Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily

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ABSTRACT

The objective of this study was to investigate how preweaning plane of milk replacer intake and age can affect insulin and glucose kinetics as well as abomasal emptying rate in dairy calves fed twice a day. A total of 12 female Holstein Friesian calves were blocked by cow parity, paired by colostrum origin, and were randomly assigned to a high plane of milk replacer intake (8 L/d, 1.2 kg of milk replacer/d; $n = 6$) or a low plane of nutrition (4 L/d, 0.6 kg of milk replacer/d; $n = 6$). All calves received 4 L of colostrum over 2 meals (1 and 6 h after birth) and were then directly transferred to their assigned feeding plans until they were stepped-down from milk by 50% during wk 7 and weaned on wk 8. Milk replacer (24% crude protein, 18% crude fat) was fed at 150 g/L twice daily (0700 and 1700 h) and all calves had ad libitum access to pelleted calf starter, chopped wheat straw, and water. Jugular catheters were placed in all calves at 4, 7, and 10 wk of age. Then, postprandial response to plasma glucose, insulin, and acetaminophen (supplied with the meal) were determined to measure abomasal emptying. The next day, a glucose tolerance test was conducted by infusing glucose via the jugular catheter. At 4 and 7 wk of age, the rate constant (%/h) for abomasal emptying of the meal was lower in high calves (0.21 ± 0.02 in wk 4; 0.27 ± 0.02 in wk 7) compared with low (0.34 ± 0.02 in wk 4; 0.47 ± 0.02 in wk 7). The postprandial plasma insulin area under the curve over 420 min was greater in high calves ($18,443 \pm 7,329$; low = $5,834 \pm 739$ $\mu\text{U/mL}$) compared with low. We found no differences in glucose tolerance test kinetics between the high and low dairy calves at 4, 7, or 10 wk of age. The findings from this study suggest that feeding dairy calves an elevated

plane of nutrition in 2 meals of milk replacer per day does not decrease insulin sensitivity.

Key words: preweaning nutrition, insulin, glucose, abomasal emptying

INTRODUCTION

Calf-management programs have traditionally restricted milk or milk replacer (MR) fed to calves (~ 4 L or 10% birth weight) to promote starter consumption, rumen development, and to decrease replacement heifer raising costs (Khan et al., 2011). Current calf research is focused on feeding elevated planes of nutrition to replacement heifers by increasing the volume of milk or MR to approximately double (~ 8 L or 20% birth weight) the amount that traditionally has been fed. Calves raised on these programs have been shown to have increased ADG, earlier onset of puberty, as well as the potential for higher milk production sustained over multiple lactations (Soberon et al., 2012). In addition to improvements in production, feeding elevated planes of nutrition has also been shown to decrease signs of hunger, which may improve animal welfare (Miller-Cushon and DeVries, 2015). These elevated feeding programs are often offered in a twice-a-day feeding plan on commercial dairy farms, which is in contrast to feeding patterns observed when the calf is allowed to suckle from the dam (Jasper and Weary, 2002) or from an automated feeder (Berends et al., 2014), where they are consuming 7 to 10 meals throughout the day. Feeding similar large volumes twice daily may have longstanding effects on digestion and metabolism.

One of the most common concerns when feeding larger volumes of milk to young calves at a low feeding frequency is the decrease of insulin sensitivity (Bach et al., 2013). Despite a generally delicate physiological regulation of glucose homeostasis, veal calves that are provided large milk volumes up to 6 mo of age often express problems characterized by postprandial hyperglycaemia, hyperinsulinaemia, and glucosuria

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(Hostettler-Allen et al., 1994; Hugi et al., 1998; Vicari et al., 2008). Impaired insulin sensitivity could lead to a reduced efficiency of protein and energy utilization (van den Borne et al., 2006) and may predispose calves to metabolic diseases later in life (Kaufhold et al., 2000; Quigley et al., 2006). It has recently been shown that insulin sensitivity is affected by plane of MR intake in dairy calves when calves are fed large meals twice daily during the preweaning period (Bach et al., 2013; Yunta et al., 2015). However, it is unclear to whether this can persist postweaning when calves are transitioned to only solid feed.

