Lactational Effect of Propionic Acid and Duodenal Glucose in Cows

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ABSTRACT

Five dairy cows were arranged in a 5 × 5 Latin square design to compare the effects of two amounts of either duodenal glucose or ruminal propionic acid (C3) on milk yield and composition. Treatments consisted of a grass silage-based diet supplemented with glucogenic nutrients either infused in the rumen as a mixture of volatile fatty acids (control) or pure C3 (1.72 and 3.45 Mcal/d) or in the duodenum as glucose (1.72 and 3.45 Mcal/d). Treatments were isoenergetic and isonitrogenous and contained 100 and 115% of energy and protein requirements according to INRA (1989), respectively. Only C3 treatments significantly modified ruminal volatile fatty acid composition and linearly increased C3 percentage (up to 25.5%). Both treatments substantially decreased milk fat yield and content, and linearly increased milk and protein yields. Although no significant differences between glucose and C3 were highlighted for milk yield and composition, it seems that mechanisms involved in milk fat decrease are different. Indeed, whereas C3 treatments decreased fatty acid production in an homogeneous way, short- and long-chain fatty acids decreased and medium-chain fatty acid production increased with glucose treatments. A bibliographical study confirmed that increasing glucogenic precursors (GP) supply curvilinearly increase milk yield, linearly increase milk protein content (+ 0.04% per Mcal of GP) and curvilinearly decrease milk fat content (– 0.14% per Mcal of GP). Thus, it appears important to account for the nature of energy supplied by the ration in formulation.

(Key words: propionic acid, glucose, dairy cow, milk composition)

Abbreviation key: C3 = propionic acid, GP = glucogenic precursors, PDI = protein truly digested in the small intestine.

INTRODUCTION

Productive responses in ruminants are largely dependent on energy supply. The amount of energy as assessed by energy systems is the most important trait, but the nature of energetic materials supplied is also important for milk production in dairy cows. Glucose is essential for milk synthesis and an increase of its supply, especially when the dietary supply of glucose precursors is limiting, i.e., grass-silage diets, has been shown to enhance milk yield (Huhtanen et al., 1998; Hurtaud et al., 2000). A lot of energy has to be provided by diets in the form of propionic acid (C3) in the rumen or glucose in the intestine. The C3 can be supplied by favorable carbohydrate fermentation in the rumen, but an excessive ingestion of amounts of readily fermented carbohydrates or starch could lead to acidosis and laminitis (Owens et al., 1998). The amount of postruminal glucose can be increased by decreasing the part of starch fermented in the rumen with an appropriate starch processing, but this could alter total tract digestibility (Knowlton et al., 1998).

Many studies on the effect of a direct supply of glucose to the duodenum or the abomasum (Lemosquet et al., 1997; Oldick et al., 1997; Hurtaud et al., 1998a, 1998b) or C3 to the rumen (Hurtaud et al., 1998a) exist, but very few direct comparisons between these two precursors of glucose have been conducted (Hurtaud et al., 1998a; Hurtaud and Rulquin, 1999). With regard to literature results, the effects of infusions of C3 in the rumen or glucose in the duodenum have led to inconsistent results on milk production (Hurtaud et al., 2000). On the other hand, postruminal glucose (Lemosquet et al., 1997; Oldick et al., 1997; Hurtaud et al., 1998b) or ruminal C3 (Miettinen and Huhtanen, 1996; Huhtanen et al., 1998; Sheperd and Combs, 1998) seem to have similar effects on milk composition. Indeed, increasing

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their supply induces an increase of milk protein yield and a decrease of milk fat content and yield. However, the composition of milk fatty acids is different depending on the precursor supplied, so the decrease of milk fat may occur by different metabolic ways (Hurtaud et al., 1998a).

Thus, an experiment was conducted to compare the effect of graded amounts of duodenal glucose and ruminal C3 on milk yield and composition in dairy cows. Other measurements on glucose and AA metabolisms were carried on and will be the subject of a future study (Lemosquet et al., unpublished). The results were compared to literature results to define a dose-response curve of these two glucogenic materials on milk yield and composition and to know if it is interesting to optimize their supply by the ration.

MATERIALS AND METHODS

Treatments and Experimental Design

The experiment was conducted according to a 5×5 Latin square design with 14-d periods (9 d of transition and adaptation and 5 d of measurement).

Treatments consisted of a control, two doses of glucose infused in the duodenum and two doses of C3 infused in the rumen. Treatments were formulated to be isoenergetic and isonitrogenous to compare the effect of energetic materials (i.e., glucose and C3) and to avoid the confounding effect of an increasing supply of energy. The control treatment consisted in a ruminal infusion of a VFA mixture: 14.6 mol/d as 53.9% acetic acid, 20.4% of C3 and 25.6% of butyric acid (acetic and butyric acids: Merck S.A., Chelles, France; C3: Langlois S.A., Saint Jacques de la Lande, France). Such high concentrations of butyric acid were necessary to obtain a sufficient energetic concentration of the solution associated with a low molar concentration to prevent ruminal problems. The two glucose treatments consisted, respectively, of a ruminal infusion of the VFA mixture (7.3 mol/d) plus a duodenal infusion of glucose (618 g/d) or a duodenal infusion of pure glucose (1238 g/d; dextrose, Roquette, Lestrem, France). The two C3 treatments consisted in a ruminal infusion of the VFA mixture (7.3 mol/d) plus C3 (6.5 mol/d) or a ruminal infusion of pure C3 (13 mol/d).

