE)	3415	3492	122	0.67	<0.01
Metabolisable energy intake (ME)	2079	2124	74	0.68	<0.01
ME:GE ratio (%)	60.7	60.8	0.6	0.90	<0.01
Methane production	179	178	9	0.96	<0.01
Heat production	1096	1124	30	0.54	<0.01
Energy in milk	1075	1173	23	0.01	<0.01
Energy retention in body mass (ER) ²	-94	-172	28	0.12	<0.01
Energy retention as body protein (ER _p)	19	18	7	0.89	<0.01
Energy retention as body fat (ER _f)	-113	-190	24	0.09	<0.01

¹ Diet×week interaction was not significant ($P > 0.05$) for any of the variables.

² ER = GE – (Energy in faeces+urine) – Methane production – Heat production – Energy in milk (all in kJ/(kg^{0.75}·d))

Milk fat composition

Of short-chain fatty acids in milk fat, C6:0, C8:0, C10:0, C11:0 and C13:0 were higher for cows fed the glucogenic diet compared with cows fed the lipogenic diet ($P < 0.05$) (Table 6). Of the medium-chain fatty acids, the concentrations of C14:0 and C14:1 in milk fat were higher for cows fed the glucogenic diet ($P < 0.05$), C16:0 was higher for cows fed the lipogenic diet ($P < 0.05$). Of the long-chain fatty acids, the concentration of C18:0 was higher and C18:3 was lower ($P < 0.05$) for cows fed the lipogenic diet compared with cows fed the glucogenic diet. Total production of milk fatty acids was 195 g fatty acids per day more for cows fed the lipogenic diet compared with cows fed the glucogenic diet. This increase in milk fatty acid production could almost entirely be explained by an increase in secretion of C16:0 (96 g/d), C18:0 (51 g/d) and C18:1 (45 g/d). The daily production of medium chain fatty acids (\geq C14 and \leq C16) in milk fat increased linearly with week in lactation (Figure 3). The production of long chain fatty acids ($>$ C16) decreased linearly from week 2 until week 9 in lactation.

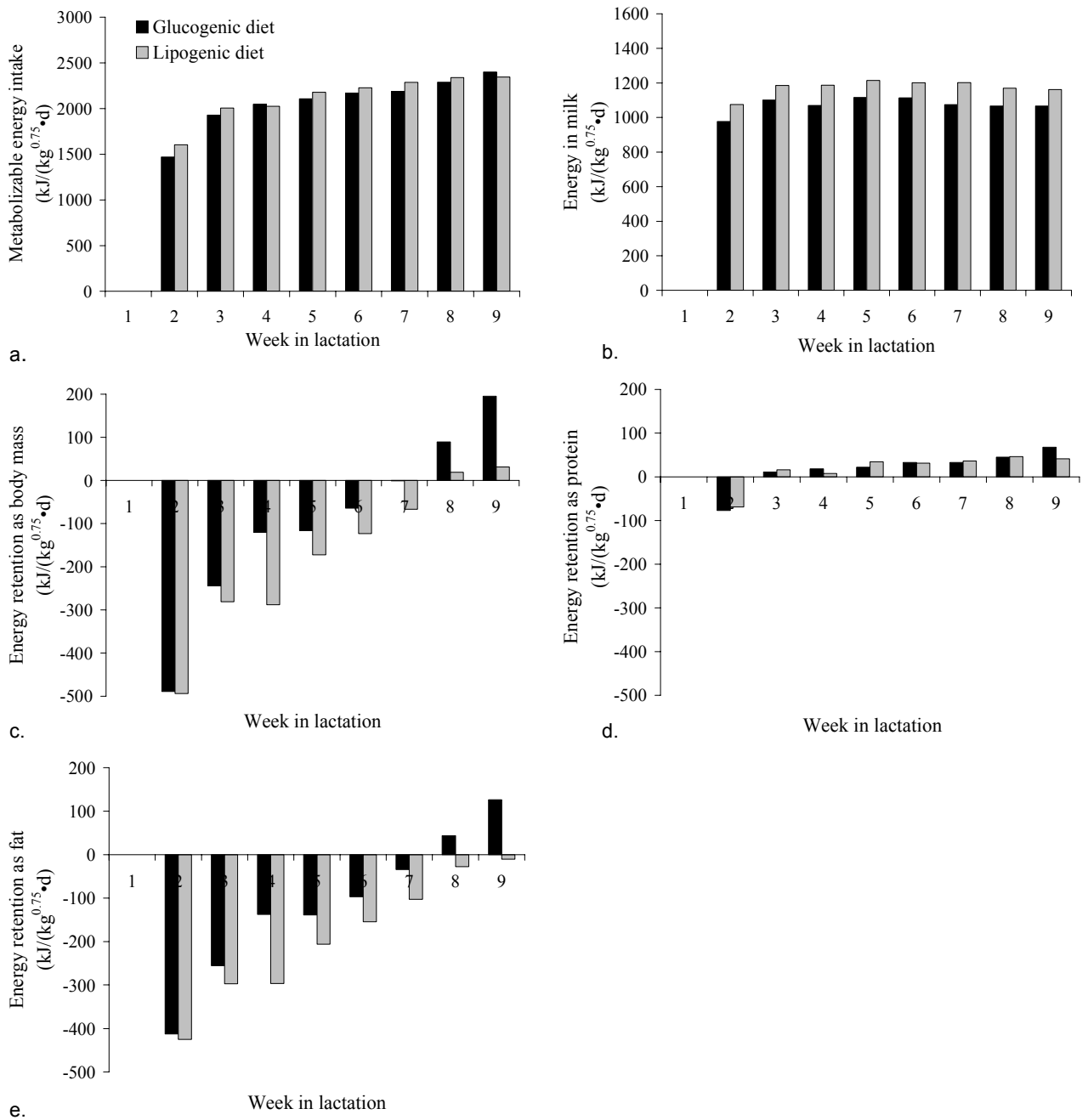


Figure 2. a. Metabolisable energy intake (ME); b. Energy in milk (NE_{milk}); c. Energy retention as body mass (ER); d. Energy retention as protein (ER_p) and e. Energy retention as fat (ER_f) of dairy cows fed a mainly glucogenic or lipogenic diet during week 2 till week 9 in lactation. Values represent means (kJ/(kg^{0.75}·d)) for 3 climate-respiration chambers with 2 cows each during 8 balance periods of 1 week. Overall SEM: ME, 74; NE_{milk}, 23; ER, 28; ER_p, 7; ER_f, 24.

Table 6. Milk fatty acid composition of dairy cows fed a mainly glucogenic or lipogenic diet during week 2 till week 9 of lactation¹.

Fatty acid	Diet		SEM	<i>P</i> -values ²	
	Glucogenic	Lipogenic		Diet	Week
	g/100g fatty acids				
4:0	6.36	6.19	0.33	0.71	0.03
6:0	2.61	2.23	0.10	0.02	<0.01
8:0	1.26	1.01	0.07	0.03	0.01
10:0	2.53	1.93	0.17	0.03	<0.01
11:0	0.24	0.16	0.02	<0.01	<0.01
12:0	2.69	2.44	0.16	0.29	<0.01
13:0	0.09	0.07	0.01	<0.01	<0.01
Total short-chain	15.80	14.00	0.66	0.07	<0.01
14:0	9.81	8.48	0.36	0.02	<0.01
14:1	0.67	0.49	0.04	0.01	<0.01
15:0	0.67	0.65	0.04	0.64	0.03
16:0	29.17	31.47	0.71	0.03	<0.01
16:1	0.98	1.30	0.20	0.26	0.07
Total medium-chain	41.34	42.38	0.97	0.44	<0.01
17:0	0.91	0.71	0.12	0.25	<0.01
17:1	0.25	0.19	0.02	0.02	<0.01
18:0	12.62	14.16	0.40	0.02	<0.01
18:1	25.74	25.41	1.05	0.82	<0.01
18:2	2.25	2.05	0.18	0.42	0.08
18:3	0.55	0.45	0.02	<0.01	0.72
20:0	0.42	0.34	0.13	0.64	0.01
20:1	0.07	0.06	0.01	0.32	0.84
20:4	0.02	0.03	0.01	0.38	<0.01
22:0	0.02	0.03	0.01	0.19	0.24
CLA	0.34	0.37	0.04	0.55	0.02
Total long-chain	42.85	43.42	1.28	0.66	<0.01

¹ Values are least square means.

² Diet×week interaction was not significant ($P > 0.05$) for any of the variables.

Discussion

This study agrees with other studies which observed an increase in milk fat percentage after feeding more lipogenic nutrients (e.g. Grum et al., 1996a; Moallem et al., 1999). However, these studies not only increased the proportion of lipogenic nutrients, but also the energy density and energy intake by adding lipogenic nutrients to the diet. Consequently, milk yield increased in the treatment group compared with the control diet. Feeding extra glucogenic nutrients, accompanied by an increase in energy intake,

resulted also in an increase in milk yield (Grum et al., 1996a; Hurtaud et al., 2000). However, extra glucogenic nutrients often decreased the milk fat percentage and increased the milk protein percentage. In contrast to these studies, the present study was designed to feed isocaloric diets with a contrast in lipogenic and glucogenic nutrient content. As a result GE and ME intake were not different between diets. Despite a similar quantity of available ME, cows fed the lipogenic diet partitioned more energy to milk compared with cows fed the mainly glucogenic diet. The increased partitioning of energy to milk resulted only in an increase in milk fat production, but had no effect on total milk yield or milk protein yield.

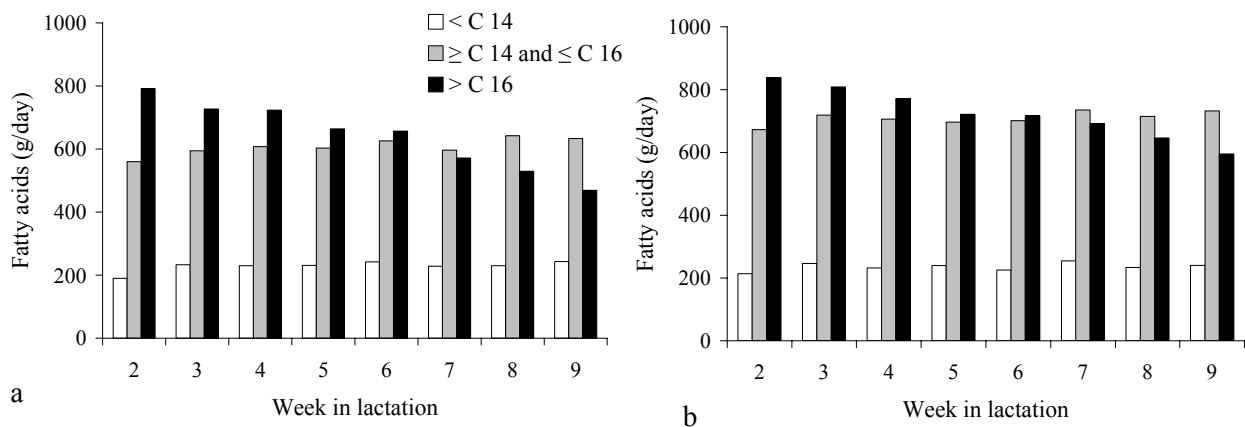


Figure 3. a. Milk fatty acid production (g/d) of dairy cows fed a mainly glucogenic diet from week 2 to week 9 in lactation; b. Milk fatty acid production (g/d) of dairy cows fed a mainly lipogenic diet from week 2 to week 9 in lactation. Values represent means per week. SEM for cows fed the glucogenic diet: < C14, 58; ≥ C14 and ≤ C16, 86; > C16, 128. SEM for cows fed the lipogenic diet: < C14, 39; ≥ C14 and ≤ C16, 63; > C16, 100.

In the current study, cows fed the glucogenic diet seem to lower the priority of milk fat production as indicated by a lower milk energy output and increase the priority of energy retention in body reserves as indicated by the numerically increased energy balance. This observation is in agreement with the review of Van Soest (1963) who suggested that milk fat depressing diets lower the priority of milk production relative to body reserves. In the present experiment, dietary energy source did not alter the body protein balance, but specifically the body fat balance was less negative with feeding a more glucogenic diet. Concerning the period of week 2 till week 9 pp, cows fed the glucogenic diet mobilised 18.6 kg body fat (1 g body fat = 39.8 kJ; Wenk et al., 2001) compared with 31.2 kg for cows fed the mainly lipogenic diet. Cows fed the lipogenic diet mobilised 226 g/d more body fat compared with cows of comparable bodyweight, body condition score and milk yield, but fed the mainly glucogenic diet (332 g vs. 558 g/d body fat respectively). The difference in body fat mobilisation is in accordance with 220 g/d more milk fat produced for cows fed the lipogenic diet compared with cows fed the glucogenic diet. After subtracting body fat mobilisation from the milk fat production, cows fed the glucogenic diet produced 1,348 g/d milk fat from ME intake compared with 1,342 g/d milk fat for cows fed the lipogenic diet.

This suggests the extra milk fat production for cows fed the lipogenic diet originates predominantly from body fat mobilisation that was increased with 68% as compared with cows fed a mainly glucogenic diet.

Energy retention as protein was positive for both treatment groups from week 3 pp onwards which is in agreement with Tamminga et al. (1997) who estimated changes in body composition with time after parturition. They reported body protein mobilisation to stabilise from week 2 pp and body protein gain from week 4 pp. Total estimated mobilisation of body reserves during the first 8 weeks of lactation was 41.6 kg and included 30.9 kg body fat and 2.4 kg body protein (Tamminga et al., 1997). This corresponds with the current study, where on average the cows mobilised 24.9 kg body fat and 5.2 kg body protein (1 g body protein = 23.6 kJ ARC, 1980) from week 2 to week 9 in lactation.

The results confirm the hypothesis that a mainly lipogenic diet during early lactation increases the secretion of long chain fatty acids in milk fat compared with a mainly glucogenic diet. Since the lipogenic diet has a higher dietary fat content, and since body fat mobilisation is higher compared with cows fed the glucogenic diet, it can be suggested that both sources of long chain fatty acids (dietary and endogenous) contribute to the increased secretion of long chain fatty acids in milk fat of cows fed the lipogenic diet. Besides the diet treatment also a negative energy status seems to have an effect on milk fat composition. A decrease from early to mid lactation in the production of long-chain fatty acids relative to medium-chain fatty acids has been reported earlier (Garnsworthy et al., 2006). This corresponds with the current study which showed that with increasing energy balance during week 2 to week 9 postpartum, the daily production of medium chain fatty acids ($\geq C14:0$ and $\leq C16:0$) increased and at the same time the production of long chain fatty acids ($\geq C18:0$) decreased (Figure 3).

Conclusions

This study confirms the hypothesis that energy partitioning between milk and body tissue can be altered by feeding isocaloric diets that differ in lipogenic and glucogenic nutrient content. While ME intake was not different, daily milk fat yield and energy in milk were higher in the lipogenic diet group. Consequently, the higher milk energy resulted in a tendency for less energy being partitioned into body fat for cows fed the lipogenic diet compared with cows fed the glucogenic diet.

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CHAPTER 4

Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones

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Abstract

Negative energy balance (NEB) related metabolic disorders suggest that the balance between available lipogenic and glucogenic nutrients is important. The objectives of this study were to compare the effects of a glucogenic or a lipogenic diet on liver triacylglycerides (TAG), metabolites and metabolic hormones in dairy cows in early lactation and to relate metabolite concentrations to the determined energy retention in body mass (ER). Sixteen dairy cows were fed either a lipogenic or glucogenic diet from week 3 prepartum to 9 postpartum (pp) and housed in climate-respiration chambers from week 2 to 9 pp. Diets were isocaloric (net energy basis). Postpartum, cows fed a lipogenic diet tended to have higher non-esterified fatty acid concentration (NEFA) (0.46 ± 0.04 vs. 0.37 ± 0.04 mMol/l) and lower insulin concentration (4.0 ± 0.5 vs. 5.5 ± 0.6 uIU/ml). No difference was found in plasma glucose, β -hydroxybutyrate, IGF-1 and thyroid hormones. Liver TAG was equal between both diets in week -2 and 2 pp. In week 4 pp cows fed the glucogenic diet had numerically lower TAG levels, although there was no significant dietary effect. Negative relationships were detected between ER and milk fat and between ER and NEFA. A positive relationship was detected between ER and insulin concentration. Overall, results suggest that insulin plays a regulating role in altering energy partitioning between milk and body tissue. Feeding lactating dairy cows a glucogenic diet decreased mobilisation of body fat compared with a lipogenic diet. The relative abundance of lipogenic nutrients, when feeding a more lipogenic diet is related to more secretion of lipogenic nutrients in milk, lower plasma insulin and higher plasma NEFA concentration.

Introduction

The negative energy balance (NEB) in early lactation is associated with metabolic disorders like fatty liver, ketosis (Grummer, 1993) and ruminal acidosis (Owens et al., 1998). Metabolites and metabolic hormones are recognized as signals in the interaction between NEB and metabolic disorders in dairy cows.

In an earlier published review (chapter 2) it was hypothesised that part of the metabolic effect of a NEB is an imbalance in C2/C3 nutrient ratio. C2 and C3 nutrients are or can split up into fragments containing two or three C atoms. C2 and C3 nutrients are directly supplied in the diet or are derived from larger compounds. This imbalance in C2/C3 nutrient ratio is induced by an excess of C2 nutrients, stimulated by body fat mobilisation, which can not be oxidized completely as a result of a deficiency in C3 nutrients. There are three major alternative pathways for C2 nutrients. Firstly, an increase in incomplete oxidation of C2 nutrients may occur, which causes an increase in plasma ketone body concentration, ultimately resulting in ketosis. Secondly, C2 nutrients can be esterified and stored in the liver as tri-acyl-glycerides (TAG), potentially causing a fatty liver. Thirdly, C2 nutrients can be transported to the mammary gland and transformed into milk fat (Palmquist and Mattos, 1978), resulting in an increased milk fat production.

It has been suggested (Drackley, 1999; chapter 2) that diets high in lipogenic nutrient content increase the severity and risk of ketosis and fatty liver by increasing the C2/C3 nutrient ratio. In dairy cows, lipogenic dietary ingredients like dietary fat, or forages that stimulate the ruminal production of acetate and butyrate, are expected to increase the availability of C2 nutrients. In contrast, glucogenic dietary ingredients are either fermented in the rumen to produce high amounts of propionate or are digested in the small intestine and absorbed as glucose. Additionally, in early lactation high-producing dairy cows experience NEB. A negative energy status promotes mobilisation of body fat (Tamminga et al., 1997) and increases the availability of C2 compounds.

The importance of an appropriate balance of lipogenic and glucogenic nutrients to optimize milk production efficiency and to decrease metabolic disorders has been addressed earlier (Adler, 1970; Drackley, 1999; Kronfeld, 1976). However, studies which test this hypothesis by feeding isocaloric diets differing in lipogenic and glucogenic nutrients are scarce. The accompanying paper showed that dietary energy source alters energy partitioning in dairy cows in early lactation (chapter 4). Although energy intake was not different between diets, cows fed a mainly lipogenic diet had a higher milk fat and milk energy output and an increased body fat mobilisation compared with cows fed a glucogenic diet. In order to obtain insight in the metabolic signals of this observed shift in energy partitioning, the objective of this study was to measure the effects of feeding a lipogenic or a glucogenic diet on liver TAG content,

metabolites and metabolic hormones. Various studies have dealt with relationships between plasma non-esterified fatty acids (NEFA), ketone bodies, glucose, insulin or milk fat and milk protein and the energy balance (EB) in dairy cattle (De Vries and Veerkamp, 2000; Kokkonen et al., 2005). Such relationships were based on the estimated EB, calculated from energy intake and milk production, and to our knowledge the measured EB has not been used in testing associations with metabolite and metabolic hormone concentrations in dairy cows in early lactation. Therefore, the second objective of this study was to relate blood metabolites, metabolic hormones and milk characteristics to the measured energy balance in early lactation dairy cattle.

Materials and methods

Animals, management and experimental design

The experiment was conducted at Wageningen University and Research Centre, The Netherlands. The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. Sixteen Holstein-Friesian dairy cows, with comparable milk production (> 9500 kg fat and protein corrected milk in 305 days), were selected from a group of 44 cows which were inseminated at synchronised ovulation (the Crestar+ method, Intervet, Boxmeer, The Netherlands). Ovulation was synchronised to obtain 4 groups of 4 natural calving cows with at maximum 4 d variation in calving date. Selection was based on calving date. Within each group, cows were assigned to either the glucogenic or lipogenic diet 3 weeks prepartum. Parity of the selected cows ranged from 2 to 4. Within 1 week postpartum (pp), 4 cows were transported and housed, paired by diet, in 2 identical, large, open-circuit, indirect climate-respiration chambers (Verstegen et al., 1987) for 8 weeks to determine energy retention in body mass (ER) as reported in the accompanying paper (chapter 3). Per group dietary treatment was assigned alternatively to one of the 2 chambers. In the climate respiration chamber, the cows were housed in a tie stall. Cows were exposed to 16 h of light and to 8 h of darkness. Cows were milked twice daily (0600h and 1700h) in the chamber using a mobile milking system.

Feeding

The complete ingredient and chemical composition of both diets are described in the accompanying paper (chapter 3). In summary, diets were fed as total mixed ration (TMR) and formulated to be isocaloric (NE basis) and equal in intestinal digestible protein and degraded protein balance (DVE/OEB system) (Tamminga et al., 1994). Postpartum, concentrate supply increased stepwise by 0.5 kg/d until concentrate intake reached 10.0 kg/d with a concentrate to forage ratio of 40:60 (DM basis). Forage consisted of grass silage, corn silage and chopped alfalfa hay in a ratio 45:45:10 (DM basis). Diets were fed twice daily in equal proportions prior to milking.

Sampling procedures

Liver biopsies were taken in week -2, 2 and 4 relative to calving. Prior to the biopsy, the biopsy site was clipped and disinfected. A stab incision was made at the location of the greater trochanter in the 11th intercostal space on the right side of the cow. Biopsy was obtained under local anesthesia (7 ml Lidocaine-HCl 2% with adrenaline, Alfasan Nederland B.V., Woerden, The Netherlands) with a 17G X 200mm biopsy needle. Approximately 300 mg wet weight of liver tissue was harvested by moving the biopsy needle several times in the direction of the contra lateral ulna. Tissue was kept at maximum 24 h on ice in a 0.9% NaCl solution. Subsequently, connective tissue was removed, the sample was weighed and stored at -20°C until analysis.

Samples of jugular blood were obtained weekly from week -2 until week 9 pp at 3 h after the a.m. feeding and immediately before liver biopsy if both were on the same day. Blood was collected in evacuated tubes (Vacuette[®], Greiner Bio-One, Kremsmünster, Austria) containing either NaF for glucose determination, Li-heparine for NEFA, BHBA, cholesterol, urea, triiodothyronine (T3) and thyroxin (T4) determination or EDTA for insulin-like growth factor 1 (IGF-1) and insulin determination. Plasma was obtained by centrifugation, aliquoted, and frozen at -20°C until analysis.

Analytical procedures

Blood and liver samples were analysed in a quality controlled veterinary laboratory (Stichting Kwaliteitsbewaking Medische Laboratoriumtechniek, Nijmegen, The Netherlands). Analyses for glucose, NEFA, BHBA and cholesterol were performed using commercial available kits (glucose: reagent 443355 for Synchron CX[®]7 analyser, Beckman Instruments B.V., Mijdrecht, The Netherlands; NEFA: FA 115 kit, RANDOX laboratories Ltd., Crumlin, UK; BHBA: Ranbut kit, RANDOX laboratories Ltd., Crumlin, UK; cholesterol: chol reagens for Synchron CX[®]5, Beckman Instruments B.V., Mijdrecht, The Netherlands). Insulin concentration was determined using a radioimmunoassay kit (Coat-a-Count[®] Insulin, Diagnostic Products Corporation, Los Angeles, USA). Triiodothyronine, T4 and IGF-1 were analysed as described by Gerrits et al. (1998). Liver tissue was handled as described by Van den Top et al. (1995) and analysed on a Synchron CX[®]5 analyser with a TAG reagent (Beckman Instruments B.V., Mijdrecht, The Netherlands). The accuracy of each assay was monitored with the use of a commercial reference serum sample (Bovine precision serum RANDOX laboratories Ltd., Crumlin, UK) and the outcome deviated <5% from the target values.

Statistical analyses

Three cows were excluded from the experiment because of left displaced abomasums. In group 2, one cow fed the lipogenic diet; and in group 3, two cows fed the glucogenic diet. Therefore, values are based on 13 cows (glucogenic diet: $n = 6$; lipogenic diet: $n = 7$) for analysis of metabolites and metabolic hormones (model 1) or 6 climate-respiration chambers ($n = 3$ per dietary treatment per lactation week) for analysis of relationships between ER and metabolites, metabolic hormones, milk fat and fat to protein ratio (model 2). Whereas multiple measurements per animal cannot be regarded as independent units of observation, repeated measures analysis of variance (PROC MIXED (Littell et al., 2006) of SAS[®] VERSION 9.1) was performed. Cow (model 1) or chamber (model 2) was included as the repeated subject. First, metabolites and metabolic hormones were analysed with diet (glucogenic or lipogenic), week (-2 until 9 pp) and their interaction included in the model as fixed effects (both as class variables) (model 1a). In case of lack of significance ($P > 0.05$), the diet \times week interaction was excluded from the model. Additionally, the same analysis with model 1 was performed for the prepartum and postpartum data separately. Secondly, to quantify metabolic relationships, plasma BHBA, insulin, liver TAG content and milk fat were analysed with NEFA and glucose included as fixed effects in the repeated measurements model 1 (model 1b) in order to obtain regression coefficients (β). Diet was not significant ($P > 0.05$) and therefore not included as a covariable. Thirdly, to test possible indicators for ER, energy retention as body fat (ER_f) and energy retention as body protein (ER_p) were analysed with either milk fat, fat to protein ratio, plasma glucose, NEFA, BHBA, insulin, IGF-1, T3, T4 or liver TAG content included as fixed effects in a repeated measurements model (model 2) to obtain regression coefficients (β). To avoid collinearity, week was not included in the model. Concerning the three mentioned analyses, a first-order autoregressive covariance structure (AR(1)) was the best fit and was used to account for within-cow (model 1a and 1b) or within-chamber variation (model 2). The only exception was liver TAG in which a compound symmetry covariance structure was best. The null model likelihood ratio test (PROC MIXED (Littell et al., 2006) of SAS[®] VERSION 9.1) confirmed that modelling the extra covariance was significant over the null model ($P < 0.001$). Model assumptions were evaluated by examining the distribution of residuals. Transformation (natural logarithm) of plasma insulin concentration was necessary to obtain normal distribution.

