J. Dairy Sci. 107:184–201
https://doi.org/10.3168/jds.2023-23368

Postprandial metabolism and gut permeability in calves fed milk replacer with different macronutrient profiles or a whole milk powder

J. N. Wilms,[1,2]* M. H. Ghaffari,[3] P. Stahel Darani,[4] M. Jansen,[1,5] H. Sauerwein,[3] M. A. Steele,[2] J. Martín-Tereso,[1] and L. N. Leal[1]
[1]Trouw Nutrition R&D, 3800 AG, Amersfoort, the Netherlands
[2]Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada N1G 1W2
[3]Institute of Animal Science, University of Bonn, 53115 Bonn, Germany
[4]Mad Barn Inc., Kitchener, ON, Canada N2R 1H2
[5]Animal Nutrition Group, Wageningen University, 6700 AH Wageningen, the Netherlands

ABSTRACT

Significant differences exist in the composition of current milk replacers (MR) and bovine whole milk. This study investigated how the macronutrient profile of 3 different MR formulations containing varying amounts of fat, lactose, and protein, and a whole milk powder (WP), affect postprandial metabolism and gut permeability in male Holstein calves. Sixty-four calves (45.4 ± 4.19 kg [mean ± SD] and 1.8 ± 0.62 d of age) were blocked in order of arrival to the facility and within each block, calves were randomly assigned to 1 of 4 treatments. Treatments included a high-fat MR (HF: 25.0% dry matter [DM] fat, 22.5% protein, 38.6% lactose; n = 14), a high-lactose MR (HL: 44.6% lactose, 22.5% protein, 18.0% fat; n = 17), a high-protein MR (HP: 26.0% protein, 18.0% fat, 41.5% lactose; n = 17), and WP (26.0% fat, 24.5% protein, 38.0% lactose; n = 16). Calves were fed 3.0 L (135 g/L) 3 times daily at 0600, 1200, and 1800 h with a teat bucket. Milk intake was recorded daily for the first 28 d after arrival, and blood sampling and body weight measurements were performed at arrival and on d 7, 14, 21, and 27. Gut permeability was estimated from fractional urinary excretion of indigestible markers (Cr-EDTA, lactulose, and D-mannitol) administered as a single dose on d 21 instead of the morning milk meal. Digestibility was determined simultaneously from a total collection of feces over 24 h. Postprandial dynamics were measured on d 28 by sequential blood sampling over 7.5 h. Dry matter intake of MR over 28 d was slightly greater in calves fed HL and HP than in WP. Recovery of Cr-EDTA and D-mannitol over a 24-h urine collection was greater in calves fed WP and HP than HL calves. Apparent total-tract digestibility of crude ash, protein, and fat did not differ among treatments; however, DM digestibility was lower in calves fed WP than in other treatment groups. In addition, abomasal emptying, as indicated by the area under the curve (AUC) for acetaminophen, was slower in calves fed WP than in calves fed HF and HL. The AUC for postprandial plasma glucose was lower in calves fed HL than WP and HF and lower in calves fed HP than WP. The AUC for postprandial serum insulin was greater in calves fed HP than WP and HF, whereas calves fed HL did not differ from the other treatments. Postprandial triglycerides were greater in calves fed WP, and postprandial adiponectin was higher in calves fed HL than other treatments. The high content of lactose and protein in MR had a major effect on postprandial metabolism. This raises the possibility of optimizing MR formulations to maintain metabolic homeostasis and influence development.

Key words: calf, macronutrient profile, milk replacer, postprandial metabolism

INTRODUCTION

Compared with bovine whole milk (**WM**), most milk replacers (**MR**) for calves currently contain higher levels of lactose (~45 vs. 35% DM) and lower levels of fat (~16 vs. 30%; Echeverry-Munera et al., 2021; Wilms et al., 2022). Although protein levels in MR and WM are rather similar, high-protein MR formulations (≥26%) are also available (Daniels et al., 2008; USDA, 2011). Low-fat formulations (<20%) for calves seem to result from the historical availability of whey and lean growth objectives set for calves. This practice has been supported by studies showing increased body fat deposition in response to the higher fat content in MR (Tikofsky et al., 2001; Bartlett et al., 2006; Hill et al., 2008). Increased fat deposition in the mammary glands of postweaning heifers has been negatively associated with milk production (Swanson, 1960; Sejrsen and Purup, 1997; Van Amburgh et al., 2019), but there is currently no evidence suggesting that fat deposition in the mammary glands of preweaning calves has adverse

Received February 12, 2023.
Accepted June 4, 2023.
*Corresponding author: juliette.wilms@trouwnutrition.com

effects on subsequent milk production. In fact, a high plane of nutrition in the preweaning phase has been associated with an increase in mammary parenchyma mass, which may partly explain differences in future milk yield (Geiger, 2019).

An imbalanced macronutrient profile in MR has implications for neonatal metabolism. Prolonged feeding of MR with high lactose content (up to 55% DM) resulted in impaired glucose homeostasis and insulin sensitivity in veal calves (Hugi et al., 1997). However, at shorter feeding durations, MR with high lactose (44 to 46% lactose) fed twice daily did not affect the insulin sensitivity of calves of 4 d (Welboren et al., 2021a) and 4 wk of age (Stahel et al., 2019). Likewise, a high protein intake from infant formula may increase the concentration of insulin-releasing AA, which may stimulate secretion of insulin and IGF-1 (Luque et al., 2016). While this promotes enhanced growth during infancy (Koletzko et al., 2009), it also elevates the risk of obesity and related metabolic disorders later in life (Michaelsen and Greer, 2014). In calves, Wilms et al. (2022) reported higher serum IGF-I in the blood of calves fed MR with high lactose and high protein compared with calves fed MR high in fat. These collective findings in infants and calves suggest that glucose-insulin homeostasis could be altered when feeding high-protein and high-lactose MR for a prolonged duration at high plane of nutrition.

Increasing the fat intake from liquid feed reduced the number of therapeutic interventions (Berends et al., 2020), the percentage of abnormal fecal scores (Amado et al., 2019), and mortality in preweaning calves (Urie et al., 2018). In contrast, previous work reported an increase in gut permeability assessed by fractional recovery of indigestible markers when feeding MR high in fat (up to 25% fat) compared with high-lactose MR (Amado et al., 2019; Welboren et al., 2021b). This was attributed to a lower mRNA expression of genes related to tight junction proteins or to an increase in chylomicron synthesis in response to an HF diet (Amado et al., 2019; Welboren et al., 2021b). Nevertheless, no established permeability reference values exist for calves fed WM, so the biological relevance of these differences remains unclear.

Breastfed infants are the gold standard for growth, health, and development in infant nutrition. Likewise, WM likely has an optimal nutrient composition; thus, formulating MR closer to WM could benefit calf health and metabolism. However, studying calves fed WM is challenging because the composition and quality of WM varies widely. Studies examining feeding of WM or MR showed improved growth in WM-fed calves (Moallem et al., 2010; Zhang et al., 2019), a different metabolic profile (Lepczyński et al., 2015; Kesser et al., 2017; Wilms et al., 2022), and differences in diarrhea incidence (Bascom et al., 2007; Bowen Yoho et al., 2013). Although extensive data are available in MR-fed calves, postprandial metabolism and gut permeability have not been studied in WM-fed calves.

It was hypothesized that feeding a MR high in fat would lead to similar postprandial dynamics and gut permeability values as feeding WM, whereas feeding MR with a high lactose or protein content would lead to different results. Therefore, this study aimed to investigate how the macronutrient profile in MR affects postprandial metabolism and gastrointestinal health of male dairy calves fed 3 times daily in the first 4 wk of life.

MATERIALS AND METHODS

This study was conducted at the Calf Research Facility of Trouw Nutrition Research & Development (Sint Anthonis, the Netherlands) between April and July 2019. All procedures described in this article complied with the Dutch Law on Experimental Animals, which complies with 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), under project application code AVD2040020173425.

Animals and Experimental Design

This experiment was conducted in a randomized complete block design. A total of 68 male Holstein Friesian calves were purchased at birth from 7 neighboring dairy farms. A standardized protocol for colostrum management was followed on the farm of origin, which included 3 feedings of colostrum in the first 24 h: 3.0 to 4.0 L within the first 3 h after birth, followed by 2 feedings of 2.0 L. Colostrum quality was monitored at the farm of origin, and a Brix value of 22% or greater, indicating an IgG content of 50 g/L or greater (NAHMS, 2007), was required. Thereafter, meals of 3.0 L of MR containing 135 g/L (13.5% solids; Sprayfo Excellent, Trouw Nutrition, Deventer, the Netherlands) were offered twice daily until the day of collection. Calves were brought to the research facility between 1 and 3 d after birth, and blood IgG concentrations were measured within 48 to 72 h after birth using a portable Multi-Test Analyzer (model RHB-32ATC; Westover Scientific, Mill Creek, WA) to monitor successful colostrum administration. The mean BW at arrival was 45.4 ± 4.19 kg (mean ± SD), and the age was 1.8 ± 0.62 d. On arrival, calves were assigned to 1 of 17 blocks based on the day of arrival and the day of birth to minimize age differences within a block. Within each block, calves were randomly assigned to 1 of 4 treatments (17 calves per treatment

group) and were exposed to their respective diet up to 4 wk after arrival. Randomization was performed using the random function [RANDBETWEEN (0,100000)] in Microsoft Excel (Microsoft 365 MSO, Version 2212, Build 16.0.15928.20278; Microsoft Corporation, Redmond, WA). Treatment allocation was performed by an individual who was not involved in treatment administration or sampling. Treatments were blinded to animal caretakers by randomly assigning a letter (A, B, C, or D) to each treatment. Health was monitored daily, and a standardized protocol was followed in case of disease. Administration of medical treatments and oral rehydration solution (Sprayfo OsmoFit, Trouw Nutrition, Burgheim, Germany) was recorded. Four calves were removed from the study after admission due to severe diarrhea requiring intravenous administration of bicarbonate and saline solutions (3 calves) and critically low milk intake (1 calf). One calf belonged to the WM powder treatment group, and 3 calves to the high-fat MR treatment. Calves were removed during the second (1 calf) and third (3 calves) weeks of the experiment, and incomplete data collected from these animals were not included in the statistical analysis.

Treatments included a high-fat MR (**HF**: 25.0% fat, 22.5% protein, 38.6% lactose; n = 14), a high-lactose MR (**HL**: 44.6% lactose, 22.5% protein, 18.0% fat; n = 17), a high-protein MR (**HP**: 26.0% protein, 18.0% fat, 41.5% lactose; n = 17), and a WM powder (**WP**: 26.0% fat, 24.5% protein, 38.0% lactose; n = 16). Ingredients and analyzed nutrient composition of MR and the WP treatment (whole milk powder 26%, Arla Foods, Denmark) are presented in Table 1. Each MR treatment (HF, HL, and HP) included the same raw materials but varying inclusion of fat, protein, and lactose (Trouw Nutrition, Deventer, the Netherlands). Skim milk powder accounted for 50% (% product) of each MR formula. The HF treatment was designed to be close to WP in terms of fat content, whereas the HL and HP treatments aimed to represent formulation strategies currently proposed in Europe and North America, respectively. To define these formulations, fat, lactose, and protein were exchanged on a weight-to-weight basis (wt/wt). The fat concentrate used was based on spray-dried fat kernels, with 65% fat derived from palm fat and 35% from coconut fat. In all 3 MR formulas, the percentage of solids was 13.5% to standardize the ash percentage across MR formulations and to remain close to industrial conditions. In addition, this allowed isonitrogenous comparisons between HL and HF, which would not be possible if the concentrations of MR were adjusted to ME density. Milk replacer and WP were prepared using a milk shuttle (Urban MS100 Wüsting Germany), reconstituted with water at a concentration of 135 g/L, and supplied in a teat bucket at 40°C. The concentration was chosen to be close to the percentage of solids of bovine WM. Treatments were administered daily through teat buckets in 3 equal meals of 3.0 L at 0600, 1200, and 1800 h, and calves were allowed to drink their milk meal for 15 min. Water was available ad libitum through plain buckets, and no solid feeds were offered during the experimental period. Calves were purchased in successive batches and incorporated into the study as the blocks were set, and measurement periods were staggered accordingly.

