Effects of energy source in milk replacer on glucose metabolism of neonatal dairy calves

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ABSTRACT

Milk replacers (MR) formulated to contain more lactose than whole milk could potentially reduce insulin sensitivity in dairy calves. This study evaluated how partially replacing lactose in MR with fat on a weight/weight basis affects glucose–insulin kinetics in neonatal dairy calves fed high quantities of MR. Thirty-four Holstein bull calves were blocked by dam parity (1.6 ± 0.3) and randomly assigned to 1 of 2 treatments ($n = 17$): a high-lactose MR (HL; 46.1% lactose, 18.0% crude fat, and 23.9% crude protein on a dry matter basis) or a high fat MR (HF; 39.9% lactose, 24.6% crude fat, and 24.0% crude protein on a dry matter basis). Calves were individually housed and fed pooled colostrum at 1.5 h and 12 h postnatal at 18 and 9% of metabolic body weight ($BW^{0.75}$), respectively. From 24 h postnatal until the end of the study (d 7), calves were transitioned to MR (prepared at 150 g/L) at 18% of $BW^{0.75}$ twice daily at 0700 and 1900 h. On d 4, an insulin-modified intravenous glucose tolerance test was conducted 8 h after the morning meal by intravenous infusion of glucose (0.54 g/kg of $BW^{0.75}$, min 0) and insulin (3.9 μ g/kg of $BW^{0.75}$, min 20). During postprandial sampling on d 6, abomasal emptying was assessed by dosing acetaminophen (0.13 g/kg of $BW^{0.75}$) with MR and measuring its appearance within plasma. Sequential blood samples were collected and analyzed for plasma glucose, insulin, and acetaminophen concentrations. Calves were killed on d 7 and liver and pancreatic tissues were collected for histomorphological analysis. Partially replacing lactose in MR with fat increased body weight gain (679.6 vs. 462.0 ± 69.68 g/d) and gain:metabolizable energy

intake (186.6 vs. 120.5 ± 20.21 g of body weight/Mcal) compared with feeding HL MR. Furthermore, abomasal emptying rate was reduced in calves fed HF MR. Calculated variables based on postprandial glucose and insulin concentrations, such as maximum concentration or area under the curve, were or tended to be lower in HF than in HL calves. Postprandial insulin sensitivity tended to be reduced in HL compared with HF, although insulin sensitivity estimated from the insulin-modified intravenous glucose tolerance test was not different. Calves fed HF MR showed enhanced glucose-stimulated insulin secretion. In conclusion, partially replacing lactose in MR with fat resulted in smaller fluctuations in postprandial glucose and insulin concentrations and tended to increase postprandial but not fasting insulin sensitivity in neonatal dairy calves.

Key words: lactose, fat, body composition, glucose homeostasis, insulin sensitivity

INTRODUCTION

Approximately 50% of all calves on US dairy farms receive milk replacer (MR) or a combination of MR and whole milk (Urie et al., 2018). Recently, calf research has focused on feeding elevated planes of milk or MR (>8 vs. 4 L/d; MacPherson et al., 2016) to improve preweaning growth performance (Khan et al., 2011); however, this practice is often implemented without considering the dietary macronutrient composition. Relative to whole milk, MR generally contains more lactose (approximately 43 vs. 38% on a DM basis) and less fat (21 vs. 28% on a DM basis; Lee et al., 2009; Pantophlet et al., 2016b; Amado et al., 2019). This is a consequence of economic restrictions that drive formulations with higher levels of protein and fat (Hof, 1980), the use of lactose-rich by-products from cheese manufacturing, and the attempt to minimize body fat deposition in growing Holstein heifers (Tikofsky et al., 2001). High lactose consumption may negatively

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affect glucose homeostasis, resulting in hyperglycemia, hyperinsulinemia, and glucosuria (Vicari et al., 2008; Pantophlet et al., 2016a). Eventually, insulin resistance may develop, as observed in milk-fed calves that received a 42% versus 29% lactose MR from wk 5 to 16 of age (Hugi et al., 1997). However, it is unclear whether the development of insulin resistance begins as early as the neonatal phase when high-lactose MR feeding commences.

Elevated feeding rates combined with a high-lactose MR will likely exacerbate any negative metabolic effects of excessive lactose consumption. A previous study by MacPherson et al. (2016) demonstrated the feasibility of feeding 8 L as opposed to 4 L of high-lactose (45% lactose, 18% crude fat, and 24% CP) MR in 2 meals per day from wk 1 to 8 of life, resulting in negligible effects on insulin sensitivity based on data collected during an intravenous glucose tolerance test. In an attempt to reduce lactose content of MR, Pantophlet et al. (2016b) investigated partial replacement of lactose with glucose, fructose, or glycerol. In response to replacing one third of dietary lactose with glucose, fructose, or glycerol, insulin sensitivity in 97-d-old Holstein-Friesian bull calves was unaffected when >10 L of MR (47.8% lactose, 20.9% crude fat, and 20.5% CP on a DM basis) per day was fed for 8 wk (Pantophlet et al., 2016b). The authors ascribe the lack of differences to the low insulin sensitivity at the start of the study and suggest that it is necessary to test the effects of a high-lactose MR in younger calves, as calves may be more sensitive to insulin at this time. Interestingly, postprandial plasma glucose and insulin concentrations and urinary excretion of glucose were increased in control (only lactose) and glucose calves compared with fructose and glycerol calves (Pantophlet et al., 2016b). The increase in urinary excretion of glucose suggests an imbalance in glucose homeostasis and an inefficient use of the nutrients consumed by the calf. Hence, it is relevant to understand the metabolic implications of feeding a high-lactose MR to calves early in life.

