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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 hypothesized to decrease the production of milk fat, 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, our objective was to evaluate the effects of a glucogenic, lipogenic, or mixed diet on EB, plasma metabolites and metabolic hormones, liver triacylglycerides (TAG), and reproductive variables in high-producing dairy cows in early lactation. Cows (n = 114) were randomly assigned to 1 of 3 diets and were fed either a mainly lipogenic diet, a mainly glucogenic diet, or a mixture of both diets (50:50 dry matter basis) from wk 3 before the expected calving date until 9 wk postpartum. 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 the lipogenic diet. Milk fat percentage was less for multiparous cows fed the glucogenic diet compared with cows fed the mixed or lipogenic diet (3.69 vs. 4.02 vs. 4.22 ± 0.07%, respectively). The calculated EB was less negative for multiparous cows fed the glucogenic diet compared with cows fed the mixed or lipogenic diet [–33 vs. –125 vs. –89 ± 21 kJ/(kg$^{0.75}$·d), respectively]. Postpartum, the glucogenic diet decreased plasma nonesterified 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 until first milk progesterone rise in multiparous cows compared with the mixed or lipogenic diet (20.4 vs. 24.4 vs. 26.4 ± 2.1 d, respectively). Diet had no effect on any of the above-mentioned variables in primiparous cows, except that milk lactose percentage was greater for primiparous cows fed the glucogenic diet. We concluded that the glucogenic diet was effective in improving the calculated EB and decreasing plasma β-hydroxybutyrate and liver TAG concentrations, suggesting a reduced risk of metabolic disorders in multiparous dairy cows fed a glucogenic diet.
Key words: dietary energy source, reproduction, parity

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 mobilize body fat to compensate for this energy deficit. The extensive body fat mobilization predisposes them to fatty liver and ketosis because of an inability to dispose of fatty acids via β-oxidation or the limited capacity to export triacylglycerides (**TAG**) in the form of very low density lipoproteins (**VLDL**) from the liver (Grummer, 1993; Bell, 1995). This is usually accompanied by a period of reduced fertility (Butler, 2003). Several strategies have been studied to reduce the severity and incidence of metabolic disorders in early lactation. 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; Reist et al., 2003; Hayirli and Grummer, 2004). To achieve this objective, an increase in energy density of the diet has been suggested. 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 has been presented as a dietary

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regimen to decrease milk energy output and thereby 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 thereby improve the EB and decrease plasma ketone body concentration in dairy cows in early lactation (Adler, 1970; Van Knegsel et al., 2005). In ruminants, lipogenic nutrients originate from either fiber, which is fermented to acetate and butyrate, or dietary fat or are derived from body reserves. Glucogenic nutrients originate from starch that has escaped rumen degradation or gluconeogenesis. Diets high in ruminally degraded starch and low in fiber typically decrease the acetate-to-propionate ratio (Bannink et al., 2006). Propionate is the major precursor for gluconeogenesis, whereas acetate is a main precursor for de novo lipogenesis. Therefore, the depression in milk fat upon feeding glucogenic diets has been explained by a shift from a 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 mobilization decreased with a glucogenic diet compared with a lipogenic diet (Van Knegsel et al., 2007a). The glucogenic diet increased plasma insulin concentration and tended to decrease plasma NEFA concentrations in early lactation compared with the lipogenic diet (Van Knegsel et al., 2007b). It has been suggested that the availability of glucogenic nutrients relative to lipogenic nutrients affects the susceptibility of cows to metabolic disorders such as fatty liver and ketosis (Adler, 1970; Kronfeld, 1976; Drackley, 1999). However, in the respiration chamber study, the design of the experiment with 16 cows did not allow sufficient power to evaluate plasma BHBA concentration and liver TAG content as indicators of ketosis and fatty liver (Van Knegsel et al., 2007b). Furthermore, the 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 to further outline the relationship between availability of different glucogenic to lipogenic nutrients 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. Holstein-Friesian dairy cows (n = 114) 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 wk 3 before the expected calving date until 9 wk pp. From these cows, blood was collected weekly from wk –3 to 9 relative to calving, and individual feed intake was determined from parturition until wk 9 pp for 72 of the 76 cows. Liver biopsies were taken in wk –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 2 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 death at parturition (glucogenic concentrate). Of the 111 cows that 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 participated 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 (0600 and 1700 h).