Table 1. Ingredient and analyzed nutrient composition of milk replacers and whole milk powder fed to calves 3.0 L, 3 times daily

	Treatment[1]			
Item	WP	HF	HL	HP
Ingredient (% product)				
Skim milk powder	—	50.0	50.0	50.0
Fat blend[2]	—	25.0	18.0	18.0
Whey powder	—	12.4	23.4	10.5
Whey protein concentrate	—	6.8	2.8	15.7
Hydrolyzed wheat protein	—	2.0	2.0	2.0
Maltodextrin	—	2.0	2.0	2.0
Premix[3]	—	1.8	1.8	1.8
Nutrient (% DM)				
DM	96.0	96.8	96.8	96.6
CP	24.5	22.5	22.5	26.0
Crude fat	26.0	25.0	18.0	18.0
Crude ash	6.5	6.3	6.9	6.6
Lactose	38.0	38.6	44.6	41.5
Glucose	0	0.5	0.5	0.5
Starch	0	1.4	1.4	1.4
Sodium	0.54	0.45	0.49	0.48
Potassium	1.4	1.2	1.3	1.3
Chloride	1.2	0.83	0.97	0.97
Calcium	1.4	0.8	0.9	0.83
Phosphorus	0.98	0.66	0.71	0.71
Magnesium	0.13	0.12	0.13	1.2
WPNI[4] (mg/g)	1.9	6.9	7.1	8.2
Osmolality[5] (mOsm/kg)	314	326	370	350
SID[6] (mEq/L)	33	44	45	40
ME[7] (MJ/kg)	21.6	21.3	19.7	20.0

[1]Treatments included a whole milk powder (WP; n = 16), a milk replacer (MR) with high fat (HF; n = 14), a MR with high lactose (HL; n = 17), and a MR with high protein (HP; n = 17).

[2]Blend of palm and coconut fats, 2:1.

[3]Contains vitamins, minerals, probiotics and prebiotics, stabilizers, AA, as well as flavoring and stability agents.

[4]WPNI = whey protein nitrogen index.

[5]Osmolality (in mol/kg of solvent, expressed in mOsm/kg) was calculated by adding osmolality of carbohydrates and minerals as described in (Wilms et al., 2020), considering a concentration of 135 g/L.

[6]Strong ion difference (SID) = sodium + potassium − chloride, considering a concentration of 135 g/L.

[7]ME content of the MR was calculated according to NRC (2001).

Housing

Calves were housed indoors in individual pens (2.34 m × 1.16 m) separated by galvanized bar fences and equipped with rubber-slatted floor in the front area (50% of the total pen area) and a lying area in the

back, which contained a mattress covered with flax straw. During the collection of total urine and feces, the calves were tethered to the front of the pen, and an elevated rubber-covered plateau was positioned at the front to raise the animals to facilitate urine collection. The temperature in the calf pen was maintained at a minimum of 12°C and a maximum of 28°C. Relative humidity was maintained between 60 and 85%. Calves were exposed to daylight and artificial light from 0530 to 2130 h.

Measurements

Milk and water intakes were recorded daily throughout the study period by weighing the unconsumed volumes. Body weight was measured weekly starting at arrival, on d 7, 14, 21, and 27 (the day before the postprandial sampling) at 1100 h with a custom scale (W2000; Welvaarts Weegsystemen, Hertogenbosch, the Netherlands). Weekly blood samples for general health evaluation were obtained by venipuncture from the jugular vein at the same time as BW measurements. Blood samples were collected in two 9-mL lithium-heparin tubes (BD Vacutainer, Becton Dickinson, Vianen, the Netherlands), two 9-mL serum/gel tubes, and one 5.0-mL sodium fluoride (NaF) tube. This means that blood parameters were likely affected by the morning meal; however, the time of weekly sampling was standardized relative to the morning meal. Fecal scoring was performed over the first 21 d after arrival through visual assessment of photos of feces taken daily after the morning meal. The scoring was performed by only one examiner using a 3-level scoring system as follows: normal feces (score 0), wet feces (score 1), and watery feces (score 2).

On d 21, gut permeability was assessed by measuring urinary recovery of indigestible markers. Lactulose (0.2 g/kg BW; Sigma-Aldrich, Zwijndrecht, the Netherlands) and D-mannitol (0.12 g/kg BW; Sigma-Aldrich) were dissolved separately in 100 mL of warm water. The volume of liquid chromium (**Cr**)-EDTA (7.0 g/L of Cr; MasterLab, Boxmeer, the Netherlands) was adjusted for each calf to provide a dose of 0.1 g/kg BW of Cr-EDTA (considering that Cr-EDTA contains 14% Cr). Both solutions were orally pulse-dosed to the calves separately using 100-mL syringes (BD Plastipak, Merkala, Alkmaar, the Netherlands) instead of the morning meal at 0630 h, to avoid interference between the dynamics of marker uptake and the macronutrient composition of the liquid feed (Amado et al., 2019; Welboren et al., 2021b). The subsequent milk meal was fed at 1230 h so that the first 6 h of urine collection would be collected without the interference of a meal. Following marker administration, urine was collected over 6 and 24 h, using urine collection bags attached with medical glue, as described in Wilms et al. (2020). Two subsequent samples were taken from the 24-h urine collection: 0 to 6 h and 6 to 24 h. Feces were quantitatively collected over 24 h at the same time as the urine collection, using fecal collection bags as described in Wilms et al. (2020), to evaluate apparent total-tract digestibility. This means that from 0630 h on d 21 to 0630 h on d 22 after arrival, calves were fed only 2 milk meals, which were accounted for when evaluating total-tract apparent digestibility of dietary treatments.

To investigate abomasal emptying rate and postprandial kinetics, all calves were sedated by i.m. injection of an anesthetic (Sedamun, Eurovet Animal Health BV, the Netherlands; xylazine 2%/20 mg; 23.3 mg xylazine hydrochloride) into the neck at 1400 h on d 27 after arrival, to alleviate the stress associated with catheter placement in the jugular vein. Water was withdrawn until the end of the procedure to avoid bloating and rumen distension. Throughout the procedure, the calf was kept in the sternal position, and the effect of sedation was reversed with an i.m. injection of an antisedative (Antisedan, Vetoquinol GmbH, Germany; atipamezole hydrochloride; 5 g/mL). Intakes of MR from the subsequent evening meal were not affected by the procedure. On d 28, sequential blood sampling was performed to evaluate postprandial dynamics. To assess abomasal emptying, a dose of acetaminophen (**Ac**; 150 mg Ac/kg BW) was mixed into the morning milk meal at 0600 h. Notwithstanding, abomasal emptying measurements have been successfully performed in previous experiments with lower doses of acetaminophen (0.13 g/kg of $BW^{0.75}$; MacPherson et al., 2016; Stahel et al., 2016; Welboren et al., 2021a). On the day of postprandial sampling, calves received their second milk meal at 1300 h rather than 1200 h, to avoid affecting postprandial dynamics. Blood samples were collected in one 9-mL LH Monovette (S-Monovette lithium heparin, 92 × 16 mm [length × diameter], Sarstedt), one 9-mL Monovette with gel for serum (S-Monovette Serum, 9 mL, 92 × 16 mm, Sarstedt), and one 5.5-mL NaF Monovette (S-Monovette Fluoride/EDTA, 5.5 mL, 75 × 15 mm, Sarstedt) per time points. Blood was collected at −30 min and 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 min relative to the morning meal offered at 0600 h. Lithium heparin and NaF tubes were placed on ice and were centrifuged within 5 min at 1,500 × *g* for 15 min at 4°C (Rotina 380R, Hettich, Tuttlingen, Germany). Serum tubes were set for 15 min at ambient temperature and centrifuged at 1,500 × *g* for 15 min at ambient temperature. Plasma, serum, and NaF aliquots were stored in 1.5-mL cryotubes. All samples were transported in boxes with cooling elements and stored at −18°C until analyses were performed.

Chemical Analysis

Feed, urine, and fecal samples were processed and analyzed at MasterLab (Boxmeer, the Netherlands). Analyses of crude fat, CP, crude ash, minerals, lactose, and DM in MR and WP samples are described in Wilms et al. (2022). The whey protein nitrogen index, which is an indicator for the severity of the heat treatment to which a milk powder was subjected during its manufacture, was analyzed as described in Wilms et al. (2022). Fecal samples were analyzed for DM, pH, crude ash, crude fat, crude protein, and macrominerals. Dry matter content was determined by drying in a 103°C oven for 4 h to a constant weight (EC 152/2009; EC, 2009). Crude ash was analyzed by incineration in a muffle furnace by combustion for 4 h at 550°C (EC 152/2009; EC, 2009). Crude fat was determined by treating the sample with hydrochloric acid followed by extraction with petroleum (EC 152/2009; EC, 2009). Crude protein content was analyzed by combustion using the Dumas method (Etheridge et al., 1998; ISO 16634-1, ISO, 2008). Macrominerals in feces (Na, K, and Ca) were analyzed via inductively coupled plasma mass spectrometry (PerkinElmer ICP-MS 300D) according to EN 15510:2017 (EN, 2017). Chloride in feces was analyzed as described by Wilms et al. (2019). Analyses of lactulose and D-mannitol in urine are described in Mellors et al. (2023). Urine osmolality was measured using a semi-micro freezing point depression osmometer (K-7400S, Knalier). Urinary urea was analyzed by 2-step enzymatic colorimetric analysis, in which urea was hydrolyzed to ammonium and CO2. Ammonium ions were analyzed using a modified Berthelot reaction (10505, Human Diagnostics). Urinary creatinine was analyzed by kinetic colorimetric analysis based on the Jaffe reaction (10051, Human Diagnostics).

Blood and urine samples were processed and analyzed at the University of Nottingham (Nottingham, UK). Sodium and K in urine were determined via inductively coupled plasma mass spectrometry (Thermo Fisher X Series II, Thermo Fisher Scientific, Waltham, MA). Weekly serum samples were analyzed for urea, total protein, nonesterified fatty acids (**NEFA**), alkaline phosphatase (**ALP**), and total triglycerides (**TG**) using colorimetric kits according to manufacturers' instructions, using the RX IMOLA (Randox Laboratories Ltd., Crumlin, UK). Postprandial serum samples were analyzed for Ac using the Paracetamol (acetaminophen) Assay Kit-K8002 (Cambridge Life Sciences Ltd., Ely, UK; MacPherson et al., 2016) and for insulin using the Mercodia Bovine Insulin ELISA Kit (Mercodia, Uppsala, Sweden; Bach et al., 2013). Postprandial NEFA and TG were analyzed in postprandial serum samples as described for weekly serum samples. Postprandial NaF samples were analyzed for glucose using the EnzyChrom Glucose Assay Kit (BioAssay Systems, Hayward, CA; Zebeli et al., 2012). Finally, the concentration of the 2 adipokines, leptin and adiponectin, were analyzed in serum at the University of Bonn (Bonn, Germany), as described in Sauerwein et al. (2004) and Mielenz et al. (2013), respectively.

Model Parameterization Using Postprandial Data

A mechanistic model incorporating glucose-insulin dynamics with intermittent gastric emptying developed by Stahel et al. (2016), allowing for the estimation of pancreatic responsiveness, whole-body insulin sensitivity, and glucose effectiveness, was used to parametrize glycemic responses. In brief, the model included pools for Ac and glucose content in the abomasum, as well as Ac, glucose, and insulin in circulation. Differential equations translating input and output fluxes for each plasma pool were then solved using a fourth-order Runge-Kutta algorithm in acslX (AEgis Technologies Group Inc., Huntsville, AL). For each calf, the differential equations describing the disappearance of Ac from the abomasum and serum Ac concentrations were solved using WolframAlpha (Wolfram Research, 2010) and then were entered in Microsoft Excel to predict values at each sampling time point. The Z-value representing the occurrence of abomasal emptying, and its speed ($Z = 0$ for no gastric emptying occurring, $Z = 1$ being slow, and $Z = 2$ being fast), was determined based on the slope of the observed successive Ac postprandial concentrations. The best-fit parameters of Ac kinetics (slow emptying rate, fast emptying rate, and utilization rate) were estimated with the Solver function of Microsoft Excel to minimize the residual sum of squares between observed and predicted serum Ac. Parameters for glucose-insulin kinetics were estimated with a differential evolution algorithm, as previously described (Stahel et al., 2016). Finally, the root mean squared prediction errors, expressed as percentage of the observed means for serum Ac, serum insulin, and plasma glucose dynamics, were calculated to indicate the goodness of fit.

