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Duodenal Glucose Increases Glucose Fluxes and Lactose Synthesis in Grass Silage-Fed Dairy Cows

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ABSTRACT

The effect of intestinal glucose supply on whole body rate of glucose appearance (WBGRa) and mammary utilization of glucose was studied in four lactating dairy cows. Glucose (0, 443, 963 and 2398 g/d) was continuously infused in the duodenum over 14-d periods using a Latin square design. A grass silage-based diet was formulated so that treatments were isoenergetic and isonitrogenous and contained 100 and 110% of energy and protein requirements according to INRA (1989). The WBGRa was measured by the [6,6-²H₂]glucose dilution technique, and mammary glucose balance by arteriovenous differences and blood flow measurements. Duodenal glucose infusion increased arterial glucose concentrations linearly, whereas arterial concentrations of insulin, growth hormone, and glucagon were not changed. The WBGRa increased linearly with increasing glucose loads. The increase represented 42% of the intestinal glucose supplement. Mammary blood flow dramatically increased (up to 45%) and was associated with a significant increase of arterial insulin-like growth factor-1 concentrations. Mammary gland rate of glucose disappearance ([6,6-²H₂]glucose measurement) increased linearly, whereas net mammary balance of glucose, lactose, and milk yields increased quadratically. Net mammary balance of glucose accounted for 60% of WBGRa, except for the greatest dose (47.6%). The decrease in milk yield with 2398 g/d of glucose may be explained by an imbalance in intracellular intermediate concentrations. The milk ratio of glucose-1-phosphate to glu-

cose-6-phosphate decreased significantly at the greatest infusion of glucose. In conclusion, exogenous glucose supply to a grass silage-based diet increased WBGRa, mammary utilization of glucose and milk synthesis.

(Key words: glucose infusion, mammary metabolism, glucose flux, dairy cows)

Abbreviation key: AV = arteriovenous, G0 = 0% of the DMI provided as glucose infused in the duodenum, G1 = 2.5% of the DMI provided as glucose infused in the duodenum, G2 = 5.1% of the DMI provided as glucose infused in the duodenum, G3 = 14.2% of the DMI provided as glucose infused in the duodenum, GH = growth hormone, MBF = mammary blood flow, MGRd = mammary gland release of glucose, MGRd = mammary gland rate of glucose disappearance, MPF = mammary plasma flow, PDI = protein truly digested in the small intestine, WBGRa = whole body rate of glucose appearance.

INTRODUCTION

High yielding dairy cows require large amounts of glucose during lactation, as it is the primary precursor for mammary lactose synthesis. The availability of glucose has an important impact on milk yield because lactose is the major osmoregulator for mammary uptake of water. Enhanced lactose synthesis and transport into the alveolar lumen is followed by enhanced water transport and thus milk yield. It has been shown that the supply of glucose can be increased by enhancing the production of propionic acid, the main precursor of gluconeogenesis (Danfaer et al., 1995), or a greater postruminal supply of glucose (Knowlton et al., 1998). A recent study (Hurtaud et al., 2000) showed that with diets providing only small amounts of starch

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postruminally, the supply of glucose to the mammary gland may be the limiting factor for milk synthesis. However, in literature, the effects of postruminal infusion of glucose on milk yield are inconsistent. As mentioned by Hurtaud et al. (2000), differences in supply of energy or amount of starch provided postruminally by the diet could explain some differences among treatments. Therefore, because glucose metabolism is closely regulated, differences in energy or postruminal glucose supplies should modify glucose metabolism, affecting the whole body rate of glucose appearance (**WBGRa**) and(or) partitioning of glucose utilization between the mammary gland and other tissues (Judson and Leng, 1973; Clark et al., 1977).

The precise response of **WBGRa** to postruminal glucose supply is not fully understood. In some studies, **WBGRa** increased less than the amount of glucose abomasally infused (Clark et al., 1977) or remained unchanged with direct intravenous infusion (Judson and Leng, 1973). An abomasal infusion of starch tended to increase **WBGRa** (Knowlton et al., 1998). When milk yield was enhanced by a glucose supplement (Hurtaud et al., 2000), **WBGRa** was probably increased because it has been well established that an increase of energy supply leads to an increase of **WBGRa** (see Wieghart et al., 1986 for a review). When milk yield was unaffected by glucose infusion, it may be hypothesized that **WBGRa** was not enhanced (Judson and Leng, 1973). Therefore, a constant **WBGRa** despite an increased supply of glucose may be due to a reduction of gluconeogenesis (Clark et al., 1977; Judson and Leng, 1973) associated with increased arterial concentrations of insulin and(or) with enhanced glucose utilization by the digestive tract (Cappelli et al., 1997).

In the absence of a positive response in milk yield to glucose administration, it cannot be excluded that factors other than **WBGRa** limit milk synthesis. Thus, when large amounts of glucose are intravenously infused (Judson and Leng, 1973), an increase of glucose utilization in insulin-dependent tissues may reduce glucose availability to the mammary gland (Lemosquet et al., 1997). A down regulation of glucose utilization by the mammary gland may also be responsible. Furthermore, a limited availability of nutrients other than glucose such as AA (Clark et al., 1977) might limit milk synthesis.

The objectives of the current study were to evaluate the response of glucose metabolism to increasing duodenal supply of glucose. Glucose metabolism variables were measured simultaneously and included **WBGRa**, net mammary balance, and intramammary metabolism of glucose.

