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Pre- and postweaning feeding strategies on growth and digestion of female Holstein calves

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ABSTRACT

This study evaluated the effects of pre- and postweaning feeding strategies on growth, digestibility, nitrogen metabolism, and ruminal fermentation in Holstein calves up to 5 mo of age. Eighty female calves were blocked by farm and week of birth and assigned to a 2 × 2 factorial arrangement: 10% (PRE_{10}) or 20% (PRE_{20}) of initial BW as milk replacer (MR) during preweaning, combined with postweaning feeding plans targeting an ADG of 500 ($POST_{500}$) or 700 g/d ($POST_{700}$). During preweaning, PRE_{20} calves consumed more MR and total DM, resulting in greater ADG, BW, and nitrogen intake per kilogram $BW^{0.75}$; however, nitrogen efficiency and nitrogen retention were similar between preweaning treatments. Calves in PRE_{20} ingested less starter feed and accumulated less NFC, whereas PRE_{10} calves exhibited lower minimum daily ruminal pH. Contrary to our initial hypothesis, postweaning solid feed digestibility was not impaired. Both preweaning treatments surpassed the 15-kg cumulative NFC from starter feed threshold, supporting adequate digestive capacity after weaning. As expected, $POST_{700}$ calves consumed more nutrients and achieved greater BW and BCS at 5 mo. No interactions among preweaning and postweaning feeding strategies were detected for body growth or diet digestibility. However, significant interactions were observed for nitrogen metabolism and ruminal fermentation shortly after weaning. Increased MR allowance enhanced nutrient supply and preweaning growth without compromising postweaning digestibility, reinforcing cumulative starter intake as the primary driver of digestive development. Restricted calves tended to retain more nitrogen and reached BW comparable to those fed greater MR when provided higher postweaning nutrition, highlighting compensatory growth. These results emphasize the importance of aligning pre- and postweaning feeding strategies to support physiological adaptation and optimize developmental outcomes of dairy heifers under postweaning forage-based diets.
Key words: dairy calves, weaning, digestibility, rumen, nitrogen metabolism

INTRODUCTION

Early-life feeding strategies in dairy calves continue to represent a major challenge for the dairy industry, because achieving an optimal balance between rapid preweaning growth and adequate rumen development is critical for postweaning performance. Increasing whole milk (**WM**) or milk replacer (**MR**) allowances increases nutrient intake and promotes faster early growth (Diaz et al., 2001; Terré et al., 2006; Hu et al., 2020), which is desirable for reducing age at first calving and enhancing lifetime productivity. However, high milk allowances frequently depress starter feed (**SF**) intake (Gelsinger et al., 2016; Hu et al., 2020), reduce the calf's capacity to digest solid feed after weaning (Terré et al., 2007; Hill et al., 2010; Chapman et al., 2016), and may lead to growth checks after weaning. Therefore, the industry faces a practical dilemma: stimulating early growth without compromising solid feed digestion and performance during the transition to functional rumination.

More recently, Quigley et al. (2019) highlighted that cumulative NFC intake from solid feed during the preweaning period is the key determinant of postweaning solid feed digestibility. These authors reported that calves accumulating at least 15 kg of NFC from SF were able

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

to extract levels of ME comparable to those predicted by equations developed for mature ruminants. Together, these observations indicate that digestive development, rather than liquid feed intake alone, plays a central role in determining postweaning performance. Thus, understanding how nutritional programs influence cumulative NFC intake and digestive readiness at weaning becomes central to the formulation of feeding strategies.

Consequently, although high MR intake during preweaning promotes early growth, limited digestive development at weaning may offset these advantages. Evidence is inconsistent, with some studies reporting persistence of early BW gains up to 4 or even 20 mo (Kiezebrink et al., 2015), whereas others indicate these gains diminish by weaning (Terré et al., 2009; Davis Rincker et al., 2011). In contrast, calves fed restricted MR allowances often display compensatory growth, driven by enhanced digestive development at weaning, activation of compensatory growth mechanisms (Choi et al., 1997), or both.

Despite this, most available studies have evaluated pre- and postweaning nutrition independently, and relatively few have evaluated their combined or interactive effects on body growth and composition (Brown et al., 2005a,b), ruminal fermentation and circulating hormones, puberty onset (Bruinjé et al., 2021; Rosadiuk et al., 2021), or long-term metabolic outcomes (Ockenden et al., 2025). This gap highlights the need for integrative, hypothesis-driven studies that evaluate interactive effects of pre- and postweaning nutrition.

To address this need, the present study was designed to test specific hypotheses regarding these interactions. We hypothesized that a greater MR allowance would improve preweaning growth and nutrient intake at the expense of SF consumption. At the same time, a higher nutritional plane after weaning would enhance postweaning growth. Furthermore, we hypothesized that the postweaning feeding level would modulate any carryover effects of preweaning nutrition on growth, digestibility, and nitrogen metabolism. Consistent with this framework, we assumed that strategies promoting earlier rumen development and greater cumulative NFC intake would enhance postweaning feed efficiency and growth. From a practical and economic standpoint, identifying feeding combinations that support both rapid early growth and efficient postweaning performance may reduce weaning stress, shorten the rearing period, and decrease feed costs per unit of BW gain, thereby contributing to a more cost-effective heifer-rearing system.

This study aimed to evaluate the effects of preweaning MR allowance on nutrient intake, body growth, and cumulative NFC intake at weaning and to determine whether preweaning treatments exert carryover effects and interact with postweaning feeding strategies to influence body growth, solid feed digestion, nitrogen metabolism, and ruminal fermentation in female dairy calves up to 5 mo of age. By clarifying how early-life nutritional strategies shape digestive development and subsequent performance, this study provides information relevant for designing feeding programs in commercial calf-rearing systems.

MATERIALS AND METHODS

The experiment was conducted in accordance with the Honorary Commission for Animal Experimentation of the Universidad de la República-Uruguay (Protocol: CEUA-FVET-No. 69). All procedures took place at the Instituto de Producción Animal de Veterinaria (**IPAV**) facilities, Facultad de Veterinaria-Universidad de la República (Libertad, San José, Uruguay). The average (±SD) ambient temperature during the study was 13°C ± 3.7°C, with a relative humidity of 75% ± 13.3%.

Experimental Design and Treatments

Eighty Holstein heifer calves (38.4 ± 0.61 kg BW; 6 ± 2 d of age) were transported from 4 commercial dairy farms to the IPAV facilities. Only healthy calves meeting adequate passive immunity transfer criteria (serum Brix ≥8.4%; Deelen et al., 2014) were enrolled. Calves born during the same week on each farm were grouped into a block of 8 animals to account for variation in origin and birth timing. Within each block, calves were randomly assigned to 1 of 4 treatment combinations defined by a 2 × 2 factorial arrangement: preweaning (from 1 to 8 wk of life) MR allowances of 10% (**PRE_{10}**) or 20% (**PRE_{20}**) of initial BW (at 12.5% of DM) and 2 early postweaning (from 9 to 21 wk of life) feeding strategies targeting ADG of 500 (**$POST_{500}$**) or 700 g/d (**$POST_{700}$**). The restricted MR level (10% of initial BW) was selected because it reflects the average liquid feeding allowance used in commercial dairy systems in several regions of South America (Hötzel et al., 2014; Schild et al., 2020) and thus served as a practical benchmark for comparison.

Within each block, calves were randomly assigned to 1 of 4 treatment combinations. The 2 calves assigned to the same treatment within a block formed a pair, which served as the experimental unit (n = 40 pairs). One pair was removed due to a health issue unrelated to the experimental treatments, resulting in 39 experimental units (n = 78 calves) included in the analysis.