Previous studies evaluated how MR composition influences insulin sensitivity after the first week of life. In the current study, calves were enrolled immediately at birth to focus on the first week of life, during which calves transition from colostrum to milk and undergo major metabolic adaptations. We hypothesized that the excess available glucose from high-lactose MR will decrease insulin sensitivity and that a high-lactose MR will result in faster abomasal emptying. Our objective was to evaluate how partially replacing lactose in MR formulations with fat affects glucose–insulin kinetics during an insulin-modified intravenous glucose tolerance test (**IM-IVGTT**) and postprandial sampling in calves during their first week of life.

MATERIALS AND METHODS

The study was conducted at the Dairy Research and Technology Centre of the University of Alberta (Edmonton, AB, Canada) from September 2017 to April 2018. Animal procedures complied with the guidelines of the Canadian Council on Animal Care (CCAC, 1993) and were approved by the Animal Care and Use Committee of the University of Alberta (AUP00002418).

Animals, Housing, and Treatments

Thirty-four Holstein bull calves with an average birth BW of 43.2 ± 0.81 kg were separated from the dam approximately 15 min after birth, weighed using an electronic scale (Digi-Star SW300, Digi-Star LLC, Fort Atkinson, WI), and moved to a pen (122×183 cm; width $\times$ length) that was bedded with shavings and straw. Calves were dried and stimulated with clean towels for approximately 10 min. At 1.5 and 12 h after birth, calves were bottle-fed pooled fresh colostrum (2.8% lactose, 4.5% crude fat, and 12.6% CP; 63.9 g of IgG/L; Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada) at 18.1 and 9.1% of their metabolic BW ($BW^{0.75}$), respectively. Before feeding, colostrum was thawed and warmed to a temperature of 39°C using a water bath. If more than 25% of a colostrum meal remained, the remainder was drenched using an esophageal tube feeder. At 24 h after birth, blood was sampled via jugular venipuncture using serum vacutainers (Becton, Dickinson and Co., Franklin Lakes, NJ) and centrifuged at $3,000 \times g$ at 4°C for 20 min. Supernatant was transferred into 1.5-mL microcentrifuge tubes and frozen at -20°C until analysis of serum IgG by radial immunodiffusion analysis (Saskatoon Colostrum Company quality assurance laboratory, Saskatoon, SK, Canada).

In a randomized complete block design, calves were blocked by dam parity and randomly assigned to 1 of 2 treatments ($n = 17$): a high-lactose MR (**HL**; 46.1% lactose, 18.0% crude fat, and 23.9% CP on a DM basis) or a high-fat MR (**HF**; 39.9% lactose, 24.6% crude fat, and 24.0% CP on a DM basis; Sloten B.V., Trouw Nutrition, Deventer, the Netherlands). Ingredients and nutrient composition of the MR (analyzed by Masterlab, Trouw Nutrition, Boxmeer, the Netherlands) are presented in Table 1. The MR was prepared at 150 g/L and bottle-fed at 18.1% of $BW^{0.75}$. The assigned MR was fed 24 h after birth; thereafter, calves were adjusted to being fed twice daily at 0700 and 1900 h. On d 4, calves were reweighed before the morning meal and meal size was adjusted accordingly. Intake and refusal of MR was recorded for each meal. At all times, fresh water was available ad libitum from plastic buckets and

Table 1. Ingredients and nutrient composition of the milk replacers¹ used

Variable	High lactose	High fat
Ingredient (%)		
Skim milk powder	29.5	29.5
Palm and coconut oil mixture (2:1)	16.7	22.6
Whey products	48.4	42.1
Hydrolyzed wheat protein	4.0	4.1
Premix and others	1.5	1.7
Nutrient (% of DM unless otherwise noted)		
DM (%)	97.5	97.5
Lactose	46.1	39.9
Crude fat	18.0	24.6
CP	23.9	24.0
Crude ash	7.9	7.7
ME (Mcal/kg of DM)	4.23	4.49

¹Sloten B.V., Trouw Nutrition (Deventer, the Netherlands).

intake was recorded daily. Calves were exposed to day-light and artificial light from 0600 to 1930 h.