Results

Animal performance

The analyses of dry matter intake (DMI), ER, milk yield and milk composition are described in the accompanying paper (chapter 3). In summary, DMI, gross energy intake (GE), metabolisable energy intake (ME), heat production, milk yield (Figure 1) and milk protein (%) did not differ between diets. Milk fat (%) and daily milk fat yield were higher ($P < 0.05$) in cows fed the lipogenic diet compared with cows fed the glucogenic diet. ER was numerically lower ($P = 0.12$) for cows fed the lipogenic diet compared with cows fed the glucogenic diet. ER_p did not differ between diets, but ER_f tended to be lower ($P = 0.08$) for cows fed the lipogenic diet compared with cows fed the glucogenic diet.

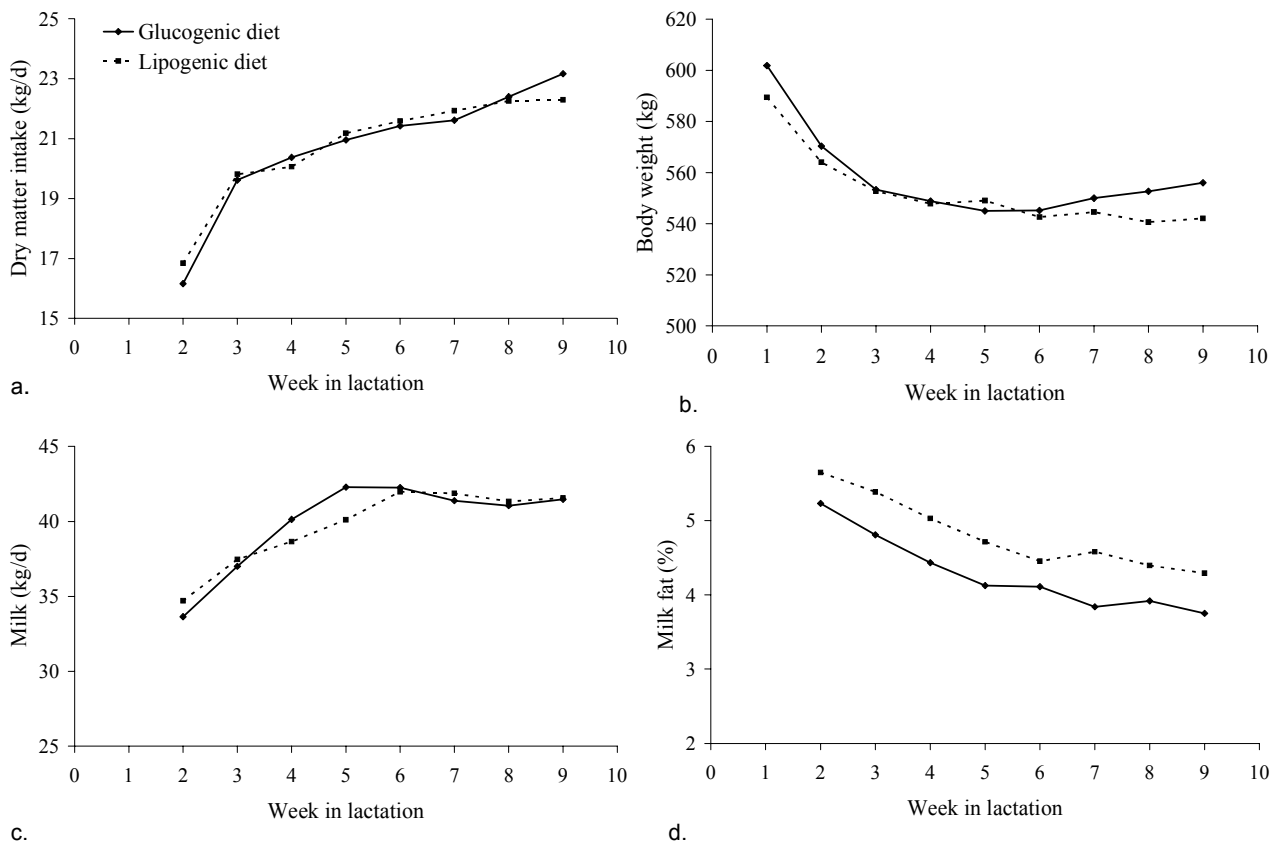


Figure 1. a. Dry matter intake (DMI) (kg/d); b. Body weight (kg); c. Milk yield (kg/d); d. Milk fat (%) from week 2 till week 9 postpartum for dairy cows fed a mainly glucogenic or lipogenic diet. Values represent lsmeans. Overall SEM: DMI, 0.05; bodyweight, 11.5; milk, 0.7; milk fat, 0.05.

Metabolites, metabolic hormones and liver TAG**Table 1.** Plasma NEFA, BHBA, cholesterol, liver TAG, plasma glucose, insulin, IGF-1, T4, T3, and urea from week -2 till week 9 postpartum for dairy cows fed a mainly glucogenic or lipogenic diet. Values represent lsmeans \pm SEM per diet, pooled prepartum or postpartum¹.

Variable	Diet ²	Prepartum	Postpartum	<i>P</i> -value ³		
				Diet	Week	D×W ⁴
		mMol/l unless otherwise stated				
NEFA	G	0.20 \pm 0.02	0.37 \pm 0.04	0.08	<0.01	<0.01
	L	0.23 \pm 0.02	0.47 \pm 0.04			
BHBA	G	0.64 \pm 0.04	1.93 \pm 0.20	0.34	<0.01	ns
	L	0.70 \pm 0.04	2.20 \pm 0.19			
Cholesterol	G	2.25 \pm 0.18	4.18 \pm 0.29	0.17	<0.01	0.01
	L	2.25 \pm 0.17	4.83 \pm 0.27			
Liver TAG (mg/g wet)	G	21.4 \pm 1.7	82.1 \pm 16.0	0.46	<0.01	ns
	L	18.1 \pm 1.6	107.3 \pm 14.2			
Glucose	G	3.46 \pm 0.10	2.51 \pm 0.12	0.85	<0.01	ns
	L	3.48 \pm 0.10	2.55 \pm 0.11			
Insulin ⁵	G	1.85 \pm 0.24	1.53 ^a \pm 0.08	0.11	<0.01	ns
	L	1.96 \pm 0.23	1.29 ^b \pm 0.07			
IGF-1 (ng/ml)	G	24.79 \pm 3.19	16.63 \pm 0.94	0.25	<0.01	ns
	L	32.14 \pm 2.98	16.83 \pm 0.88			
T4 (ng/ml)	G	16.02 \pm 1.07	11.41 \pm 0.50	0.52	<0.01	ns
	L	16.44 \pm 1.02	11.75 \pm 0.46			
T3 (ng/ml)	G	0.87 \pm 0.09	0.62 \pm 0.04	0.83	<0.01	ns
	L	0.86 \pm 0.09	0.63 \pm 0.03			
Urea	G	3.81 \pm 0.12	4.07 ^a \pm 0.16	<0.01	<0.01	ns
	L	4.10 \pm 0.11	4.87 ^b \pm 0.15			

¹ Prepartum = week -2 and -1; Postpartum = week 0, 1, ...9.² G = Glucogenic diet (n = 6 cows); L = Lipogenic diet (n = 7 cows).³ Analysed with model 1 with week = -2, -1, ...9 included.⁴ Diet×week interaction was excluded from the model if not significant (*P* > 0.05).⁵ Natural logarithm of plasma insulin concentration (uIU/ml).^{a,b} Values within a variable and within period (prepartum or postpartum) with different superscripts differ significantly (*P* < 0.05).

Plasma BHBA, cholesterol, glucose, IGF-1, T3 and T4 concentrations were not different between diets (Table 1). Plasma BHBA, cholesterol, glucose and IGF-1 concentrations were different (*P* < 0.01) over time (Figure 2). Plasma NEFA concentration tended (*P* = 0.08) to be lower for cows fed the glucogenic diet

compared with cows fed the lipogenic diet. A diet×week interaction ($P < 0.01$) was detected for plasma NEFA and cholesterol. Plasma NEFA concentration increased earlier (week 0) and decreased later for cows fed the lipogenic diet compared with cows fed the glucogenic diet. Plasma insulin concentration tended ($P = 0.11$) to be higher for cows fed the glucogenic diet compared with cows fed the lipogenic diet. Postpartum, the plasma insulin concentration was significantly higher ($P < 0.05$) for cows fed the glucogenic diet. Plasma urea was lower ($P < 0.01$) for cows fed the glucogenic diet compared with cows fed the lipogenic diet. Liver TAG content increased from week 2 prepartum till week 2 pp, but the increase was similar for both diets (Figure 2). From week 2 to week 4 pp liver TAG content decreased again. In week 4 liver TAG content was numerically lower ($P > 0.05$) for cows fed the glucogenic diet.

Relationships between metabolites and metabolic hormones

Plasma NEFA concentration was positively related to liver TAG content ($P < 0.01$) and milk fat percentage ($P < 0.05$) and negatively to plasma insulin concentration (Table 2). Plasma glucose was negatively related to plasma BHBA, liver TAG ($P < 0.01$) and milk fat ($P < 0.05$). There was a positive relationship ($P < 0.01$) between plasma glucose and insulin concentration.

Table 2. Regression coefficients (β) of NEFA and glucose related to plasma BHBA, TAG, insulin, milk fat and milk fat production.

	BHBA (mMol/l)	TAG (mg/g wet liver weight)	NEFA (mMol/l)	Insulin ¹	Milk fat (%)	Milk fat (kg/d)
Glucose (mMol/l)	-1.28 ^a	-29.3 ^b	-0.01	0.33 ^a	-0.38 ^a	-0.06 ^b
NEFA (mMol/l)	0.30	131.0 ^a		-0.82 ^a	0.90 ^a	0.14 ^b

¹ Natural logarithm of plasma insulin concentration (uIU/ml).

^a $P < 0.01$; ^b $P < 0.05$

Relationships with energy retention

ER, determined by indirect calorimetry in the climate-respiration chambers and reported in the accompanying paper, was negatively associated ($P < 0.01$) with milk fat (%) and plasma NEFA concentration (Table 3). There was a tendency ($P = 0.10$) for a positive relation between ER and plasma insulin. No significant relationships ($P > 0.05$) existed between ER and milk fat to protein ratio, liver TAG content, plasma

glucose, BHBA, IGF-1, T3 or T4. ER_f and ER_p showed a similar relationship as with overall ER.

Table 3. Regression coefficients (β) of energy retention in body mass (ER), energy retention as body fat (ER_f), energy retention as body protein (ER_p) related to milk fat, fat to protein ratio, plasma glucose, NEFA, BHBA, liver TAG, plasma insulin, IGF-1, T3 and T4.

	ER (kJ/kg ^{0.75} /d)	ER_f (kJ/kg ^{0.75} /d)	ER_p (kJ/kg ^{0.75} /d)
Milk fat (%)	-374.2 ^a	-298.7 ^a	-73.1 ^a
Fat to protein ratio	-297.9	-379.6	75.6
Glucose (mMol/l)	0.5	1.1	2.3
NEFA (mMol/l)	-597.0 ^a	-515.5 ^a	-84.4 ^c
BHBA (mMol/l)	-49.8	-43.6	-7.3
Liver TAG (mg/g wet weight)	-1.8	-1.3	-0.4
Insulin ¹	109.3 ^c	99.3 ^c	17.3
IGF-1 (ng/ml)	-0.6	-0.4	-0.1
T3 (ng/ml)	100.0	91.3	2.8
T4 (ng/ml)	-11.7	-8.8	-3.4

¹ Natural logarithm of plasma insulin concentration (uIU/ml).

^a $P < 0.01$; ^c $P < 0.10$

Discussion

The objective of this study was to obtain insight into the metabolic signals of a shift in energy partitioning (chapter 3) after altering dietary energy source in dairy cows in early lactation. The second objective was to relate metabolites, metabolic hormones and milk characteristics to the energy balance, as measured by indirect calorimetry. In the discussion we focus on how the measured metabolic effects of dietary energy source may be related to the effects on energy partitioning of dietary energy source.

It was shown (chapter 3) that feeding early lactation dairy cows a diet high in lipogenic nutrients resulted in an increase ($P < 0.05$) in milk fat yield. This increase was mainly caused by an increase in daily secretion of C16:0, C18:0 and C18:1 fatty acids. Dietary fat as well as mobilised body fat were higher for cows fed the lipogenic diet, both fat sources likely contribute to this increase in long chain fatty acids. Furthermore, the contrast in total fat available from both mobilised body fat reserves and dietary fat for maintenance and milk production increased during week 2 to 4 pp.

Based on observed dietary fat intake and mobilised body fat and assuming a fat digestibility of 0.86 (NRC, 2001), we estimated that in week 2 pp cows fed the glucogenic diet had 1.66 kg fat available per day for maintenance and milk

production. Cows fed the lipogenic diet had in week 2 pp 2.04 kg fat available per day. From week 2 to 4 pp, cows fed the glucogenic diet had a 42% decrease in fat availability to 0.96 kg per day in week 4. However, fat availability for cows fed the lipogenic diet decreased much less in this period (13% to 1.79 kg per day in week 4).

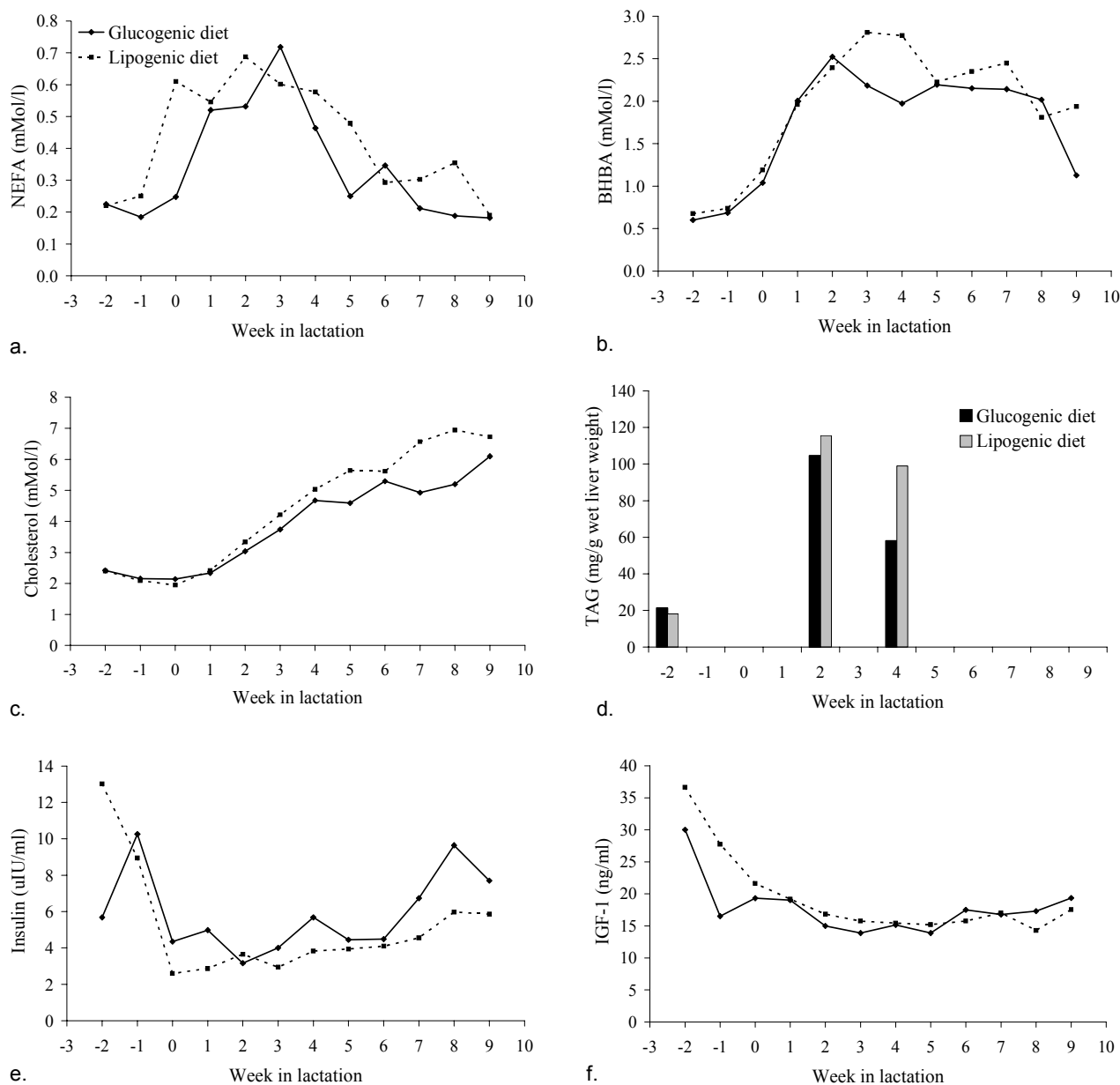


Figure 2. a..Plasma NEFA (mMol/l); b. Plasma BHBA (mMol/l); c. Plasma cholesterol (mMol/l) ; d. Liver TAG (mg/g wet liver weight); e. Plasma insulin (uIU/ml); f. Plasma IGF-1 (ng/ml) from week - 2 till week 9 postpartum for dairy cows fed a mainly glucogenic or lipogenic diet. Values represent lsmeans per diet per week. Overall SEM: NEFA, 0.03; BHBA, 0.16; cholesterol, 0.25; TAG, 10.0; Insulin 0.07 (natural logarithm); IGF-1, 18.63.

Thus, differences in fat availability could explain the dietary difference in plasma cholesterol concentration, a tendency for a difference between diets in plasma NEFA

concentration and possibly the numerical difference between diets in liver TAG in week 4. However, liver TAG content was not statistically different between diets. The rate of decrease in fat availability was equal for both groups after week 4. In addition, body fat mobilisation was negative in week 9 for cows fed the glucogenic diet and the BHBA concentration was lower ($P < 0.05$) compared with cows fed the lipogenic diet.

The difference in mobilised body fat and EB between diets, as reported in the accompanying paper (chapter 3), may be explained by effects of dietary energy source on plasma insulin concentration. In this study, a trend was found for a higher plasma insulin concentration in cows fed the glucogenic diet compared with cows fed the lipogenic diet. The difference between diets in plasma insulin concentration was significant when only the postpartum data were taken into account. Dietary effects on plasma insulin concentration were more pronounced pp, which can be explained by an increase in contribution of the experimental concentrates to the TMR pp. Insulin is known as a key regulator of nutrient repartitioning from body tissue to milk in the transition period from pregnancy to lactation (Laarveld et al., 1981). Low plasma insulin concentrations reduce glucose uptake by muscle and adipose tissue and facilitate increased uptake of glucose by the mammary gland, which is not insulin-responsive (Bauman and Elliot, 1983). A hyperinsulinemic-euglycemic clamp in dairy cows in early lactation was shown to decrease milk yield and positively affect the EB (Butler et al., 2003). In the current study, the negative relationship ($P < 0.05$) between plasma NEFA and insulin and a tendency for a negative relationship between ER_f and insulin confirm the importance of insulin status in relationship to the EB in dairy cows in early lactation. In addition, insulin has been suggested to restore hepatic growth hormone (GH) responsiveness in dairy cows in a NEB (Butler et al., 2003). Whereas no diet effect or increase with week pp on plasma IGF-1 concentration was found, it can be speculated that the diet effect on insulin concentration or the increase in insulin concentration with week pp for cows fed the glucogenic diet was not enough to restore GH responsiveness during the first nine weeks of lactation.

Considering the lipogenic effect of insulin, the low insulin concentration in cows fed the lipogenic diet corresponds with a tendency for higher plasma NEFA concentration. An increase in NEFA concentrations for cows fed the lipogenic diet compared with the glucogenic diet corresponds with other studies that increased the dietary fat content and also found an increase in plasma NEFA concentration (e.g. Grum et al., 1996a; Salado et al., 2004). However, these studies did not feed isocaloric diets, but increased the dietary energy content with the fat addition. Other studies released a decrease in DMI with dietary fat addition (Beam and Butler, 1998; Simas et al., 1995) without compromising milk production, which resulted in more body weight loss. In the current study, the higher plasma NEFA concentration in the lipogenic diet group likely originated from the mobilised body fat reserves, as indicated by the relationship between ER_f and plasma NEFA concentration.

NEFA, originating from body fat stores, are mobilised to compensate for a NEB and to be oxidized in the Krebs' cycle and respiratory chain reaction to make energy available to the body (Webster, 1993). In the Krebs' cycle, oxaloacetate, a C3 nutrient, plays a crucial role. However, during periods of relative excess of NEFA (e.g. during NEB), NEFA can follow several alternative pathways. First, dairy cows in early lactation exhibit incomplete oxidation of fatty acids as indicated by high concentrations of ketone bodies (Guretzky et al., 2006). The second alternative pathway for mobilised NEFA is incorporation of NEFA into milk fat, directly or indirectly via esterification to very-low density lipoproteins (VLDL), which causes an increase in long-chain fatty acids in milk (Belyea and Adams, 1990). The third alternative for NEFA is esterification to TAG in the liver, possibly causing a fatty liver (Bobe et al., 2004). Fatty liver occurs when the rate of fatty acid esterification exceeds the rate of TAG disappearance via export from the liver as a component of VLDL. It has been suggested that hepatic secretion of TAG is positively correlated with the lipogenic capacity of the liver (Pullen et al., 1990). The predominant site for lipogenesis in ruminants is adipose tissue, which might explain the low rate of hepatic secretion of TAG in ruminants (Emery et al., 1992) compared with rat (Kleppe et al., 1988) or chicken and fish (Pullen et al., 1990). Consequently, this makes ruminants more susceptible to TAG accumulation in the liver during a period of NEB, eventually causing a fatty liver.

Plasma BHBA was high (> 2.0 mMol/l) for cows in both dietary treatment groups from week 1 to 8 pp (Figure 2). This suggests this alternative pathway is used irrespective of dietary treatment in early lactation. In support, the non-significant regression coefficient of NEFA on BHBA suggests that NEFA concentrations do not determine the extent of incomplete oxidation and production of BHBA in periparturient dairy cows. In contrast, the regression coefficient of plasma glucose on BHBA was significant. This suggests that plasma glucose had a negative relationship with the plasma BHBA concentration. It has been reported that feeding cows glucogenic nutrients in early lactation decreases plasma BHBA and acetate concentrations (Pickett et al., 2003). Although, we discussed the existence of a difference in fat availability from diet and mobilised body reserves between diets in the current study, the increase in lipogenic nutrients with the lipogenic diet did not result in an increase in plasma BHBA concentration as suggested earlier (Drackley, 1999, chapter 2).

The second alternate pathway for NEFA seems to be used throughout the experimental period. A positive relationship was detected between plasma NEFA and milk fat yield. Body fat mobilisation and NEFA were both higher in cows fed the lipogenic diet and simultaneously milk fat yield was increased by 220 g per cow per day. Glucogenic nutrients lower milk fat percentage and often also daily milk fat yield (e.g. Pickett et al., 2003).

With regard to the third alternative pathway the current results do not support our hypothesis (chapter 2) that a diet high in glucogenic nutrients decreases hepatic TAG accumulation. This might be caused by the fact that the dietary treatment was established by feeding concentrates differing in lipogenic and glucogenic nutrient content, while the contribution of concentrates to the diet increased from parturition until 3 weeks pp. In week 2 pp, liver TAG content was high, but not different between diets. In week 4 pp liver TAG was numerically lower in cows fed the glucogenic diet, but did not differ significantly from cows fed the lipogenic diet. This observation corresponds with the relative reduction in fat load in cows fed the glucogenic diet compared with cows fed the lipogenic diet as discussed above.

Conclusions

In conclusion, the results of this study suggest that plasma insulin is an intermediate in altering energy partitioning in dairy cows in early lactation. Feeding lactating dairy cows a diet high in glucogenic nutrients resulted in a less negative NEB due to anabolic effects of a high insulin concentration. The relative abundance of lipogenic nutrients, when feeding a more lipogenic diet, resulted in more excretion of lipogenic nutrients in milk and an increased recovery time from a NEB. Although it can be expected that cows with an improved EB suffer less from hepatic TAG accumulation and elevated plasma ketone concentrations the current experiment could not confirm this hypothesis.

Acknowledgements

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CHAPTER 5

Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation

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Abstract

Increasing the availability of glucogenic nutrients relative to lipogenic nutrients has been hypothesised to decrease the milk fat production, to improve the energy balance (EB) and to decrease the incidence and severity of metabolic and reproductive disorders in dairy cows in early lactation. Therefore, the objective was to evaluate the effect of glucogenic, lipogenic or mixed diet on EB, plasma metabolites and metabolic hormones, liver tri-acyl-glyceride (TAG) and reproductive variables in high-producing dairy cows in early lactation. One hundred fourteen cows were assigned randomly to one of three diets. Cows were fed either a mainly lipogenic, a mainly glucogenic or a mixed of both diets (50:50 DM basis) from week 3 before the expected calving date until 9 weeks postpartum (pp). Diets were isocaloric (net energy basis) and equal in intestinal digestible protein. Dry matter intake, net energy intake, milk yield and milk protein percentage did not differ among diets. Milk lactose percentage was less for cows fed lipogenic diet. Milk fat percentage was less for multiparous cows fed glucogenic diet compared with cows fed mixed or lipogenic diet (3.69 vs. 4.02 vs. 4.22 \pm 0.07 %, respectively) ($P < 0.05$). The calculated EB (EBc) was less negative for multiparous cows fed glucogenic diet compared with cows fed mixed or lipogenic diet (-33 vs. -125 vs. -89 \pm 21 kJ/(kg^{0.75}·d), respectively) ($P < 0.05$). Postpartum, glucogenic diet decreased plasma non-esterified fatty acids, β -hydroxybutyrate and liver TAG concentrations and increased insulin concentration in multiparous cows. The glucogenic diet tended to decrease the number of days till 1st P4 rise in multiparous cows compared with mixed or lipogenic diet (20.4 vs. 24.4 vs. 26.4 \pm 2.1 d, respectively) ($P < 0.10$). Diet had no effect on any of the above mentioned variables in primiparous cows, except milk lactose percentage was greater for primiparous cows fed glucogenic diet. We concluded that glucogenic diet was effective in improving the EBc and decreasing plasma BHBA and liver TAG concentrations, suggesting a reduced risk of metabolic disorders in multiparous dairy cows fed glucogenic diet.