Calculations and Statistical Analysis

The apparent total-tract digestibility of DM and nutrients (fat, protein, ash, minerals) was calculated using MR intakes and fecal output over a 24 h period on d 21, as follows:

$$\text{Digestibility} = 100 - \text{in feces (g)}/\text{in feed ingested (g)} \times 100.$$

Continuous variables were analyzed using mixed-effects model with PROC MIXED in SAS (SAS 9.4M6; SAS Institute, 2018). The calf was the experimental unit, and the statistical model was as follows:

$$Y_{ijk} = \mu + T_i + V_j + W_k + TW_{ik} + \varepsilon_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of the ith treatment; V_j is the random effect of the jth block; W_k is the fixed effect of the kth time point entering the model as a repeated measure; TW_{ik} is the fixed effect interaction between the ith treatment and the kth time point; and ε_{ijk} is the random error associated with the jth block at the kth day with the ith treatment. In the case of BW and ADG, arrival BW was used as a baseline covariate (μ_0), as this parameter was measured before calves were exposed to their respective dietary treatments. For variables with equally spaced time points, the covariance structure (autoregressive covariance [AR(1)] or the heterogeneous autoregressive covariance [ARH(1)]) with the lowest corrected Akaike information criterion was used. The heterogeneous Toeplitz (TOEPH) covariance structure was used for variables with unequally spaced time points. Data that did not meet the assumptions of normality of residuals were log-transformed (base 10). After the log-transformation, the data distribution was tested again to ensure a normal distribution. Significant effects of treatment were explored with the LSMEANS statement using the PDIFF option of the MIXED procedure of SAS. Significant interactions between treatment and time were explored with the SLICE option of the LSMEANS statement using the PDIFF option of the MIXED procedure of SAS. Results in tables and figures are presented as least squares means with standard error of the means. Discrete variables (e.g., therapeutic interventions, fecal scores) were analyzed using logistic regressions. These analyses were conducted with PROC GENMOD in SAS. Significance was declared at $P \leq 0.05$, and the trend threshold was set at $0.05 < P \leq 0.10$.

Correlations, Principal Components Analysis, and Hierarchical Clustering

Heat maps for Pearson correlations were used to determine relationships between variables using an online platform for multivariate analysis (MVApp; Julkowska et al., 2019). Only variables measured in wk 3 were considered for this analysis, as urinary and fecal collection took place in wk 3. For macronutrient intakes, the total intake expressed in kg DM over the first 28 d was considered. Correlations were defined as weakly positive (r = |0.1| to |0.3|), moderately positive (r = |0.3| to |0.7|), or strongly positive (r = |0.7| to |1.0|). Furthermore, the correlation structure was plotted as a principal component analysis (**PCA**) to determine correlation patterns among the individual traits using the MVApp online platform. Significance of correlations was declared at $P \leq 0.05$.

RESULTS

General Health and Milk Intake

Four calves, one from WP and 3 from HF, were removed shortly after admission due to severe diarrhea (3 calves) and critically low milk intake (1 calf). Despite adequate randomization, the concentration of IgG measured between 48 and 72 h after birth was lower in HF calves than in the other treatment groups ($P = 0.03$; Table 2). All 4 calves removed post-inclusion from the experiment had blood IgG lower than 18 g/L. In the first week after arrival, the percentage of days with diarrhea (defined as a fecal score of 2) was lower in calves fed HP (28%) than in the other groups (44%; $P < 0.01$). In addition, in wk 3, the percentage of days with diarrhea was higher in calves fed WP (13%) than in the other groups (4%; $P = 0.02$). Despite these differences, the number of calves receiving therapeutic interventions for diarrhea and respiratory diseases did not differ across treatment groups. Daily milk intakes (as volume fed) throughout the experimental period of 28 d were not analyzed due to infinite likelihood in wk 3 and 4, as calves would consume the full 9.0 L (Supplemental Figure S1; https://doi.org/10.6084/m9.figshare.24187458; Wilms et al., 2023). When considering only the first 14 d after arrival, milk intakes (as volume fed) were lower in calves fed WP than other treatment groups ($P < 0.01$) and lower in calves fed HP than HL ($P < 0.01$). Milk replacer DM intakes over the first 28 d were lower in calves fed WP than other treatments and higher in calves fed HL than HP ($P < 0.01$). As expected from MR composition, fat intake was higher in calves fed HF than in the other treatments and in calves fed WP than HL and HP ($P < 0.01$). Protein intake was higher in calves fed HP than other treatments ($P < 0.01$). Lactose intake was the highest in calves fed HL, followed by HP, HF, and at last WP ($P < 0.01$). Total ME intake over the first 28 d was higher in calves fed HF than other treatments ($P < 0.01$). Body weight and ADG did not differ across treatment groups when considering the entire experimental period of 28 d. Serum urea concentration was greater in calves fed WP than HF and HL, and greater in calves fed HP than HL ($P < 0.01$). Serum NEFA concentration was greater in

Table 2. Parameters describing calves before and after treatment initiation; calves were fed milk replacers differing in macronutrient profile or a whole milk powder 3.0 L, 3 times daily (n = 64)

	Treatment[2]					*P*-value		
Item[1]	WP	HF	HL	HP	Pooled SEM	Treat	Week	Treat × Week
Blood IgG (g/L)	19.3[a]	15.6[b]	19.6[a]	20.8[a]	1.36	0.03	—	—
Arrival BW (kg)	45.0	45.4	47.2	44.2	1.00	0.19	—	—
Arrival age (d)	1.71	1.76	1.73	1.82	0.151	0.94	—	—
Calves treated[3] (%)	12 (2/17)	24 (4/17)	6 (1/17)	6 (1/17)	—	0.36	—	—
Total milk intake[4] (kg)								
DM	28.3[c]	31.1[ab]	31.8[a]	30.3[b]	0.46	<0.01	—	—
Fat	7.38[b]	7.77[a]	5.71[c]	5.50[c]	0.106	<0.01	—	—
Protein	6.95[b]	6.99[b]	7.15[b]	7.94[a]	0.111	<0.01	—	—
Lactose	10.8[d]	12.0[c]	14.2[a]	12.7[b]	0.179	<0.01	—	—
ME (MJ)	613[b]	663[a]	624[b]	610[b]	9.4	<0.01	—	—
Water intake[5] (L/d)	0.94	0.88	0.63	0.59	0.159	0.10	<0.01	<0.01
BW[6] (kg)	54.8	55.6	56.9	57.1	0.91	0.17	<0.01	0.90
Final BW at wk 4 (kg)	62.4	63.4	64.4	64.2	1.18	0.36	—	—
ADG[6] (g/d)	693	693	686	685	54.3	0.99	<0.01	0.48
Blood metabolites[7]								
Urea[8] (mmol/L)	3.61[a]	2.89[bc]	2.99[c]	3.45[ab]	0.055	<0.01	<0.01	0.12
TP (g/L)	57.2	55.6	54.8	56.0	1.08	0.34	<0.01	0.80
NEFA[8] (mmol/L)	0.076[ab]	0.088[a]	0.043[c]	0.054[bc]	0.1443	<0.01	0.09	0.36
ALP (IU/L)	220[b]	324[a]	342[a]	283[a]	24.4	<0.01	<0.01	0.21
TG (mmol/L)	0.32[a]	0.27[ab]	0.26[ab]	0.21[b]	0.025	0.02	<0.01	0.99

[a–d]Means with a different superscript are significantly different ($P \leq 0.05$).

[1]TP = total protein; NEFA = nonesterified fatty acids; ALP = alkaline phosphatase; TG = triglycerides.

[2]Treatments (Treat) included a whole milk powder (26% fat; 21.6 MJ/kg; WP; n = 16), a MR with high fat (25% fat; 21.3 MJ/kg; HF; n = 14), a MR with high lactose (44% lactose; 19.7 MJ/kg; HL; n = 17), and a MR with high protein (26% protein; 20 MJ/kg; HP; n = 17), fed at 135 g/L. It should be noted that for blood IgG, arrival age, and BW, as well as the percentage of calves treated, all 17 calves per treatment group were included in the statistical analyses.

[3]Total of number of calves treated for diarrhea and respiratory diseases. Values in parentheses represent the number of calves treated out of the total number of calves in that treatment group. These values were used to calculate the percentage of calves treated.

[4]Total milk intake, expressed in kg of DM, was calculated by adding the daily consumption of calves over a 28-d period.

[5]Water intake refers to the plain water that was offered ad libitum to calves.

[6]BW was measured at arrival and weekly thereafter from wk 1 to 4. Arrival BW entered the statistical model as baseline covariate.

[7]Blood samples were collected on d 7, 14, and 21 from the jugular vein.

[8]SEM is expressed as log, whereas LSM was back-transformed after the log transformation.

calves fed WP and HF than HL and greater in calves fed HF than HP ($P < 0.01$). The enzymatic activity of ALP was lower in WP-fed calves compared with the other treatments ($P < 0.01$). Serum concentration of TG was greater in calves fed WP compared with HP ($P = 0.02$).

Urine Chemistry, Digestibility, and Gut Permeability

Results for urine chemistry and apparent total-tract digestibility are shown in Table 3. Urinary urea output was lower in WP-fed calves than in the other groups ($P < 0.01$), whereas creatinine did not differ among treatments. Urinary sodium content was lower in calves fed WP than in the other groups ($P < 0.01$). Urinary potassium content was higher in calves fed HL than WP and HF and higher in calves fed HP than WP ($P < 0.01$). This resulted in a lower urine osmolality in WP-fed calves than in the other treatments for the urine sample collected between 6 and 24 h after initiation of total collection ($P = 0.03$). Apparent total-tract digestibility of DM was lower in WP-fed calves than in the other groups ($P = 0.02$). During the first 6 h of urine collection, urinary lactulose recovery was lower in calves fed HL than in the other treatments ($P = 0.02$; Table 4). Recovery of D-mannitol was lower in calves fed HL than WP and HP calves ($P = 0.01$), and recovery of Cr-EDTA was higher in calves fed WP than other treatments in the first 6 h of urine collection ($P = 0.01$). When considering the total 24-h urine collection, urinary D-mannitol recovery was lower in calves fed HL than other treatments and higher in calves fed HP than HF ($P < 0.01$). The 24-h urinary recovery of Cr-EDTA was lower in calves fed HL than WP and HP and higher in claves fed WP than HF ($P < 0.01$). For the 6- and 24-h urine collection, the intestinal permeability (**IP**) index, defined as the ratio of urinary recovery (%) of lactulose to D-mannitol, did not differ between treatment groups.