IM-IVGTT

On d 4, a catheter (16 G × 2 in., Terumo IV Catheter Surfash, Tokyo, Japan) was inserted into the jugular vein 2 h after the morning meal and kept in place for 3 d. Heparinized saline (20 IU/mL; Fresenius Kabi, Bad Homburg, Germany) was used to flush the catheter line. On d 4, an IM-IVGTT was conducted 8 h after the morning meal. Glucose was dosed at 0.54 g/kg of BW^{0.75} by infusing a 50% dextrose solution (DMVét, Western Drug Distribution Center, Edmonton, AB, Canada) via the jugular catheter over 60 s, followed 20 min later by an infusion of bovine insulin (Hybri-Max, Millipore Sigma, Oakville, ON, Canada) at 3.9 µg/kg of BW^{0.75}. Sequential blood samples were collected before and after glucose infusion in a lithium heparin Vacutainer (Becton, Dickinson and Co.). Immediately after sampling, a competitive serine protease inhibitor (Aprotonin, Sigma-A1153, Millipore-Sigma) was added to blood at 5 µg/mL. Blood was centrifuged at 3,000 × g at 4°C for 20 min and supernatant was transferred into 1.5-mL microcentrifuge tubes and frozen at −20°C until analysis of glucose and insulin concentrations.

Postprandial Abomasal Emptying and Glucose and Insulin Concentrations

Postprandial abomasal emptying and plasma glucose and insulin curves were determined on d 6. Acetaminophen was added to the morning MR meal at 0.13 g/kg of BW^{0.75} and its appearance in plasma was used as an indicator of abomasal emptying (Schaer et al., 2005; MacPherson et al., 2016). Plasma was analyzed for concentrations of acetaminophen [Paracetamol (ac-

etaminophen) Assay Kit-K8002; Cambridge Life Sciences Ltd., Cambridgeshire, UK] and insulin (Mercodia Bovine Insulin ELISA; Mercodia, Uppsala, Sweden) as described by MacPherson et al. (2016). Plasma glucose was determined using an enzymatic assay with peroxidase glucose oxidase and dianisidine dihydrochloride (Sigma-Aldrich, St. Louis, MO). Inter- and intra-assay coefficients of variation for plasma acetaminophen, insulin, and glucose were 0.9 and 1.3%, 4.7 and 4.6%, and 3.3 and 0.9%, respectively.

Postmortem Analysis

Calves were killed 3 h after the morning meal on d 7 to collect the liver and pancreas. Pentobarbital (Euthanyl, Vetoquinol, Lavaltrie, QC, Canada) was infused intravenously at 0.125 mL/kg of BW to achieve a surgical plane of anesthesia, after which the calf was exsanguinated. The pancreas and liver were weighed, and subsamples collected from the tail of the pancreas and the center of the right lobe of the liver were fixed for a minimum of 24 h in 10% buffered formalin solution (4% formaldehyde; Fisher Scientific, Hampton, NH) and snap frozen in liquid nitrogen, respectively. Then, the carcass was split at the spine and the right carcass half was stored at −20°C until analysis. The fixed pancreatic tissue was processed (VIP 6, Somagen, Edmonton, AB, Canada) and embedded in paraffin (Tissue Tek, Sakura, Torrance, CA), and, subsequently, sections of 5 µm were processed onto glass slides (2 sections per slide; Leica RM2255, Leica Biosystems, Buffalo Grove, IL) and stained with a hematoxylin and eosin stain (Leica ST5020, Leica Biosystems) by the Animal Health Laboratory histotechnology laboratory (University of Guelph, Guelph, ON, Canada). Images were captured using a Leica ICC50W microscope at 100× magnification (Leica Microsystems, Wetzlar, Germany). Measurements were performed using ImageJ 1.46r software (National Institutes of Health, Bethesda, MD). The size and cell count of 10 islets of Langerhans (approximately 4 images) per calf were determined to evaluate pancreatic endocrine function. Liver glycogen content was determined using a fluorometric assay (glycogen assay kit, Cayman Chemical, Ann Arbor, MI; Mann et al., 2018). Approximately 125 mg of frozen tissue was homogenized in 1 mL of assay buffer and diluted 300-fold using the assay buffer. Fluorescence was measured using a Cytation5 plate reader (Biotek, Winooski, VT) with 530 nm excitation and 585 nm emission wavelengths. The inter- and intra-assay coefficients of variation were 3.51 and 2.60%, respectively.

The body composition of the half-carcass was estimated using dual energy X-ray absorptiometry (DXA) as described by López-Campos et al. (2017). The right

half-carass was thawed and scanned with a Lunar iDXA unit (Lunar Prodigy Advance, General Electric, Madison, WI; Agriculture and Agri-Food Canada, Lacombe Research and Development Centre, Lacombe, AB, Canada) using the “small animal” option on large mode (>20 kg; ~7 min per whole DXA table scan). Based on the different attenuation of low- and high-energy X-rays, the fat, lean, and bone tissue mass were estimated. The DXA unit was calibrated using the automated 6-point calibration and quality assurance system of Lunar iDXA twice daily to ensure there was no variation within and between experimental batches.

Calculations and Statistics

Feed efficiency was calculated as gram of BW gained per gram of DMI or MJ of ME intake. The area under the curve (AUC) for glucose and insulin was calculated using the trapezoid rule (Le Floch et al., 1990). For the IM-IVGTT, the positive (above baseline) AUC was calculated before the insulin infusion (0–20 min) and after insulin infusion (20–240 min). For the postprandial measurements, the total AUC was calculated over 600 min.