To compare the effects of glucose and C3 on milk yield and composition, an energetic equivalence was adopted, assuming that glucose provides 2.75 Mcal of NE_L /kg (Armstrong and Blaxter, 1961) and C3 0.266 Mcal of NE_L /mol (Institut National de la Recherche Agronomique, 1978). This equivalence corresponds to a molar equivalence of 1 mole of glucose for 1.86 mole of C3.

Cows, Feeding, and Infusions

Surgical preparation was reviewed and approved by the animal care committee of the French Ministry of Agriculture. The procedure was the same as described by Hurtaud et al. (1998b). Five duodenum- and rumen-fistulated Holstein cows (613 ± 62 kg of BW, 32 ± 4 kg/d of milk at 53 ± 12 DIM) were used for the experiment. They were individually fed restricted amounts of a basal diet (58.6% big bale grass silage, 24.8% energy concentrate, 9.1% formaldehyde-treated soybean meal; DM basis) supplemented with minerals and vitamins (200 g/d) so that treatments (diet plus infusions) provide 100% of energy and 115% of protein requirements (Institut National de la Recherche Agronomique, 1989). Diets were also supplemented with 11 g/d of L-Lys HCl (Ajinomoto Co. Inc., Tokyo, Japan) and 15 g/d of DL-Met (Rhône-Poulenc, Commentry, France) according to the recommendations of Rulquin et al. (2001) to meet the requirement for the most limiting AA (Lys and Met) in dairy cows. The L-Lys HCl and DL-Met were dissolved with glucose in water and infused in the duodenum. Table 1 shows the chemical and nutrient composition of soybean meal, energy concentrate and grass silage and Table 2 shows the composition and nutritive value of the diets. Grass silage was fed three times per d (25% at 0700 h, 25% at 1300 h, 50% at 1900 h) and concentrate eight times a day in equal portions. Access to feed was limited to 1 h every 3 h from 0700 h onwards.

Each cow was continuously infused with three separate solutions: VFA mixture, C3 or water, and buffer in the rumen, and glucose or water in the duodenum (Table 3). The five infusions were isoenergetic and provided 3.45 Mcal/d (Table 3). The solutions were infused continuously by means of peristaltic pumps for 24 h. A special device prevented direct contact between the ruminal wall and infused VFA.

Measurements and Sampling

The amounts of feed offered and refusals were measured daily. The DM content of grass silage was determined daily to adjust offered amounts.

Ruminal fluid was assayed for pH, VFA, and ammonia on d 12 of each period. Individual samples were collected at 0.5, 2.5, 4.5, 6.5, 8.5, and 10.5 h after morning milking. The pH was measured immediately. Then, after filtration through six layers of muslin, subsamples were composited to obtain a single daily pool for VFA and ammonia measurements. For VFA analysis, a preservative (H_3PO_4 5% vol/vol + $HgCl_2$ 1% wt/vol) was added to one subsample (10% vol/vol), and for ammonia analysis, another preservative (NaCl 200% wt/vol) was added to a second subsample (vol/vol). The VFA concentrations were then determined by gas chromatography

Table 1. Chemical composition and nutritive value of feedstuffs (DM basis).

	DM	CP	OM	ADF	NDF	Starch	EE ¹	Energy	PDIE ²
	% of DM							(Mcal/kg)	(g/kg)
Big bale grass silage	55.5	15.4	90.7	29.9	56.3	2.2	2.5	1.70	83
Soybean meal ³	87.8	51.3	92.5	8.1	17.8	5.6	2.9	2.01	385
Energy concentrate ⁴	88.6	11.3	92.0	13.1	32.7	37.4	5.1	1.70	101

¹Ether extract.²Protein truly digested in the small intestine delimited by energy supply.³Soybean meal spray-treated with 1% formaldehyde on an as fed basis.⁴Contained 20% barley, 21.5% wheat, 37% dehydrated beet pulps, 15% fine wheat bran, 2% coprah fat, 2% beet molasses, 0.5% calcium carbonate, 1% sodium bicarbonate, 1% salt (DM basis).

(Hurtaud et al., 1998a) and ammonia concentrations by the reaction of Berthelot (Sahnoune et al., 1991) adapted to an auto-analyzer (Technicon, Domont, France).

Jugular blood was sampled on d 12 of each period at 0.5, 2.5, 4.5, 6.5, 8.5, and 10.5 h after morning milking, using syringes containing heparin at 12 to 30 IU/ml (S-Monovette, 7.5 ml; Sarstedt, Nümbrecht, Germany) for glucose (Bondar and Mead, 1974), acetate (Gynn and Veech, 1975), NEFA (Duncombe, 1964), β -hydroxybutyrate (McMurray et al., 1984), and total glycerides (Bucolo and David, 1973). Plasma metabolites were measured on a multiparameter analyzer (KONE Instruments Corporation, Espoo, Finland) using a KONE kit for glucose, a Biotrol kit (Biotrol Diagnostic, Chennevières les Louvres, France) for total glycerides and a Wako kit (Oxoid S.A., Dardilly, France) for NEFA determinations.