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; it was supplied ad libitum and consisted prepartum of grass silage, corn silage, and wheat straw in a ratio of 45:45:10 (DM basis). Postpartum, forage consisted of grass silage, corn silage, chopped alfalfa hay, and rapeseed meal in a ratio of 48:45:3:3 (DM basis). For cows of parity 3 and greater, concentrate supply was 3.5 kg/d at d 1 pp and increased stepwise by 0.5 kg/d pp. From d 16 to 22 pp, concentrate supply increased by 0.25

Table 1. Distribution of cows per diet, parity,[1] and monitoring protocol[2]

	Diet						
	Glucogenic		Mixed		Lipogenic		
Cows, n	P	M	P	M	P	M	Total
Milk production, milk composition, BW, 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.

[2]Number of cows of total number of cows completing the experiment (n = 111).

kg/d until the 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 was 70.8% (maximum supply, 8.5 kg/d) of the schedule for cows of parity 3 and greater. The ingredients and calculated chemical composition of concentrates are presented in Table 2. Chemical composition of the diets, based on the realized total feed intake, is presented in Table 3. Diets were isocaloric (net energy basis, VEM system; Van Es, 1975) and were 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 was equally divided over 3 meals/d.

Sampling and Analytical Procedures

Body condition was scored (1 to 5 scale) in wk –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, ISO, 1999; Melkcontrolestation, Zutphen, the Netherlands) were collected 4 times/wk (Monday p.m., Tuesday a.m., Wednesday p.m., and Thursday a.m.). At 3 p.m. milkings weekly (Monday, Wednesday, Friday), milk was sampled and stored at –20°C until analysis of milk progesterone (**P4**). Milk P4 concentration was determined as described by Roelofs et al. (2006). The intraassay 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 was 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

Table 2. Ingredient and calculated[1] chemical composition of glucogenic and lipogenic concentrates

	Concentrate	
Item	Glucogenic	Lipogenic
Ingredient, g/kg		
Rapeseed meal	127.2	198.1
Corn	265.6	
Milo corn	250.0	
Palm kernel, expeller	31.6	125.0
Citrus pulp		161.5
Peas	131.5	
Sunflower seed, solvent-extracted		89.3
Soybean hulls		124.0
Soybean meal, formaldehyde-treated	12.40	
Rapeseed meal, formaldehyde-treated		189.2
Bergafat F100[2]		21.3
Cane molasses	40.0	50.0
Palm oil		25.0
Calcium carbonate	13.6	1.3
Magnesium oxide	0.7	
Sodium chloride	8.3	7.9
Mineral-vitamin mixture[3]	7.5	7.5
Calculated chemical composition, g/kg of DM unless otherwise stated		
DM, g/kg of product	874	890
CP	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, MJ/kg of DM	7.69	7.62

[1]Based on the table of the 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]NE_L calculated with the VEM system (Van Es, 1975).

Table 3. Calculated chemical composition of the glucogenic, lipogenic, and mixed diets[1]

Calculated chemical composition, g/kg of DM unless otherwise stated	Diet[2]		
	Glucogenic	Mixed	Lipogenic
DM, g/kg of product	495	500	490
CP	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_L,[6]	6.88	6.84	6.81

[1]Based on realized feed intake.

[2]Corn silage: DM, 311 g/kg of product; OM, 954 g/kg of DM; CP, 76 g/kg of DM; crude fat, 29 g/kg of DM; NDF, 400 g/kg of DM; starch, 343 g/kg of DM; NE_L, 6.49 MJ/kg of DM; DVE, 46 g/kg of DM; OEB, –28 g/kg of DM (Blgg, Oosterbeek, the Netherlands).

Grass silage: DM, 461 g/kg of product; OM, 873 g/kg of DM; CP, 169 g/kg of DM; crude fat, 32 g/kg of DM; NDF, 463 g/kg of DM; sugars, 68 g/kg of DM; NE_L, 6.25 MJ/kg of DM; DVE, 77 g/kg of DM; OEB, 30 g/kg of DM (Blgg).