During the preweaning phase (1–8 wk), calves were housed individually indoors (1.5 m^2/calf), with visual and physical contact. Milk replacer was offered via nipple buckets twice daily (0800 and 1600 h), and all calves had free access to a finely ground pelleted SF (Table 1), while no forage was offered before weaning. Because MR was gradually increased during the first 2 wk and progres-

sively reduced during the 6-d weaning period, the effective full allowance was provided only from 2 to 7 wk. Therefore, average MR intake across wk 1 to 8 reflects the planned step-up and step-down schedule rather than refusals, as no refusals of MR were detected. Weaning was conducted over 6 d (8 wk) by reducing MR volume by 16.6%/d, completed by 57 d of age. Calves remained individually housed from wk 1 to 11 to allow accurate measurement of feed intake, prevent cross-feeding, and ensure precise determination of apparent nutrient digestibility and nitrogen metabolism.

From 9 to 21 wk, all calves received the same SF plus chopped mixed legume–grass hay offered separately. The SF-to-hay ratio was standardized across treatments, being set at 70:30 (DM basis) at 9 wk and 50:50 thereafter. Because calves did not receive forage during the preweaning period, forage was introduced at 30% of total DM starting at 9 wk across all treatment combinations to facilitate a gradual transition toward a higher-forage diet after weaning. This feeding strategy allowed evaluation of postweaning nutritional planes under diets with comparable ingredient composition and forage-to-concentrate ratios. Importantly, postweaning treatments differed exclusively in the amount of feed offered (DM basis), which was adjusted to achieve targeted postweaning ADG, whereas diet composition and forage-to-concentrate ratios were identical for all animals.

From 12 to 21 wk, calves were moved to outdoor pair pens with their pre-established partner to facilitate adaptation to group housing, minimizing both dietary transition stress and the social stress associated with relocation. Given this management sequence, the temporary housing change was unlikely to have had confounding effects on postweaning performance. Postweaning diets were assigned according to NASEM (2021) to support the targeted ADG of 500 (n = 19 pairs) or 700 g/d (n = 20 pairs). These target ADG levels represent typical growth rates in pasture-based rearing systems (Costa et al., 2010), with 700 g/d being the minimum required to reach ~55% of mature BW (assumed at 635 kg) by 15 mo of age.

Consequently, 4 nutritional treatment groups were established: PRE_{10}-$POST_{500}$, PRE_{10}-$POST_{700}$, PRE_{20}-$POST_{500}$, and PRE_{20}-$POST_{700}$. Calves remained for 2 additional wk in their individual pens and were then grouped with their pre-established pair at wk 12. This management strategy aimed to separate the dietary transition stress associated with weaning from the social stress associated with relocation. From wk 12 to 21, calves were housed in outdoor pens (15 m^2/calf) in pairs. During this period, forage and concentrate were offered separately in different feed bunks. Sufficient bunk space was provided, and feeders were in different areas of the pen to minimize competition and ensure equal access to feed, although feeding behavior was not recorded.

Table 1. Chemical composition and particle size of feeds

Item	Milk replacer[1]	Starter feed[2]	Mixed hay[3]
Chemical composition, % DM			
DM, %	91.9	92.0	90.8
CP	24.9	21.0	14.8
Ether extract	20.1	2.2	1.4
NDF	—	15.1	56.9
ADF	—	5.1	38.4
Ash	7.9	6.0	10.1
NFC[4]	47.1	55.7	20.7
Lactose[5]	47.1	—	—
ME[6]	4.58	3.00	1.96
Particle size,[7] % DM			
Long, >20 mm	—	0.0	78.3
Medium, 8–20 mm	—	0.0	2.1
Short, <8 mm	—	100.0	19.6

[1]The milk replacer premix (on DM basis) contained 1.3% Ca, 0.6% P, 0.4% Na, 0.5% Cl, 27,000 IU vitamin A, 5,300 IU vitamin D_3, 50,000 IU vitamin E, 11 mg Cu, 44 mg Zn, 111 mg Fe, 2.7% lysine, and 0.9% methionine.

[2]Starter feed concentrate, composed on a DM basis of 50% corn grain, 35% solvent-extracted soybean meal, 12% soybean hulls, and 3% vitamin–mineral premix. The vitamin–mineral premix contained 0.8% to 1.1% Ca, 0.7% to 0.9% P, 4,800 IU vitamin A, 600 IU vitamin D_3, 120 mg vitamin E, 28.8 mg vitamin B_1, 7.2 mg biotin, 5,760 mg Cu, 18,000 mg Mn, 0.228 mg I, 18,000 mg Zn, 0.062 mg Co, 0.115 mg Se, and 16,800 mg Fe.

[3]Mixed grass-legume hay chopped with a mill with a mesh size of 20 mm to achieve a theoretical fiber length <20 mm.

[4]Nonfiber carbohydrates = [100 − (CP + ash + ether extract + NDF)].

[5]Estimated according to Drackley (2008).

[6]Metabolizable energy was calculated based on NASEM (2021) and is reported as megacalories per kilogram of DM.

[7]Particles were separated into 3 fractions using a Penn State Particle Separator.

Sample Size

An a priori, model-oriented power analysis was conducted following current recommendations for experiments in animal science (Kaps and Lamberson, 2017; Wang et al., 2025). The experiment was designed as a 2 × 2 factorial arrangement of pre- and postweaning feeding strategies implemented as a randomized complete block design, with block included as a random effect and the pair of heifers defined as the experimental unit.

For final BW, the effect of interest was defined as a biologically meaningful difference of 15 kg, based on previous literature and practical relevance in postweaning heifer feeding trials (e.g., De La Quintana et al., 2018). The SD at the experimental unit level was assumed to be 10 kg, derived from comparable studies conducted under similar management and nutritional conditions (De La Quintana et al., 2018). Under these assumptions, and accounting for the factorial structure and blocking in the mixed-model framework, 10 experimental units (pairs) per treatment combination were considered sufficient to provide reasonable power to detect treatment effects and interactions at a 2-sided $\alpha = 0.05$.

For the digestibility trial, the minimum detectable difference was set at 5.0 percentage points with an assumed SD of 3.0 percentage points, resulting in a requirement of 7 experimental units per treatment combination under the same statistical assumptions. Power was evaluated within the full mixed-model framework, acknowledging that the achievable power depends on plausible variance-component assumptions inherent to blocked designs.

Measurement and Calculation

In the preweaning phase, MR and SF intake were recorded daily, whereas BW, hip height (**HH**), hip width (**HW**), and BCS (1–5 scale) were measured weekly for each calf. Actual MR intake was recorded individually at each feeding by weighing refusals, which were subtracted from the amount offered. Postweaning, SF and hay intake were recorded daily until 70 d, then by pair, whereas BW, HH, HW, and BCS were measured weekly and averaged per pair of heifers. Empty BW (**EBW**) and empty BW gain (**EBWg**) were estimated following Jahn and Chandler (1976) as BW − (BW × gut fill), where gut fill (% of BW) was estimated as 10.4 − [(0.39 × CP % from solid feeds) + (0.41 × ADF % from solid feeds)].

Both ME for growth (**MEg**) and MP for growth (**MPg**) were calculated according to NASEM (2021) by subtracting maintenance requirements from the total ME and MP intake. Maintenance ME was estimated as $0.137 \times BW^{0.75}$, and maintenance MP as $\{(2.75 \times BW^{0.75}) + [(0.22 \times BW^{0.6}) + (MFCP)]/0.68\}$. Metabolic fecal CP (**MFCP**) was estimated as [(11.9 × DMI of MR) + (20.6 × DMI of SF)].

Serum IGF-1 concentration was measured in jugular blood samples collected at 55 ± 2 and 145 ± 2 d of life. These times corresponded to the end of the pre- and postweaning phases, respectively, to characterize the cumulative metabolic response to each feeding period. Samples were centrifuged (1,500 × *g*, 10 min, at room temperature) and stored at −20°C before RIA analysis (IGF1-RIACT, Cisbio Bioassays, France; Adrien et al., 2012). Sensitivity was 0.46 ng/mL, with intra-assay CV of 2.1% (34 ng/mL) and 4.4% (335 ng/mL).