MATERIALS AND METHODS

Treatments and Experimental Design

The experiment was conducted according to a 4×4 Latin square design over 14-d periods. Treatments consisted of continuous duodenal infusions of graded amounts of glucose with equal supplies of NE_L and protein truly digested in the small intestine (**PDI**; Institut National de la Recherche Agronomique, 1989). Doses of glucose were calculated to account for 0, 2.5, 5.1, and 14.2% of DMI for each cow (**G0**, **G1**, **G2**, and **G3**, respectively) and corresponded in average to 0, 443, 963, and 2398 g/d of glucose infused. The **G2** and **G3** doses were chosen to ensure that treatments of duodenal infusions of glucose experiments with grass silage- and corn silage-based diets overlap each other. Indeed, **G2** and **G3** treatments correspond to the amount of glucose (% DMI) of control and greatest treatment, respectively, with corn silage-based diets (Hurtaud et al., 1998b). Diet plus infusion were formulated to provide 100 and 110% of energy and protein requirements, respectively (Institut National de la Recherche Agronomique, 1989). Diets were formulated based on the theoretical requirements of each cow by applying a weekly reduction of 2% to the reference milk yield. The reference milk yield was the mean of daily yield recorded during wk 3 and 2 before the start of infusions. To avoid the confounding effect of an increasing supply of energy from glucose and to keep energy and protein constant among treatments, 0.46 kg of grass silage and 1.62 kg of energy concentrate were replaced with 1.0 kg of glucose and 0.50 kg of soybean meal, assuming that 1 kg of glucose provided 2.75 Mcal of NE_L and 0 g of **PDI** (Armstrong and Blaxter, 1961). Grass silage was made from perennial ryegrass (*Lolium perenne*). The grass was chopped with a disc mower, wilted for 1.5 to 2 d to obtain a DM of 50 to 60%, harvested, pressed, and wrapped with four layers of plastic. 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 diets.

Feeding and Infusions

Four multiparous Holstein cows (635 ± 52 kg of BW; 81 ± 21 DIM; 32 ± 2 kg/d of milk yield) were managed in individual tie stalls and were fed grass silage immediately after calving. Two weeks before the start of infusions, cows were adapted to the basal diet (63.9% grass silage, 7.1% formaldehyde-treated soybean meal, 27.7% energy concentrate; DM basis) supplemented with minerals and vitamins (300 g/d) and with L-Lys HCl (11 g/d; Ajinomoto Co. Inc., Tokyo, Japan)

Table 1. Chemical composition and nutritive value of feedstuffs that composed the basal diet (DM basis).

	DM	CP	OM	ADF	NDF	Starch	EE ¹	Energy	PDI ²
	(%)	(% of DM)						(Mcal/kg DM)	(g/kg DM)
Grass silage	55.1	15.5	90.4	30.4	56.9	1.85	2.45	1.69	83
Soybean meal ³	87.8	51.8	93.1	10.1	20.2	2.01	1.90	1.99	389
Energy concentrate ⁴	88.1	11.2	92.4	12.9	30.7	34.09	4.65	1.87	98

¹Ether extract.

²Protein truly digested in the small intestine.

³Soybean meal spray-treated with 1% formaldehyde on an as fed basis.

⁴Contained 20% barley, 21.5% wheat, 37% dehydrated beet pulp, 15% fine wheat bran, 2% animal fat, 2% beet molasses, 0.5% calcium carbonate, 1% sodium bicarbonate, 1% salt (DM basis).

and DL-Met (16 g/d; Rhône-Poulenc, Commentry, France) according to recommendations of Rulquin et al. (1993). Grass silage was allocated 3 times per day (25% at 0700 h, 25% at 1300 h, 50% at 1900 h) and concentrate in eight equal portions per day. All along the experiment, access to feed was limited to 1 h every 3 h from 0700 h onwards (i.e., 7, 10, 13, 16, 19, 22, 1, 4, ...). Doses of monohydrated glucose (Dextrose, Rocquette Frères, Lestrem, France), L-Lys HCl, and DL-Met were weighed out every 7 d, chilled at 4°C, and dissolved daily in 20 kg of water. The solution was then infused continuously in the duodenum with a peristaltic Ismatec pump (Bioblock Scientific, Illkirch, France). Fresh solution was replaced and used every 24 h.

Surgical Preparation

The surgical preparations were reviewed and approved by the animal care committee of the French Ministry of Agriculture. Six months before calving, the four cows were fitted with a proximal T-shaped duodenal cannula placed 10 to 15 cm from the pylorus.

Two or three months before the beginning of the experiment, cows were surgically prepared to estimate the net mammary balance of nutrients according to the methods described by Guinard et al. (1994). Two indwelling catheters were placed in the left carotid artery and the left subcutaneous abdominal vein to measure udder arteriovenous (AV) differences. A transit time ultrasonic flow probe with a Dacron-reinforced silicone U-shaped bracket (Probe 20 S; i.d., 20 mm; cable length, 2.5 m; Transonic Systems Inc., Ithaca, NY) was positioned around the left external pudic artery before the S-shape to measure mammary blood flow (MBF). The flow probe cable, protected in a silastic tubing (i.d., 3.35 mm; o.d., 4.65 mm; Dow Corning Corp., Midland, MI) was exteriorized with s.c. tunnelling to the left upper part of the pelvis, between the ischial and the coxal tuberosities. A ring of Dacron (Mersutures TS 53, Ethicon, Neuilly, France) was placed at the level of exteriorization around the flow probe cable under the skin to anchor the cable and reduce the risk of infection. An indwelling catheter (length, 1.70 m; i.d., 1.27 mm; o.d., 2.23 mm; Tygon microbore tubing; Norton Performance Plastics,

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

	Glucose ¹			
	G0	G1	G2	G3
Diet (% DM)				
Grass silage	60.8	59.9	61.4	56.6
Soybean meal	7.6	9.4	10.4	15.9
Energy concentrate	29.8	26.1	21.4	11.4
Minerals and vitamins	1.6	1.7	1.6	1.8
Lysine	0.06	0.06	0.05	0.06
Methionine	0.08	0.09	0.07	0.10
NE _L , ² Mcal/kg	1.74	1.69	1.64	1.49
CP, g/kg	167	171	173	183
PDI, ³ g/kg	109	112	112	120

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Without energy provided by glucose infusions.

