Metabolic Changes and Net Portal Flux in Dairy Cows Fed a Ration Containing Rumen-Protected Fat as Compared to a Control Diet

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ABSTRACT

Feeding rumen-protected fat (RPF) is an alternative to increase energy density of the diet and therefore energy intake in dairy cows. To investigate metabolic and endocrine changes in dairy cows fed either a diet containing RPF (FD) or a control diet with an increased amount of cornstarch (SD), 3 Holstein cows (83 ± 1 d in milk) were fitted with catheters in the portal vein, a mesenteric artery, and 2 mesenteric veins. Cows were fed consecutively SD and FD for 3 wk, respectively. In FD, cornstarch [92 g/kg of dry matter (DM)] was replaced by 50 g of RPF/kg of DM (mainly C16:0 and C18:1). Tracer infusions of $\text{NaH}^{13}\text{CO}_3$ and $\text{D-[U-}^{13}\text{C}_6\text{]glucose}$ were performed into a jugular vein to measure rate of appearance and oxidation of glucose. Arterial and portal blood samples were collected to measure concentrations of glucose, lactate, volatile fatty acids, nonesterified fatty acids, β -hydroxybutyrate, triglycerides, AA, insulin, and glucagon. Concomitantly, para-aminohippurate was infused into a mesenteric vein for measurement of portal plasma flow. Although DM intake was slightly lower in FD, protein and energy intakes were unaffected by diets. Milk and lactose yields were higher in FD than SD. Arterial plasma glucose concentration was lower with FD than SD, whereas nonesterified fatty acid and triglyceride concentrations were higher in FD. Glucagon concentration and glucagon-to-insulin ratio were both augmented by FD feeding. When feeding FD, greater milk and lactose yields, but not energy-corrected milk, were associated with elevated lipid status and higher glucagon concentrations but occurred despite lower plasma glucose concentration and were not linked with changes in whole body glucose rate of appearance. This study

suggests a glucose-sparing effect allowing an enhanced lactose synthesis when feeding RPF.

Key words: rumen-protected fat, glucose metabolism, dairy cow, portal flux

INTRODUCTION

Sufficient energy intake is critical in high-yielding dairy cows to meet energy demands with the onset of lactation. Diets containing large amounts of unprotected fat or starch without adequate fiber supply may lead to digestive problems and impaired rumen functions (Emery and Herdt, 1991; Huntington, 1997; Annisson and Bryden, 1998). Therefore, feeding rumen-protected fat (**RPF**) is a strategy widely used to enhance energy density of the ration and consequently increase energy supply to dairy cows without negative impacts on gastrointestinal tract functions (Palmquist and Jenkins, 1980; Emery and Herdt, 1991; Grummer and Carroll, 1991). Although feeding RPF increases milk yield, inconsistent changes in milk components are observed (Emery and Herdt, 1991; Chilliard, 1993; Weiss and Wyatt, 2004). There is indeed controversy on the benefits of feeding RPF to high-yielding dairy cows to improve the energy status of the dairy cow, because the supply of lipids does on a net basis not substitute glucose precursors from which glucose can be generated for lactose synthesis in the mammary gland (Chilliard, 1993; van Knegsel et al., 2005).

Adequate supply of glucose to the mammary gland is essential to sustain milk production in high-yielding dairy cows (Rigout et al., 2002; Brockman, 2005). However, feeding RPF slightly enhanced milk and lactose yields despite a reduction in plasma glucose concentration (Grummer and Carroll, 1991; van Knegsel et al., 2005; Voigt et al., 2005). Supply and mammary uptake of long-chain fatty acids (**LCFA**) that are directly incorporated into milk fat combined with a reduction of de novo mammary fat synthesis could spare glucose from oxidative metabolism and partition it toward lactose synthesis (Grummer and Carroll, 1991; Chilliard, 1993;

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Voigt et al., 2005). However, this is speculative, because little is known on the effect of feeding RPF on glucose metabolism in dairy cows.

In ruminants, glucose mainly originates from gluconeogenesis (85% from the liver and the remainder from the kidney and gut) with very limited net portal absorption (Brockman, 2005). Therefore substituting an energy source such as starch by an isoenergetic amount of RPF could negatively impact glucose supply to the lactating cow. This could potentially occur through a reduction in net portal absorption of glucose precursor originating from rumen carbohydrate fermentation, such as propionate, and a modest decrease in glucose absorption originating from starch escaping rumen digestion (Huntington, 1997; van Kneegsel et al., 2005). In the present study, we have therefore investigated an isoenergetic substitution of cornstarch by RPF in a dairy ration on rate of appearance of glucose (**RaGluc**), net portal flux of nutrients, as well as on metabolic and endocrine parameters relating to glucose metabolism.