Diet digestibility, microbial nitrogen yield (**MNY**), nitrogen metabolism, and ruminal fermentation variables were assessed at wk 7, 9, and 11 in 7 experimental units per treatment combination. Total fecal output was collected over 6 d using plastic bags glued to the perianal area and replaced every 6 h (Terré et al., 2007). A textile patch was adhered using a synthetic adhesive to the perineal area, and no glue was applied directly to the skin surrounding the vulva to avoid irritation. The textile device incorporated a nonadhered fold designed to prevent contact between urine and feces, and routine checks performed every 6 h confirmed that the fecal-collection bags did not interfere with urination or voiding behavior. Digestibility of DM (**dDM**), CP (**dCP**), and NDF (**dNDF**) was calculated as [(Ingested − Excreted)/Ingested] × 100, and true digestibility of CP (**tdCP**) = {[CP ingested − (CP excreted − MFCP)]/CP ingested} × 100. Nitrogen retention [nitrogen ingested/kg $BW^{0.75}$ − (fecal nitrogen/kg $BW^{0.75}$ + urinary nitrogen/kg $BW^{0.75}$)] and nitrogen efficiency were [(g nitrogen retained/kg $BW^{0.75}$)/(g nitrogen ingested/kg $BW^{0.75}$) × 100].

Spot urine samples (15 mL each) were collected 4 times daily (0800, 1200, 1600, and 2000 h) over 3 consecutive days in 7, 9, and 11 wk. Aliquots of urine (15 mL) were diluted and acidified with 60 mL of 0.072 N H_2SO_4 and subsequently stored at −20°C (Broderick et al., 2009). After thawing, samples were pooled for creatinine analysis (Vitalab Selectra II autoanalyzer, Wiener Laboratories, Argentina; detection limit = 0.09 mg/dL, intra-assay CV <10%), and creatinine concentration was used to estimate daily urine volume output (26.8 × BW/urine creatinine concentration in mg/L), according to Lascano and Heinrichs (2011). The urinary nitrogen concentration was determined via Kjeldahl (method 955.04; AOAC International, 1990).

Postweaning urine samples were also analyzed for purine derivatives (uric acid and allantoin) by HPLC to estimate total purine derivatives (**PD**) according to general procedures described by Balcells et al. (1992). Analyses were performed on a Dionex Ultimate 3000 system (Thermo Scientific) equipped with an Acclaim C18 reversed-phase column (5 µm, 4.6 × 250 mm). Separation was isocratic with UV detection at 205 nm. Allopurinol served as an internal standard. Microbial nitrogen yield per kilogram of digestible OM intake (**DOMI**) estimated PD digestibility of 0.83, PD nitrogen content of 70 mg/mmol, and purine-to-microbial nitrogen ratio of 0.116 (Chen and Gomes, 1992). An endogenous PD excretion of 0.705 mmol/kg $BW^{0.75}$/d (Funaba et al., 1997) was subtracted from total PD.

Ruminal fluid was collected on a single day at wk 7, 9, and 11 using a stomach tube at 0800, 1200, 1600, and 2000 h. A double oroesophageal tubing system was used, in which a second inner tube was inserted through the main tube to further minimize saliva contamination. In addition, the first 10 mL of ruminal fluid were discarded before sample collection to reduce the risk of saliva carryover and ensure sample integrity. Ruminal pH was measured immediately in all samples using a portable pH meter (Oakton Instruments, Vernon Hills, IL) equipped with a pH electrode (EW-05991-36, Cole-Parmer, Vernon Hills, IL). Two subsamples from the 0800 and 1200 h collections were preserved in specific solutions and stored at −20°C: a 200-µL subsample was mixed with 1,800 µL of 3.6 *M* H_2SO_4 for subsequent analyses of NH_3-N concentration by phenol-hypochlorite method

(Weatherburn, 1967), and another 500-µL subsample was mixed with 0.1 *M* $HClO_4$ for VFA and lactate determination by HPLC (Dionex Ultimate 3000; Adams et al., 1984). Before analysis, all preserved samples were thawed and centrifuged at 10,000 × *g* at 4°C for 15 min.

Chemical Composition

Feed and feces samples were dried at 60°C for 48 h and ground through a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA). Feed and feces samples were analyzed for DM, ash, CP, and ether extract (AOAC International, 1990; methods 934.01, 942.05, 955.04, and 920.39, respectively). For NDF, heat-stable α-amylase and sodium sulfite were used, whereas ADF was not sequentially analyzed. Both values were expressed as discounted residual ash (Van Soest et al., 1991; Mertens, 2003). The concentration of ME and NFC of each feed was estimated according to NASEM (2021).

Statistical Analyses

All data were analyzed using mixed-effects models in SAS (version 9.4, University Edition; SAS Institute Inc., Cary, NC) with the GLIMMIX procedure. The experimental unit was the pair of calves (n = 39 pairs), because treatments were randomized within the block to pairs of calves, not to individual animals. Thus, calves assigned to the same treatment within a block represent a single nonindependent replicate. Accordingly, calf-level measurements were averaged within each pair before analysis to preserve the randomization structure and avoid pseudoreplication.

For the preweaning phase, the model included the fixed effects of preweaning treatment, week, and their interaction, with block as a random effect. For the postweaning phase, the model included preweaning, postweaning treatment, week, and all 2- and 3-way interactions, with block as a random effect. Digestibility, nitrogen metabolism, MNY, and ruminal fermentation variables were analyzed with the same model, except that preweaning data were evaluated without time effects.

For repeated measurements, because BW and intake were measured at regular weekly intervals within each phase and adjacent observations were expected to be more strongly correlated than distant ones, an autoregressive covariance structure [AR(1)] was selected. This structure provided a biologically coherent correlation pattern and yielded stable model estimation while remaining more parsimonious than heterogeneous or unstructured alternatives (e.g., ARH(1) or UN).

Least squares means were reported ± SEM and were compared using the Tukey–Kramer adjustment. Because treatment groups were slightly unbalanced (one treatment included 9 vs. 10 pairs), the Tukey–Kramer method was used, as it provides appropriate control of type I error under unequal sample sizes and maintains a suitable balance between type I and type II error rates. Significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a tendency. Correlation and regression analyses between IGF-1 and MEg or MPg were performed with the CORR and REG procedures, and only associations with $P < 0.05$ were reported.

RESULTS AND DISCUSSION

Feed Intake and Performance

Calves in PRE_{20} consumed more MR from wk 2 to 8 ($P < 0.01$), more total DMI ($P < 0.01$), ME ($P < 0.01$), CP ($P < 0.01$), MEg ($P < 0.01$), and MPg from wk 2 to 7 than PRE_{10} calves (Table 2 and Figure 1A–1C). As expected from the planned feeding schedule, the average MR intake across the whole preweaning period was lower than the theoretical peak allowance. The PRE_{20} calves received ~0.87 kg of MR DM/d at the full 20% BW allocation, whereas the average intake across wk 1 to 8 was 0.77 kg DM/d. The PRE_{10} showed a similar pattern, with average intake slightly exceeding the theoretical 0.43 kg DM/d due to the progressive increase during the first 2 wk.

In contrast, PRE_{20} calves had lower SF ($P < 0.01$), NFC ($P < 0.01$), and NDF ($P < 0.01$) at 4, 6, and 8 wk in comparison with PRE_{10} calves ($P < 0.01$; Table 2). These results support Gelsinger et al. (2016), who found a strong inverse relationship (r = −0.82) between liquid feed and voluntary SF intake. Nevertheless, we observed a numerically smaller treatment difference (0.105 kg/d) compared with the predicted value (0.200 kg/d) according to Gelsinger et al. (2016).

