Effect of milk replacer feeding rate and frequency of preweaning dairy calves in the southeastern United States: Glucose metabolism

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ABSTRACT

The objective of this experiment was to examine the effect of milk replacer (MR) feeding rate (FR) and frequency (FF) on glucose metabolism before and after weaning during summer and winter in the subtropical climate of the southeastern United States. Holstein calves ($n = 48/\text{season}$) were enrolled at 8 d of age (DOA) in the summer (June to August, body weight = 40.6 ± 0.7 kg) and winter (November to January, body weight = 41.9 ± 0.8 kg). In each season, calves were randomly assigned to 1 of 4 treatments in a 2×2 factorial arrangement including 2 FR [0.65 (low) or 0.76 kg of solids/d (high) of a 26% CP and 17% fat MR] and 2 FF [2 $\times$ (0700 and 1600 h) or 3 $\times$ (0700, 1600, and 2200 h)]. Calves were managed similarly and housed in polyethylene hutches bedded with sand. Milk replacer (12.5%) was fed based on treatments until 42 DOA when FR was reduced by half and offered 1 $\times$ /d (0700 h) for 7 d. Plasma was collected weekly at 1400 h for analyses of glucose and insulin concentrations in all calves. Pre- and postprandial glucose and insulin concentrations of a subset of calves ($n = 10/\text{treatment per season}$) were measured on 20 DOA. A subset of calves ($n = 8/\text{treatment per season}$) was subjected to an intravenous glucose tolerance test (GTT) on 27 and 57 DOA and insulin challenge on 28 and 58 DOA at 1030 h. Average ambient temperature was $26.1 \pm 2.2^\circ\text{C}$ in summer and $12.9 \pm 5.4^\circ\text{C}$ in winter. During the preweaning period in both seasons, feeding high increased plasma glucose concentrations compared with low, and increasing FF reduced basal insulin concentrations. Compared with 2 $\times$, feeding 3 $\times$ did not affect postprandial glucose but lowered insulin in the summer, whereas in the winter, increased glucose from 30 to 180 min but lowered insulin from 240 to 420 min

after MR feeding. Following GTT before weaning in both seasons, 3 $\times$ reduced insulin increment and area under the curve compared with 2 $\times$ without affecting glucose disposal. After weaning, treatment did not affect glucose disposal or insulin responses after GTT during winter, but calves fed 3 $\times$ had faster glucose disposal and stronger insulin responses than 2 $\times$ during summer. In both summer and winter, preweaned calves fed 3 $\times$ had greater decrement and area under the curve of plasma glucose after insulin challenge, suggesting enhanced peripheral tissue insulin response compared with 2 $\times$. This effect persisted after weaning only during summer. Increasing FR had no effect on metabolic responses in both seasons. In conclusion, increasing MR FF from 2 to 3 times per day reduced insulin secretion but enhanced insulin response on peripheral tissues of preweaned calves regardless of season.

Key words: milk replacer, season, calf, glucose metabolism

INTRODUCTION

Calves raised during summer have lower ADG partially due to reduced ME available for growth (Stull et al., 2008; Broucek et al., 2009). This is mainly because of the lower starter intake and the increased energy requirement for maintenance due to heat stress (Gebremedhin et al., 1981; Chaves, 2011). Increasing milk replacer (MR) feeding rate (FR) has been reported to improve ADG for preweaned dairy calves (Chapman et al., 2016; Hill et al., 2016) and, therefore, is a potential approach to improve calf growth during summer. Our preliminary study (Orellana Rivas et al., 2020) reported that calves fed 0.66 kg of DM/d of a MR containing 26% CP and 17% fat (26:17, as-fed basis) twice daily had greater preweaning ADG compared with calves fed 0.55 kg of DM/d a MR containing 20% CP and 20% fat during summer. However, feeding more than 0.66 kg of DM/d of the 26:17 MR did not support any additional increase in ADG and led to increased incidence of ab-

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omasal bloating (Orellana Rivas et al., 2020). One potential mechanism for this lack of improvement in body growth is the lower energy and nutrient utilization due to delayed abomasal emptying, which may result from increased meal size and slower gastric motility caused by heat stress (Orellana Rivas et al., 2020).

Altered glucose metabolism by MR FR and heat stress may also influence body growth. Previous research suggests that feeding larger quantities of MR increases insulin response without influencing glucose disposal after an intravenous glucose tolerance test (**GTT**, Bach et al., 2013a). Similarly, we observed that increasing MR FR during summer increased plasma insulin concentration but did not affect glucose concentration (Orellana Rivas et al., 2020). These data suggest that increasing MR FR stimulates insulin release and possibly reduced insulin responsiveness on peripheral tissues, lowering the efficiency of glucose utilization by muscle and adipose tissue. These effects may also be exaggerated by heat stress during summer, leading to lower growth efficiency. Indeed, Tao et al. (2014) and Monteiro et al. (2016) reported that calves born to late gestation heat-stressed cows had increased glucose utilization by tissue other than muscle and adipose tissue.

Therefore, strategies need to be developed to minimize insulin resistance and improve glucose utilization by peripheral tissues when a large amount of MR is fed to preweaned calves, especially during summer. Increasing MR feeding frequency (**FF**) is reported to influence glucose metabolism. Increasing milk or MR FF to veal calves from 2×/d to 4–6×/d decreased plasma glucose and insulin concentrations after a meal (Kaufhold et al., 2000; Vicari et al., 2008). This suggested well-controlled postprandial hyperglycemia. However, if increased MR FF could improve calf growth partially by sensitizing insulin action on peripheral tissue has never been investigated under heat-stressed condition in the subtropical climate. Therefore, our hypothesis was that calves fed increased quantities of MR and raised during summer have blunted insulin action on peripheral tissues and that increasing MR FF will increase glucose partition to peripheral tissues by increasing peripheral tissue insulin responsiveness. The objective of this study was to evaluate the effect of MR FR and FF on glucose metabolism during pre- and postweaning periods of dairy calves in the summer and winter in the southeastern United States.