MATERIALS AND METHODS

Animals, Husbandry, Feeding, and Experimental Procedures

The experimental procedures were carried out according to the animal care guidelines and were approved by the relevant authorities of the State Mecklenburg-Vorpommern, Germany (LVL-MV/TSD/7221.3-1.1-036/04). Three Holstein cows of second parity, averaging 560 ± 30 kg of BW and 83 ± 1.2 DIM at the start of the experiment, were housed in tie stalls of the animal experimental facility of the Research Institute in Dummerstorf. Chronic indwelling catheters were surgically implanted as described by Huntington et al. (1989). Briefly, Teflon catheters sheathed in silicone tubing were implanted into a hepatic vein and the portal vein, whereas Tygon catheters were inserted into 2 mesenteric veins and a mesenteric artery. The right carotid artery was surgically raised to a s.c. position to provide access to arterial blood if the catheter for the mesenteric artery would fail, but this was not the case. One temporary catheter was inserted into a jugular vein 1 d before blood sampling. The study started 4 wk after the surgery. Due to problems with the hepatic catheter in 2 cows, we omitted the data on liver metabolism.

After recovery, cows were fed a diet containing cornstarch, but no RPF (SD) for 3 wk, followed by a diet containing RPF (FD) for an additional 3-wk period (Table 1; Figure 1). The 2 diets were formulated based on the recommendations of the German Society of Nutrition Physiology (2001) for lactating cows and were calculated to be isonitrogenous and isoenergetic: they dif-

Table 1. Ingredients and chemical composition of the starch (SD) and fat diet (FD) fed to dairy cows

Item	Diet	
	SD	FD
DM, g/kg of wt	466	455
Corn silage, g/kg of DM	255	273
Grass silage, g/kg of DM	236	252
Grass pellets, g/kg of DM	93	100
Concentrate mix, g/kg of DM	88	93
Cornstarch, g/kg of DM	92	—
Extracted soybean meal, g/kg of DM	63	—
Extracted soybean meal, protected, ¹ g/kg of DM	—	123
Sugar beet pulp, g/kg of DM	149	94
Protected fat, ² g/kg of DM	—	50
Urea, g/kg of DM	4.0	—
Limestone, g/kg of DM	80	—
Vitamin-mineral premix, g/kg of DM	12	14
CP, g/kg of DM	169	175
Utilizable protein, ³ g/kg of DM	156	164
NE _L , ³ MJ/kg of DM	6.9	7.4
Crude fiber, g/kg of DM	175	180
Starch, g/kg of DM	184	104
Crude fat, g/kg of DM	23.8	72.2
Crude ash, g/kg of DM	88.6	94.5

¹Byoprofin from Wulfamast, Dinklage-Wulfenau, Germany. Protection of extracted soybean meal was through formaldehyde treatment.

²Hajenol from Harles und Jentzsch GmbH, Uetersen, Germany, contained 840 g/kg of DM crude fat (1.5% myristic acid, 44% palmitic acid, 5% stearic acid, 40% oleic acid, and 9.5% linoleic acid), 20.2 MJ of NE_L/kg of DM, 120 g/kg of DM crude ash, and 95 g/kg of DM Ca.

³German Society of Nutrition Physiology, 2001.

ferred in their energy sources, primarily by substituting cornstarch in the SD ration with RPF in the FD ration. To supply a similar amount of utilizable proteins with the 2 diets, rumen-protected soybeans substituted soybean meal in the FD treatment, because removing rapidly fermentable starch from the ration would theoretically decrease microbial protein (Table 1). Diets were fed ad libitum as TMR during the first week of the experimental periods, and refusals were weighed every day to determine feed intake. From the second week of each experimental period onward, cows were fed at 97% of their respective ad libitum intake in 12 equal meals at 2-h intervals during the day and in double portions every 4 h during the night (2000 to 0600 h) to minimize postprandial variations of nutrient absorption. Cows had free access to water and were milked twice daily, at 0500 and 1700 h. Milk production was recorded each day, and milk samples were taken weekly (Wednesday) at morning milkings.

Diet and Milk Analyses

Dry matter, crude fat, crude fiber, and crude ash of freeze-dried dietary components and TMR were determined according to the Weender standard procedure (Naumann and Bassler 1993). Nitrogen was deter-

Experimental periods, d

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Starch diet																					Fat diet																				
Ad lib feeding									97% of ad lib feeding												Ad lib feeding									97% of ad lib feeding											

Figure 1. Experimental design. Days 16, 17, 37, and 38 = days of blood sampling for measurement of CO₂ production (2 cows/d); days 18, 19, 39, and 40 = days of blood sampling for measurement of glucose kinetics and net portal fluxes of metabolites and hormones (2 cows/d).

mined by combustion analysis (Leco, CNS-2000, St. Joseph, MI), and CP was then calculated by multiplying the N content with 6.25. The NE_L in the diets were calculated according to German Society of Nutrition Physiology (2001).

Milk protein, fat, and lactose concentrations were measured by the infrared spectrophotometric method (Milkoscan, Foss Germany, Rellingen, Germany) according to the MGVO (Milch-Güteverordnung milk quality by-law, Germany) at the local dairy-control association (LKV Mecklenburg-Vorpommern, Güstrow, Germany). Energy-corrected milk was calculated according to Reist et al. (2003).