Although insulin production in the pancreas and cellular glucose uptake have received most of the attention for mechanisms that control blood glucose, the rate of nutrient delivery to the lower gut after a meal may play a pivotal role in the regulation of blood glucose (Tong and D'Alessio, 2014). In calves, the rate of abomasal emptying may control nutrient delivery to the small intestine (Schaer et al., 2005; Sen et al., 2006; Constable et al., 2009). Meals with high glucose concentration have been shown to delay abomasal emptying when compared with electrolyte solutions, which can lead to slower nutrient delivery (Wittek et al., 2005; Sen et al., 2006). When larger meals are fed, abomasal emptying may be slowed down as a means to decrease the rate of nutrient delivery that can help reduce dramatic increases in blood glucose concentrations (Coradini et al., 2015). Therefore, abomasal emptying may be an important factor affecting plasma glucose levels and insulin action when feeding larger meals; however, the effect of plane of nutrition on abomasal emptying in this scenario has not yet been investigated.

The objective of our study was to investigate the effect of feeding plane of milk replacer, when provided twice daily, on abomasal emptying preweaning, as well as insulin and glucose kinetics preweaning (wk 4 and 7 of age) and postweaning (wk 10 of age). Our hypothesis was that an elevated plane of MR intake preweaning, fed 2 times a day, would decrease insulin sensitivity, but no carryover effects would be observed after weaning. In addition, we expected that larger meal sizes of milk would result in a delayed abomasal emptying rate as an adaptive response to minimize large fluctuations in blood glucose levels.

MATERIALS AND METHODS

Animals

Twelve Holstein Friesian female calves from Trouw Nutrition Ruminant Research facility (Boxmeer, the Netherlands) born between October 2014 and January 2015 were selected for this study. They were housed in

individual hutches (1.07×1.60 m) bedded with wheat straw and outfitted with a metal roof attachment to protect feed and water from precipitation. Procedures complied with the Dutch Law on Experimental Animals, and the ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee from Utrecht University.

Feed Intakes and BW

All calves received 4 L of pasteurized colostrum that had been frozen and reheated from a bottle in 2 feedings of 2 L each at 1 and 6 h after birth. Calves were blocked in pairs by colostrum (same colostrum for each pair of calves) and each pair had similar parity of dam. Calves were randomly allocated to 1 of 2 treatments: 8 L of MR/d for an elevated plane of MR intake (high) and 4 L of MR/d for the low plane of MR intake (low). Calves were fed MR (150 g/L; 24% CP, 18% crude fat, and 45% lactose; Sloten B.V., Deventer, the Netherlands) twice daily at 0700 and 1700 h via nipple buckets. The raw material inclusion of the calf MR was 50% skim milk powder, 20% sweet whey powder, 17% vegetable oils, 11% delactosed whey powder, and 2% premix.

Low plane calves immediately began their treatment from birth after 2 colostrum feedings. Milk replacer volumes fed to calves in the high treatment were gradually stepped-up in the first week of life, consisting of 2 daily meals of 2.5 L for d 2 and 3, 3 L for d 4 and 5, and 3.5 L for d 6 and 7. From d 8 onward, calves were fed 8 L of MR/d. Calves had ad libitum access to water, calf starter (18.2% CP, 11.2% crude fiber, 2.2% crude fat; 3-mm pellet; AgruniekRijnvallei, Wageningen, the Netherlands), and wheat straw (3 cm chop length), each provided in separate buckets at the front of the hutch during the same time as morning milk feeding (0700 h). Individual intakes of all feeds were recorded daily and weaning entailed a step-down reduction of the amount of MR in each meal by 50% on wk 7 and finished by wk 8. Body weights were recorded at birth, d 2, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and on the day of jugular catheter placement. All weights were taken 2 to 4 h after the morning meal.

Blood Samples

Jugular catheters (Intraflon 2 13G, Ecouen, France) were placed on wk 4, 7, and 10 (Figure 1) of age and remained for ~48 h. The catheters were flushed with 2 mL of heparinized saline (2% solution) before and after sample collection. Blood samples were collected using 20-mL syringes and then allocated into an EDTA vacutainer (Becton Dickinson, Franklin Lakes, NJ) and a sodium fluoride vacutainer that contained a glycolysis

Figure 1. Weekly averages of daily ME from milk replacer, starter, and total in calves fed an elevated intake of milk replacer (MR; A) or a low plane of nutrition MR (B). Data are LSM $\pm$ SEM, $n = 6$ per group.

inhibitor. All blood samples were centrifuged immediately at $2,800 \times g$ for 30 min at room temperature, and 1.5 mL of plasma was pipetted into 2-mL cryotubes and stored immediately at -20°C .