Table 3. Urine chemistry and apparent total-tract digestibility measured at 21 d after arrival by collection of urine and feces over a 24 h period; calves were fed milk replacers differing in macronutrient profile or a whole milk powder 3.0 L, 3 times daily (n = 64)

	Treatment[2]					*P*-value
Item[1]	WP	HF	HL	HP	Pooled SEM	Treat
Urine volume (g/kg BW per 24 h)	85.0	75.9	79.9	78.5	5.51	0.71
Urine chemistry						
Creatinine (mg/kg BW per 24 h)	15.4	15.5	14.8	13.9	0.71	0.28
Urea (g/kg BW per 24 h)	1.19^{b}	1.52^{a}	1.68^{a}	1.78^{a}	0.171	<0.01
Sodium (mg/kg BW per 24 h)	34.0^{b}	47.2^{a}	55.9^{a}	53.8^{a}	5.47	<0.01
Potassium (mg/kg BW per 24 h)	145^{c}	159^{bc}	175^{a}	162^{ab}	6.6	<0.01
Urine osmolality (mOsm/kg)						
0 to 6 h[3]	336	343	334	395	0.12	0.67
6 to 24 h[3]	172^{b}	201^{a}	204^{a}	209^{a}	0.056	0.03
Fecal DM (%)	15.6	16.0	16.9	18.5	1.10	0.24
Fecal volume[3] (g/kg BW per 24 h)	1.22	0.75	0.79	0.80	0.166	0.11
Apparent total-tract digestibility (%)						
DM digestibility	89.3^{b}	92.5^{a}	92.1^{a}	91.8^{a}	0.014	0.02
Crude fat[3]	95.3	95.9	94.5	95.5	0.010	0.48
CP[3]	85.6	88.5	88.7	88.7	0.014	0.15
Crude ash[3]	98.5	98.7	98.6	98.8	0.002	0.51
Calcium[3]	92.4	89.5	86.3	86.6	0.026	0.13
Sodium[3]	92.6	93.9	94.9	95.0	0.011	0.22
Potassium[3]	97.8	97.3	97.3	98.2	0.005	0.50
Chloride[3]	95.1	95.9	96.8	96.0	0.006	0.28

[a–c]Means with a different superscript are significantly different ($P \leq 0.05$).

[1]Total urine and fecal collection were initiated at the same time than administration of indigestible markers at 0630 h. Calves received their milk meal at 1230 h, and total collection ended at 0630 h on the following day. Body weight measured in wk 3 was used to expressed selected parameters as mg/kg BW per 24 h collection.

[2]Treatments (Treat) included a whole milk powder (26% fat; WP; n = 16), a milk replacer (MR) with high fat (25% fat; HF; n = 14), a MR with high lactose (44% lactose; HL; n = 17), and a MR with high protein (26% protein; HP; n = 17), fed at 135 g/L.

[3]SEM is expressed as log, whereas LSM was back-transformed after the log transformation.

Table 4. Gut permeability assessed by fractional urinary recovery of lactulose, D-mannitol, and Cr-EDTA measured at 21 d after arrival in calves fed milk replacers differing in macronutrient profile or a whole milk powder 3.0 L, 3 times daily (n = 64)

	Treatment[2]					*P*-value
Item[1]	WP	HF	HL	HP	Pooled SEM	Treat
Lactulose						
0 to 6 h	2.59^{a}	2.21^{a}	1.51^{b}	2.19^{a}	0.240	0.02
0 to 24 h	3.67	3.06	2.77	3.37	0.375	0.25
D-mannitol						
0 to 6 h	6.75^{a}	5.45^{ab}	4.10^{b}	6.10^{a}	0.637	0.01
0 to 24 h	10.7^{ab}	8.97^{b}	7.59^{c}	11.94^{a}	0.894	<0.01
IP index[3]						
0 to 6 h	0.38	0.35	0.34	0.33	0.029	0.60
0 to 24 h[4]	0.34	0.31	0.28	0.32	0.106	0.50
Cr-EDTA						
0 to 6 h	4.38^{a}	2.84^{b}	2.92^{b}	3.39^{b}	0.450	0.01
0 to 24 h[4]	9.64^{a}	6.99^{bc}	6.90^{c}	8.45^{ab}	0.092	<0.01

[a–c]Means with a different superscript are significantly different ($P \leq 0.05$).

[1]Expressed in percentage oral dose. Markers were orally pulse dosed at 0630 h instead of the morning meal in wk 3. Lactulose (0.2 g/kg BW; Sigma-Aldrich, Zwijndrecht, the Netherlands) and D-mannitol (0.12 g/kg BW; Sigma-Aldrich) were dissolved separately in 100 mL of warm water. The volume of liquid Cr-EDTA (7.0 g/L of Cr; MasterLab, Boxmeer, the Netherlands) was adjusted for each calf to provide an individual dose of 0.1 g/kg BW of Cr-EDTA (considering the Cr-EDTA contains 14% Cr).

[2]Treatments (Treat) included a whole milk powder (26% fat; WP; n = 16), a milk replacer (MR) with high fat (25% fat; HF; n = 14), a MR with high lactose (44% lactose; HL; n = 17), and a MR with high protein (26% protein; HP; n = 17), fed at 135 g/L.

[3]The intestinal permeability (IP) index was determined as the ratio between urinary recovery (%) of lactulose and D-mannitol.

[4]SEM is expressed as log, whereas LSM was back-transformed after the log transformation.

Correlations and Principal Component Analysis

A heatmap for Pearson correlations matrix between macronutrient intakes, blood metabolites, urinary parameters, and apparent total-tract nutrient and DM digestibility measured in wk 3 is presented in Supplemental Figure S2 (https://doi.org/10.6084/m9.figshare.24187458; Wilms et al., 2023). Serum urea was positively correlated with total serum protein (r = 0.51, $P < 0.01$) and with protein intakes (r = 0.43, $P = 0.01$). Urinary lactulose recovery over 24-h collection was positively correlated with Cr-EDTA recovery (r = 0.78; $P < 0.01$) and with urine osmolality over 6-h collection (r = 0.36; $P = 0.04$). Supplemental Figure S3A (https://doi.org/10.6084/m9.figshare.24187458; Wilms et al., 2023) shows the biplot of the PCA analysis with the contributions of different variables, including macronutrient intakes, blood metabolites, urinary parameters, and apparent total-tract nutrient and DM digestibility measured in wk 3. Results showed that PCA 1 explained 20.5% of the observed variation, corresponding mainly to fecal volume and nutrient digestibility (Supplemental Figure S3B). Moreover, PCA 2 explained 14.3% of the observed variation, corresponding primarily to the recovery of indigestible gut permeability markers (Supplemental Figure S3C).

Postprandial Metabolism

The results for postprandial dynamics are shown in Table 5. Serum postprandial NEFA concentration was higher in calves fed HF than other treatments ($P = 0.04$; Supplemental Figure S4A; https://doi.org/10.6084/m9.figshare.24187458; Wilms et al., 2023). Consistently, the maximum concentration ($\mathbf{C_{max}}$) was greater in calves fed HF than HL ($P = 0.02$). Basal TG concentration was lower in calves fed HP compared with all other treatments and higher in calves fed HF as compared with WP and HL ($P < 0.01$). Accordingly, TG C_{max} was greater in calves fed WP and HF than HP ($P = 0.01$). When considering only time points from 210 to 420 min after the meal, postprandial TG was higher in calves fed WP than all other treatments ($P < 0.01$; Figure 1A). The baseline concentration of adiponectin was greater in calves fed HL than other treatments ($P < 0.01$). In addition, there was an interaction between treatment and time for the postprandial adiponectin concentration ($P = 0.05$; Figure 1B), in which adiponectin concentration was greater in calves fed HL than in calves fed WP between 90 and 210 min after the meal. At 60, 90, 150, and 420 min following the meal, adiponectin concentration was greater in calves fed HL than HP. Finally, at 90, 150, and 420 min after the meal, adiponectin concentration was greater in calves fed HL than HF. No differences were observed in the time to reach the maximum concentration ($\mathbf{T_{max}}$) or in C_{max}. Baseline leptin concentrations tended to be lower in calves fed HL than other treatments ($P = 0.06$; Supplemental Figure S4B). The ratio between adiponectin and leptin was consistently higher in HL-fed calves than in other treatment groups in the postprandial samples ($P < 0.01$; Supplemental Figure S4C). A significant interaction between treatment and time was detected for postprandial Ac ($P < 0.01$; Figure 1C), with serum Ac lower in WP-fed calves between 240 and 420 min after the meal than in the other treatment groups. At 360 min after the meal, serum Ac was lower in calves fed HP than HL. At 420 min following the meal, serum Ac was lower in calves fed HP than HL and HF. The area under the curve (**AUC**) for postprandial serum Ac was lower in calves fed WP than HF and HL ($P = 0.02$), whereas calves fed HP did not differ with other treatments. The T_{max} of Ac was shorter in calves fed WP than other treatments ($P = 0.01$), whereas C_{max} was lower in calves fed WP than HF and HL and lower in calves fed HP than HF ($P = 0.02$). For postprandial glucose dynamics (Figure 1D), the interaction between treatment and time tended to be significant ($P = 0.08$). Between 150 and 180 min after the meal, glucose concentration was higher in calves fed HF than WP and HP ($P \leq 0.01$). At 210 min after the meal, glucose was higher in calves fed HF than HL ($P = 0.04$) and HP ($P = 0.03$), whereas at 240 min after the meal, glucose was higher in calves fed HF than WP ($P = 0.02$) and HL ($P = 0.04$). Finally, between 360 and 420 min after the meal, glucose concentration was higher in calves fed WP than HF ($P \leq 0.05$). In addition, the AUC for glucose was lower in calves fed HL than WP and HF and lower in calves fed HP than WP ($P = 0.03$). An interaction occurred between treatment and time for postprandial insulin concentrations ($P < 0.01$; Figure 1E). Serum insulin concentration was greater in WP-fed calves at 60 min after the meal than in calves fed HF and HL ($P = 0.05$). At 180 min following the meal, serum insulin was lower in calves fed WP than HP and HL, whereas at 210 min following the meal, serum insulin was lower in calves fed WP than HP ($P = 0.02$) and HL ($P = 0.05$). At 240 min after the meal, serum insulin was lower in calves fed WP than all other treatments ($P = 0.01$). At 360 and 420 min after the meal, serum insulin was slightly greater in WP-fed calves than HF ($P < 0.02$). The delta insulin concentration, defined as the C_{max} insulin concentration minus the basal insulin concentration, was lower in calves fed HF than WP and HP ($P = 0.05$). The AUC for insulin was greater in calves fed HP than WP and HF, whereas calves fed HL did not differ from the other treatments ($P = 0.05$).

Table 5. Postprandial dynamics measured at 4 wk of age in calves fed milk replacers differing in macronutrient profile or a whole milk powder 3 times daily (n = 64); blood was collected at −30 min and at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 330, and 420 min relative to the morning milk meal of 3.0 L

	Treatment[2]					*P*-value
Item[1]	WP	HF	HL	HP	Pooled SEM	Treat
NEFA						
Basal concentration (mmol/L)	0.30	0.31	0.27	0.37	0.045	0.15
T_{max} (min)	243	252	263	337	35.10	0.18
C_{max} (mmol/L)	0.47^{ab}	0.57^{a}	0.39^{b}	0.45^{ab}	0.049	0.02
TG						
Basal concentration (mmol/L)	0.32^{b}	0.37^{a}	0.34^{b}	0.24^{c}	0.022	<0.01
T_{max} (min)	334	332	339	321	18.71	0.92
C_{max} (mmol/L)	0.89^{a}	0.83^{a}	0.76^{ab}	0.63^{b}	0.056	0.01
Adiponectin						
Basal concentration (ng/mL)	8.72^{b}	7.80^{b}	10.1^{a}	8.48^{b}	0.455	<0.01
T_{max} (min)	173	163	155	245	32.76	0.18
C_{max} (ng/mL)	11.5	10.6	12.0	10.7	0.87	0.47
Leptin						
Basal concentration (ng/mL)	2.15^{A}	2.19^{A}	1.83^{B}	2.29^{A}	0.130	0.06
T_{max} (min)	202	229	242	219	0.137	0.15
C_{max} (ng/mL)	2.81	2.88	2.82	2.76	0.195	0.95
Adiponectin:leptin ratio[3]	4.37^{ab}	3.99^{b}	5.32^{a}	3.77^{b}	0.081	0.02
Acetaminophen						
AUC (mmol/L × 420 min)	80.3^{b}	92.1^{a}	92.2^{a}	85.6^{ab}	3.33	0.02
T_{max} (min)	150^{b}	186^{a}	184^{a}	199^{a}	10.90	0.01
C_{max} (mmol/L)	0.27^{c}	0.31^{a}	0.30^{ab}	0.28^{bc}	0.009	0.02
Glucose						
Basal concentration (mmol/L)	5.86	5.84	5.77	5.71	0.158	0.90
AUC (× 10 mmol/L × 420 min)	284^{a}	281^{ab}	267^{c}	273^{bc}	48.7	0.03
T_{max} (min)	68	103	94	78	12.5	0.21
C_{max}[4] (mmol/L)	9.45	9.12	8.80	9.13	0.016	0.57
Delta[4,5] (mmol/L)	3.47	2.87	3.15	3.28	0.090	0.52
Insulin						
Basal concentration (mmol/L)	0.37	0.22	0.28	0.31	0.046	0.16
AUC (μg/L × 420 min)	588^{b}	588^{b}	675^{ab}	799^{a}	63.8	0.05
T_{max} (min)	84	118	114	96	11.3	0.13
C_{max}[4] (mmol/L)	6.65	5.04	5.38	7.08	0.767	0.13
Delta[5] (ng/mL)	6.31^{a}	4.38^{b}	5.10^{ab}	6.77^{a}	0.741	0.05
Glucose $AUC_{0–420}$:insulin $AUC_{0–420}$[4]	4.70	5.08	4.62	3.82	0.109	0.28

[a–c]Means with a different superscript are significantly different ($P \leq 0.05$).