Infusions, Blood Sampling, and Analyses

To determine the rate of appearance of CO₂ (RaCO₂) as an indirect measure of energy expenditure, cows received an i.v. bolus of NaH¹³CO₃ (2.5 μmol/kg of BW, 99 atom % ¹³C; Chemotrade Ltd., Leipzig, Germany) on d 16 or 17 of each feeding period (Figure 1). The NaH¹³CO₃ was dissolved in 0.9% saline and filtered through a 0.2-μm filter before i.v. administration. Blood samples from the jugular vein were taken 10 min before and at 2, 5, 10, 20, 30, 60, 120, 240, and 360 min after NaH¹³CO₃ bolus injection. To determine the ¹³C enrichment in blood CO₂ (representing all acid-volatile CO₂ and being a proximate measure for breath ¹³CO₂ enrichment; Junghans et al., 2007), 0.5 mL of heparinized whole blood in a 10-mL glass tube (Exetainer, Labco Ltd., Buckinghamshire, United Kingdom) was treated with 1 mL of lactic acid (10% wt/wt) and sealed airtight by a screwed cap with a rubber septum for 2 h at room temperature. After this time period, the isotopic ratio of the released CO₂ was measured in the headspace by gas isotope ratio mass spectrometry (DELTA Plus XL, Thermo Quest, Bremen, Germany) coupled with the Gas Bench II (Finnigan, Bremen, Germany). Enrichments are expressed as atom percent excess (APE). The APE was calculated by difference between the enrichments of each blood sample and the preinfusion baseline abundance.

Glucose oxidation (GO_x) and RaGluc were determined on d 18 or 19 of each feeding period (Figures 1 and 2). A primed (5.4 μmol/kg of BW) continuous D-[U-¹³C₆]glucose infusion (6.3 μmol/kg of BW per h; 99 atom % ¹³C; Chemotrade Ltd.) was conducted for 4.5 h into a jugular vein. The D-[U-¹³C₆]glucose was dissolved in 0.9% saline and filtered through a 0.2-μm filter before administration. Arterial blood samples were taken 10 min before and 10, 40, 70, 100, 130, 160, 190, and 220 min after the initiation of the D-[U-¹³C₆]glucose infusion. For the determination of the ¹³C enrichment in blood CO₂, 0.5 mL of whole blood was treated as outlined for the bicarbonate infusion. Isotopic enrichments of plasma glucose were measured in heparinized plasma that was harvested and frozen for subsequent analyses. Glucose was converted into the aldonitrile pentaacetate derivative by a modified method of Pfaffenberger et al. (1975). Briefly, acetonitrile (300 μL) was added to a plasma sample (50 μL), and samples were centrifuged at 18,500 × g for 20 min. The superna-

Figure 2. Isotopic enrichments of plasma glucose [left axis, mole percent excess (MPE), solid circles] and blood ¹³CO₂ [right axis, atom percent excess (APE), open circles] during a primed continuous infusion of [U-¹³C₆]glucose. Mean data (±SEM) represent the average of the 3 cows fed the starch and the fat diets.

tant was dried under N₂ gas at 60°C, and 100 µL of hydroxylamine hydrochloride solution was added to the vial. The sample was then vortexed and kept at 90°C for 60 min. Acetic anhydride (100 µL) and pyridine (50 µL) were added, and heating was continued at 90°C for another 60 min. Thereafter, samples were dried under N₂ at 60°C, and isooctane (100 µL) was added. Samples were then transferred to capped vials and injected into a GC-MS system. Chemicals used were of analytical grade and were purchased from several suppliers (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; Fluka Chemie AG, Buchs, Switzerland).

The ¹³C enrichment of plasma [U-¹³C₆]glucose was measured by means of GC-MS (GCMS QP 2010, coupled with GC 2010, Shimadzu Deutschland GmbH, Duisburg, Germany). Splitless injections of 1 µL were made with the injector held at 260°C. Separation of glucose derivative was achieved on a 50 m × mm i.d. Ultra 2 US column with a film thickness of 0.52 µm (J&W Scientific, Folsom, CA). The gas chromatograph was operated in the constant flow mode with the flow rate of the He carrier gas of 35 cm/s. The temperature program started at 150°C, which was held for 1 min, and was then ramped at 40°C/min, to 280°C, which was held for 3 min. The mass spectrometer was operated in positive chemical ionization mode, and the ion fragments *m/z* 328 (MH⁺ – CH₃COOH: tracee) and *m/z* 334 (tracer) were monitored. Calculations of enrichments were based on tracer-tracee peak area ratios converted to [U-¹³C₆]glucose mole percent excess (**MPE**) using a regression curve based on known standard mixtures of D-[U-¹³C₆]glucose with unlabeled glucose (0 to 5 MPE).