Introduction

High-producing dairy cows are challenged postpartum (pp) with large metabolic demands caused by the sudden increase in energy requirements due to the start of the lactation, which cannot be met by feed intake alone. Cows mobilise body fat to compensate for this energy deficit. The extensive body fat mobilisation predisposes fatty liver and ketosis due to an inability to dispose of fatty acids via β -oxidation or the limited capacity to export tri-acyl-glycerides (TAG) in the form of very-low density lipoproteins (VLDL) from the liver (Bell, 1995; Grummer, 1993). This is usually accompanied by a period of reduced fertility (Butler, 2003). Several strategies to reduce the severity and incidence of metabolic disorders in early lactation have been studied. Most studies aimed at increasing energy intake in the periparturient period were conducted to increase the energy balance (EB) and thereby reduce the risk of metabolic disorders (Drackley et al., 2003; Hayirli and Grummer, 2004; Reist et al., 2003a). To achieve this objective it was suggested that the energy density of the diet be increased. An alternative approach is to help the cow maintain a positive EB in early lactation by decreasing the caloric demand of milk production. Recently, feeding calcium salts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) has been presented as a dietary regimen to decrease milk energy output and hereby improve the EB in transition cows (Castaneda-Gutierrez et al., 2005). Alternatively, decreasing the lipogenic to glucogenic nutrient ratio has been suggested to decrease the milk fat content and hereby improve the EB and decrease plasma ketone body concentration in dairy cows in early lactation (Adler, 1970; Chapter 2). In ruminants, lipogenic nutrients either originate from fibre, which is fermented to acetate and butyrate, or originate from dietary fat or are derived from body reserves. Glucogenic nutrients originate from starch escaped from rumen degradation or gluconeogenesis. Diets high in ruminally degraded starch and low in fibre typically decrease the acetate to propionate ratio (Bannink et al., 2006). Propionate is the major precursor for gluconeogenesis, while acetate is a main precursor for *de novo* lipogenesis. Therefore, the milk fat depression upon feeding glucogenic diets has been explained by a shift from high availability of fat precursors to glucose and by a shift from lipogenesis to gluconeogenesis.

In an earlier experiment, we tested the effect of a mainly lipogenic vs. glucogenic diet on energy partitioning in climate-respiration chambers with multiparous dairy cows in early lactation. Milk fat content, milk energy output and body fat mobilisation decreased with a glucogenic diet compared with a lipogenic diet (chapter 3). The glucogenic diet increased plasma insulin concentration and tended to decrease plasma NEFA concentrations in early lactation compared with lipogenic diet (chapter 4). It has been suggested that the availability of glucogenic nutrients relative to lipogenic nutrients affects the susceptibility of cows to metabolic disorders like fatty liver and ketosis (Adler, 1970; Drackley, 1999; Kronfeld, 1976). However, in the respiration chamber study the design of the experiment with 16 cows did not allow sufficient power to evaluate the plasma BHBA concentration and liver TAG content, as

indicators for ketosis and fatty liver (chapter 4). Furthermore, potential effects of dietary energy source on reproductive performance could not be assessed. Therefore, the objective of this study was to evaluate the effects of a mainly glucogenic or lipogenic diet on calculated EB (EBc), plasma metabolites and metabolic hormones, liver TAG content and reproductive variables in high-producing dairy cows in early lactation in a larger experiment. Further, to test a possible parity-effect both primiparous as multiparous cows were included in this experiment. Additionally, an intermediate diet was added in order to further outline the relation between different glucogenic to lipogenic nutrient availability and energy metabolism.

Materials and methods

Experimental design, animals and housing

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. One hundred fourteen Holstein-Friesian dairy cows were selected from the Schothorst Feed Research dairy herd (Lelystad, The Netherlands) for this experiment. The experiment consisted of an intensive part (76 cows) and an extensive part (38 cows). Treatment of the intensive part consisted of either a mainly glucogenic diet (n=18 multiparous and n=7 primiparous cows), a mainly lipogenic diet (n=19 multiparous and n=7 primiparous cows) or a mix (50:50 DM basis) of both diets (n=18 multiparous and n=7 primiparous cows) from week 3 before the expected calving date until 9 weeks pp. From these cows, blood was collected weekly from week -3 till week 9 relative to calving and individual feed intake was determined from parturition until week 9 pp for 72 of the 76 cows. Liver biopsies were taken in week -2, 2, 4 and 6 relative to calving in 42 multiparous cows of the 76 cows. Additionally, the remaining 38 cows in the extensive part of the experiment were divided randomly over the two extreme diets (lipogenic and glucogenic) to study dietary energy source effects on reproduction variables. All 114 cows were monitored for milk production, milk composition and reproduction variables. Three cows were excluded from the experiment because of a left displaced abomasum (lipogenic concentrate), serious mastitis (glucogenic concentrate) or dead at parturition (glucogenic concentrate). From the 111 cows, who completed the experiment, 33 cows were primiparous and 78 multiparous (parity ranged from 2 to 10). From these 111 cows, 76 cows participated in the intensive part of the experiment and 35 cows in the extensive part of the experiment (no blood sampling). Table 1 shows the distribution of cows per diet, parity and monitoring protocol. Cows were housed in a free-stall with slatted floor and boxes. Cows were milked twice daily (0600h and 1700h).

Diets

Three weeks prepartum, cows were fed 1 kg/d of the experimental concentrates, followed by 2 kg/d in the last week prepartum. Forage did not differ among diets and was supplied ad libitum and consisted prepartum of grass silage, corn silage and wheat straw in a ratio 45:45:10 (DM basis). Postpartum, forage consisted of grass silage, corn silage, chopped alfalfa hay, and rapeseed meal in a ratio 48:45:3:3 (DM basis). For cows of parity 3 and greater concentrate supply was 3.5 kg/d at day 1 pp and increased stepwise by 0.5 kg/d pp. From day 16 until day 22 pp concentrate supply increased by 0.25 kg/d, until concentrate supply reached 12.0 kg/d for cows of parity 3 and greater. Concentrate supply for second parity cows was 95.8 % (maximum supply 11.5 kg/d) and for first parity cows 70.8% (maximum supply 8.5 kg/d) of the schedule for cows of parity 3 and greater. Ingredient and calculated chemical composition of concentrates are presented in Table 2. Chemical composition of the diets based on the realised total feed intake is presented in Table 3. Diets were isocaloric (net energy basis; VEM system) (Van Es, 1975) and equal in intestinal digestible protein and degraded protein balance (DVE/OEB system) (Tamminga et al., 1994). Concentrate and forage were supplied separately. Forage was supplied twice daily, after milking, in equal proportions in individual (n=72) or group (n=39) feeding stations. Concentrate was supplied in individual (n=111) automatic feeding stations and equally divided over 3 meals per day.

Table 1. Distribution of cows per diet, parity and monitoring protocol (no of cows of total no of cows completed experiment, n=111).

Parity ¹	Diet						Total
	Glucogenic		Mixed		Lipogenic		
	P	M	P	M	P	M	
Milk production, milk composition, body weight, BCS, reproduction	13	29	7	18	12	32	111
+ blood sampling	7	18	7	18	7	19	76
+ feed intake	7	16	7	18	7	17	72
+ liver biopsies		12		17		13	42

¹ P = primiparous; M = multiparous.

Table 2. Ingredient and calculated¹ chemical composition of glucogenic and lipogenic concentrate.

Item	Concentrate	
	Glucogenic	Lipogenic
Ingredient, g/kg		
Rapeseed meal	12.72	19.81
Corn	26.56	
Milocorn	25.00	
Palmkernel, expeller	3.16	12.50
Citruspulp		16.15
Peas	13.15	
Sunflower seed, extracted		8.93
Soyabean hulls		12.40
Soyabean meal, formaldehyde treated	12.40	
Rapeseed meal, formaldehyde treated		18.92
Bergafat F100 ²		2.13
Molasses-cane	4.00	5.00
Palmoil		2.50
Calcium carbonate	1.36	0.13
Magnesium oxide	0.07	
Sodium chloride	0.83	0.79
Mineral-vitamin mixture ³	0.75	0.75
Calculated chemical composition, g/kg of DM unless otherwise stated		
Dry matter, g/kg	874	890
Crude protein,	204	226
Crude fat	36	62
NDF	151	348
ADF	86	242
Starch	420	8
Sugars ⁴	62	115
Ash	70	83
DVE ⁵	138	139
OEB ⁶	14	22
NE ⁷ , MJ/kg DM	7.69	7.62

¹ Based on CVB table (Centraal Veevoederbureau, 2005).

² Fractionated palm fatty acids (Berg+Schmidt, Hamburg, Germany).

³ Premix 2031, PreMervo, Utrecht, The Netherlands.

⁴ (van Vuuren et al., 1993).

⁵ Intestinal digestible protein (Tamminga et al., 1994).

⁶ Degraded protein balance (Tamminga et al., 1994).

⁷ Net energy for lactation calculated with VEM system (Van Es, 1975).

Table 3. Calculated chemical composition of glucogenic, lipogenic or mixed diet^{1,2}.

Item	Diet		
	Glucogenic	Mixed	Lipogenic
Dry matter, g/kg	495	500	490
Crude protein, g/kg DM	158	163	164
Crude fat	31	41	50
NDF	320	360	398
ADF	186	218	247
Starch	266	179	104
Sugars ³	51	62	70
Ash	66	73	70
DVE ⁴	94	94	92
OEB ⁵	10	11	13
NE ⁶ , MJ/kg DM	6.88	6.84	6.81

¹ Based on realised feed intake.

² Corn silage: DM 311 g/kg product, OM 954 g/kg DM, crude protein 76 g/kg DM, crude fat 29 g/kg DM, NDF 400 g/kg DM, starch 343 g/kg DM, NE 6.49 MJ/kg DM, DVE 46 g/kg DM, OEB -28 g/kg DM (Blgg, Oosterbeek, The Netherlands).

Grass silage: DM 461 g/kg product, OM 873 g/kg DM, crude protein 169 g/kg DM, crude fat 32 g/kg DM, NDF 463 g/kg DM, sugars 68 g/kg DM, NE 6.25 MJ/kg DM, DVE 77 g/kg DM, OEB 30 g/kg DM (Blgg, Oosterbeek, The Netherlands).

Chopped alfalfa hay: DM 876 g/kg product; OM 905 g/kg DM, crude protein 156 g/kg DM, crude fat 14 g/kg DM, NDF 487 g/kg DM, NE 5.27 MJ/kg DM, DVE 82 g/kg DM, OEB -1 g/kg DM (Blgg, Oosterbeek, The Netherlands).

Rapeseed meal: DM 873 g/kg product; OM 923 g/kg DM, crude protein 384 g/kg DM, crude fat 30 g/kg DM, NDF 334 g/kg DM, NE 6.70 MJ/kg DM, DVE 148 g/kg DM, OEB 159 g/kg DM (Centraal Veevoederbureau, 2005).

³ (Van Vuuren et al., 1993).

⁴ Intestinal digestible protein (Tamminga et al., 1994).

⁵ Degraded protein balance (Tamminga et al., 1994).

⁶ Net energy for lactation calculated with VEM system (Van Es, 1975).

Sampling and analytical procedures

Body condition was scored (1-5 scale) in weeks -3, 1, 4 and 8 relative to week of calving. Body weight was recorded after each milking and averaged per week. Milk production was recorded daily per cow. Milk samples for fat, protein, lactose and SCC analyses (ISO 9622, Melkcontrolestation, Zutphen, The Netherlands) were collected 4 times per week (Monday afternoon, Tuesday morning, Wednesday afternoon and Thursday morning). At 3 p.m. milkings weekly (Monday, Wednesday, Friday), milk was sampled and stored at -20°C until analysis of milk P4. P4 concentration in milk was determined as described by Roelofs et al. (2006). The intra-assay coefficient of

variation was 9.9%. The chosen threshold, which indicated a luteal phase, was at least more than 2 succeeding samples > 5 ng/ml.

For 72 cows feed intake was determined daily, by measuring feed supply and refusals for concentrate and forage separately, and averaged per week. Energy balance was calculated according to the Dutch net energy evaluation system for dairy cows (VEM system) (Van Es, 1975; Centraal Veevoederbureau, 2005) as the difference between VEM supplied with feed and VEM required for maintenance and milk production. Animal maintenance requirements are $42.4 \text{ VEM/kg}^{0.75} \cdot \text{d}$ (1000 VEM = 6.9 MJ NE). The VEM required for milk production is 442 VEM/kg fat and protein corrected milk (Van Es, 1975). In calculating the maintenance and milk energy requirements, a correction factor to scale requirements to an average cow was applied as described by Van Es (1975). Forage samples were taken weekly and stored at -20°C until analysis. Before analyses (Blgg, Oosterbeek, the Netherlands), forage samples were pooled per batch.

Samples of jugular blood were obtained weekly for 76 cows from week -3 until week 9 pp 2-3 hours after the a.m feeding and immediately before liver biopsy if both were on the same day. Blood was collected in three evacuated tubes (Vacuette[®], Greiner Bio-One, Kremsmünster, Austria) containing either NaF for glucose determination, Li-heparin for NEFA, BHBA, cholesterol, and urea determination or EDTA for insulin determination. Blood samples were stored on ice for maximal 2 hours. Plasma was obtained by centrifugation (10 min at 2900 Xg), aliquoted, and frozen at -20°C until analysis. Blood and liver samples were analysed in a quality controlled veterinary laboratory (Veterinary Diagnostic Laboratory, Utrecht University, Utrecht, The Netherlands). Analyses for glucose, NEFA, BHBA and cholesterol were performed using commercial available kits on a Unicel[®] Dx C 600 analyser (Beckman Instruments B.V., Mijdrecht, The Netherlands) (glucose: reagent 443355 Beckman Instruments B.V., Mijdrecht, The Netherlands; NEFA: FA 115 kit, RANDOX laboratories Ltd., Crumlin, UK; BHBA: Ranbut kit, RANDOX laboratories Ltd., Crumlin, UK; cholesterol: cholesterol reagents, Beckman Instruments B.V., Mijdrecht, The Netherlands). Insulin concentration was determined using a radioimmunoassay kit (Coat-a-Count[®] Insulin, Diagnostic Products Corporation, Los Angeles, USA). The intra-assay coefficients of variation were: glucose 1.7%; NEFA 3.0%, BHBA 4.5%, cholesterol 4.6%, urea 0.4%, insulin 3.4%. The accuracy of each assay was monitored with the use of a commercial reference serum sample (Bovine precision serum RANDOX laboratories Ltd., Crumlin, UK) and the outcome deviated $<5\%$ from the target values.

Liver biopsies were taken in week -2, 2, 4 and 6 relative to week of calving in 42 multiparous cows. Prior to the biopsy, the biopsy site was clipped and disinfected. A stab incision was made at the location of the greater trochanter in the 11th intercostal space on the right side of the cow. Biopsy was obtained under local anaesthesia (7 ml Lidocaine-HCl 2% with adrenaline, Alfasan Nederland B.V., Woerden, The Netherlands) with a 17G X 200 mm biopsy needle. Approximately 300 mg wet weight of liver tissue was harvested by moving the biopsy needle several times in the direction of the contra lateral olecranon. Tissue was kept at maximum 24 h on ice in a

0.9% NaCl solution. Subsequently, connective tissue was removed, the sample was weighed and stored at -20°C until analysis. Liver tissue was handled as described earlier (Van den Top et al., 1995) and analysed on a Unicel[®] DxC 600 analyser with a TAG reagent (Beckman Instruments B.V., Mijdrecht, The Netherlands). The intra assay coefficient of variation was 6.5%.

Statistical analysis

Since multiple measurements per animal cannot be regarded as independent units of observation, repeated measures analysis of variance (PROC MIXED (Littell et al., 1996) of SAS[®] VERSION 9.1; SAS Institute, Inc., Cary, NC) was performed for milk production, milk composition, EBc, metabolites and metabolic hormone variables. Cow was considered as the repeated effect. Diet (glucogenic, lipogenic or mixed), week (-3 till 9 pp) and the interaction between diet and week were included in the model as fixed effects (model 1). All cows (n=111) were included in the analyses for milk production and composition variables, a selection of cows were included for plasma metabolites and metabolic hormones (n=76), feed intake and EBc variables (n=72) and liver TAG content (n=42) (Table 1). Data were analysed for the primiparous and multiparous cows and for the prepartum and postpartum data separately. A first-order autoregressive structure (AR(1)) was the best fit and was used to account for within-cow variation. With exception of liver TAG concentration, the compound symmetry (CS) structure was the best fit, and was used to account for within-cow variation. For body weight and BCS at start and end of the experiment, body weight loss and reproduction variables, analysis of variance was performed (PROC MIXED of SAS[®] VERSION 9.1) for primiparous and multiparous cows separately with diet included in the model as fixed effect (model 2). Model assumptions for both models were evaluated by examining the distribution of residuals. Values are presented as LSM with their SEM.

To study the risk of ketosis and fatty liver, plasma BHBA and liver TAG concentration were plotted as categorical data. Chi-square analysis (PROC FREQ of SAS[®] VERSION 9.1) was used to investigate differences between percentages of observations of cows fed the different diets for BHBA and TAG for the different BHBA (≤ 0.6 , 0.6-0.8, 0.8-1.0, 1.0-1.2, 1.2-1.4, >1.4 mMol/l) and TAG (≤ 25 , 25-50, 50-75, 75-100, >100 mg/g wet weight) classes. Classes were arbitrarily chosen.

Results

Milk production

Milk yield and fat-and-protein corrected milk (FPCM) did not differ among diets, but both increased with time pp ($P < 0.05$) (Table 4). For primiparous cows, milk yield increased from 23.9 kg/d (± 0.5 kg/d) in week 1 to 31.7 kg/d (± 0.5 kg/d) in week 9 pp. For multiparous cows, milk yield increased from 36.2 kg/d (± 0.6 kg/d) in week 1

to 44.6 kg/d (± 0.7 kg/d) in week 9 pp. Milk fat content and milk protein content did not differ among diets for primiparous cows. Milk lactose content was greater ($P < 0.05$) for both primiparous and multiparous cows fed glucogenic diet than for cows fed lipogenic diet. Milk fat content, daily milk fat yield and the fat to protein ratio were greater ($P < 0.05$) for multiparous cows fed lipogenic diet or mixed diet compared with cows fed glucogenic diet. Multiparous cows fed lipogenic diet had a greater SCC ($P < 0.05$) than multiparous cows fed mixed or glucogenic diet. There were 9 cows detected and treated for mastitis during the experimental period: 1 cow fed glucogenic diet, 1 cow fed mixed diet and 7 cows fed lipogenic diet. After exclusion of these cows the SCC for multiparous cows fed glucogenic diet or mixed diet was still less compared with cows fed lipogenic diet (68 vs. 65 vs. $220 \pm 59 \times 10^3/\text{ml}$; $P < 0.05$).

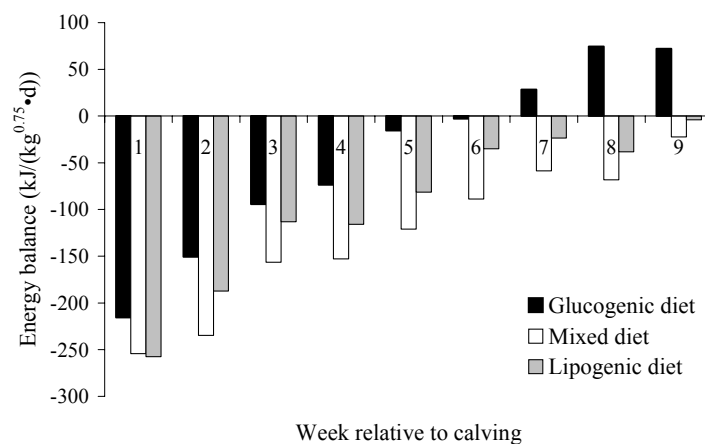


Figure 1. Calculated energy balance for multiparous cows fed a glucogenic (n=16), a lipogenic diet (n=17) or a mixed (50:50) of both diets (n=18) for the first 9 weeks of lactation. Values represent means per diet per week. Overall SEM was 9 kJ/(kg^{0.75}·d).

Energy balance

The body condition score 3 weeks prepartum (3.26 ± 0.04) or body weight (634 ± 7 kg) and body condition score in week 1 pp (3.24 ± 0.04) did not differ among diets. At the end of the experiment, week 9 pp, body weight (624 ± 7 kg) and body condition score (2.90 ± 0.04) also did not differ among dietary treatments. There were no diet effects on the weekly determined body weights or total body weight loss for multiparous cows. During the first 9 weeks after calving, primiparous cows fed lipogenic diet had a greater body weight than primiparous cows fed glucogenic diet. Dry matter intake and energy intake were not different among diets for both primiparous and multiparous cows (Table 5). Concentrate intake was less for multiparous cows fed lipogenic diet and forage intake was greater for primiparous cows fed lipogenic diet compared with the other diets. There was no diet effect on forage intake for primiparous cows when the forage intake was expressed per kg metabolic body weight (102 vs. 102 vs. 105 ± 3 g/kg^{0.75}·d; $P > 0.05$). The EBc did not differ among diets for primiparous cows. The EBc was less negative ($P < 0.05$) for multiparous cows fed glucogenic diet compared with cows fed mixed diet. EBc

became positive in week 7 pp for multiparous cows fed glucogenic diet and in week 9 pp for cows fed mixed diet or lipogenic diet (Figure 1).

Table 4. Milk production and milk composition of dairy cows in early lactation¹ fed a glucogenic, a lipogenic diet or a mixed of both diets (LSM ± SEM).

Variable	Diet			SEM	P-values ²		
	Glucogenic	Mixed	Lipogenic		Diet	Week	D×W
Primiparous cows, n	13	7	12				
Milk yield, kg/d	30.0	29.2	29.8	0.8	0.78	<0.01	0.76
FPCM ³ , kg/d	30.0	30.3	30.6	0.8	0.84	<0.01	0.35
Lactose, %	4.73 ^a	4.76 ^{a,b}	4.67 ^b	0.02	0.03	<0.01	0.63
Fat, %	4.07	4.39	4.30	0.11	0.10	<0.01	0.97
Protein, %	3.23	3.28	3.27	0.06	0.78	<0.01	0.84
Lactose, kg/d	1.42	1.39	1.40	0.04	0.82	<0.01	0.65
Fat, kg/d	1.22	1.27	1.27	0.04	0.47	<0.01	0.42
Protein, kg/d	0.97	0.95	0.97	0.02	0.84	<0.01	0.94
Fat/protein ratio	1.27	1.34	1.31	0.03	0.17	<0.01	0.97
SCC (×10 ³ /ml) ⁴	91	192	68	26	0.11	0.02	0.73
Multiparous cows, n	29	18	32				
Milk yield, kg/d	43.4	44.0	43.2	1.1	0.88	<0.01	0.05
FPCM ³ , kg/d	41.5	43.8	44.1	1.1	0.18	<0.01	0.24
Lactose, %	4.58 ^a	4.56 ^{a,b}	4.49 ^b	0.02	0.01	<0.01	0.81
Fat, %	3.69 ^a	4.02 ^b	4.22 ^b	0.07	<0.01	<0.01	0.82
Protein, %	3.25	3.24	3.23	0.03	0.94	<0.01	0.09
Lactose, kg/d	1.99	2.01	1.95	0.05	0.69	<0.01	0.09
Fat, kg/d	1.59 ^a	1.76 ^{a,b}	1.83 ^b	0.05	<0.01	<0.01	0.54
Protein, kg/d	1.40	1.42	1.40	0.04	0.92	0.01	0.18
Fat/protein ratio	1.14 ^a	1.24 ^b	1.31 ^b	0.01	<0.01	<0.01	0.66
SCC (×10 ³ /ml) ⁴	77 ^a	65 ^a	303 ^b	26	<0.01	<0.01	0.41

^{a,b} Values in the same row with different superscripts differ significantly ($P<0.05$).

¹ Week 1, 2, ...9 relative to calving.

² nm: not included in the model.

³ Fat and protein corrected milk.

⁴ P-values based on natural logarithm of somatic cell count.

Table 5. Dry matter intake and energy balance of dairy cows in early lactation¹ fed a glucogenic, a lipogenic diet or a mixed of both diets (LSM \pm SEM).

Variable	Diet			SEM	P-values ²		
	Glucogenic	Mixed	Lipogenic		Diet	Week	D×W
Primiparous cows, n	7	7	7				
Dry matter intake, kg/d	18.0	18.4	18.9	0.4	0.28	<0.01	0.52
Concentrate, kg DM/d	6.7	6.7	6.3	0.2	0.16	<0.01	0.43
Forage, kg DM/d	11.3 ^a	11.7 ^{a,b}	12.6 ^b	0.3	0.04	<0.01	0.04
Energy intake ³ , kJ/(kg ^{0.75} ·d)	1108	1092	1072	22	0.55	<0.01	0.87
Energy balance ³ , kJ/(kg ^{0.75} ·d)	-28	-35	-33	25	0.98	<0.01	0.40
Body weight ⁴ , kg	536 ^a	560 ^{a,b}	591 ^b	13	0.02	<0.01	0.46
Body weight loss ⁵ , kg	33	29	16	7	0.22	nm	nm
Body weight loss ⁶ , kg	24	12	4	10	0.19	nm	nm
Multiparous cows, n	16	18	17				
Dry matter intake, kg/d	23.8	23.6	24.2	0.5	0.74	<0.01	0.49
Concentrate, kg DM/d	9.4 ^a	9.6 ^a	9.2 ^b	0.1	<0.01	<0.01	0.13
Forage, kg DM/d	14.4	14.1	15.1	0.4	0.29	<0.01	0.52
Energy intake ³ , kJ/(kg ^{0.75} ·d)	1291	1284	1287	22	0.97	<0.01	0.55
Energy balance ³ , kJ/(kg ^{0.75} ·d)	-33 ^a	-125 ^b	-89 ^{a,b}	21	0.02	<0.01	0.37
Body weight ⁴ , kg	641	637	653	14	0.74	<0.01	0.73
Body weight loss ⁵ , kg	26	24	29	6	0.79	nm	nm
Body weight loss ⁶ , kg	10	6	14	8	0.79	nm	nm

^{a,b} Values in the same row with different superscripts differ significantly ($P < 0.05$).

¹ Week 1, 2, ...9 relative to calving.

² nm: not included in the model.

³ Calculated with VEM system (Van Es, 1975; Centraal Veevoederbureau, 2005).

⁴ Body weight data are based on 13 vs. 7 vs. 12 primiparous cows and 28 vs. 18 vs. 32 multiparous cows.

⁵ Total body weight loss during negative energy balance.

⁶ Body weight loss from week 1 till week 9 pp.

Metabolites, metabolic hormones and liver TAG

Prepartum, there were no differences among diets in plasma NEFA, BHBA, glucose, urea or liver TAG concentrations (Table 6). Prepartum plasma cholesterol was greater ($P < 0.05$) for multiparous cows fed lipogenic diet compared with glucogenic diet. Prepartum plasma insulin concentration was greater ($P < 0.01$) for multiparous cows fed mixed diet compared with glucogenic or lipogenic diet.

Postpartum, multiparous cows fed glucogenic diet had less ($P < 0.01$) plasma NEFA, plasma BHBA, plasma cholesterol and liver TAG ($P < 0.05$) than cows fed mixed diet or lipogenic diet (Table 7). From week 1 till 9 pp plasma NEFA decreased, while dietary differences continued to exist (Figure 2). Additionally, liver TAG content decreased from week 2 till 6 pp, but dietary differences continued to exist. Plasma insulin was greater for multiparous cows fed glucogenic diet compared with lipogenic diet and increased with week pp. No dietary differences were detected for plasma glucose and plasma urea concentration. There was no diet effect pp on plasma NEFA, BHBA, glucose, insulin or urea concentration for primiparous cows. Plasma cholesterol was less ($P < 0.05$) for primiparous cows fed glucogenic diet.