[A,B]Means with a different superscript include a trend ($0.05 < P \leq 0.10$).

[1]NEFA = nonesterified fatty acids; T_{max} = time to C_{max}; C_{max} = maximal concentration; AUC = positive incremental area under the curve; TG = triglycerides.

[2]Treatments (Treat) included a whole milk powder (26% fat; WP; n = 16), a milk replacer (MR) with high fat (25% fat; HF; n = 14), a MR with high lactose (44% lactose; HL; n = 17), and a MR with high protein (26% protein; HP; n = 17), fed at 135 g/L.

[3]The adiponectin-to-leptin ratio was calculated using basal concentrations.

[4]SEM is expressed as log, whereas LSM was back-transformed after the log transformation.

[5]Delta insulin concentration was defined as the maximum change from baseline and was calculated by subtracting basal insulin concentrations to C_{max}.

Modeling of Insulin-Glucose Kinetics

The results of estimated postprandial insulin-glucose kinetics are shown in Table 6. The first-order, slow gastric emptying rate constant ($\mathbf{k_{SP,2}}$), and fast gastric emptying rate constant ($\mathbf{k_{SP,3}}$) did not differ across treatment groups. In contrast, there was a trend toward a greater first-order Ac utilization rate constant in calves fed WP than HF and HP ($\mathbf{k_{Ac,UAc}}$; $P = 0.07$). The time in which abomasal emptying was fast (Z = 2, time fast) was shorter in calves fed WP than HF and HL ($P = 0.03$), whereas calves fed HP did not differ from the other groups. In contrast, the parameters for which abomasal emptying was slow (Z = 1, time slow) or off (Z = 0, time off) did not differ among treatments. Postprandial glucose kinetics parameters showed that the initial rate of endogenous glucose production ($\mathbf{iP\text{-}Gl_{end}}$) was lower in calves fed WP than in other treatment groups ($P < 0.01$). Similarly, the absorption lag time from stomach to plasma ($\mathbf{T_{lag,SP}}$) was lower in WP calves than in MR-fed calves ($P < 0.01$). The Hill coefficient for glucose-dependent insulin secretion ($\mathbf{exp_{PIn}}$)

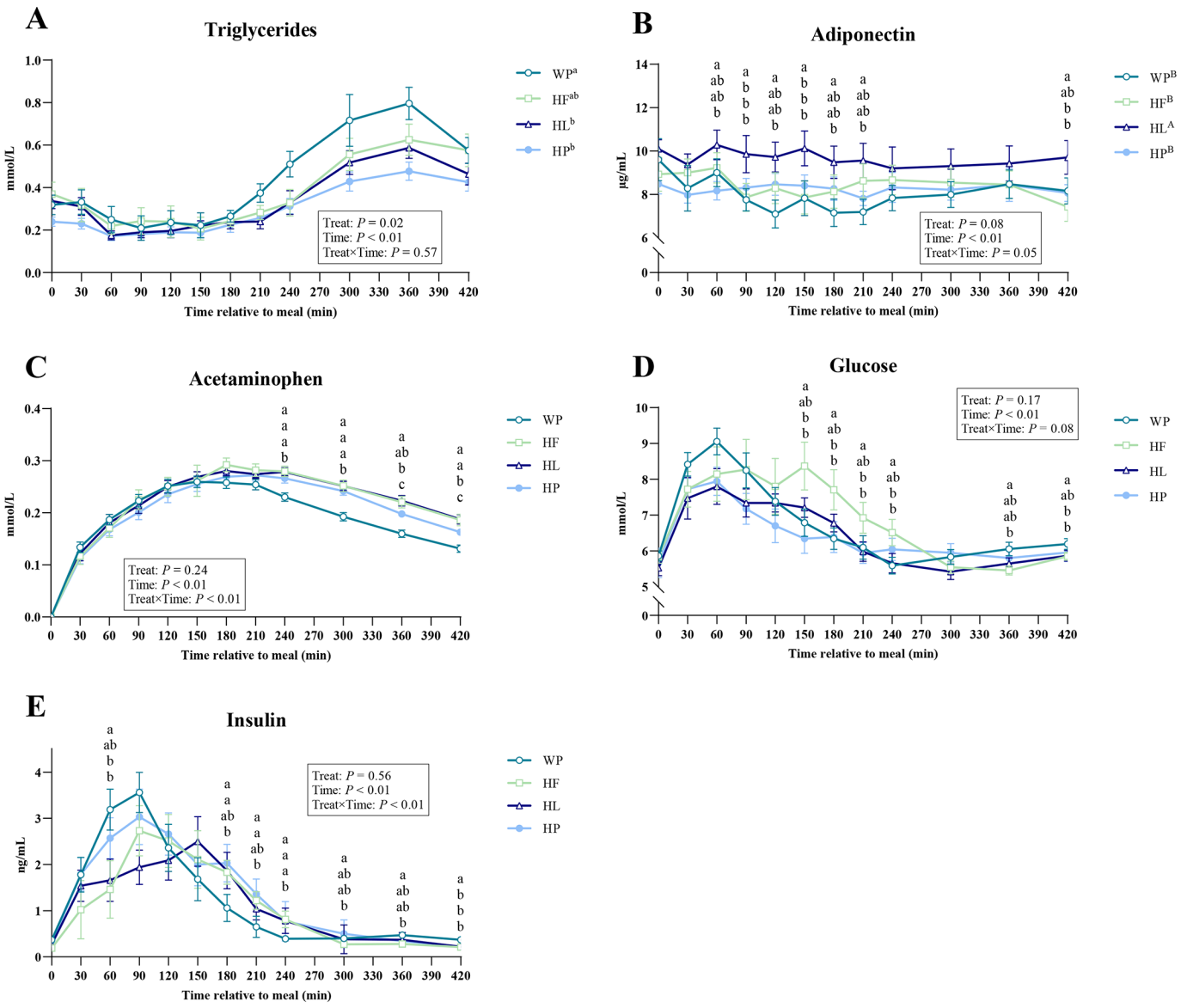

Figure 1. Postprandial dynamics of triglycerides (A), adiponectin (B), acetaminophen (C), glucose (D), and insulin (E) in calves. Blood samples were collected at −30 min and at 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 min relative to the morning meal of 3.0 L (135 g/L) on d 28 after arrival. Treatments (Treat) included a whole milk powder (26% fat; WP; n = 16) and a milk replacer (MR) with high fat (25% fat; HF; n = 14), a MR with high lactose (44% lactose; HL; n = 17), and a MR with high protein (26% protein; HP; n = 17). Error bars represent SEM. a–c: Means with a different lowercase letter are significantly different ($P \leq 0.05$). A,B: Means with a different uppercase letter include a trend ($0.05 < P \leq 0.10$). Letters indicating significance follow the height of the dots per time point.

tended to be greater in calves fed HP than other treatment groups ($P = 0.08$). Finally, the root mean squared prediction error for insulin ($\mathbf{rMSPE_{ln}}$) was greater in calves fed HL than other treatment groups ($P < 0.01$).

DISCUSSION

This experiment examined the effect of macronutrient profile in MR on gastrointestinal health and postprandial metabolism in male dairy calves fed 3 times daily. Despite the greater ME intake in HF-fed calves throughout the experimental period, growth within the first 28 d after arrival did not differ across treatment groups. In the current study, gut permeability was the highest in calves fed WP followed by calves fed HP, and lowest in calves fed HL. Postprandial dynamics were largely influenced by macronutrient composition, with calves fed WP and HP exhibiting slower abomasal emptying rates, likely due to higher protein and casein contents of the meals. Finally, the AUC for insulin was

Table 6. Parameter values derived from parametrization of a mechanistic model of abomasal emptying and glucose-insulin kinetics; the model uses postprandial acetaminophen, glucose, and insulin in calves fed milk replacers differing in macronutrient profile or a whole milk powder 3.0 L, 3 times daily (n = 64)

	Treatment[2]					*P*-value
Item[1]	WP	HF	HL	HP	Pooled SEM	Treat
BW at wk 4 (kg)	62.4	63.4	64.4	64.2	1.18	0.36
Abomasal emptying						
$k_{SP,2}$[3] ($min^{-1} \times 10^{-3}$)	0.81	1.28	1.20	1.14	0.314	0.73
$k_{SP,3}$ ($min^{-1} \times 10^{-3}$)	3.77	3.26	3.40	3.02	0.335	0.45
$k_{Ac,UAc}$[3] ($min^{-1} \times 10^{-3}$)	4.70^{A}	2.84^{B}	3.74^{AB}	3.50^{B}	0.127	0.07
Time fast (min)	124^{b}	152^{a}	164^{a}	146^{ab}	9.7	0.03
Time slow (min)	178	154	129	139	24.8	0.52
Time off (min)	110	114	125	135	20.1	0.81
$rMSPE_{ac}$[3] (% of mean)	2.81	2.64	3.22	3.53	0.099	0.16
Glucose						
$iPGl_{end}$[3] (mmol/min)	0.212^{b}	1.023^{a}	0.830^{a}	0.588^{a}	0.3743	<0.01
$k_{Gl,UGl}$ (L/min)	0.103	0.189	0.167	0.179	0.0419	0.32
$k_{IS,UGl}$[3] (L^2/μg per min)	0.044	0.052	0.051	0.025	0.3484	0.37
$T_{lag,SP}$[3] (min)	0.003^{b}	2.46^{a}	1.26^{a}	0.95^{a}	1.309	<0.01
$rMSPE_{Gl}$[3] (% of mean)	8.17	7.62	8.92	10.45	0.0953	0.11
Insulin						
V_{PIn}[3] (mg/min)	642	721	2,633	2,200	0.633	0.25
$k_{Gl,PIn}$[3] (L/min)	15.1	16.8	15.3	12.2	0.151	0.48
exp_{PIn}[3]	8.46^{B}	8.17^{B}	8.67^{B}	13.43^{A}	0.156	0.08
iIs[3] (μg/L)	0.247	0.196	0.222	0.135	0.2562	0.22
$T_{lag,IS}$[3] (min)	15.79	11.36	3.98	6.38	0.737	0.54
$k_{In,UIn}$[3] (L/min)	2.82	1.45	4.11	3.08	0.751	0.80
$rMSPE_{In}$ (% of mean)	31.4^{b}	38.3^{b}	47.6^{a}	34.8^{b}	3.69	<0.01

[a,b]Means with a different superscript are significantly different ($P \leq 0.05$).

[A,B]Means with a different superscript include a trend ($0.05 < P \leq 0.10$).

[1]Blood was collected at −30 min and at 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 420 min relative to the morning meal of 3.0 L. Ac = acetaminophen; $k_{SP,2}$ = first-order slow gastric emptying rate constant; $k_{SP,3}$ = first-order fast gastric emptying rate constant; $k_{Ac,UAc}$ = first-order Ac utilization rate constant; $rMSPE_{ac}$ = root mean squared prediction error for Ac; $iPGl_{end}$ = initial rate of endogenous glucose production; $k_{Gl,UGl}$ = first-order glucose-dependent glucose utilization rate constant; $k_{IS,UGl}$ = first-order insulin-dependent glucose utilization rate constant; $T_{lag,SP}$ = absorption lag time from stomach to plasma; $rMSPE_{Gl}$ (% of mean) = root mean squared prediction error for glucose; V_{Pin} = maximal rate of insulin secretion; $k_{Gl,Pin}$ = glucose-dependent insulin secretion Michaelis constant; exp_{Pin} = Hill coefficient for glucose-dependent insulin secretion; iIs = basal insulin signal mass before meal; $T_{lag,IS}$ = signaling lag time for insulin; $k_{In,UIn}$ = first-order insulin utilization rate constant; $rMSPE_{In}$ = root mean squared prediction error for insulin.