Chopped alfalfa hay: DM, 876 g/kg of product; OM, 905 g/kg of DM; CP, 156 g/kg of DM; crude fat, 14 g/kg of DM; NDF, 487 g/kg of DM; NE_L, 5.27 MJ/kg of DM; DVE, 82 g/kg of DM; OEB, –1 g/kg of DM (Blgg).

Rapeseed meal: DM, 873 g/kg of product; OM, 923 g/kg of DM; CP, 384 g/kg of DM; crude fat, 30 g/kg of DM; NDF, 334 g/kg of DM; NE_L, 6.70 MJ/kg of DM; DVE, 148 g/kg of DM; OEB, 159 g/kg of 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]Calculated with the VEM system (Van Es, 1975).

maintenance requirements are 42.4 VEM/$kg^{0.75}$·d (1,000 VEM = 6.9 MJ of net energy). The VEM required for milk production is 442 VEM/kg of fat- and protein-corrected milk (**FPCM**; 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 wk –3 to 9 pp at 2 to 3 h after the a.m. feeding and immediately before liver biopsy if both were on the same day. Blood was collected in 3 evacuated tubes (Vacuette, Greiner BioOne, 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 a maximum of 2 h. Plasma was obtained by centrifugation (10 min at 2,900 $\times g$), aliquoted, and frozen at –20°C until analysis. Blood and liver samples were analyzed in a quality-controlled veterinary laboratory (Veterinary Diagnostic Laboratory, Utrecht University, Utrecht, the Netherlands). Analyses for glucose, NEFA, BHBA, and cholesterol were performed using commercially available kits on a Unicel DxC 600 analyzer (Beckman Instruments B.V., Mijdrecht, The Netherlands; glucose: reagent 443355, Beckman Instruments B.V.; NEFA: FA 115 kit, Randox Laboratories Ltd., Crumlin, UK; BHBA: Ranbut kit, Randox Laboratories Ltd.; cholesterol: cholesterol reagent, Beckman Instruments B.V.). Insulin concentration was determined using an RIA kit (Coat-a-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA). The intraassay coefficients of variation were as follows: 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.) and the outcome deviated <5% from the target values.

Liver biopsies were taken in wk –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. The biopsy was obtained under local anesthesia (7 mL of lidocaine-HCl 2% with adrenaline, Alfasan Nederland B.V., Woerden, the Netherlands) with a 17G × 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 contralateral olecranon. Tissue was kept a maximum of 24 h on ice in a 0.9% NaCl solution. Subsequently, connective tissue was removed, and 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 analyzed on a Unicel D×C 600 analyzer with a TAG reagent (Beckman Instruments B.V.). The intraassay coefficient of variation was 6.5%.

Statistical Analysis

Because multiple measurements per animal cannot be regarded as independent units of observation, repeated-measures ANOVA (PROC MIXED of SAS, version 9.1; Littell et al., 1996) 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 to 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 was included for plasma metabolites and metabolic hormones (n = 76), feed intake and EBc

Table 4. Milk production and milk composition of dairy cows in early lactation[1] fed a glucogenic diet, a lipogenic diet, or a mix of both diets (LSM ± SEM)

	Diet				*P*-value		
Variable	Glucogenic	Mixed	Lipogenic	SEM	Diet	Week	Day × week
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,[2] kg/d	30.0	30.3	30.6	0.8	0.84	<0.01	0.35
Lactose, %	4.73[a]	4.76[ab]	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[3] ($\times 10^3$/mL)	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,[2] kg/d	41.5	43.8	44.1	1.1	0.18	<0.01	0.24
Lactose, %	4.58[a]	4.56[ab]	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[ab]	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[3] ($\times 10^3$/mL)	77[a]	65[a]	303[b]	26	<0.01	<0.01	0.41

[a,b]Values in the same row with different superscripts differ ($P < 0.05$).

[1]Weeks 1 to 9 relative to calving.

[2]Fat- and protein-corrected milk.