Despite the reduced voluntary SF intake, PRE_{20} calves showed greater nutrient intake and were heavier at weaning (Table 2 and Figure 1D), consistent with previous findings (Hu et al., 2020). No effect of preweaning treatments (**Tpre**) × wk interaction was detected for ADG, however, calves in PRE_{20} gained an additional 150 g of EBWg ($P < 0.01$) and 168 g of ADG ($P < 0.01$) compared with PRE_{10}. Also, we detected that calves in PRE_{20} tended ($P = 0.067$) to gain more HH per day than PRE_{10} (Table 2). These differences align with greater MEg ($P < 0.01$) and MPg ($P < 0.01$) availability in PRE_{20} calves from wk 2 to 7 (Table 2). This agrees with previous reports showing that calves cannot fully compensate for lower MR intake by increasing SF intake (Williams and Frost, 1992; Silva et al., 2015; Khan et al., 2016), particularly when no step-down strategy is implemented before weaning (Khan et al., 2007). Our findings support previous studies indicating that growth during preweaning is driven

Table 2. Preweaning treatment effect on feed intake, nutrient intake, performance, and IGF-1 concentration in female Holstein calves

Item	Preweaning treatment[1]		SEM	*P*-value[2]		
	PRE_{10}	PRE_{20}		Tpre	Wk	Tpre × Wk
Feed and nutrient intake						
Milk replacer, g of DM/d	468	768	19.4	<0.01	<0.01	<0.01
Starter feed, g of DM/d	573	468	22.5	<0.01	<0.01	<0.01
Total DMI, g/d	1,044	1,237	29.2	<0.01	<0.01	<0.01
Total ME intake, Mcal/d	4.0	5.1	0.11	<0.01	<0.01	<0.01
Total CP intake, g/d	238	290	6.6	<0.01	<0.01	<0.01
Total NDF intake, g/d	86	71	3.39	<0.01	<0.01	<0.01
Total NFC intake, g/d	319	261	12.5	<0.01	<0.01	<0.01
g CP/Mcal ME	58.7	56.7	0.14	<0.01	<0.01	<0.01
Cumulative NFC at weaning,[3] kg	19.2	14.6	0.578	<0.01	—	—
MEg,[4] Mcal/d	1.59	2.55	0.069	<0.01	<0.01	<0.01
MPg,[4] g/d	163	221	4.7	<0.01	<0.01	<0.01
Performance						
Initial BW, kg	38.3	38.5	0.605	0.847	—	—
BW at weaning, kg	63.5	71.4	1.55	<0.01	—	—
BW, kg	50.7	54.6	1.45	0.030	<0.01	0.003
EBW,[5] kg	45.5	48.9	1.30	0.030	<0.01	0.003
ADG, g/d	498	666	65.4	<0.01	0.093	0.747
EBWg,[5] g/d	447	597	58.6	0.005	0.093	0.746
Hip height, cm	83.7	85.0	0.77	0.240	<0.01	0.191
Hip height gain, cm/d	0.19	0.25	0.032	0.067	<0.01	0.505
Hip width, cm	18.6	18.7	0.30	0.728	<0.01	0.999
Hip width gain, cm/d	0.063	0.068	0.0147	0.734	<0.01	0.969
BCS (1−5)	2.66	2.77	0.105	0.335	0.024	0.283
Efficiency, kg BW/kg DM	0.416	0.439	0.0265	0.383	<0.01	0.335
IGF-1, ng/mL	148.3	152.8	11.84	0.694	—	—

[1]Calves in PRE_{10} and PRE_{20} had access to 10% or 20% of milk replacer and free access to starter feed before weaning (wk 1–8).

[2]Tpre = effect of preweaning treatments; Wk = week effect; Tpre × Wk = interaction effect of treatment and week.

[3]NFC = nonfiber carbohydrate [NFC% = DM – (ash + CP + ether extract + NDF)].

[4]MEg = ME available for gain (ME intake − ME for maintenance), and MPg = MP available for gain (MP intake − MP for maintenance), both estimated according to NASEM (2021).

[5]EBW = empty BW and empty BW gain (EBWg), both corrected for gut fill according to Jahn and Chandler (1976).

by WM or MR intake due to limited SF intake in the first 4 to 5 wk of life (Williams and Frost, 1992; Khan et al., 2016). As feed efficiency did not differ between preweaning treatments (0.428 ± 0.0265 kg BW/kg DMI, $P = 0.383$), performance differences were primarily driven by nutrient intake rather than differences in digestive or metabolic efficiency (Table 2).

Predicted EBW gain from the NASEM (2021) model, using young calf animal type module, closely matched observed values during the preweaning phase in both PRE_{20} (625 vs. 597 ± 58.6 g/d) and PRE_{10} calves (464 vs. 447 ± 58.6 g/d). According to NASEM (2021), growth in PRE_{10} calves was primarily limited by ME intake, whereas in PRE_{20} calves, both ME and MP were limited. The higher dietary CP to ME ratio in PRE_{10} calves from wk 3 to 8 ($P < 0.01$) indicates a greater ME deficiency in comparison with PRE_{20}. Nevertheless, both treatments exceeded the optimal CP to ME ratio proposed by Hill et al. (2013; 51–55 g CP/Mcal ME), with values ranging from 54 to 61 in PRE_{20} and 55 to 64 g CP/Mcal ME in PRE_{10} calves. Contrary to previous studies (Brown et al., 2005a; Bartlett et al., 2006, 2024; Rosadiuk et al., 2021), no differences in IGF-1 concentrations were observed among preweaning treatments (Table 2). Supporting this, weak and nonsignificant correlations were observed between IGF-1 and ME for gain (r = 0.27; $P = 0.13$), MP for gain (r = 0.28; $P = 0.12$), and empty BW gain (r = 0.03; $P = 0.87$), indicating no clear association between nutrient supply, growth, and IGF-1 concentrations during the preweaning period. This may be due to the timing of sampling, as IGF-1 was measured only once during the week of weaning, when previous studies reported convergence of IGF-1 levels across nutritional planes around weaning (Brown et al., 2005a; Haisan et al., 2018).

After weaning, we detected a significant effect of postweaning treatment (**Tpost**) × wk interaction effect ($P < 0.01$, Table 3). As we expected, $POST_{700}$ calves consumed more SF from 11 to 21 wk ($P < 0.01$), hay ($P < 0.01$), total DM ($P < 0.01$), ME ($P < 0.01$), CP ($P < 0.01$), NDF ($P < 0.01$), MEg ($P < 0.01$), and MPg from 9 to 21