Cows were milked twice daily at 0630 and 1800 h. Milk yield was recorded, and fat and protein contents were determined by infrared analysis (Milkoscan, Foss Electric, Hillerød, Denmark) at each milking. On d 13, 2 L of milk was taken from each cow at morning milking. Milk samples were stored at -20°C for chemical analy-

ses in the laboratory. Milk was analyzed for lactose, casein, fatty acids, soluble proteins (α -lactalbumin, β -lactoglobulin) according to Hurtaud et al. (1993), and milk metabolites (glucose-6-P, glycerol-3-P, 2-oxoglutarate, citrate, isocitrate, lactate and malate after milk deproteinization with HClO_4 , vol/vol) according to Hurtaud et al. (2000). The measurement of milk metabolites is interesting in the sense that their concentrations reflect their Golgi-vesicular and cytosolic levels (Faulkner, 1980).

Statistical Analysis

Daily milk yields during the last 7 d of each period were averaged for statistical analysis.

Each parameter was analyzed using PROC GLM of SAS (1990); cow, period, treatment and residual effects were the factors of variation. Results were expressed as least mean squares with standard errors of the means because of missing values for one period of one cow. VFA treatment was considered as control. Differences among treatment least mean squares were compared using the method of orthogonal polynomial contrasts. The contrast A was used to test linear effects of increas-

Table 2. Composition and nutritive value of the diets (DM basis).

	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)	
		1.72	3.45	1.72	3.45
Diet (% DM)					
Grass silage	53.5	55.8	55.2	54.7	55.2
Energy concentrate	28.7	26.9	26.8	27.9	27.9
Soybean meal	10.5	9.8	9.8	10.1	10.2
Minerals and vitamins	1.21	1.14	1.13	1.19	1.16
Lysine	0.07	0.06	0.06	0.07	0.06
Methionine	0.09	0.09	0.09	0.09	0.09
NE _L , ² Mcal/kg	1.61	1.60	1.59	1.61	1.62
CP, g/kg	18.1	17.9	17.8	18.0	18.1
PDI, ³ g/kg	115	113	112	114	115

¹Propionic acid.²Without energy provided by infusions.³Protein truly digested in the small intestine.

Table 3. Nature and composition of infusions in the rumen and duodenum, and NE_L (Mcal/d) supplied by infusions.

			Glucose (Mcal/d)		C3 ¹ (Mcal/d)	
		Control	1.72	3.45	1.72	3.45
Vol. of water						
Site of infusion						
Rumen	40 L	VFA ²	VFA ³	— ⁷	VFA ³ + C3 ⁴	C3 ⁵
Rumen	10 L	Buffer ⁶	Buffer ⁶	Buffer ⁶	Buffer ⁶	Buffer ⁶
Duodenum	10 L	— ⁷	Glucose ⁸	Glucose ⁹	— ⁷	— ⁷
NE _L supply (Mcal/d)						
VFA		3.45	1.73	—	1.73	—
C3		—	—	—	1.72	3.45
Glucose		—	1.72	3.45	—	—
Total		3.45	3.45	3.45	3.45	3.45

¹Propionic acid.²14.6 mol/d of VFA as 53.9% acetic acid, 20.4% propionic acid and 25.6% butyric acid.³7.3 mol/d of VFA.⁴6.5 mol/d of propionic acid.⁵13 mol/d of pure propionic acid.⁶707 g of NaHCO₃ and 367 g of KHCO₃.⁷pure water.⁸618 g/d of pure glucose.⁹1238 g/d of pure glucose.

ing amounts of glucogenic materials whatever glucose or C3; the contrast B was used to test any difference between glucose and C3 in such a linear effect. The significance threshold was set at $P < 0.05$ and the tendency threshold, at $P < 0.10$, unless otherwise specified.

RESULTS

Effect of Infusions on Intake and Digestive Parameters

The control had an unexpectedly high level of feed refusal (Table 4). As a consequence of DMI, NE_L, and protein (protein truly digested in the small intestine [PDI] and CP) intakes increased linearly with increasing amounts of glucogenic materials. However, except for the control treatment, NE_L and PDI intakes were comparable for the other treatments. The NE_L balance was increased linearly ($P < 0.01$) by glucogenic materials, whereas no effect was observed on PDI balance.

Increasing the amounts of infused C3 linearly increased ($P < 0.001$) the percentage of ruminal C3 (from 17.1% for control treatment to 25.5%) at the expense of ruminal acetic (from 66.7% for control treatment to 61.1%) and butyric (from 12.6% for control treatment to 9.5%) acids. Compared with control treatment, glucose treatments did not modify the percentage of acetic and propionic acids but slightly decreased butyric acid and increased isobutyric acid. Valeric acid increased linearly with increasing amounts of both glucogenic materials. The ratio of (acetic acid + butyric acid) to C3

remained unchanged with glucose treatments, whereas it decreased linearly from 4.67 to 2.80 with C3 treatments. Glucose had no significant effect on ruminal pH, but C3 decreased ruminal pH for 3.45 Mcal/d infused. Treatments had no significant effect on total VFA and ammonia concentrations (Table 5).

Milk Yield and Composition

Milk yield tended to increase linearly ($P = 0.09$) with increasing amounts of glucogenic materials without significant difference between glucose and C3 (Table 6). Yields of milk, lactose, and protein increased or tended to increase linearly with increasing amounts of glucogenic materials up to 4% ($P = 0.09$), 6% ($P = 0.01$) and 7% ($P = 0.07$), respectively, without significant differences between glucose and C3 treatments. Lactose and protein contents and milk protein fractions (casein, α -lactalbumin, and β -lactoglobulin) were unaffected (Table 6). Milk fat was more markedly modified than other milk parameters. Milk fat content was decreased linearly ($P < 0.001$) by glucose (−0.39%) and by C3 (−0.43%) treatments. Milk fat yield was also decreased linearly by glucose (−5%) and C3 (−8%).

