



Incremental supply of fat, lactose, or protein influences the diurnal pattern of heat production and substrate oxidation in preweaning calves

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ABSTRACT

Increasing nutrient supply to dairy calves has well known benefits; however, the effects of milk replacer (MR) composition when supplied in higher amounts are not fully understood, particularly in the first weeks of life. To better understand the metabolism of macronutrient supply in young calves (21 d old), we investigated diurnal patterns of heat production and substrate oxidation in young calves fed MR with an incremental supply of fat, lactose, or protein. Thirty-two groups of 3 mixed-sex Holstein-Friesian newborn calves (3.4 ± 1.6 d of age), were randomly assigned to one of 4 dietary treatments and studied for 21 d. Diets consisted of a basal MR (23.3% CP, 21.2% EE, and 48.8% lactose of DM) fed at 550 kJ/kg BW^{0.85} per day (CON; n = 24), or the basal MR incrementally supplied with 126 kJ of digestible energy/BW^{0.85} per day as milk fat (+FAT; n = 23), lactose (+LAC; n = 24), or milk protein (+PRO; n = 23). Calves were fed MR in 2 daily meals and had ad libitum access to water, but were not supplied with any calf starter nor forage. After 2 weeks of adaptation to the diets, groups of 3 calves were placed for 1 wk in an open-circuit respiration chamber for nitrogen and energy balance measurements (lasting 7 d). On d 3, glucose oxidation kinetics was estimated by using [U-¹³C]glucose. Measurements included total heat production (total energy [HP], activity [H_{act}] expenditure, resting metabolic rate [RMR]), respiration quotient (RQ), carbohydrate (COX) and fat oxidation (FOX) in 10 min. intervals and averaging these values per hour over days. Incremental supply of lactose and fat increased body fat deposition, with observed patterns in RMR indicating that this increase occurred primarily after the meals. Specifically, the average daily RMR was highest in the +PRO group and lowest in the CON treatment. The HP was higher in the +PRO group and throughout the day, hourly means of HP were higher in

this treatment mainly caused by an increase in H_{act} . The recovery of ¹³CO₂ from oral pulse-dosed [U-¹³C]glucose was high (77%), and not significantly different between treatments, indicating that ingested lactose was oxidized to a similar extent across treatments. Increasing lactose supply in young calves increased fat retention by reduction in fatty oxidation. Calves fed a MR with additional protein or fat raised RMR persistently throughout the day, whereas the extra lactose supply only affects RMR after the meal. Dietary glucose was almost completely oxidized (77% based on (¹³C) glucose measurement) regardless of nutrient supplementation. Extra protein supply increased HP and FOX compared with similar intakes of fat and lactose. Fasting heat production (FHP) of young, group-housed calves is comparable to literature values and unaffected by energy intake. Overall, these findings deepen our understanding of how different nutrients affect metabolic processes, fat retention, and energy expenditure in young dairy calves.

Key words: calf, heat partitioning, glucose and fat oxidation, milk replacer

INTRODUCTION

Traditionally, dairy calves have been fed either whole milk (WM) or milk replacer (MR), but in either case restricted at an approximate rate of 8% to 10% of their birth BW, aiming to encourage starter intake and anticipate rumen development (Drackley, 2008). Minimizing milk supply can spare milk for human consumption and MR being more expensive than concentrate by weight, is perceived as more costly way to feed calves, although this depends on costs and feed conversion efficiencies (Bach et al., 2013). However, ad libitum WM or MR intake of calves is naturally much higher (20% of BW; Jasper and Weary, 2002), and most recent nutritional understanding accepts that higher supplies of WM or MR have short-term benefits other than growth, and long-term positive effects on the productivity of cows (Davis Rincker et al., 2011; Gelsing et al., 2016).

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

This paper presents new data from a study that was reported previously by Amado et al. (2024), which reported the incremental efficiencies of energy and protein deposition in very young calves (21 d old) fed with a MR incrementally supplemented with isoenergetic amounts of fat, lactose, or protein. Incremental fat and lactose both resulted in increased fat deposition as compared to incremental protein. In most nonruminants, such as humans and pigs, *de novo* fatty acid synthesis mainly derives from glucose supply (Bergen and Mersmann, 2005). In swine, glucose abundance in the postabsorptive state enhances *de novo* fatty acid synthesis and lipogenesis (van Erp et al., 2018, 2020). The contribution of glucose to fat deposition increases with increasing feeding level and explains approximately two-thirds of total fat deposition in ad libitum fed pigs (van den Borne et al., 2006). In contrast, dietary glucose is almost completely oxidized in heavy pre-ruminant calves (veal calves), and therefore glucose is not deposited as body fat. An increase in feed intake increased glucose but decreased fatty acid oxidation (van den Borne et al., 2007). This mechanism is also observed in adult ruminants where fatty acid synthesis from glucose is limited (Laliots et al., 2010). Furthermore, it has been suggested that changes in insulin sensitivity during early life, as observed in the study by Pantophlet et al. (2016), could potentially lead to alterations in how nutrients are distributed within the body. For instance, studies have shown high insulin sensitivity in calves younger than 3 wk of age. It has been described that high insulin sensitivity in calves decreases with age, regardless of the feeding strategy, and reaches very low levels compared with other species (Stanley et al., 2002; Pantophlet et al., 2016). Moreover, it has been shown that the prolonged intake of lactose can have adverse effects on glucose homeostasis and insulin sensitivity in veal calves older than 4 mo of age, leading to conditions such as hyperglycemia, hyperinsulinemia, and insulin resistance (Hugi et al., 1997). Understanding the effect of energy sources, such as lactose, on insulin sensitivity and glucose metabolism in calves is crucial for optimizing their growth and overall health.

In terms of protein deposition, the gross efficiency at which digested nitrogen intake was retained by young calves (60–100 kg of BW) was ~60%. However, as BW increases above 150 kg, the gross efficiency drops below 50% (Gerrits et al., 1996; Diaz et al., 2001; van den Borne et al., 2006; Labussière et al., 2008; Berends et al., 2012). The metabolic mechanism by which these efficiencies are achieved and how nutrient and energy partitioning are modulated remain unknown. Daily patterns of substrate oxidation and fasting heat production (FHP) can help with gaining understanding during the process of nutrient partitioning in young calves.

The present article describes the diurnal patterns of energy expenditure by partitioning total heat production (HP) into heat production related to physical activity (H_{act}) and the resting metabolic rate (RMR) of young calves fed MR supplied with incremental fat, lactose, and protein. In addition, the diurnal patterns of the respiratory quotient (RQ), net rates of carbohydrate and fat oxidation, and the FHP of these calves were also measured.

MATERIALS AND METHODS

This study was conducted at Carus, Research Facility of the Department of Animal Sciences, Wageningen University and Research (Wageningen, the Netherlands) from April 2018 to June 2018. All procedures described in this article complied with the Dutch Law on Experimental Animals, which meets European guidelines defined by ETS123 (Council of Europe 1985 and the 86/609/EEC Directive) and were approved by the animal welfare authority (Centrale Commissie Dierproeven, CCD, the Netherlands) and the Animal Care and Use Committee of Wageningen University. The project application code is AVD2040020173425.

Experimental Design

A detailed description of the experimental design has been described by Amado et al. (2024). Briefly, 94 Holstein-Friesian calves (37 females, 57 males) were selected based on clinical health, age, BW, and sex uniformity and arrived in 9 different batches over a period of 3 mo. The average age and weight at arrival were 3.5 ± 1.4 d and 44.5 ± 0.3 kg, respectively. The experiment consisted of 3 consecutive periods: an adaptation period of 14 d after arrival; the first measurement period (HP, which lasted 5 d); and the second measurement period (FHP), which lasted 2 d. Before the FHP period, 1 calf from each group was randomly selected and euthanized for the collection of gastrointestinal tract tissues (A. Welboren, University of Guelph, Guelph, Canada; unpublished data). Each group ($n = 3$ for HP and $n = 2$ for FHP) was housed in an indirect calorimetric chamber without bedding material. This schedule was the same for all batches.