³Protein truly digested in the small intestine.

Cleveland, OH) was inserted against the bloodstream at 30 cm into the left carotid and exteriorized on the left shoulder. A provisional catheter (PVC, length, 21 cm; i.d., 1.02 mm; o.d., 1.78 mm; Tygon microbore tubing; Norton Performance Plastics, Cleveland, OH) was inserted against the bloodstream where the left s.c. abdominal vein appears from the udder under local anesthesia at the onset of each sampling session. Cows were fitted with another provisional silicone catheter (length, 21 cm; i.d., 1.02 mm, o.d., 2.16 mm; Silclear Tubing, Degania, Israel) inserted in the right jugular vein for tracer infusion during the measurement of WBGRa. Catheter maintenance was as described by Guinard et al. (1994).

Measurement, Sampling, and Analyses

The amount of feed offered and refusals were weighed daily. The DM content of grass silage was determined daily to adjust offered amounts. To allow adaptation, all reported measurements were made during the second week of each period only. The effect of duodenal infusion of glucose on WBGRa was measured on d 10 or 11. A 98.0% pure solution of [6,6-²H₂]glucose (59.43 ± 0.70 g/L; Euriso-top, Saint Aubin, France) was prepared with sterile saline and sterilized by filtration through a 0.22- μ m sterile disk filter (Millipore, Saint-Quentin en Yvelines, France). A priming dose of 50 ml of this solution was injected through the jugular vein before the beginning of measurements. The solution of [6,6-²H₂]glucose was then continuously infused at a constant rate of 1.3 ml/min for 120 min with a syringe pump (Harvard apparatus, Les Ulis, France). Blood samples were collected simultaneously from arterial and venous catheters with syringes containing heparin at 12 to 30 IU/ml (S-Monovette, 7.5 ml; Sarstedt, Nümbrecht, Germany) 20, 15, and 10 min before injection of [6,6-²H₂]glucose solution to measure the natural abundance, and during the plateau period, at 90, 100, 110, and 120 min after injection.

To measure [6,6-²H₂]glucose enrichments, plasma samples were deproteinized with an equal volume of 1.2 M perchloric acid and filtered. The supernatant was neutralized with 3.2 M K₂CO₃ and centrifuged. After centrifugation, the fraction containing glucose was extracted from the second supernatant by a rapid sequential anion and cation exchange chromatography according to the procedure of Bier et al. (1977). The fraction containing glucose was then dried before derivatization with butaneboronic acid followed by addition of acetic anhydride (Wiecko and Sherman, 1976). Glucose enrichment was then determined by gas chromatography/mass spectrometry in electron

impact ionization mode (GC 8060 chromatograph coupled to a VG Platform II, Fisons Instruments, Altrincham, England). The gas chromatography injector temperature was set at 260°C and the transfer line was held at 280°C. One microliter of the derivative mixture was injected in the split (1:20) mode. A 30-m DB 5-MS, 0.25-mm i.d. and 0.1- μ m film thickness capillary column (J&W Scientific, Courtaboeuf, France) was used in this study. Each molar ratio (the ratio of [6,6-²H₂]glucose to glucose) was determined in triplicate from areas of the corresponding peaks (the ratio of m/z = 299 to m/z = 297), and the glucose enrichment was then calculated (Wolfe, 1992).

The MBF was continuously measured for the duration of the experiment. Average blood flow was measured 18 times/s; each cow was scanned every 4 min for 60 s. Means were therefore calculated for every 60-s period and recorded on a computer connected to the two flow meters (T208D, Transonic Systems Inc., Ithaca, NY) that integrated the output signal from the flow probes.

To determine the concentrations of glucose, L-lactate and hormones, we sampled blood on d 13 at 0.5, 2.5, 4.5, 6.5, 8.5, and 10.5 h after morning milking. Blood samples were collected simultaneously from arterial and venous catheters with heparinized syringes (Sarstedt) except for hormonal determination, only blood from the arterial catheter was collected. Samples for insulin, growth hormone (GH) and IGF-1 were collected with syringes containing 1.2 to 2 mg/ml of K₂-EDTA (S-Monovette, 7.5 ml; Sarstedt). Syringes containing K₂-EDTA (S-Monovette, 2.6 ml; Sarstedt) and 260 μ l (2600 KIU) of the protease inhibitor aprotinin (Antagosan, Hoechst Marion Roussel GmbH, Marbourg, Germany) were used to collect blood for determination of glucagon concentrations. Blood samples were kept on ice and centrifuged at 2500 \times g for 10 min at 4°C. Insulin and GH were analyzed according to the methods described by Lemosquet et al. (1997), and glucagon and IGF-1 according to Hammon and Blum (1998). Glucose and L-lactate were determined from a pooled sample from each sampling session with a multiparameter analyzer (KONE Instruments Corporation, Espoo, Finland) using a KONE kit for glucose and a Bio-Mérieux kit (Bio-Mérieux S.A., Marcy-l'Étoile, France) for L-lactate.

Individual samples of ruminal liquid from one cow on the experiment equipped with a ruminal cannula were obtained on d 12 of each period at 0.5, 2.5, 4.5, 6.5, 8.5, and 10.5 h after morning milking. A preservative (H₃PO₄ 5%, vol/vol, + HgCl₂ 1%, wt/vol) was added to each sample (10%, vol/vol), and the samples were composited to obtain a single daily pool. The VFA were

then measured by gas chromatography (Hurtaud et al., 1998a).

Cows were milked twice daily at 0630 and 1830 h. Each half udder was milked separately and milk yield for each half was recorded at each milking.