[3]*P*-values based on the natural logarithm of SCC.

variables (n = 72), and liver TAG content (n = 42; Table 1). Data were analyzed for primiparous and multiparous cows and for prepartum and pp data separately. A first-order autoregressive structure [AR(1)] was the best fit and was used to account for within-cow variation. With the exception of liver TAG concentration, the compound symmetry structure was the best fit and was used to account for within-cow variation. For BW and BCS at the start and end of the experiment, BW loss, and reproduction variables, ANOVA was performed (PROC MIXED of SAS, version 9.1) for primiparous and multiparous cows separately, with diet included in the model as a fixed effect (model 2). Model assumptions for both models were evaluated by examining the distribution of residuals. Values are presented as least squares means with their standard errors of the mean.

To study the risk for ketosis and fatty liver, plasma BHBA and liver TAG concentrations were plotted as categorical data. A chi-squared 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 to 0.8, 0.8 to 1.0, 1.0 to 1.2, 1.2 to 1.4, >1.4 mmol/L) and TAG (≤25, 25 to 50, 50 to 75, 75 to 100, >100 mg/g of wet weight) classes. Classes were arbitrarily chosen.

RESULTS

Milk Production

Milk yield and 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 wk 1 to 31.7 kg/d (± 0.5 kg/d) in wk 9 pp. For multiparous cows, milk yield increased from 36.2 kg/d (± 0.6 kg/d) in wk 1 to 44.6 kg/d (± 0.7 kg/d) in wk 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 the glucogenic diet than for cows fed the lipogenic diet. Milk fat content, daily milk fat yield, and fat-to-protein ratio were greater ($P < 0.05$) for multiparous cows fed the lipogenic diet or the mixed diet compared with cows fed the glucogenic diet. Multiparous cows fed the lipogenic diet had a greater SCC ($P < 0.05$) than multiparous cows fed the mixed or glucogenic diet. Nine cows were detected and treated for mastitis during the experimental period: 1 cow fed the glucogenic diet, 1 cow fed the mixed diet, and 7 cows fed the lipogenic diet. After excluding these cows, the SCC for multiparous cows fed the glucogenic or mixed diet was still lower compared with cows fed the lipogenic diet (68 vs. 65 vs. 220 ± 59 $\times 10^3$/mL; $P < 0.05$).

Table 5. Dry matter intake and energy balance of dairy cows in early lactation[1] fed a glucogenic diet, a lipogenic diet, or a mix of both diets (LSM ± SEM)

	Diet				*P*-value[2]		
Variable	Glucogenic	Mixed	Lipogenic	SEM	Diet	Week	Diet × week
Primiparous cows, n	7	7	7				
DMI, kg/d	18.0	18.4	18.9	0.4	0.28	<0.01	0.52
Concentrate, kg of DM/d	6.7	6.7	6.3	0.2	0.16	<0.01	0.43
Forage, kg of DM/d	11.3[a]	11.7[ab]	12.6[b]	0.3	0.04	<0.01	0.04
Energy intake,[3] kJ/(kg$^{0.75}$·d)	1,108	1,092	1,072	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
BW,[4] kg	536[a]	560[ab]	591[b]	13	0.02	<0.01	0.46
BW loss,[5] kg	33	29	16	7	0.22	NM	NM
BW loss,[6] kg	24	12	4	10	0.19	NM	NM
Multiparous cows, n	16	18	17				
DMI, kg/d	23.8	23.6	24.2	0.5	0.74	<0.01	0.49
Concentrate, kg of DM/d	9.4[a]	9.6[a]	9.2[b]	0.1	<0.01	<0.01	0.13
Forage, kg of DM/d	14.4	14.1	15.1	0.4	0.29	<0.01	0.52
Energy intake,[3] kJ/(kg$^{0.75}$·d)	1,291	1,284	1,287	22	0.97	<0.01	0.55
Energy balance,[3] kJ/(kg$^{0.75}$·d)	–33[a]	–125[b]	–89[ab]	21	0.02	<0.01	0.37
BW,[4] kg	641	637	653	14	0.74	<0.01	0.73
BW loss,[5] kg	26	24	29	6	0.79	NM	NM
BW loss,[6] kg	10	6	14	8	0.79	NM	NM

[a,b]Values in the same row with different superscripts differ ($P < 0.05$).