From the sequential glucose and insulin measurements during the IM-IVGTT, 2 indices of insulin sensitivity were estimated for the period before and after insulin infusion. According to the simplified model of Pantophlet et al. (2016c),

$$\text{Insulin sensitivity} = K_g / (\text{AUC}_{\text{ins}} / T), \quad [1]$$

where K_g (glucose disappearance rate constant; min^{-1}) was estimated by converting the glucose concentration at each time point to its natural logarithm and determining the slope in Microsoft Excel (Microsoft Corp., Redmond, WA); K_g was determined before (4–20 min) and after (24–50 min) the insulin infusion. The positive AUC for insulin (AUC_{ins}) was calculated before (0–20 min) and after (18–50 min) insulin infusion. Second, the quantitative insulin sensitivity check index (QUICKI) was calculated with the following equation (Katz et al. (2000):

$$\begin{aligned} \text{QUICKI} = & [1 / \log \text{baseline glucose (mg/dL)}] \\ & + \log \text{baseline insulin (mU/L)}. \end{aligned} \quad [2]$$

To assess treatment effects on abomasal emptying and postprandial glucose–insulin kinetics, we applied a mathematical model that allows for estimation of pancreatic responsiveness, whole-body insulin sensitivity, and glucose effectiveness (Stahel et al., 2016). Briefly,

the model contains pools for abomasal acetaminophen and glucose and plasma acetaminophen, glucose, and insulin. Differential equations that describe the inputs and outputs of each pool were solved with a fourth-order Runge-Kutta algorithm in acslX (Aegis Technologies Group Inc., Huntsville, AL). Acetaminophen parameters (slow emptying rate, fast emptying rate, and utilization rate) were fit by analytically integrating the differential equation for plasma acetaminophen in Microsoft Excel and estimated for each calf using Excel Solver to minimize residual sum of squares between observed and predicted plasma acetaminophen. The parameters of glucose–insulin kinetics (endogenous synthesis, glucose effectiveness, insulin sensitivity, absorption lag time, maximum secretion, pancreatic responsiveness, glucose-dependent secretion, basal insulin mass, signaling lag time, and insulin utilization) were estimated with a differential evolution algorithm (Storn and Price, 1997) applied to the numerical simulation in acslX to minimize residual sum of squares for plasma glucose and insulin concentrations. Root mean squared prediction error as a percentage of the observed mean was calculated to indicate goodness of curve fits.

All observations were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). We adjusted the response distribution in the model statement to lognormal when it improved the fit of the model according to the distribution of studentized residuals and Shapiro-Wilk statistic. For data consisting of proportions, a β distribution was used. The model included the fixed effect of treatment and random effect of block. The experimental unit was the calf. For glucose and insulin concentrations during the IM-IVGTT and postprandial on d 6, time and treatment $\times$ time interaction were included and the covariance structure used was compound symmetry based on the lowest Akaike information criterion. Means were separated using the PDIF statement of SAS. One HF calf missed a meal the evening before postprandial sampling; therefore, data points were removed from analysis if considered an outlier (studentized residual < -3.4 or > 3.4). All values reported are least squares means, with significance declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Intake, Passive Transfer of Immunity, and Growth

Passive transfer of immunity was balanced across treatment groups based on serum IgG concentration at 24 h after birth (24.1 and 25.1 ± 0.73 mg/mL for HL and HF, respectively; $P = 0.370$; data not shown). Dry

Table 2. Least squares means of milk replacer intake, BW, and body composition of neonatal dairy calves fed a high-lactose or high-fat milk replacer until 7 d of age

Variable	Milk replacer ¹		SEM	P-value
	High lactose	High fat		
Total milk replacer intake until d 7				
kg of DM	4.4	4.5	0.01	0.283
Mcal of ME	19.2	20.9	0.42	0.001
BW (kg)				
At birth	44.3	42.3	1.12	0.208
At d 7	46.8	46.2	1.11	0.724
ADG (g/d)	402.0	679.6	69.68	0.008
Gain:feed (g of BW/kg)	521.7	860.3	89.50	0.010
Gain:ME intake (g of BW/Mcal)	120.5	186.6	20.21	0.022
Body composition ² (%)				
Fat tissue	24.8	26.2	0.78	0.266
Lean tissue	70.6	69.3	0.82	0.248
Bone tissue	4.6	4.5	0.14	0.754

¹Sloten B.V., Trouw Nutrition (Deventer, the Netherlands). High lactose = 46.1% lactose and 18.0% fat on DM basis; high fat = 39.9% lactose and 24.6% fat on a DM basis.

²Body composition was estimated using dual energy X-ray absorptiometry.

matter intake did not differ ($P = 0.283$) between treatment groups; however, by design, ME intake was 9% higher ($P = 0.001$) for HF compared with HL until d 7 (Table 2). During the 7-d study, ADG, gain:feed, and gain:ME intake were approximately 1.5-fold greater ($P = 0.008$, $P = 0.010$, and $P = 0.022$, respectively) for HF compared with HL. Body composition was not significantly different for fat, lean, and bone tissue ($P = 0.266$, $P = 0.248$, and $P = 0.754$, respectively).