After distribution of each plasma BHBA concentration observation and liver TAG concentration observation over classes, multiparous cows fed glucogenic diet had a greater percentage ($P < 0.05$) of observations in the lowest BHBA and TAG class (Figure 3). Cows fed mixed or lipogenic diet had proportional more observations in the higher BHBA and TAG classes compared with cows fed glucogenic diet.

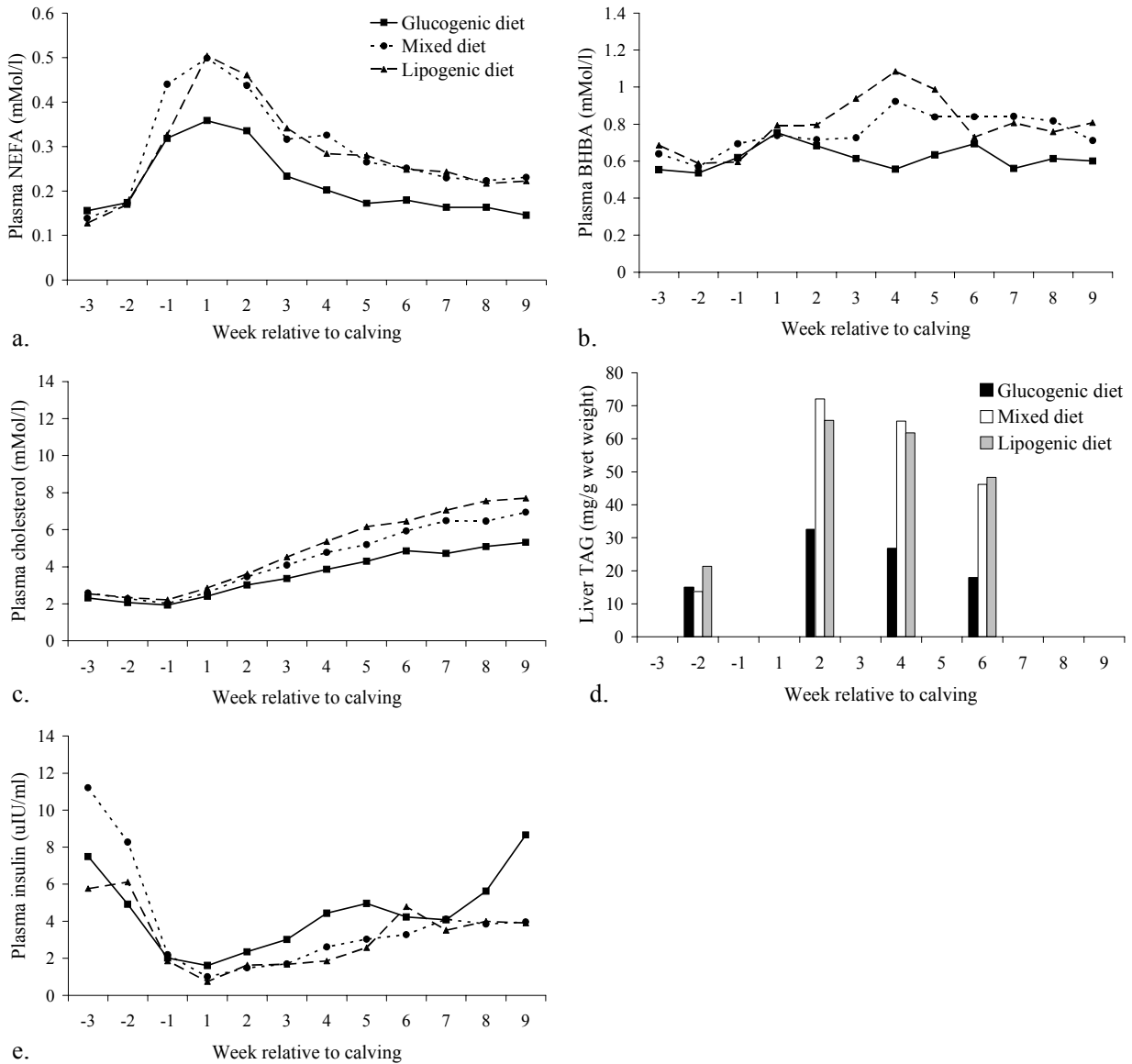


Figure 2. Plasma NEFA (a), plasma BHBA (b), plasma cholesterol (c), liver TAG content (d) and plasma insulin (e) concentration for multiparous cows fed a glucogenic, a lipogenic diet or a mixed (50:50) of both diets during week -3 till week 9 of lactation. Values represent means per diet per week. Overall SEM: NEFA, 0.01 mMol/l; BHBA, 0.02 mMol/l; cholesterol, 0.12 mMol/l; Liver TAG, 5.0 mg/g wet liver weight; plasma insulin, 0.29 uIU/ml.

Table 6. Blood metabolites, metabolic hormones and liver TAG prepartum¹ of dairy cows fed a glucogenic, a lipogenic diet or a mixed of both diets (LSM ± SEM).

Variable	Diet			SEM	<i>P</i> -values ²		
	Glucogenic	Mixed	Lipogenic		Diet	Week	D×W
Primiparous cows, n	7	7	7				
NEFA, mMol/l	0.35	0.31	0.27	0.05	0.61	<0.01	0.11
BHBA	0.58	0.51	0.53	0.04	0.38	0.26	0.95
Cholesterol	2.35	2.70	2.56	0.11	0.10	0.25	0.98
Glucose	4.01	3.92	4.00	0.16	0.90	0.61	0.95
Urea	3.23	3.24	3.01	0.17	0.58	0.80	0.22
Insulin, uIU/ml	4.56	4.39	5.41	0.83	0.71	<0.01	0.71
Multiparous cows, n	18	18	19				
NEFA, mMol/l	0.22	0.25	0.23	0.02	0.61	<0.01	0.17
BHBA	0.57	0.64	0.62	0.03	0.27	<0.01	0.27
Cholesterol	2.11 ^a	2.31 ^{a,b}	2.44 ^b	0.08	0.02	<0.01	0.53
Glucose	3.34	3.53	3.36	0.07	0.11	0.40	0.81
Urea	4.00	3.93	4.12	0.16	0.78	0.24	0.72
Insulin, uIU/ml	4.82 ^b	7.24 ^a	4.30 ^b	0.55	<0.01	<0.01	0.06
Liver TAG, mg/g wet weight	15.0	13.7	21.3	4.6	0.42	nm	nm

^{a,b} Values in the same row with different superscripts differ significantly ($P<0.05$)

¹ Week -3, -2 and -1 relative to calving

² nm: not included in the model.

Table 7. Blood metabolites, metabolic hormones and liver TAG postpartum¹ of dairy cows fed a glucogenic, a lipogenic diet or a mixed of both diets (LSM ± SEM).

Variable	Diet			SEM	<i>P</i> -values		
	Glucogenic	Mixed	Lipogenic		Diet	Week	D×W
Primiparous cows, n	7	7	7				
NEFA, mMol/l	0.25	0.28	0.29	0.02	0.36	<0.01	0.98
BHBA	0.68	0.69	0.79	0.05	0.26	0.07	0.07
Cholesterol	4.02 ^a	4.96 ^b	4.84 ^b	0.22	0.02	<0.01	0.04
Glucose	3.57	3.68	3.65	0.07	0.54	0.69	0.22
Urea	3.84	3.98	3.87	0.14	0.74	<0.01	0.47
Insulin, uIU/ml	4.24	4.65	4.87	0.55	0.83	<0.01	0.79
Multiparous cows, n	18	18	19				
NEFA, mMol/l	0.22 ^a	0.31 ^b	0.31 ^b	0.02	<0.01	<0.01	0.97
BHBA	0.64 ^a	0.79 ^{a,b}	0.84 ^b	0.05	<0.01	0.46	0.08
Cholesterol	4.10 ^a	5.10 ^b	5.83 ^b	0.22	<0.01	<0.01	<0.01
Glucose	3.35	3.29	3.26	0.04	0.22	<0.01	0.30
Urea	4.16	4.01	4.21	0.14	0.58	<0.01	0.26
Insulin, uIU/ml	4.19 ^a	2.78 ^b	2.88 ^b	0.33	<0.01	<0.01	0.14
Liver TAG, mg/g wetweight ²	25.7 ^a	62.7 ^b	58.6 ^b	10.4	0.04	<0.01	0.85

^{a,b} Values in the same row with different superscripts differ significantly ($P<0.05$)

¹ Week 1, 2, ...9 relative to calving

² Glucogenic diet: n=12; Mixed diet: n=17; Lipogenic diet: n=13

Reproduction

There was no diet effect on any of the reproduction variables in primiparous cows (Table 8). First P4 rise tended ($P < 0.10$) to be earlier after parturition for multiparous cows fed glucogenic diet compared with cows fed mixed diet or lipogenic diet. No dietary differences were detected in luteal phase length, cycle length or days till second P4 rise. The mean P4 concentration during the second luteal phase tended to be higher ($P < 0.10$) for cows fed mixed diet compared with cows fed glucogenic diet.

Table 8. Reproduction variables of dairy cows fed a glucogenic diet, a lipogenic diet or a mixed of both diets (LSM \pm SEM).

Variable	Diet			SEM	P-value Diet
	Glucogenic	Mixed	Lipogenic		
Primiparous cows, n	13	7	12		
No of cows ovulated	12	7	12		
Days till 1 st P4 rise	31.7	20.9	25.6	4.4	0.25
Luteal phase length, d	9.7	16.7	11.4	2.3	0.12
Cycle length, d	17.7	22.8	21.1	1.9	0.23
P4, max ² , ng/ml	32.7	35.6	45.0	6.4	0.30
P4 mean ³ , ng/ml	22.3	25.8	27.2	4.2	0.64
Days till 1 st estrous	35.8	30.8	42.7	5.7	0.38
Cows with 2 nd P4 rise, n	6	6	11		
Days till 2 nd P4 rise	40.8	43.2	47.4	3.5	0.37
P4, max ² , ng/ml	52.1	41.1	48.9	9.0	0.70
P4, mean ³ , ng/ml	32.8	25.4	30.7	10.6	0.30
Multiparous cows, n	29	18	32		
No of cows ovulated	29	18	31		
Days till 1 st P4 rise	20.4	24.4	26.1	2.1	0.10
Luteal phase length, d	17.0	17.2	15.4	1.8	0.73
Cycle length, d	24.3	26.3	27.0	1.9	0.52
P4, max ² , ng/ml	34.5	44.2	39.0	3.4	0.17
P4, mean ³ , ng/ml	21.8	25.2	26.5	2.0	0.17
Days till 1 st estrous	33.0	40.6	39.4	4.5	0.43
Cows with 2 nd P4 rise, n	24	14	26		
Days till 2 nd P4 rise	44.3	45.5	49.1	2.4	0.26
P4, max ² , ng/ml	34.8	48.1	42.8	5.4	0.23
P4, mean ³ , ng/ml	22.5	31.4	27.0	2.6	0.07

^{a,b} Values in the same row with different superscripts differ significantly ($P < 0.05$)

¹ #: $P < 0.10$; ns: $P > 0.10$

² Maximal P4 concentration during the first/second luteal phase pp.

³ Mean P4 concentration during the first/second luteal phase pp.

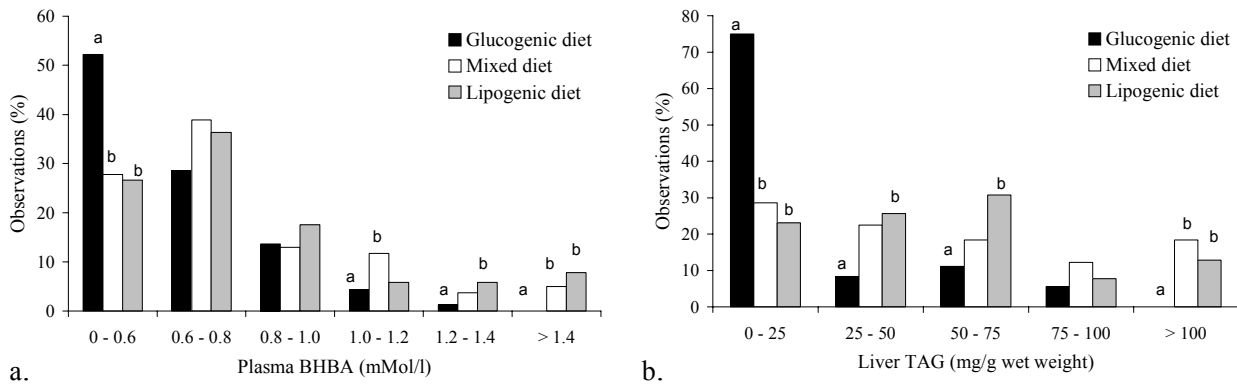


Figure 3. Frequency distribution postpartum per plasma BHBA and liver TAG concentration class for multiparous cows fed a glucogenic, lipogenic or mixed diet. Columns within class with different symbols differ significantly ($P < 0.05$). Total observations for plasma BHBA concentration: glucogenic diet, $n = 161$; mixed diet, $n = 162$; lipogenic diet, $n = 153$. Total observations for liver TAG: glucogenic diet, $n = 39$; mixed diet, $n = 49$; lipogenic diet, $n = 39$.

Discussion

The present study demonstrates that dietary energy source affects EBC in multiparous high-producing dairy cows in early lactation. A glucogenic diet reduces the partitioning of energy to milk fat and decreases body fat mobilisation compared with a lipogenic diet. Multiparous cows fed glucogenic diet produced 170 g/d less milk fat than cows fed mixed diet and 240 g/d less milk fat than cows fed lipogenic diet. This corresponds with our earlier study, where cows fed glucogenic diet had a decrease in milk fat production of 220 g/d compared with cows fed a mainly lipogenic diet (1.68 vs. 1.90 kg/d) (chapter 3). In the earlier study, the decrease in milk fat production resulted in a decrease in milk energy output (1075 vs. 1173 ± 23 kJ/(kg^{0.75}·d)), which resulted in an improved EB (-94 vs. -172 ± 28 kJ/(kg^{0.75}·d)), measured by indirect calorimetry. In particular, energy mobilised from body fat reserves was decreased for cows fed glucogenic diet. This matches with the results of this study, showing that the EBC was improved for multiparous cows fed glucogenic diet compared with cows fed mixed or lipogenic diet (Table 5). The EBC was numerically, but not significantly, lower for multiparous cows fed mixed diet than for cows fed lipogenic diet. This was partly caused by the higher FPCM (43.8 vs. 43.1 ± 1.2 kg/d; $P > 0.05$) and partly by the lower body weight (637 vs. 653 ± 14 ; $P > 0.05$) for cows fed mixed diet ($n = 18$) compared with cows fed lipogenic diet and monitored for individual feed intake ($n = 17$). Although cows fed mixed diet are expected to have a higher availability of glucogenic nutrients compared with cows fed lipogenic diet, there was no significant difference in milk fat production, milk energy output or EBC between both diets. This can possibly be explained by limited animal numbers, as the power calculation of the current experiment was based on the difference between lipogenic and glucogenic diet

in our earlier experiment (chapters 3 & 4), while no information on mixed diet was available.

The body weight of the primiparous cows differed among diets and was highest for lipogenic group. This higher body weight and the lower lipogenic concentrate intake, contributes to the observed significantly higher forage intake of cows fed lipogenic diet. Furthermore, differences in body weight loss among diets were not significant, and these differences numerically did not match the differences in EBc. In line with an earlier study (Oldick et al., 1997), the results of this study suggest that body weight changes may not be valid indicators of EB status in dairy cows.

A possible explanation for milk fat depression (MFD) caused by decreasing the lipogenic-to-glucogenic nutrient ratio is the glucogenic theory. This theory was first proposed by McClymont and Vallance (1962). They suggested that an increase in insulin, induced by an increase in propionic acid or glucose, would decrease lipolysis and decrease the availability of milk fat precursors, which may reduce milk fat and milk energy output. Other researchers suggest that the depression in milk fat might be caused by an accumulation of trans-fatty acids in the rumen caused by a low pH on glucogenic diets (Kalscheur et al., 1997). However, studies on abomasal (Oldick et al., 1997) or duodenal (Lemosquet et al., 1997) infusion of glucose indicate that trans-fatty acid production in the rumen may not always be the cause of MFD, observed after increasing glucogenic nutrient supply. They reported a trend for lower milk fat and improved energy status and significant lower plasma NEFA concentration and increased insulin concentration with the glucose infusion compared with the abomasal infusion of fat (Oldick et al., 1997) or duodenal infusion of water (Lemosquet et al., 1997).

Hypoinsulinemia in early lactation dairy cows is part of an adaptation process around parturition in support of lactation. Low plasma insulin concentration reduces glucose uptake by muscle and adipose tissue and makes glucose available for uptake by the mammary gland, which is not insulin-responsive (Bauman and Elliot, 1983). Conversely, a hyperinsulinemic-euglycemic clamp in dairy cows in early lactation was shown to partition energy from milk to body tissue and improve the EBc (Butler et al., 2003). This corresponds with the observation that glucogenic diet in this study increased the plasma insulin concentration pp and improved the EBc in multiparous cows. The results suggest that glucogenic diet realised an increase in plasma insulin and as a consequence of the antilipolytic effect of insulin, the contribution of mobilised body fat to milk fat is reduced and milk fat yield and milk energy output are depressed.

The lack of diet effect on plasma insulin concentration or EBc in primiparous cows in this study can most likely be attributed to limited animal numbers in this parity class. However recently, it has been suggested that in primiparous cows insulin might be

less important in controlling the relative partitioning of nutrients between body tissue and milk synthesis possibly due to prevailing higher IGF-1 concentrations compared with multiparous cows (Wathes et al., 2006). Additionally, those authors suggested that primiparous cows, because of a lower milk production, spare glucose for uptake by other tissues and consequently have a higher plasma glucose concentration peripartum. This suggestion is in agreement with both the current study and Santos et al. (2001), who reported higher plasma glucose concentrations for primiparous cows compared with multiparous cows peripartum.

In the current study, the plasma NEFA concentrations were less for multiparous cows fed glucogenic diet compared with mixed or lipogenic diet, consistent with the less extensive NEB. Additionally, plasma BHBA concentrations and particularly liver TAG content were less for cows fed glucogenic diet compared with mixed or lipogenic diet. In concert with the lack of difference in EB between mixed and lipogenic diet, there was no difference between mixed and lipogenic diet in plasma NEFA and plasma insulin concentration. Plasma cholesterol and plasma BHBA were numerically lower for cows fed mixed diet compared with cows fed lipogenic diet. Figure 3 shows that cows fed glucogenic diet had a greater proportion of the observations in the lowest concentration class for plasma BHBA (≤ 0.6 mMol/ml) as well as for liver TAG (≤ 25 mg/g wet weight). Conversely, all observations which can be classified as severe liver fattening (>100 mg/g wet liver weight) (Gaal et al., 1983) and most of the observations which can be classified as subclinical ketosis (> 1.2 mMol/l) (Duffield et al., 1997) were for cows fed mixed or lipogenic diet. This indicates a reduced risk of metabolic disorders like ketosis and fatty liver with a glucogenic diet compared with a more lipogenic diet. Studies which compared lipogenic and glucogenic nutrients as dietary energy source and their effect on metabolic disorders are scarce and in most cases included a confounding effect of altering dietary energy content or energy intake (Drackley et al., 2003; Grum et al., 1996b). Most studies on feeding more glucogenic nutrients confirm the observation of a decrease in plasma NEFA and BHBA concentration (Lemosquet et al., 1997; Reist et al., 2003). On the other hand, lipogenic nutrients increased the plasma NEFA and BHBA concentration in a majority of the cases (Drackley et al., 2003; Moallem et al., 1997; Ponter et al., 2006), except when CLA served as lipogenic supplement (Castaneda-Gutierrez et al., 2005). The lack of difference between the mixed and lipogenic diet in the current study is consistent with the lack of difference in EBc between cows fed mixed diet and cows fed lipogenic diet.

Dietary effects on the incidence and severity of reproductive disorders in early lactation can be divided in caloric and non-caloric effects. Beneficial effects of polyunsaturated fatty acids in the reduction of uterine PGF 2α secretion (Danet-Desnoyers et al., 1993) or fat supplementation on progesterone concentration (Spicer et al., 1993) have non-caloric effects. Dietary treatments with caloric effects in most cases increased the energy intake and hereby improved the EBc (Minor et al., 1998).

However, various studies found a depression in feed intake and/or an increase in milk production with fat supplementation resulting in no significant improvement of the EBc (Lucy et al., 1993; Spicer et al., 1993). Although a diet effect on EBc was detected in this study, no diet effects on reproduction variables were observed, apart from a trend for an effect on day till first P4 rise and mean P4 concentration. The trend for a diet effect on days till first P4 rise pp was most probably established by decreasing the milk energy output by milk fat depression with the glucogenic diet. The numerically, but not significantly, greater maximal and mean P4 concentration during the first luteal phase and the trends observed for days till first P4 rise and mean P4 concentration suggest that animal numbers were limited to detect diet effects on reproduction variables.

Most studies on feeding extra glucogenic nutrients did not find an effect on milk lactose (Hoedemaker et al., 2004; Krause et al., 2003; Peterson et al., 2003) or reported an increase in milk yield and consequently also an increase in lactose yield (Eriksson et al., 2004)(Eriksson et al., 2004). In the current study there was no diet effect on lactose yield for both primiparous (1.41 ± 0.01 kg/d) and multiparous cows (1.98 ± 0.01 kg/d). However, in contrast to our previous study where lipogenic diet tended ($P < 0.10$) to increase milk lactose percentage (chapter 3), in the present study milk lactose percentage was greater for multiparous cows fed glucogenic diet, which can possibly be explained by a lower SCC for cows fed glucogenic diet compared with lipogenic diet. Mastitis has been associated with mammary tissue damage, open-up of tight junctions between secretory cells and increased permeability of blood capillaries (Kitchen, 1981). This results in diffusion of ions down their respective concentration gradients and decreases milk lactose percentage in order to maintain a constant osmolarity in the milk (Kaufmann and Hagemester, 1987). The greater SCC for multiparous cows fed lipogenic diet can not be completely attributed to a higher proportion of cows with clinical mastitis in lipogenic diet group. After exclusion of the cows with clinical mastitis the SCC for multiparous cows fed glucogenic diet or mixed diet was still less compared with cows fed lipogenic diet.

Conclusions

Increase in dietary glucogenic nutrients in multiparous cows during the transition period and early lactation improved the energy status, as reflected in EBc, plasma NEFA and BHBA concentration and liver TAG content. Higher plasma insulin concentration pp in multiparous cows fed glucogenic diet indicated insulin to be an intermediate in altered energy partitioning through different dietary energy sources. The results of the current study suggest that multiparous cows fed a mainly glucogenic diet have a decreased risk of metabolic and reproductive disorders, like ketosis, fatty liver and an increased number of days pp till first ovulation. Further studies are needed to confirm this hypothesis. In particular large-scale studies which monitor

cows from the transition period till mid-lactation could present the relationship between dietary energy source and pregnancy rates.

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CHAPTER 6

Natural antibodies related to energy balance in early lactation dairy cows

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Abstract

The objectives of this study were to determine the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows and to study the relation between NAb concentrations and energy balance (EB) and dietary energy source. Cows ($n=76$) were either fed a mainly glucogenic, lipogenic or mixture of both diets (50:50 dry matter basis) from wk 3 before the expected calving date until wk 9 postpartum (pp). Diets were isocaloric (net energy basis) and equal in intestinal digestible protein. Blood and milk were sampled weekly. Liver biopsies were taken in week -2, 2, 4 and 6 relative to calving. Data are expressed as LSM \pm SEM. NAb titers are expressed as the $^2\log$ values of the highest dilution giving a positive reaction. NAb concentration in plasma binding either keyhole limpet hemocyanin (KLH) or *Escherichia coli* lipopolysaccharide (LPS) increased with parity ($P<0.01$). NAb concentration binding KLH was greater ($P<0.01$) for cows fed the glucogenic diet (9.63 ± 0.08) compared with the lipogenic diet (9.26 ± 0.08). In milk, cows fed the glucogenic diet had smaller ($P<0.01$) NAb concentrations binding KLH (3.98 ± 0.18) and LPS (2.88 ± 0.17) compared with cows fed the mixed diet (KLH: 4.93 ± 0.18 ; LPS: 3.70 ± 0.17). NAb concentration in plasma had a positive relation ($P<0.05$) with energy balance variables: EB, dry matter intake (DMI), milk yield, and plasma cholesterol, whereas NAb concentration in milk had a negative relation ($P<0.05$) with energy balance variables: EB, DMI, and plasma cholesterol. Additionally, NAb concentrations in milk had a positive relation ($P<0.05$) with plasma non-esterified fatty acid concentration and milk fat and protein percentage. There was a tendency ($P<0.10$) for a positive relation of NAb concentration binding LPS in plasma and somatic cell count (SCC) in milk. No significant relations were detected between NAb concentrations in milk or plasma and plasma β -hydroxybutyrate concentration and liver tri-acyl glyceride content. In conclusion, NAb are present in both milk and plasma of dairy cows peripartum and NAb concentrations increase with parity. Furthermore, our data indicate that a negative energy balance in dairy cows in early lactation can be associated with compromised innate immune function as indicated by decreased NAb concentration in plasma.

Introduction

Lactation imposes serious metabolic demands for high-producing dairy cows in the periparturient period. A failure to meet the energy needs for milk production by energy intake results in a negative energy balance (NEB), which is in particular accompanied by metabolic disorders like fatty liver and ketosis (Grummer, 1993). Feeding extra glucogenic nutrients rather than lipogenic nutrients improved energy balance (EB) (chapter 3) and decreased plasma β -hydroxybutyrate (BHBA) and liver tri-acyl-glyceride (TAG) concentrations, suggesting a decreased risk of ketosis and fatty liver (Adler, 1970). Additionally, the early lactation period has been related with increased incidences of infectious diseases, like mastitis, endometritis and laminitis (Collard et al., 2000; Heuer et al., 1999). These diseases have been related to suboptimal immune function in the periparturient period (Mallard et al., 1998), as illustrated by diminished mitogen-induced lymphocyte proliferation (Kehrli et al., 1989), decreased serum IgG concentrations (Dettileux et al., 1995) and lowered antibody responses (Mallard et al., 1997). Furthermore, several studies investigated the relation between NEB-related metabolic disorders and peripartum immune suppression in dairy cows. *In vitro*, it was shown that ketone bodies negatively affect the chemotactic and proliferative capacity of lymphocytes (Nonnecke et al., 1992; Suriyasathaporn et al., 1999; Targowski and Klucinski, 1983). Both ketone bodies (Franklin et al., 1991) and non-esterified fatty acids (NEFA) (Lacetera et al., 2004) were reported to decrease mitogen-induced IgM secretion by leukocytes *in vitro*. *In vivo*, high BHBA concentration during a status of ketosis has been related to an increased severity of mastitis as indicated by bacterial counts (Kremer et al., 1993).