[1]Weeks 1 to 9 relative to calving.

[2]NM = not included in the model.

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

[4]BW data are based on 13 vs. 7 vs. 12 primiparous cows and 28 vs. 18 vs. 32 multiparous cows.

[5]Total BW loss during negative energy balance.

[6]BW loss from wk 1 to 9 postpartum.

EB

The BCS at 3 wk prepartum (3.26 ± 0.04) or the BW (634 ± 7 kg) and BCS in wk 1 pp (3.24 ± 0.04) did not differ among diets. At the end of the experiment, wk 9 pp, BW (624 ± 7 kg) and BCS (2.90 ± 0.04) also did not differ among the dietary treatments. There were no diet effects on the weekly determined BW or total BW loss for multiparous cows. During the first 9 wk after calving, primiparous cows fed the lipogenic diet had a greater BW than primiparous cows fed the 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 the lipogenic diet, and forage intake was greater for primiparous cows fed the 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 kilogram of metabolic BW (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 the glucogenic diet compared with cows fed the mixed diet. The EBc became positive in wk 7 pp for multiparous cows fed the glucogenic diet and became positive in wk 9 pp for cows fed the mixed or lipogenic diet (Figure 1).

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 the lipogenic diet compared with the glucogenic diet. Prepartum plasma insulin concentration was greater ($P < 0.01$) for multiparous cows fed the mixed diet compared with the glucogenic or lipogenic diet.

Postpartum, multiparous cows fed the glucogenic diet had less ($P < 0.01$) plasma NEFA, plasma BHBA, plasma cholesterol, and liver TAG ($P < 0.05$) than cows fed the mixed diet or the lipogenic diet (Table 7). From wk 1 to 9 pp, plasma NEFA decreased, whereas dietary differences continued to exist (Figure 2). Additionally, liver TAG content decreased from wk 2 to 6 pp, but dietary differences continued to exist. Plasma insulin was greater for multiparous cows fed the glucogenic diet compared with the 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 the glucogenic diet.

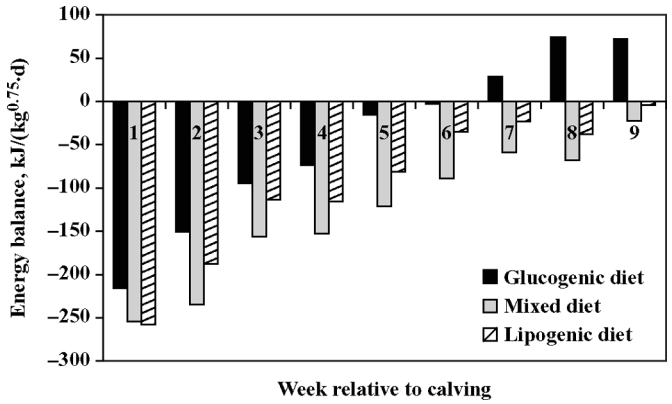

Figure 1. Calculated energy balance for multiparous cows fed a glucogenic diet (n = 16), a lipogenic diet (n = 17), or a mix (50:50) of both diets (n = 18) for the first 9 wk of lactation. Values represent means per diet per week. Overall SEM was 9 kJ/(kg$^{0.75}$·d).

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

Reproduction

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

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 mobilization compared with a lipogenic diet. Multiparous cows fed the glucogenic diet produced 170 g/d less milk fat than cows fed the mixed diet and 240 g/d less milk fat than cows fed the lipogenic diet. This corresponds with our earlier study, in which cows fed the 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; Van Knegsel et al., 2007a). In the earlier study, the decrease in milk fat production resulted in a decrease in milk energy output [1,075 vs. 1,173 ± 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 mobilized from body fat reserves was decreased for cows fed the glucogenic diet. This matches with the results of this study showing that the EBc was improved for multiparous cows fed the glucogenic diet compared with cows fed

Table 6. Blood metabolites, metabolic hormones, and liver triacylglycerides (TAG) prepartum[1] of dairy cows fed a glucogenic diet, a lipogenic diet, or a mix of both diets (LSM ± SEM)

	Diet				*P*-value[2]		
Variable	Glucogenic	Mixed	Lipogenic	SEM	Diet	Week	Diet × week
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, μIU/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[ab]	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, μIU/mL	4.82[b]	7.24[a]	4.30[b]	0.55	<0.01	<0.01	0.06
Liver TAG, mg/g of 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 ($P < 0.05$).