MATERIALS AND METHODS

Animal Handling and Experimental Design

The University of Georgia Institutional Animal Care and Use Committee approved the experimental design

and animal handling before beginning the experiment. The experiment was conducted at the Dairy Research Center on the University of Georgia–Tifton Campus during the summer (May to October 2018) and the subsequent winter (November 2018 to April 2019). Detailed experimental procedures are reported in the companion paper (Orellana Rivas et al., 2022). Briefly, in each season, 48 Holstein calves were enrolled. After birth, all calves were fed a colostrum replacer containing at least 200 g of IgG (bovine IgG colostrum replacer, Land O'Lakes Inc.) and managed in the same manner according to the standard operating procedures of the dairy research center. The day of birth was considered as 1 d of the age (**DOA**). From 2 to 7 DOA, calves were fed 0.65 kg of DM/d of a 26:17 MR (Provimi North America Inc.) twice daily (0700 and 1600 h). The MR was composed of dried whey, dried milk protein, dried whey products, and vegetable and animal fat, and the actual CP and fat concentrations (of DM) were 27.98 and 19.01% during summer, and 26.22 and 18.37% in the winter. From 2 DOA, a textured calf starter (18% CP, Godfrey's Warehouse Inc.) was offered ad libitum. At 8 DOA, calves were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement, including 2 FR [0.65 (low) or 0.76 (high) kg of DM/d of the 26:17 MR] and 2 FF [2× [0700 and 1600 h] or 3×/d (0700, 1600, and 2200 h)]. The FR (0.65 vs. 0.76 kg of DM/d) were selected based on our previous study. Orellana Rivas et al. (2020) reported that feeding 0.66 kg of DM/d of a 26:17 MR improved ADG of preweaned calves raised in summer compared with those fed 0.55 kg of DM/d a MR containing 20% CP and 20% fat. However, further increasing FR from 0.66 to 0.77 kg of DM/d of the 26:17 MR had no additional improvement in ADG, and feeding an aggressive amount of MR (0.87 kg of DM/d) was associated with increased incidence of abomasal bloating (Orellana Rivas et al., 2020). The BW of calves at 8 DOA (before the treatments) averaged 40.6 ± 0.7 kg in the summer and 41.9 ± 0.8 kg in the winter, and was not different ($P > 0.40$) between treatments in either season. Calves were weaned by reducing the MR FR by half and fed once daily (0700 h) from 43 to 49 DOA. Calves remained on the experiment until 63 DOA. During the entire experiment, intake of MR and calf starter was recorded daily and BW was measured weekly. Water was offered ad libitum during the entire experiment. The water buckets were cleaned twice each day and refilled as needed. Investigators were not blinded to treatment information.

Blood Sampling and Analyses

Blood samples were collected from all calves at 1400 h on 7, 14, 21, 28, 35, 42, 49, 56, and 63 DOA from

the jugular vein into sodium-heparinized Vacutainer tubes (Becton Dickinson) and immediately placed on ice. Samples were centrifuged at $1,700 \times g$ at 4°C for 30 min to collect plasma. Plasma concentrations of glucose (Autokit glucose, Wako Chemicals USA Inc.) and insulin (Bovine insulin ELISA, Mercodia AB) were determined using commercially available kits; the inter- and intraassay coefficients of variation were 4.2 and 8.2% for glucose, and 5.6 and 4.2% for insulin, respectively.

Pre- and Postprandial Metabolism

Pre- and postprandial glucose and insulin concentrations of a subset ($n = 10/\text{treatment per season}$) of calves were measured at 20.1 ± 1.3 DOA (mean $\pm$ SD) during summer and at 20.2 ± 1.2 DOA during winter. On the day before sample collection, an indwelling jugular vein catheter (Terumo Corporation) was inserted (~ 1700 h). Blood samples were collected through the catheter into Vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson) at -15 , 15 , 30 , 60 , 90 , 120 , 180 , 240 , 300 , and 420 min relative to the starting point of MR morning feeding (0700 h). Samples were immediately placed on ice and then centrifuged at $1,700 \times g$ at 4°C for 30 min to obtain plasma. Plasma concentrations of glucose and insulin were analyzed using the same approaches described above. The inter- and intraassay coefficients of variation were 7.4 and 3.6% for glucose and 6.7 and 6.0% for insulin, respectively.

Metabolic Tests

A subset of calves ($n = 8/\text{treatment per season}$) was subjected to GTT and insulin challenge (IC) during the pre- and postweaning periods. The maximal insulin concentration after a GTT (41.5 vs. $98.7 \mu\text{U/mL}$, SD = $26.3 \mu\text{U/mL}$, respectively) of calves fed 0.479 and 0.957 kg/d MR (25:19; Bach et al., 2013a) were used to calculate the sample size. A sample size of 6 calves per treatment per season was calculated using a level of significance of 0.05 and 90% power. Therefore, 8 animals per treatment per season will provide enough power. At each period, GTT and IC were conducted on 2 adjacent days with GTT performed on the first day and IC on the following day. The actual day when GTT was performed was 26.9 ± 1.1 and 57.2 ± 1.3 DOA during summer and 27.3 ± 1.1 and 57.1 ± 1.1 DOA during winter. The IC was conducted on 27.9 ± 1.1 and 57.9 ± 1.1 DOA during summer and 28.3 ± 1.1 and 58.1 ± 1.1 DOA during winter. On the day of GTT, immediately after MR feeding (~ 0730 h), a catheter was inserted into the jugular vein of calves and remained until the end of IC on the following day. At 3.5 h following the

morning MR feeding (~ 1030 h), calves received a bolus of glucose [0.3 g/kg of BW (Tao et al., 2014; Monteiro et al., 2016), dextrose 50%, wt/vol; Phoenix Scientific Inc.] or insulin [0.1 IU/kg of BW (Tao et al., 2014; Monteiro et al., 2016), Novolin R, 100 IU/mL, recombinant human insulin, Novo Nordisk Inc.] through the catheter inserted in the jugular vein followed by 10 mL of sterile saline containing 10 IU/mL heparin to flush the catheter and prevent clotting. The first 2 to 3 mL of blood collected was discarded before each sample. Blood samples were collected through the catheter into Vacutainer tubes containing sodium fluoride and potassium oxalate (Becton Dickinson) at -15 , -5 , 0 , 5 , 10 , 15 , 20 , 30 , 40 , 50 , 60 , 75 , 90 , and 120 min relative to the glucose infusion during GTT, and at -15 , -5 , 0 , 15 , 20 , 30 , 40 , 50 , 60 , 75 , 90 , and 120 min relative to insulin infusion during IC. Immediately after sampling, blood tubes were placed on ice and then centrifuged at $1,700 \times g$ at 4°C for 30 min to collect plasma. Plasma concentrations of glucose and insulin were determined as described before. For samples collected during GTT, the inter- and intra-CV were 7.9 and 3.0% for glucose, and 6.8 and 4.9% for insulin, respectively. For samples collected during IC, the inter- and intraassay coefficients of variation were 3.3 and 6.2% for glucose, and 7.1 and 4.5% for insulin, respectively.