On d 18 or 19 of each feeding period, additional blood samples from the portal vein and the artery were taken simultaneously and transferred into tubes containing EDTA at 30-min intervals for 2.5 h, beginning 70 min after the onset of D-[U-¹³C₆]glucose infusion, to measure glucose, lactate, NEFA, BHBA, triglycerides, acetate, propionate, n-butyrate, free AA, insulin, and glucagon concentrations to calculate net flux across the portal-drained viscera (**PDV**; Figure 1). The tubes were immediately put on ice, centrifuged (20 min, 1,500 × *g* at 4°C), and aliquots were stored at –20°C (–80°C for free AA) until analyzed. Para-aminohippurate (**pAH**) was infused at a dose of 14.4 g/h (10%: wt/vol) into a mesenteric vein to determine portal plasma flow, starting at least 90 min before the first sampling time.

Plasma concentrations of glucose, lactate, and triglycerides were measured using kits from Labor und Technik Lehmann, Berlin, Germany (GL 0103, LC 0013, and GL TR 0015, respectively) analogous to Reist et al. (2003). Plasma concentrations of NEFA and BHBA were measured using kits from Randox, Krefeld, Germany (FA 115 and RA-RB 1007) analogous to Reist

et al. (2003). Analyses were performed at the Landesanstalt für Landwirtschaft, Rostock, Germany, using the automatic analyzer Cobas Mira Plus (Roche, Basle, Switzerland). Volatile fatty acids were analyzed using GC with flame-ionization detection as previously described (Thivierge et al., 1998). Plasma pAH concentrations were measured with an automatic analyzer (Technicon Autoanalyser II, Technicon Instruments Corporation, Tarrytown, NY; Huntington, 1984).

Insulin was measured by RIA using a porcine kit (PI-12K, Linco Research, St. Charles, MO; Bellmann et al., 2004). Cross-reactivity with bovine insulin was 90%. Intra- and interassay CV were 4.3 and 8.2%, respectively. Glucagon was measured by RIA using a kit from Linco (GL-32K, Linco Research; Hammon and Blum, 1998). Intra- and interassay CV were 7.8 and 13.6%, respectively. The assay is specific for the determination of pancreatic glucagon in plasma in most mammals. Cross-reactivity to oxyntomodulin, which is the primary gut glucagon, is less than 0.1%.

Plasma AA concentrations were measured by isotope dilution using GC-MS (Calder et al., 1999). Briefly, 1 g of plasma was added to 0.2 g of an internal standard containing AA labeled with stable isotopes, and then the samples were frozen at –80°C. The internal standard solution was prepared with labeled AA diluted in water with the following concentrations (µM): DL-His-¹⁵N (180), L-Ile-¹⁵N (708), L-Leu-1-¹³C (864), DL-Lys-2-¹⁵N-2HCl (486), DL-Met-1-¹³C (86), L-Phe-1-¹³C (247), L-Thr-¹⁵N (375), L-Val-¹⁵N (835), L-Ala-1-¹³C (1,060), L-Glu-1-¹³C (273), L-Gln-1-¹³C (949), L-Gly-1-¹³C (1,131), L-Ser-1-¹³C (491), and L-Tyr-¹⁵N (245). Labeled AA (95 to 99 atom %) were supplied by CDN isotopes (Montreal, Québec, Canada) for His, Leu, Lys, Met, and Phe and Cambridge Isotope Lab (Andover, MA) for others.

Calculations

From the single bolus dose of NaH¹³CO₃, RaCO₂ was calculated as follows (Elia et al., 1992; Junghans et al., 2007):

$$\text{RaCO}_2 = \text{D/A}^{(13\text{C})}, \quad [1]$$

where the RaCO₂ excreted in blood is in moles during 6 h, the ¹³C dose administered is D (mol), and the area under the blood CO₂ ¹³C enrichment-time curve is A(¹³C). The 6-h period corresponds to the time when blood CO₂ isotopic enrichment returned to baseline. The 6-h value was scaled up to 1 d by multiplying by 4 considering the animals were in nutritional steady state induced by frequent meal feeding.

Determination of the RaGluc, determined from the constant infusion of the D-[U-¹³C₆]glucose was calculated as follows (Tserng and Kalhan, 1983):

$$\text{RaGluc} = \text{IR}/(1/E_{\text{glucose}} - 1) \quad [2]$$

where RaGluc (mol/h) is the rate of appearance of glucose, IR (mol/h) is the infusion rate of D-[U- $^{13}\text{C}_6$]glucose, and E_{glucose} (MPE) is the steady-state isotopic enrichment of plasma [U- $^{13}\text{C}_6$]glucose (Figure 2). The hourly value was scaled up to 1 d.

Glucose oxidation was calculated as follows (Kien, 1989):

$$\text{GOx} = \text{RaCO}_2 \times E_{\text{CO}_2}/(6 \times E_{\text{glucose}}) \quad [3]$$

where GOx (mol/h) is the rate of glucose oxidation, E_{CO_2} (APE) is the steady-state ^{13}C enrichment of CO_2 from oxidized glucose in blood, E_{glucose} (MPE) is the steady-state ^{13}C enrichment of [U- $^{13}\text{C}_6$]glucose in plasma, and RaCO_2 is the rate of CO_2 appearance as calculated in equation [1]. Steady-state isotopic enrichments of glucose and CO_2 sampled from 70 to 220 min after the onset of the labeled glucose infusion were averaged and used in this calculation (Figure 2).