Diets and Feeding

Calves were fed according to their metabolic BW ($BW^{0.85}$), adjusted weekly as they grew at an individual animal level. The power of 0.85 ($BW^{0.85}$) was chosen to minimize variation in heat and fasting production parameters measured by calorimetry in Holstein veal calves, as previously determined by Labussière et al. (2008). Groups of calves ($n = 3$) were blocked as they arrived and assigned to 1 of 4 dietary treatments: a control

diet (CON; basal MR; Trouw Nutrition, Deventer, the Netherlands), or the control diet supplemented with butterfat (+FAT; anhydrous milk fat; Royal VIV Buisman BV, Zelhem, the Netherlands), lactose (+LAC; lactose powder; Arla Foods Ingredients Group, Viby, Denmark), or protein (+PRO; milk protein concentrate powder 80; Fonterra Ltd., Auckland, New Zealand), and the groups were adapted to their respective diets for 14 d before the measurement period started. The incremental nutrient treatments were defined on an isoenergetic basis (digestible energy, 675 MJ/kg BW^{0.85} per day) and provided in 2 equal daily portions. The ingredient and nutrient composition of the experimental MR is shown in Table 1. Milk replacer was reconstituted with water at 150 g/L and supplied with a teat bucket at ~40°C. Feeding times were set at 0600 and 1800 h. Calves had ad libitum access to water but did not have access to calf starter nor forage.

Measurements and Calculations

During the 7 d in the respiration chambers, nitrogen and energy balances were monitored, and exchanges of oxygen (O₂), carbon dioxide (CO₂), and methane (CH₄) were measured over 10-min intervals as described by Heetkamp et al. (2015). The production of ¹³CO₂ was measured during 10-min intervals using a nondispersive infrared spectrometer (Advance Optima Uras 14, ABB group, Switzerland) as described by Alferink et al. (2003). The total energy expenditure (HP) was calculated using the formula of Brouwer (1965) based on gaseous exchange. The HP was divided into activity energy expenditure (H_{act}) and RMR by use of penalized β-spline regression procedures (van Klinken et al., 2012) in Matlab (MathWorks), with 8 knots. For each group of calves, FHP was estimated as the asymptotic RMR during 24 h (including last feeding) of feed deprivation:

$$\text{RMR} = \text{FHP} + [b_0 \cdot c \cdot t^{(-c-1)} \cdot b_1^c] / [1 + (b_1/t)^c]^2,$$

where RMR = FHP (kJ·kg of BW^{0.85}) at time t (h); b₀, b₁, and c (all >0) are parameters that define the curve. The nonlinear least squares regression procedure (PROC NLIN, SAS Institute) was used for curve fitting. The RQ was determined by calculating the ratio of CO₂ production to O₂ consumption. The net rates of carbohydrate oxidation (COX) and fat oxidation (FOX) were calculated from gaseous exchanges (L) as described by van den Borne et al. (2015).

On the third day in the chambers, an oral dose of 11 μmol/kg BW of [U-¹³C]glucose (99.0 atom%; Cambridge Isotope Laboratories) was added to the MR fed at 1800 h to calculate the glucose oxidation from the amount of ¹³CO₂ exhaled. Bicarbonate sequestration was measured on d 4 and determined from the amount of ¹³CO₂ exhaled

Table 1. Ingredient and analyzed nutrient composition of the experimental milk replacers

Item	Treatment ¹			
	CON	+FAT	+LAC	+PRO
Ingredient, % DM				
Milk replacer ²	100	88.2	77.7	82.7
Anhydrous milk fat	—	11.8	—	—
Milk protein powder	—	—	—	17.3
Lactose powder	—	—	22.3	—
Nutrient composition, % DM				
DM, %	96.2	96.5	96.9	95.6
CP	23.4	20.7	18.2	34.5
Crude fat	21.4	30.6	16.6	18.0
Lactose	49.0	43.2	60.2	41.4
Crude ash	6.2	5.5	4.8	6.4
DE, MJ/kg DM	20.6	22.3	19.6	20.6
Gross energy, MJ/kg DM	21.1	23.0	19.9	21.0
Intake, g/kg BW ^{0.85}				
DM	27	30	34	33
CP	6	6	6	11
Crude fat	6	9	6	6
Lactose	13	13	21	13
Crude ash	2	2	2	2
DE, MJ/d	548	674	674	674

¹CON = 23.3% CP, 21.2% crude fat, and 48.8% lactose on a DM basis, provided at 550 kJ/kg BW^{0.85} per day; or the basal MR incrementally supplied with 125 kJ of DE/BW^{0.85} per day as milk fat (+FAT), lactose (+LAC), or milk protein (+PRO).

²Premix, provided per kilogram of milk replacer: 25,000 IU/kg vitamin A; 5,000 IU/kg vitamin D₃; 90 mg/kg vitamin E; 158 mg/kg vitamin C; 94 mg/kg iron; 47 mg/kg manganese; 124 mg/kg zinc; 11 mg/kg copper; and 0.2 mg/kg selenium.

after a bolus dose of 32 μmol of [¹³C] sodium bicarbonate (NaHCO₃, 99.0 atom%; Cambridge Isotope Laboratories) injected into an ear vein. The injection took place 2 h after feeding and was completed within 2 min at 1800 h. The measurement of [¹³C]-carbon dioxide enrichment was taken on d 2 (background) and d 3. Because within-day patterns in background enrichment were observed, the enrichment levels in excess to background were determined by subtracting hourly background levels determined on d 2, which were subtracted from the measured enrichment of the corresponding hour on d 3. The recovery of ¹³C was calculated by dividing the amount of ¹³C exhaled in the form of carbon dioxide by the oral dose received on a per-hour basis.

A model described by van den Borne et al. (2007) was fitted to the 60-min means of ¹³CO₂ excretion in breath of each group of calves (corrected for background enrichment) after ingestion of [U-¹³C]glucose, and after infusion of [¹³C]sodium bicarbonate:

$$y = [b_0 \cdot c \cdot t^{(-c-1)} \cdot b_1^c] / [1 + (b_1/t)^c]^2,$$

where y = ¹³CO₂ production (micromoles per minute) at time t (min); b₀, b₁, and c (all >0) are parameters that define the curve. The nonlinear least squares regression

procedure (PROC NLIN, SAS Institute) was used for curve fitting. Oxidation of the tracer metabolites was calculated by integration of the area under the $^{13}\text{CO}_2$ excretion curve over a period of 24 h after administration of the isotope, $b_0/(1 + b_1 \cdot 1,440^{-c})$ and expressed relative to the amount of ingested or infused isotope by dividing the area under the curve by the dose of isotope corrected for enrichment and chemical purity. The time to peak was calculated as $t_{\max} = [b_1 \cdot c \cdot (1 - c) / (-c - 1)]^{(1/c)}$. The maximum $^{13}\text{CO}_2$ excretion (mmol/h) was calculated as $y_{\max} = b_0 \cdot c \cdot t_{\max}^{(-c-1)} \cdot b_1 \cdot c / [1 + (b_1/t_{\max})^c]^2$ and was expressed as percentage of the dose. Recoveries and maxima for the $^{13}\text{CO}_2$ excretion after the ingestion of glucose tracer were corrected for bicarbonate sequestration, using the average of sodium bicarbonate recovery value as $^{13}\text{CO}_2$, % of dose (86.9%) to be subtracted from the glucose recovery test.