[2]Treatments (Treat) included a whole milk powder (26% fat; WP; n = 16), a milk replacer (MR) with high fat (25% fat; HF; n = 14), a MR with high lactose (44% lactose; HL; n = 17), and a MR with high protein (26% protein; HP; n = 17), fed at 135 g/L.

[3]SEM is expressed as log, whereas LSM was back-transformed after the log transformation.

greater in calves fed HP than in calves fed WP and HF, but the ratio of insulin to glucose AUC did not differ across treatment groups.

Gastrointestinal Health

The percentage of days with diarrhea was lower in wk 1 for calves fed HP and higher in wk 3 for calves fed WP than other treatments. The numbers of therapeutic interventions for diarrhea and respiratory diseases and calf removal were numerically higher in calves fed HF than in the other treatment groups. These results are inconsistent with those of Wilms et al. (2022), in which calves fed ad libitum levels of the same HF MR did not exhibit more health disorders than other treatment groups. In the current study, despite adequate randomization, blood IgG measured upon arrival at the facility was significantly lower in HF calves. This may explain the numerically higher removal rate, as IgG concentrations below 15.0 g/L have been associated with higher morbidity and mortality in calves (Weaver et al., 2000).

Indigestible permeability markers are commonly used to assess the mucosal integrity of the gastrointestinal tract (**GIT**; Wilms et al., 2019; Welboren et al., 2021b). In the current study, urinary recovery of Cr-EDTA and lactulose measured in wk 3 after arrival were greater in calves fed WP and HP compared with calves fed HL. Transport of molecules such as Cr-EDTA (344 g/mol) and lactulose (342 g/mol) through the intestinal epithelium is paracellular and occurs via tight junction proteins (Linsalata et al., 2020). The greater recovery of these markers in urine may indicate decreased intestinal barrier function associated with structural changes such as the opening of tight junction

proteins. Consistent with other markers, urinary recovery of D-mannitol, a small-size marker (182 g/mol) that is absorbed both transcellularly and paracellularly (Linsalata et al., 2020), was higher in calves fed WP and HP than in the other treatments. However, the absence of differences across treatment groups for the IP index, defined as the ratio of urinary recovery (%) of lactulose to D-mannitol, does not indicate damage to the intestinal mucosa, but rather an overall increase in paracellular permeability. Dietary proteins are largely digested in the small intestine; however, a high protein intake may result in more proteins entering the large intestine and colon. Because Cr-EDTA passes through the intestinal wall in both the small and the large intestines (Maxton et al., 1986; Elia et al., 1987), protein fermentation in the large intestine may influence Cr-EDTA recovery.

The amount of protein that reaches the large intestine is influenced not only by protein intake but also by protein source and quality. Highly digestible proteins such as casein are digested in the proximal intestine, resulting in less microbial fermentation than with plant proteins (Ma et al., 2017). In the current study, only highly digestible milk proteins were used (Mellors et al., 2023), but DM digestibility was lower in calves fed the WP treatment. However, it is important to highlight that the total collection period in the current experiment might have been too short to accurately determine the apparent total-tract digestibility. This lower DM digestibility is consistent with the lower whey protein nitrogen index in the WP treatment compared with other treatments. This likely indicates denaturation of the milk proteins during the pasteurization or evaporation processes due to high heat (Wilms et al., 2022; Mellors et al., 2023). These results suggest that a greater quantity of undigested proteins may have reached the large intestine, which may explain the higher gut permeability in WP-fed calves. This also aligns with the higher percentage of days with diarrhea in wk 3 in calves fed WP as compared with other treatment groups. Denaturation of the protein fraction leads to a change in the tertiary structure of the protein and other chemical modifications that negatively affect the accessibility of AA to proteolytic enzymes, thus reducing digestibility (van Lieshout et al., 2020). Alternatively, Mellors et al. (2023) showed that the fatty acid composition of the proximal jejunum and ileum differed between calves fed WP and HF and largely mirrored that of the fat fraction of the milk diets. The higher ratio of n-6 to n-3 PUFA in the gut tissue of calves fed HF as compared with calves fed WP may have modulated gut permeability (Usami et al., 2001).

Blood Metabolites Measured Weekly

Urea is the major end product of protein metabolism and the major solute in urine (Bankir et al., 1996). Dietary AA are either used for anabolic functions or broken down in the liver and converted into urea (Weiner et al., 2015). The lower urinary urea and electrolyte content of WP-fed calves resulted in lower urine osmolality in this group in the 6 to 24 h after initiation of urine collection. Serum urea was greater in calves fed HP than HL and higher in calves fed WP than HF and HL. These results align with those of Wilms et al. (2022), in which serum urea was higher in calves fed WP and HP over a 12-wk rearing period. The authors attributed the higher serum urea in HP-fed calves to higher protein intake, which is consistent with the positive correlation between these parameters observed in the current study. A greater protein intake results in more protein being degraded to AA, which in turn is converted to urea and released into the bloodstream. The higher serum urea concentration in WP-fed calves could be attributable to protein denaturation from the WP treatment, which may affect protein biological value and activity. However, the lower urea urinary output in WP calves could be due to a better protein utilization than in calves fed other treatments.

In the current study, calves fed HF had greater serum NEFA concentration measured weekly than calves fed HL and HP, which aligns with results from Wilms et al. (2022). In addition, calves fed WP had higher serum NEFA concentrations than HL-fed calves. Calves fed HF and WP MR also had the highest fat intake; however, the correlation between fat intake and serum NEFA was low. Serum TG measured weekly was lower in calves fed HP than in WP-fed calves, whereas calves fed HF and HL did not differ from the other treatment groups. These results contrast those of Wilms et al. (2022), where no differences in serum TG were found among treatment groups. This could be related to the timing of blood collection relative to the milk meal, which was controlled in the current study but not in Wilms et al. (2022), as calves were fed ad libitum. Common assays for serum ALP enzymatic activity do not differentiate between placental ALP, intestinal ALP, and liver, bone, kidney ALP, making it difficult to interpret differences across treatments. In the current experiment, WP-fed calves had a lower serum ALP enzymatic activity compared with the other treatment groups. Overall, the enzymatic activity of ALP in blood correlated negatively with serum urea and recovery of Cr-EDTA over 24-h urine collection. Previous studies have shown that the activity of ALP in the gut is significantly reduced when gut integrity is compromised

(Pearce et al., 2013). Nevertheless, the correlation between intestinal and blood ALP enzymatic activity remains to be evaluated.

Abomasal Emptying and Postprandial Metabolism

Dietary fat from liquid feed is ingested as TG, which needs to be hydrolyzed into fatty acids and monoglycerides by digestive enzymes in the upper GIT and in the intestines before absorption (Noble, 1979; Lambert and Parks, 2012). In the current study, postprandial TG concentration increased from 180 min after meal ingestion onward. Although the time to reach the maximum postprandial concentration (T_{max}) did not differ for NEFA and TG, the maximum postprandial concentration (C_{max}) for NEFA was greater in calves fed HF than HL, and TG C_{max} was greater in calves fed WP and HF than HP. The magnitude of the response of postprandial TG depends on the amount and composition of dietary fats (Lambert and Parks, 2012). In the current study, overall postprandial TG concentrations were greater in calves fed WP and HF than HL and HP. In addition, calves fed WP had higher postprandial TG concentrations than calves fed HF when considering the sampling period starting from 180 min after the meal onward. This may indicate that milk fat leads to different fat absorption dynamics and utilization than the same amount of vegetable oils.

Leptin and adiponectin are adipokines whose circulating concentrations are positively and negatively related to body fat content in cattle, respectively (Häussler et al., 2022), although the association of adiponectin with body fat is limited to a study in dry cows (De Koster et al., 2017). Leptin is known to reduce appetite, enhance fatty acid oxidation, and decrease glucose, thus reducing body fat, whereas adiponectin is regarded as insulin sensitizer; however, the situation in cattle, in particular calves, is less clear (Sauerwein and Häussler, 2016; Kesser et al., 2017). In human studies, the postprandial concentration of both adipokines hardly changed during 120 min after oral glucose or fat challenge tests in normal-weight adults (Larsen et al., 2019). In 4-mo-old children, no difference was observed in the plasma concentrations of leptin and adiponectin before and 1 h after a meal (Tomasik et al., 2011). This aligns with the findings of Blum et al. (2005), in which postprandial plasma leptin measured in young calves remains stable, which suggest that plasma leptin is not involved in the immediate partitioning of nutrients following a meal. When assessing the response to high-fat, high-protein, or high-carbohydrate and medium-protein diets for 9 h in overweight dogs, Blees et al. (2020) did not observe any change in adiponectin, but leptin increased until 6 h. The type of diet affected the baseline values of adiponectin but not leptin; the AUC of leptin was the lowest in the high-protein diet; for the adiponectin AUC, all tested diets yielded lower values than the basal maintenance diet (Blees et al., 2020). For cattle, to the best of our knowledge, the present study is the first to report the postprandial time course of adiponectin. In the current experiment, the basal concentration of adiponectin was greater, whereas the basal leptin concentration tended to be lower in calves fed HL than in other treatment groups. Postprandial dynamics also consistently showed higher adiponectin concentrations in HL calves, leading to a greater postprandial adiponectin-to-leptin ratio. This is likely due to the higher lactose intake in this group, resulting in an increased need for glucose homeostasis regulation in calves fed HL. Interestingly, when considering the first 120 min after meal ingestion, calves fed HL seemed to have the least glucose response with the lowest insulin concentrations. This supports a greater insulin sensitivity (Frühbeck et al., 2018) in this group and is consistent with the greater postprandial adiponectin concentration, which is thought to enhance the body's sensitivity to insulin. Nevertheless, when considering the entire sampling period of 420 min relative to the milk meal, the AUC for insulin and the glucose-to-insulin ratio in HL calves were not different from other treatment groups. Low leptin concentrations associated with high adiponectin concentrations could also indicate increased hunger or decreased satiety in calves fed MR with high lactose content. These findings may partially explain why these animals consistently consume higher volume of high-lactose MR when fed ad libitum than other treatment groups (Berends et al., 2020; Echeverry-Munera et al., 2021; Wilms et al., 2022).

Gastric emptying depends on several characteristics of the meal, such as meal size (MacPherson et al., 2016), caloric density (Hunt and Stubbs, 1975; Calbet and MacLean, 1997), and protein content and composition (Burn-Murdoch et al., 1978; Wittek et al, 2016), in humans and calves. In the current study, abomasal emptying was slower in WP- and HP-fed calves. Consistently, the time in which abomasal emptying was fast was lower in calves fed WP than other treatment groups throughout the 420 min of postprandial sampling. As described in Stahel et al. (2016), serum Ac arises from the emptying of the abomasum and disappears according to the first-order elimination constant ($k_{Ac,UAc}$; Stahel et al., 2016). The $k_{Ac,UAc}$ constant tended to be greater in calves fed WP than in calves fed HF and HP, whereas calves fed HL showed no differences from the other treatment groups. This slower abomasal emptying in calves fed WP is likely related to differences in protein characteristics between treatments. The protein fraction of the WP treatment consisted of 82% casein

proteins and 18% whey proteins, equivalent to a ratio of 4.6 to 1. In contrast, MR treatments (HF, HL, and HP) contained a greater proportion of whey proteins than WP, resulting in a casein-to-whey ratio of 2.15 for HF, 2.06 for HL, and 1.47 for HP. For the same protein intake, a lower ratio of casein to whey protein in the total protein fraction resulted in less curd formation in the abomasum and, thus, faster passage rate through the GIT (Longenbach and Heinrichs, 1998). In addition to protein composition, a higher protein intake may also decrease gastric emptying rate because the end products of protein hydrolysis affect duodenal receptors that regulate gastric emptying in humans (Burn-Murdoch et al., 1978).