Plasma flow across the PDV was calculated from downstream dilution of pAH infusion (Katz and Bergman, 1969). If, within 1 sampling day, the CV of the measured plasma flow for a cow was greater than 25% due to only 1 sample, then this value was removed: this only occurred for 1 sampling time in 1 cow (period 1). We assumed that this high CV was due to an improper mixing of the pAH infused, and therefore, the concentration data for this sampling time were not discarded. Net portal fluxes of metabolites and hormones were calculated for each cow and feeding period as the product of the average venous-arterial concentration difference with the average plasma flow. A negative flux indicates utilization or removal, whereas a positive flux indicates net production or release of the nutrient across tissue bed.

Statistical Analyses

Data for nutrient intake, milk parameters, and measurements in blood are presented as $\text{LSM} \pm$ pooled standard error of the means. Due to the loss of 1 cow, the originally planned Latin square could not be conducted as expected. Because cows were on a SD during the recovery period, the first experimental period tested this diet on the 3 remaining cows, and the FD diet was tested in the second period, acknowledging that treatment and period effects would then be confounded, but after 100 DIM, when changes in feed intake and milk production with time are small, and with 21-d experimental periods, a time effect was unlikely to occur, as also suggested in the literature (Huntington, 1999). Also, during the second period, the portal cath-

Table 2. Nutrient intake of cows fed the starch (SD) and the fat diets (FD)¹

Item	Diet		SEM	P-value
	SD	FD		
DM intake, kg/d	17.2	16.4	0.9	0.05
NE _L , MJ/d	119	121	6	NS ²
CP, kg/d	2.91	2.88	0.15	NS
Utilizable protein, kg/d	2.70	2.69	0.16	NS
Crude fiber, kg/d	3.01	2.93	0.17	NS
Starch, kg/d	3.17	1.64	0.14	0.01
Crude fat, kg/d	0.41	1.19	0.05	0.01

¹Data are presented as $\text{LSM} \pm \text{SEM}$.

²NS = not significant at $P > 0.1$.

ter of 1 cow was not patent, so flux data for period 2 only include 2 cows. Main effects of treatments on plasma concentrations of metabolites and hormones were assessed using the repeated methods of the MIXED procedure (SAS, 1995). Diet and time of blood sampling were used as fixed effects and individual cows as random effects. Differences were defined by Tukey t test with $P < 0.1$ for a trend and $P < 0.05$ for significant difference. Net portal flux data, averaged per cow $\times$ period and glucose kinetics were tested using the MIXED procedure (SAS, 1995). Diet was used as fixed effect and individual cows as random effect. Differences were localized by LSD t test with $P < 0.1$ for a trend and $P < 0.05$ for a significant difference.

RESULTS

Feed Intake, Milk Production, and Glucose Production

The DMI was greater ($P = 0.05$) in cows fed SD than FD, but because, after analyses of ingredients used in the experiment, FD turned out to contain more CP and energy than planned, CP and energy intakes were not different between treatments (Table 2). As planned, starch intake was higher ($P < 0.01$) in cows fed SD, whereas crude fat intake was higher ($P < 0.01$) in cows fed FD. During the 2-h interval feeding periods, feeding residuals per day were on average $8.2 \pm 2.5\%$ for SD and $0.6 \pm 0.5\%$ for FD of the amount offered, based on 97% of ad libitum intake measured during the first week of each experimental period.

In cows fed SD, milk and lactose yields were decreased ($P < 0.05$) in comparison to cows fed FD, but ECM yield was not different between treatments (Table 3) as a consequence of reduced milk fat content ($P < 0.05$). Milk protein yield tended to be lower ($P < 0.1$) in cows fed SD as compared with FD. The RaGluc, GOx, and RaCO_2 were not affected by diet (SD vs. FD $\pm$ pooled SEM: RaGluc: 547 vs. 540 ± 49 mmol/h; GOx: 76 vs. 69 ± 7 mmol/h; RaCO_2 : 5.89 vs. 6.82 ± 0.57 mol/h).

Table 3. Milk yield and composition in cows fed the starch (SD) and the fat diets (FD)¹

Item	Diet		SEM	P-value
	SD	FD		
Milk yield, kg/d	26.7	28.5	2.6	0.05
ECM ² , kg/d	26.9	27.1	2.0	NS ³
Milk fat, %	4.36	3.95	0.14	0.05
Milk fat, kg/d	1.16	1.12	0.08	NS
Milk protein, %	2.96	2.89	0.12	NS
Milk protein, kg/d	0.78	0.82	0.06	0.1
Lactose, %	4.79	4.78	0.04	NS
Lactose, kg/d	1.28	1.37	0.14	0.05

¹Data are presented as LSM $\pm$ SEM.