Statistical Analysis

All statistical analyses were performed using SAS Studio (version 3.2, SAS institute, Cary, NC). The group of calves housed in one respiration chamber were considered the experimental unit with treatment and batch as fixed effects. Continuous variables (i.e., figures presented of HP, activity-related heat production, RMR, RQ, FHP, and the net rates of COX and FOX) were analyzed using mixed-model analysis with PROC MIXED in SAS (SAS 9.4M6, SAS Studio, SAS Institute). Time entered the model as a repeated statement in case of repeated measurements, and then the interactions between time and treatment and the SLICE command from SAS Studio (version 3.2, SAS Institute, Cary, NC) to control Type I error were included. The effect of the diet on daily averages over the entire experimental period of HP, activity-related HP, RMR, ^{13}C recovery, RQ, FHP, and the net rates of COX and FOX were analyzed by ANOVA using a general linear model with treatment, batch, chamber, and their interactions as fixed effects. The normal distribution of the residuals was checked to verify the model assumptions. Treatment effects were studied by pairwise comparisons using the Tukey method. All values are presented as $\text{LSM} \pm \text{SEM}$. Significance was declared when $P \leq 0.05$ and trends were declared when $P < 0.10$.

RESULTS

The data set included 32 observations, 8 replicates per treatment in which 2 replicates were based on 2 calves per treatment, instead of 3 calves per treatment. For FHP measurement, each replicate was based on 2 calves per treatment.

Daily averages of HP parameters are presented in Table 2, and hourly patterns are shown in Figure 1. The total

HP over the 5-d period was lowest in the CON calves ($P < 0.01$; Table 2), and this difference was consistently observed throughout the day, with an HP peak following meal ingestion for all treatments (Figure 1A). The daily average of H_{act} was numerically higher in the +LAC group than in the other treatments (Table 2). Circadian patterns of H_{act} revealed meal-related behaviors, with calves displaying increased activity before meals (anticipation) and +LAC calves exhibiting higher activity levels, especially during the night ($P < 0.01$; Figure 1C). The average daily RMR was significantly higher in the +PRO group when compared with the +CON, +LAC, and the +FAT calves ($P < 0.001$; Table 2). Hourly RMR means were lower in the +CON group than in +PRO calves, whereas consistently higher values were observed for +FAT and +PRO compared with CON and +LAC ($P = 0.006$; Figure 1D). Circadian patterns of RMR also displayed meal-related responses across all treatments, though these patterns were less clear for +PRO calves and most pronounced for +LAC calves, particularly after the morning meal. The RMR maximum (RMR_{\max}) following the morning meal was met 3 h after the meal in the +CON, +FAT, and +PRO groups, but it did not significantly differ between the groups. For the +LAC group, the RMR_{\max} was reached 2 h later compared with the other treatments, but it was not significantly different between the groups ($P > 0.10$; Figure 1D). The RMR_{\max} in response to the afternoon meal was met at 1900 h in the +PRO and +FAT groups and at 2000 h in the +LAC and +CON groups, and the results were significantly different between the groups at these 2 time points ($P < 0.05$).

The average RQ over the 5-d period was highest among the +LAC calves ($P < 0.01$; Table 2), a difference that consistently persisted throughout the day (Figure 1B). Hourly RQ means were significantly greater in the +LAC treatment following both meals ($P < 0.01$; Figure 1B). The RQ always remained consistently below 1 across all treatments and time periods. Circadian patterns of net carbohydrates and fat oxidation correspond with patterns observed for RQ, with greater average daily net rates of COX in the +LAC group ($P < 0.01$; Table 2) and lower average daily net rates for +FAT calves, and the opposite was observed for fat oxidation (Table 2; Figure 2). Hourly means of net rates of COX were significantly greater in the +LAC calves after the morning meal and lower in the +FAT and +CON group before the morning and afternoon meal ($P < 0.01$; Figure 2A). Average daily net rates of FOX over the 5-d period of measurements were lower in the +LAC treatment when compare with the other treatments ($P < 0.01$; Table 2). Hourly means of net rates of FOX were significantly less for all the treatments after the morning and afternoon meal. All hourly FOX values were lower in the +LAC ($P < 0.01$; Figure 2B).

Table 2. Heat production parameters and net rates of carbohydrate (COX) and fat oxidation (FOX) over a 5-d measurement period and RQ and net rates of COX and FOX during the fasting period in young calves fed a milk replacer supplemented with additional fat, lactose, or protein at 21 d of age

Item	Treatment ¹				SEM ²	P-value
	CON	+FAT	+LAC	+PRO		
Total heat production, kJ/kg BW ^{0.85} per day	382 ^b	411 ^a	417 ^a	424 ^a	5.1	<0.01
Resting metabolic rate, kJ/kg BW ^{0.85} per day	337 ^d	363 ^b	342 ^c	382 ^a	9.4	<0.001
Activity-related heat production, kJ/kg BW ^{0.85} per day	44	47	75	43	10.1	0.09
RQ	0.89 ^b	0.87 ^c	0.95 ^a	0.89 ^b	0.002	<0.01
COX, kJ/d	9.76 ^c	9.61 ^c	14.29 ^a	10.57 ^b	0.06	<0.01
FOX, kJ/d	5.40 ^b	6.66 ^a	2.38 ^c	6.30 ^a	0.15	<0.01
Fasting period						
FHP, kJ/kg BW ^{0.85} per day	291	311	312	313	8.4	0.22
RQ	0.85 ^b	0.84 ^b	0.89 ^a	0.85 ^b	0.003	<0.01
COX, kJ/d	7.28 ^c	7.64 ^c	10.45 ^a	8.29 ^b	0.23	<0.01
FOX, kJ/d	6.97 ^b	7.63 ^a	5.03 ^c	7.59 ^a	0.15	<0.01

^{a-d}Means that do not share a common letter within a row differ ($P < 0.05$).

¹CON = 23.3% CP, 21.2% crude fat, and 48.8% lactose on a DM basis, provided at 550 kJ/kg BW^{0.85} per day; or the basal MR incrementally supplied with 125 kJ of DE/BW^{0.85} per day as milk fat (+FAT), lactose (+LAC), or milk protein (+PRO).

²Pooled SEM.

No differences were found between the treatments in the estimate FHP at the end of the 24-h measurement period (Table 2). The FHP was 20 kJ/BW^{0.85} lower for CON calves compared with the other groups, but this difference did not reach statistical significance (Table 2). During the period of measurement of FHP, calves of all treatments exhibited a response to the last meal similar to the meal responses shown in Figures 1 and 2. During the final 10 h of the fasting period, treatment differences were absent, except for the lower RMR in CON calves ($P < 0.01$; Figure 3A). Hourly means of RMR were lower in the +LAC calves and higher in the +PRO calves during the fed state ($P < 0.01$; Figure 3A). Hourly means of RQ were significantly higher in the +LAC group after the last meal, between 0600 h and 2200 h ($P < 0.01$; Figure 3B). However, RQ was not significantly different among the treatments after 2200 h, when it decreased and reached an average value of 0.80 for all treatments (Figure 3B). Hourly means of net rates of COX over the 24-h fasting period were higher in the +LAC treatment than in the other treatments, particularly between 0600 and 2200 h ($P < 0.01$; Figure 3C) and followed the same pattern as the RQ. Hourly means of net rates of FOX over the 24-h fasting period were higher in the +FAT and +PRO calves than in the +LAC and CON calves, specially 8 h after the last meal, and followed the same pattern for all treatments after 2200 h ($P < 0.01$; Figure 3D).

Corrected recoveries of orally supplied [U-¹³C]glucose dose as ¹³CO₂ were high (mean 77%) and were unaffected by treatment ($P = 0.30$; Table 3). The maximum ¹³CO₂ production was similar across the treatments and was unaffected by the experimental diets. The time to

maximum ¹³CO₂ production for the +PRO calves was 30 min shorter compared with CON- and +LAC-fed calves ($P < 0.05$), with values of +FAT calves being intermediate (Table 3).

DISCUSSION

We investigated the diurnal patterns of heat production, RQ, FHP, and net rates of carbohydrate and fat oxidation of Holstein-Friesian calves fed a MR supplemented with additional fat, lactose, or protein to better understand the regulation and metabolism of macronutrient supply in 21-d old calves. Our study shows that calves receiving a higher protein intake had the highest HP and RMR. The circadian patterns of RMR for CON, +FAT, and +PRO treatments followed a similar pattern over time before and after feeding. However, notable distinctions in energy metabolic profiles were observed among the treatments, particularly with an increase in RMR during the postprandial state. The +LAC treatment displayed larger within-day variations in RMR, indicating substantial lactose oxidation between meals. In contrast, the circadian RMR patterns for the other treatments consistently remained higher throughout the day. The +PRO treatment exhibited less variation after the morning meal compared with +FAT and +LAC treatments, possibly due to differences in abomasal emptying kinetics.