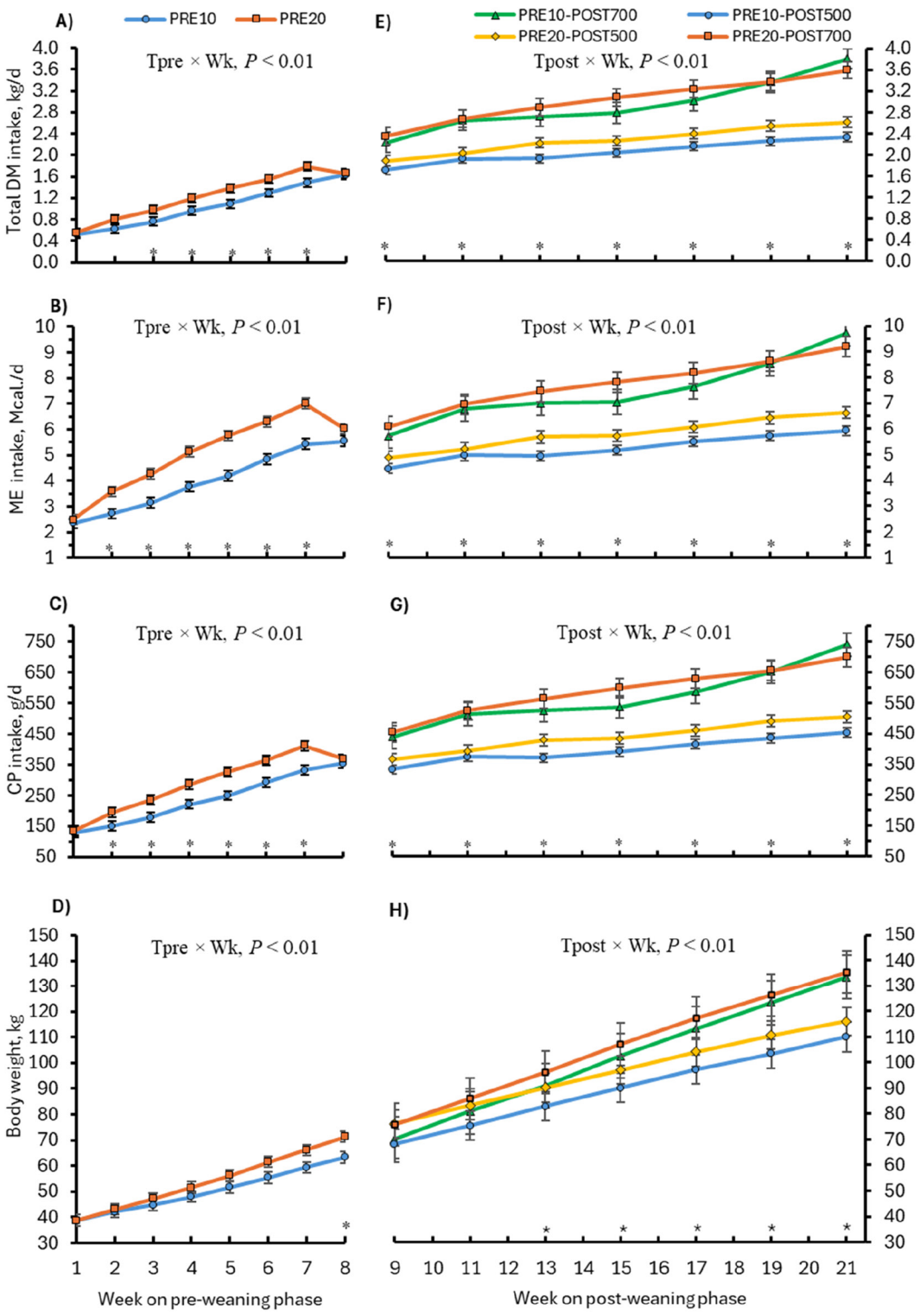

Figure 1. Total DM intake (A and E), ME intake (B and F), CP intake (C and G), and BW (D and H) during preweaning (A–D) and postweaning (E–H) phases. Calves were fed milk replacer at 10% (PRE_{10}) or 20% (PRE_{20}) of birth BW preweaning. Postweaning, calves were fed to target ADG of 500 ($POST_{500}$) or 700 ($POST_{700}$) g/d. Error bars represent SEM. Asterisks (*) on panels A to D denote a significant effect of preweaning treatment (PRE_{10} vs. PRE_{20}) at a specific time point ($P < 0.01$). Asterisks (*) on panels E to H denote a significant effect of postweaning treatment ($POST_{500}$ vs. $POST_{700}$) at a specific time point ($P < 0.01$). No significant interactions between pre- and postweaning treatments were detected for these variables.

wk ($P < 0.01$) compared with $POST_{500}$ (Figure 1E–1G). We also detected a Tpost × wk interaction effect on BW after weaning, and calves in $POST_{700}$ were heavier from wk 13 to 21 ($P < 0.01$; Figure 1H) and had greater BCS from wk 19 to 21 ($P = 0.033$) and were 21 kg heavier ($P < 0.01$) than $POST_{500}$ calves at wk 21 of life (Table 3). As in the preweaning phase, NASEM (2021) model predictions using young calf animal type module closely matched observed EBWg in $POST_{700}$ (680 vs. 658 ± 36.6 g/d) and $POST_{500}$ (390 vs. 451 ± 36.6 g/d) calves. Growth was similarly limited by ME and MP, consistent with the comparable CP to ME ratios across treatment combinations (75.4 ± 1.25 g CP/Mcal ME; Table 3).

After weaning, $POST_{700}$ calves showed higher IGF-1 concentrations than $POST_{500}$ calves ($P = 0.017$; Table 3). Although absolute values were lower than those reported in other studies, this may be attributed to differences in postweaning growth rates and nutritional supply. Supporting this, moderate positive correlations were observed between IGF-1 and ME for gain (r = 0.51; $P = 0.002$) and MP for gain (r = 0.52; $P = 0.002$), whereas the association with empty BW gain was weaker and did not reach statistical significance (r = 0.30; $P = 0.086$), reinforcing the link between nutrient availability and IGF-1 concentrations during the postweaning period.

During the postweaning phase, a carryover effect of preweaning treatments was detected, as calves receiving PRE_{20} had greater BW ($P = 0.018$), EBW ($P = 0.019$), and HH than PRE_{10} ($P = 0.033$). However, no Tpre × Tpost interaction was observed for any postweaning performance variable (Table 3). The postweaning ADG in $POST_{700}$ calves was 745 ± 28.7 g/d, regardless of preweaning feeding strategy. This rate meets the estimated requirement (~710 g/d) for heifers to reach 55% of mature BW (635 kg) by 15 mo of age, assuming a birth BW of 38 kg (NASEM, 2021). In contrast, $POST_{500}$ calves showed a lower ADG of 510 ± 27.6 g/d and would require compensatory growth between 5 and 15 mo to reach the target BW for first service.

Diet Digestibility

Calves in PRE_{20} had greater dDM ($P < 0.01$), dCP ($P < 0.01$), and dNDF than PRE_{10} ($P < 0.01$), whereas tdCP did not differ between preweaning treatments (Table 4). These differences in digestibility were expected given the composition of the preweaning diets and the higher metabolizability of MR compared with SF (Marcondes and Silva, 2021; NASEM, 2021). In wk 7, when the digestibility trial was conducted, SF accounted for 66% of the diet in PRE_{10} but only 48% of DM in PRE_{20}. This difference is consistent with the greater contribution of MR to total DM intake in PRE_{20}, as MR has substantially higher digestibility than SF (Silva et al., 2015). The higher digestibility of DM ($P < 0.01$; Table 4), combined with greater ME ($P < 0.01$) and CP intake ($P < 0.01$), resulted in a higher intake of digestible nutrients in PRE_{20} calves, which likely contributed to their greater ADG ($P < 0.01$) and BW ($P < 0.01$) during the preweaning phase (Table 2).

A main Tpre effect was detected for dNDF, which was greater in PRE_{20} than in PRE_{10} calves ($P < 0.01$). This difference can be partly explained by a less acidified ruminal environment in PRE_{20} calves, as indicated by a tendency for higher average ruminal pH ($P = 0.052$) and a significantly higher minimum pH ($P < 0.01$). Silva et al. (2015) also reported that greater WM intake increased OM and NDF digestibility before weaning, likely through increased fatty acid intake that stimulated cholecystokinin release and prolonged digesta retention. Taken together, the lower NDF intake ($P < 0.01$), a longer digesta retention time, and a ruminal pH more favorable to fibrolytic activity provide a plausible explanation for the higher dNDF observed in PRE_{20} calves (Table 4).