Glucose and Insulin Responses During IM-IVGTT

As shown in Table 3, glucose baseline concentration tended to be greater for HL compared with HF (7.46 vs. 7.02 mmol/L; $P = 0.091$), whereas baseline insulin concentration did not differ ($P = 0.451$) between groups. Before the insulin infusion, time of maximum concentration (**Tmax**) for insulin tended ($P = 0.086$) to be earlier for HF calves than for HL calves. Furthermore, the AUC for insulin was greater ($P = 0.036$) in HF compared with HL calves. After the insulin infusion, the starting concentration of glucose (at 18 min) tended to be greater ($P = 0.061$) and the maximum change from baseline (delta) glucose concentration was greater (3.77 vs. 3.28 ± 0.166 mmol/L; $P = 0.035$) for HL compared with HF. No differences were found for any of the other variables calculated. As indicated in Figure 1, insulin and glucose concentrations during the IM-IVGTT were unaffected by MR treatment, although a treatment $\times$ time interaction ($P < 0.001$) was found for insulin concentration during the first 20 min of the test.

Postprandial Glucose, Insulin, and Acetaminophen Responses

A treatment $\times$ time interaction ($P = 0.003$, Figure 2) was observed for postprandial concentrations of acetaminophen. Abomasal emptying rate was lower in calves fed the HF MR ($P = 0.039$, Table 4) compared with calves fed HL. A tendency for an interaction of treatment $\times$ time ($P = 0.065$, Figure 2) was observed for postprandial concentrations of glucose. Similarly, a treatment $\times$ time interaction was observed ($P = 0.005$, Figure 2) for postprandial plasma insulin concentrations. In addition, as shown in Table 5, the baseline, maximum plasma concentration (**Cmax**), and AUC for plasma glucose were or tended to be lower by 5% ($P = 0.046$), 9% ($P = 0.031$), and 7% ($P = 0.071$), respectively, in HF compared with HL. The HF treatment decreased Cmax, delta, Cmax:Tmax, and AUC of plasma insulin by 32% ($P = 0.032$), 37% ($P = 0.032$), 37% ($P = 0.035$), and 25% ($P = 0.022$), respectively, compared with HL. No differences were found for other variables calculated.

Pancreas and Liver

Pancreas weight did not differ ($P = 0.353$) between treatment groups (Table 6). Furthermore, the number of cells in and size of the islets of Langerhans were similar ($P = 0.572$ and $P = 0.638$, respectively) for both treatment groups. However, the liver was 10% heavier (1,378 vs. 1,239 g; $P = 0.002$) and contained 50% more glycogen (26.5 vs. 17.7 mg/g; $P = 0.002$) in HL compared with HF calves.

DISCUSSION

In recent years, there has been a shift toward feeding dairy calves more milk to more closely meet their nutrient requirements for their biological potential for BW gain (>8 vs. 4 L/d or >1,000 vs. 500 g of MR/d; MacPherson et al., 2016). However, the macronutrient composition of the formulation should also be considered. In the current study, lactose was replaced with fat on a weight/weight basis, resulting in a higher ME content of the HF compared with the HL MR (4.49 vs. 4.23 Mcal/kg of DM). Neither meal size nor amount of solids were adjusted to achieve isocaloric intakes. This approach allows for studying responses to different nutrient profiles of the solids fraction of the milk supplied to calves. In essence, the differences in solids composition between the most commonly available MR formulations (high lactose and low fat) and whole milk are contrasted. Additionally, this approach prevents effects of meal volume on postprandial variables, such

as abomasal emptying (MacPherson et al., 2016). On that note, previous work shows that partially replacing lactose in MR with fat can reduce DMI from MR when fed ad libitum, potentially as a means to regulate ME intake (Echeverry-Munera et al., 2020). The calves in the current study were restricted to ensure similar DMI in both treatment groups. Therefore, despite a similar total DMI for both treatment groups, total ME intake was, by study design, greater for HF compared with HL. A greater ME intake results in higher growth rates (Khan et al., 2011), as reflected by the 60% increase in growth over the 7-d period for HF compared with HL calves (3.90 vs. 2.44 kg, respectively). Calculating the growth per megacalorie intake (gain:ME intake) shows a 55% increase in feed efficiency for HF compared with HL. Tikofsky et al. (2001) indicated the importance of isocaloric and isonitrogenous intakes when evaluating effects of macronutrient composition on growth. Because the MR in the current study were isonitrogenous but not isocaloric, the HF calves may have had

Table 3. Least squares means of glucose and insulin responses measured during an insulin-modified intravenous glucose tolerance test on d 4 of life in neonatal dairy calves fed a high-lactose or high-fat milk replacer