[1]Weeks –3, –2, and –1 relative to calving.

[2]NM = not included in the model.

Table 7. Blood metabolites, metabolic hormones, and liver triacylglycerides (TAG) postpartum[1] of dairy cows fed a glucogenic diet, a lipogenic diet, or a mix of both diets (LSM ± SEM)

	Diet				*P*-value		
Variable	Glucogenic	Mixed	Lipogenic	SEM	Diet	Week	Diet × week
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, μIU/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[ab]	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, μIU/mL	4.19[a]	2.78[b]	2.88[b]	0.33	<0.01	<0.01	0.14
Liver TAG, mg/g of wet weight[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 ($P < 0.05$).

[1]Weeks 1 to 9 relative to calving.

[2]Glucogenic diet: n = 12; mixed diet: n = 17; lipogenic diet: n = 13.

the mixed or lipogenic diet (Table 5). The EBc was numerically, but not significantly, lower for multiparous cows fed the mixed diet than for cows fed the 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 BW (637 vs. 653 ± 14; $P > 0.05$) for cows fed the mixed diet (n = 18) compared with cows fed the lipogenic diet and monitored for individual feed intake (n = 17). Although cows fed the mixed diet were expected to have a higher availability of glucogenic nutrients compared with cows fed the lipogenic diet, there was no significant difference in milk fat production, milk energy output, or EBc between the 2 diets. This can possibly be explained by the limited animal numbers, because the power calculation of the current experiment was based on the difference between the lipogenic and glucogenic diets in our earlier experiment (Van Knegsel et al., 2007a,b), whereas no information on the mixed diet was available.

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

A possible explanation for milk fat depression 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 suggested that the depression in milk fat might be caused by an accumulation of *trans* fatty acids in the rumen because of the 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 the milk fat depression observed after increasing the glucogenic nutrient supply. They reported a trend toward lower milk fat and improved energy status, significantly 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. A 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 responsive to insulin (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 the glucogenic diet in this study