Calculation and Statistical Analyses

For data collected from metabolic tests, the average concentration of plasma glucose or insulin for the samples collected at -15 , -5 , and 0 min relative to infusion was considered as baseline. The baseline values were subtracted from the maximum or minimum concentrations to obtain the increment or decrement change in glucose or insulin concentrations when appropriate. The AUC between times was calculated using the trapezoidal method, where the concentrations of glucose or insulin were calculated by subtracting the baseline value from the actual glucose or insulin concentrations. The accumulated AUC of glucose and insulin were calculated from 5 to 30 min, 5 to 60 min, and 5 to 120 min for GTT, and from 15 to 30 min, 15 to 60 min, and 15 to 120 min for IC. Exponential curves of glucose concentration from 5 to 60 min of the GTT and from 15 to 40 min for the IC were fitted using PROC NLIN of SAS (SAS Institute Inc.), and the clearance rate (CR) and time to reach half of the maximal concentration ($T_{1/2}$) were calculated using the following equations: $\text{CR, \%}/\text{min} = 100 \times (\ln|t_a| - \ln|t_b|)/(t_b - t_a)$; and $T_{1/2, \text{ min}} = 100 \times [\ln(2)]/\text{CR}$, where $|t_a|$ and $|t_b|$ are the concentrations at time a and b , respectively (Hayirli et al., 2001).

Basal and pre- and postprandial plasma concentrations of glucose and insulin were analyzed by PROC MIXED of SAS 9.4 as repeated measures data. The model included sex, FR, FF, time, and their interactions with calf within treatment ($FR \times FF$) as a random variable. Data collected from metabolic tests during pre- and postweaning periods were analyzed separately. Covariance structures of PROC MIXED models were selected based on goodness of fit criteria of SAS (Littell et al., 1998). The GLM procedure of SAS was used to analyze baseline, maximum, minimum, increment or decrement, CR, $T_{1/2}$, and accumulated AUC of glucose and insulin in each test. The model included sex, FR, FF, and their interaction. Plasma concentrations of glucose and insulin during GTT and IC were analyzed as repeated measures data using PROC MIXED of SAS. The statistical model included sex, FR, FF, minute relative to infusion, and their interactions with calf within the treatment ($FR \times FF$) as the random variable. The least squares means $\pm$ standard error of the means are reported. Significance and tendency were declared when $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

RESULTS

Environment, Intake, and Growth

The detailed performance data were reported in the companion paper (Orellana Rivas et al., 2022). During summer and winter, average daily ambient temperature was $26.14 \pm 2.24^\circ\text{C}$ and $12.85 \pm 5.42^\circ\text{C}$ (mean $\pm$ SD), and relative humidity averaged $83.85 \pm 7.78\%$ and $81.54 \pm 15.18\%$, respectively. These data suggest that calves during summer were exposed to heat stress. In both seasons, feeding high increased ($P \leq 0.01$) intake of MR compared with low supporting improved ($P = 0.02$) preweaning BW gain. Increased MR FR also resulted in greater ($P \leq 0.10$) feed efficiency, as expressed by ADG/DMI, ADG/CP, or ADG/ME during the experimental period in both seasons. During summer, feeding MR $3\times/d$ supported greater intake of starter and DM at wk 8 and 9 of age compared with $2\times/d$ ($FR \times \text{time}$: $P \leq 0.08$). But MR FF did not affect ADG during the experimental period or BW gain before and after weaning during summer. In contrast, during winter, feeding MR more frequently supported greater ($P \leq 0.08$) intake of starter, DMI, CP, and ME, resulting in increased ($P \leq 0.08$) ADG and gains in BW during the entire experimental period. Additionally, feeding MR $3\times/d$ tended to support greater ($P = 0.07$) BW gain during the preweaning period compared with $2\times/d$ during winter. Milk replacer FF did not affect efficiency measures in both seasons regardless of FR.

Basal and Pre- and Postprandial Glucose and Insulin

Prewaning plasma glucose concentration was higher ($P \leq 0.04$) for calves fed high compared with low during both summer and winter (Table 1). However, MR FR did not affect ($P > 0.15$) plasma insulin concentration before weaning or circulating glucose or insulin concentrations after weaning. During the preweaning period, increasing FF from 2 to $3\times/d$ lowered ($P \leq 0.01$) plasma insulin concentrations in both seasons without ($P \geq 0.17$) affecting plasma glucose concentrations (Table 1). After weaning, calves previously fed $3\times/d$ had higher ($P = 0.03$) plasma glucose concentrations compared with those previously fed $2\times/d$ only during winter (Table 1). Milk replacer FF had no effect ($P \geq 0.59$) on plasma insulin concentrations after weaning in both seasons. Regardless of seasons, feeding MR more times increased ($P \leq 0.01$) the ratio of glucose to insulin before weaning. However, FR did not ($P \geq 0.40$) affect the ratio of glucose to insulin before and after weaning.

Pre- and postprandial glucose concentrations at ~ 20 DOA were not affected by FR in both seasons ($FR \times \text{time}$: $P \geq 0.30$; Supplemental Table S1, Supplemental Figure S1A, S1C; <https://doi.org/10.5281/zenodo.4613068>, Orellana Rivas and Tao, 2021). No differences were observed for pre- and postprandial insulin concentrations due to FR during winter ($FR \times \text{time}$: $P = 0.74$; Supplemental Table S1), but calves fed high had higher ($P = 0.04$) plasma insulin concentrations at 15 min after MR feeding compared with those fed low during summer ($FR \times \text{time}$: $P = 0.01$, Supplemental Figure S1B). Before MR feeding, calves fed $3\times/d$ had higher ($P \leq 0.03$) plasma concentrations of glucose and insulin compared with those fed $2\times/d$ in both seasons ($FF \times \text{time}$: $P \leq 0.03$; Figure 1). Feeding $3\times/d$ in the winter compared with $2\times$ increased ($P \leq 0.07$) plasma glucose concentrations from 30 to 180 min after MR feeding ($FF \times \text{time}$: $P = 0.03$; Figure 1C). In contrast, plasma insulin concentrations from 15 to 180 min were not different among FF but were lower ($P \leq 0.07$) from 240 to 420 min for $3\times/d$ compared with $2\times/d$ after MR feeding ($FF \times \text{time}$: $P \leq 0.01$, Figure 1D). During the summer, feeding $3\times/d$ did not affect postprandial glucose concentration, but reduced plasma insulin concentration from 30 to 420 min after MR feeding compared with $2\times/d$ ($FF \times \text{time}$: $P < 0.01$, Figure 1A, 1B).