²Energy-corrected milk = [(0.038 $\times$ g of crude fat + 0.024 $\times$ g of CP + 0.017 $\times$ g of lactose) $\times$ kg of milk]/3.14.

³NS = not significant at $P > 0.1$.

Arterial Concentrations and Portal Net Flux of Metabolites and Hormones

Portal plasma flow was reduced ($P < 0.05$) in cows fed SD in comparison to FD (1,296 vs. 1,469 $\pm$ 31 L/h). Arterial plasma glucose concentrations were higher ($P < 0.01$) in cows fed SD than FD, but net portal flux of glucose was not different between treatments (Table 4). In contrast, SD feeding tended to decrease arterial lactate concentrations ($P = 0.06$) compared with FD. Arterial plasma concentrations and net PDV fluxes of VFA were not altered by the type of energy substrate. Plasma lipid components were altered with dietary energy substrates; arterial NEFA and triglyceride concentrations were both increased with FD ($P < 0.05$). Feeding the SD increased the arterial concentrations of Gln, Glu, and Ser ($P < 0.05$; Table 5), whereas Ile and Leu were reduced ($P < 0.05$). Net PDV flux of Ile tended to be lower ($P < 0.1$) in SD than FD. Feeding FD increased arterial glucagon concentration and the glucagon-to-insulin ratio ($P < 0.01$).

DISCUSSION

Milk Production and Rate of Appearance of Glucose

It is well recognized that dietary fatty acid supplementation, especially unsaturated fatty acid, can depress feed intake in dairy cows (Benson et al., 2001; Allen et al., 2005; Litherland et al., 2005) due to elevated fat oxidation in the liver, changes in gastrointestinal hormones, or impact on rumen digestion (Chilliard, 1993; Benson and Reynolds, 2001; Allen et al., 2005). Although DMI was slightly lower in cows fed FD than SD in this study, energy and protein intake did not differ between treatments due to a slightly greater energy and protein concentration in FD. Differences in energy intake components, however, were as targeted,

Table 4. Plasma arterial concentrations and portal net flux of metabolites and hormones in cows fed the starch (SD) and the fat diets (FD)¹

Item	Diet		SEM	P-value
	SD	FD		
Glucose				
Artery, mM	3.70	3.36	0.1	0.01
PDV, ² mmol/h	-3.4	-76.8	17.5	NS ³
Lactate				
Artery, mM	0.29	0.35	0.01	0.06
PDV, mmol/h	165	161	20.9	NS
Acetate				
Artery, μ M	1,262	1,434	86	NS
PDV, mmol/h	811	931	164	NS
Propionate				
Artery, μ M	43.4	42.7	12.2	NS
PDV, mmol/h	322	365	34	NS
n-Butyrate				
Artery, μ M	17.4	17.0	4.1	NS
PDV, mmol/h	44.4	51.0	3.9	NS
NEFA				
Artery, μ M	0.12	0.16	0.04	0.05
PDV, mmol/h	12.5	-10.0	18.2	NS
BHBA				
Artery, mM	0.85	0.94	0.07	NS
PDV, mmol/h	5.8	86.6	190	NS
Triglycerides				
Artery, mM	1.47	1.74	0.1	0.05
PDV, mmol/h	-96	14	138	NS
Insulin				
Artery, μ g/L	0.74	0.64	0.06	NS
PDV, μ g/h	262	93	129	NS
Glucagon				
Artery, ng/L	89.7	130.4	11.4	0.01
PDV, μ g/h	30.9	37.8	3.9	NS
Glucagon:insulin ratio	0.22	0.46	0.04	0.01

¹Data are presented as LSM $\pm$ SEM.

²PDV = portal-drained viscera net flux; negative value = uptake; positive value = release.

³NS = not significant at $P > 0.1$.

namely a higher starch intake in SD-fed cows and a higher fat intake in FD-fed cows. In previous studies, feeding Ca soaps of fatty acids to dairy cows increased milk and lactose yields and reduced fat content (Rodriguez et al., 1997; Voigt et al., 2005), as observed in this study. Because energy intake was not different between treatments, the main components causing the reduction in milk fat with the FD diet were the 2 energy substrates fed in this study. We speculate that substituting protected fat for starch in cows in positive energy balance alters mammary metabolism and that this was likely accompanied with an inhibition of de novo fatty acid synthesis by LCFA (Emery and Herdt, 1991). However, changes in milk fat content with RPF feeding are inconsistent and have also been reported to increase: this may depend on the type and amount of fat added to the diet and lactation stage (Benson et al., 2001; Drackley et al., 2003; Weiss and Wyatt, 2004). Also, in

Table 5. Arterial plasma concentrations and PDV flux of amino acids in cows fed the starch (SD) and the fat diets (FD)¹