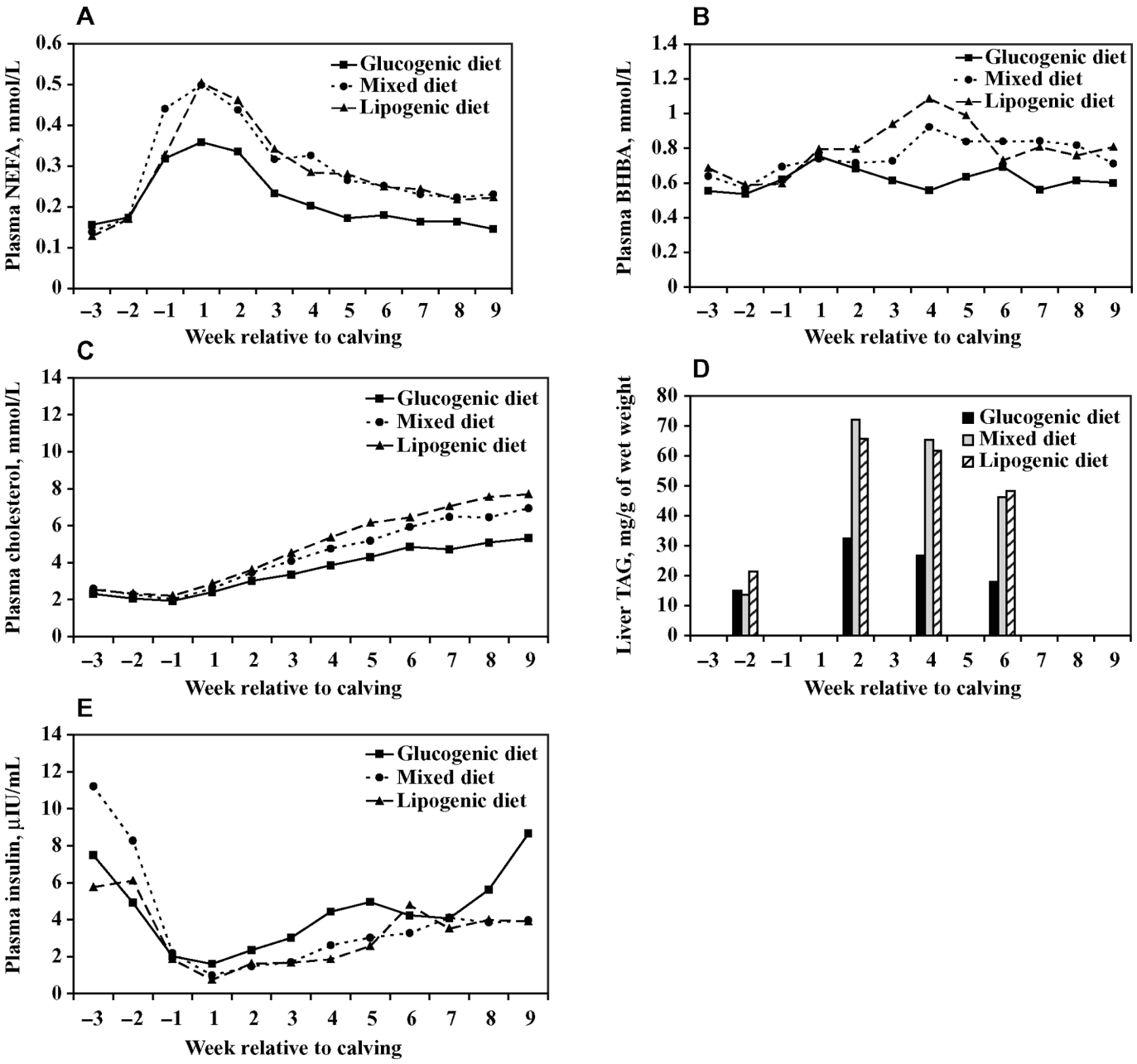

Figure 2. Plasma NEFA (A), plasma BHBA (B), plasma cholesterol (C), liver triacylglyceride (TAG) content (D), and plasma insulin (E) concentration for multiparous cows fed a glucogenic diet, a lipogenic diet, or a mix (50:50) of both diets during wk –3 to 9 of lactation. Values represent means per diet 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 of wet liver weight; plasma insulin, 0.29 μIU/mL.

increased plasma insulin concentration pp and improved the EBc in multiparous cows. This suggests that the glucogenic diet realized an increase in plasma insulin and, as a consequence of the antilipolytic effect of insulin, the contribution of mobilized body fat to milk fat was reduced and milk fat yield and milk energy output were depressed.