Metabolic Tests

During the preweaning period, CR, $T_{1/2}$, and AUC of glucose following GTT were not different ($P \geq 0.11$) among treatments in either season except that calves fed low tended ($P = 0.07$) to have higher AUC_{30} than

Table 1. Milk replacer (MR) feeding rate (FR) and frequency (FF) effects on basal plasma glucose and insulin concentrations during summer and winter

Item	Treatment ¹				SEM	P-value		
	Low		High			FR	FF	FR × FF
	2×	3×	2×	3×				
Summer								
Glucose, mg/dL								
wk 1–6	86.9	83.1	93.3	89.6	2.7	0.02	0.17	0.98
wk 6–9	71.1	76.1	72.1	74.1	2.6	0.86	0.18	0.57
Insulin, µg/L								
wk 1–6	0.26	0.17	0.30	0.14	0.03	0.78	<0.01	0.22
wk 6–9	0.31	0.29	0.23	0.28	0.04	0.16	0.59	0.30
Glucose/insulin								
wk 1–6	26	501	307	622	51	0.51	<0.01	0.24
wk 6–9	225	250	280	252	34	0.40	0.99	0.44
Winter								
Glucose, mg/dL								
wk 1–6	98.4	95.9	101.4	102.2	2.19	0.04	0.70	0.46
wk 6–9	76.0	82.1	75.4	80.4	2.41	0.62	0.03	0.81
Insulin, µg/L								
wk 1–6	0.30	0.21	0.35	0.21	0.04	0.68	0.01	0.64
wk 6–9	0.30	0.26	0.27	0.35	0.05	0.56	0.66	0.20
Glucose/insulin								
wk 1–6	327	461	294	491	65	0.90	0.01	0.61
wk 6–9	234	318	292	233	36	0.75	0.77	0.06

¹Low = 0.65 kg of DM/d of a MR containing 26% CP and 17% fat; high = 0.76 kg of DM/d of a MR containing 26% CP and 17% fat; 2× = feeding the MR twice a day (0700 and 1600 h); 3× = feeding the MR 3 times a day (0700, 1600, and 2200 h); n = 12/treatment per season.

those fed high during winter (Tables 2 and 3, Supplemental Figure S2, <https://doi.org/10.5281/zenodo.4613068>, Orellana Rivas and Tao, 2021). In response to intravenous glucose infusion, preweaned calves fed 3×/d had lower ($P \leq 0.05$) maximal concentration, increment, and AUC of plasma insulin compared with calves fed 2×/d in both winter and summer (Tables 2 and 3, Figure 2B, 2D). After weaning, the response to GTT among treatments differed during summer and winter. In the winter, FR or FF did not affect ($P \geq 0.10$) CR, $T_{1/2}$, and AUC of plasma glucose, or the increment and AUC of circulating insulin following GTT of postweaned calves (Table 3, Figure 3C, 3D, Supplemental Figure 3C, 3D, <https://doi.org/10.5281/zenodo.4613068>, Orellana Rivas and Tao, 2021). In contrast, during summer, calves previously fed 3×/d tended ($P = 0.10$) to have faster glucose CR, greater ($P \leq 0.05$) glucose AUC₆₀ and AUC₁₂₀, and higher ($P \leq 0.04$) increment and AUC of circulating insulin compared with those previously fed 2×/d (Table 2, Figure 3A, 3B). Milk replacer FR had no effect ($P \geq 0.11$) on CR, $T_{1/2}$, and AUC of plasma glucose, or the increment and AUC of plasma insulin after GTT of postweaned calves during summer (Table 2, Supplemental Figure S3A, S3B).

During summer, treatment did not affect ($P \geq 0.07$) insulin clearance after IC before weaning. After weaning in the summer, calves previously fed low 3×/d had greater ($P < 0.05$) CR but lower $T_{1/2}$ than those fed low

2×/d with calves fed high intermediate (FR × FF: $P \leq 0.02$); however, treatment had no effect ($P > 0.10$) on insulin AUC after intravenous insulin infusion (Table 4). In the winter, FR, FF, or their interaction had no ($P \geq 0.09$) effect on CR, $T_{1/2}$, and AUC of plasma insulin following IC before and after weaning (Table 5, Figure 4D and 5D, Supplemental Figure S4D and S5D, <https://doi.org/10.5281/zenodo.4613068>, Orellana Rivas and Tao, 2021). Compared with those fed 2×/d, preweaned calves fed 3×/d had greater ($P \leq 0.03$) decrement and AUC of plasma glucose after IC in both summer and winter (Tables 4 and 5, Figure 4A, 4C). During the postweaning period in the summer, calves previously fed 3×/d had greater ($P = 0.04$) glucose AUC₃₀ compared with those previously fed 2×/d (Table 4). Additionally, interactions of FR and FF ($P \leq 0.05$) were observed for decrement, AUC₆₀, and AUC₁₂₀ of circulating glucose. These were because calves previously fed high 3×/d had the greatest values compared with other treatments (Table 5). In contrast, treatment did not ($P \geq 0.08$) affect parameters related to glucose responses following IC of postweaned calves raised during winter.

DISCUSSION

Both nutrition and environment significantly affect an animal's metabolism, which might partially

Figure 1. Effect of milk replacer feeding frequency [twice (2×, $n = 20$) versus 3 times (3×, $n = 20$) per d] on plasma glucose and insulin concentrations relative to morning milk replacer feeding (0700 h) on 20.2 ± 1.3 d of age during summer and 20.2 ± 1.2 d of age during winter. Error bars represent SEM. ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

explain the responses in growth performance. In this current experiment, regardless of season, increasing FR increased basal plasma glucose concentration before weaning without affecting insulin concentration, possibly due to the higher lactose consumption with greater MR intake. In contrast, our previous study conducted in summer (Orellana Rivas et al., 2020) reported that calves fed high had similar plasma glucose and higher insulin concentrations compared with calves fed low. The discrepancy between studies may be due to the time of blood sample collection [1400 h in this current study vs. 1100 h in Orellana Rivas et al. (2020) relative to the morning feeding (0700 h in both studies)]. Increasing FF from 2 to 3×/d did not affect plasma glucose concentration but decreased circulating insulin concentrations during the preweaning period in both seasons. This, coupled with a higher glucose to insulin ratio, may suggest that feeding MR more frequently reduced insulin release and enhances peripheral tissue insulin responsiveness before weaning. Neither FR nor FF altered basal glucose and insulin concentrations after weaning during summer. In contrast, calves previously fed MR more frequently in the winter had greater

plasma glucose concentrations after weaning. This may suggest increased hepatic gluconeogenesis, reduced glucose utilization, or both. However, because growth was not affected by treatment in both seasons, basal glucose concentrations do not seem to be associated with postweaning body growth in this experiment.