Postprandial Glucose, Insulin, and Abomasal Emptying

To determine postprandial glucose and insulin patterns and abomasal emptying, a total of 12 blood samples were collected at -30 , 30 , 60 , 90 , 120 , 150 , 180 , 210 , 240 , 300 , 360 , and 420 min relative to feeding of the morning MR meal, 1 d after catheter placement on wk 4 and 7. The meal of 2 or 4 L for low or high treatments, respectively, was consumed within 5 min and contained acetaminophen at 150 mg/kg of $\text{BW}^{0.75}$ (metabolic BW) to estimate gastric emptying from kinetics of acetaminophen appearance in plasma. The method and dose for using acetaminophen in blood as a marker for gastric emptying is well established in calves (Schaer et al., 2005) and in many other species (Clements et al., 1978). During sampling, calves had

no access to calf starter and chopped straw, but had access to water at all times. Calves did have access to their bedding, but it was assumed that the intake was marginal and would not influence the precision of data collection in the study. Blood samples were analyzed for acetaminophen using the Paracetamol (Acetaminophen) Assay Kit-K8002 (Cambridge Life Sciences Ltd., Ely, UK; Berends, 2014), for glucose using the EnzyChrom Glucose Assay Kit (BioAssay Systems, Hayward, CA; Zebeli et al., 2012), and for insulin using the Mercodia Bovine Insulin ELISA kit (Mercodia, Uppsala, Sweden; Bach et al., 2013) in the Trouw Nutrition research and development laboratory (Boxmeer, the Netherlands).

Glucose Tolerance Test

Calves were administered an intravenous glucose tolerance test (GTT) the day after abomasal emptying was evaluated. As acetaminophen has been shown to have no long-term effect on glucose metabolism (Basu et al., 2016), it was assumed that the dose a day before the GTT had no marginal influence over the results. The GTT encompassed an intravenous infusion of glucose (30% glucose solution, Eurovet, Bladel, the Netherlands) at a dose of 540 mg/kg of $\text{BW}^{0.75}$ via the jugular catheter (administered over 1 min). The GTT occurred during the time of the morning feeding (0700 h) at 4, 7, and 10 wk of age. The dose was based on previous studies reported in calves (Bach et al., 2013; Yunta et al., 2015); therefore, the renal threshold was not evaluated for the dose in our study. Blood samples were collected at -15 , 0 , 5 , 10 , 20 , 30 , 45 , 60 , 90 , 120 , 180 , and 240 min relative to the glucose infusion. To ensure basal plasma glucose levels were achieved before the infusion, calves were fasted overnight (12 h) with no access to starter and chopped straw, and their morning MR was delayed until sampling was finished (4 h). During sampling, calves were restricted from access to calf starter and chopped straw, but had access to water at all times. Blood samples were analyzed for glucose and insulin as previously described.

Calculations and Statistics

Body weight, ADG, feed intake (weekly average), abomasal emptying, and postprandial and GTT glucose and insulin data were all summarized and analyzed as one measurement per week and also over both the pre- and postweaning periods. For glucose and insulin during the GTT and postprandial measurements, time to reach maximum concentration (**Tmax**), maximum concentration (**Cmax**), ratio of Cmax-to-Tmax, basal levels, and the change in concentration (delta change) were calculated from the raw data. The positive incre-

mental area under the curve (**AUC**) for glucose and insulin was evaluated using the trapezoid rule for values above baseline and was calculated over 420 min for the postprandial responses and 240 min for the GTT. The clearance rate of glucose and insulin during the GTT was calculated using the method established in Pires et al. (2007):

$$\text{clearance rate (\%/min)} = \{(\ln[ta] - \ln[tb]) / (ta - tb)\} \times 100,$$

where [ta] and [tb] are the concentration of insulin or glucose at time ta or tb, respectively. Furthermore, insulin sensitivity was estimated using a simplified model described by Christoffersen et al. (2009).

To calculate gastric emptying rate, it was assumed that absorption of acetaminophen before the small intestine was zero, outflow from the abomasum followed first-order kinetics according to the rate constant (k_{AB}), absorption from the small intestine into blood was instantaneous, and elimination from blood plasma followed first-order kinetics according to the rate constant (k_{el}). Another assumption was that MR did not enter the rumen. The differential equations describing acetaminophen mass in abomasum (A) and blood (B) are

$$\begin{aligned} \frac{dA}{dt} &= -k_{AB}A \quad [1] \text{ and} \\ \frac{dB}{dt} &= k_{AB}A - k_{el}B, \end{aligned} \quad [2]$$

respectively. Integrating these equations, and assuming a volume of distribution of acetaminophen of 90% of BW (Rawlins et al., 1977; Forrest et al., 1982), the concentration (c) of acetaminophen in blood plasma at time t becomes

$$cB_t = \frac{\text{dose} \cdot k_{AB}}{0.9BW(k_{AB} - k_{el})} (e^{-k_{el}t} - e^{-k_{AB}t}). \quad [3]$$