Milk Metabolites

Glucose and C3 treatments induced a linear ($P < 0.001$) increase of glucose-6-P concentration in milk (Table 6). Concentrations of citrate, oxoglutarate, and malate decreased linearly with glucose and C3 treatments,

Table 4. Effect of infusions of glucogenic materials on feed intake and nutrient balance.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
Diet (kg DM/d)								
DMI	15.6	16.6	16.7	16.0	16.3	0.22	0.011	0.288
Grass silage	8.95	9.89	9.90	9.35	9.53	0.200	0.010	0.227
Energy concentrate	4.76	4.75	4.78	4.73	4.80	0.029	0.382	0.640
Soybean meal	1.74	1.75	1.76	1.72	1.76	0.012	0.276	1.000
Mineral-vitamin	0.2	0.2	0.2	0.2	0.2	—		
Lysine	0.011	0.011	0.011	0.011	0.011	—		
Methionine	0.015	0.015	0.015	0.015	0.015	—		
NE _L , ⁴ Mcal/d	30.4	31.8	31.8	31.2	31.8	0.37	0.011	0.947
NE _L Balance, %	102	107	108	106	109	1.6	0.008	0.435
CP intake, g/d	2830	2977	2987	2878	2933	39.2	0.014	0.317
PDI, ⁵ g/d	1919	1998	2006	1942	1978	21.2	0.016	0.382
PDI balance, %	121	121	121	121	120	1.8	0.939	0.758

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is 1.19 × SEM presented in the table.³Contrast A: linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate in such a linear effect.⁴Including infusions.⁵Protein truly digested in the small intestine.

whereas concentration of isocitrate remained unchanged. Both glucogenic materials tended to increase ($P = 0.05$) milk concentration of glycerol-3-P and did not change milk lactate concentration (Table 6).

The ratios of lactose to glucose-6-P, and glycerol-3-P to glucose-6-P were decreased linearly by glucose and C3 treatments. The ratios of citrate, isocitrate, and oxoglutarate to glucose-6-P also decreased linearly with glucose and C3 treatments, whereas the ratios of isoci-

trate to citrate and oxoglutarate to isocitrate remained unchanged (Table 6).

Milk Fatty Acid Composition and Production

The proportion and the production of even short-chain fatty acids (C_{4:0} to C_{8:0}) decreased linearly with increasing doses of glucogenic materials without difference between glucose and C3 treatments (Tables 7 and

Table 5. Effect of infusions of glucogenic materials on rumen pH and volatile fatty acid and ammonia concentrations.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
VFA concentrations, mol/L	112.1	112.8	112.3	103.4	116.1	3.53	0.629	0.466
VFA, mol/100 mol								
Acetic	66.7	67.0	67.7	63.8	61.1	0.33	<0.001	<0.001
Propionic	17.1	17.6	17.1	21.2	25.5	0.20	<0.001	<0.001
Isobutyric	0.59	0.67	0.74	0.66	0.60	0.016	0.002	<0.001
Butyric	12.6	11.6	10.8	11.0	9.5	0.34	<0.001	0.017
Isovaleric	1.43	1.42	1.61	1.62	1.45	0.115	0.498	0.360
Valeric	1.18	1.40	1.58	1.38	1.57	0.051	<0.001	0.945
Caproic	0.34	0.40	0.49	0.37	0.32	0.046	0.274	0.023
Ratio (C2+C4)/C3	4.67	4.52	4.65	3.58	2.80	0.066	<0.001	<0.001
pH	6.40	6.37	6.44	6.46	6.21	0.056	0.267	0.014
NH ₃ , mg/L	57.6	56.0	68.7	59.8	52.9	7.35	0.729	0.156

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is 1.19 × SEM presented in the table.³Contrast A: linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate in such a linear effect.