During the preweaning period in both seasons, calves fed 3×/d had similar glucose clearance but reduced insulin responses following GTT than those fed 2×/d. These data indicate that increasing FF diminished pancreatic insulin release after glucose stimulation without affecting glucose disposal. Moreover, calves fed MR more frequently had stronger glucose responses following IC. This, coupled with similar insulin clearance, suggests that increasing FF enhanced insulin mediated glucose entry into the peripheral tissues, such as muscle and adipose tissues, of preweaned calves. This may be a negative feedback response to the reduced insulin release and explains similar glucose disposal after GTT between calves fed MR 2 and 3×/d. In addition, season did not affect these responses. In contrast, Stanley et al. (2002) reported that increasing FF from 1 to 2×/d increased insulin release in response to intravenous

Table 2. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to a glucose tolerance test of calves during the pre- (26.9 ± 1.1 d of age) and postweaning (57.2 ± 1.3 d of age) periods in the summer

Parameter ¹	Prewaning						Postweaning									
	Treatment ²			P-value			Treatment			P-value						
	Low			High			Low			High						
	2×	3×	2×	3×	SEM	FR	FF	FR × FF	2×	3×	2×	3×	SEM	FR	FF	FR × FF
Glucose, mg/dL																
Baseline	83.7	87.2	87.5	86.5	3.9	0.69	0.75	0.57	73.2	78.4	66.6	74.7	3.8	0.19	0.10	0.70
Maximum	157.3	170.7	169.5	167.9	6.2	0.45	0.36	0.24	177.6	181.2	171.9	174.9	4.4	0.18	0.46	0.94
Increment	73.7	83.5	82.1	81.4	4.7	0.50	0.34	0.27	104.4	102.8	105.3	100.1	3.9	0.83	0.40	0.65
CR, %/min	1.43	1.48	1.38	1.44	0.14	0.77	0.70	0.98	1.32	1.49	1.28	1.61	0.15	0.80	0.10	0.61
T _{1/2} , min	49.4	49.1	51.4	49.6	4.5	0.79	0.82	0.87	53.5	47.5	55.5	45.5	5.0	1.00	0.12	0.69
AUC, mg × min/dL																
30 min	1,337	1,507	1,452	1,525	103	0.52	0.25	0.64	1,893	1,832	2,048	1,768	103	0.66	0.11	0.29
60 min	1,417	1,631	1,646	1,732	231	0.48	0.53	0.78	2,662	2,380	3,016	2,182	270	0.77	0.05	0.31
120 min	1,242	861	1,532	1,252	418	0.42	0.44	0.90	2,575	2,103	3,418	1,941	394	0.39	0.02	0.21
Insulin, µg/L																
Baseline	0.78	0.34	1.01	0.42	0.14	0.28	<0.01	0.95	0.17	0.23	0.10	0.15	0.03	0.02	0.10	0.91
Maximum	8.66	6.43	10.06	7.30	0.98	0.25	0.02	0.79	1.96	3.17	1.38	2.30	0.43	0.10	0.02	0.94
Increment	7.78	5.98	9.01	6.76	0.89	0.26	0.03	0.80	1.81	2.94	1.27	2.15	0.41	0.11	0.02	0.92
AUC, mg × min/dL																
30 min	153.1	112.6	185.6	129.3	19.6	0.22	0.02	0.69	37.4	57.4	26.6	45.4	8.6	0.19	0.03	0.94
60 min	177.4	128.9	230.7	159.9	24.3	0.09	0.02	0.65	50.6	73.1	38.3	56.7	9.0	0.12	0.03	0.82
120 min	161.4	113.8	195.5	147.1	22.9	0.15	0.05	0.99	51.2	73.5	42.5	57.4	8.5	0.15	0.04	0.66

¹Baseline = average glucose and insulin concentrations at -15, -5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment = concentration difference between maximum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; T_{1/2} = time to reach half maximal glucose concentration; AUC = area under the curve.

²Low = 0.65 kg of DM/d of a MR containing 26% CP and 17% fat; high = 0.76 kg of DM/d of a MR containing 26% CP and 17% fat; 2× = feeding the MR twice a day (0700 and 1600 h); 3× = feeding the MR 3 times a day (0700, 1600, and 2200 h); n = 8/treatment.

Table 3. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to a glucose tolerance test of calves during the pre- (27.3 ± 1.0 d of age) and postweaning (57.1 ± 1.0 d of age) periods in the winter

Parameter ¹	Prewaning						Postweaning									
	Treatment ²			P-value			Treatment			P-value						
	Low			High			Low			High						
	2×	3×	2×	3×	SEM	FR	FF	FR × FF	2×	3×	2×	3×	SEM	FR	FF	FR × FF
Glucose, mg/dL																
Baseline	82.8	100.9	94.1	99.0	3.9	0.24	0.01	0.10	75.3	81.0	75.5	81.5	3.3	0.91	0.09	0.97
Maximum	177.0	184.1	179.3	182.0	7.9	0.99	0.54	0.78	181.8	182.1	181.1	191.3	6.5	0.52	0.42	0.45
Increment	94.2	83.2	85.2	83.0	7.2	0.53	0.37	0.55	106.6	101.2	105.6	109.8	6.1	0.53	0.92	0.44
CR, %/min	2.2	1.7	2.1	2.0	0.3	0.64	0.27	0.35	2.0 ^a	1.7 ^{ab}	1.4 ^b	1.8 ^{ab}	0.2	0.16	0.68	0.06
T _{1/2} , min	32.94	48.73	34.97	35.99	5.99	0.45	0.19	0.26	37.1	42.7	54.3	43.3	5.2	0.10	0.60	0.12
AUC, mg × min/dL																
30 min	1,402	1,451	1,181	1,165	132	0.07	0.90	0.81	1,804	1,739	1,945	1,795	123	0.43	0.39	0.73
60 min	1,099	1,379	764	661	317	0.11	0.78	0.55	2,022	2,033	2,692	2,136	302	0.21	0.38	0.36
120 min	812	80	−263	−160	498	0.20	0.53	0.41	1,836	2,050	2,788	1,782	447	0.45	0.38	0.18
Insulin, µg/L																
Baseline	0.85	0.54	1.33	0.65	0.27	0.34	0.08	0.69	0.17	0.19	0.15	0.19	0.04	0.73	0.46	0.83
Maximum	10.48	6.76	12.91	8.22	1.78	0.29	0.02	0.97	6.12	5.33	3.86	4.81	1.01	0.18	0.94	0.39
Increment	9.73	7.03	12.20	8.56	1.51	0.20	0.04	0.76	5.93	5.09	3.69	4.59	1.00	0.18	0.98	0.39
AUC, mg × min/dL																
30 min	200.7	130.5	236.2	160.4	29.7	0.28	0.02	0.93	101.3	89.2	63.7	82.4	17.8	0.22	0.85	0.39
60 min	236.4	127.2	265.5	174.4	31.3	0.24	<0.01	0.77	118.8	106.4	87.0	95.4	20.4	0.30	0.92	0.62
120 min	208.1	154.2	254.0	127.0	41.0	0.82	0.04	0.38	118.3	105.4	89.2	91.7	20.4	0.31	0.80	0.71

^{a,b}Within a row, means without a common superscript differ ($P \leq 0.05$).¹Baseline = average glucose and insulin concentrations at -15, -5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment = concentration difference between maximum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; T_{1/2} = time to reach half maximal glucose concentration; AUC = area under the curve.²Low = 0.65 kg of DM/d of a MR containing 26% CP and 17% fat; high = 0.76 kg of DM/d of a MR containing 26% CP and 17% fat; 2× = feeding the MR twice a day (0700 and 1600 h); 3× = feeding the MR 3 times a day (0700, 1600, and 2200 h); n = 8/treatment.

glucose infusion without influencing peripheral tissue insulin sensitivity. When raised on an automated calf feeder and fed larger quantities of MR (1.2 kg solids of MR/d containing 26% CP and 18% fat), calves given MR access 4×/d had similar glucose clearance after GTT without influencing insulin secretion and tissue response compared with those given MR access 2×/d (MacPherson et al., 2019). The reasons for the discrepancy among studies may include, but are not limited to, amount of MR offered, composition of MR, and feeding method.