Plasma glucose dynamics are determined by exogenous appearance and endogenous glucose production and utilization, as described in the model developed by Stahel et al. (2016). Interestingly, the absorption lag time from the GIT to plasma, referred to as movement from stomach to plasma in the model, was slower in WP- than in MR-fed calves. As mentioned above, curd formation in the abomasum was likely greater in WP- than in MR-fed calves due to the greater proportion of casein in the protein fraction. Although casein remains longer in the abomasum, the liquid phase containing lactose and whey proteins separates from the curd and flows rapidly into the intestines (Petit et al., 1987; Radostits and Bell, 1970). This is consistent with the greater insulin concentrations observed in calves fed WP at 60 min postmeal compared with calves fed HF and HL, followed by lower insulin concentrations between 180 and 240 min compared with calves fed HP and HF. The higher root mean squared prediction error for insulin ($rMSPE_{in}$) in calves fed HL than other treatments suggests that the model developed by Stahel et al. (2016) may not predict postprandial insulin dynamics as accurately in calves fed HL.

The AUC for insulin was greater in calves fed HP than WP and HF, whereas calves fed HL did not differ from the other treatment groups. This is consistent with previous studies showing that high protein intake leads to increased concentrations of insulin-releasing AA, which can result in greater insulin and IGF-I concentrations (Luque et al., 2016; Socha et al., 2011). Consistently, Wilms et al. (2022) showed that ad libitum feeding of MR high in protein and lactose resulted in an increase in serum IGF-I concentrations during a 12-wk rearing period, compared with calves fed WP. Because the ratio of postprandial glucose to insulin did not differ between groups, conclusions regarding insulin sensitivity cannot be drawn in this study. Further work is needed to determine the effects of a high-protein MR formulation on long-term glucose-to-insulin homeostasis in calves.

Results from the current study indicate that postprandial nutrient signaling differs greatly depending on the macronutrient inclusion and the macronutrient composition. The higher curd formation in calves fed WP resulted in slower abomasal emptying. In addition, postprandial TG concentrations were greater in calves fed WP, which could be due to different digestion and absorption dynamics of dietary fats. Nevertheless, calves fed WP may not be representative of calves fed fresh WM because processing steps of WM disrupted fat globule membranes and decreased protein quality. Although colostrum management at birth was standardized, differences in blood IgG at arrival may have negatively affected health of calves fed HF.

CONCLUSIONS

Despite its optimal nutrient composition, the WP treatment resulted in lower DM digestibility and increased gut permeability. Differences in the macronutrient profile and composition of liquid feed led to distinct metabolic and endocrine profiles in calves. The HP treatment resulted in a higher AUC for insulin than WP and HF, although the glucose-to-insulin ratio did not differ from other treatment groups. Calves fed HL had an increased postprandial ratio of adiponectin to leptin, indicating an increased need for glucose homeostasis regulation. Although it is unclear whether the HP and HL diets negatively affected insulin sensitivity in calves, the absence of differences between calves fed HF and WP in the AUC for insulin, as well as in postprandial leptin and adiponectin, suggest that high-fat MR are preferable for maintaining hormonal homeostasis in calves.

ACKNOWLEDGMENTS

This study was funded by, and several of the authors (J. N. Wilms, L. N. Leal, and J. Martín-Tereso) are employed by, Trouw Nutrition (Amersfoort, the Netherlands), a company with commercial interests in milk replacers for calves. Trouw Nutrition R&D adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2012). The authors express their gratitude to Natasja Boots, Akke ten Berge (Trouw Nutrition, Amersfoort, the Netherlands), and the personnel of the Trouw Nutrition's Calf Research Facility for conducting this study. The authors also acknowledge Thomas Blees and Katharina Kustwan from the University of Bonn (Bonn, Germany) for their technical assistance. In addition, the authors thank the technical team of Trouw Nutrition (Deventer, the Netherlands), and especially Gert Klaassen, Henk Oldenziel, and Erik Boterman for formulating the milk

replacers used in the experiment and for sharing their technical expertise. The authors have not stated any other conflicts of interest.

REFERENCES

Amado, L., H. Berends, L. N. Leal, J. Wilms, H. Van Laar, W. J. J. Gerrits, and J. Martín-Tereso. 2019. Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. J. Dairy Sci. 102:3994–4001. https://doi.org/10.3168/jds.2018-15847.

Bach, A., L. Domingo, C. Montoro, and M. Terré. 2013. Short communication: Insulin responsiveness is affected by the level of milk replacer offered to young calves. J. Dairy Sci. 96:4634–4637. https://doi.org/10.3168/jds.2012-6196.

Bankir, L., N. Bouby, M. M. Trinh-Trang-Tan, M. Ahloulay, and D. Promeneur. 1996. Direct and indirect cost of urea excretion. Kidney Int. 49:1598–1607. https://doi.org/10.1038/ki.1996.232.

Bartlett, K. S., F. K. McKeith, M. J. VandeHaar, G. E. Dahl, and J. K. Drackley. 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. J. Anim. Sci. 84:1454–1467. https://doi.org/10.2527/2006.8461454x.

Bascom, S. A., R. E. James, M. L. McGilliard, and M. Van Amburgh. 2007. Influence of dietary fat and protein on body composition of Jersey bull calves. J. Dairy Sci. 90:5600–5609. https://doi.org/10.3168/jds.2007-0004.

Berends, H., H. van Laar, L. N. Leal, W. J. J. Gerrits, and J. Martín-Tereso. 2020. Effects of exchanging lactose for fat in milk replacer on ad libitum feed intake and growth performance in dairy calves. J. Dairy Sci. 103:4275–4287. https://doi.org/10.3168/jds.2019-17382.

Blees, N. R., J. Wolfswinkel, H. S. Kooistra, and R. J. Corbee. 2020. Influence of macronutrient composition of commercial diets on circulating leptin and adiponectin concentrations in overweight dogs. J. Anim. Physiol. Anim. Nutr. (Berl.) 104:698–706. https://doi.org/10.1111/jpn.13285.

Blum, J. W., Y. Zbinden, H. M. Hammon, and Y. Chilliard. 2005. Plasma leptin status in young calves: Effects of preterm birth, age, glucocorticoid status, suckling, and feeding with an automatic feeder or by bucket. Domest. Anim. Endocrinol. 28:119–133. https://doi.org/10.1016/j.domaniend.2004.06.011.

Bowen Yoho, W. S., V. A. Swank, M. L. Eastridge, K. M. O'Diam, and K. M. Daniels. 2013. Jersey calf performance in response to high-protein, high-fat liquid feeds with varied fatty acid profiles: Intake and performance. J. Dairy Sci. 96:2494–2506. https://doi.org/10.3168/jds.2012-6099.

Burn-Murdoch, R. A., M. A. Fisher, and J. N. Hunt. 1978. The slowing of gastric emptying by proteins in test meals. J. Physiol. 274:477–485. https://doi.org/10.1113/jphysiol.1978.sp012161.

Calbet, J. A., and D. A. MacLean. 1997. Role of caloric content on gastric emptying in humans. J. Physiol. 498:553–559. https://doi.org/10.1113/jphysiol.1997.sp021881.

Daniels, K. M., S. R. Hill, K. F. Knowlton, R. E. James, M. L. McGilliard, and R. M. Akers. 2008. Effects of milk replacer composition on selected blood metabolites and hormones in preweaned Holstein heifers. J. Dairy Sci. 91:2628–2640. https://doi.org/10.3168/jds.2007-0859.

De Koster, J., C. Urh, M. Hostens, W. Van den Broeck, H. Sauerwein, and G. Opsomer. 2017. Relationship between serum adiponectin concentration, body condition score, and peripheral tissue insulin response of dairy cows during the dry period. Domest. Anim. Endocrinol. 59:100–104. https://doi.org/10.1016/j.domaniend.2016.12.004.

Drenth, P. J. 2012. A European code of conduct for research integrity. Promoting research integrity in a global environment. ALLEA (All European Academies). 161.

Echeverry-Munera, J., L. N. Leal, J. N. Wilms, H. Berends, J. H. C. 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.

Elia, M., R. Behrens, C. Northrop, P. Wraight, and G. Neale. 1987. Evaluation of mannitol, lactulose and 51Cr-labelled ethylenediaminetetra-acetate as markers of intestinal permeability in man. Clin. Sci. (Lond.) 73:197–204. https://doi.org/10.1042/cs0730197.

EC (European Commission). 2009. No 152/2009. Commission regulation laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L 54:1–130.

EN (European Standard). 2017. EN 15510:2017. Animal feeding stuffs: Methods of sampling and analysis—Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES. European Committee for Standarization.

Etheridge, R., G. Pesti, and E. Foster. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. Anim. Feed Sci. Technol. 73:21–28. https://doi.org/10.1016/S0377-8401(98)00136-9.

Frayn, K. N., F. Karpe, B. A. Fielding, I. A. Macdonald, and S. W. Coppack. 2003. Integrative physiology of human adipose tissue. Int. J. Obes. Relat. Metab. Disord. 27:875–888. https://doi.org/10.1038/sj.ijo.0802326.

Frühbeck, G., V. Catalán, A. Rodríguez, and J. Gómez-Ambrosi. 2018. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte 7:57–62. https://doi.org/10.1080/21623945.2017.1402151.

Geiger, A. J. 2019. Review: The pre-pubertal bovine mammary gland: Unlocking the potential of the future herd. Animal 13:s4–s10. https://doi.org/10.1017/S1751731119001204.

Häussler, S., H. Sadri, M. H. Ghaffari, and H. Sauerwein. 2022. Symposium review: Adipose tissue endocrinology in the periparturient period of dairy cows. J. Dairy Sci. 105:3648–3669. https://doi.org/10.3168/jds.2021-21220.

Hill, S. R., K. F. Knowlton, K. M. Daniels, R. E. James, R. E. Pearson, A. V. Capuco, and R. M. Akers. 2008. Effects of milk replacer composition on growth, body composition, and nutrient excretion in preweaned Holstein heifers. J. Dairy Sci. 91:3145–3155. https://doi.org/10.3168/jds.2007-0860.

Hugi, D., S. H. Gut, and J. W. Blum. 1997. Blood metabolites and hormones—especially glucose and insulin—in veal calves: Effects of age and nutrition. J. Vet. Med. A Physiol. Pathol. Clin. Med. 44:407–416. https://doi.org/10.1111/j.1439-0442.1997.tb01126.x.

Hunt, J. N., and D. F. Stubbs. 1975. The volume and energy content of meals as determinants of gastric emptying. J. Physiol. 245:209–225. https://doi.org/10.1113/jphysiol.1975.sp010841.

ISO. 2008. Food products—Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content—Part 1: Oilseeds and animal feeding stuffs. ISO 16634-1:2008. International Organization for Standardization (ISO).

Julkowska, M. M., S. Saade, G. Agarwal, G. Gao, Y. Pailles, M. Morton, M. Awlia, and M. Tester. 2019. MVApp-multivariate analysis application for streamlined data analysis and curation. Plant Physiol. 180:1261–1276. https://doi.org/10.1104/pp.19.00235.

Kesser, J., M. Korst, C. Koch, F. J. Romberg, J. Rehage, U. Müller, M. Schmicke, K. Eder, H. M. Hammon, H. Sadri, and H. Sauerwein. 2017. Different milk feeding intensities during the first 4 weeks of rearing dairy calves: Part 2: Effects on the metabolic and endocrine status during calfhood and around the first lactation. J. Dairy Sci. 100:3109–3125. https://doi.org/10.3168/jds.2016-11595.

Khajuria, A., N. Thusu, and U. Zutshi. 2002. Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: Influence on brush border membrane fluidity, ultrastructure and enzyme kinetics. Phytomedicine 9:224–231. https://doi.org/10.1078/0944-7113-00114.

Koletzko, B., R. von Kries, R. Closa, J. Escribano, S. Scaglioni, M. Giovannini, J. Beyer, H. Demmelmair, D. Gruszfeld, A. Dobrzanska, A. Sengier, J. P. Langhendries, M. F. Rolland Cachera,

and V. Grote. 2009. Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. Am. J. Clin. Nutr. 89:1836–1845. https://doi.org/10.3945/ajcn.2008.27091.