On d 13 of each period, 100 ml of the evening milk from the left udder was taken, prepared for chemical analysis of lactose (Hurtaud et al., 2000), and stored at -20°C . On d 14, 100 ml was taken from the left udder of each cow at morning milking, and samples were prepared for the determination of glucose, glucose-6-P, and glucose-1-P after milk deproteinization with 5 M HClO_4 according to the methods described by Faulkner (1980) before storage at -20°C .

Calculations and Statistical Analysis

Daily milk yields were averaged for the last 7 d of each period. The MBF averaged for the same time period was used to estimate the ratio of MBF to milk yield. The net mammary balance of glucose (g/h) was calculated with the following equation:

$$\text{Net balance} = \text{AV} \times \text{MPF},$$

where AV (g/L) is AV difference of glucose and MPF (L/h) is mammary plasma flow estimated by the MBF (recorded on d 13 from 0630 to 1830 h) averaged and corrected for hematocrit.

The WBGRa (g/min), equal to hepatic plus renal production of glucose plus intestinal absorption of glucose, was determined by the equation:

$$\text{WBGRa} = F \times (\text{IEp}/\text{IEinf} - 1),$$

where F is the $[6,6\text{-}^2\text{H}_2]$ glucose infusion rate (ml/min), IEinf is the isotopic enrichment of the infusate (i.e., 98 mol% excess) and IEp (also in mol% excess) is the plasma $[6,6\text{-}^2\text{H}_2]$ glucose enrichment.

The mammary gland rate of glucose disappearance (**MGRd**, g/h) represents the total amount of glucose removed by the udder. A part of this glucose is utilized for lactose and glycerol synthesis and to supply energy for fat synthesis. A part of glucose not utilized by the udder and transferred into the venous circulatory system represented the mammary gland release of glucose (**MGR_e**, g/h). The MGRd and MGR_e were calculated as described by Boirie et al. (1995). The MGR_e was determined with the following equation:

$$\text{MGR}_e = \text{MPF} \times [\text{Gluc}]_v \times [1 - (\text{RM}_v/\text{RM}_A)]$$

where MPF (L/min) is measured during the infusion of tracer (d 10 or 11), $[\text{Gluc}]_v$ (g/L) is the venous concen-

tration of glucose during the plateau and, RM_v and RM_A (%) are the molar ratios of glucose in the vein and artery. Then, the MGRd was calculated as:

$$\text{MGRd} = \text{MGR}_e + \text{Net balance}.$$

To evaluate the efficiency of glucose utilization for lactose synthesis, the ratio of net balance to output for glucose was calculated from the glucose net mammary balance estimated over 12 h on one half udder and from the corresponding yield of milk lactose. The ratio of net balance to WBGRa was calculated to analyze partitioning of glucose between the mammary gland and the other tissues. The calculation was made on the whole udder by multiplying net balance of glucose by two, because milk yield was identical between the two half udders throughout the experiment.

The data were analyzed using the following model for a Latin square: cow, period, treatment, and residual. The residual error term was used to test for significance of cow, period, and treatment. In addition, the linear, quadratic, and cubic effects of glucose infusion were determined, using the residual error term. Because the treatment doses were not at equal intervals, specific coefficients for each infusion amount (0, 1, 2, 6) were used to avoid a biased comparison described by Gill (1986). The calculations were done using PROC GLM of SAS (1990). Results were expressed as least squares means with root means squares errors because of missing values for one period of one cow and two missing values for the glucose-6-P and its associated ratios.

RESULTS

Nutrient Supply, Milk, and Lactose Yields

According to the experimental design, feed intake decreased linearly with increasing infusion of glucose to 4 kg of DMI between the two extreme treatments (Table 3). However, despite a statistical linear decrease, feed DMI remained unchanged for G0, G1, and G2 (-0.7 kg/d between G0 and G1 or G2) and decreased for G3 (-4 kg/d). Nevertheless, as was originally planned, the combinations of diet and infusion provided the same amount of energy (32.7 Mcal/d on average) and protein (2131 g of PDI/d on average) for all treatments.

Yields of milk and lactose increased quadratically ($P < 0.05$) with increasing duodenal infusions of glucose (Table 3). Yields of milk and lactose increased by 2.3 kg/d and 142 g/d, respectively, between G0 and G2, whereas milk lactose content remained unchanged for G0, G1, and G2, and slightly but not significantly decreased for G3 (Table 3).

Table 3. Effect of duodenal infusions of glucose on feed intake, nutrient intake, milk yield, and milk lactose content and yield.

	Glucose ¹				RMSE ²	Effect			
	G0	G1	G2	G3		Linear	Quadratic	Cubic	
								<i>P</i>	
Total DMI, ³ kg/d	18.7	18.4	19.0	17.1	1.09	0.082	0.336	0.563	
Feed DMI, kg/d	18.7	18.0	18.0	14.7	0.95	0.002	0.381	0.559	
Grass silage, kg/d	11.3	11.1	11.6	9.8	1.05	0.074	0.330	0.548	
Energy concentrate, kg/d	5.57	4.88	4.08	1.92	0.233	< 0.001	0.136	0.608	
Soybean meal, kg/d	1.43	1.68	1.98	2.69	0.116	< 0.001	0.151	0.692	
NE _L , ³ Mcal/d	32.4	32.4	33.9	32.1	1.99	0.743	0.317	0.566	
CP intake, g/d	3140	3155	3303	3146	192.4	0.937	0.285	0.574	
PDI, ⁴ g/d	2066	2076	2157	2071	106.6	0.955	0.282	0.591	
Milk, kg/d	25.5	26.6	27.8	25.0	1.48	0.314	0.043	0.791	
Lactose, %	4.62	4.68	4.68	4.53	1.468	0.266	0.343	0.849	
Lactose, g/d	1229	1315	1371	1200	97.2	0.315	0.053	0.952	

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³Including infusions of glucose.

⁴Protein truly digested in the small intestine.