When pre-ruminant calves are fed MR that is formulated with skim milk, this can lead to a difference in the kinetics of protein and glucose digestion with variation in nutrient absorption. This is because casein, which accounts for 80% of the skim milk protein, coagulates in

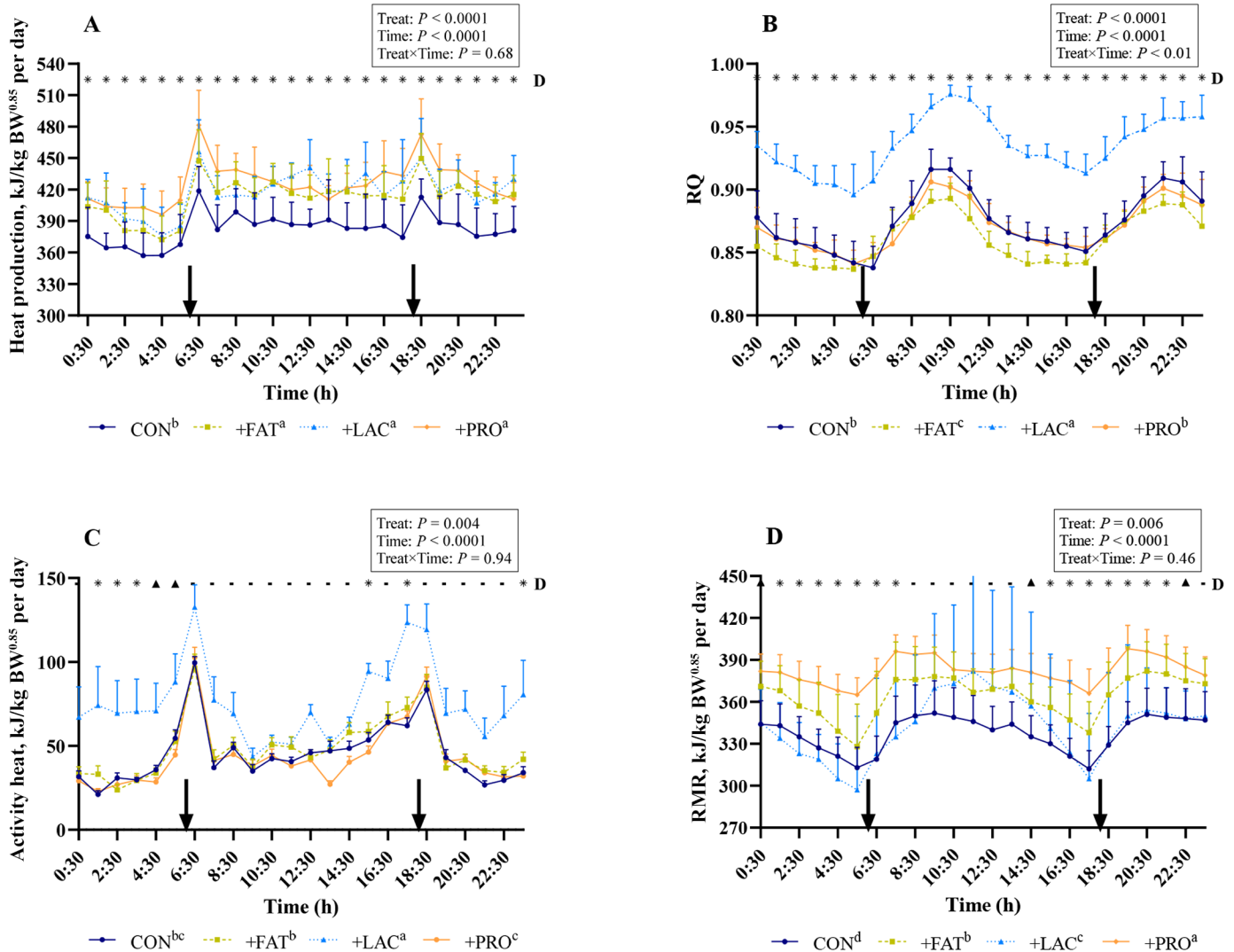


Figure 1. Circadian patterns of total heat production (A), RQ (B), activity-related heat production (C), and RMR (D) in young calves fed a milk replacer supplemented with additional fat, lactose, or protein at 21 d of age. Arrows represent feeding times. Values are LSM \pm pooled SD (* represents $P < 0.05$, \blacktriangle represents $0.05 < P < 0.1$, $-$ represents $P > 0.1$). D = dietary treatment effect. Treatments that do not share a common letter (a–d) differ ($P < 0.05$).

the abomasum and is gradually released during the day. In contrast, glucose and galactose are absorbed soon after milk ingestion, and their levels peak within 1 h after feeding (Verdonk et al., 1999). Lactose is rapidly hydrolyzed and absorbed, with a small quantity of triglyceride absorption (Gilbert et al., 2016). The remaining amount of the ingested nutrients is retained in the abomasal coagulum for a longer period and released more progressively (van den Borne et al., 2006; Ortigues et al., 1994).

Furthermore, calves in the +FAT, +PRO, and CON treatments exhibited a consistent pattern of physical activity before 6 p.m., displaying a gradual increase common to all treatments, possibly indicating the anticipation of a meal. No differences were observed between

the CON, +FAT, and +PRO groups. However, the +LAC group displayed higher activity levels. In the study by van den Borne et al. (2006), calves housed individually and fed MR with varying feeding frequencies and levels showed no significant effects on physical activity patterns. The proportion of HP spent on activity averaged 11% in that study, a value similarly observed for the +FAT, +PRO, and CON calves in our experiment (11%). The +LAC calves exhibited higher activity levels (18%) in comparison. Although in our study, the calves were housed in groups, we found similar values for H_{act} (as a percentage of total HP) as those reported by van den Borne et al. (2006). In group-housed pigs, 65% to 70% of the variation in HP within a day was related to physi-