Estimates of k_{AB} and k_{el} were obtained by fitting equation [3] to observed cB_t curves from each calf at 4 and 7 wk of age using PROC NLIN of SAS (SAS Institute Inc., Cary, NC). It is important to acknowledge that calves were drinking different meals sizes as a proportion of their BW. However, according to the law of mass action embodied in equations [1] and [2], the abomasal emptying rate constant (k_{AB}) and elimination rate constant (k_{el}) are independent of acetaminophen dose and concentration. Neither the dose of acetaminophen nor the intake of water and DM affect the ability to estimate these parameters with precision.

To examine the effect of treatment, data were analyzed using the MIXED procedure of the statistical analysis system (SAS Institute Inc.), with observations taken at different ages taken as a repeated measure. The model included the fixed effects of treatment, age, treatment by age interaction, and block. For responses within a day, such as individual time points of the postprandial and GTT data, the same model was used to compare between treatments at specific time points. The covariance structure with the minimum values of Akaike's information criterion was determined to be compound symmetry and used for all variables. A Tukey test was used to correct for multiple comparisons and all values reported are least squares means. When age and treatment interactions were detected, a Tukey test was used to correct for multiple comparisons between treatments by each week using the PDIF statement in SAS. Significance was declared when $P \leq 0.05$ and trends were declared when $P < 0.10$.

RESULTS

BW and Intakes

The BW, ADG, and feed intake results are presented in Table 1 and the ME of intake for each treatment are presented in Figure 1. Within the first week of life, high calves were consuming their full allotment of MR. The high calves had a higher ADG preweaning and the low calves consumed more starter before weaning (no differences in straw intake detected). The BW at weaning was greater in high calves and persisted as a trend until d 70 of the experiment.

Postprandial Insulin, Glucose, and Gastric Emptying

The postprandial glucose and insulin concentrations over a meal were followed for 420 min in accordance with abomasal emptying measurements and are presented in Figure 2 and Table 2. The glucose baseline in high calves was 27% higher during wk 4 and 12% higher at wk 7 compared with low calves. We noted no age effects on postprandial measurements. The Cmax and maximum change in concentration for glucose were greater (18 and 21%, respectively) in high calves compared with low calves. In addition, the Cmax and maximum change in concentration for insulin were greater (165 and 174%, respectively) in high calves compared with low calves. The postprandial AUC at 420 min for insulin was 221% greater in high calves whereas, for glucose, it was not different between treatments. We found no differences for Tmax and Cmax-to-Tmax ratio for glucose and insulin as well as no difference for baseline insulin levels. For abomasal emptying, it was determined using

Table 1. Milk intake, BW, starter intake, and ADG of calves fed a low plane of nutrition (4 L of milk replacer/d) and high plane of nutrition (8 L of milk replacer/d); data are means $\pm$ SEM, $n = 6$ per group

Item	Treatment		SEM	<i>P</i> -value
	Low	High		
Milk intake (L/d; d 1 to 56)	3.72	6.99	0.08	<0.01
BW (kg)				
Initial, d 1	41.4	39.3	1.9	0.47
Weaning, d 56	71.9	81.2	2.4	0.03
Final, d 70	85.3	92.0	2.4	0.07
Starter intake (g/d)				
Prewaning (d 7 to 56)	586.8	194	19	<0.01
Postweaning (d 57 to 70)	2,526	2,010	53	0.01
Overall (d 7 to 70)	1,004	585	33	<0.01
ADG (kg/d)				
Prewaning (d 14 to 56)	0.61	0.77	0.04	0.01
Postweaning (d 57 to 70)	0.94	0.79	0.07	0.27
Overall (d 14 to 70)	0.68	0.78	0.04	0.06

plasma acetaminophen concentrations that the high calves had 40% slower gastric emptying compared with low calves, as shown in Figure 2E and 2F. The rate constant (%/h) of abomasal emptying was positively correlated ($r = 0.41$; $P = 0.04$) with glucose AUC at 420 min and tended to be correlated with Tmax for glucose ($r = 0.37$; $P = 0.07$). No additional correlations between emptying rate and blood glucose and insulin kinetics were uncovered in postprandial data.