Arterial Concentrations

Duodenal infusions of glucose linearly increased ($P < 0.01$) arterial plasma glucose concentrations by up to 10.2 mg/100 ml (Figure 1). L-Lactate arterial concentrations (Table 4) decreased quadratically ($P < 0.05$) with increasing infusions of glucose. At the same time, arterial plasma insulin concentrations tended to increase quadratically ($P = 0.120$). Plasma insulin concentration reached a plateau at G1 and remained at this level for G2 and G3, whereas whole plasma glucose concentration continued to increase (Figure 1). Arterial plasma concentrations of glucagon were not affected by treatments, whereas plasma GH decreased, although not significantly, with duodenal infusions of glucose. In contrast, arterial plasma concentrations of IGF-1 increased linearly ($P < 0.05$).

Fluxes of Glucose

Whole body rate of glucose appearance. During the day of measurements of plasma [6,6-²H₂]glucose enrichments (d 10 or 11), arterial plasma glucose concentrations exhibited similar values to those measured on the day of kinetic measurement (d 13). Concentrations and enrichments of plasma glucose were both in a steady state during the last 30 min of [6,6-²H₂]glucose infusion corresponding to the plateau measurement. The coefficients of variations for concentrations and enrichments of plasma glucose ranged from 0.65 to 3.72% and 1.39 to 4.86%, respectively. When no glucose was infused to the duodenum (G0), WBGRa reached 2591 g/d for cows producing 25.5 kg/

d of milk on average. In response to graded infusions of glucose, WBGRa increased linearly (Figure 2) up to 3570 g/d for the greatest dose infused (G3 = 14.2% DMI). The linear response fitted the following equation WBGRa (g/d) = 0.42 × Ginf_{duod.} + 2604 ($R^2 = 0.94$, SD = 191, $P < 0.001$), where Ginf_{duod.} represented the amount of glucose infused (g/d).

Glucose utilization. The MBF and MPF increased curvilinearly with treatments by up to 45 and 48%, respectively, between G0 and G3 (Table 5). As a result, the ratio of MBF to milk yield increased linearly ($P < 0.001$) up to 46% (Table 5) with duodenal infusions of glucose. As MBF increased, AV differences and extraction rates of glucose (Table 4) decreased linearly with the graded infusions of glucose ($P < 0.01$ and $P < 0.05$, respectively).

Net mammary balance of glucose increased quadratically ($P < 0.01$) with treatments, reached a maximum with G2, and decreased to values obtained with G1. For the first three treatments (0 to 5.1% DMI of glucose infused in the duodenum), net mammary balance of glucose represented 60% of WBGRa on average, and decreased to 47.6% for G3. The MGRd increased linearly ($P = 0.054$) with treatments, whereas MGR_e tended to increase linearly, the difference between both resulting in the quadratic response of net mammary balance. For all treatments, the glucose removal from the udder was sufficient to sustain milk lactose synthesis. Furthermore, the ratio of net mammary balance to milk output for glucose increased but did not reach the significance threshold.

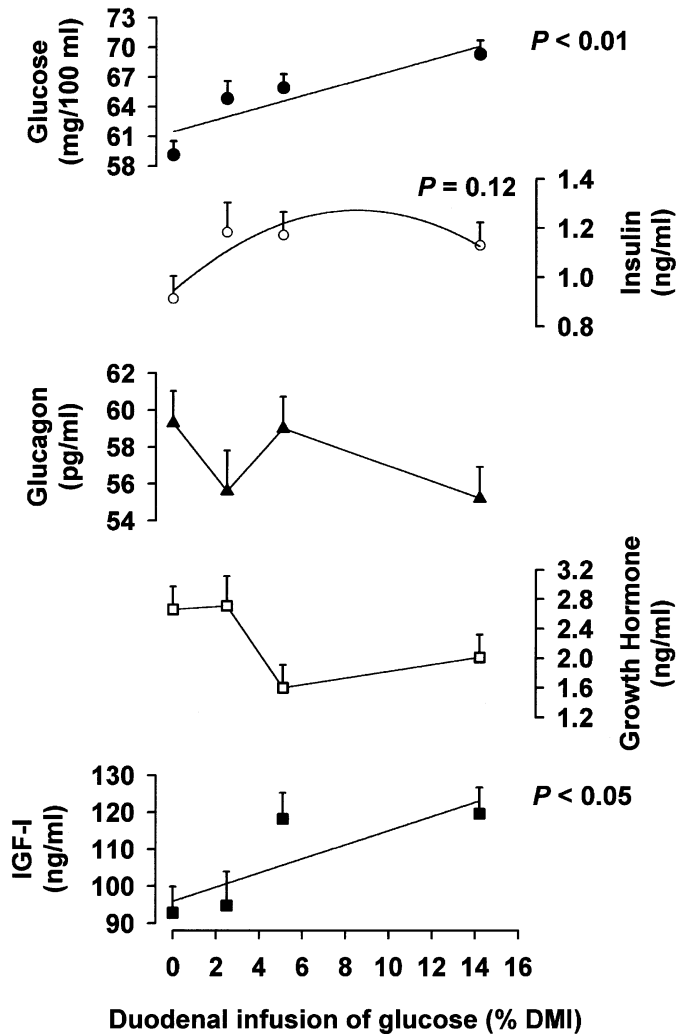


Figure 1. Effect of duodenal infusion of glucose (% of the DMI) on arterial concentrations of glucose (●), insulin (○), glucagon (▲), growth hormone (□) and IGF-I (■) measured on d 13. Arterial glucose concentrations increased linearly with treatments (regression equation: arterial concentrations of glucose (mg/100 ml) = 0.61 × [% DMI of glucose infused per day] + 61.46; R² = 0.79; SD = 2.38; P < 0.01), IGF-I increased linearly (regression equation: arterial concentrations of IGF-I (ng/ml) = 95.95 + 1.90 × [% DMI of glucose infused per day]; R² = 0.66; SD = 10.33; P < 0.05) and insulin tended to increase quadratically (regression equation: arterial concentrations of insulin (ng/ml) = 0.94 + 0.077 × [% DMI of glucose infused per day] - 0.005 × [% DMI of glucose infused per day]²; R² = 0.82; SD = 0.094; P = 0.120). Plasma growth hormone and glucagon concentrations were not significantly affected by glucose infusions.