Item	Diet		SEM	P-value
	SD	FD		
Ala				
Artery, μM	205.0	200.6	20.8	NS ³
PDV, $\frac{\text{mmol}}{\text{h}}$	54.6	74.6	9.2	NS
Gln				
Artery, μM	215.5	193.0	18.3	0.05
PDV, $\frac{\text{mmol}}{\text{h}}$	-1.6	-2.1	8.4	NS
Glu				
Artery, μM	60.1	54.9	4.2	0.05
PDV, $\frac{\text{mmol}}{\text{h}}$	4.3	8.5	1.8	NS
Gly				
Artery, μM	293.4	280.8	18.1	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	30.6	36.4	7.5	NS
His				
Artery, μM	50.0	50.4	2.3	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	9.0	9.3	1.7	NS
Ile				
Artery, μM	142.3	175.8	17.1	0.05
PDV, $\frac{\text{mmol}}{\text{h}}$	22.4	25.8	3.7	0.1
Leu				
Artery, μM	144.0	173.2	14.3	0.05
PDV, $\frac{\text{mmol}}{\text{h}}$	33.8	37.6	3.6	NS
Lys				
Artery, μM	85.5	82.5	8.3	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	30.0	34.7	2.7	NS
Met				
Artery, μM	15.7	13.0	1.7	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	8.3	9.2	1.0	NS
Phe				
Artery, μM	49.7	1.9	11.4	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	19.8	26.1	2.4	NS
Ser				
Artery, μM	89.6	75.2	11.4	0.05
PDV, $\frac{\text{mmol}}{\text{h}}$	31.4	42.2	5.3	NS
Thr				
Artery, μM	93.3	85.6	6.4	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	21.8	24.6	3.5	NS
Tyr				
Artery, μM	43.8	43.3	7.9	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	18.5	23.4	2.2	NS
Val				
Artery, μM	288.4	308.7	31.1	NS
PDV, $\frac{\text{mmol}}{\text{h}}$	21.6	27.4	4.6	NS

¹Data are presented as LSM $\pm$ SEM.²PDV = portal-drained viscera net flux; negative value = uptake; positive value = release.³NS = not significant at $P > 0.1$.

the current study, FD tended to increase milk protein output by 5%, whereas previous studies frequently observed a reduction in this parameter (Emery and Herdt, 1991; Chilliard, 1993; Drackley et al., 2003). However, in our study, rumen-protected protein was supplied with the FD to ensure no decrease in estimated utilizable protein with a reduced starch supply, because this would theoretically decrease microbial protein. This

might, however, have resulted in an overall greater supply in utilizable protein and could be a reason for a slightly greater milk protein yield in FD-fed cows.

The rate of glucose appearance (the sum of glucose absorption, gluconeogenesis, and glucose turnover from the glycogen pool) was not affected by diets and was in the range of values previously reported (Bauman et al., 1988; Knowlton et al., 1998; Lemosquet et al., 2004). In cows abomasally infused with LCFA, glucose postliver supply tended to decrease, but LCFA also reduced DMI to a greater extent than in the current study (Benson et al., 2002). Lactose yield relative to the RaGluc averaged 57 vs. 62% for SD vs. FD, respectively. This suggests that with FD, a greater proportion of available glucose was used for lactose synthesis compared with SD-fed cows. Indeed, when LCFA are supplemented, they are preferentially taken up by the mammary gland and directly incorporated into milk fat: a decrease in de novo synthesis of palmitic acid and shorter fatty acids from acetate contributes to reduce the oxidative use of glucose required for producing NADPH and therefore increases glucose partitioning toward lactose production (Palmquist and Jenkins, 1980; Emery and Herdt, 1991; Grummer and Carroll, 1991). In addition, the lower milk fat content in FD-fed cows allowed for even greater use of glucose for lactose synthesis. Because milk yield strongly depends on mammary availability of glucose and on lactose production (Rigout et al., 2002; Brockman, 2005), the mammary metabolic changes outlined above may explain the slightly enhanced milk yield observed with FD feeding. However, a reduced whole body GluOx was not observed with the FD feeding, and it represented on average only 13% of RaGluc for both dietary treatments. Whole body measurements might not be sensitive enough for the fine mammary adjustments likely occurring in this study. Furthermore, the oxidation rate measured through the recovery of glucose carbons in CO_2 is a minimum estimate, because it accounts only for the carbons of glucose that are recovered in CO_2 , and not for all the other carbons of glucose that have been incorporated into intermediate products.

Arterial Concentrations and Net Portal Flux

Portal plasma flow increased in FD-fed cows as observed in response to abomasal infusion of LCFA in dairy cows (Benson et al., 2002). Reasons for this finding are presently unknown, particularly because ME intake that affects portal blood flow in cattle (Reynolds, 1995) was not different between treatments.