GTT

The results of the GTT are displayed in Figure 3 and descriptions of the curves are in Table 3. Blood glucose levels for the GTT reached maximum concentration within 5 to 10 min after the infusion; Cmax levels ranged between 7.4 and 14.5 mmol/L and all calves returned to basal levels within an hour of the infusion. We found no significant difference between treatment and age or across ages for Tmax, Cmax, and insulin sensitivity index, respectively. The AUC at 240 min had a trend ($P = 0.08$) for an interaction between treatment and age, with the high having a lower AUC at 240 min at wk 7, and AUC at 240 min increasing in the low group from wk 4 to 7. We observed no significant difference in the change in glucose over the infusion or the basal glucose level before each infusion. Clearance rate of blood glucose decreased with age, with calves 10 wk of age having a slower clearance rate than 4-wk-old calves.

Insulin response to the GTT was highly variable across calves, with Cmax ranging from 12 to 129 μ U/mL and Tmax ranging from 10 to 240 min. Neither Cmax nor Tmax was significantly different between treatment groups. Area under the curve at 240 min ranged from 52 to 4,064 μ U/mL and was not different between treatments. The basal plasma insulin levels,

the changes in insulin, and the insulin clearance rates were similar between treatment groups, which may be due to the large variability in insulin sensitivity between calves. Overall, no difference was noted in blood insulin levels despite a large degree of variable insulin responses to a GTT.

DISCUSSION

In recent years, the dairy industry has moved to feeding an elevated plane of milk intake (≥ 8 L of MR/d) to dairy calves preweaning to improve growth and lifetime milk production (Bar-Peled et al., 1997; Jasper and Weary, 2002; Soberon et al., 2012). Dairy farms typically feed calves manually twice per day (Vasseur et al., 2010), which dramatically contrasts the 7 to 10 meals per day that occur when a calf is left to suckle the dam (Jasper and Weary, 2002) or from an automated feeder (Berends et al., 2014). Early-life feeding schemes have been shown to have a short- and long-term effect on insulin sensitivity in humans and rodents (Duque-Guimarães and Ozanne, 2013). However, in dairy calves, the effect of common feeding strategies practiced in our industry on overall metabolism pre- and postweaning are largely undescribed. Thus, the objective of this study was to investigate the effect of elevated preweaning planes of milk level offered in 2 meals per day on postprandial insulin and glucose kinetics and abomasal emptying preweaning, as well as insulin responsiveness to a GTT pre- and postweaning.

In the current study, glucose metabolism was examined using postprandial measurements and a GTT simultaneously during sampling weeks. In accordance with earlier studies (Kaufhold et al., 2000; Terré et al., 2009; Yunta et al., 2015), basal glucose was higher in calves fed an elevated plane of MR intake during

Figure 2. Postprandial blood glucose (A, B; mmol/L), insulin (C, D; $\mu\text{U/mL}$), and acetaminophen (E, F; mg/mL) in dairy calves at wk 4 and 7 of age for 420 min. The dotted line represents calves fed the low plane of nutrition and the solid line represents calves fed an elevated plane of nutrition. Data are LSM $\pm$ SEM, $n = 6$ per group. $*P < 0.05$. kAB = rate constant.

postprandial measurements. This finding was expected, as high calves were fed a 2-fold greater level of dietary lactose, which has been shown to increase blood glucose levels (Palmquist et al., 1992). We found no effect of age on basal glucose, which contrasts previous studies in veal calves showing that extended feeding of large volumes of milk for several months leads to hyperglycemia and glucosuria (Hostettler-Allen et al., 1994; Hugi et al., 1997, 1998). There were, however, key discrepancies between these studies and the current study. In these studies (Hostettler-Allen et al., 1994; Hugi et al., 1997, 1998) the total feeding level of MR was greater than 2 kg/d (DM basis) with expected gains of greater than 1.4 kg/d (2 $\times$ the high calves and

4 $\times$ the low calves in this experiment). Furthermore, the majority of the other studies were conducted after the first 2 mo of life, which constitutes a different experimental model compared with the current study. It has been well documented that insulin resistance develops with age when calves are maintained primarily on a diet consisting of milk for more than 2 mo of life. This prolonged feeding of a predominately milk diet, high in lactose in older calves, is causally associated with reduced insulin receptor number in skeletal muscle as the calf ages past 2 mo (Hugi et al., 1998). After 2 mo of age, specific gene expression changes occur in the rumen and liver to support the use of energy derived from ruminal microbial fermentation (Baldwin et al.,

Table 2. The effect of low (4 L of milk replacer/d) and high plane of nutrition (8 L of milk replacer/d) on pre- and postprandial glucose and insulin responses in dairy calves