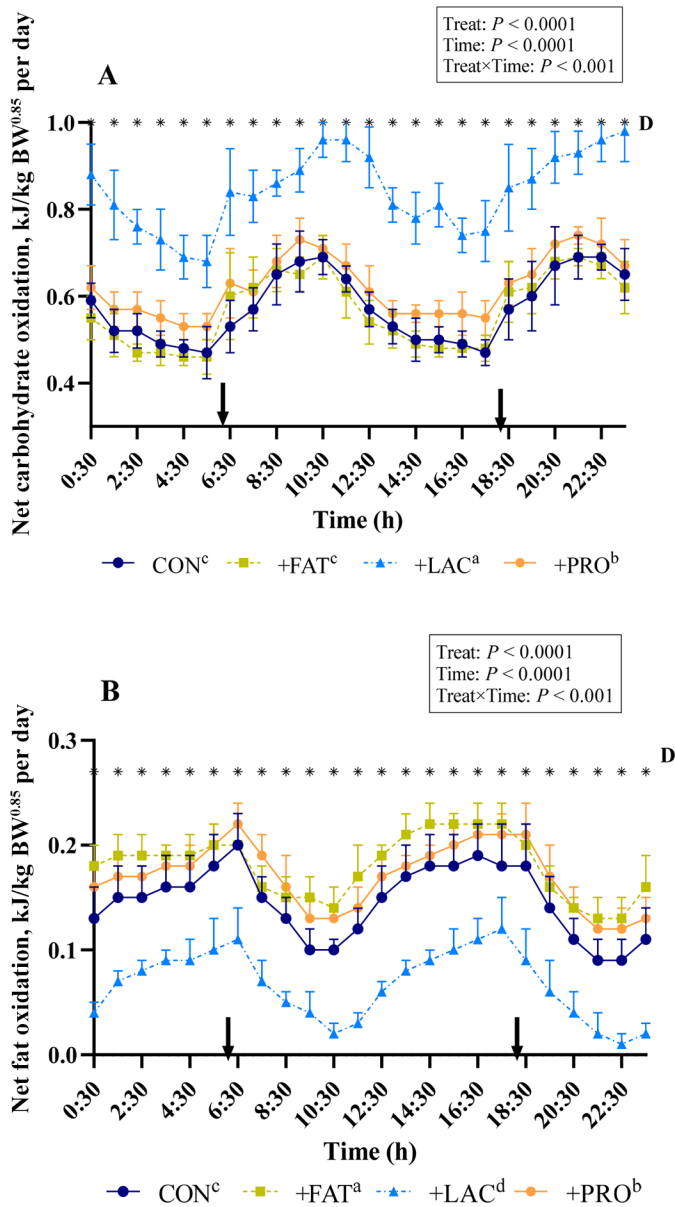


Figure 2. Circadian patterns of net carbohydrate (A) and fat oxidation (B) in young Holstein calves fed a milk replacer supplemented with additional fat, lactose, or protein at 21 d of age. Arrows represent feeding times. Values are LSM \pm pooled SD (* represents $P < 0.05$). D = dietary treatment effect. Treatments that do not share a common letter (a–d) differ ($P < 0.05$).

cal activity (van Erp et al., 2020). Moreover, it is worth noting that the difference in activity levels observed in +LAC calves persisted throughout the day and became even more pronounced during the night. Calves within the +LAC group exhibited the lowest fecal DM content at 14% and consistently had lower apparent total-tract digestibility of DM, crude fat, and CP compared with the other treatments (data shown in Amado et al., 2024). We attribute this to the high lactose concentration (60.2% of

DM) in their diet. This high concentration likely exceeds the absorptive capacities of the calves and affects nutrient digestibility. Despite the low fecal DM content of 14% in the +LAC group—indicative of softer feces—clinical diarrhea, typically associated with fecal DM below 10%, was not observed. The high lactose content may cause an osmotic effect in the intestines, yet it did not result in diarrhea (Hof, 1980). Although these calves did not present with clinical diarrhea, calves experiencing digestive issues might display signs of abdominal discomfort, such as kicking or increased vocalization, due to discomfort or distress (Stull and Reynolds, 2008). Furthermore, it has been reported that calves fed restricted amounts of MR exhibit higher non-nutritive sucking and engage in unrewarded visits to the automatic feeder, both described as behavioral signs of hunger (De Paula Vieira et al., 2008). In a recent study comparing the effects of high lactose or high fat MR on ad libitum calves, calves fed the high lactose formulation had ~41% more unrewarded visits to the automatic feeder (Echeverry-Munera et al., 2021). It may be possible that +LAC calves in our study were showing more hunger-related behaviors than the other calves, and this could influence H_{act} .

The RQ values reflect the net rates of oxidation of carbohydrates, fats, and proteins, and are ~1.0, 0.7, and 0.8, respectively, with slight variations depending on the specific nutrient source. These values are important because they provide insight into metabolic processes, such as lipogenesis and carbohydrate oxidation. When the RQ value exceeds 1.0, it suggests the simultaneous occurrence of lipogenesis and carbohydrate oxidation. An RQ exceeding 1.0, corresponding to negative FOX values, indicates that fatty acids are synthesized from carbohydrates (van den Borne et al., 2015). A high intake of energy from carbohydrates, mainly when animals are fed high levels of rapidly digestible sources of carbohydrates, such as in growing pigs, has been found to stimulate this process (Gerrits et al., 2012). Our study examined the effects of different treatments on RQ values and heat production in calves. Specifically, it was found that the circadian patterns of RQ and heat production followed the ingestion of meals for all treatments. Calves fed a MR with extra lactose had a higher RQ value (0.95) than those on other treatments, but it did not exceed 1.0. This finding suggests little synthesis of de novo fatty acids from glucose in the postprandial period because COX was almost complete. The increase in postprandial RQ coincided with reduced FOX and increased COX after the meals. Additionally, we observed that diurnal patterns for carbohydrate and fat oxidation were affected by treatment, with +LAC calves having the highest COX (14.31 kJ/d) and CON and +FAT having the lowest (9.66 and 9.76 kJ/d, respectively). However, it is important to note that the calculation of substrate oxidation from

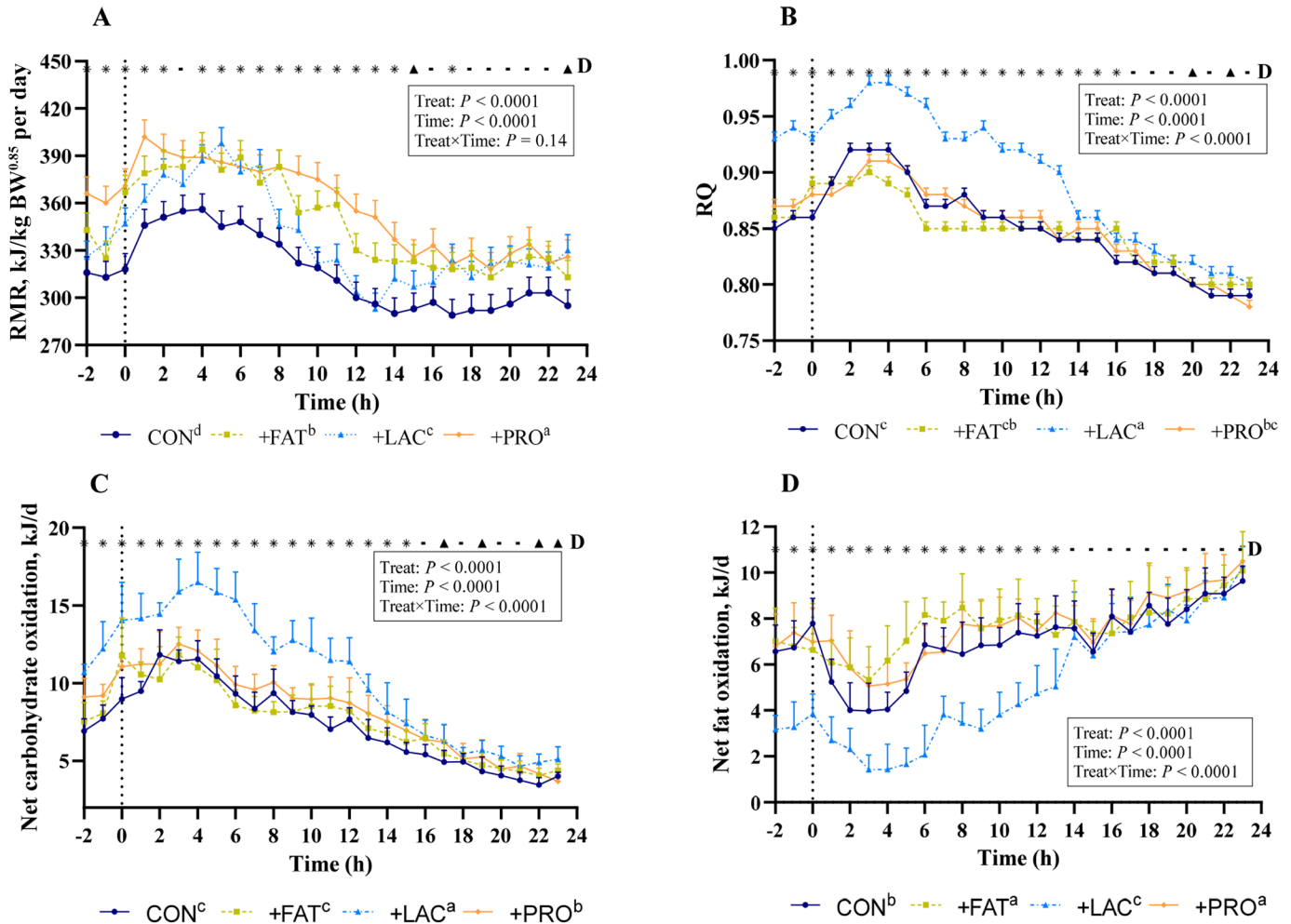


Figure 3. Twenty-four-hour patterns of RMR (A), RQ (B), net carbohydrate (C), and fat oxidation (D) during the fasting period in pair-housed, young Holstein calves following a single milk replacer meal supplemented with additional fat, lactose, or protein at 4 wk of age. Dotted lines represent feeding time and start time of the fasting period. Fasting heat production was calculated from these data as described in the text. Values are LSM \pm pooled SD (* represents $P < 0.05$, \blacktriangle represents $0.05 < P < 0.1$, – represents $P > 0.1$). Treatments that do not share a common letter (a–d) differ ($P < 0.05$).