Natural antibodies (NAb) are defined as antigen-binding antibodies present in non-immunized individuals and can be considered as a humoral part of the innate immune system. In mammals, NAb are preferentially derived from CD5⁺ B (B1) cells (Casali and Notkins, 1989) located in the peritoneal cavity (Ochsenbein et al., 1999), and along the intestinal tract (Quan et al., 1997). Natural antibodies are characterized by a broad specificity repertoire, with usually low binding affinity. In mammals NAb are mostly of the IgM isotype class (Boes, 2000). In cooperation with the complement system, NAb might act as a first line of defense (Thornton et al., 1994), and can be regarded as the specific part of the innate immune system. Antigen uptake, processing, and presentation via B cells or dendrites may be enhanced by NAb, that provide initial protection against infection. Natural antibody concentrations increased during aging in dairy cows (Srinivasan et al., 1999) and chickens (Parmentier et al., 2004). This indicates that NAb are the cumulative result of antigenic stimulation of the poly-specific receptors of B1 B cells (Tomer and Shoenfeld, 1988).

Little is known about the existence and function of NAb in dairy cows in the peripartum period. The objectives of the present study were firstly to determine the presence of NAb binding keyhole limpet hemocyanin (KLH) and *Escherichia coli*

lipopolysaccharide (LPS) in plasma and milk of high-producing dairy cows in the transition period. The second objective was to study the relation between NAb concentration and energy balance (EB) and indicators of metabolic disorders, like BHBA and liver tri-acyl glyceride (TAG) concentration. The third objective was to study the effect of dietary energy source on NAb concentration. The overall experiment had been designed to study the effect of dietary energy source during the transition period and early lactation on EB, metabolites, metabolic hormones and days till first ovulation in high-producing dairy cows. These results were described earlier (chapter 5).

Materials and Methods

Experimental design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. The experimental design, ingredient and chemical composition of the diets, calculation of EB and analytical procedures for determination of plasma NEFA, BHBA, cholesterol, glucose and insulin concentration and liver TAG content were previously reported (chapter 5). Cows (n=76) were blocked by parity, calving date and milk production in the previous lactation (multiparous cows) or expected milk production based on pedigree (primiparous cows) and assigned to either a mainly lipogenic (n=26), a mainly glucogenic (n=25) or a mixed diet (50:50 DM basis) (n=25) from week 3 before the expected calving date until week 9 postpartum (pp). Basic diet did not differ among treatments and consisted of grass silage, corn silage, chopped alfalfa hay, and rapeseed meal (48:45:3:3 DM basis). Corn and milocorn or rumen protected fat and citrus pulp were the main concentrate ingredients of the glucogenic and lipogenic concentrate, respectively. Concentrate and forage was supplied separately (38:62 DM basis). Diets were isocaloric (net energy basis; VEM system) (Van Es, 1975) and equal in intestinal digestible protein and degradable protein balance (DVE/OEB system) (Tamminga et al., 1994). Cows were housed in a free-stall with slatted floor and cubicles and milked twice daily (0600h and 1700h).

All cows (n=76) were monitored for milk production, body weight and plasma metabolites and metabolic hormones. A random subset of cows was monitored for feed intake (n=72) and liver TAG content (n=42). Blood samples were obtained weekly from week -3 (minimal 3 days after feeding first experimental diet) until week 9 pp, 2-3 hours after the a.m. feeding. Blood samples were stored on ice for maximal 2 hours. Plasma was obtained by centrifugation (10 min at 2900 Xg), aliquoted, and frozen at -20°C until analysis. From week 2 until week 9 pp, at the same day as the blood samples, milk was sampled during the p.m. milking and stored at -20°C until analysis of NAb. Liver biopsies were obtained in week -2, 2, 4 and 6 relative to week of calving.

Table 1. Distribution of cows (n=76) per diet per parity class.

	Diet			Total
	Glucogenic	Mixed	Lipogenic	
Parity				
1	7	7	7	21
2	8	7	7	22
3	5	5	7	17
≥ 4	5	6	5	16
Total	25	25	26	76

Analytical procedures

Total immunoglobulin titres (NAb titres) binding keyhole limpet hemocyanin (KLH) (Cal Biochem-Novabiochem Co., San Diego, CA) and *Escherichia coli* O55:B5 LPS (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were determined by indirect ELISA in plasma and milk of all cows. Ninety-six-well plates were coated with 1 µg of KLH/ml or 4 µg of LPS/ml (100 µl/well) dissolved in carbonate buffer (pH 9.6). The plates were incubated overnight at 4°C and then washed twice with water and 0.05% Tween. Plates for NAb determination in milk were blocked with 2.5% rabbit serum in PBS and 0.05% Tween. Serial dilutions of plasma (1:4) or milk (1:3) in PBS, 0.05% Tween, and 2.5% rabbit serum were added, dilutions started at 1/40 for plasma and 1/30 for milk samples. Plates were incubated for 1 hr at room temperature. After being washed with water and 0.05% Tween, binding of antibodies was detected using 1:10,000 diluted rabbit-anti-bovine IgG (whole molecule) conjugated to peroxidase (RAB/IgG_{H+L}/PO) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After being washed, tetramethylbenzidine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as a substrate, and 0.05% H₂O₂ were added and incubated for 10 min at room temperature. The reaction was stopped by adding 1.25 M H₂SO₄. Extinctions were measured with a Multiskan reader (Labsystems, Helsinki, Finland) at a wavelength of 450 nm. Titres were expressed as the ²log values of the highest dilution giving a positive reaction.

Statistical analysis

Repeated measures analysis of variance (PROC MIXED (Littell et al., 1996) of SAS[®] VERSION 9.1; SAS Institute, Inc., Cary, NC) was performed for EB, DMI, milk yield and composition, metabolites and metabolic hormones, NAb concentration binding KLH and NAb concentration binding LPS measured in milk or plasma. Diet (glucogenic, lipogenic or mixed), week (-3 till 9 pp), parity (1, 2, 3 and ≥ 4) and a diet × parity interaction (D×P) were included in the model as fixed effects (model 1). Parity was distributed over 4 classes, to obtain a balanced distribution of cows per

class (Table 1). Preliminary analysis showed no significant effect of diet \times week interaction and was therefore not included in model 1. A first-order autoregressive structure (AR(1)) was the best fit and was used to account for within-cow variation. Model assumptions were evaluated by examining the distribution of residuals. Values are presented as LSM with their SEM. Secondly, to test relations between NAb concentration (binding KLH or LPS) and EB, DMI, milk production and metabolites, these variables were included as fixed effects in a repeated measurements model (model 2) to obtain regression coefficients (β). To avoid collinearity, week, diet and parity were not included in model 2.

Table 2. Energy balance (EB), dry matter intake (DMI), milk production and composition, metabolites and metabolic hormones for dairy cows fed a glucogenic (n=25), a lipogenic (n=26) or mixed diet (n=25) (LSM \pm SEM). Energy balance and milk variables represent lsmeans for wk 1 till wk 9 relative to calving. Metabolic variables represent lsmeans for wk -3 till wk 9 relative to calving

	Diet			SEM	P-values			
	Glucogenic	Mixed	Lipogenic		Diet	Week	Parity	D \times P
EB (kJ/kg ^{0.75} /d) ¹	-38 ^a	-103 ^b	-76 ^{a,b}	15	0.01	<0.01	<0.01	0.03
DMI (kg/d) ¹	20.2	20.0	20.5	0.3	0.40	<0.01	<0.01	0.92
Milk yield (kg/d)	40.2	40.1	39.7	0.8	0.88	<0.01	<0.01	0.63
Milk fat (%)	3.86 ^a	3.10 ^{a,b}	4.16 ^b	0.07	<0.01	<0.01	<0.01	0.65
Milk protein (%)	3.26	3.26	3.26	0.04	0.99	<0.01	0.56	0.78
Milk lactose (%)	4.62 ^a	4.60 ^a	4.53 ^b	0.02	<0.01	<0.01	<0.01	0.28
SCC ² , $\times 10^3$	3.60 ^a	3.67 ^a	4.25 ^b	0.16	<0.01	<0.01	<0.01	<0.01
NEFA (mMol/l)	0.23 ^a	0.29 ^b	0.29 ^b	0.01	<0.01	<0.01	<0.01	<0.01
BHBA (mMol/l)	0.63 ^a	0.73 ^b	0.76 ^b	0.03	<0.01	<0.01	0.40	0.02
Cholesterol (mMo)	3.55 ^a	4.44 ^b	4.73 ^b	0.16	<0.01	<0.01	0.16	0.12
Glucose (mMol/l)	3.43	3.45	3.40	0.03	0.44	<0.01	<0.01	0.73
Insulin (uIU/ml)	4.39	4.04	3.64	0.28	0.16	<0.01	0.11	0.18
Liver TAG (mg/g wet weight) ³	25.8	49.0	49.8	7.6	0.06	<0.01	0.35	0.03

^{a,b} Values in the same row with different superscripts differ ($P < 0.05$)

¹ Energy balance and DMI data are based on 23 vs. 25 vs. 24 cows.

² Somatic cell count, natural logarithm $\times 10^3$ /ml

³ Liver tri-acyl glycerides (data are based on 12 vs. 17 vs. 13 cows).

Results

Energy balance and metabolites

Earlier we presented the effects of glucogenic vs. lipogenic and mixed diet on EB, DMI, milk yield and composition, metabolites and metabolic hormones, for the

prepartum and postpartum period and multiparous and primiparous cows separately (chapter 5). Table 2 presents a summary of these results. While DMI, milk yield and milk protein content did not differ among diets, cows fed the glucogenic diet had lower milk fat content, less negative EB, lower plasma NEFA, BHBA and cholesterol concentration and postpartum a greater plasma insulin concentration ($P<0.01$). Somatic cell count in milk was higher for cows fed the lipogenic diet ($P<0.05$) compared with the other diet groups and decreased ($P<0.01$) with week pp, in particular for cows fed the lipogenic diet.

NAb related to diet and parity

NAb activity was present in both plasma and milk for all cows. In plasma, NAb concentration binding KLH was lower ($P<0.05$) for cows fed the lipogenic diet compared with cows fed the glucogenic diet (Table 3). In plasma, NAb concentrations binding to KLH and LPS decreased prepartum and increased from week 2 pp (Figure 1). In milk, NAb concentrations binding KLH and LPS were higher for cows fed the mixed diet compared with the other diet groups ($P<0.05$) and decreased from week 2 until week 9 pp (Figure 2). In plasma, NAb concentrations binding KLH increased with parity. NAb concentration binding LPS increased with parity for cows fed lipogenic and glucogenic diet, but decreased with parity for cows fed the mixed diet (Figure 3). Somatic cell count in milk was greater for cows fed the lipogenic diet ($P<0.05$) compared with the other diet groups and decreased with week pp, in particular for cows fed the lipogenic diet.

Table 3. Natural antibody in plasma and milk binding KLH¹ and LPS² and SCC³ in milk in the transition period for dairy cows fed a glucogenic, a lipogenic or a mixed diet (LSM ± SEM).

	Diet				P-values			
	Glucogenic	Mixed	Lipogenic	SEM	Diet	Week	Parity	D×P
In plasma, week -3 until 9								
KLH	9.63 ^a	9.40 ^{a,b}	9.26 ^b	0.08	<0.01	<0.01	<0.01	0.11
LPS	8.53	8.56	8.40	0.07	0.22	<0.01	0.13	0.01
In milk, week 2 until 9								
KLH	3.98 ^a	4.93 ^b	4.39 ^a	0.18	<0.01	<0.01	0.81	0.80
LPS	2.88 ^a	3.70 ^b	3.33 ^a	0.17	<0.01	<0.01	0.44	0.19
SCC	3.60 ^a	3.67 ^a	4.25 ^b	0.16	<0.01	<0.01	<0.01	<0.01

^{a,b} Values in the same row with different superscripts differ ($P<0.05$).

¹ Keyhole limpet hemocyanin.

² Lipopolysaccharide.

³ Somatic cell count, natural logarithm × 10³/ml.

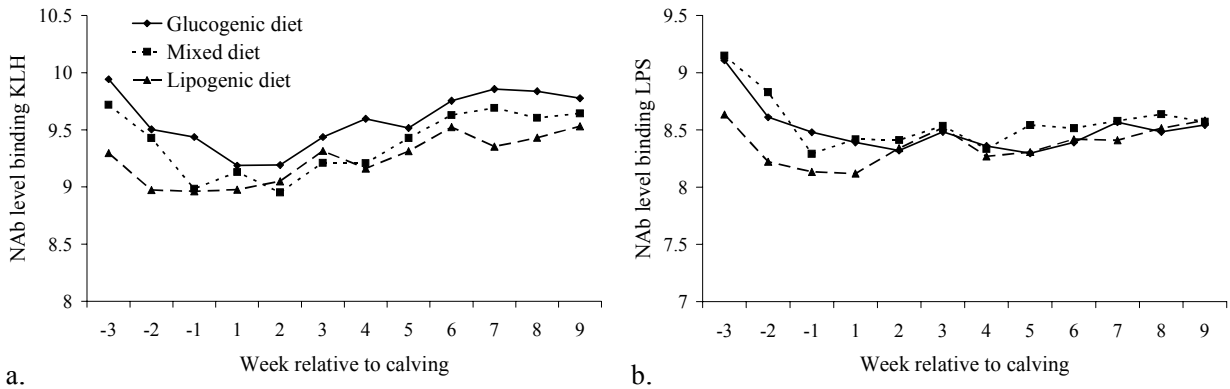


Figure 1. NAb concentration binding keyhole limpet hemocyanin (KLH) (a) or lipopolysaccharide (LPS) (b) in plasma for all cows from wk -3 till wk 9 relative to calving. Cows were fed a glucogenic, a lipogenic or a mixed (50:50 DM basis) diet. Values represent means per diet per week.

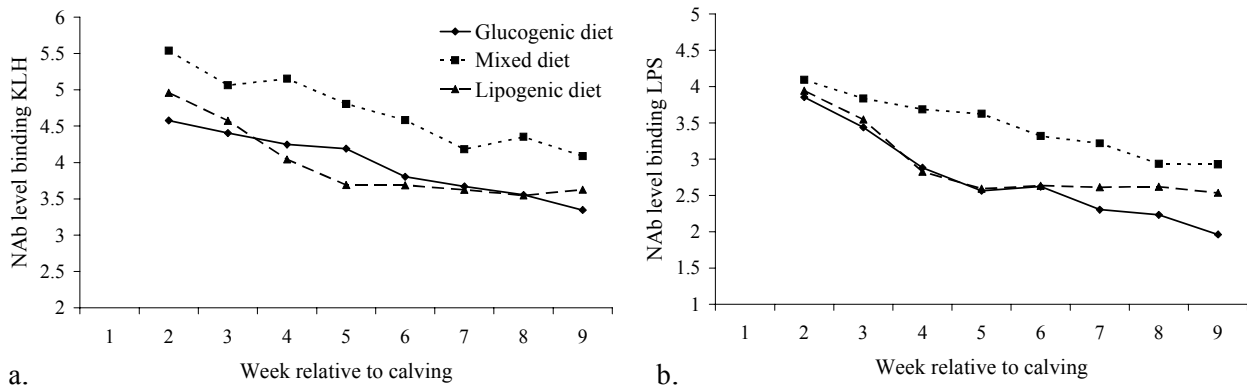


Figure 2. NAb concentration binding keyhole limpet hemocyanin (KLH) (A) or lipopolysaccharide (LPS) (B) in milk for cows from wk 2 till wk 9 relative to calving. Cows were fed a glucogenic, a lipogenic or a mixed (50:50 DM basis) diet. Values represent means per diet per week.

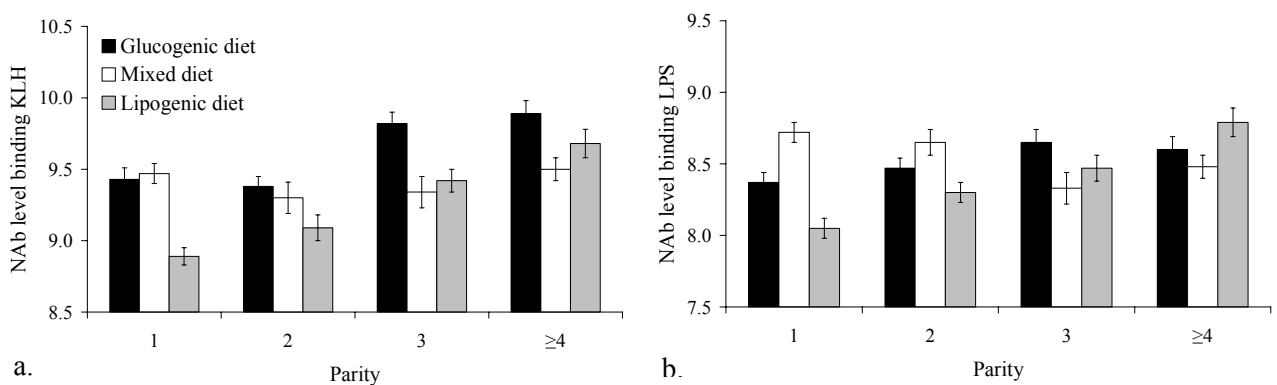


Figure 3. NAb concentration binding keyhole limpet hemocyanin (KLH) (A) and lipopolysaccharide (LPS) (B) in plasma for cows from wk -3 till wk 9 relative to calving per diet per parity class. Cows were fed a glucogenic, a lipogenic or a mixed (50:50) diets. Values represent weekly observations averaged per diet per parity class.

NAb related to EB and EB-related metabolites

In plasma, NAb concentration binding KLH had a positive relation ($P<0.05$) with EB, DMI, milk yield, milk lactose and plasma cholesterol and a negative relation with milk protein percentage (Table 3). NAb concentration binding LPS tended ($P<0.10$) to have a positive relation with EB, SCC and plasma cholesterol. Relations of EB and EB-related variables of NAb concentrations in milk were opposite to relations found in plasma, except for plasma glucose and insulin. In milk, NAb concentrations binding KLH had a negative relation ($P<0.05$) with DMI, EB, plasma cholesterol, glucose and insulin and a positive relation with milk fat and protein percentage and plasma NEFA. In milk, NAb concentrations binding LPS had a negative relation with EB, DMI and plasma cholesterol and a positive relation with milk protein percentage and plasma NEFA.

Relation between NAb concentrations in plasma and milk

There was a tendency for negative relation between NAb in plasma and NAb in milk for both NAb binding KLH ($\beta = -0.101$; $P=0.07$) and LPS ($\beta = -0.100$; $P=0.12$).

Table 3. Regression coefficients (β)¹ of natural antibody concentrations binding KLH¹ and LPS², related to energy balance (EB), dry matter intake (DMI), milk production and composition, metabolites and metabolic hormones in dairy cows from week -3 till week 9 relative to calving.

	Plasma		Milk	
	KLH	LPS	KLH	LPS
Energy balance (kJ/kg ^{0.75} /d)	0.001**	0.0004*	-0.002**	-0.002**
DMI (kg/d)	0.039**	0.013	-0.059**	-0.054**
Milk yield (kg/d)	0.015**	0.006	-0.016	-0.013
Milk fat (%)	-0.032	-0.058	0.207**	0.196*
Milk protein (%)	-0.287**	-0.210*	1.197**	1.297**
Milk lactose (%)	0.536**	0.087	0.006	-0.092
SCC ³ , $\times 10^3$	-0.015	0.042*	0.020	0.046
NEFA (mMol/l)	-0.252	-0.028	0.686**	1.079**
BHBA (mMol/l)	-0.120	-0.067	0.179*	0.001
Cholesterol (mMol/l)	0.102**	0.038*	-0.205**	-0.196**
Glucose (mMol/l)	-0.051	-0.023	-0.212**	-0.116
Insulin (uIU/ml)	-0.003	0.003	-0.070**	-0.008
Liver TAG (mg/g wet weight)	-0.002	-0.001	0.005	0.001

¹ Keyhole limpet hemocyanin.

² Lipopolysaccharide.

³ Somatic cell count, natural logarithm $\times 10^3$ /ml.

** : $P<0.05$; * : $P<0.10$.

Discussion

In the present study we detected binding of antibodies to the model antigens KLH (glycoprotein) and LPS (lipopolysaccharide) in plasma and milk of dairy cows in the peripartum period that had not been intentionally immunized with these components. In general, antibodies from non-immunized individuals, when detected in specific binding assays such as ELISA, are regarded as 'background' (Ochsenbein et al., 1999). Conversely, it is known that next to antigen-specific antibodies, non-specific antibodies, with no or unknown binding specificity (NAb) are present in the blood of healthy people and laboratory rodents (Dacie, 1950), fish (Gonzalez et al., 1988) and poultry (Parmentier et al., 2004). NAb are probably derived from CD5+ B(1) cells (Casali and Notkins, 1989). Since approximately 20% of the peripheral B cells are CD5+, NAb form an important part of the antibody repertoire as well as the mass of immunoglobulins in mammals. As earlier discussed for poultry (Star et al., 2007), the major difference between responses of NAb binding KLH or NAb binding LPS, is that cows did not, and probably will not encounter KLH, thus reflecting a capacity to respond, whereas cows probably did encounter LPS, thus the latter reflects an active status of the innate immune system.

NAb activity was detected in all cows and NAb concentrations in plasma increased with parity. This indicates a relation between parity and the capacity of cows to produce NAb in plasma and milk. Indeed, Srinivasan et al. (1999) found greater concentrations of NAb binding mannans in adult cows compared with calves and newborn calves. The origin of NAb is subject of debate. The NAb repertoire and concentrations may be either shaped by continuous polyclonal stimulation by exogenous microbes initiating cross reactivity driven responses of auto-reactive B cells, or correspond with the secretion of naturally occurring (auto-)reactive B cell clones, or both (Avrameas, 1991). The positive effect of parity on NAb concentration in cows corresponds with the idea that exogenous stimuli enhance the NAb formation (Prokesova et al., 1996).

The function of NAb in bovine milk, produced during wk 2 until wk 9 relative to calving, as reported in the current study, remains unspecified. It can be hypothesized that if calves are able to absorb IgG, or NAb in particular, after the intestinal closure at about 24 h of age (Rajala and Castren, 1995), NAb in milk might contribute to the immune competence of young calves. Although concentration of IgG in milk rapidly declines during the first 3 d pp (Stott et al., 1981), a possible immunological value for NAb in milk in later stages of lactation is confirmed by the expression of the FcRn receptor, neonatal IgG transporter, in the small intestine of adult cows (Kacskovics et al., 2000). This indicates that also after the intestinal closure soon after birth, the ruminant is still able to selectively transport IgG across the intestinal wall via the FcRn receptor. This is in accordance with the human FcRn receptor, that has been detected in both fetal and adult intestinal epithelial cells (Israel et al., 1997), whereas in rodents expression of the FcRn in intestinal cells is limited to the suckling period (Martin et al., 1997). Alternatively, it can be speculated that NAb in milk are a residue of the

immune barrier in the mammary gland and therefore contribute to the resistance to mammary infections.

The relation between nutrition and immune function in dairy cows is complicated and remains subject of interest (Goff, 2006). Recently, beneficial effects have been reported of glutamine supplementation (Doepel et al., 2006) and poly-unsaturated fatty acids (PUFA) (Lessard et al., 2003, 2004) on the immune response in dairy cows in early lactation. Also parity x supplement interactions were observed. These effects, however, were limited and not always unambiguous. Further, grain-induced subacute ruminal acidosis (SARA) has been related to increased lysis of gram-negative bacteria and increased concentration of LPS in the rumen of lactating dairy cows (Gozho et al., 2007). Earlier, increased ruminal LPS concentration has been related to an increase in peripheral plasma LPS concentration in calves (Aiumlamai et al., 1992). In the current study, we aimed for increasing the glucogenic nutrient content in the diet by feeding rumen resistant starch, trying to avoid cows suffering from SARA. In an earlier experiment (Van Knegsel et al., 2007a), we reported with similar diets no enhancing effect of the glucogenic diet on the secretion of short chain fatty acids in milk, indicating no increase in de novo fatty acid synthesis as established by lowering of ruminal pH associated with SARA. This might imply that, although we fed a glucogenic diet, because this diet was especially high in rumen resistant starch, cows had not an increased risk for SARA and therefore did not experience an increase in ruminal LPS concentration. With high concentrations of dietary rumen resistant starch, starch fermentation is high in the intestine and cows are possibly more at risk for an increase in LPS at the intestine level. Nevertheless, there were no indications for high LPS concentration in the intestine and no diet-related effect on NAb binding LPS could be detected. We found greater NAb concentrations binding KLH for cows fed the glucogenic diet compared with cows fed the lipogenic diet. As unexpected, this diet effect was already present in the first plasma sample that was taken 3 days after feeding the first experimental diet. Information on the NAb concentration before feeding is lacking, but rapid effects at 5 days after start of dietary supplementation (PUFA) on immune variables were reported earlier (Lessard et al., 2003). Cows fed the glucogenic diet had not only greater NAb concentrations binding KLH, but also a significant improved EB compared with the other diet groups, that supports the hypothesis that in early lactation the severity of the NEB can be related to innate immune function.

NAb concentrations binding LPS increased with parity for cows fed the lipogenic and glucogenic diet, in contrast to cows fed the mixed diet that had smaller NAb concentrations for the greater parity classes (Figure 3). Furthermore, third and greater parity cows fed the mixed diet had an extreme NEB, compared with the other diet groups (Figure 4). Confirmed by the presented relation between NAb concentration binding LPS and EB, the decrease with parity in NAb concentration binding LPS for the mixed diet might be related to the extreme negative energy status as calculated for these diet x parity groups. In accordance with this indication, figure 3a shows that also NAb binding KLH hardly increased ($P>0.05$) with parity for cows fed the mixed diet, in contrast to the other diets, although a diet x parity interaction was not detected for NAb binding KLH.

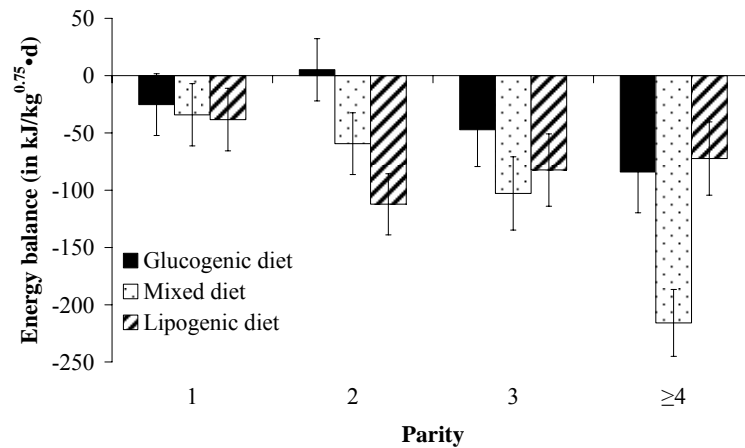


Figure 4. Calculated energy balance (in kJ/kg^{0.75}·d) for cows from wk 1 till wk 9 relative to calving per diet per parity class. Cows were fed a glucogenic, a lipogenic or a mixed (50:50) of both diets. Values represent LSM \pm SEM.