Milk Metabolites

The effects of glucose infusion on milk metabolites of the lactose synthesis pathway and their ratios are depicted in Table 6. Milk glucose and glucose-6-P concentrations tended to increase by about 33% (P = 0.078) between G0 and G2 and by 83% (P = 0.079) between G0 and G3, respectively. Glucose-1-P increased qua-

dratically (P < 0.05), and reached similar concentrations for G0 and G3 and for G1 and G2. Infusions of glucose had no significant effect on the ratio of glucose-6-P to glucose, although a greater value for G3 was observed. Treatments tended to linearly decrease (P = 0.091) the ratio of glucose-1-P to glucose-6-P, but, the values for G0, G1, and G2 were similar, and an important decrease was only observed for G3 (down to 49% between G2 and G3). The ratio of lactose to glucose-1-P decreased quadratically (P < 0.05) and reached a minimum at G2.

DISCUSSION

This experiment demonstrates that in the presence of a stable supply of energy, increasing amounts of glucose, when up to about 1 kg/d was infused to the duodenum, linearly increased the WBGRa and milk yield.

The increase in milk yield in response to graded duodenal infusions of glucose was similar to that reported by Hurtaud et al. (2000) using identical experimental conditions (i.e., isoenergetic and isonitrogenous feeding with protein in excess). Differences in milk yield observed in these experiments were probably due to genetic differences between cows. However, when data were corrected for experiment effects other than the amounts of glucose infused, there was a curvilinear dose-response for milk yield (Figure 3). The decrease of milk yield obtained when the greatest

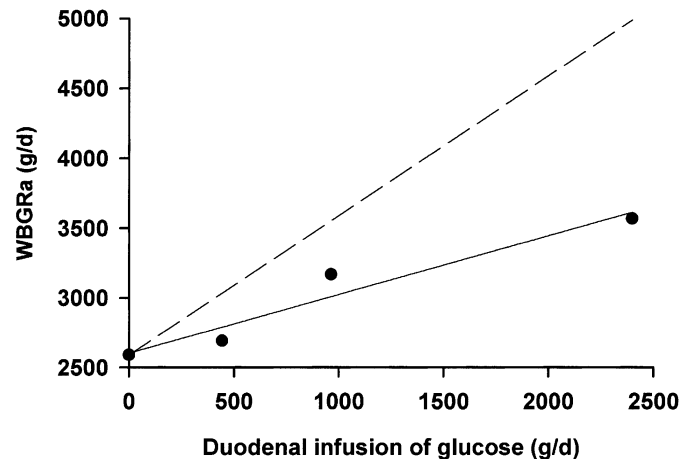


Figure 2. Effect of duodenal infusions of glucose on whole body rate of glucose appearance (WBGRa). Each point represents least squares means of WBGRa for each treatment. Regression equation: WBGRa (g/d) = 0.42 × [grams of glucose infused per day] + 2604 (R² = 0.94; SD = 191; P < 0.001). The dotted line for which the equation is: WBGRa (g/d) = [grams of glucose infused per day] + 2591, represented the theoretical total WBGRa increase, i.e., endogenously produced glucose (2591 g/d) plus the amount of glucose infused.

Table 4. Effect of duodenal infusions of glucose on arterial concentration of glucose and lactate and utilization of glucose by the udder.

	Glucose ¹				RMSE ²	Effect			
	G0	G1	G2	G3		Linear	Quadratic	Cubic	
								<i>P</i>	
Lactate									
Arterial concentrations, mg/100 ml	3.32	2.32	2.19	2.61	0.59	0.448	0.045	0.489	
Glucose									
Arterial concentrations, mg/100 ml	59.1	64.8	65.9	69.3	2.81	0.006	0.073	0.409	
AV ³ differences, mg/100 ml	14.0	11.9	12.3	9.9	1.31	0.010	0.476	0.281	
Extraction rates, %	24.2	18.1	18.6	14.2	3.24	0.013	0.198	0.267	
Net mammary balance									
g/h ⁴	32.1	34.6	38.6	34.8	1.88	0.267	0.006	0.338	
%WBGRa ⁵	59.6	60.2	58.7	47.6	3.26	0.002	0.148	0.803	
MGR _e ⁶ , g/h	6.8	13.6	10.4	17.9	6.74	0.095	0.808	0.370	
MGR _d ⁷ , g/h	38.9	48.1	49.0	52.7	6.44	0.054	0.169	0.504	
Net Balance/Output, ⁸ g/g	1.45	1.46	1.50	1.63	0.19	0.191	0.877	0.916	

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³Arteriovenous differences.

⁴Expressed for one half udder.

⁵WBGRa represents the percentage of whole body rate of glucose appearance measured which was taken up by the mammary gland, and was calculated from the net mammary balance of glucose estimated over 24 h on the entire udder and from the whole body rate of glucose appearance estimated over 24 h.

⁶Mammary gland release of glucose.

⁷Mammary gland rate of glucose disappearance.

⁸Calculated from the net mammary balance of glucose estimated over 12 h on one half udder and from the corresponding milk lactose yield.

amount of glucose was infused (treatment G3), confirmed data of Hurtaud et al. (2000). However, in this experiment, the decrease was less important, likely because of the greatest amount of glucose infused (expressed as percentage of DMI) was smaller than in our study (12 vs. 14%).