Table 6. Effect of infusions of glucogenic materials on milk yield and composition and milk metabolites.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
Milk yield, kg/d	25.2	26.3	26.8	26.0	25.9	0.51	0.090	0.257
FCM, kg/d	25.8	25.6	25.7	25.5	24.8	0.49	0.384	0.233
Fat								
%	4.17	3.86	3.78	3.90	3.74	0.06	<0.001	0.633
g/d	1050	1007	999	1009	965	21.1	0.023	0.277
True protein								
%	3.04	3.09	3.07	3.00	3.11	0.029	0.190	0.426
g/d	764	809	818	775	804	19.0	0.068	0.610
Lactose, %	4.97	5.01	4.95	5.03	4.96	0.029	0.572	0.796
Lactose, g/d	1246	1328	1352	1319	1318	23.8	0.011	0.334
Casein, g/kg	24.1	24.6	23.9	24.0	24.8	0.36	0.525	0.140
Casein/true protein, %	79.1	79.5	77.9	80.1	79.6	1.53	0.845	0.458
α -Lactalbumin, g/kg	1.51	1.48	1.55	1.57	1.50	0.029	0.562	0.254
β -Lactoglobulin, g/kg	4.55	4.48	4.65	4.68	4.84	0.140	0.278	0.375
Milk metabolites, mmol/l								
Glucose-6-P	0.030	0.046	0.059	0.042	0.055	0.004	<0.001	0.573
Citrate	9.13	7.28	7.46	8.00	7.16	0.320	<0.001	0.523
Oxoglutarate	0.138	0.106	0.094	0.104	0.080	0.019	0.052	0.611
Isocitrate	0.248	0.223	0.239	0.220	0.225	0.024	0.595	0.672
Glycerol-3-P	0.076	0.103	0.099	0.101	0.098	0.008	0.053	0.922
Malate	0.223	0.133	0.120	0.149	0.125	0.012	<0.001	0.778
Lactate	0.155	0.184	0.211	0.183	0.190	0.036	0.321	0.687
Ratios (mmol/mmol)								
Lactose/glucose-6-P	5124	4124	2937	4077	3234	432.2	0.007	0.638
Glycerol-3-P/glucose-6-P	3.839	3.032	2.027	2.997	2.183	0.478	0.013	0.822
Citrate/glucose-6-P	319.9	204.0	153.3	220.6	168.1	24.74	0.001	0.680
Isocitrate/citrate	0.032	0.031	0.032	0.029	0.032	0.002	0.806	0.855
Isocitrate/glucose-6-P	9.45	6.36	5.04	6.37	5.13	0.766	<0.001	0.934
Oxoglutarate/isocitrate	0.454	0.463	0.396	0.443	0.364	0.080	0.520	0.784
Oxoglutarate/glucose-6-P	4.73	3.13	1.92	3.11	2.03	0.566	0.006	0.899

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is $1.19 \times \text{SEM}$ presented in the table.³Contrast A: linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate is such a linear effect.

8). The same trend occurred for the increase of even medium-chain fatty acids ($C_{10:0}$ to $C_{14:0}$) as proportion, but their production tended to be different depending on the glucogenic material: a slight increase with glucose treatments and a linear decrease with C3 treatments. The C_{16} fatty acid group had a different response between glucose and C3 treatments: it increased linearly with glucose but not with C3 (contrast B: $P < 0.01$). As a consequence, their production was unmodified with glucose but linearly decreased with C3 (contrast B: $P < 0.05$). On the contrary, C_{18} fatty acid group as proportion decreased linearly with glucose treatments but remained unchanged with C3 treatments (contrast B: $P < 0.01$). The percentage and production of *trans*- C_{18} fatty acid group decreased linearly with glucogenic materials without difference between glucose and C3 treatments. The percentage of unsaturated fatty acid group decreased slightly with glucose treatments, whereas it increased linearly with C3 treatments. The proportion and the production of odd short- ($C_{5:0}$ to $C_{9:0}$) and medi-

um- ($C_{11:0}$ to $C_{15:0}$) chain fatty acids increased linearly with increasing amounts of glucogenic materials. However, the linear increase was greater for C3 than for glucose (contrast B: $P < 0.001$). The percentage of C_{17} fatty acid group remained unchanged with glucose treatments, whereas it increased linearly with C3 treatments. As a consequence, their production decreased linearly with glucose treatments, whereas it remained unchanged with C3 treatments. As a result for the glucose treatment, the decrease in fatty acid yield could be accounted for 80% by C_{18} fatty acids and for 17% by short-chain fatty acids. In contrast, for C3 treatments the decrease in milk fatty acid yield was constituted by similar decrease of short- and medium-chain fatty acids (27%), C_{16} fatty acids group (38%) and C_{18} fatty acids group (35%).

Plasma Metabolites

Even though glucogenic materials tended to linearly increase plasma glucose concentration (Table 9, con-