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

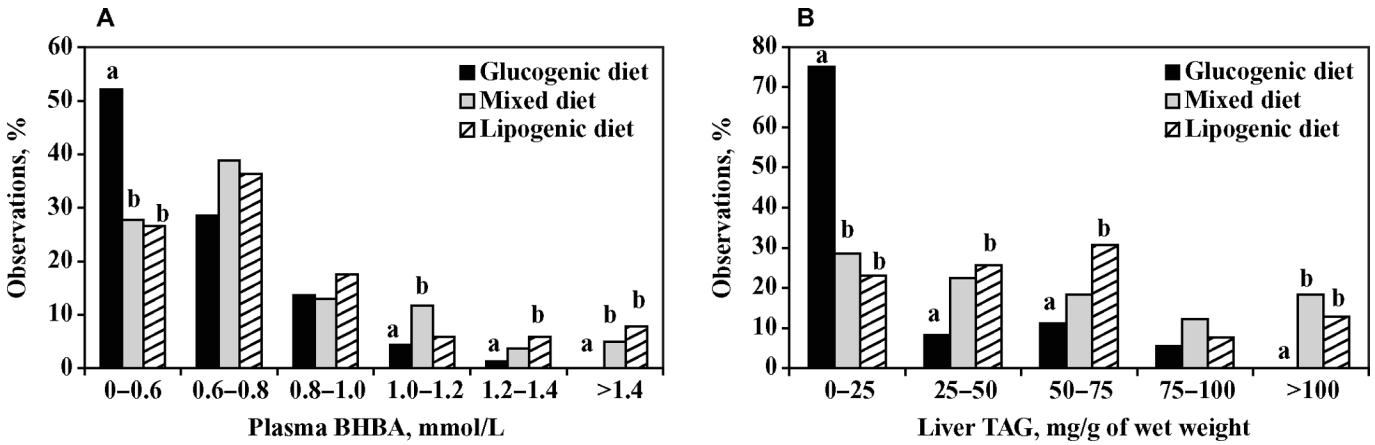

Figure 3. Frequency distribution postpartum per plasma BHBA and liver triacylglyceride (TAG) concentration class for multiparous cows fed a glucogenic diet, a lipogenic diet, or mixed (50:50) diet. Columns within a class with different letters differ ($P < 0.05$). Total observations for plasma BHBA concentration: 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.

because of prevailing higher IGF-I concentrations compared with multiparous cows (Wathes et al., 2006). Additionally, those authors suggested that, because of a lower milk production, primiparous cows 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, plasma NEFA concentrations were lower for multiparous cows fed the glucogenic diet

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

Variable	Diet			SEM	*P*-value, diet
	Glucogenic	Mixed	Lipogenic		
Primiparous cows, n	13	7	12		
Cows ovulated, n	12	7	12		
Days until first P4[1] 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 until first estrous	35.8	30.8	42.7	5.7	0.38
Cows with second P4 rise, n	6	6	11		
Days until second 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		
Cows ovulated, n	29	18	31		
Days until first 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 until first estrous	33.0	40.6	39.4	4.5	0.43
Cows with second P4 rise, n	24	14	26		
Days until second 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

[1]P4 = milk progesterone.

[2]Maximal P4 concentration during the first and second luteal phase postpartum.

[3]Mean P4 concentration during the first and second luteal phase postpartum.

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

Dietary effects on the incidence and severity of reproductive disorders in early lactation can be divided in caloric and noncaloric effects. Beneficial effects of polyunsaturated fatty acids in the reduction of uterine $PGF_{2\alpha}$ secretion (Danet-Desnoyers et al., 1993) or fat supplementation on progesterone concentration (Spicer et al., 1993) have noncaloric effects. Dietary treatments with caloric effects in most cases have increased the energy intake and thereby improved the EBc (Minor et al., 1998). However, various studies have found a depression in feed intake, an increase in milk production, or both 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 until first P4 rise and mean P4 concentration. The trend for a diet effect on days until 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 concentrations during the first luteal phase and the trends observed for days until first P4 rise and mean P4 concentration suggest that animal numbers were limited for detecting diet effects on reproduction variables.

Most studies on feeding extra glucogenic nutrients have found no effect on milk lactose percentage (Krause et al., 2003; Peterson et al., 2003; Hoedemaker et al., 2004) or reported an increase in milk yield and consequently also an increase in lactose yield (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 in which the lipogenic diet tended ($P < 0.10$) to increase milk lactose percentage (Van Knegsel et al., 2007a), in the present study milk lactose percentage was greater for multiparous cows fed the glucogenic diet, which can possibly be explained by a lower SCC for cows fed the glucogenic diet compared with the lipogenic diet. Mastitis has been associated with mammary tissue damage, opening 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 to maintain a constant osmolarity in the milk (Kaufmann and Hagemeister, 1987). The greater SCC for multiparous cows fed the lipogenic diet cannot be completely attributed to a higher proportion of cows with clinical mastitis in the lipogenic diet group. After excluding the cows with clinical mastitis, the SCC for multiparous cows fed the glucogenic diet or mixed diet was still less compared with cows fed the lipogenic diet.

CONCLUSIONS

An 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 concentrations, and liver TAG content. The higher plasma insulin concentration pp in multiparous cows fed the 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

for metabolic and reproductive disorders such as ketosis and fatty liver, and an increased number of days pp until first ovulation. Further studies are needed to confirm this hypothesis. In particular, large-scale studies that monitor cows from the transition period until mid-lactation could present the relationship between dietary energy source and pregnancy rates.

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