Before the morning MR feeding in both seasons, calves fed 3×/d had higher plasma glucose concentrations due to the shorter interval from last MR feeding (9 vs. 15 h, respectively) compared with calves fed 2×/d. This also explains the higher preprandial plasma insulin concentration observed for calves fed 3×/d compared with those fed 2×/d. Similarly, MacPherson et al. (2019) reported higher preprandial plasma glucose concentrations for calves given access 4×/d to an automated calf feeder compared with those at 2×/d. Al-

though numerically higher in calves allowed MR more frequently, plasma insulin concentrations before feeding was not affected by FF (MacPherson et al., 2019). It is important to note that the number of animals per FF (5 vs. 20) in the experiment conducted by MacPherson et al. (2019) is lower than this current experiment.

The postprandial glucose metabolism of the milk-fed calves is also affected by FF. Compared with those fed 1×/d, preweaned dairy calves fed MR 2×/d had similar postprandial plasma glucose concentrations but reduced insulin concentrations (Stanley et al., 2002). Similarly, MacPherson et al. (2019) reported that preweaned dairy calves raised on an automated calf feeder and allowed MR access 4×/d had similar postprandial plasma glucose concentrations but lower insulin concentrations than those allowed MR access 2×/d at wk 4 of age. In contrast, in large veal calves (BW $\approx$ 110–140 kg), feeding milk or MR more frequently than 2×/d was associated with reduced postprandial plasma glucose and insulin concentrations (Kaufhold et al., 2000; Vicari et al., 2008). The discrepancy between studies may

Figure 2. Effect of milk replacer feeding frequency [FF, twice (2×, $n = 16$ /season) versus 3 times (3×, $n = 16$ /season) per d] on plasma glucose and insulin concentrations during a glucose tolerance test performed on calves at 26.9 ± 1.1 d of age during summer and 27.3 ± 1.1 d of age during winter. Error bars represent SEM. During summer for the glucose concentration, effect of FF ($P = 0.82$) and FF by minute interaction ($P = 0.24$). During summer for the insulin concentration, effect of FF ($P < 0.01$) and FF by minute interaction ($P < 0.01$). During winter for the glucose concentration, effect of FF ($P = 0.05$) and FF by minute interaction ($P = 0.40$). During winter for the insulin concentration, effect of FF ($P = 0.01$) and FF by minute interaction ($P = 0.10$). ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

be due to the higher MR FR and older age (11–14 vs. 8 wk of age) of veal calves than preweaned dairy calves. Interestingly, in this current study, distinct patterns of postprandial circulating glucose and insulin concentration were observed between calves with different MR FF in different seasons.

During summer, calves with different FF had similar postprandial plasma glucose concentrations, consistent with results reported by Stanley et al. (2002) and MacPherson et al. (2019). However, calves fed MR more frequently had higher plasma glucose concentrations from 30 to 180 min after morning feeding during winter. This is particularly surprising because calves fed 3×/d received less MR, and thus lower lactose intake, per meal relative to calves fed 2×/d. Because no differences were observed for glucose disposal following GTT, the higher postprandial plasma glucose concentrations may suggest faster nutrient delivery to the small intestine and intestinal glucose absorption because of the faster abomasal emptying rate of calves

fed MR more frequently in the winter. The reasons for the distinct patterns of postprandial plasma glucose concentration between FF in different seasons are not clear but may reflect the increased glucose usage for maintenance during heat stress. During lactation, heat-stressed dairy cows had increased glucose utilization as an energy source in nonmammary gland tissues or cells (Baumgard and Rhoads, 2012). Additionally, previous studies (Tao et al., 2014; Monteiro et al., 2016) observed that maternal heat stress during the last 45 d of gestation increased glucose disposal after intravenous glucose infusion of the preweaned calf. Presumably, similar increases in glucose utilization by pre- and postnatal calves raised during summer would occur and mask potential differences in postprandial plasma glucose concentrations observed with different FF. Similar to glucose concentrations, postprandial plasma insulin concentrations displayed distinct patterns in calves fed 2 vs. 3×/d in different seasons. However, the circulating insulin concentration seemed to be dependent on

Figure 3. Effect of milk replacer feeding frequency [FF, twice (2×, $n = 16$ /season) versus 3 times (3×, $n = 16$ /season) per d] on plasma glucose and insulin concentrations during a glucose tolerance test performed on calves at 57.2 ± 1.3 d of age during summer and 57.1 ± 1.1 d of age during winter. Error bars represent SEM. During summer for the glucose concentration, effect of FF ($P = 0.91$) and FF by minute interaction ($P = 0.10$). During summer for the insulin concentration, effect of FF ($P = 0.04$) and FF by minute interaction ($P = 0.19$). During winter for the glucose concentration, effect of FF ($P = 0.52$) and FF by minute interaction ($P = 0.31$). During winter for the insulin concentration, effect of FF ($P = 0.73$) and FF by minute interaction ($P = 0.15$). † $P \leq 0.10$.

Table 4. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to an insulin challenge of calves during the pre- (27.9 ± 1.1 d of age) and postweaning (58.2 ± 1.3 d of age) periods in the summer