Lambert, J. E., and E. J. Parks. 2012. Postprandial metabolism of meal triglyceride in humans. Biochim. Biophys. Acta 1821:721–726. https://doi.org/10.1016/j.bbalip.2012.01.006.

Larsen, M. A., V. T. Isaksen, E. J. Paulssen, R. Goll, and J. R. Florholmen. 2019. Postprandial leptin and adiponectin in response to sugar and fat in obese and normal weight individuals. Endocrine 66:517–525. https://doi.org/10.1007/s12020-019-02102-9.

Lepczyński, A., A. Herosimczyk, M. Ożgo, and W. F. Skrzypczak. 2015. Feeding milk replacer instead of whole milk affects blood plasma proteome and lipid profile in preruminant calves. Pol. J. Vet. Sci. 18:91–99. https://doi.org/10.1515/pjvs-2015-0012.

Linsalata, M., G. Riezzo, C. Clemente, B. D'Attoma, and F. Russo. 2020. Noninvasive biomarkers of gut barrier function in patients suffering from diarrhea predominant-IBS: An update. Dis. Markers 2020:1–12. https://doi.org/10.1155/2020/2886268.

Longenbach, J. I., and A. J. Heinrichs. 1998. A review of the importance and physiological role of curd formation in the abomasum of young calves. Anim. Feed Sci. Technol. 73:85–97. https://doi.org/10.1016/S0377-8401(98)00130-8.

Luque, V., R. Closa-Monasterolo, J. Escribano, and N. Ferré. 2016. Early programming by protein intake: The effect of protein on adiposity development and the growth and functionality of vital organs. Nutr. Metab. Insights 8 (Suppl. 1):49–56. https://doi.org/10.4137/NMI.S29525.

Ma, N., Y. Tian, Y. Wu, and X. Ma. 2017. Contributions of the interaction between dietary protein and gut microbiota to intestinal health. Curr. Protein Pept. Sci. 18:795–808. https://doi.org/10.2174/1389203718666170216153505.

MacPherson, J. A. R., H. Berends, L. N. Leal, J. P. Cant, J. Martín-Tereso, and M. A. Steele. 2016. Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. J. Dairy Sci. 99:8007–8017. https://doi.org/10.3168/jds.2015-10826.

Maxton, D. G., I. Bjarnason, A. P. Reynolds, S. D. Catt, T. J. Peters, and I. S. Menzies. 1986. Lactulose, 51Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethyleneglycol 400 [corrected] as probe markers for assessment in vivo of human intestinal permeability. Clin. Sci. (Lond.) 71:71–80. https://doi.org/10.1042/cs0710071.

Mellors, S. C., J. N. Wilms, A. C. Welboren, M. H. Ghaffari, L. N. Leal, J. Martín-Tereso, H. Sauerwein, and M. A. Steele. 2023. Gastrointestinal structure and function of preweaning dairy calves fed a whole milk powder or a milk replacer high in fat. J. Dairy Sci. 106:2408–2427. https://doi.org/10.3168/jds.2022-22155.

Michaelsen, K. F., and F. R. Greer. 2014. Protein needs early in life and long-term health. Am. J. Clin. Nutr. 99:718S–722S. https://doi.org/10.3945/ajcn.113.072603.

Mielenz, M., B. Mielenz, S. P. Singh, C. Kopp, J. Heinz, S. Häussler, and H. Sauerwein. 2013. Development, validation, and pilot application of a semiquantitative Western blot analysis and an ELISA for bovine adiponectin. Domest. Anim. Endocrinol. 44:121–130. https://doi.org/10.1016/j.domaniend.2012.10.004.

Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650. https://doi.org/10.3168/jds.2009-3007.

NAHMS (National Animal Health Monitoring System). 2007. Dairy 2007. Part 1: Reference of Dairy Cattle Health and Management in the United States. USDA Animal and Plant Health Inspection Service (USDA-APHIS) Veterinary Services, Ft. Collins, CO.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academies Press.

Noble, R. C. 1979. Lipid metabolism in the neonatal ruminant. Prog. Lipid Res. 18:179–208. https://doi.org/10.1016/0163-7827(79)90004-3.

Pearce, S. C., V. Mani, T. E. Weber, R. P. Rhoads, J. F. Patience, L. H. Baumgard, and N. K. Gabler. 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J. Anim. Sci. 91:5183–5193. https://doi.org/10.2527/jas.2013-6759.

Petit, H. V., M. Ivan, and G. J. Brisson. 1987. Duodenal flow of digesta in preruminant calves fed clotting or nonclotting milk replacer. J. Dairy Sci. 70:2570–2576. https://doi.org/10.3168/jds.S0022-0302(87)80326-0.

Radostits, O. M., and J. M. Bell. 1970. Nutrition of the pre-ruminant dairy calf with special reference to the digestion and absorption of nutrients: A review. Can. J. Anim. Sci. 50:405–452. https://doi.org/10.4141/cjas70-063.

SAS Institute. 2018. SAS 9.4M6, SAS Studio User's Guide. SAS Institute Inc.

Sauerwein, H., and S. Häussler. 2016. Endogenous and exogenous factors influencing the concentrations of adiponectin in body fluids and tissues in the bovine. Domest. Anim. Endocrinol. 56(Suppl):S33–S43. https://doi.org/10.1016/j.domaniend.2015.11.007.

Sauerwein, H., U. Heintges, M. Hennies, T. Selhorst, and A. Daxenberger. 2004. Growth hormone induced alterations of leptin serum concentrations in dairy cows as measured by a novel enzyme immunoassay. Livest. Prod. Sci. 87:189–195. https://doi.org/10.1016/j.livprodsci.2003.08.001.

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: A review. J. Anim. Sci. 75:828–835. https://doi.org/10.2527/1997.753828x.

Socha, P., V. Grote, D. Gruszfeld, R. Janas, H. Demmelmair, R. Closa-Monasterolo, J. E. Subías, S. Scaglioni, E. Verduci, E. Dain, J. P. Langhendries, E. Perrin, and B. Koletzko. 2011. Milk protein intake, the metabolic-endocrine response, and growth in infancy: Data from a randomized clinical trial. Am. J. Clin. Nutr. 94:S1776–S1784. https://doi.org/10.3945/ajcn.110.000596.

Stahel, P., H. Berends, L. N. Leal, and J. Martin-Tereso. 2019. Effect of replacing lactose with fat in milk replacer on abomasal emptying and glucose–insulin kinetics in male dairy calves. Appl. Anim. Sci. 35:586–595. https://doi.org/10.15232/aas.2019-01896.

Stahel, P., J. P. Cant, J. A. MacPherson, H. Berends, and M. A. Steele. 2016. A mechanistic model of intermittent gastric emptying and glucose-insulin dynamics following a meal containing milk components. PLoS One 11:e0156443 https://doi.org/10.1371/journal.pone.0156443.

Swanson, E. W. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377–387. https://doi.org/10.3168/jds.S0022-0302(60)90172-7.

Tikofsky, J. N., M. E. Van Amburgh, and D. A. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim. Sci. 79:2260–2267. https://doi.org/10.2527/2001.7992260x.

Tomasik, P. J., A. Wędrychowicz, I. Rogatko, K. Fyderek, and K. Sztefko. 2011. Peptides from adipose tissue in monitoring energy balance in infants. J. Pediatr. Endocrinol. Metab. 24:939–943. https://doi.org/10.1515/JPEM.2011.328.

Urie, N. J., J. E. Lombard, C. B. Shivley, C. A. Kopral, A. E. Adams, T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244. https://doi.org/10.3168/jds.2017-14019.

Usami, M., K. Muraki, M. Iwamoto, A. Ohata, E. Matsushita, and A. Miki. 2001. Effect of eicosapentaenoic acid (EPA) on tight junction permeability in intestinal monolayer cells. Clin. Nutr. 20:351–359. https://doi.org/10.1054/clnu.2001.0430.

USDA. 2011. Dairy Heifer Raiser—An overview of operations that specialize in raising dairy heifers. Animal and Plant Health Inspection Service–Veterinary Services. Accessed Oct. 25, 2023. https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairyheifer11/HeiferRaiser_1.pdf.

Van Amburgh, M. E., F. Soberon, M. J. Meyer, and R. A. Molano. 2019. Symposium review: Integration of postweaning nutrient requirements and supply with composition of growth and mammary development in modern dairy heifers. J. Dairy Sci. 102:3692–3705. https://doi.org/10.3168/jds.2018-15270.

van Lieshout, G. A. A., T. T. Lambers, M. C. E. Bragt, and K. A. Hettinga. 2020. How processing may affect milk protein digestion and overall physiological outcomes: A systematic review. Crit. Rev. Food Sci. Nutr. 60:2422–2445. https://doi.org/10.1080/10408398.2019.1646703.

Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569–577. https://doi.org/10.1111/j.1939-1676.2000.tb02278.x.

Weiner, I. D., W. E. Mitch, and J. M. Sands. 2015. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin. J. Am. Soc. Nephrol. 10:1444–1458. https://doi.org/10.2215/CJN.10311013.

Welboren, A. C., B. Hatew, O. López-Campos, J. P. Cant, L. N. Leal, J. Martín-Tereso, and M. A. Steele. 2021a. Effects of energy source in milk replacer on glucose metabolism of neonatal dairy calves. J. Dairy Sci. 104:5009–5020. https://doi.org/10.3168/jds.2020-19405.

Welboren, A. C., B. Hatew, J. B. Renaud, L. N. Leal, J. Martin-Tereso, and M. A. Steele. 2021b. 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.

Wilms, J., H. Berends, and J. Martín-Tereso. 2019. Hypertonic milk replacers increase gastrointestinal permeability in healthy dairy calves. J. Dairy Sci. 102:1237–1246. https://doi.org/10.3168/jds.2018-15265.

Wilms, J. N., J. Echeverry-Munera, L. Engelking, L. N. Leal, and J. Martín-Tereso. 2020. Tonicity of oral rehydration solutions affects water, mineral and acid–base balance in calves with naturally occurring diarrhoea. J. Anim. Physiol. Anim. Nutr. (Berl.) 104:1655–1670. https://doi.org/10.1111/jpn.13405.

Wilms, J. N., M. H. Ghaffari, M. A. Steele, H. Sauerwein, J. Martín-Tereso, and L. N. Leal. 2022. Macronutrient profile in milk replacer or a whole milk powder modulates growth performance, feeding behavior, and blood metabolites in ad libitum-fed calves. J. Dairy Sci. 105:6670–6692. https://doi.org/10.3168/jds.2022-21870.

Wilms, J., M. Hosseini Ghaffari, P. Stahel Darani, M. Jansen, H. Sauerwein, and M. Steele. 2023. Postprandial metabolism and gut permeability in calves fed milk replacer with different macronutrient profiles or a whole milk powder. figshare. Figure. https://doi.org/10.6084/m9.figshare.24187458.

Wittek, T., M. Ernstberger, M. Muckenhuber, and M. Flock. 2016. Effects of wheat protein in milk replacers on abomasal emptying rate in calves. J. Anim. Physiol. Anim. Nutr. (Berl.) 100:264–270. 10.1111/jpn.12363.

Wolfram Research. 2010. WolframAlpha, Wolfram language function (updated 2017). Accessed Jun. 25, 2023. https://reference.wolfram.com/language/ref/WolframAlpha.html.

Zebeli, Q., S. J. Terrill, A. Mazzolari, S. M. Dunn, W. Z. Yang, and B. N. Ametaj. 2012. Intraruminal administration of *Megasphaera elsdenii* modulated rumen fermentation profile in mid-lactation dairy cows. J. Dairy Res. 79:16–25. https://doi.org/10.1017/S0022029911000707.

Zhang, R., W. B. Zhang, Y. L. Bi, Y. Tu, Y. Beckers, H. C. Du, and Q. Y. Diao. 2019. Early feeding regime of waste milk, milk, and milk replacer for calves has different effects on rumen fermentation and the bacterial community. Animals (Basel) 9:443. https://doi.org/10.3390/ani9070443.