Variable ¹	Milk replacer ²		SEM	<i>P</i> -value
	High lactose	High fat		
Before insulin infusion				
Glucose				
Baseline (mmol/L)	7.46	7.02	0.177	0.091
Cmax ³ (mmol/L)	12.00	11.96	0.477	0.967
Delta ³ (mmol/L)	4.42	4.76	0.390	0.628
AUC _{0-20 min} ³ (mmol/L × min)	41.34	42.64	2.075	0.677
Disappearance rate (min ⁻¹)	0.0010	0.0079	0.00104	0.179
Insulin				
Baseline (μg/L)	0.24	0.28	0.037	0.451
Cmax ³ (μg/L)	0.70	0.95	0.108	0.149
Tmax (min)	14.24	11.88	0.886	0.086
Delta ³ (μg/L)	0.45	0.67	0.088	0.103
AUC _{0-20 min} ³ (μg/L × min)	3.90	6.91	0.967	0.036
Insulin sensitivity ³ [L/(μg × min)]	0.028	0.030	0.0085	0.883
Insulin sensitivity (QUICKI)	0.37	0.36	0.011	0.448
After insulin infusion				
Glucose				
Starting concentration (mmol/L)	9.33	8.61	0.255	0.061
Cmin ³ (mmol/L)	5.41	5.25	0.187	0.594
Delta ³ (mmol/L)	3.77	3.28	0.166	0.035
AUC _{18-240 min} ³ (mmol/L × min)	18.45	14.73	3.531	0.473
Disappearance rate (min ⁻¹)	0.0113	0.0117	0.00136	0.797
Insulin				
Starting concentration ³ (μg/L)	0.57	0.64	0.088	0.614
Cmax (μg/L)	9.71	9.45	1.035	0.862
Delta ³ (μg/L)	8.99	8.69	1.002	0.834
AUC _{18-240 min} ³ (μg/L × min)	60.99	59.12	5.912	0.829
Insulin sensitivity [L/(μg × min)]	0.0058	0.0064	0.00068	0.543

¹Cmax = maximum plasma concentration; delta = maximum change from baseline; AUC = positive incremental area under the curve; Tmax = time of maximum concentration; QUICKI = quantitative insulin sensitivity check index; Cmin = minimum plasma concentration.

²Sloten B.V., Trouw Nutrition (Deventer, the Netherlands). High lactose = 46.1% lactose and 18.0% fat on DM basis; high fat = 39.9% lactose and 24.6% fat on DM basis.

³Least squares means and SEM are back transformed to the original scale.

more energy available for retention after maintenance requirements were met compared with HL (Tikofsky et al., 2001). However, if more energy became available for retention in calves fed the HF MR, this did not result in statistically detectable differences in body composition; this is in contrast with previous studies (Donnelly, 1983; Tikofsky et al., 2001; Bascom et al., 2007) that found that increasing the fat content of MR increased fat deposition in Holstein-Friesian and Jersey bull calves during the first 2 mo of life. The discrepancies between the current study and previous studies could be due to unexpectedly high variation in the measurements of body composition, resulting in insufficient statistical power to detect a difference in body composition (0.24; POWER procedure using SAS). In addition, in previous studies, treatment diets contained up to 32% fat on a DM basis (compared with 25% of DM in our study) and a slightly higher CP content (27–29% compared with 24% of DM in our study), and calves were fed for a longer time (e.g., for 28–55 d; Donnelly, 1983; Tikofsky et al., 2001; Bascom et al., 2007), potentially allowing greater differences in fat deposition to develop.

Although body composition was unaffected by MR treatment in the current study, altering the macronutrient composition did result in digestive and metabolic consequences. The delayed abomasal emptying in HF was in agreement with what we expected based on the higher caloric density of HF. Osmolality of the MR was not likely a contributing factor because osmolalities did not differ greatly (490 vs. 457 mOsm/kg for HL vs. HF; Advanced Instruments 3300 Micro-Osmometer, Norwood, MA). Similarly, Bell and McLeay (1978) found that duodenal fat infusion resulted in a stronger reduction in abomasal emptying compared with a duodenal lactose infusion in 3-wk-old dairy calves. A potential mechanism involves the hormone cholecystokinin, as ingesting fat stimulates release of cholecystokinin and intravenously infusing cholecystokinin slows abomasal emptying (Bell and McLeay, 1978; Liddle et al., 1985, 1986). A delay in abomasal emptying might be beneficial because it could increase abomasal clotting and, subsequently, delay digestion of protein and fat, allowing for a steady supply of absorbable nutrients (Bell and McLeay, 1978). However, the same MR used in

Figure 1. Plasma glucose and insulin concentrations during an insulin-modified intravenous glucose tolerance test on d 4 of life in calves fed a high-lactose (46.1% lactose and 18.0% fat on a DM basis) or high-fat (39.9% lactose and 24.6% fat on a DM basis) milk replacer. (A) Glucose concentrations before insulin infusion, (B) glucose concentrations during entire test, (C) insulin concentrations before insulin infusion, and (D) insulin concentrations during entire test. Data points are mean $\pm$ SE; * $P < 0.05$, † $P < 0.10$.

the current study were evaluated in dairy bull calves, and at 4 wk of age no differences in apparent total-tract digestibility of DM, CP, crude fat, and crude ash between treatment groups were detected (Amado et al., 2019). Furthermore, the delay in abomasal emptying found in the current study could be beneficial in terms of regulating glucose homeostasis as discussed below.

Dietary glucose intake and the subsequent inflow of dietary glucose into the duodenum, which is regu-

lated by abomasal emptying, play a role in maintaining glucose homeostasis. Therefore, in HL calves, greater quantities of MR containing more lactose entered the small intestine per unit of time, causing a greater increase in postprandial glucose and insulin concentrations in plasma. Moreover, the greater lactose intake in HL increased the supply of energy precursors for storage in the liver in the form of glycogen. Potentially, lactose-derived galactose may have played a major role,

Figure 2. Plasma (A) glucose, (B) insulin, and (C) acetaminophen concentrations during postprandial sampling on d 6 of life in calves fed a high-lactose (46.1% lactose and 18.0% fat on a DM basis) or high-fat (39.9% lactose and 24.6% fat on a DM basis) milk replacer. Data points are mean $\pm$ SE; * $P < 0.05$, † $P < 0.10$.