indirect calorimetry may lead to an underestimation of COX and an overestimation of FOX due to the contribution of noncarbohydrate precursors to gluconeogenesis (van den Borne et al., 2015). To address this limitation, we used a stable isotope tracer in combination with indirect calorimetry to measure substrate oxidation. Specifically, we measured breath $^{13}\text{CO}_2$ excretion and calculated the proportion of the oxidized substrate, corrected for bicarbonate sequestration (van Hall, 1999). We found that the recovery of [^{13}C]bicarbonate averaged 86.9%, after an intravenous single dose, independently of the diet. This value is higher than the one reported by van den Borne et al. (2007), where calves with different feeding level of MR had a recovery of [^{13}C]bicarbonate of 72%. Additionally, the average recovery rate of 77% for [U- ^{13}C]glucose as $^{13}\text{CO}_2$ suggests that ingested carbohydrates

are predominantly oxidized independent of the diet. Although glucose oxidation by the tracer method was higher than that calculated from the gas exchange, this can be explained by the contribution of gluconeogenesis to gas exchange at a low RQ and that of lipogenesis at a high RQ (van den Borne et al., 2015). Based on the high recovery rate of [U- ^{13}C]glucose as $^{13}\text{CO}_2$ and the high COX, little fat deposition appears to be from carbohydrates, which is consistent with the low fat oxidation found after feeding. This scenario is also true for milk-fed heavy calves (150 kg of BW) as shown by van den Borne et al. (2007), where dietary glucose was almost completely oxidized (80% based on [^{13}C]glucose and 94% from indirect calorimetry measurements), regardless of feeding level. Furthermore, Pantophlet et al. (2016) conducted a comparison between the effects of a

Table 3. Recovery of ^{13}C from $[\text{U-}^{13}\text{C}]$ glucose, provided with the afternoon meal, in exhaled carbon dioxide in calves at 21 d of age fed a milk replacer supplemented with additional fat, lactose, or protein

Item	Treatment ¹				SEM ²	P-value
	CON	+FAT	+LAC	+PRO		
$[\text{U-}^{13}\text{C}]$ glucose oxidation						
Recovery as $^{13}\text{CO}_2$, % of dose	67.2	65.7	69.2	66.1	1.55	0.38
Maximum $^{13}\text{CO}_2$ production, % of dose/h	6.7	6.5	7.0	7.3	0.23	0.09
Time to peak, min	351 ^a	335 ^{ab}	352 ^a	318 ^b	8.4	0.03
^{13}C sodium bicarbonate						
Recovery as $^{13}\text{CO}_2$, % of dose	76.3	89.1	93.6	88.6	5.72	0.30
Maximum $^{13}\text{CO}_2$ production, % of dose/h	22.5	22.7	25.3	23.2	2.36	0.81
Time to peak, min	111	123	97	109	9.0	0.47
$[\text{U-}^{13}\text{C}]$ glucose oxidation						
Recovery as $^{13}\text{CO}_2$, % of dose, corrected for carbon sequestration ³	77.2	75.5	79.6	76.0	1.78	0.37

^{a,b}Means that do not share a common letter differ ($P < 0.05$).

¹CON = 23.3% CP, 21.2% crude fat, and 48.8% lactose on a DM basis, provided at 550 kJ/kg BW^{0.85} per day; or the basal MR incrementally supplied with 125 kJ of DE/BW^{0.85} per day as milk fat (+FAT), lactose (+LAC), or milk protein (+PRO).

²Pooled SEM.

³Corrected for the average ^{13}C sodium bicarbonate recovery as $^{13}\text{CO}_2$, percentage of dose.

high lactose (59% DM) and high fat (30% DM) MR on heavy milk-fed calves (120 kg BW), analyzing glucose homeostasis and insulin sensitivity, alongside energy and nitrogen balance measurements (unpublished data). In these unpublished results, the RQ of calves in the high lactose treatment, temporarily exceeded 1 during certain periods of the day. This coincided with an increase in the expression of genes related to lipogenesis in adipose tissue, liver, and muscle (as evidenced by mRNA expression of genes DGAT1-DGAT2). These findings suggest that calves have the capability to store glucose as body fat when exposed to high lactose intakes. However, calves in the +LAC treatment stored very little lactose as fat, even though they should theoretically be in an insulin-sensitive phase and exposed to high lactose intake (60% DM). Our findings are consistent with the conclusions of the NRC (NRC, 2021), indicating that pre-ruminant calves use glucose similarly to adult ruminants, with minimal utilization of glucose for fatty acid synthesis and a significant amount used to fuel protein accretion.

Furthermore, the rate of nutrient delivery to the lower gut after a meal has been proposed to be a critical determinant of blood glucose regulation (Tong and D'Alessio, 2014; Gerrits et al., 2008). Although we have not studied insulin metabolism in these calves, the oxidation patterns in +LAC calves suggest that more lactose entered the small intestine per unit of time, causing a greater increase in postprandial glucose and insulin concentrations in plasma (Welboren et al., 2021; Palmquist et al., 1992). Others have observed that prolonged feeding of a predominantly milk diet with high protein intake is associated with the development of hyperglycemia, hyperinsulinemia, and glucosuria in older milk-fed calves

(Gerrits et al., 2008; Hugi et al., 1998). Pantophlet et al. (2016) showed that during early life, insulin sensitivity has a significant decrease that is not influenced by diet composition. This reduction in sensitivity results in a change in the available substrate but does not prevent oxidative carbohydrate metabolism. Further research is needed to elucidate the mechanisms underlying the effects of nutrient intake on glucose metabolism and insulin regulation in very young milk-fed calves.

This paper presents new data from a study that was reported previously by Amado et al. (2024), which reported the incremental efficiencies of energy and protein deposition in very young calves (21 d old). In that study, calves were fed with a MR incrementally supplemented with isoenergetic amounts of fat, lactose, or protein. The study found differences between the additional protein supply and the supply of lactose or fat in terms of incremental efficiencies for protein deposition, and these distinctions could potentially be attributed to variations in fat or carbohydrate oxidation. Specifically, calves in the +PRO treatment were more efficient at protein deposition and had a higher FOX rate than those in the CON or +LAC treatments. Additionally, the study found that body fat deposition increased when fat or lactose supply increased. However, our results in FOX suggest that additional lactose supply reduces fatty acid oxidation rather than fatty acid deposition.

In our experiment, we used nonlinear regression modeling to estimate FHP, which involved calculating the horizontal asymptotic value of the decreasing HP kinetics during starvation and excluding the energy expenditure associated with physical activity. By using this method, we obtained an estimate of FHP that was

not influenced by any behavioral disturbances that may have occurred during the starvation period (Gerrits et al., 2015; Labussière et al., 2011). No significant differences were observed in the estimation of FHP among the treatments, indicating that additional supply of fat, lactose, or protein did not affect the overall metabolic rate of the calves. These values agree with previously observed estimates by Labussière et al. (2011) for veal calves with a BW of 73 kg and fed with different levels of MR. They obtained FHP values ranging from 276 and 310 kJ/(kg of BW^{0.85}/d). As concluded by Labussière et al. (2011), the FHP value can be considered an estimate of the net energy requirement for maintenance and is independent of diet composition. However, studies involving pigs showed that FHP increased with higher feeding levels before the onset of fasting. This increase could potentially be linked to the occurrence where previous feeding leads to an increase in organ mass, particularly metabolically active tissues such as intestinal tissues (Labussière et al., 2011), as also numerically observed in our study. In our trial, FHP was determined over a 23-h fasting period following a morning meal. Although this fasting period was relatively short, and the values did not reach a plateau, a longer period of fasting may increase abnormal behaviors that can affect the results (Labussière et al., 2008).