Parameter ¹	Prewaning						Postweaning									
	Treatment ²						Treatment									
	Low			High			Low			High						
	2×	3×	2×	3×	SEM	FR	FF	FR × FF	2×	3×	2×	3×	SEM	FR	FF	FR × FF
Insulin, µg/L																
Baseline	0.68 ^a	0.45 ^{ac}	1.05 ^b	0.31 ^c	0.10	0.27	<0.01	0.02	0.17	0.23	0.10	0.15	0.03	0.02	0.10	0.91
Maximum	8.66	6.43	10.06	7.30	0.98	0.25	0.02	0.79	1.96	3.17	1.38	2.30	0.43	0.10	0.02	0.94
Increment	7.78	5.98	9.01	6.76	0.89	0.26	0.03	0.80	1.81	2.94	1.27	2.15	0.41	0.11	0.02	0.92
CR, %/min	7.02	9.41	7.76	6.80	1.01	0.36	0.49	0.11	6.32 ^a	15.57 ^b	11.51 ^{ab}	9.19 ^{ab}	2.11	0.78	0.11	0.01
T _{1/2} , min	10.39 ^{xy}	8.34 ^x	8.97 ^{xy}	10.74 ^y	1.01	0.63	0.89	0.07	9.95 ^a	5.15 ^b	6.24 ^{ab}	8.18 ^{ab}	1.39	0.99	0.31	0.02
AUC, µg × min/L																
30 min	187	141	161	187	34	0.77	0.76	0.29	148	196	163	217	37	0.63	0.18	0.93
60 min	215	158	180	212	38	0.80	0.75	0.25	184	228	179	260	43	0.75	0.16	0.67
120 min	188	171	157	189	40	0.87	0.85	0.54	190	228	162	267	48	0.91	0.15	0.50
Glucose, mg/dL																
Baseline	74.4	80.2	73.6	90.6	4.0	0.23	0.01	0.16	66.1	67.4	65.3	75.1	2.4	0.16	0.03	0.08
Minimum	36.4	26.1	40.3	36.1	3.6	0.06	0.06	0.40	24.5	26.6	23.5	25.7	1.6	0.56	0.20	0.99
Decrement	-38.1	-54.1	-33.3	-54.5	3.9	0.57	<0.01	0.51	-41.7 ^a	-40.8 ^a	-41.8 ^a	-49.5 ^b	1.7	0.02	0.06	0.02
AUC, mg × min/ dL																
30 min	-527	-797	-423	-803	83	0.56	<0.01	0.51	-593	-654	-597	-738	48	0.36	0.04	0.41
60 min	-1,458	-2,158	-1,129	-2,227	190	0.50	<0.01	0.30	-1,700 ^a	-1,692 ^a	-1,686 ^a	-2,019 ^b	79	0.06	0.05	0.04
120 min	-1,985	-3,698	-1,140	-3,650	381	0.25	<0.01	0.30	-2,861 ^{ab}	-2,419 ^a	-2,736 ^{ab}	-2,996 ^b	168	0.19	0.59	0.05

^{a-c}Means within a row without a common superscript differ ($P \leq 0.05$).^{xy}Mean values in the same row with different superscripts tend to differ ($P \leq 0.10$).¹Baseline = average glucose and insulin concentrations at -15, -5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment or decrement = concentration difference between maximum or minimum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; T_{1/2} = time to reach half maximal glucose concentration; AUC = area under the curve.²Low = 0.65 of kg of DM/d of a MR containing 26% CP and 17% fat; high = 0.76 of kg of DM/d of a MR containing 26% CP and 17% fat; 2× = feeding the MR twice a day (0700 and 1600 h); 3× = feeding MR 3 times a day (0700, 1600, and 2200 h); n = 8/treatment.

Table 5. Milk replacer (MR) feeding rate (FR) and feeding frequency (FF) effect on glucose and insulin response to an insulin challenge of calves during the pre- (28.3 ± 1.0 d of age) and postweaning (58.1 ± 1.0 d of age) periods in the winter

Parameter ¹	Prewaning						Postweaning									
	Treatment ²			P-value			Treatment			P-value						
	Low			High			Low			High						
	2×	3×	2×	3×	SEM	FR	FF	FR × FF	2×	3×	2×	3×	SEM	FR	FF	FR × FF
Insulin, µg/L																
Baseline	0.85	0.54	1.33	0.65	0.27	0.34	0.08	0.69	0.17	0.19	0.15	0.19	0.04	0.73	0.46	0.83
Maximum	10.48	6.76	12.91	8.22	1.78	0.29	0.02	0.97	6.12	5.33	3.86	4.81	1.01	0.18	0.94	0.39
Increment	9.57	6.18	11.21	7.30	1.52	0.37	0.02	0.98	5.93	5.09	3.69	4.59	1.00	0.18	0.98	0.39
CR, %/min	7.58	6.78	6.82	6.65	0.75	0.56	0.53	0.68	7.89	8.18	8.04	7.50	0.71	0.72	0.86	0.56
T _{1/2} , min	9.31	11.02	10.37	10.03	1.16	0.98	0.56	0.39	9.04	8.65	8.92	9.63	0.91	0.65	0.87	0.55
AUC, µg × min/L																
30 min	213	268	194	195	29	0.12	0.35	0.36	153	160	156	143	21	0.75	0.90	0.63
60 min	232	342	226	219	37	0.09	0.18	0.13	184	190	189	181	24	0.93	0.97	0.78
120 min	200	328	211	184	45	0.15	0.27	0.10	185	196	203	190	25	0.80	0.96	0.64
Glucose, mg/dL																
Baseline	73.4 ^a	92.5 ^b	85.7 ^b	87.1 ^b	3.9	0.39	0.01	0.03	71.0	75.5	76.3	79.5	2.8	0.11	0.18	0.82
Minimum	39.4	38.1	42.1	35.5	3.2	0.99	0.23	0.41	26.2	31.8	28.7	28.7	2.1	0.89	0.19	0.20
Decrement	-34.0	-54.4	-43.6	-51.6	4.9	0.49	0.01	0.22	-44.8	-43.7	-47.6	-50.7	2.7	0.08	0.71	0.44
AUC, mg × min/dL																
30 min	-510	-782	-658	-733	115	0.67	0.14	0.40	-696	-665	-695	-750	58	0.47	0.84	0.46
60 min	-1,289	-2,136	-1,747	-2,069	253	0.45	0.03	0.31	-1,865	-1,801	-1,956	-1,990	120	0.25	0.90	0.69
120 min	-1,442	-3,631	-2,282	-2,962	491	0.86	0.01	0.14	-2,911	-2,554	-3,053	-2,984	190	0.15	0.27	0.46

^{a,b}Within a row, means without a common superscript differ ($P \leq 0.05$).¹Baseline = average glucose and insulin concentrations at -15, -5, and 0 min relative to glucose infusion; maximum = the maximal glucose and insulin concentrations; increment or decrement = concentration difference between maximum or minimum and baseline; CR = clearance rate of glucose during the first 60 min after glucose infusion; T_{1/2} = time to reach half maximal glucose concentration; AUC = area under the curve.²Low = 0.65 kg of DM/d of a MR containing 26% CP and 17% fat; high = 0.76 kg of DM/d of a MR containing 26% CP and 17% fat; 2× = feeding the MR twice a day (0700 and 1600 h); 3× = feeding the MR 3 times a day (0700, 1600, and 2200 h); n = 8/treatment.

plasma glucose concentrations and reflected reduced insulin secretion in response to glucose stimulation of calves fed MR more frequently.