Duodenal Infusions of Glucose Increased Whole Body Rate of Glucose Appearance

The parallel increase of WBGRa and milk yield with increasing glucose loads between G0 and G2 confirms that endogenous production of glucose was insufficient

to provide enough glucose for a high rate of milk synthesis. Essentially composed of grass silage, the basal diet provided almost no postruminal glucose (Van Vuuren et al., 1999), i.e., WBGRa for treatment G0 represented the endogenously produced amount of glucose, supplied mainly by gluconeogenesis. For cows producing 25.5 kg/d of milk, an endogenous production of glucose of 2.59 kg/d was required. This quantity was similar to the value calculated by Danfaer et al. (1995) for a cow yielding 25 kg/d of milk.

The WBGRa increased linearly with duodenal infusions of glucose, but at a slower rate than the duodenal supply of glucose (i.e., the slope of the linear response

Table 5. Effect of duodenal infusions of glucose on mammary blood flow (MBF), mammary plasma flow (MPF) and on ratio of mammary blood flow to milk yield (MBF/Milk).

	Glucose ¹				RMSE ²	Effect			
	G0	G1	G2	G3		Linear	Quadratic	Cubic	
								<i>P</i>	
MBF, L/min	5.22	6.33	6.89	7.56	0.552	0.003	0.042	0.711	
MPF, L/min	3.96	4.89	5.26	5.88	0.416	0.002	0.039	0.561	
MBF/Milk, L/kg	653	729	743	956	37.4	<0.001	0.886	0.298	

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

Table 6. Effect of duodenal infusions of glucose on concentrations of milk metabolites of lactose synthesis pathway and their ratios.

	Glucose ¹				RMSE ²	Effect			
	G0	G1	G2	G3		Linear	Quadratic	Cubic	
								<i>P</i>	
Concentration, mmol/L									
Glucose	0.43	0.53	0.57	0.52	0.089	0.408	0.078	0.853	
Glucose-6-phosphate	0.024	0.034	0.033	0.044	0.010	0.079	0.674	0.467	
Glucose-1-phosphate	0.021	0.033	0.031	0.024	0.005	0.573	0.015	0.148	
Ratios, mmol/mmol									
Glucose-6-P/Glucose	0.064	0.069	0.056	0.088	0.017	0.120	0.303	0.441	
Glucose-1-P/Glucose-6-P	0.83	0.89	1.00	0.51 ³	0.229	0.091	0.175	0.739	
Lactose/Glucose-1-P	8823	6173	5024	7509	1733	0.876	0.023	0.809	

¹Duodenal infusions of glucose: G0 = 0% of the DMI; G1 = 2.5% of the DMI; G2 = 5.1% of the DMI; G3 = 14.2% of the DMI.

²Root mean squares error.

³Significantly different from 1 ($P < 0.05$).

was lower than 1; Figure 2). The increment was 42% of the duodenal supply independent of the amounts of glucose infused. Knowlton et al. (1998) obtained an increase of WBGRa equal to 36% of the abomasal supply of starch. The linear increase of WBGRa indicated that the intestinal capacity for glucose absorption was not saturated. Van Vuuren et al. (1999) also demonstrated that the small intestine of a dairy cow could fully digest up to 4 kg/d of starch, suggesting total absorption of glucose in the small intestine. The linear increase of WBGRa was lower than what was infused;

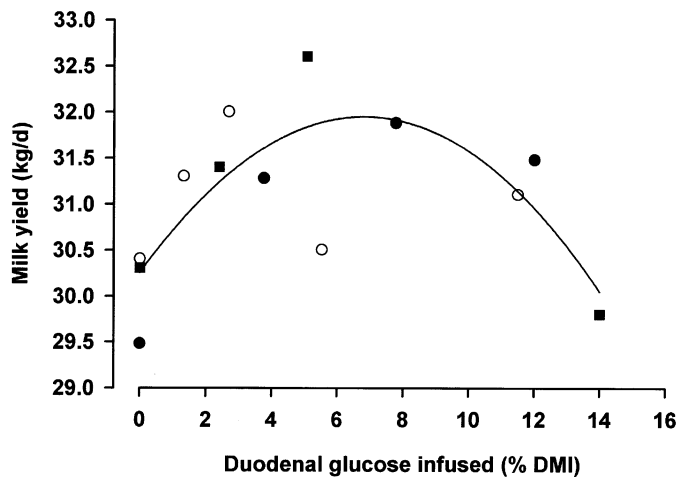


Figure 3. Effect of duodenal glucose infusions on statistically corrected data of milk yield. Data of the present experiment (■) were compared with data from the literature concerning duodenal infusion of glucose substituting a part of the diet based on grass silage (●: Hurtaud et al., 2000, trial 1; ○: Hurtaud et al., 2000, trial 2). Data were analyzed by analysis of covariance with duodenal glucose infused as covariable and corrected for experiment effect. Regression equation: Milk yield (kg/d) = 30.25 + 0.50 × [% DMI of glucose infused per day] - 0.04 × [% DMI of glucose infused per day]² ($R^2 = 0.56$; SD = 0.65; $P < 0.05$).

therefore, this suggests a linear increase in glucose utilization by portal-drained viscera.

According to Huntington (1997), only 55% of starch entering the small intestine reaches the liver. For treatment G3, this would mean that 1319 g/d (55% × 2398 g/d) of glucose was absorbed. If the endogenous production of glucose remained unchanged, the WBGRa would have been 3910 g/d (2591 + 1319 g/d). However, WBGRa was only 3570 g/d. Therefore, in accordance with Huntington (1997), it could be hypothesized that hepatic production of glucose was reduced by 13%: (3910 - 3570 g/d)/2591 g/d.