Furthermore, during the fasting period measurements, the hourly means of RQ were significantly higher in the +LAC group after the last meal, indicating a greater utilization of carbohydrates as the primary substrate for oxidation in pre-ruminants calves (Kuhla et al., 2015). This finding is consistent with the higher hourly means of net rates of COX observed in the +LAC treatment between 0600 and 2200 h and the decrease in FOX during the same hours, demonstrating a reciprocal relationship between these measurements (Kuhla et al., 2015). After the meal, FOX declined for all treatments, with similar values in +FAT and +PRO calves without reaching a negative value. This finding indicates that the utilization of fat as an energy source was higher in these treatments, potentially due to differences in the availability and utilization of lipid substrates. However, after 2200 h, when RQ decreased and reached a value of 0.80 for all treatments, the hourly means of net rates of FOX followed the same pattern for all treatments. Between 3 to 4 h after the meal, FOX started to increase again and remained increasing until the end of the fasting period. This indicates a shift in energy substrate utilization toward lipid oxidation during the later stages of fasting, regardless of the treatment.

CONCLUSIONS

In our study, the additional supply of lactose was observed to increase fat retention in young calves. This

increase was not primarily attributed to an increase in fatty acid synthesis from glucose but was predominantly caused by a reduction in the oxidation of fatty acids. Moreover, the provision of extra lactose led to an increase in RMR during the postprandial state, whereas the supplementation of fat or protein resulted in a persistent increase of RMR throughout the day. Glucose oxidation remained almost complete regardless of nutrient supplementation. Calves that were fed a MR with additional milk protein showed the highest HP, surpassing similar intakes of fat and lactose. This increase in HP was partly due to the oxidation of fatty acids. Additionally, the FHP of young, group-housed calves was found to be comparable to values in the literature, and this parameter appeared to be independent of energy intake. Overall, our results underscore the importance of considering the macronutrient profile of MR, as changes in nutrient supply can significantly affect energy expenditure patterns in calves. These findings have practical implications for optimizing calf nutrition and management strategies.

NOTES

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Nonstandard abbreviations used: BW^{0.85} = metabolic BW; CON = control diet; COX = the net rate of

carbohydrate oxidation; DE = digestible energy; +FAT = control diet supplemented with butterfat; FHP = fasting heat production; FOX = the net rate of fat oxidation; H_{act} = heat production related to physical activity; HP = heat production; +LAC = control diet supplemented with lactose; MR = milk replacer; +PRO = control diet supplemented with protein; RMR = resting metabolic rate; RMR_{max} = resting metabolic rate maximum; RQ = respiration quotient; WM = whole milk.

REFERENCES

- Alferink, S. J. J., J. van den Borne, W. J. J. Gerrits, S. C. W. Lammers-Wienhoven, and M. J. W. Heetkamp. 2003. On-line, continuous determination of $^{13}CO_2/^{12}CO_2$ ratios by non-dispersive infrared absorption in indirect calorimetry facilities. Pages 465–468 in *Progress in Research on Energy and Protein Metabolism*. W. B. Souffrant and C. C. Metges, ed. EAAP Publication no. 109. Wageningen Academic Publishers.
- Amado, L., L. N. Leal, H. Berends, P. van Keulen, J. Martín-Tereso, and W. J. J. Gerrits. 2024. Responses to incremental nutrient supply on energy and protein metabolism in pre-weaning dairy calves. *J. Dairy Sci.* 107:5616–5625. <https://doi.org/10.3168/jds.2024-24338>.
- Bach, A., M. Terré, and A. Pinto. 2013. Performance and health responses of dairy calves offered different milk replacer allowances. *J. Dairy Sci.* 96:7790–7797. <https://doi.org/10.3168/jds.2013-6909>.
- Berends, H., J. J. G. C. Van den Borne, S. J. J. Alferink, C. G. Van Renen, E. A. M. Bokkers, and W. J. J. Gerrits. 2012. Low-protein solid feed improves the utilization of milk replacer for protein gain in veal calves. *J. Dairy Sci.* 95:6654–6664. <https://doi.org/10.3168/jds.2012-5463>.
- Bergen, W. G., and H. J. Mersmann. 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. *J. Nutr.* 135:2499–2502. <https://doi.org/10.1093/jn/135.11.2499>.
- Brouwer, E. 1965. Report of sub-committee on constants and factors. Pages 441–443 in *Energy Metabolism*. K. L. Blaxter, ed. Academic Press.
- Davis Rincker, L. E., M. J. VandeHaar, C. A. Wolf, J. S. Liesman, L. T. Chapin, and M. S. Weber Nielsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. *J. Dairy Sci.* 94:3554–3567. <https://doi.org/10.3168/jds.2010-3923>.
- De Paula Vieira, A., V. Guesdon, A. M. de Passillé, M. A. G. von Keyserlingk, and D. M. Weary. 2008. Behavioural indicators of hunger in dairy calves. *Appl. Anim. Behav. Sci.* 109:180–189. <https://doi.org/10.1016/j.applanim.2007.03.006>.
- Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. *J. Dairy Sci.* 84:830–842. [https://doi.org/10.3168/jds.S0022-0302\(01\)74541-9](https://doi.org/10.3168/jds.S0022-0302(01)74541-9).
- Drackley, J. K. 2008. Calf nutrition from birth to breeding. *Vet. Clin. North Am. Food Anim. Pract.* 24:55–86. <https://doi.org/10.1016/j.cvfa.2008.01.001>.
- Drenth, P. J. 2011. A European code of conduct for research integrity. *Promoting Research Integrity in a Global Environment*. 2012:161. Accessed Jul. 25, 2020. <https://www.researchgate.net/publication/265237063>.
- Echeverry-Munera, J., L. N. Leal, J. N. Wilms, H. Berends, J. H. Costa, M. Steele, and J. Martín-Tereso. 2021. Effect of partial exchange of lactose with fat in milk replacer on ad libitum feed intake and performance in dairy calves. *J. Dairy Sci.* 104:5432–5444. <https://doi.org/10.3168/jds.2020-19485>.
- Gelsinger, S. L., A. J. Heinrichs, and C. M. Jones. 2016. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. *J. Dairy Sci.* 99:6206–6214. <https://doi.org/10.3168/jds.2015-10744>.
- Gerrits, W. J. J., M. W. Bosch, and J. J. G. C. Van den Borne. 2012. Quantifying resistant starch using novel, in vivo methodology and the energetic utilization of fermented starch in pigs. *J. Nutr.* 142:238–244. <https://doi.org/10.3945/jn.111.147496>.
- Gerrits, W. J. J., G. H. Tolman, J. W. Schrama, S. Tamminga, M. W. Bosch, and M. W. A. Verstegen. 1996. Effect of protein and protein free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight. *J. Anim. Sci.* 74:2129–2139. <https://doi.org/10.2527/1996.7492129x>.
- Gerrits, W. J. J., J. J. G. C. van den Borne, and J. W. Blum. 2008. Low-dietary protein intake induces problems with glucose homeostasis and results in hepatic steatosis in heavy milk-fed calves. *Domest. Anim. Endocrinol.* 35:121–129. <https://doi.org/10.1016/j.domaniend.2007.10.002>.
- Gerrits, W. J. J., J. J. G. C. Van den Borne, and E. Labussière. 2015. Deriving heat production from gaseous exchange: Validity of the approach. Pages 19–34 in *Indirect Calorimetry. Techniques, Computations and Applications*. W. J. J. Gerrits, and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Gilbert, M. S., A. J. Pantophlet, J. J. G. C. van den Borne, W. H. Hendriks, H. A. Schols, and W. J. J. Gerrits. 2016. Effects of replacing lactose from milk replacer by glucose, fructose, or glycerol on energy partitioning in veal calves. *J. Dairy Sci.* 99:1121–1132. <https://doi.org/10.3168/jds.2015-10062>.
- Heetkamp, M. J. W., S. J. J. Alferink, T. Zandstra, P. Hendriks, H. van den Brand, and W. J. J. Gerrits. 2015. Design of climate respiration chambers, adjustable to the metabolic mass of subjects. Pages 35–56 in *Indirect Calorimetry*. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Hof, G. 1980. An investigation into the extent to which various dietary components, particularly lactose, are related to the incidence of diarrhoea in milk-fed calves. PhD thesis. Wageningen University, Wageningen, the Netherlands.
- Hugi, D., R. M. Bruckmaier, and J. W. Blum. 1997. Insulin resistance, hyperglycemia, glucosuria, and galactosuria in intensively milk-fed calves: Dependency on age and effects of high lactose intake. *J. Anim. Sci.* 75:469–482. <https://doi.org/10.2527/1997.752469x>.
- Hugi, D., L. Tappy, H. Sauerwein, R. M. Bruckmaier, and J. W. Blum. 1998. Insulin-dependent glucose utilization in intensively milk-fed veal calves is modulated by supplemental lactose in an age-dependent manner. *J. Nutr.* 128:1023–1030. <https://doi.org/10.1093/jn/128.6.1023>.
- Jasper, J., and D. M. Weary. 2002. Effects of ad libitum milk intake on dairy calves. *J. Dairy Sci.* 85:3054–3058. [https://doi.org/10.3168/jds.S0022-0302\(02\)74391-9](https://doi.org/10.3168/jds.S0022-0302(02)74391-9).
- Kuhla, B., M. Derno, and C. C. Metges. 2015. Indirect calorimetry for elucidating dynamics in energy metabolism of farm animals. Pages 35–56 in *Indirect Calorimetry: Techniques, Computations and Applications*. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers.
- Labussière, E., S. Dubois, J. van Milgen, G. Bertrand, and J. Noblet. 2008. Effects of dietary crude protein on protein and fat deposition in milk-fed veal calves. *J. Dairy Sci.* 91:4741–4754. <https://doi.org/10.3168/jds.2008-1203>.
- Labussière, E., J. van Milgen, C. F. de Lange, and J. Noblet. 2011. Maintenance energy requirements of growing pigs and calves are influenced by feeding level. *J. Nutr.* 141:1855–1861. <https://doi.org/10.3945/jn.111.141291>.
- Laliots, G. P., I. Bizelis, and E. Rogdakis. 2010. Comparative approach of the de novo fatty acid synthesis (lipogenesis) between ruminant and non ruminant mammalian species: from biochemical level to the main regulatory lipogenic genes. *Curr. Genomics* 11:168–183. <https://doi.org/10.2174/138920210791110960>.
- NRC. 2021. *Nutrient Requirements of Dairy Cattle*. 8th rev. ed. Natl. Acad. Press.
- Ortigue, I., C. Martin, M. Vermorel, and Y. Anglaret. 1994. Energy cost of standing and circadian changes in energy-expenditure in the pre-ruminant calf. *J. Anim. Sci.* 72:2131–2140. <https://doi.org/10.2527/1994.7282131x>.
- Palmquist, D. L., J. Doppenberg, K. L. Roehrig, and D. J. Kinsey. 1992. Glucose and insulin metabolism in ruminating and veal calves fed