Earlier, several studies associated EB or EB-related variables in early lactation dairy cows with indicators of humoral (Mallard et al., 1997) or cellular (Kehrl et al., 1989; Lacetera et al., 2005) adaptive immune function. Not only a negative energy status, but also NEB-related metabolic disorders like ketosis (Franklin et al., 1991; Suriyasathaporn et al., 1999) and fatty liver (Bobe et al., 2004) have been suggested to be related to impaired immune function, as indicated by decreased antibody responses (Wentink et al., 1997), impaired neutrophil function (Hoeben et al., 2000), decreased capacity for phagocytosis by macrophages (Zerbe et al., 2000) and a depression of interferon production by leukocytes (Szuster-Ciesielska et al., 1995). The objective of the current study was to relate EB and plasma metabolites to innate immune function *in vivo* as indicated by NAb concentrations in order to find evidence for NEB associated innate immune suppression in early lactation dairy cows. Indeed, we found relations between NAb concentrations and EB, DMI and milk yield. In addition, plasma NEFA, that is considered as an indicator for body fat mobilization and NEB, was related to NAb concentrations in milk. Although, decreased plasma insulin concentration stimulates lipolysis and NEB, insulin was not related to NAb concentrations. Furthermore, in contrast to earlier studies that related immune suppression to metabolic disorders (e.g. Wentink et al., 1997), no relations were detected between NAb concentrations and indicators for NEB-associated metabolic disorders ketosis (BHBA) and fatty liver (liver TAG content). This might imply that a cow in a status of NEB might not be able to maintain its concentrations of humoral natural immunity, though the innate immunity seems not to be affected by postpartum metabolic disorders, like ketosis and fatty liver. On the other hand, the important role of NAb in the direction of either TH-1 or TH-2 mediated specific immunity (Bayry et al., 2004, 2005), activation of B-cells and adjuvating effect on cellular responses (Stager et al., 2003), may be related with the increase in infectious diseases in early lactation. In this respect it is noteworthy that cows fed a glucogenic diet were characterized by greater NAb concentrations binding KLH in plasma and smaller SCC, whereas cows fed a lipogenic diet were characterized by smaller NAb concentrations binding KLH and a greater SCC.

Indicators for immune competence or health status are of importance in dairy practice. Somatic cell count has been considered as an indicator for udder health and therefore the trend for a positive relation between NAb concentration binding LPS and SCC is interesting, but should be confirmed in further studies. There were 9 incidences of clinical mastitis detected during the first 9 weeks of lactation. Clinical mastitis was related to a numerically, but not significantly, increased concentration of NAb binding LPS compared with non-mastitis cows in both plasma (8.48 ± 0.02 vs. 8.57 ± 0.32 (mean \pm SE); $P=0.84$) and milk (2.98 ± 0.06 vs. 3.40 ± 0.54 (mean \pm SE); $P=0.14$). If NAb concentration would turn out to be a good indicator for immune function in early lactation dairy cows, it can still be questioned how to interpret the negative relation between NAb concentration in plasma and NAb concentration in milk. Furthermore, whereas NAb in plasma had a positive relation with several energy balance indicators (EB, DMI, milk yield and plasma cholesterol), concentration of NAb in milk turned out to have a negative relation with the majority of determined energy balance variables (EB, DMI, and plasma cholesterol). This is in accordance with tendencies for an inverse relation between NAb concentration in plasma and NAb concentration in milk. Based on these observations, it can be hypothesized that the partitioning of NAb between plasma (cow) and milk (calf) parallels the partitioning of energy between body reserves and milk as observed in early lactation dairy cows. It has been suggested that the current high-producing dairy cow has a tremendous priority in early lactation for her calf at the cost of cow's body condition, health and fertility (Friggens, 2003), as indicated by high milk yield, that results in a NEB and extensive body fat mobilization. The current data indicate that this extensive priority for her current calf, seems not only to be reflected in milk yield, but also in high NAb concentrations in milk in the first weeks of lactation while simultaneously NAb concentrations in plasma are decreased. The physiological mechanism, that implements this suggested priority for milk yield is considered to be a status of hypoinsulinemia in early lactation (Bonczek et al., 1988) that reduces glucose uptake by muscle and adipose tissue and makes glucose available for the mammary gland, that is not insulin-responsive (Bauman and Elliot, 1983). The physiological mechanism, that implements this hypothesized priority for high NAb concentrations in milk is unknown, although the reverse relation between NAb in plasma and milk indicates an active mechanism in regulating the concentration of NAb in milk. A possible candidate might be the FcRn receptor, as transcripts of this receptor were identified in the bovine mammary gland and suggested to play a role in regulating IgG transfer into milk (Kacskovics et al., 2000). In spite of this, theories concerning physiological mechanisms, that establish the partitioning of NAb to milk are as yet speculative.

Conclusions

From the present data, we conclude that NAb are present in dairy cows in early lactation. Apart from the increase with parity, indicating environmental sensitization,

NAb concentrations binding KLH and LPS in plasma had a positive relation with EB. Hereby, our data indicate that a NEB in dairy cows in early lactation can be associated with compromised innate immune function. The possible role of NAb in regulation of the immune response raises the question whether effects on the concentration of NAb underlie immune related health problems in dairy cows during early lactation. This implies that feeding diets to dairy cows to maintain NAb concentrations may favour maintenance of health. Further studies are in progress to unravel the relations among nutrition, NAb, somatic cell counts and various biochemical plasma variables.

CHAPTER 7

General discussion

Introduction

The hypothesis of this thesis was that increasing the availability of glucogenic nutrients relative to lipogenic nutrients in the diet of dairy cows in early lactation improves the energy balance (EB), health and fertility. The first study, a literature review, suggested that alteration in lipogenic and glucogenic nutrient content in the diet affected the EB and resulted in modifications in blood metabolic profiles and milk fat yield (chapter 2). To test these indications, the effect of two diets, differing in lipogenic and glucogenic nutrient availability, on energy partitioning in dairy cows in NEB was studied in experiment 1 (chapter 3 and 4). These studies showed that a mainly glucogenic diet compared with a lipogenic diet reduced body fat mobilisation by decreasing milk energy output by depressing milk fat. This positive effect of a glucogenic diet on the EB confirms the first part of the hypothesis. Experiment 2 (chapter 5) focused on the second part of the above mentioned hypothesis and results suggested that a glucogenic diet compared with a more lipogenic diet had potential to reduce the risk of ketosis and fatty liver in multiparous dairy cows in early lactation. Finally, the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows was determined and relations were detected between NAb and EB and indicators of metabolic disorders (chapter 6). These data showed that alterations in immune competence peripartum are reflected in the humoral part of the innate immune system. These alterations in the innate immune system may play a role in the increased incidence of infectious disease peripartum.

This discussion first focuses on increasing dietary glucogenic nutrient content relative to lipogenic nutrient content as a strategy to reduce body fat mobilisation and improve the EB, health and fertility. The second part of this chapter discusses, besides nutritional strategies, management and selection strategies as tools to reduce milk energy output and their potential for improving the EB and health in early lactation dairy cows.

Glucogenic nutrients to reduce body fat mobilisation and improve health

Mobilisation of body reserves

Both the first and the second experiment showed that a glucogenic diet compared with a lipogenic diet stimulate partitioning of energy to body reserves at the cost of milk energy output, which results in an improved EB and a reduction in body fat mobilisation in multiparous cows.

Multiparous cows fed a mainly glucogenic diet had less milk fat yield compared with the lipogenic diet (experiment 1: 1.68 vs. 1.90 ± 0.06 kg/d; experiment 2: 1.59 vs. 1.83 ± 0.05 kg/d) and had an improved EB (experiment 1: -94 vs. -172 ± 28 kJ/(kg^{0.75}·d)) or calculated EB (EBc) (experiment 2: -33 vs. -89 ± 21 kJ/(kg^{0.75}·d)). The improvement in EB did not affect the mobilisation of body protein, but body fat mobilisation tended

to be less ($P < 0.10$) in cows fed the glucogenic diet than in cows fed the lipogenic diet (332 ± 110 vs. 558 ± 96 g/d; Figure 1) in experiment 1. The difference in body fat mobilisation was in accordance with 220 g/d less milk fat produced in cows fed the glucogenic diet compared with cows fed the lipogenic diet. Milk fatty acid profiles indicated that the decrease in milk fat production in cows fed the glucogenic diet could be attributed to a decrease in milk fat from body reserves (Chilliard and Ferlay, 2004; Ward et al., 2002) as it was explained largely by a reduction in the secretion of long-chain fatty acids: C16:0 (96 g/d), C18:0 (51 g/d) and C18:1 (45 g/d).

During NEB, mobilisation of body protein contributed little to total mobilisation of body reserves in cows in the current study and overall energy retention in body protein (ERp) was positive during the NEB period, which is in agreement with Tamminga et al. (1997). Comparing body protein mobilisation in cows with lactating sows, both for week 2 till 3 pp, led to the conclusion that dairy cows mobilise body protein (-30 kJ/(kg^{0.75}·d)) besides body fat (-347 kJ/(kg^{0.75}·d)) during these early weeks of lactation and values were comparable between the current study on cows and the earlier report on lactating sows (body protein: 32 kJ/(kg^{0.75}·d); body fat: -466 kJ/(kg^{0.75}·d)) (van den Brand et al., 2000). Concerning week 2 and 3 pp, the relative contribution of body protein to mobilisation of body reserves compared with body fat seemed to be higher for cows than for sows. This might be explained by the use of primiparous sows compared with multiparous cows in the current study. Primiparous sows (Langendijk et al., 2007) and cows (Wathes et al., 2006) can be expected to have an additional priority besides lactation for growth, which is reflected by higher IGF-1 levels, also during NEB.

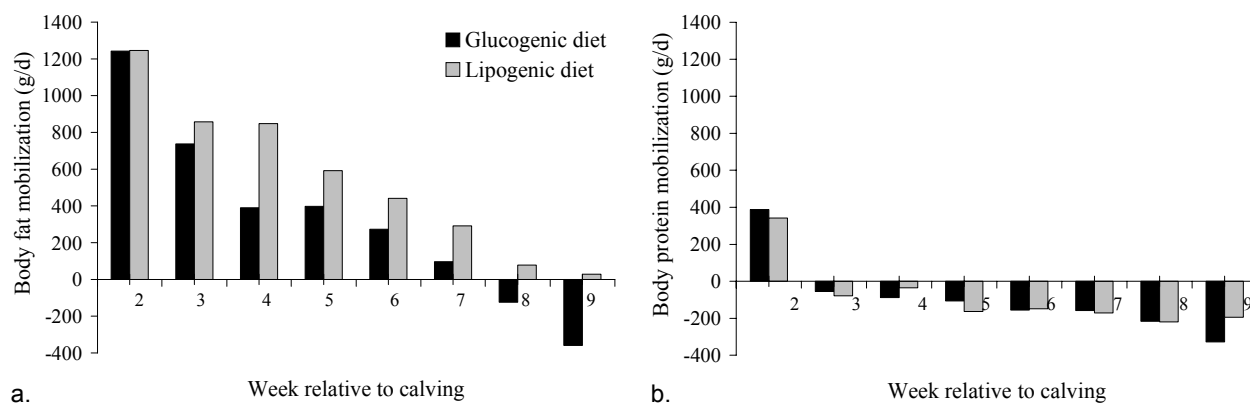


Figure 1. Body fat mobilisation (a) and body protein mobilisation (b) for dairy cows fed a mainly glucogenic (n=6) or a mainly lipogenic (n=7) diet from week 2 until week 9 relative to calving. Overall SEM: body fat mobilisation 74 g/d; body protein mobilisation 30 g/d.

Health aspects

The availability of glucogenic nutrients relative to lipogenic nutrients has been suggested to affect the susceptibility of cows to metabolic disorders like fatty liver and ketosis in early lactation (Adler, 1970; Kronfeld, 1976; chapter 2). Additionally, with an improvement of the EB with feeding a mainly glucogenic diet, as presented in these experiments, it can be expected that risks of metabolic and reproductive disorders (Butler, 1993) were reduced. Whereas experiment 1 seemed to have lack of power to illustrate a reduction in risk of fatty liver or ketosis, experiment 2 showed that multiparous dairy cows fed the glucogenic diet had lower plasma BHBA and liver TAG concentrations and additional a low percentage of cows in the highest BHBA and TAG classes, indicating a reduced risk of ketosis and fatty liver.

Suggested relation between NEB and the incidence of infectious diseases in early lactation (Collard et al., 2000; Heuer et al., 1999) implies that an improvement of the EB by a predominantly glucogenic diet in multiparous cows has potential to reduce the incidence of infectious diseases. These diseases have been related to suboptimal immune function in the periparturient period (Mallard et al., 1998) and consequently several studies focused firstly on describing the nature of this peripartum immune suppression (e.g. Kehrlı et al., 1989; Lacetera et al., 2004) and secondly on dietary treatments which were hypothesised to improve the immune competence of dairy cows in this period of lactation (Doepel et al., 2006; Lessard et al., 2004). However, nutritional effects on immune function in early lactation dairy cows were limited and not always unambiguous. The present study (Chapter 6) showed a significant positive relation between NAb levels and EB in early lactation, which suggests that the innate immune response is compromised during NEB in dairy cows. Secondly, this study presented a difference in natural antibody (NAb) levels for cows on different dietary treatments. With the current experimental set-up, however, the ability to draw definite conclusions on the relation between dietary energy source and NAb levels is debatable, so further research is needed.

Insulin as the metabolic signal

It can be hypothesised that dairy cows in a negative energy balance (NEB) suffer from an unbalanced availability of C3 (glucogenic) relative to C2 (lipogenic) nutrients, as supplied by dietary ingredients and mobilisation of body reserves (Chapter 2). Mobilisation of body reserves results in elevated availability of lipogenic nutrients, while glucogenic nutrients are driven towards lactose for the production of milk. We illustrated that the availability of C2 and C3 nutrients, when manipulated by ingredients in the diet, indeed affected body fat mobilisation as indicated by plasma metabolites. Lipogenic diets resulted in an increase in availability of C2 nutrients from dietary origin, but, due to enhanced mobilisation of body fat, also of C2 nutrients from body reserves. In conclusion, feeding glucogenic nutrients, compared with lipogenic

nutrients, to dairy cows in NEB can be beneficial to energy status in this period of lactation (Chapter 3 and 5).

Both experiment 1 and experiment 2 thus illustrated an improvement of EB through feeding a glucogenic diet compared with a lipogenic diet. The improvement of EB was established by a depression of milk fat and milk energy output. A possible explanation for milk fat depression by decreasing the lipogenic to glucogenic nutrient ratio is the glucogenic theory (McClymont and Vallance, 1962). According to this theory, an increase in insulin, induced by an increase in propionic acid or glucose, would decrease lipolysis and decrease the availability of milk fat precursors, which in turn may reduce milk fat and milk energy output. With an equal energy intake the depression in milk energy is likely to favour body reserves and improve the EB. However, more recently, evidence has been provided against a role for insulin in the depression of milk fat synthesis by high-concentrate diets (Bauman and Griinari, 2003; Neville and Picciano, 1997). Those authors based this conclusion in their reviews firstly on studies utilising a glucose clamp to maintain high plasma insulin concentration for four days found no effect on milk fat synthesis (Griinari et al., 1997; McGuire et al., 1995). Secondly, another study reported no change in plasma glucose and insulin concentration of cows that showed a substantial decrease in milk fat in response to a high-concentrate diet (Gaynor et al., 1995). Possibly, a major difference between the above-mentioned studies and the current experiments is the period of lactation. While cows in the current experiments were in NEB during the major part of the experimental period, cows were 109 DIM (Gaynor et al., 1995), 147 DIM (McGuire et al., 1995) and 184 DIM (Griinari et al., 1997) in above-mentioned studies, implying cows to be in a positive EB. Hence, it can be hypothesised that a NEB and a NEB-related relative shortage of plasma insulin would be essential for establishing a milk fat depression and improvement of the EB by glucogenic diets according to the glucogenic theory. This hypothesis is further confirmed by a recent study in which a hyperinsulinemic-euglycemic clamp resulted in a decrease in milk energy and improvement of the EB of dairy cows in early lactation (Butler et al., 2003).

A proposed alternative for the glucogenic theory which could explain the depression in milk fat and milk energy output in cows fed a glucogenic diet is a theory on alterations in rumen function (Bauman and Griinari, 2003; Neville and Picciano, 1997). This theory states that an increase in rumen-fermentable glucogenic nutrients may induce a decrease in ruminal pH. The altered rumen pH results in incomplete biohydrogenation of C18:3 and C18:2 and an increased formation of *trans*-C18:1, which is suggested to directly inhibit milk fat synthesis at the mammary gland (Baumgard et al., 2002). This proposed mechanism is in particular associated with a decrease in fatty acids synthesised *de novo* and results mainly in a decrease in short- and medium-chain fatty acids (Bauman and Griinari, 2003). Conversely, in experiment 1 (Chapter 3) the depression in milk fat could largely be explained by a decrease in the secretion of

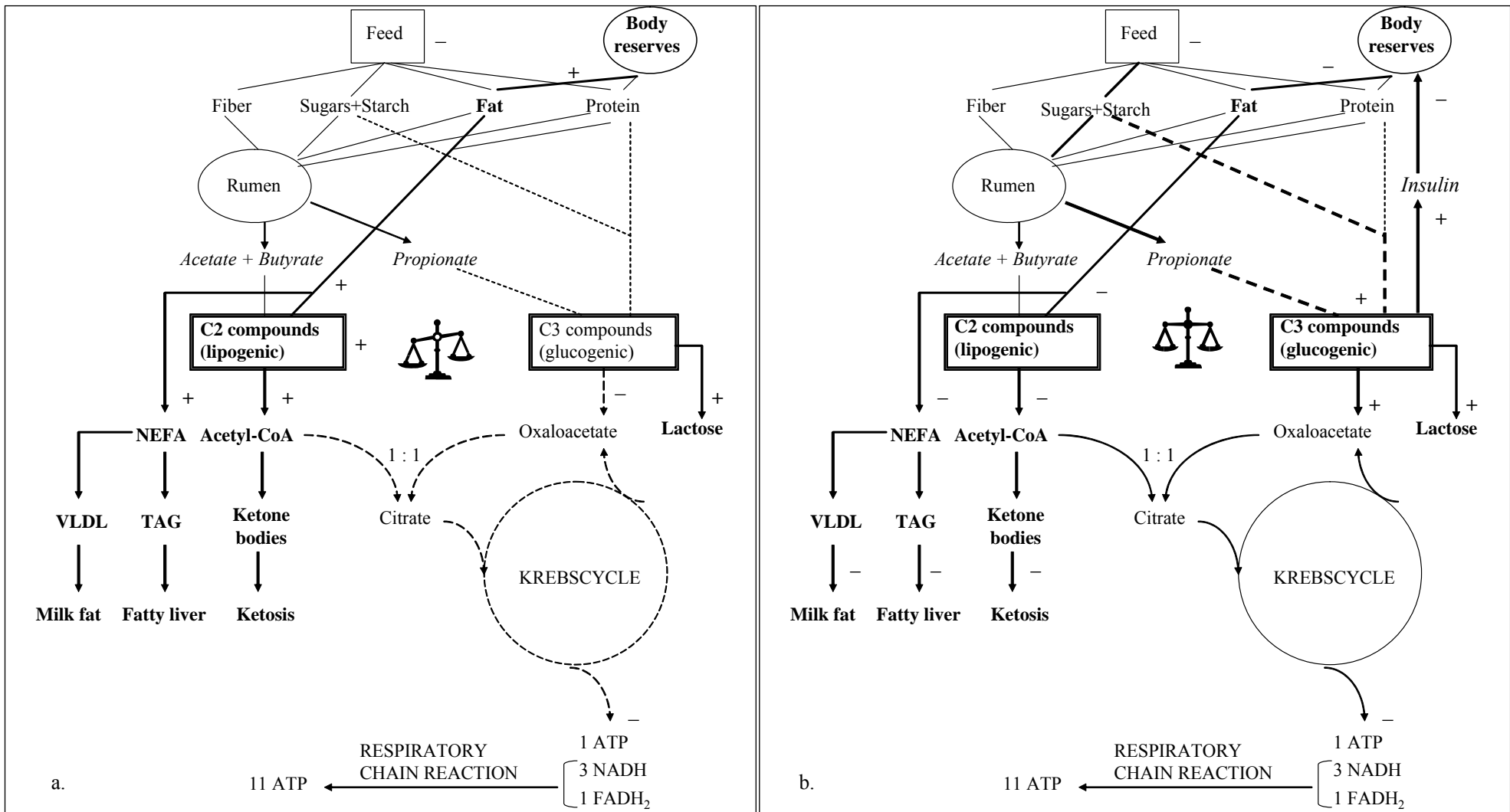


Figure 2. Energy metabolism of lactating dairy cows in a negative energy balance (a) (adjusted from Chapter 2); Energy metabolism of lactating dairy cows in a negative energy balance fed a mainly glucogenic diet (b).

long-chain fatty acids, while no differences were observed in the secretion of short and medium chain fatty acids in milk fat. This suggests that the depression in milk fat might be explained by a depression in milk fatty acids that originate largely from palm fatty acids in the diet or from mobilised body reserves. Therefore, both current experiments confirmed the glucogenic theory stating that insulin is an important metabolic signal in establishing the shift in energy partitioning from milk fat to body reserves realised by a glucogenic diet compared with a lipogenic diet (Figure 2).

Furthermore, insulin is known for its direct and indirect antiketogenic effects. These effects include decreasing liver NEFA uptake through stimulating lipogenesis and inhibiting lipolysis in adipose tissue, enhancing peripheral tissue ketone utilisation and altering enzyme activities and availability of substrates that are involved in hepatic ketogenesis (Brockman and Laarveld, 1986). Intramuscular injection of insulin has been proposed as a treatment to decrease lipolysis, ketogenesis and liver TAG content without compromising plasma glucose concentrations (Hayirli, 2002). Alternatively, the current results show a dietary strategy which increased plasma insulin concentration and decreased plasma BHBA, NEFA and liver TAG content and had no effect on glucose concentrations.

Glucogenic nutrients and fertility

Although in experiment 2 the experimental period during which a glucogenic, lipogenic or mixed diet was fed ended at 9 weeks pp, fertility data of cows were available during the complete lactation. Dairy cows fed the glucogenic diet tended to have an earlier ovulation postpartum compared with cows fed the lipogenic diet, no relations were detected between dietary energy source and number of inseminations per conception or days open (Table 1). Besides that, the effect on resumption of ovarian activity can be expected to be too small (6 days) to cause an effect on fertility in the long term. Moreover cows were randomly assigned to a new dietary treatment after 9 weeks pp, which might extinguish the trend for a diet effect on ovarian activity. With the currently discussed experiments, it still seems difficult to draw definite conclusions on the relation between glucogenic and lipogenic nutrients and reproductive performance, though expectations can be discussed. Firstly, suggestions have been made concerning beneficial effects of plasma glucose and insulin (Gong et al., 2002) and detrimental effects of high plasma NEFA and BHBA concentrations on fertility (Leroy et al., 2005; Walters et al., 2002). Secondly, an early postpartum return to cyclicity, as reported for multiparous cows fed the glucogenic diet, has been shown to be beneficial to overall reproductive performance in dairy cows (Staples et al., 1990; Stevenson and Britt, 1979). Delay in first postpartum ovulation (Darwash et al., 1997) and a reduced number of oestrous cycles before onset of breeding (Lucy et al., 1992c) is associated with reduced conception rates. It can be hypothesised that an improved EB, reduced risk of ketosis and fatty liver and an earlier resumption of ovarian activity, as presented for multiparous cows fed the glucogenic diet in

experiment 2, have potential to be beneficial for overall fertility. Full assessment of dietary energy source effects on fertility in dairy cows should be confirmed in large-scale studies, which monitor cows from the transition period till mid-lactation.

Table 1. Days till 1st P4 rise, days open and no of inseminations per conception of dairy cows fed a glucogenic diet, a lipogenic diet or a mixed of both diets (LSM \pm SEM).

	Diet			SEM	<i>P</i> -value Diet
	Glucogenic	Mixed	Lipogenic		
Primiparous cows, n	13	7	12		
No of cows ovulated	12	7	12		
Days till 1 st P4 rise	31.7	20.9	25.6	4.4	0.25
Days open	128	125	98	20	0.42
No of inseminations per conception	2.2	1.7	1.9	0.4	0.46
Multiparous cows, n	29	18	32		
No of cows ovulated	29	18	31		
Days till 1 st P4 rise	20.4	24.4	26.1	2.1	0.10
Days open	117	127	118	10	0.45
No of inseminations per conception	2.3	2.0	2.0	0.3	0.74

Reduction of body fat mobilisation: type of lipogenic and glucogenic nutrients

Lipogenic nutrients

Specific lipogenic nutrients can, like glucogenic nutrients, also induce milk fat depression and help the cow to maintain EB in early lactation. Fatty acid intermediates, formed during rumen biohydrogenation of poly-unsaturated fatty acids (PUFA), are suggested to directly inhibit milk fat synthesis at the mammary gland (Bauman and Griinari, 2003). *Trans*-10, *cis*-12 conjugated linoleic acid (one of the CLA isomers) has been identified as a rumen biohydrogenation intermediate that is involved in milk fat depression by decreasing the expression of genes that are involved in fatty acid uptake and transport, *de novo* fatty acid synthesis, triglyceride synthesis and desaturation of fatty acids (Baumgard et al., 2002). Recently, feeding calcium salts of CLA has been presented as a dietary regimen to decrease milk energy output and hereby improve the EB in transition cows (Castaneda-Gutierrez et al., 2005; Odens et al., 2007). Furthermore, an improvement of the EB after CLA supplementation tended to result in lower plasma non-esterified fatty acid (NEFA) concentration, fewer days pp to first ovulation and fewer days open (Castaneda-Gutierrez et al., 2005).

This is in contrast to an earlier study, which reported that although CLA did reduce milk fat content, milk yield tended to increase in response to CLA, resulting in no reduction in milk energy NEB (Bernal-Santos et al., 2003). Concerning the carbohydrate status, reported results of dietary CLA supplementation seem to be conflicting. While Castaneda-Gutierrez et al (2005) found no effect on plasma glucose

or insulin concentration in response to an improvement of the EB by CLA, recently the same research group did report an increase in plasma glucose in cows where CLA significantly improved the EB (Odens et al., 2007).