Feeding the FD reduced plasma glucose concentrations, as reported previously in most (Benson et al., 2002; Drackley et al., 2003; Voigt et al., 2005) but not

all studies (Smith et al., 1978; Blum et al., 1999) with LCFA supplementation. In our study, the decrease in plasma glucose concentration was not associated with decreased postabsorptive availability of glucose, because RaGluc was not affected by the diet. Therefore, the reduced glucose concentration was likely due to an increased utilization of glucose. First, the increased lactose yield with the FD reflects increased glucose use, but this was only equivalent to 21 mmol/h (i.e., approximately 4% of the RaGluc). With both diets, there was no net portal absorption of glucose but rather an uptake by the PDV, as often reported in dairy cows (Reynolds, 1995; Benson et al., 2002). Although not significant, the net PDV uptake of glucose increased with FD feeding, and this increment represented up to 14% of the RaGluc. Because an infusion of LCFA does not alter net glucose portal absorption (Benson et al., 2002), the smaller PDV glucose uptake in cows fed SD may be due to utilization by the small intestine of glucose available from starch intestinal digestion, thereby decreasing PDV utilization of arterial glucose and increasing the net portal absorption of glucose.

Plasma lactate concentrations increased with the FD, with no effect of the diets on its net portal absorption. Similarly, Benson et al. (2002) did not observe any effect of LCFA infusion on lactate net portal absorption, but they observed an increased hepatic uptake. Elevated plasma concentrations of triglycerides in FD-fed cows were expected (Smith et al., 1978; Palmquist and Jenkins, 1980; Emery and Herdt, 1991; van Knegsel et al., 2005). In addition, plasma NEFA increased in FD-fed cows when compared with SD-fed cows, in line with previous findings (Smith et al., 1978; Benson et al., 2002; Voigt et al., 2005). Obviously, fatty acids are hydrolyzed from triglycerides by lipoprotein lipases, and those escaping uptake by tissue beds contribute to elevated plasma NEFA concentrations (Grummer and Carroll, 1991; Chilliard, 1993). In addition, hepatic lipolytic activities are potentially enhanced during fat feeding (Chilliard, 1993). Reduced insulin plasma concentrations, as discussed later, or impaired insulin action due to high fat intake may also support NEFA release from adipose tissues in dairy cows (Chilliard, 1993). However, fat feeding did not affect insulin action as indicated by glucose clamp studies in dairy cows (Blum et al., 1999). Higher plasma NEFA concentrations may lead to elevated fatty acid oxidation, preferentially in the muscle and liver, which seems to have an impact on feed intake (Allen et al., 2005) and therefore might partly explain lower DMI in FD-fed cows in the current study.

Free AA concentrations in plasma and net portal absorption were not affected by treatments, with the exception of Ile and Leu arterial concentrations, which

increased, whereas Gln, Glu, and Ser concentrations decreased with FD. Concentrations of the branched-chain AA are more closely related with protein supply, because they are barely removed by the liver (Raggio, 2006), and altogether, this suggests that feeding protected soy in FD increased utilizable protein supply, as discussed above. In addition, there was a tendency for increased insulin concentrations in cows fed SD, with the difference being significant ($P < 0.05$) at the portal level (portal insulin plasma concentrations LSM for SD vs. FD $\pm$ pooled SEM: 0.95 vs. 0.63 ± 0.07 mmol/h). Increased insulin concentrations are usually linked with decreased branched-chain AA concentration (Mackle et al., 2000). Lower plasma insulin concentrations were observed in some previous studies feeding RPF to dairy cows (van Knegsel et al., 2005; Voigt et al., 2005) but not in all (Blum et al., 1999). Benson and Reynolds (2001) reported a trend for reduced insulin concentrations and a reduced portal-arterial difference of insulin concentrations with infusion of LCFA to dairy cows, but net PDV flux for insulin did not change. Because arterial glucose concentration was reduced due to fat feeding, this might have lowered pancreatic insulin secretion. On the other hand, plasma glucagon increased with fat feeding, resulting in a significantly greater glucagon-to-insulin ratio when fed FD compared with SD. Benson and Reynolds (2001) also observed a trend for higher arterial glucagon concentrations with LCFA infusion in the abomasum, with a numerical increment in net PDV flux of glucagon, as observed in the present study. Glucagon stimulates endogenous glucose production (gluconeogenesis and glycogenolysis) in ruminants, and glucagon administration improves the glucose status in lactating dairy cows (Drackley et al., 2001; Bobe et al., 2003). However, our results indicated no changes in RaGluc, and even lower plasma glucose concentrations were observed in spite of elevated glucagon-to-insulin ratios. In addition, Benson et al. (2002) have found a trend for reduced hepatic glucose release with LCFA infusion, although arterial pancreatic glucagon concentrations were elevated (Benson and Reynolds, 2001). Therefore, it remains to be resolved whether and how the increased glucagon-to-insulin ratio affects endogenous glucose production in FD-fed cows.

In conclusion, substituting starch by RPF in a dairy ration increased milk and lactose yields but decreased plasma concentrations of glucose. Our study clearly showed that whole body rate of glucose appearance was not affected by RPF feeding. This suggests a more efficient use of available glucose for milk lactose synthesis when feeding RPF. Whether changes in glucose availability are related to endocrine regulation of nutrient partitioning during lactation, with the large increment

of the ratio of glucagon to insulin observed in this study in cows fed the FD, needs further investigations.

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