It has been suggested that when the absorption of glucose from the gut increases, gluconeogenesis generally decreases in ruminants (Huntington, 1997) and even preruminating cattle (Hugi et al., 1998), although the inhibition is not complete (Hostettler-Allen et al., 1994). A decrease of gluconeogenesis proportional to exogenous supply of glucose has been observed in sheep and lactating dairy cows (Clark et al., 1977; Judson and Leng, 1973). This decrease was probably due to an increase in concentrations of insulin that is known to inhibit gluconeogenesis (Judson and Leng, 1973; Hostettler-Allen et al., 1994; Hugi et al., 1998). In the present study, plasma concentrations of insulin, GH and glucagon were not significantly affected by treatments, although insulin concentrations numerically increased. Thus, the overall hormonal profile did not favor hepatic production of glucose, and hence possibly this hormonal arrangement might have slightly reduced hepatic production of glucose.

It is rather unlikely that decreased availability of glucogenic precursors caused a decrease of gluconeogenesis if large amounts of glucose were infused. Main glucogenic precursors are propionate, lactate, and nonessential AA. Feed DMI decreased with treat-

ments, especially with the greatest infusion of glucose. The estimated amount of propionate produced in the rumen remained unchanged for G0, G1, and G2 (17.2 moles/d in average) and decreased to 15.2 moles/d for G3 (calculations of production of propionate according to Rulquin, 1982). The decrease in arterial concentrations of L-lactate may induce a decrease in hepatic production of glucose. However, Baird et al. (1983) reported that only 5% of glucose produced is derived from lactate in well-fed lactating ewes. The diet was formulated to exceed protein requirements (110%), so it can be hypothesized that nonessential AA supply was not limiting for hepatic glucose production. However, glucose infusion could have directly reduced hepatic production of glucose. Indeed, an increase of glycemia at a basal level of insulin already decreased endogenous and total production of glucose in monogastric mammals (Hother-Nielsen et al., 1993). Thus, the numeric variations in the profile of glucogenic hormones and the supply of glucogenic precursors were in favor of a slight reduction of hepatic production of glucose estimated at 13%.

The Increase of Mammary Blood Flow Allowed an Increase of Net Mammary Balance of Glucose

The increase of WBGRa allowed an increase in net mammary balance of glucose, except for the G3 treatment. Despite the increase in arterial concentrations of glucose, the increase in net mammary balance of glucose was mainly due to the increase of MBF, because AV differences of glucose decreased linearly with the graded infusions of glucose. The decrease of AV differences of glucose was in agreement with the increase of MBF, as stated by the law of diffusion (Baron et al., 1994). However, changes of MBF were not related to milk yield, because the ratio of MBF to milk yield increased linearly, even when G3 milk yield was reduced.

It is the first time that such a dramatic and consistent increase of MBF is observed with a supply of a single nutrient at physiological amounts. This may have been caused by the elevation of arterial concentrations of IGF-1 (Prosser et al., 1990). The MBF increased up to 45% between G0 and G3, which is in agreement with observations by Mackle et al. (2000) and by Bequette et al. (2001) during 3- or 4-d hyperinsulinemic-euglycemic clamps. Based on McGuire et al. (1995) and Bequette et al. (2001), large circulating insulin levels can increase plasma IGF-1 levels, which in turn may have caused an increase of MBF. In the current study, the variations in the hormonal profile suggest the important role of IGF-1 in the increase of MBF.

A Mammary Intracellular Imbalance Caused a Decrease in Milk Yield

Milk and lactose yields followed the same trend as the net mammary balance of glucose. Net mammary balance of glucose accounted for 60% of the WBGRa on average from G0 to G2, as previously observed for lactating dairy cows (Annison, 1983). Interestingly, this ratio decreased to 47.6% for the G3 treatment. However, the supply of glucose to the udder was not limiting because WBGRa, MBF, and arterial glucose concentrations continued to increase with G3 treatment. According to Clark et al. (1977), the availability of precursors other than glucose, such as AA, may affect milk production. However, AA were most likely not limiting because, in our experimental design, protein feeding of cows was in excess of requirements (110%) and was supplemented with limiting AA, Lys and Met (Rulquin et al., 1993). The decrease of net mammary balance of glucose could result from a down regulation of glucose uptake. Glucose transporters, especially GLUT-1, are important for glucose entry into mammary epithelial cells, but physiological blood concentrations of glucose should not saturate GLUT-1 (Zhao et al., 1996). Moreover, glucose removal measured by MGRd increased linearly with treatments, indicating a progressive removal from the udder. Nevertheless, more glucose was released from the udder with the G3 treatment compared with the other treatments, because the MGRe tended to increase especially with this treatment. In addition, the mammary gland removed greater amounts of glucose than needed for lactose synthesis. Thus, the ratio of glucose net mammary balance to lactose output was not significantly enhanced during G3. The above remarks indicate that the decrease of milk yield may be the result of an imbalance in intracellular intermediate concentrations. In this respect, the analysis of metabolites of lactose synthesis in milk is interesting because their concentrations reflect their Golgi-vesicular and cytosolic levels (Faulkner, 1980). When glucose concentrations in milk reached a plateau, concentrations of glucose-6-P tended to increase linearly with treatments simultaneously, whereas concentrations of glucose-1-P decreased during G3. The ratio of glucose-1-P to glucose-6-P also decreased with this treatment, suggesting the possibility of a blocking in the conversion of glucose-6-P in glucose-1-P, which consequently would negatively affect lactose production, hence milk production. This blocking could be due to the decrease of P-glucose mutase activity, as observed by Opstvedt et al. (1967) with all-concentrate diets.

CONCLUSIONS

With the grass silage-based diet fed in this study, duodenal infusions of glucose improved milk synthesis quadratically. Exogenous supply of glucose increased WBGra and MBF, therefore more glucose was available for mammary removal and lactose synthesis. However, if in excess, glucose supply may have negative effects on milk production, as it may inhibit lactose synthesis by reducing the glucose-1-P synthesis.

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