Item ¹	Treatment				SEM	<i>P</i> -value ²		
	Low		High			T	W	T × W
	wk 4	wk 7	wk 4	wk 7				
Glucose Cmax (mmol/L)	6.41	6.85	8.14	7.49	0.36	<0.01	0.90	0.15
Insulin Cmax (μU/mL)	43.7	67.6	163	132	42.0	0.03	0.41	0.44
Glucose Tmax (min)	95	80	105	115	24.6	0.90	0.86	0.71
Insulin Tmax (min)	90	95	130	105	18.9	0.42	0.50	0.28
Glucose Cmax/Tmax (mmol/L per minute)	0.08	0.09	0.13	0.11	0.03	0.65	0.90	0.57
Insulin Cmax/Tmax (μU/mL per minute)	0.55	0.73	1.52	1.97	0.48	0.21	0.37	0.95
Glucose AUC (mmol/L × 240 min)	251	262	284	203	68.0	0.89	0.87	0.40
Insulin AUC (μU/mL × 240 min)	4,289	6,831	21,458	14,241	5,096	0.01	0.68	0.21
Glucose delta (mmol/L)	1.84	2.06	2.33	2.05	0.32	0.40	0.99	0.49
Insulin delta (μU/mL)	39.7	64.3	158.3	126.9	41.8	0.03	0.38	0.41
Glucose baseline (mmol/L)	4.57	4.79	5.81	5.38	0.28	0.01	0.71	0.27
Insulin baseline (μU/mL)	3.98	3.29	4.68	5.17	0.90	0.17	0.91	0.52

¹Cmax = maximum plasma concentration; Tmax = time of maximum concentration observed; AUC = area under the concentration-time curve; delta = the maximum change from baseline.

²Statistical comparisons: T = low vs. high; W = week; T × W = treatment by time interaction.

2004). These may be indicators that the calf may have a fixed time to use lactose effectively from milk as the dominant energy source.

Although the highest postprandial blood glucose and insulin concentrations, as well as the highest glucose delta (baseline to maximum concentration), were found

in the calves fed an elevated plane of MR, the levels always returned to baseline by 360 min postprandially. No differences were found between treatments in the AUC at 420 min for glucose, which suggests that the calves fed larger meals can regulate blood glucose concentrations as there was no evidence of hyperglycemia in this

Figure 3. Blood glucose (A, B, C) and insulin (D, E, F) concentration relative to intravenous glucose tolerance test in calves at wk 4, 7, and 10 of life in dairy calves fed a low (4 L of milk replacer/d) and high plane of nutrition (8 L of milk replacer/d). The dotted line represents calves fed the low plane of nutrition and the solid line represents calves fed an elevated plane of nutrition. Data are LSM ± SEM, n = 6 per group.

Table 3. The effect of feeding a low (4 L of milk replacer/d) and high plane of nutrition (8 L of milk replacer/d) on glucose and insulin responses to an intravenous glucose tolerance test in dairy calves

Item ¹	Low plane			High plane			P-value ²			
	wk 4	wk 7	wk 10	wk 4	wk 7	k 10	SEM	T	W	T × W
Glucose Cmax (mmol/L)	8.50	9.92	9.90	10.00	9.68	9.03	0.69	0.81	0.73	0.22
Insulin Cmax (μU/mL)	62.2	41.0	47.8	33.8	45.3	54.2	11.7	0.54	0.80	0.26
Glucose Tmax (min)	5.00	5.83	6.67	5.00	6.67	5.83	0.78	1.00	0.19	0.57
Insulin Tmax (min)	23.3	20.0	13.3	26.7	26.7	50.0	17.0	0.27	0.87	0.57
Glucose Cmax/Tmax (mmol/L)	1.72	1.77	1.68	2.00	1.62	1.68	0.20	0.78	0.61	0.54
Insulin Cmax/Tmax (μU/mL)	4.27	2.29	4.58	1.83	2.92	4.67	1.25	0.33	0.55	0.53
Glucose AUC (mmol/L × 240 min)	73.2	185	155	121	95.6	172	30.2	0.75	0.10	0.08
Insulin AUC (μU/mL × 240 min)	1,398	614	1,099	1,129	810	1,207	338	0.42	0.14	0.44
Glucose delta (mmol/L)	4.30	5.43	5.15	4.88	4.62	5.10	0.59	0.85	0.63	0.50
Insulin delta (μU/mL)	57.7	37.0	43.1	29.7	41.4	48.8	11.1	0.45	0.76	0.23
Glucose baseline (mmol/L)	4.37	4.45	4.75	5.17	5.05	3.92	0.57	0.44	0.49	0.29
Insulin baseline (μU/mL)	4.33	3.77	4.83	4.17	3.83	5.00	0.90	0.98	0.47	0.98
Glucose clearance rate (%/min)	3.23	1.72	1.37	2.12	2.43	1.30	0.61	0.70	0.05	0.29
Insulin clearance rate (%/min)	5.17	4.53	7.93	4.88	4.02	4.46	2.23	0.29	0.70	0.97
Insulin sensitivity (mmol/min × μU/mL)	0.31	0.79	0.56	0.96	0.53	0.51	0.08	0.21	0.34	0.43