In the postweaning phase, no Tpre × Tpost × wk or Tpre × wk interaction effect was detected for digestibility (Table 5). However, a significant Tpost × wk interaction effect was detected with $POST_{500}$ calves showing greater dDM ($P < 0.01$), dCP ($P < 0.01$), and tdCP ($P < 0.01$) than $POST_{700}$ calves in 9 wk, whereas no differences were observed in 11 wk (Figure 2A–2C). No treatment or interaction effects were detected for dNDF (Table 5), although values increased ($P < 0.01$) from 47.4% ± 2.21% in 9 wk to 67.7% ± 1.99% in 11 wk across all treatment combinations, despite the increase in dietary forage proportion from 30% to 50%, respectively. These values are within the range (42.3%–70.7%) reported in 17 studies reviewed by NASEM (2021) for weaned calves.

No Tpre × wk, Tpre × Tpost interaction, or main Tpre effects were detected for digestibility, indicating no evidence of carryover from preweaning treatments into the postweaning phase in any fraction (Table 5). Contrary to previous reports suggesting that higher MR intake reduces starter intake and impairs postweaning digestibility (Terré et al., 2007; Hill et al., 2010; Hu et al., 2020), no such effects were observed in our study. However, examination of previous studies (e.g., Terré et al., 2007; Hill et al., 2010; Chapman et al., 2016; van Niekerk et al., 2020) indicates that in at least one treatment group of each study, cumulative NFC intake was below 15 kg, which could account for discrepancies with our findings. Collectively, these results support the proposal by Quigley et al. (2019) that cumulative NFC intake from solid feed is the key determinant of postweaning digestive capacity.

It is important to note that postweaning digestibility outcomes were more variable than anticipated, and treatment differences were smaller than the expected 5 percentage

Table 3. Effects of pre- and postweaning treatments on nutrient intake, performance, and IGF-1 concentration during the postweaning phase

	Pre- and postweaning treatments[1]					*P*-value[2]						
	PRE_{10}		PRE_{20}									
Item	$POST_{500}$	$POST_{700}$	$POST_{500}$	$POST_{700}$	SEM	Tpre	Tpost	Wk	Tpre × Tpost	Tpre × Wk	Tpost × Wk	Tpre × Tpost × Wk
Feed and nutrient intake												
Starter feed, kg DM/d	1.04	1.47	1.14	1.52	0.056	0.012	<0.01	<0.01	0.416	0.838	<0.01	0.636
Hay, kg DM/d	1.02	1.47	1.14	1.51	0.039	0.037	<0.01	<0.01	0.341	0.406	<0.01	0.380
Total DMI, kg/d	2.05	2.94	2.28	3.03	0.075	0.016	<0.01	<0.01	0.281	0.277	<0.01	0.240
Total ME intake, Mcal/d	5.2	7.5	5.8	7.8	0.26	0.016	<0.01	<0.01	0.383	0.417	<0.01	0.328
Total CP intake, g/d	396	571	440	590	28.4	0.041	<0.01	<0.01	0.420	0.328	<0.01	0.359
Total NDF intake, g/d	613	861	681	897	17.1	<0.01	<0.01	<0.01	0.267	0.412	<0.01	0.101
g CP/Mcal ME	75.3	75.4	75.4	75.3	1.25	0.927	0.777	<0.01	0.577	0.984	0.734	0.987
ME for growth,[3] Mcal/d	1.50	3.39	1.93	3.76	0.317	0.019	<0.01	<0.01	0.869	0.492	<0.01	0.492
MP for growth g,[3] g/d	228	338	255	351	20.2	0.045	<0.01	<0.01	0.474	0.344	<0.01	0.479
Performance												
BW, kg	89.6	102.1	96.8	106.3	2.26	0.018	<0.01	<0.01	0.521	0.867	<0.01	0.979
EBW,[4] kg	79.0	90.0	85.3	93.6	2.64	0.019	<0.01	<0.01	0.498	0.868	<0.01	0.975
ADG, g/d	521	782	499	709	39.9	0.196	<0.01	<0.01	0.494	0.944	0.727	0.352
EBWg,[4] g/d	460	690	441	626	36.6	0.209	<0.01	<0.01	0.495	0.945	0.735	0.379
Hip height, cm	94.0	96.7	97.2	97.9	1.22	0.033	0.087	<0.01	0.295	0.924	0.532	0.446
Hip height gain, cm/d	0.16	0.17	0.14	0.16	0.018	0.411	0.331	0.013	0.672	0.850	0.909	0.766
Hip width, cm	22.8	23.4	23.2	23.6	0.37	0.410	0.154	<0.01	0.692	0.724	0.843	0.929
Hip width gain, cm/d	0.057	0.058	0.054	0.056	0.0069	0.690	0.834	<0.01	0.952	0.772	0.934	0.975
BCS (1–5)	2.64	2.94	2.79	2.91	0.120	0.603	0.073	0.208	0.409	0.527	0.033	0.980
Efficiency, kg BW/kg DM	0.200	0.235	0.246	0.200	0.0203	0.807	0.433	<0.01	0.132	0.810	0.866	0.879
IGF-1, ng/mL	85.6	114.5	94.3	131.1	13.90	0.303	0.017	—	0.747	—	—	—

[1]Calves in PRE_{10} and PRE_{20} had access to 10% or 20% of milk replacer and had free access to starter feed before weaning (1–8 wk). After weaning (9–21 wk), calves were fed with starter feed and hay to achieve 500 ($POST_{500}$) or 700 g of BW/d ($POST_{700}$).

[2]Tpre = effect of preweaning treatment; Tpost = effect of postweaning treatment; Wk = week effect; Tpre × Tpost = effect of interaction of pre- and postweaning treatments on postweaning phase; Tpre × Wk = effect of interaction of preweaning treatment and week on postweaning phase; Tpost × Wk = effect of interaction of postweaning treatment and week; Tpre × Tpost × Wk = effect of pre- and postweaning treatment and week on postweaning phase.

[3]ME available for gain = ME intake minus ME for maintenance, and MP available for gain = MP intake minus MP for maintenance, both according to NASEM (2021).

[4]EBW = empty BW and empty BW gain (EBWg), both corrected for gut fill according to Jahn and Chandler (1976).

Table 4. Preweaning treatment effect on diet digestibility, nitrogen metabolism, and ruminal fermentation variables before weaning

Item	Preweaning treatments[1]		SEM	*P*-value[2]
	PRE_{10}	PRE_{20}		Tpre
Diet digestibility				
DM digestibility, %	89.0	92.0	0.32	<0.01
CP digestibility, %	88.0	90.5	0.53	<0.01
True CP digestibility,[3] %	96.6	97.4	0.42	0.175
NDF digestibility, %	45.3	53.5	0.91	<0.01
Nitrogen metabolism				
Intake, g/kg $BW^{0.75}$/d	2.21	2.56	0.027	<0.01
Urine output, g/kg $BW^{0.75}$/d	0.95	1.43	0.033	<0.01
Fecal output, g/kg $BW^{0.75}$/d	0.28	0.22	0.004	<0.01
Retained,[4] g/kg $BW^{0.75}$/d	0.99	0.92	0.037	0.171
Retained/ingested, %	40.3	35.4	1.41	0.012
Ruminal fermentative variables				
Ruminal pH	5.35	5.47	0.057	0.052
Minimum ruminal pH[5]	4.95	5.10	0.045	<0.01
Total VFA,[6] m*M*	123.7	117.6	3.99	0.293
Acetate, m*M*	64.9	68.5	2.19	0.258
Propionate, m*M*	42.7	35.6	1.67	<0.01
Butyrate, m*M*	17.4	13.4	0.88	<0.01
Acetate, %	52.3	58.2	0.80	<0.01
Propionate, %	33.8	30.2	0.59	<0.01
Butyrate, %	13.9	11.6	0.64	0.012
NH_3-N, mg/dL	16.4	23.6	0.79	<0.01
Lactate, m*M*	17.1	26.1	0.91	<0.01

[1]Calves in PRE_{10} and PRE_{20} had access to 10% or 20% of milk replacer and had free access to starter feed before weaning (wk 1–8).