Table 7. Effect of infusions of glucogenic materials on milk fatty acid composition.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
Fatty acids, %								
C _{4:0}	2.67	2.47	2.28	2.44	2.24	0.064	<0.001	0.674
C _{5:0}	0.017	0.023	0.020	0.028	0.042	0.003	0.005	<0.001
C _{6:0}	2.23	2.08	1.97	2.03	1.90	0.042	<0.001	0.312
C _{7:0}	0.017	0.027	0.029	0.029	0.049	0.003	<0.001	<0.001
C _{8:0}	1.48	1.46	1.44	1.39	1.38	0.024	0.025	0.145
C _{9:0}	0.029	0.047	0.053	0.047	0.085	0.005	<0.001	0.001
C _{10:0}	3.50	3.69	3.78	3.39	3.59	0.071	0.056	0.082
C _{11:0}	0.048	0.089	0.099	0.096	0.168	0.011	<0.001	<0.001
C _{12:0}	4.00	4.50	4.74	4.00	4.42	0.091	<0.001	0.029
C _{13:0}	0.085	0.123	0.135	0.134	0.206	0.008	<0.001	<0.001
C _{14:1}	1.17	1.40	1.41	1.25	1.39	0.047	0.003	0.743
C _{14:0}	13.2	13.6	13.6	13.1	13.3	0.20	0.361	0.429
<i>iso</i> -C _{15:0}	0.28	0.26	0.25	0.28	0.28	0.007	0.054	0.036
C _{15:1}	0.52	0.50	0.50	0.54	0.55	0.023	0.994	0.128
C _{15:0}	1.10	1.34	1.43	1.45	1.95	0.058	<0.001	<0.001
C _{16:1}	2.30	2.71	2.83	2.48	2.58	0.126	0.025	0.180
C _{16:0}	37.8	38.9	39.7	38.4	37.1	0.517	0.407	0.004
<i>iso</i> -C _{17:0}	0.33	0.31	0.30	0.35	0.33	0.014	0.302	0.148
C _{17:1}	0.74	0.72	0.72	0.81	0.81	0.022	0.314	0.016
C _{17:0}	0.55	0.54	0.54	0.62	0.68	0.018	0.013	<0.001
C _{18:2}	1.58	1.57	1.53	1.55	1.65	0.030	0.769	0.017
C _{18:3}	0.48	0.49	0.49	0.45	0.50	0.027	0.680	0.834
C _{18:1}	15.5	14.5	14.2	15.5	16.0	0.34	0.368	0.004
<i>trans</i> -10 C _{18:1}	0.77	0.69	0.65	0.71	0.70	0.021	0.003	0.081
<i>trans</i> -11 C _{18:1}	1.37	1.30	1.24	1.33	1.26	0.051	0.086	0.746
C _{18:0}	8.19	6.77	6.06	7.53	6.91	0.330	0.001	0.098
Fatty acids, %								
C _{4:0} to C _{8:0}	6.38	6.02	5.68	5.86	5.53	0.119	<0.001	0.374
C _{5:0} to C _{9:0}	0.063	0.097	0.102	0.105	0.175	0.010	<0.001	<0.001
C _{10:0} to C ₁₄	21.9	23.2	23.5	21.8	22.7	0.34	0.013	0.135
C _{11:0} to C _{15:0}	2.04	2.30	2.41	2.50	3.15	0.069	<0.001	<0.001
C _{16:0} + C _{16:1}	40.2	41.6	42.6	40.9	39.6	0.59	0.219	0.005
<i>iso</i> -C _{17:0} + C _{17:1} + C _{17:0}	1.62	1.57	1.56	1.78	1.82	0.047	0.236	0.003
C _{18:2} + C _{18:3} + C _{18:1} + C _{18:0}	25.7	23.3	22.3	25.1	25.0	0.57	0.013	0.007
C _{18:1} (<i>trans</i> -10 + <i>trans</i> -11)	2.14	1.99	1.88	2.04	1.96	0.068	0.026	0.425
Odd, %	3.72	3.97	4.08	4.38	5.14	0.109	<0.001	<0.001
Unsaturated, %	24.4	23.8	23.6	24.6	25.4	0.42	0.880	0.011

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is 1.19 × SEM presented in the table.³Contrast A: linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate in such a linear effect.

trast A: $P = 0.06$), the evolution of plasma glucose was different between glucose and C3 treatments (contrast B: $P < 0.05$). Indeed, glucose treatments linearly increased plasma glucose concentration by 6.6 mg/100 ml, whereas C3 treatments had no effect. Plasma concentrations of acetate and BHBA decreased linearly by 50% with glucogenic materials. Plasma NEFA concentrations decreased linearly with both treatments. With C3 infusions, NEFA concentrations decreased significantly only for the highest dose of C3, mainly due to a lower value measured in one cow. Plasma total glyceride concentrations decreased linearly without difference between glucose and C3.

DISCUSSION

Effects of Glucose and C3 Infusions on Milk Yield and Composition

Linear responses of milk production and composition were obtained with increasing amounts of glucogenic materials. Glucose and C3 substantially decreased milk fat yield and content and increased in a lesser extent milk, lactose, and protein yields. However, no difference between glucose and C3 could have been highlighted in the tested range of glucogenic materials amounts.

To avoid a confounding effect between the level of energy supply and changes in the nature of materials

Table 8. Effect of infusions of glucogenic materials on milk fatty acid production.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
Fatty acids, g/d								
C _{4:0} to C _{8:0}	58.2	52.4	49.1	50.8	46.0	1.95	0.001	0.276
C _{5:0} to C _{9:0}	0.57	0.82	0.86	0.89	1.41	0.078	<0.001	<0.001
C _{10:0} to C ₁₄	199	203	204	189	189	5.5	0.784	0.075
C _{11:0} to C _{15:0}	18.3	19.8	20.5	21.7	25.8	0.57	<0.001	<0.001
C _{16:0} + C _{16:1}	360	357	363	353	329	9.5	0.241	0.027
iso-C _{17:0} + C _{17:1} + C _{17:0}	14.6	13.6	13.3	15.5	15.0	0.43	0.422	0.021
C _{18:2} + C _{18:3} + C _{18:1} + C _{18:0}	233	202	192	219	208	6.5	0.002	0.112
trans-10 C _{18:1} + trans-11 C _{18:1}	19.2	17.2	16.1	17.7	16.2	0.64	0.003	0.925
Odd, g/d	33.5	34.2	34.7	38.1	42.2	0.94	0.001	<0.001
Unsaturated, g/d	220	206	201	215	211	4.9	0.038	0.194

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is 1.19 × SEM presented in the table.³Contrast A: Linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate in such a linear effect.

supplied in the experiment, isoenergetic treatments were used to compare graded amounts of duodenal glucose and ruminal C3 infusions. However, feed intake was depressed with the VFA infusion for the control treatment (Table 4). A decrease in feed DMI has already been observed with increasing infusions of VFA in the rumen (Faverdin et al., 1992) and with ruminal butyrate infusion (Huhtanen et al., 1998). Thus, the decrease of feed DMI could be due to the high percentage of butyric acid (25.6%) infused in the rumen. Consequently, the control treatment provided slightly less energy supply than the other treatments, and this could have softened the conclusions of the experiment.