¹Cmax = maximum plasma concentration; Tmax = time of maximum concentration observed; AUC = area under the concentration-time curve; delta = the maximum change from baseline.

²Statistical comparisons: T = low vs. high; W = week; T × W = treatment by time interaction.

study. We noted greater AUC at 420 min for insulin and greater variation in the concentration of insulin for high calves compared with low calves, especially during wk 4 (Figure 2C) corresponding to differences in lactose load from the meal. Grütter and Blum (1991) showed that the mechanisms behind insulin secretion in the pancreas in response to blood glucose levels during the neonatal period are not fully developed until months after birth, which might explain the large variability in insulin concentration in high and low calves.

The homeostasis of blood glucose relies on the interplay between pancreatic insulin and glucagon production, as well as gluconeogenesis and glucose utilization (Jones et al., 1995). However, the role of gastric motility and emptying is often overlooked despite having a significant effect on the rate of glucose appearance in the bloodstream. Preweaned calves do not yet have a functional rumen; therefore, most milk passes directly into the abomasum, which behaves similar to a simple stomach, effectively making the calf a pseudo-monogastric animal.

The volume of a fluid meal, its caloric content, composition, and osmolality, as well as the abomasal and duodenal pH can alter the rate of abomasal emptying in a calf (Sen et al., 2006). The phenomenon of ruminal drinking where milk may leak to the rumen is commonly reported in veal calves, and could therefore have substantial influence on gastric emptying (Labussière et al., 2014). However, it has recently been shown that calves between the ages of 19 and 23 d of life were able consume larger meal volumes compared with the current study without evidence of ruminal drinking before emptying into the small intestine (Ellingsen et al., 2016).

In monogastrics, it has been shown that minor delays in gastric emptying rate have a notable effect on the glycemic response after a meal (Tong and D'Alessio, 2014). For example, when gastric emptying is delayed, a small amount of glucose enters the blood as a means of reducing large spikes in blood glucose postprandially (Fraser et al., 1990). In the current study, the abomasal emptying rate for the entire meal was shown to be slower for the high calves compared with low; however, due to higher MR intake, more lactose was probably flowing into the small intestine per unit of time in the high calves. This suggests that in high calves the emptying rate of the meal from the abomasum to the lower gut may be altered as a means of stabilizing blood metabolite levels. In accordance with our results, high glucose concentration and osmolality in a meal have been shown to lead to a slower gastric emptying rate in calves (Schaer et al., 2005; Sen et al., 2006). Traditionally, a reduced abomasal emptying rate was considered unfavorable, as it was associated with reduced aboma-

sal pH and ulcers (Ahmed et al., 2002); however, when large meals are fed twice daily, a delayed abomasal emptying may positively influence blood metabolite synchrony. It is well established in multiple species that larger meals are emptied more slowly from the stomach (Delgado-Aros et al., 2004; Jackson et al., 2004; Métayer et al., 2004). To our knowledge, we are the first to demonstrate this phenomenon in calves. From a mechanistic standpoint, the delay may be related to detection of high nutrient concentrations in the ileum and large intestine, leading to glucagon-like peptide-1 release and a slowdown of gastric emptying (Tong and D'Alessio, 2014).

Treatment differences were noted for postprandial glucose and insulin kinetics. However, no differences between high and low calves were detected by a GTT pre- or postweaning. These results suggest that feeding plane has little effect on insulin sensitivity and contrasts recent studies using a GTT to assess insulin sensitivity in preweaning calves fed similar rations of 4 and 8 L of MR/d (Bach et al., 2013; Yunta et al., 2015). In these studies, calves had similar glucose AUC across treatment groups, indicating a tight regulation of blood glucose kinetics. However, calves fed 8 L of MR/d needed higher levels of insulin to control blood glycaemia during a GTT performed preweaning, which suggests reduced insulin sensitivity.