To our knowledge, research on the relation between a CLA-induced improvement of the EB and an altered risk of ketosis and fatty liver, as indicated by plasma BHBA and liver TAG concentrations, is very limited. In vitro, an increase in TAG concentrations in response to CLA was reported in bovine hepatocytes from neonatal origin (Mashek and Grummer, 2003). It can be speculated that because the EB is improved in response to CLA, body fat mobilisation, plasma BHBA and liver TAG concentrations and the risk of lipid-related metabolic disorders decrease. Nevertheless, glucogenic nutrients are still essential in metabolising lipogenic nutrients. Despite the fact that lipogenic nutrient availability, originating from body reserves, is decreased with CLA supplementation, the cow still experiences a relative shortage of glucogenic nutrients and the related risk of ketosis and fatty liver in early lactation would still likely depend on the glucogenic nutrient availability in the diet. Furthermore, if CLA supplementation is moderate, milk production and hence lactose production may increase and further aggravate the relative glucogenic nutrient shortage.

Glucogenic nutrients

In our experiments glucogenic nutrient supply was increased by increasing the availability of rumen resistant starch (corn) in order to increase the availability of glucogenic nutrients at the intestine level. Feeding highly fermentable carbohydrates is a common approach to increase the energy density of the diet and increase the availability of glucogenic nutrients at the rumen level (e.g. Grum et al., 1996a; O'Mara et al., 1997). Greater ruminal propionate production in response to greater non-fibre carbohydrate (NFC) concentration increases serum insulin secretion, which promotes papillae growth (Sakata et al., 1980). Hereby, NFC can be beneficial to rumen function and dry matter intake (Allen, 2000). However, feeding dairy cattle diets high in ruminally fermentable carbohydrates associated with a low NDF availability in the diet results in growth of lactate-producing bacteria and shift away from producing VFA towards producing lactic acid of these bacteria. An increase in lactic acid production results in accumulation of lactic acid, lowering of the rumen pH, which compromises rumen function and possibly lead to (subclinical) acidosis (Owens et al., 1998), displaced abomasum (Cameron et al., 1998) and laminitis (Nocek, 1997). The incidence of acidosis is highest in the first month pp and almost non-existent three months pp (Grohn and Bruss, 1990). Onset of acidosis is associated with a relatively abrupt shift to high concentrate diets containing more highly fermentable carbohydrates than when cows are accustomed to utilising concentrates during the dry period (Nocek, 1997). As symptoms of acidosis include diminished appetite and body weight loss, it can be hypothesised that increasing glucogenic nutrient availability by feeding excessive amounts of highly fermentable carbohydrates entails a serious risk of aggravating the NEB. Therefore, it can be suggested to increase the availability of rumen resistant glucogenic nutrients when applying a low lipogenic/glucogenic

nutrient ratio as a nutritional tool to improve the EB in early lactation dairy cows, as performed in the current studies.

Determined EB related to calculated EB

In experiment 1 the EB was determined as energy retention in body mass using the formula:

$$ER = \text{Gross energy intake} - E_{\text{faeces+urine}} - E_{\text{methane}} - \text{heat production} - E_{\text{milk}} \quad (\text{formula 1})$$

In experiment 2, in accordance with Dutch dairy practice, EB was estimated using the Dutch feed evaluation system for energy (VEM; Centraal Veevoederbureau, 2005; Van Es, 1975). In order to evaluate the correspondence between EB determinations in the climate-respiration chambers (EB) with the Dutch feed evaluation system (EBc), EBc was calculated with the VEM system for 6 chambers (2 cows each) from week 2 till 9 relative to calving as the difference between calculated net energy (NEc) intake and NEc required. Concerning 2 chambers, EB was determined for week 1 till 9 relative to calving. Resulting, 25 balance periods were available for comparing EB and EBc. NEc intake was based on the Dutch feeding table (Centraal Veevoederbureau, 2005). NEc required was estimated using formulas 2 and 3 (Centraal Veevoederbureau, 2005; Van Es, 1975). Table 2 shows the ME intake, NE in milk and EB as determined in the climate-respiration chambers and the NE intake, NE in milk and EB as calculated with the VEM system for cows in the balance experiment.

$$\text{NEc required} = ((42.4 \times W^{0.75} + 442 \times \text{FPCM}) \times (1 + (\text{FPCM} - 15) \times 0.00165) \times 6.9) / W^{0.75} \\ (\text{in kJ} / (\text{kg}^{0.75} \cdot \text{d})) \quad (\text{formula 2})$$

$$\text{FPCM} = (0.337 + 0.116 \times \% \text{fat} + 0.06 \times \% \text{protein}) \times \text{milk} \quad (\text{in kg/d}) \quad (\text{formula 3})$$

Whereas in an ideal situation EBc is equal to EB, formula 4 (Table 3) suggests that EBc underestimates the EB when $EB < -360 \text{ kJ} / (\text{kg}^{0.75} \cdot \text{d})$ and EBc overestimates the EB when $EB > -360 \text{ kJ} / (\text{kg}^{0.75} \cdot \text{d})$ (Figure 3a). Comparing the relation between EB and EBc within dietary treatments does increase the proportion of declared variability per relation (Figure 3b), although the intercept was lower for the lipogenic diet compared with the glucogenic diet. Further, for the regression coefficients in Figure 3a as well as the regression coefficients for both lines in figure 3 counts that they are significantly different from zero and significantly different from 1.

Table 2. Energy intake, energy in milk and energy balance (EB) as determined in the climate-respiration chambers and energy intake, energy in milk and energy balance (EBc) as calculated with the VEM system for cows in experiment 1. Values are means \pm SE in $\text{kJ}/(\text{kg}^{0.75}\cdot\text{d})$.

	Diet	
	Glucogenic	Lipogenic
<i>Climate-respiration chambers¹</i>		
Gross energy intake (GE)	3355 \pm 100	3449 \pm 92
Metabolisable energy intake (ME)	2038 \pm 70	2094 \pm 62
Energy in milk	1069 \pm 9	1170 \pm 13
Energy balance (EB)	-119 \pm 35	-194 \pm 31
<i>VEM system</i>		
Net energy intake (NEc)	1204 \pm 38	1203 \pm 33
Calculated energy in milk	1062 \pm 16	1140 \pm 17
Calculated energy balance (EBc)	-207 \pm 29	-296 \pm 24

¹ Based on 25 balance periods (1 week) per diet.

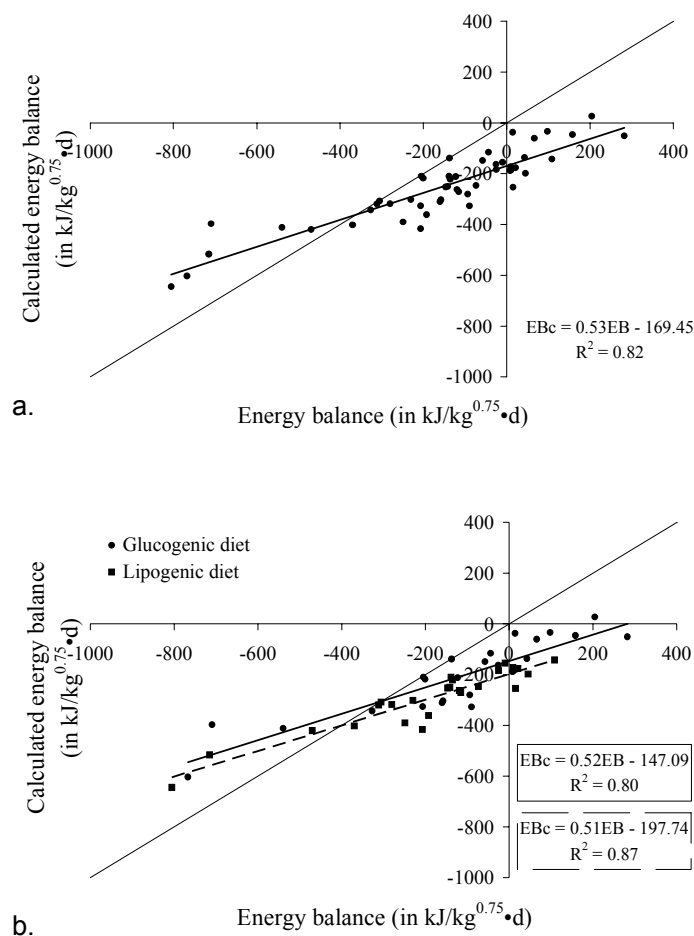


Figure 3. Relation between energy balance as determined in the climate-respiration chambers and energy balance as calculated with the VEM system across dietary treatments (a) and within dietary treatments (b).

From Figure 3a it can be suggested that for the extreme negative EB ($< -360 \text{ kJ (kg}^{0.75} \cdot \text{d)}$) the VEM system does not take into account an ‘energy-required-factor’ which did contribute to the EB for cows in the climate-respiration chambers. The extreme negative EB’s are all measured during the first week in the climate-respiration chambers, which make it persuasive to hypothesise that adaptation to housing in the chambers results in greater energy requirements, which is not accounted for in the VEM system. On the other hand, for the less negative and positive EB ($> -360 \text{ kJ (kg}^{0.75} \cdot \text{d)}$), Figure 3a suggests that the VEM system accounts for an ‘energy-required-factor’, which is not present for the cows in the climate-respiration chambers. It can be questioned whether this missing ‘energy-required-factor’ is either an efficiency factor (e.g. utilisation of ME) or it is caused by a lack of activity for cows in balance experiments compared with cows in standard feeding experiments.

Table 3. Statistics of estimated regression formulas for the relation between EB and EBc across dietary treatments (formula 4) and for the glucogenic (formula 5) and lipogenic diet (formula 6) separately.

Formula	Estimates	Statistics			
		R ²	SE	95% Confidence limits	
				lower	upper
(4)	EBc = $0.53 \times \text{EB} - 169.45$ Intercept -169.45 Regression coefficient 0.53	0.82	10.25 0.04	-190.05 0.46	-148.84 0.60
(5)	EBc = $0.52 \times \text{EB} - 147.09$ Intercept -147.09 Regression coefficient 0.52	0.80	14.78 0.05	-177.67 0.41	-116.51 0.63
(6)	EBc = $0.51 \times \text{EB} - 197.74$ Intercept -197.74 Regression coefficient 0.51	0.87	11.77 0.04	-222.09 0.42	-173.39 0.59

Furthermore, it should be realised that the VEM system was developed and evaluated with dairy cows in positive energy balance (Van der Honing et al., 1977; Van Es, 1975). This means that all the estimated VEM originates from GE in feed intake, while in the current study cows obtain additional energy from mobilised body reserves. Moreover, it has been suggested that efficiencies of energy conversion have been changed considerably over the last four decades (Kebreab et al., 2003). So, for now it seems only possible to hypothesise that besides uncommon housing conditions, limited number of animals, inclusion of extreme diets, also the origin of the VEM system and changed energy conversion efficiencies contribute to the observed difference between EB and EBc in this study.

Comparison of the NEB between studies

Multiparous cows in experiment 2 had, according to VEM calculations (Van Es, 1975), a less negative EB (-61 ± 8 kJ/(kg^{0.75}·d); Chapter 5) compared with cows in experiment 1 (-245 ± 15 kJ/(kg^{0.75}·d)), while diet compositions were comparable between both experiments. At least part of the difference in EBc between experiment 2 and 1 could be attributed to a greater DMI (24.0 ± 0.2 vs. 20.4 ± 0.3 kg/d) and greater energy intake (160.0 ± 1.0 vs. 139.8 ± 1.7 MJ/d) in experiment 2, possibly related to transport and housing in the climate-respiration chambers of cows in experiment 1. This greater energy intake in experiment 2 did not result in greater milk production in experiment 2 (42.0 ± 0.3 kg FPCM) compared with experiment 1 (41.7 ± 0.3 kg FPCM). This suggests that the greater energy intake in experiment 2, compared to experiment 1, was beneficial to body reserves, which could explain the difference in EB between experiment 1 and 2.

In most studies, EBc is expressed in Mcal/d, without correcting for metabolic body weight (Chapter 2). For the current studies, it would come down to an average EB across dietary treatments of -4.37 ± 0.67 Mcal/d (EB) or -6.92 ± 0.43 Mcal/d (EBc) for experiment 1 and -1.92 ± 0.24 Mcal/d (EBc) for experiment 2. In literature, estimations for the EB in Holstein dairy cows in early lactation vary from -7.50 Mcal/d (Harrison et al., 1995) till 8.15 Mcal/d (Spicer et al., 1993), which implies that cows in experiment 1 were at the lower end, while multiparous cows in experiment 2 seem to approach the mean of the observed range in EB in early lactation. More direct comparisons of the current values for EB with literature data are difficult, as besides variations in DMI, diet composition and milk yield, also variations in experimental period likely contribute to differences in EB between studies.

Depression in milk energy as a tool to improve health and fertility in dairy cows

The current study shows that increasing the glucogenic nutrient availability in the diet can be a nutritional strategy to reduce milk fat and milk energy output and hereby improve the EB in dairy cows in early lactation. Strategies to reduce milk energy output should be distinguished in strategies that are accompanied with a reduction in energy intake and strategies that result in milk energy depression without compromising energy intake. The first are likely not to be beneficial to EB, health and fertility, while the second have potential to repartition energy from milk to body reserves and hereby improve the EB and consequently reduce the incidence and severity of health and fertility disorders in early lactation. The objective of the current discussion is to focus on the second type of strategy and discuss nutritional, management and selection methods to reduce milk energy output and improve the EB. In addition,

economic aspects of applying milk energy reduction to improve the EB in early lactation are discussed shortly.

Nutrition

Both decreasing the lipogenic/glucogenic nutrient ratio (this thesis) as well as feeding CLA (Odens et al., 2007) to dairy cows in early lactation have been discussed above as nutritional strategies which decrease milk fat content, reduce milk energy and hereby improve the EB. The effect of decreasing the lipogenic/glucogenic ratio can most likely be attributed to increasing plasma insulin, hereby decreasing body fat mobilisation and reducing the availability of lipogenic nutrients. CLA are suggested to inhibit milk fat synthesis directly at the mammary gland (Baumgard et al., 2002). An increased lipogenic/glucogenic ratio has been suggested to decrease the risk for ketosis and fatty liver, relations between CLA supplementation and metabolic disorders are quite rare. Additionally, both for CLA (Castaneda-Gutierrez et al., 2005) and the lipogenic/glucogenic nutrient ratio (chapter 5) a potential for improved fertility has been reported. As the experimental period and animal numbers were limited, full assessment of these nutritional strategies for improving fertility in dairy cows should be confirmed in large-scale studies which monitor cows from the transition period till mid-lactation.

Management

Stress can be considered as a management factor involved in incidence and severity of NEB in high-producing dairy cows in early lactation, though probably more by reducing DMI than reducing milk energy output. The transition period (3 week pre calving to 3 week pp) and early lactation do not only involve transition from pregnancy to lactation, but additionally the cow's adaptive capacity is challenged by several diet and group transitions (Drackley, 1999). The early lactation cow might be particularly vulnerable to limitations in dry matter intake imposed by hormonal changes peripartum as well as group transitions (Grant and Albright, 1995), hereby aggravating the NEB. The effect of group and housing transitions on the EB can be illustrated with the results of the climate-respiration chamber study. The original aim of this study was, as calving in the chambers was not considered as an option, to transport and house cows in the chambers soon post calving as carried out in batch 1. In batch 1, cows calved at the experimental dairy facility, where they were used to housing in a free stall, and were transported to the climate respiration facility within three days post calving. Because of a very limited DMI and time spend lying in the first week in the chambers, both suggesting cows were not able to cope with the transition from the dairy to the respiration unit, the transport-strategy for batch 2 and 3 was adapted. In batch 2 and 3 cows were assigned to a recovery period at the dairy facility and transported to the chambers not earlier than 1 week post calving. Whereas success of this modification was limited, as illustrated with 3 cases of left displaced

abomasums in batch 2 and 3, further modification of the transport-strategy was considered to be inevitable for batch 4. In batch 4, cows were housed in a tie-stall 2 till 4 weeks pre calving, cows calved in the tie-stall and were transported to the chambers not earlier than 1 week post calving. This resulted in an improvement of the DMI and EB in the first week in the chambers (Figure 5). Although anecdotic more than scientific, this suggests that the effect of transport-strategy on EB has disappeared in week 2 in the chambers (batch 1: week 2 post calving; batch 2 till 4: week 3 post calving). Still these observations illustrate the significance of an adaptation period in the chambers and the vulnerability of high-producing dairy cows in early lactation.

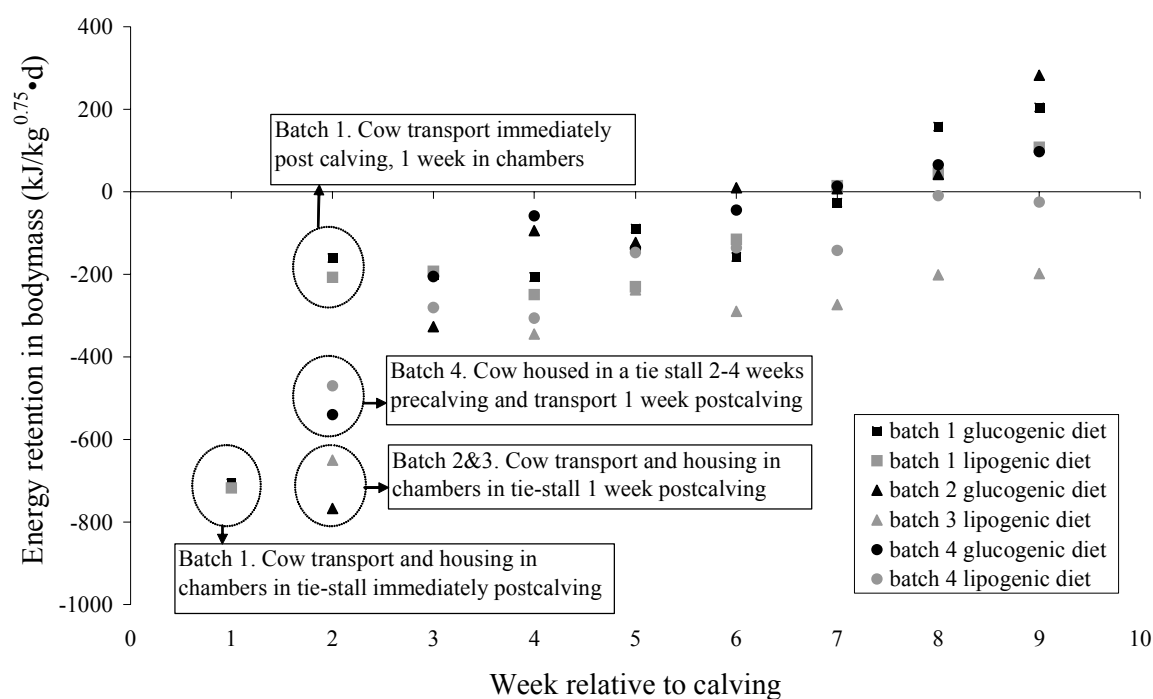


Figure 5. Energy retention (in $\text{kJ/kg}^{0.75}\cdot\text{d}$) for dairy cows fed a glucogenic or a lipogenic diet per batch.

Milking frequency

Several studies reported temporary once-daily milking in early lactation as a successful regimen to reduce body weight loss and improve the EB compared with cows milked twice or three times daily, without affecting somatic cell counts or the incidence of clinical mastitis (Patton et al., 2006; Remond et al., 2002). A reduction in milk energy was explained mainly by a reduction in milk yield, though Patton et al (2006) also reported reducing effects on fat and protein percentage. Furthermore, cows with an improved EB in response to once-daily milking had lower plasma NEFA and BHBA concentrations, higher plasma insulin concentration and had an earlier resumption of ovarian activity. It can be questioned whether the effect of milking frequency on milk yield is reversible as after the four-weeks-treatment period cows were transferred from a three- or four-times-milking-protocol to a standard two-times-

milking-protocol and the significant effect of increased milking frequency on daily milk yield and composition disappeared. However, a numerical difference of 2 kg/d was still present and three-times-milking in early lactation resulted in a higher 305-d milk yield compared with once-daily milking in early lactation (Patton et al., 2006).

Dry period length

Despite an extensive exploration of a numerous amount of nutritional and management strategies during the last decades, the high genetic merit in Holstein Friesian dairy cows and the inevitable feed intake depression peripartum still results in a NEB in early lactation. In this perspective, shortening the dry period seems to give a new and promising alternative. In recent studies, shortening the dry period significantly improved the EB, while omitting the dry period even resulted in no significant negative EB (Rastani et al., 2005; 2007) (Figure 6). In addition to a greater feed intake around calving, which contributed to an improved EB, this improved EB could in particular be attributed to a reduction in daily milk yield of around 20% in the first months of lactation (Annen et al., 2004; Rastani et al., 2005). Consequently, reported benefits from continuous milking are reduced plasma NEFA concentration and lower liver TAG content (Andersen et al., 2005; Rastani et al., 2005) and an earlier resumption of ovarian activity (Gumen et al., 2005). A recent study showed that increasing prepartum milking frequency during continuous milking reduced the milk production loss in the subsequent lactation for cows in their third or greater lactation without significantly affecting the EB postpartum (Rastani et al., 2007). Further research is needed to examine the long-term effects of continuous milking and shortened dry periods on total lactation milk yield, fertility and longevity in dairy cows.

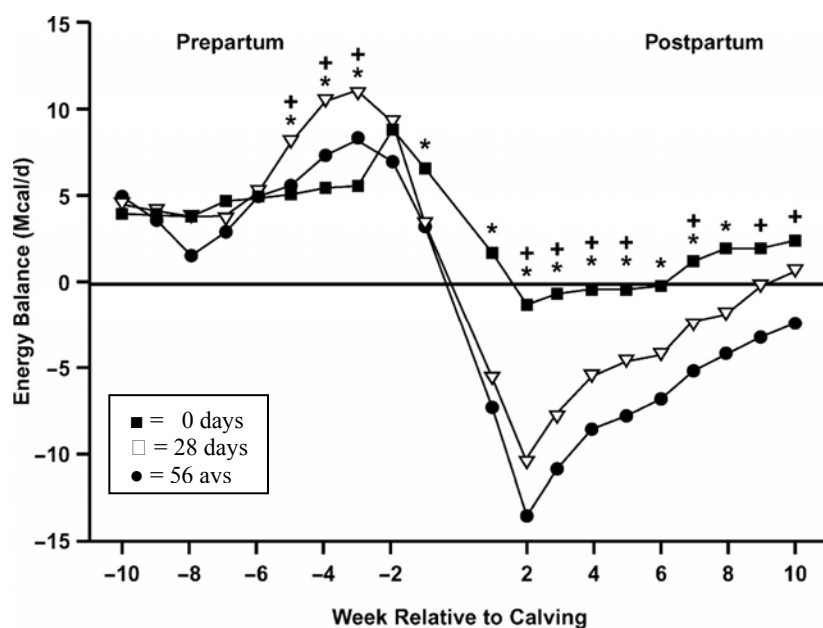


Figure 6. Effect of 0, 28 or 56 days dry on the energy balance in the subsequent lactation (Rastani et al., 2005).

Genetic selection for low milk energy output

In The Netherlands, depressing milk fat yield by selecting cows for low milk fat percentage is an interesting and applied strategy to maximise herd milk yield within a certain milk fat quota. Subsequently, depressing genetic merit for milk fat for this purpose is accompanied with increasing genetic merit for milk volume. However this might not definitely reduce milk energy output, improve the EB and have beneficial effects for health and fertility. Furthermore, selection for low milk energy seems not to be desirable in practice for economic reasons, although low milk fat contents may be beneficial in milk fat quota-systems. However, breed and line differences do exist in milk production, milk composition (Nederlands Rundvee Syndicaat, 2005), peak milk yield, lactation curves (Hansen et al., 2006) and feed intake (Grainger and Goddard, 2004; Oldenbroek, 1989), suggesting the existence of variation essential for genetic selection for decreased milk energy output or improved EB. An application of this variation is known in a subset of the dairy cattle population, where crossbreeding is applied to improve health, fertility and longevity in dairy cows, although this strategy compromises milk volume, (Weigel and Barlass, 2003). Whenever selection for reduced milk energy output will be accepted in a greater part of the dairy cattle population will most probably depend on the severity of NEB-related disorders and insight in the relation between NEB and net herd returns.

Economic feasibility

Economics generally favour prevention of milk fat depression on commercial dairy farms, but there are some scenarios in which reduced output of milk fat could be advantageous (Perfield et al., 2002). These include markets where producers are regulated by a quota system based on milk fat and situations in which cows cannot consume sufficient energy to meet requirements such as occurs during heat stress or when only relatively poor quality diets are available. Furthermore, the early lactation period is characterised by limited feed intake to meet requirements for milk production and cows experience a negative energy status. An improvement of the EB in early lactation can be expected to be beneficial for health and welfare of high-producing dairy cows and hereby would enlighten the workload of the dairy farmer by reduction of time spend on (veterinary) treatment of cows suffering from NEB-related disorders. Moreover, it would be interesting to describe the economic feasibility of an improved energy status in early lactation at the cost of milk fat yield. Aiming for reduction of the severity of NEB can be considered to be related to understanding the costs associated with NEB or NEB-related disorders and understanding the cost-benefit of a certain EB-improving measurement. However, knowledge about the costs of NEB-related disorders is limited and estimations are characterised by a large variation between studies (De Vries, 2006; Kossaibati and Esslemont, 1997).

Conclusion and remaining questions

From the current thesis, it can be concluded that glucogenic nutrients, compared with lipogenic nutrients, stimulate partitioning of energy to body reserves at the cost of milk energy and hereby improve the EB for dairy cows in early lactation. Results indicate that insulin might be an important mediator in the relation between glucogenic nutrients and energy partitioning between milk and body reserves. Furthermore, glucogenic nutrients have potential to decrease the risk of metabolic disorders like ketosis and fatty liver and tend to stimulate earlier resumption of ovarian activity in multiparous dairy cows.

Remaining questions

- Although a tendency for an earlier resumption of ovarian activity is suggested to be beneficial for fertility, large scale studies are needed, which monitor cows from the transition period till mid-lactation to confirm the relation between dietary energy source and fertility.
- Cows fed the mixed diet do not differ from cows fed the lipogenic diet for EB and plasma metabolites and metabolic hormones; although they are expected to have a higher availability of glucogenic nutrients compared with cows fed the lipogenic diet. Studies on the dose-effect relation of dietary energy source and EB could further clarify this observation.
- In contrast to multiparous cows, we were not able to repartition energy from milk to body reserves with a glucogenic diet in primiparous cows. The lack of any numerical differences for EB or plasma metabolites suggests this is not only caused by low animal numbers for primiparous cows. Additional research on energy metabolism in primiparous cows could assist in interpreting these results.
- Results of this thesis indicate a relation between EB and natural antibody (NAb) concentration in both plasma and milk and raise the question whether dietary energy source affects NAb levels and immune function in transition dairy cows. Further studies are needed to unravel the relations among EB, nutrition, NAb, somatic cell counts and various plasma variables.
- A uniform method and approach to estimate and quantify the availability of glucogenic and lipogenic nutrients in dairy rations would be useful in optimising dairy rations in practice. However, besides that several assumptions on e.g. rumen resistancy and efficiency, have to be included in these calculations; numerous approaches are used in dairy practice until now. Further research could assist in determining these assumptions and proposing a uniform approach in quantifying lipogenic and glucogenic nutrient availability.