Table 4. Least squares means of abomasal emptying and glucose–insulin dynamics during postprandial sampling on d 6 of life in neonatal dairy calves fed a high-lactose or high-fat milk replacer¹

Variable ²	Abbreviation	High lactose	SE	High fat	SE	P-value
Acetaminophen						
Slow emptying rate (min ⁻¹)	k _{SP,2}	0.0025	0.00037	0.0015	0.00022	0.039
Fast emptying rate (min ⁻¹)	k _{SP,3}	0.0037	0.00029	0.0030	0.00024	0.117
Utilization rate (min ⁻¹)	k _{Ac,UAc}	0.0034	0.00036	0.0029	0.00031	0.206
Glucose						
Endogenous synthesis (mmol·min ⁻¹)	PGI _{end}	0.0421	0.03038	0.0369	0.02666	0.876
Glucose effectiveness (L·min ⁻¹)	k _{GI,UGI}	0.0022	0.00149	0.0013	0.00089	0.348
Insulin sensitivity (L ⁻² ·µg ⁻¹ ·min ⁻¹)	k _{In,UGI}	0.1159	0.01785	0.1845	0.02841	0.068
Absorption lag time (min ⁻¹)	T _{lag,SP}	35.80	5.937	32.41	5.937	0.591
rMSPE _G (% of mean)		9.8	0.88	10.3	0.88	0.723
Insulin						
Maximum secretion (µg·min ⁻¹)	V _{PIIn}	1,307.85	657.305	1,845.89	927.716	0.678
Glucose-dependent secretion (mM)	K _{GI,PIIn}	8.04	0.863	10.27	1.103	0.251
Pancreatic responsiveness	exp _{PIIn}	16.91	3.835	14.49	3.288	0.654
Basal insulin mass (µg)	iIn	1.61	0.005	1.74	0.005	0.849
Signaling lag time (min ⁻¹)	T _{lag,In}	1.36	0.955	0.90	0.631	0.685
Insulin utilization (L·min ⁻¹)	k _{In,UGI}	28.96	14.815	8.94	4.575	0.150
rMSPE _I (% of mean)		46.5	4.70	48.1	4.70	0.826

¹Sloten B.V., Trouw Nutrition (Deventer, the Netherlands). High lactose = 46.1% lactose and 18.0% fat on DM basis; high fat = 39.9% lactose and 24.6% fat on DM basis.

²Least squares means and SE of all variables are back transformed to the original scale, except for T_{lag,SB}, root mean squared prediction error of glucose (rMSPE_G), iIn and root mean squared prediction error of insulin (rMSPE_I).

as Barosa et al. (2012) found that essentially all milk galactose was converted to hepatic glycogen in healthy human subjects that consumed milk during breakfast.

The higher baseline glucose in HL compared with HF calves on d 6 may imply a greater challenge to maintain glucose homeostasis. However, on average, calves did not experience hyperglycemia, as defined by the renal threshold for glucosuria in Holstein-Friesian

and Simmental × Red Holstein bull calves at blood concentrations of 8.3 to 11.1 mmol/L (Wijayasinghe et al., 1984; Hostettler-Allen et al., 1994). Seven calves (5 HL and 2 HF) experienced plasma glucose concentrations greater than 8.3 mmol/L during postprandial sampling; however, none of the calves exceeded 10.0 mmol/L. Moreover, postprandial baseline and Cmax plasma concentrations of glucose and insulin in the

Table 5. Least squares means of glucose and insulin responses measured during postprandial sampling on d 6 of life in neonatal dairy calves fed a high-lactose or high-fat milk replacer

Variable ¹	Milk replacer ²		SEM	<i>P</i> -value
	High lactose	High fat		
Glucose				
Baseline (mmol/L)	5.89	5.61	0.102	0.046
Cmax (mmol/L)	8.01	7.30	0.210	0.031
Delta (mmol/L)	2.12	1.85	0.199	0.378
Tmax (min)	234.71	254.12	33.112	0.619
Cmax:Tmax ³	0.04	0.03	0.005	0.549
AUC _{0-600 min} (mmol/L × min)	4,027.77	3,744.73	102.790	0.071
Insulin				
Baseline ³ (µg/L)	0.12	0.14	0.025	0.528
Cmax ³ (µg/L)	2.48	1.69	0.219	0.032
Delta (µg/L)	2.57	1.62	0.271	0.032
Tmax (min)	134.12	162.35	15.666	0.135
Cmax:Tmax	0.019	0.012	0.002	0.035
AUC _{0-600 min} (ug/L × min)	491.79	368.07	31.201	0.022
Glucose AUC ₀₋₆₀₀ :insulin AUC ₀₋₆₀₀ ³	8.52	10.62	0.750	0.115

¹Cmax = maximum plasma concentration; delta = maximum change from baseline; Tmax = time of maximum concentration; AUC₀₋₆₀₀ = area under the curve from 0 to 600 min relative to meal.