These opposing results may be explained by several different factors. First, the nutritional supply, including colostrum management, was implemented and controlled from the first hours of life in the current experiment as opposed to the second or third week of life observed in the other studies (Bach et al., 2013; Yunta et al., 2015). Recent research in calves suggests that diet quantity and quality during the first days of life are positively associated with growth performance, development, and health during the preweaning period (Blum and Hammon, 1999; de Passillé et al., 2014). It may be that metabolic programming occurs from dietary factors present in the first days or weeks of life (Soberon et al., 2012; Bartol et al., 2013). It may also be possible that high calves had a larger window of time to adapt to larger meals, which decreased the risk of reduced insulin sensitivity compared with previous studies.

Another key difference between the current study and other studies evaluating glucose tolerance was that the GTT infusions were conducted in this study after a 12-h fast, whereas other studies conducted measurements 4 to 5 h after the morning meal (Bach et al., 2013; Yunta et al., 2015). As shown in Figure 2, the postprandial glucose and insulin concentrations took longer than 4 h to return to basal levels; therefore, a GTT at this time would affect the results in the GTT

and be confounded with differences in abomasal emptying between treatments in the current study. In most human diabetes studies, patients are fasted overnight (~12 h; Matsuda and DeFronzo, 1999) to establish basal glucose levels before a GTT, after which a higher basal glucose level and a high insulin response become indicators of impaired glucose metabolism (Fraser et al., 1990). When the GTT was performed preprandially, there were lower levels of glucose before and after the infusion, shorter glucose half-lives, lower insulin response levels, and Tmax for insulin was 15 and 60 min for pre- and postprandial infusions, respectively (Hostettler-Allen et al., 1994). It has been suggested that, in the postprandial state, endogenous glucose production and glucose utilization are constantly being balanced, whereas, in the preprandial or postabsorptive state, glucose is mostly used by insulin-independent tissues (Bergman, 1989). As such, it is imperative that adequate hours fasted before a GTT be taken into consideration when interpreting results.

One final point to consider is that unlike most studies evaluating insulin sensitivity, we used female calves instead of bull calves (Bach et al., 2013). It has been shown in other species that mature females are less susceptible to developing decreased insulin responsiveness and insulin sensitivity (Stubbins et al., 2012; Wintrob et al., 2014). In the study by Yunta et al. (2015), female calves fed 8 L of MR/d demonstrated a moderate decrease in insulin responsiveness compared with calves fed 6 and 4 L of MR/d on d 42, but they did not show evidence of reduced insulin sensitivity postweaning, a finding which agrees with the current study. It is well characterized how specific hormones, such as leptin, can be regulated differently between prepubertal male and females which ultimately influences insulin sensitivity (Kennedy et al., 1996). Sex can clearly alter metabolism in early life and should be considered when interpreting calf metabolism results.

For all treatments and ages, calves in the current study were able to control glycaemia within an hour of the GTT infusion. The only age-related differences were found in low calves where plasma glucose AUC at 240 min and glucose clearance rate were reduced from 4 to 10 wk of age. This result agrees with previous reports that indicate that the ability to clear glucose decreases as the calf matures and is weaned (Palmquist et al., 1992; Yunta et al., 2015). The preweaning treatments caused no carry-over effects postweaning, which is in agreement with the measurements from Yunta et al. (2015), who found no treatment effect after weaning. Interestingly, the calves in Yunta et al. (2015) fed 4, 6, and 8 L of MR/d preweaning had an additional GTT on d 300, which showed that calves fed an elevated plane of milk intake (8 L of MR/d) had greater

insulin sensitivity than calves fed 4 and 6 L of MR/d per day preweaning. These results highlight the paucity of information investigating preweaning plane of nutrition effects on metabolism, especially postweaning and beyond. In addition to plane of MR nutrition, factors such as the composition of the milk or MR and feeding frequency during the preweaning period can program metabolism later on in life (Bartol et al., 2013). More research in this area is required for the dairy industry to properly assess and optimize the short- and long-term implications of early-life nutrition on biological outcomes later in life.

CONCLUSIONS

Feeding 8 L of MR/d in 2 meals per day may offer preweaning growth advantages compared with 4 L of MR/d, but also has minimal effect on glucose metabolism and insulin sensitivity, in contrast to our first hypothesis. The minimal effect on glucose and insulin kinetics as determined by GTT may have partially benefited from the calf's ability to slow down the delivery of large meals from the abomasum to the lower gut, which is in agreement with our secondary hypothesis.

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