- Both CLA and glucogenic nutrients have been shown to decrease milk energy output and have potential to improve the EB in early lactation. While the current studies, among others, suggest a reduced risk of ketosis and fatty liver with a mainly glucogenic diet, research on lipid-related metabolic disorders associated with dietary supplementation of CLA is scarce. A comparison between these two nutritional strategies which both decrease milk energy output, might be interesting, especially because a difference in the mechanism of altering energy metabolism could be hypothesised.
- In the current discussion, the suggestion is made that estimating the EB with the VEM system underestimates the EB at extremely negative EB and overestimates the EB at less negative and positive EB for cows in a balance experiment. For a justified comparison far more data are needed. Additional studies could clarify this suggestion.
- Besides nutritional strategies, also management and genetic selection tools could result in reduction in milk energy, without compromising energy intake, and improve the EB in early lactation dairy cows. An exploration of these strategies, comparing their value and applicability or even combining two or more milk energy reducing strategies might be a fascinating subject of research.

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Summary/Samenvatting

Summary

Introduction

Selection on high genetic merit for milk yield is only partially compensated by an increase in feed intake resulting in an ongoing increase in negative energy balance (NEB) for dairy cows in early lactation. A negative energy balance has been related with metabolic disorders, like fatty liver and ketosis, a decrease in reproductive performance, like prolonged anoestrous period post calving, and an increased incidence in infectious diseases. Several nutritional strategies to improve the energy balance (EB) and reduce the severity and incidence of metabolic and reproductive disorders in early lactation have been studied. Most studies aimed at improving the EB by increasing energy intake in the periparturient period, hereby reducing the risk of metabolic and reproductive disorders in early lactation. Possibilities to improve the dry matter intake in order to increase the energy intake are limited in peripartum dairy cows. Therefore, a common approach is to enhance the energy density of the diet by e.g. increasing the concentrate to forage ratio or by dietary supplementation of energy dense ingredients like fat or non-fibre carbohydrates. However, increasing the dietary energy density has been shown to entail a risk of compromising dry matter intake, which results in a limited increase in energy intake and improvement of the EB. An alternative approach is to improve the EB in early lactation by decreasing the caloric demand of milk production. The main hypothesis of this thesis was that increasing the availability of glucogenic nutrients relative to lipogenic nutrients in the diet of dairy cows in early lactation improves the energy balance (EB), by decreasing milk fat content and milk energy output, and decreases the risk of metabolic and reproductive disorders. Additionally, it can be hypothesised that an improved EB, induced by a decreased lipogenic relative to glucogenic nutrient availability, has potential to reduce the incidence of infectious diseases.

The first study, a literature survey (chapter 2), hypothesised that a metabolic effect of a NEB in dairy cows is an unbalanced availability of C2 (lipogenic) relative to C3 (glucogenic) nutrients. In ruminants, lipogenic nutrients (C2) either originate from fibre, which is fermented to acetate and butyrate, or originate from dietary fat or are derived from body reserves. Glucogenic nutrients (C3) originate from starch escaped from rumen degradation or from gluconeogenesis. Furthermore, chapter 2 indicated that alteration in lipogenic and glucogenic nutrient content in the diet may affect the EB and result in modifications in blood metabolic profiles and milk fat yield. However, it was difficult to draw conclusions on the effects of dietary energy source on EB and fertility. Firstly, reported effects seem to be rather incoherent, possibly due to the type of lipogenic and glucogenic nutrients. Secondly, in most studies, the contrast in dietary energy source was confounded with a contrast in energy intake level. Thirdly, studies on the relation between glucogenic nutrients and energy metabolism are scarce.

Mobilisation of body reserves affected by dietary energy source

Therefore, experiment 1 was set up to test the effect of two diets, differing in dietary energy source, on energy partitioning in dairy cows in NEB (chapter 3). Sixteen dairy cows were fed either a mainly lipogenic or a mainly glucogenic diet from week 3 prepartum until week 9 postpartum (pp) and housed in climate-respiration chambers from week 2 till 9 pp. While there was no effect of diet on dry matter intake (DMI), metabolisable energy intake or milk yield, cows fed a mainly glucogenic diet had a lower milk fat content (4.27 ± 0.15 vs. 4.81 ± 0.15 %), milk fat yield (1.68 ± 0.03 vs. 1.90 ± 0.02 kg/d) and partitioned less energy to milk (1075 ± 12 vs. 1173 ± 18 kJ/(kg^{0.75}·d)) than cows fed a mainly lipogenic diet. No difference was found in body protein mobilisation, but body fat mobilisation tended to be less for cows fed the glucogenic diet (332 ± 110 g/d) than for cows fed the lipogenic diet (558 ± 96 g/d). This study showed that a mainly glucogenic diet compared with a mainly lipogenic diet improved the EB by decreasing milk energy output in the form of milk fat depression and hereby confirmed the first part of the hypothesis of this thesis.

Health and fertility aspects of dietary energy source

It has been suggested that the availability of glucogenic nutrients relative to lipogenic nutrients affects the susceptibility of cows to metabolic disorders like fatty liver and ketosis. The glucogenic diet in experiment 1 increased plasma insulin concentration and tended to decrease plasma non-esterified fatty acid (NEFA) concentration in early lactation compared with a mainly lipogenic diet (chapter 4). However, the design of experiment 1 with 16 cows did not have sufficient power to draw definite conclusion on dietary energy source effects on plasma β -hydroxybutyrate (BHBA) concentration and liver tri-acyl glyceride (TAG) content, as indicators for ketosis and fatty liver. Furthermore, potential effects of dietary energy source on reproductive performance could not be assessed. Therefore, the objective of the second experiment (chapter 5) was to evaluate the effects of a mainly glucogenic or lipogenic diet on calculated EB (EBc), plasma metabolites and metabolic hormones, liver TAG content and reproduction variables in high-producing dairy cows in early lactation in a larger experiment. Furthermore, to test a possible parity-effect both primiparous as multiparous cows were included in this experiment. Additionally, an intermediate diet was added in order to further outline the relation between different glucogenic to lipogenic nutrient availability and energy metabolism. Like in the first experiment, also in this experiment DMI, net energy intake, milk yield and milk protein percentage did not differ among diets. Milk fat percentage was less for multiparous cows fed glucogenic diet (G) compared with cows fed mixed (M) or lipogenic diet (L). The EBc was less negative for multiparous cows fed the glucogenic diet compared with cows fed mixed or lipogenic diet. Postpartum, glucogenic diet decreased plasma NEFA, BHBA and liver TAG concentrations and increased insulin concentration in multiparous cows. The glucogenic diet tended to decrease the number of days till 1st rise in milk progesteron concentration in multiparous cows compared with mixed or lipogenic diet (20.4 vs. 24.4 and 26.4 \pm 2.1 d, for G, M and L diet, respectively). Diet

had no effect on any of the above mentioned variables in primiparous cows. We concluded from the results of experiment 2 that a mainly glucogenic diet was effective in improving the EBc by decreasing milk fat yield compared with more lipogenic diets in early lactation. Furthermore, the glucogenic diet decreased plasma BHBA and liver TAG concentrations and seemed to stimulate resumption of ovarian activity, suggesting a reduced risk of metabolic disorders and a potential for improved fertility in multiparous dairy cows fed glucogenic diet.

While chapter 2 till 5 focused on the EB, metabolic disorders and some fertility measurements, chapter 6 focuses on the relation between EB, dietary energy source and potential risk of infectious diseases, as indicated by some immune variables, which were measured in plasma and milk samples of cows in experiment 2. In this chapter, the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows is discussed. Nab concentration in plasma tended to be inversely related to NAb concentration in milk for early lactation dairy cows. Moreover, this study indicated a positive relation between NAb in plasma and EB and a negative relation between NAb in milk and EB. These results indicate that the innate immune function is compromised during NEB in dairy cows in early lactation.

Insulin as the metabolic signal?

The main conclusion of the current thesis was that glucogenic nutrients, compared with lipogenic nutrients, stimulate partitioning of energy to body reserves at the cost of milk energy and hereby improve the EB for dairy cows in early lactation. Furthermore, glucogenic nutrients had potential to reduce the risk of metabolic disorders like ketosis and fatty liver and tended to stimulate earlier resumption of ovarian activity in multiparous dairy cows. Results of both experiments indicated that insulin might be an important metabolic signal in establishing the shift in energy partitioning away from milk fat and towards body reserves realised by a glucogenic diet compared with a lipogenic diet. Improvement of the EB, through feeding the glucogenic diet, was established by a depression of milk fat (MFD) and milk energy output. A possible explanation for MFD by decreasing the lipogenic to glucogenic nutrient ratio is the glucogenic theory. According to this theory, an increase in insulin, induced by an increase in propionic acid or glucose, would decrease lipolysis and decrease the availability of milk fat precursors, which in turn may reduce milk fat and milk energy output.

Besides discussing the glucogenic theory as a possible mechanism for the observed effects of a mainly glucogenic diet on milk energy output and EB in early lactation dairy cows, the general discussion (chapter 7) focuses on the relation between calculated EB and EB as determined in the climate-respiration chambers, and indicates that other nutritional, management and selection strategies might have potential to reduce milk energy output and improve the EB for dairy cows in early lactation as well.

Samenvatting

Inleiding

Melkvee is decennialang geselecteerd op een hoge melkproductie, terwijl de koe in het begin van de lactatie niet in staat is voldoende energie voor deze melkproductie op te nemen via het rantsoen. Dit resulteert in een negatieve energiebalans (NEB) bij melkvee in de vroege lactatie, die kan leiden tot zowel stofwisselings- als vruchtbaarheidsstoornissen, zoals slepende melkziekte, leververvetting, pensverzuring, een verlengd interval van afkalven tot eerste ovulatie en lagere bevruchtingspercentages. Om de energiebalans (EB) in vroege lactatie te verbeteren zijn tot op heden al veel voedingsmaatregelen bestudeerd. De meeste studies hadden als doel de EB te verbeteren door middel van het verhogen van de energieopname en hierdoor het risico op stofwisselings- en vruchtbaarheidsstoornissen in vroege lactatie te verlagen. Mogelijkheden om de voeropname te verhogen en hiermee ook de energieopname zijn echter beperkt voor melkvee rond afkalven. Vandaar dat een gebruikelijke benadering is om de energieopname in de vroege lactatie te verhogen door de energiedichtheid van het rantsoen te vergroten. Dit kan door middel van het verhogen van de krachtvoer/ruwvoer verhouding in het rantsoen of door middel van het toevoegen van ingrediënten met een hoge energie-inhoud, zoals vet of niet-vezelrijke koolhydraten. Echter het verhogen van de energiedichtheid in het rantsoen van melkvee heeft het risico dat de voeropname verlaagd wordt, zodat de energieopname en de EB slechts in geringe mate verbeteren.

Een alternatieve benadering om de EB in de vroege lactatie te verbeteren is om de hoeveelheid energie die het lichaam met de melk verlaat, te verlagen. De hypothese van dit proefschrift was dat een toename in de beschikbaarheid van glucogene nutriënten ten opzichte van lipogene nutriënten in het rantsoen van koeien in vroege lactatie de hoeveelheid vet en energie in de melk verlaagt, waardoor de EB verbetert en het risico op stofwisselings en reproductiestoornissen vermindert. Daarnaast mag verondersteld worden dat een verbeterde EB ook het risico op infectieziekten die gerelateerd zijn met de NEB verlaagt.

De eerste studie, een literatuuroverzicht (Hoofdstuk 2), bediscussieert dat een effect van de NEB bij melkvee een onbalans is tussen beschikbaarheid van C2 (lipogene) ten opzichte van C3 (glucogene) nutriënten. In herkauwers zijn lipogene nutriënten (C2) ofwel afkomstig uit voedingsvezels, die gefermenteerd worden in de pens en omgezet worden in azijnzuur en boterzuur, ofwel afkomstig van vet uit het rantsoen of uit lichaamsreserves. Glucogene nutriënten zijn afkomstig van pensbestendig zetmeel of worden geproduceerd tijdens de gluconeogenese in de lever. De resultaten uit de literatuurstudie wezen erop dat wijzigingen in de beschikbaarheid van lipogene en glucogene nutriënten in het rantsoen de EB leek te beïnvloeden en ook resulteerde in veranderingen in melkvetproductie en concentraties van metabolieten in het bloed. Echter, het bleek moeilijk conclusies te trekken over het effect van energiesoort in het rantsoen op de EB en vruchtbaarheid. Ten eerste waren de gerapporteerde effecten niet eenduidig. Ten tweede was in de meeste studies het contrast tussen proefgroepen in

energiesoort verstrengeld met een contrast in energieniveau. Ten derde zijn studies naar de relatie tussen glucogene nutriënten en energiemetabolisme en vruchtbaarheid bij melkkoeien schaars.

Lichaamsvetmobilisatie beïnvloed door energiesoort in het rantsoen

Experiment 1 is opgezet om het effect van twee rantsoenen, verschillend in energiesoort, te testen op het energiemetabolisme bij melkvee in vroege lactatie (Hoofdstuk 3). Zestien melkkoeien werden ofwel een hoofdzakelijk lipogeen ofwel een hoofdzakelijk glucogeen rantsoen gevoerd van week 3 voor afkalven tot en met week 9 na afkalven. De koeien werden gehuisvest in klimaat-respiratiekamers van week 2 tot en met week 9 na afkalven. Er was geen rantsoeneffect op de drogestofopname, metaboliseerbare energieopname en melkproductie, maar de koeien op het glucogene rantsoen hadden wel een lager melkvetgehalte (4.27 ± 0.15 vs. $4.81 \pm 0.15\%$), een lagere melkvetproductie (1.68 ± 0.03 vs. 1.90 ± 0.02 kg/d) en minder energie in de melk (1075 ± 12 vs. 1173 ± 18 kJ/(kg^{0.75}·d)) vergeleken met koeien op het lipogene rantsoen. Er was geen verschil in mobilisatie van lichaamseiwit, maar de mobilisatie van lichaamsvet was lager voor koeien op het glucogene rantsoen (332 ± 110 g/d) dan voor koeien op het lipogene rantsoen (558 ± 96 g/d). Dit experiment laat zien dat een hoofdzakelijk glucogeen rantsoen vergeleken met een hoofdzakelijk lipogeen rantsoen, de mobilisatie van lichaamsvet vermindert en de EB verbetert door de hoeveelheid energie in de melk te verminderen. Hiermee lijkt het eerste deel van de hypothese te bevestigd.

Gezondheids -en vruchtbaarheidsaspecten van energiesoort in het rantsoen

Het idee bestaat dat de hoeveelheid glucogene nutriënten in het rantsoen ten opzichte van lipogene nutriënten een effect heeft op de gevoeligheid van koeien voor stofwisselingsstoornissen, zoals leververvetting en slepende melkziekte. Het glucogene rantsoen in experiment 1 verhoogde ook de plasma insuline concentratie en neigde tot een verlaging van de concentratie niet-veresterde vetzuren (NEFA) in begin lactatie in vergelijking met het lipogene rantsoen (Hoofdstuk 4). Het aantal dieren in experiment 1 was echter niet voldoende om uitspraken te kunnen doen over het effect van energiesoort in het rantsoen op de concentratie β -hydroxyboterzuur (BHBA) in het plasma of lever tri-acyl glyceride (TAG) in de lever, die indicatoren zijn voor respectievelijk slepende melkziekte en leververvetting. Verder konden ook mogelijke effecten van energiesoort in het rantsoen op de vruchtbaarheid niet worden bepaald. Daarom was het doel van het tweede experiment (Hoofdstuk 5) om met een groter aantal dieren de effecten te onderzoeken van een glucogeen of een lipogeen rantsoen op de berekende EB (EBc), plasma metabolieten en metabole hormonen, lever TAG gehalte en ovulatie- en tochtkenmerken bij hoogproductief melkvee in de vroege lactatie. Zowel eerstekalfs als meerderekalfs koeien werden meegenomen in dit experiment om te zien of het effect van rantsoen mede bepaald wordt door de pariteit van de dieren. Om het verband tussen de beschikbaarheid van glucogene en lipogene nutriënten en energiemetabolisme beter te begrijpen, werd bovendien een extra

behandelingsgroep toegevoegd, waarvan de koeien een mixrantsoen (50%:50% op drogestofbasis) van beide rantsoenen gevoerd kregen. Net als in het eerste experiment verschilden ook in experiment 2 de drogestofopname, energieopname, melkproductie en melkeiwitgehalte niet tussen rantsoenen. Het melkvetgehalte was lager voor meerderekalfs koeien op het glucogene rantsoen (G) vergeleken met koeien op het mixrantsoen (M) of lipogene rantsoen (L). De EBc was minder negatief voor meerderekalfs koeien op het glucogene rantsoen vergeleken met het mix en het lipogene rantsoen (-33 vs. -125 en -89 ± 21 kJ/(kg^{0.75}·d), voor G, M en L rantsoen). Meerderekalfs koeien gevoerd met het glucogene rantsoen hadden na afkalven lagere concentraties NEFA, BHBZ en lever TAG en een hogere insuline concentratie in het plasma dan koeien gevoerd met het mixrantsoen of lipogene rantsoen. Meerderekalfs koeien op het glucogene rantsoen tenderden naar minder dagen tot eerste progesteron (P4) stijging vergeleken met koeien op het mix en het lipogene rantsoen (20.4 vs. 24.4 en 26.4 ± 2.1 d, voor G, M and L rantsoen). Het rantsoen had geen effect op één van bovengenoemde variabelen bij eerstekalfs koeien. Concluderend kan gezegd worden dat in experiment 2 het hoofdzakelijk glucogene rantsoen effectief was in het verbeteren van de EBc door middel van een verlaging van de melkvetproductie vergeleken met meer lipogene rantsoenen in het begin van de lactatie. Verder verminderde het glucogene rantsoen de concentraties BHBZ en lever TAG en leek het een eerdere ovulatie na afkalven te stimuleren. Dit suggereert een verlaging van het risico op stofwisselings- en vruchtbaarheidsstoornissen voor meerderekalfs koeien op een glucogeen rantsoen.

Terwijl hoofdstuk 2 tot en met 5 voornamelijk focussen op de energiesoort in het rantsoen, EB, stofwisselingsstoornissen en enkele vruchtbaarheidsvariabelen, behandelt hoofdstuk 6 de relatie tussen EB, energiesoort in het rantsoen en een mogelijk risico op infectieziekten zoals aangeduid door enkele immuunvariabelen, gemeten in zowel bloed als melk van koeien uit experiment 2. In dit hoofdstuk wordt de aanwezigheid van natuurlijke antilichamen (NAb) in plasma en melk van individuele koeien bediscussieerd. De NAb concentratie in plasma was omgekeerd gerelateerd met de NAb concentratie in melk. Bovendien was er een positieve relatie tussen NAb in plasma en EB en een negatieve relatie tussen NAb in melk en EB. Deze resultaten suggereren dat gedurende een NEB bij melkvee in vroege lactatie de innate immuunrespons onderdrukt is.

Is insuline het metabole signaal?

De voornaamste conclusie uit dit proefschrift is dat een rantsoen met veel glucogene nutriënten, in vergelijking met lipogene nutriënten, de hoeveelheid melkvet en energie in de melk verlaagt en hiermee de EB verbetert voor meerderekalfs melkkoeien in het begin van de lactatie. Bovendien leek het erop dat glucogene nutriënten het risico op stofwisselingsstoornissen, zoals leververvetting en slepende melkziekte kunnen verlagen en het interval tussen afkalven en eerste ovulatie bij meerderekalfs koeien kunnen verminderen. Resultaten van beide experimenten duiden erop dat insuline een

belangrijk metabool signaal kan zijn voor het bewerkstelligen van de verschuiving van de verdeling van energie vanuit melkvet naar lichaamsreserves bij een glucogeen rantsoen in vergelijking met een lipogeen rantsoen. Verbetering van de EB door middel van een glucogeen rantsoen werd veroorzaakt door melkvetdepressie en vermindering van de hoeveelheid energie in de melk. Een mogelijke verklaring voor melkvetdepressie door glucogene nutriënten is de glucogene theorie. Volgens deze theorie, resulteert een toename in insuline, veroorzaakt door een toename in propionzuur of glucose, in een afname van de vetafbraak en een vermindering van de beschikbaarheid van melkvetprecursors. Een vermindering in melkvetprecursors resulteert in een afname van melkvetproductie en in een afname van energie in de melk

Naast het bediscussiëren van de glucogene theorie als een mogelijk mechanisme achter de effecten van een hoofdzakelijk glucogeen rantsoen op de energie in de melk en EB bij melkkoeien in het begin van de lactatie, focust de discussie in Hoofdstuk 7 ook op de relatie tussen berekende EB en EB zoals bepaald in de klimaat-respiratiekamers. Daarnaast wordt het voeren van een glucogeen rantsoen vergeleken met andere voedings-, management- en selectiestrategieën als mogelijkheden om de hoeveelheid energie in de melk te verlagen en de EB te verbeteren voor melkvee in het begin van de lactatie.

Curriculum vitae

Curriculum vitae

Arnoldina Theodora Maria (Ariëtte) van Knegsel werd geboren op 27 december 1977 te Eindhoven en groeide op in Veldhoven. In juni 1996 behaalde zij haar VWO diploma aan het Van Maerlantlyceum te Eindhoven. In september van datzelfde jaar begon zij aan de studie Zoötechniek aan de toenmalige Landbouwwuniversiteit in Wageningen. Deze studie werd in maart 2002 cum laude afgerond, met afstudeervakken Gezondheidsleer & Reproductie en Fokkerij en stages bij Holland Genetics in Arnhem en Massey University in Palmerston North, Nieuw Zeeland. Van februari tot en met december 2002 werkte ze als onderzoeker bij het Institute for Pig Genetics (IPG) in Beuningen. In januari 2003 werd zij aangesteld bij de leerstoelgroep Adaptatiefysiologie van Wageningen Universiteit als assistent in opleiding (AIO) aan het in dit proefschrift beschreven onderzoek. Sinds mei 2007 is zij werkzaam als onderzoeker bij de leerstoelgroep Adaptatiefysiologie van Wageningen Universiteit.

Arnoldina Theodora Maria (Ariëtte) van Knegsel was born on 27 December 1977 in Eindhoven and grew up in Veldhoven. In June 1996, she graduated from Van Maerlantlyceum in Eindhoven. In the same year she started BSc Animal Science at the former Agricultural University in Wageningen. During her MSc, she completed theses on Health & Reproduction and Animal Breeding and internships at Holland Genetics in Arnhem and Massey University in Palmerston North, New Zealand. She graduated with distinction in March 2002. From February until December 2002 she worked as a researcher at the Institute for Pig Genetics (IPG) in Beuningen. In January 2003 she started her PhD at the Adaptation Physiology Group of Wageningen University, resulting in the current dissertation. Since May 2007, she works as a researcher at the Adaptation Physiology Group of Wageningen University.

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Training and Supervision Plan		Graduate school WIAS	
<i>Name:</i> Ariëtte van Kneegsel <i>Group:</i> Adaptation Physiology and Animal Nutrition <i>Daily supervisors:</i> Dr. Ir. H. van den Brand and Dr. Ir. J. Dijkstra <i>Supervisors:</i> Prof. Dr. Ir. B. Kemp and Prof. Dr. Ir. S. Tamminga			
		Year	Credits ¹
The Basic Package			
WIAS Introduction Course		2003	1.5
WIAS Course Philosophy of science and ethics		2003	1.5
International conferences			
International Congress on Animal Reproduction (ICAR) , Porto Seguro, Brazil		2004	1.5
Nottingham Feed Conference, Nottingham, UK		2005	0.3
American Dairy Science Association Annual Meeting (ADSA), Minneapolis, USA		2006	1.5
European Society for Domestic Animal Reproduction (ESDAR), Portoroz, Slovenia		2006	0.9
Dairy Solutions Symposium, Dublin, Ireland		2006	0.6
International Conference on Farm Animal Reproduction (ICFAR), Rolduc, The Netherlands		2007	1.5
Seminars and workshops			
(Patho)physiology of the dairy cow in the transition period, Lelystad, The Netherlands		2003	0.1
Metabolic and nutritional effects on ovarian activity and fertility, Utrecht, The Netherlands		2003	0.2
Koeien in Beweging, Wageningen, The Netherlands		2003	0.2
Contactdag Platform Voortplanting, Utrecht, The Netherlands		2003	0.3
Themadag Platform Voortplanting, Wageningen, The Netherlands		2004	0.3
PhD retreat, Nijmegen, The Netherlands		2004	0.6
Endo-Neuro meeting, Doorwerth, The Netherlands		2005	0.6
Studiedag Nederlandstalige Voedingsonderzoekers (NVO) (3x)		2005 - 2007	0.9
WIAS Science Day - Wageningen, The Netherlands (5x)		2003 - 2007	1.4
Presentations			
Oral presentation PhD retreat, Nijmegen, The Netherlands		2004	1.0
Poster presentation ICAR, Porto Seguro, Brazil		2004	1.0
Oral presentation WIAS Science Day, Wageningen, The Netherlands		2006	1.0
Oral presentation NVO Meeting, Rotterdam, The Netherlands/ Ghent, Belgium		2006 - 2007	2.0
Oral presentation ADSA, Minneapolis, USA		2006	1.0
Oral presentation ESDAR, Portoroz, Slovenia		2006	1.0
Oral presentation ICFAR, Rolduc, The Netherlands		2007	1.0
In-depth studies			
Rundveevoeding - Wageningen, The Netherlands		2003	0.6
Nutrition and Sports, Zeist/Arnhem, The Netherlands		2004	1.5
Ecophysiology of the Gastrointestinal Tract, Wageningen, The Netherlands		2005	1.5
Biological basis for improved management and selection tools, Wageningen, The Netherlands		2005	1.5
Regulation of food intake, and its implications for nutrition and obesity, Wageningen, NL		2006	1.5
Professional Skills Support Courses			
Course Supervising MSc thesis work		2003	0.6
WGS course Time planning and project management		2004	0.9
WIAS Course Techniques for Scientific Writing		2005	1.2
WIAS Midterm job assessment		2005	0.6
Research Skills Training			
Writing postdoc proposal 'Shortening the dry period'		2006 - 2007	5.0
Didactic Skills Training: teaching and supervising			
MSc theses (7 students)		2003 - 2007	11.0
Practical Inleiding Dierwetenschappen		2004	0.5
Practical Use of laboratory animals		2005	0.5
Management Skills Training			
Organisation WIAS Science Day		2005	0.9
Chairman WAPS Council		2003 - 2004	10.0
Total			59.6

¹ One ECTS (European Credit Transfer System) credit equals a study load of approximately 28 hours

Colophon

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