[2]Tpre = preweaning treatment effect.

[3]True CP digestibility = [CP ingested − (CP in feces − metabolic fecal CP)]/CP ingested × 100. Metabolic fecal CP = (11.9 × milk DM intake) + (20.6 × starter DM intake), according to NASEM (2021).

[4]Nitrogen retained = nitrogen ingested − (nitrogen in feces + nitrogen in urine), per kilogram of $BW^{0.75}$.

[5]Minimum daily ruminal pH was the lowest pH value recorded among the 4 daily measurements (0800, 1200, 1600, and 2000 h).

[6]Total VFA (m*M*) = acetate + propionate + butyrate.

points. Biologically, such variation is plausible given the transition from liquid to solid feed, including the introduction of forage, when physiological and behavioral adaptations are still ongoing. This variability, together with the limited number of experimental units, reduced the power to detect subtle effects. Thus, postweaning digestibility results should be interpreted with caution, recognizing that minor treatment effects may have occurred but remain undetected under the present conditions.

Nitrogen Metabolism

Calves in PRE_{20} ingested 16% more nitrogen per kilogram of $BW^{0.75}$ ($P < 0.01$), excreted 51% more urinary nitrogen ($P < 0.01$), and 21% less fecal nitrogen ($P < 0.01$) than those in PRE_{10}, resulting in similar nitrogen retention per kilogram of $BW^{0.75}$ between preweaning treatments (Table 4). The greater nitrogen intake in PRE_{20} was expected because these calves consumed more total DM ($P < 0.01$; Figure 1A), whereas BW did not differ among treatments at wk 7 (Figure 1D). The lower fecal nitrogen excretion is consistent with the typically higher digestibility of CP from MR, its distinct site of digestion (primarily intestinal rather than ruminal), and its AA profile, which more closely matches the EAA requirements of calves compared with SF. Together, these factors would enhance the biological value of dietary protein in PRE_{20} calves, in which MR accounted for a larger proportion of total DMI.

Contrary to our expectations, nitrogen retention was similar between treatments, whereas urinary nitrogen excretion was greater ($P < 0.01$) in PRE_{20} calves (Table 4). Moreover, nitrogen use efficiency [(g nitrogen retained/kg $BW^{0.75}$)/(g nitrogen ingested/kg $BW^{0.75}$) × 100] in both PRE_{10} and PRE_{20} was lower than the maximum value of 48% reported by Zanton and Heinrichs (2008), with PRE_{20} calves showing significantly lower efficiency than PRE_{10} ($P = 0.012$). Within the framework proposed by Zanton and Heinrichs (2008), the proportion of ME derived from CP in PRE_{20} was ~24.5%, exceeding the ~22.5% threshold associated with maximal nitrogen use efficiency. This protein–energy imbalance promoted AA oxidation and urea formation, thereby explaining both the greater urinary nitrogen losses ($P < 0.01$) and the absence of differences in nitrogen retention per kilogram of $BW^{0.75}$ between treatments.

In the postweaning phase, a significant Tpre × wk interaction effect was detected for nitrogen intake ($P < 0.01$; Table 5). At 9 wk, calves from PRE_{10} treatments ingested more nitrogen ($P < 0.01$) than those from PRE_{20} treatments. Although nitrogen intake increased from 9 to 11 wk in calves from both preweaning treatments, PRE_{10} calves continued to ingest more nitrogen per kilogram of $BW^{0.75}$ than PRE_{20} calves at 11 wk (Figure 3A). A significant Tpost × wk interaction was also observed for nitrogen intake ($P < 0.01$; Table 5). At 9 wk, nitrogen intake was similar between $POST_{500}$ and $POST_{700}$ calves, but by 11 wk, $POST_{700}$ calves ingested more nitrogen than $POST_{500}$ calves (Figure 3B).

For urinary nitrogen excretion after weaning, significant Tpre × wk ($P < 0.01$) and Tpost × wk ($P < 0.01$) interactions were detected, but no Tpre × Tpost interaction effect (Table 5). At 9 wk, urinary nitrogen excretion was greater in PRE_{10} than in PRE_{20} calves ($P < 0.01$). By 11 wk, urinary nitrogen excretion increased in PRE_{20} calves and was greater than PRE_{10} ($P < 0.01$), whereas PRE_{10} calves showed similar values to 9 wk (Figure 3C). Likewise, urinary nitrogen excretion was greater in $POST_{500}$ than in $POST_{700}$ calves at 9 wk ($P < 0.01$). However, while excretion did not change between 9 and 11 wk in $POST_{500}$ calves, it increased in $POST_{700}$ calves and became significantly greater than $POST_{500}$ at 11 wk ($P < 0.01$; Figure 3D). The relatively low ME-to-CP ratio of the postweaning diets probably forced calves to oxidize

Table 5. Effects of pre- and postweaning treatments on digestibility, nitrogen metabolism, and ruminal fermentation variables after weaning

	Preweaning and postweaning treatments[1]					*P*-value[2]						
	PRE_{10}		PRE_{20}									
Item	$POST_{500}$	$POST_{700}$	$POST_{500}$	$POST_{700}$	SEM	Tpre	Tpost	Wk	Tpre × Tpost	Tpre × Wk	Tpost × Wk	Tpre × Tpost × Wk
Diet digestibility												
DM digestibility, %	76.8	76.6	78.4	74.2	1.79	0.810	0.236	<0.01	0.263	0.142	<0.01	0.443
CP digestibility, %	77.9	74.6	78.3	73.9	1.97	0.944	0.063	<0.01	0.763	0.568	<0.01	0.144
True CP digestibility,[3] %	89.4	85.7	88.7	84.9	1.87	0.745	0.068	<0.01	0.884	0.379	<0.01	0.158
NDF digestibility, %	57.4	59.5	58.3	55.9	3.08	0.530	0.835	<0.01	0.332	0.169	0.213	0.522
Nitrogen metabolism												
Intake, g/kg $BW^{0.75}$/d	1.76[c]	2.43[a]	1.70[c]	2.11[b]	0.083	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.120
Urine output, g/kg $BW^{0.75}$/d	1.24	1.17	1.19	1.14	0.102	0.017	0.011	<0.01	0.697	<0.01	<0.01	0.499
Fecal output, g/kg $BW^{0.75}$/d	0.29[d]	0.54[a]	0.37[c]	0.48[b]	0.027	0.030	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Retained,[4] g/kg $BW^{0.75}$/d	0.25[z]	0.70[w]	0.13[y]	0.47[x]	0.115	<0.01	<0.01	<0.01	0.070	0.005	<0.01	0.398
Retained/ingested, %	16.1	14.3	18.5	14.9	3.93	0.324	0.188	<0.01	0.590	0.059	<0.01	0.680
TPD excretion,[5] mmol/d	40.1	45.4	50.3	51.2	1.80	0.565	0.042	0.206	0.266	0.923	0.370	0.287
MNY,[6] g/kg DOMI	28.6	33.9	42.0	36.3	5.65	0.117	0.962	<0.01	0.271	0.136	0.082	0.134
Ruminal fermentation												
Average ruminal pH	6.28	6.12	6.21	6.17	0.080	0.913	0.142	<0.01	0.357	0.695	<0.01	0.149
Minimum ruminal pH[7]	5.64	5.59	5.28	5.41	0.152	0.023	0.706	<0.01	0.494	0.421	<0.01	<0.01
Total VFA,[8] m*M*	98.3	105.7	85.4	94.7	4.38	<0.01	0.024	0.607	0.806	0.294	0.141	0.018
Acetate, m*M*	54.5	58.5	49.5	52.3	2.99	0.033	0.173	0.220	0.808	0.987	0.645	0.065
Propionate, m*M*	30.4	34.2	26.4	29.3	1.47	<0.01	<0.01	<0.01	0.694	0.056	0.131	0.075
Butyrate, m*M*	12.6	14.4	9.5	13.8	1.64	0.047	0.026	0.310	0.068	0.051	<0.01	0.016
Acetate, %	57.0	54.3	56.9	57.1	1.54	0.307	0.338	<0.01	0.266	0.047	0.071	0.373
Propionate, %	29.5	32.0	31.2	29.5	1.39	0.744	0.748	<0.01	0.084	0.410	0.845	0.111
Butyrate, %	13.4	13.7	11.9	13.4	0.91	0.271	0.230	<0.01	0.429	0.031	<0.01	0.333
NH_3-N, mg/dL	6.6	6.4	7.9	7.0	0.70	0.126	0.332	0.951	0.501	0.601	0.966	0.881
Lactate, m*M*	11.9	15.6	12.0	10.1	2.29	0.194	0.645	0.080	0.152	0.544	0.206	0.531

[a–d]Superscripts indicate significant Tpre × Tpost differences ($P \leq 0.05$); [w–z]superscripts indicate tendencies ($0.05 < P < 0.10$).