²Sloten B.V., Trouw Nutrition (Deventer, the Netherlands). High lactose = 46.1% lactose and 18.0% fat on DM basis; high fat = 39.9% lactose and 24.6% fat on DM basis.

³Least squares means and SEM are back transformed to the original scale.

Table 6. Least squares means of pancreas and liver variables in 7-d-old dairy calves fed a high-lactose or high-fat milk replacer

Variable	Milk replacer ¹		SEM	P-value
	High lactose	High fat		
Pancreas weight ² (g)	29.16	27.96	1.706	0.510
Islet of Langerhans size (μm^2)	7,067.38	6,548.86	658.245	0.572
Islet of Langerhans cell count	4.35	4.30	0.094	0.638
Liver weight (g)	1,377.82	1,239.24	34.529	0.002
Liver glycogen (mg/g of wet tissue)	26.50	17.69	2.041	0.002

¹Sloten B.V., Trouw Nutrition (Deventer, the Netherlands). High lactose = 46.1% lactose and 18.0% fat on DM basis; high fat = 39.9% lactose and 24.6% fat on DM basis.

²Least squares means and SEM are back transformed to the original scale.

current study were similar to those in previous studies conducted with neonatal dairy calves fed ≥ 8 L/d of MR containing 45% lactose on a DM basis (MacPherson et al., 2016; Pantophlet et al., 2016b). Therefore, the differences between the treatment groups can be considered minor as the glucose and insulin concentrations return to normal baseline levels.

In the current study, an IM-IVGTT was performed to evaluate pancreatic responsiveness and insulin sensitivity in response to altering macronutrient composition of MR. The pancreatic response (i.e., the quantity of insulin secreted in response to a glucose stimulus) was evaluated from plasma insulin concentrations during the first 20 min of the test. Then, insulin was infused to suppress hepatic glucose synthesis and improve estimation of insulin sensitivity from glucose clearance out of plasma (Yang et al., 1987). The pancreatic response was increased in HF compared with HL (greater positive AUC for plasma insulin after intravenous glucose and before insulin injection), although this did not alter the size and cell count of islets of Langerhans. The effect of dietary fat directly on pancreatic β -cells might have caused this response, as demonstrated in rats fed a high-fat diet compared with a low-fat diet (22 or 40% vs. 4%; Holness, 1996; Dobbins et al., 2002). A potential mechanism behind this response could be pancreatic exposure to elevated free fatty acids that increase de novo synthesis of islet phospholipids involved in insulin release (Vara and Tamarit-Rodriguez, 1986; Vara et al., 1988).

Postprandial insulin sensitivity tended to be reduced and the ratio of glucose AUC:insulin AUC over 600 min was numerically lower in HL compared with HF. This suggests that calves fed HL tended to require more insulin per unit of dietary glucose in response to their meal to reduce plasma glucose concentrations compared with HF. Interestingly, feeding the same HL (44% of DM) or HF (23% of DM) MR during the first 4 wk of life did not affect postprandial insulin sensitivity at 30 d of age (Stahel et al., 2019), potentially due to

the ability to adapt to an HL diet (Hof, 1980). Therefore, this study is highly unique as it evaluates animals during their first week of life, a phase in which intake, animal growth, and metabolism can be highly variable. However, our data from the IM-IVGTT indicate that the macronutrient compositions of the MR used in the current study did not affect fasting insulin sensitivity. This finding agrees with Pantophlet et al. (2016a), who demonstrated that isocaloric replacement of lactose in MR with fat (34% vs. 59% lactose on a DM basis) did not alter fasting insulin sensitivity in 99-d-old Holstein-Friesian bull calves when fed for 13 wk at $>1,000$ g of MR/d. The authors ascribed the lack of difference to the low insulin sensitivity due to age in their calves compared with younger ones. Potentially, in our study the difference in macronutrient composition of the MR may not have been broad enough (46% vs. 40% lactose on a DM basis) or the exposure not long enough to elicit an effect on fasting insulin sensitivity. The discrepancy between insulin sensitivity estimated from the IM-IVGTT and postprandial sampling is due to the acute influence of fats, proteins, and other constituents in the MR on enzyme and signaling activities in tissues, including the gastrointestinal tract, and therefore, the postprandial test better represents the physiological challenge calves face after consuming a meal (Berthiaume and Zinker, 2002).

CONCLUSIONS

The results of this study demonstrate that partially replacing lactose in MR formulation with fat increased BW gain as well as gain:ME intake in neonatal dairy calves during the first week of life. Furthermore, feeding a high-fat MR slowed abomasal emptying and reduced postprandial glucose and insulin concentrations. Postprandial insulin sensitivity tended to be reduced in HL compared with HF. However, there was no evidence of hyperglycemia in the calves fed the high-lactose MR, and the glucose and insulin concentrations returned to

baseline concentrations. Additionally, fasting insulin sensitivity, as determined from IM-IVGTT data, was not different between treatments, but HF enhanced glucose-stimulated insulin secretion. Overall, partially replacing lactose with fat to mimic the macronutrient composition of whole milk may benefit growth with minor alterations in glucose homeostasis in neonatal dairy calves.

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