Similar experiments from literature with glucose infusion (Lemosquet et al., 1997; Oldick et al., 1997; Hurtaud et al., 1998a, 1998b, 2000; Hurtaud and Rulquin, 1999) led to inconsistent results, as mentioned by Hurtaud et al. (2000). This may arise from the variability of postruminal starch amount in control treatments and

could be accounted for considering postruminal supply of "bypass" starch plus glucose infusion (Hurtaud et al., 2000). Our present results fit well with literature data with a curvilinear increase of milk ($R^2 = 0.58$) and protein ($R^2 = 0.85$) yields and a linear decrease of milk fat yield and content ($R^2 = 0.70$).

Results from C3 infusion in the literature (Miettinen and Huhtanen, 1996; Huhtanen et al., 1998; Hurtaud et al., 1998a; Sheperd and Combs, 1998; Hurtaud and Rulquin, 1999) lead to an increase of milk and protein yield and a decrease of milk fat yield when mean doses were infused (Miettinen and Huhtanen, 1996; Huhtanen et al., 1998; Hurtaud et al., 1998a; Hurtaud and Rulquin, 1999), and no effect on milk yield with great doses infused (Sheperd and Combs, 1998). Here again, differences between experiments in the composition of basal diet may have affected ruminal C3 supply and accounted for the differences in responses to extra C3. Indeed, when expressed on the basis of the percentage

Table 9. Effect of infusions of glucogenic materials on blood metabolites.

Treatments	Control	Glucose (Mcal/d)		C3 ¹ (Mcal/d)		SEM ²	Contrast ³	
		1.72	3.45	1.72	3.45		A	B
Glucose, mg/100 ml	64.1	65.7	70.7	60.9	65.1	1.46	0.057	0.021
NEFA, μ mol/L	101.2	76.7	73.0	89.2	76.4	6.78	0.009	0.728
Acetate, mg/100 ml	9.0	5.6	5.0	6.1	5.7	0.53	<0.001	0.348
BHBA, mg/100 ml	8.3	5.2	4.0	5.0	4.1	0.37	<0.001	0.938
Total glycerides, mg/L	75.3	59.2	61.0	72.7	65.5	4.20	0.040	0.463

¹Propionic acid.²Standard error of the means. For the treatment of 1.72 Mcal/d of propionic acid infused (one missing cell), the SEM is 1.19 × SEM presented in the table.³Contrast A: linear effect of increasing amount of glucogenic materials whatever duodenal glucose or ruminal propionic acid; Contrast B: difference between duodenal glucose and ruminal propionate in such a linear effect.

of total C3 in the rumen, responses in milk yield fit an increasing curvilinear curve ($R^2 = 0.53$), milk protein yield shows an increasing linear relationship ($R^2 = 0.74$), and milk fat yield and content fit a linear decrease ($R^2 = 0.89$).

However, such "classical" observations did not allow either direct comparisons between glucose and C3 nor account for concomitant variations or differences in C3 and glucose. In that way, we tried to assess the amount of energy provided by glucogenic precursors (GP) available from the rumen and the intestine, using the following equations:

$$\text{Rumen GP (Mcal/d)} = (\text{FOM} \times 9 \times \% \text{C3}_{\text{rum}} + \text{C3}_{\text{inf}}) \times 0.266$$

$$\text{Intestinal GP (Mcal/d)} = (\text{Starch}_{\text{ID}} \times \text{dig.} + \text{G}_{\text{inf}}) \times 2.75,$$

where $\text{FOM} \times 9$ (moles/d) is the amount of VFA produced per gram of fermentable OM (Sauvant et al., 1996), $\% \text{C3}_{\text{rum}}$ the percentage of C3 in the rumen, $\text{Starch}_{\text{ID}} \times \text{dig.}$ (kg/d) is the amount of intestinal glucose provided by the diet and calculated using the Dutch tables of Centraal Veevoederbureau (1995), G_{inf} (kg/d) and C3_{inf} are respectively the amount of glucose infused in the duodenum and C3 infused in the rumen and assuming that 1 kg of glucose provides 2.75 Mcal (Armstrong and Blaxter, 1961), and 1 mole of C3 provides 0.266 Mcal (Institut National de la Recherche Agronomique, 1978).

Data from 10 experiments (Miettinen and Huhtanen, 1996; Lemosquet et al., 1997; Oldick et al., 1997; Huhtanen et al., 1998; Hurtaud et al., 1998a, 1998b, 2000; Sheperd and Combs, 1998; Hurtaud and Rulquin, 1999) were analyzed against each fraction or total GP accounting also for intertrials effects (possibly linked to cows and experiment characteristics). It appears that we were still unable to detect any differences between glucose and C3, but indeed it does not mean that differences does not exist. In summary, milk yield increased by 0.2 kg/d when 1 Mcal/d of energy intake is substituted by GP (Figure 1). The relationship is curvilinear but the increase has a limited extent. For milk fat yield and content, 1 Mcal of GP ingested leads to a curvilinear decrease of 0.14% of fat. Milk fat content unexpectedly decreased at a slower rate for great GP supply. Moreover, C3 may decrease milk fat content more markedly than glucose. Milk protein content is increased linearly by 0.035% by 1 Mcal of GP.