- high and low fat diets. *Domest. Anim. Endocrinol.* 9:233–241. [https://doi.org/10.1016/0739-7240\(92\)90037-X](https://doi.org/10.1016/0739-7240(92)90037-X).
- Pantophlet, A. J., M. S. Gilbert, J. J. G. C. Van Den Borne, W. J. J. Gerrits, M. G. Priebe, and R. J. Vonk. 2016. Insulin sensitivity in calves decreases substantially during the first 3 months of life and is unaffected by weaning or fructo-oligosaccharide supplementation. *J. Dairy Sci.* 99:7602–7611. <https://doi.org/10.3168/jds.2016-11084>.
- Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bate-man II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, and G. E. Goodier. 2002. Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. *J. Dairy Sci.* 85:2335–2343. [https://doi.org/10.3168/jds.S0022-0302\(02\)74313-0](https://doi.org/10.3168/jds.S0022-0302(02)74313-0).
- Stull, C., and J. Reynolds. 2008. Calf welfare. *Vet. Clin. North Am. Food Anim. Pract.* 24:191–203. <https://doi.org/10.1016/j.cvfa.2007.12.001>.
- Tong, J., and D. D'Alessio. 2014. Give the receptor a brake: Slowing gastric emptying by GLP-1. *Diabetes* 63:407–409. <https://doi.org/10.2337/db13-1764>.
- van den Borne, J. J. G. C., M. J. W. Heetkamp, S. J. J. Alferink, and W. J. J. Gerrits. 2015. Moving from a complete energy balance towards substrate oxidation: Use of stable isotopes. Pages 87–114 in *Indirect Calorimetry. Techniques, Computations and Applications*. W. J. J. Gerrits and E. Labussière, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- van den Borne, J. J. G. C., G. E. Lobley, M. W. A. Verstegen, J. M. Muij-laert, S. J. J. Alferink, and W. J. J. Gerrits. 2007. Body fat deposition does not originate from carbohydrates in milk-fed calves. *J. Nutr.* 137:2234–2241. <https://doi.org/10.1093/jn/137.10.2234>.
- van den Borne, J. J. G. C., J. M. A. J. Verdonk, J. W. Schrama, and W. J. J. Gerrits. 2006. Reviewing the low efficiency of protein utilization in heavy prerinant calves—A reductionist approach. *Reprod. Nutr. Dev.* 46:121–137. <https://doi.org/10.1051/rnd:2006011>.
- van Erp, R. J. J., S. de Vries, T. van Kempen, L. A. Den Hartog, and W. J. J. Gerrits. 2020. Circadian misalignment imposed by nocturnal feed-ing tends to increase fat deposition in pigs. *Br. J. Nutr.* 123:529–536. <https://doi.org/10.1017/S0007114519003052>.
- van Erp, R. J. J., H. M. J. van Hees, R. T. Zijlstra, T. van Kempen, J. B. van Klinken, and W. J. J. Gerrits. 2018. Reduced feed intake, rather than increased energy losses, explains variation in growth rates of normal-birth-weight piglets. *J. Nutr.* 148:1794–1803. <https://doi.org/10.1093/jn/nxy200>.
- van Hall, G. 1999. Correction factors for 13C-labelled substrate oxidation at whole-body and muscle level. *Proc. Nutr. Soc.* 58:979–986. <https://doi.org/10.1017/S0029665199001299>.
- Van Klinken, J. B., S. A. A. van den Berg, L. M. Havekes, and K. Wil- lems Van Dijk. 2012. Estimation of activity related energy expendi- ture and resting metabolic rate in freely moving mice from indirect calorimetry data. *PLoS One* 7:e36162. <https://doi.org/10.1371/ journal.pone.0036162>.
- Verdonk, J. M. A. J., W. J. J. Gerrits, G. M. Beelen, and A. J. M. Jans- man. 1999. Effect of protein source on portal nutrient fluxes in pre- ruminant calves. Page 47 in *The VIIIth International Symposium on Protein Metabolism and Nutrition*. G. E. Lobley, A. White, and J. C. MacRae, ed. Wageningen Pers, the Netherlands.
- Welboren, A. C., B. Hatew, J. B. Renaud, L. N. Leal, J. Martín-Tereso, and M. A. Steele. 2021. Intestinal adaptations to energy source of milk replacer in neonatal dairy calves. *J. Dairy Sci.* 104:12079– 12093. <https://doi.org/10.3168/jds.2021-20516>.

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