Peripheral tissue insulin response and pancreatic insulin release are critical components of glucose metabolism in dairy calves and are proposed to influence hepatic function, body growth and composition, and future performance (Gerrits et al., 2008; Van Eetvelde and Opsomer, 2017). It is imperative to consider if altered insulin action by nutrition or management in the preweaning period could persist after weaning. During summer, the greater glucose response after IC suggested that the increased peripheral tissue insulin response of calves fed MR 3×/d persisted after weaning compared with those fed 2×/d, especially for calves with greater MR FR. Following GTT after weaning, calves previously fed MR 3×/d had greater insulin response compared with calves previously fed 2×/d in the summer. This contrasted with the observations with different FF before weaning and suggested that the pancreas might develop a compensatory response after the treat-

ment ceased. The greater insulin release in response to glucose stimulation and stronger insulin response on peripheral tissues explains the faster glucose disposal after GTT of the postweaned calves previously fed MR more frequently during summer. Future research needs to examine the enhanced tissue insulin response by FF could persist beyond 8 wk of age, and how it would affect postweaning body growth, fat deposition, health, and future performance. In contrast, no difference in glucose or insulin responses were observed in the winter due to FF in both metabolic tests after weaning. These suggest that the altered metabolic response by increased FF diminished after the treatment ceased during winter. The mechanisms for altered peripheral tissue insulin response between calves with different FF only persisted after weaning in calves raised in summer is not clear but warrants further investigation.

In both seasons, FR had no effect on glucose or insulin response to either GTT or IC before or after weaning. In contrast, feeding larger quantities of MR at 2×/d to veal calves caused hyperglycemia, hyperinsulinemia,

Figure 4. Effect of milk replacer feeding frequency [FF, twice (2×, $n = 16$ /season) versus 3 times (3×, $n = 16$ /season) per d] on plasma glucose and insulin concentrations during an insulin challenge performed on calves at 27.9 ± 1.1 d of age during summer and 28.3 ± 1.1 d of age during winter. Error bars represent SEM. During summer for the glucose concentration, effect of FF ($P = 0.46$) and FF by minute interaction ($P = 0.01$). During summer for the insulin concentration, effect of FF ($P < 0.01$) and FF by minute interaction ($P = 0.01$). During winter for the glucose concentration, effect of FF ($P = 0.73$) and FF by minute interaction ($P = 0.02$). During winter for the insulin concentration, effect of FF ($P = 0.23$) and FF by minute interaction ($P = 0.17$). ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

and insulin resistance in peripheral tissues as a negative feedback mechanism of prolonged elevation of plasma insulin (Doppenberg and Palmquist, 1991; Hostettler-Allen et al., 1994; Hugi et al., 1997). In preweaned dairy calves, increasing FR increased insulin response after GTT without affecting glucose clearance, suggesting insulin resistance (Bach et al., 2013b; Yunta et al., 2015; MacPherson et al., 2016). In this present experiment, no effect of FR was observed on pre- and postprandial glucose and insulin concentrations. In heavier veal calves, increasing FR of a MR containing 19.5% CP and 20.0% fat from 1.5 to 2.5 $\times$ ME requirements for maintenance increased preprandial insulin but not glucose concentrations, and substantially increased plasma glucose and insulin concentrations after feeding (Vicari et al., 2008). In preweaned dairy calves, feeding a MR containing 24% CP and 18% fat at a rate of 1.2 kg of solids/d increased postprandial insulin responses but not glucose responses compared with those fed 0.6 kg of solids/d (MacPherson et al., 2016). The lack of differences in insulin secretion and glucose metabolism

between calves with different FR in the current study may be due to the smaller differences in FR used in this study. Compared with previous studies where calves with higher FR consumed 1.5 to 2 times more MR than control animals (Bach et al., 2013b; Yunta et al., 2015; MacPherson et al., 2016), high calves only consumed 14% to 16% more MR than calves fed low. Our previous study (Orellana Rivas et al., 2020) reported that increasing FR more than high 2 $\times$ /d during summer in southern Georgia could lead to increased incidence of abomasum bloating. This prevented us from feeding an aggressive amount of MR (>0.76 kg of solids/d) to calves enrolled in this current study.

In conclusion, increasing MR FR from 0.65 to 0.76 kg of DM/d had no effect on glucose metabolism before or after weaning in both summer and winter. Regardless of the FR and season, feeding MR 3 $\times$ /d reduced insulin secretion and improved insulin responses on peripheral tissues compared with 2 $\times$ /d before weaning. In this current experiment, feeding high improved preweaning BW gain compared with low regardless of

Figure 5. Effect of milk replacer feeding frequency [FF, twice (2 $\times$, $n = 16$ /season) versus 3 times (3 $\times$, $n = 16$ /season) per d] on plasma glucose and insulin concentrations during an insulin challenge performed on calves at 57.9 ± 1.1 d of age during summer and 58.1 ± 1.1 d of age during winter. Error bars represent SEM. During summer for the glucose concentration, effect of FF ($P = 0.04$) and FF by minute interaction ($P = 0.01$). During summer for the insulin concentration, effect of FF ($P = 0.17$) and FF by minute interaction ($P = 0.98$). During winter for the glucose concentration, effect of FF ($P = 0.06$) and FF by minute interaction ($P = 0.23$). During winter for the insulin concentration, effect of FF ($P = 0.11$) and FF by minute interaction ($P = 0.48$). ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

season, and feeding MR more frequently only improved preweaning BW gain during winter but not summer. The discrepancies between metabolic responses and growth performance suggest that altered insulin release and peripheral tissue insulin action may not significantly influence ADG. It is important to note that the methods (GTT and IC) used in this experiment can only assess the release and peripheral tissue response of insulin, but is unable to evaluate insulin sensitivity. Insulin sensitivity is determined by the insulin concentration to reach half of its maximal biological responses of an insulin-dependent tissue, and cannot be evaluated without assessing multiple insulin doses (De Koster and Opsomer, 2013). It is possible that altered insulin sensitivity has a greater effect on calf growth. Future research using hyperinsulinemic euglycemic clamp tests is needed to confirm the results obtained in this experiment and to evaluate the altered insulin sensitivity by MR FR and FF.

Changes in peripheral insulin responsiveness by FF in early life may also alter body composition of the growing animal, which have a significant implication on the cow's future performance. Indeed, Kaufhold et al. (2000) and Nussbaum et al. (2002) reported that veal and preruminant dairy calves fed MR more frequently had increased plasma IGF-I concentration. Circulating IGF-I concentration is strongly correlated with depositions of body protein and fat in preweaned Holstein calves (Bartlett et al., 2006). Interestingly, we observed a carryover effect of FF on peripheral tissue insulin response after weaning only during summer but not winter. It is important to examine if this carryover effect could persist in the calf's future life and its impact on subsequent reproductive and lactation performance.

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