Mechanisms of Milk Fat Decrease

The mechanisms of milk fat depression are different between duodenal glucose and ruminal C3. The C3

Figure 1. Effect of the amount of energy provided by glucogenic precursors (GPE) available from the rumen and the intestine on milk yield (a), milk fat (b) and milk protein contents (c). Data of the present experiment (● propionic acid treatments; ■: glucose treatments) were compared with literature data (○: Hurtaud et al., 1998ab; Hurtaud et al., 2000; Hurtaud and Rulquin, 1999; Lemosquet et al., 1997; Oldick et al., 1997 for glucose treatments and □: Huhtanen et al., 1998; Hurtaud et al., 1998a; Hurtaud and Rulquin, 2000; Miettinen and Huhtanen, 1996; Sheperd et al., 1998 for propionic acid treatments). Regression equations: Milk yield (kg/d) = $23.8 + 1.3 \times [\text{GPE}] - 0.07 \times [\text{GPE}]^2$ ($R^2 = 0.99$; $\text{SEM} = 0.62$; $P < 0.001$); Milk fat content (%) = $5.5 - 0.3 \times [\text{GPE}] + 0.01 \times [\text{GPE}]^2$ ($R^2 = 0.93$; $\text{SEM} = 0.13$; $P < 0.001$); Milk protein content (%) = $2.8 + 0.035 \times [\text{GPE}]$ ($R^2 = 0.96$; $\text{SEM} = 0.05$; $P < 0.001$).

treatments decreased milk fatty acid production homogeneously whatever the length of fatty acid chain (except the increased production of odd fatty acids). In contrast, for glucose treatments, the production of even short- and long-chain fatty acids decreased linearly and the production of medium-chain fatty acids increased. As a result, the profile of fatty acid percentage remained unchanged with C3 treatments compared with glucose treatments for which the percentage of short- and long-chain fatty acids decreased and that of medium-chain fatty acids slightly increased.

The decrease in even short-chain fatty acids resulted from the linear decrease of plasma concentrations of their precursors. In the case of C3 treatments, the decrease of plasma concentrations of acetate and BHBA could be a consequence of the modification of VFA profile in the rumen (Huhtanen et al., 1993). For glucose treatments, the decrease of plasma concentrations of acetate and BHBA cannot be explained by the same phenomenon: the ratio of (acetic acid + butyric acid) to C3 and ruminal acidity remained unchanged (Table 5). On the other hand, glucose itself could have decreased plasma acetate and BHBA, as previously observed (Storry and Rook, 1965).

The increase in *de novo* synthesis of medium-chain fatty acids could be due to a possible lower inhibition of acetyl CoA carboxylase (Palmquist et al., 1993) for glucose treatments and favored by a greater supply of NADPH for both glucogenic materials. A part of the increased amount of glucose-6-P could have been utilized to provide energy because it was not fully utilized for lactose and glycerol-3-P syntheses, as stated by the linear decrease of the ratio of lactose and glycerol-3-P to glucose-6-P (Table 6). The Krebs cycle could not have provided a greater supply of energy because milk concentrations of citrate, oxoglutarate, and malate and their ratios to glucose-6-P decreased linearly (Table 6). However, a part of glucose-6-P could have provided energy through the pentose phosphate pathway. For C3 treatments, the total amount of C3 infused is not converted in glucose through gluconeogenesis (Brockman, 1993). Indeed, a part of C3 is taken up by the udder (Laarveld et al., 1981) and utilized for odd fatty acids synthesis (Massart-Leen et al., 1983). Moreover, plasma concentrations of glucose remained unchanged and plasma concentrations of acetate decreased, suggesting that compared to glucose treatments, fewer energetic substrates (glucose and acetate) were provided to the mammary gland; this could explain the lesser extent of *de novo* synthesis of fatty acids with C3 than with glucose.

The main difference between glucose and C3 treatments lies in the linear decrease of long-chain fatty acids percentage with glucose and its constancy with

C3. The decrease in the production of long-chain fatty acids observed for glucose and C3 treatments resulted from a linear decrease of plasma concentrations of NEFA and total glycerides. For glucose treatments, these decreased plasma concentrations could be related to a decrease in the balance lipolysis/lipogenesis (Griinari et al., 1997) and may be caused by a sufficient supply of energetic substrate (i.e., glucose) to the mammary gland contrary to C3 treatments.

CONCLUSIONS

In this study, duodenal infusions of glucose and ruminal infusions of C3 decreased milk fat yield and content and increased milk and milk protein yields to a lesser extent. However, the increase in milk yield suggests that glucogenic material supply is insufficient with grass silage-based diets to ensure maximal milk yield when energy and protein supplies meet the requirements. The bibliographical study showed that increasing GP supply leads to a curvilinear increase in milk yield, a linear increase of milk protein content (+0.04% per Mcal of GP) and a curvilinear decrease of milk fat content (−0.14% per Mcal of GP).

Neither the present experiment nor the bibliographical study demonstrated any differences between glucose and C3. However, differences between glucose and C3 exist in mechanisms involved in milk fat depression (i.e., differences in milk fatty acid composition) and could exist in mechanisms involved in milk protein increase. Nevertheless, it appears important in formulation to account for the nature of energetic material supplied by the ration.

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