[1]PRE_{10} and PRE_{20} received 10% or 20% of BW as milk replacer with free access starter (wk 1–8). Postweaning (9–21 wk), calves were fed starter and hay to target 500 ($POST_{500}$) or 700 g/d BW gain ($POST_{700}$).

[2]Tpre = effect of preweaning treatment; Tpost = effect of postweaning treatment; Wk = week effect; Tpre × Tpost = interaction effect of pre- and postweaning treatments; Tpre × Wk = interaction effect of preweaning treatment and week on postweaning phase; Tpost × Wk = effect of interaction of postweaning treatment and week; Tpre × Tpost × Wk = effect of preweaning treatment, postweaning treatment, and week on postweaning phase.

[3]True CP digestibility = [CP ingested − (CP in feces − metabolic fecal CP)]/CP ingested × 100. Metabolic fecal CP = (11.9 × milk DMI) + (20.6 × starter DMI), according to NASEM (2021).

[4]Nitrogen retained = nitrogen ingested − (nitrogen in feces + nitrogen in urine), per kilogram of $BW^{0.75}$.

[5]TPD = total purine derivatives excretion was estimated according to Chen and Gomes (1992).

[6]Microbial nitrogen yield (MNY) was estimated according to Chen and Gomes (1992) divided per kilogram of digestible OM intake (DOMI).

[7]Minimum daily ruminal pH recorded among the 4 daily measurements (0800, 1200, 1600, and 2000 h) taken for each calf in each week.

[8]Total VFA = acetate + propionate + butyrate.

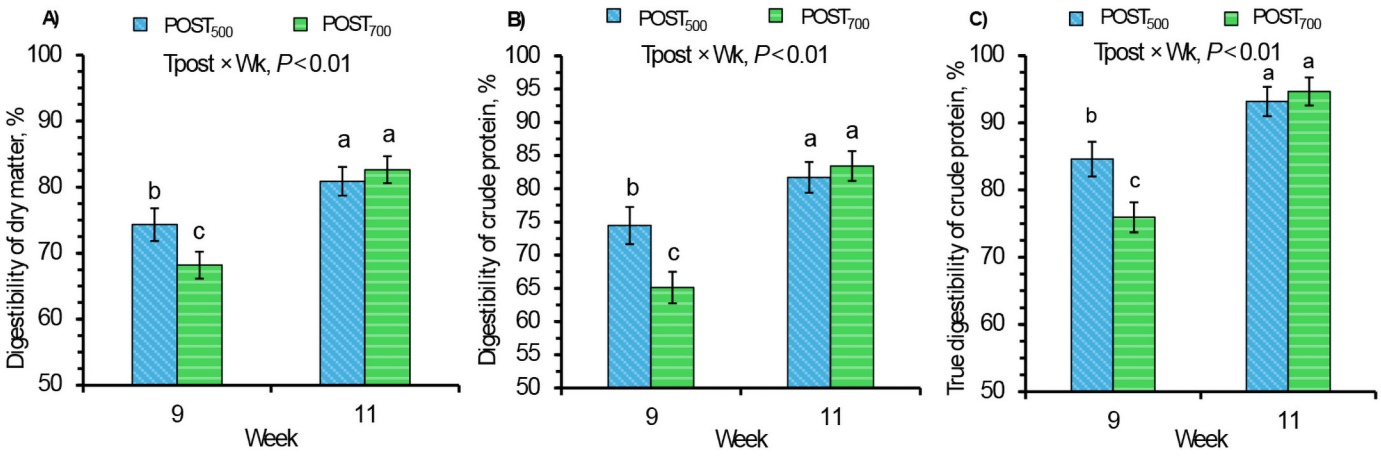

Figure 2. Postweaning digestibility of DM (A), CP (B), and true digestibility of CP (C) of Holstein calves at 9 and 11 wk of age. Calves received milk replacers at 10% (PRE_{10}) or 20% (PRE_{20}) of birth BW during the preweaning phase and were fed diets formulated to target an ADG of 500 ($POST_{500}$) or 700 g/d ($POST_{700}$) postweaning. A significant postweaning treatment × week interaction (Tpost × Wk; $P < 0.01$) was detected; therefore, data are presented as LSM ± SEM collapsed across preweaning treatments. Bars with different letters (a–c; Tukey-adjusted, $P < 0.05$) differ significantly.

some of the absorbed AA to meet energy demands, thus increasing urinary nitrogen losses ($P < 0.01$), particularly at $POST_{700}$, which were growing at a higher rate ($P < 0.01$; Table 3).

A significant Tpre × Tpost × wk interaction effect ($P < 0.01$) was detected for fecal nitrogen excretion, whereas no such interaction was observed for the other nitrogen metabolism variables (Table 5). At 9 wk, fecal nitrogen excretion was lower ($P < 0.01$) in PRE_{10}–$POST_{500}$ and PRE_{20}–$POST_{500}$ calves compared with the other 2 treatment combinations. However, by 11 wk, fecal nitrogen excretion increased in PRE_{20}–$POST_{500}$ calves, and a similar rise was observed in PRE_{20}–$POST_{700}$ calves relative to their own at 9 wk. Nevertheless, fecal nitrogen excretion per kilogram of $BW^{0.75}$ remained numerically slightly higher than preweaning values and was lower than urinary nitrogen excretion, which was the primary route of nitrogen excretion (Figure 3E).

Significant Tpre × wk ($P < 0.01$) and Tpost × wk ($P < 0.01$) interactions were detected for nitrogen retention (Table 5). Retention was near zero at 9 wk but increased in all groups by 11 wk ($P < 0.01$), with greater values in PRE_{10} than PRE_{20} calves ($P < 0.01$) and in $POST_{700}$ than $POST_{500}$ calves ($P < 0.01$; Figure 3F and 3G). A tendency for a Tpre × Tpost interaction ($P = 0.070$) suggests that higher retention in PRE_{10}–$POST_{700}$ calves reflected compensatory growth relative to PRE_{20}–$POST_{700}$ under similar diets. Conversely, PRE_{20}–$POST_{500}$ calves retained less nitrogen than PRE_{10}–$POST_{500}$, due to lower digestive utilization, as indicated by greater fecal nitrogen excretion ($P < 0.01$).

We also detected a Tpost × wk interaction ($P < 0.01$) for nitrogen efficiency (retained/ingested) after weaning (Table 5). Nitrogen efficiency at 9 wk was significantly lower in $POST_{700}$ than in $POST_{500}$ calves ($P < 0.01$). By 11 wk, however, $POST_{700}$ calves showed the greatest nitrogen efficiency, exceeding both $POST_{500}$ calves and their own values at 9 wk (Figure 3I). Overall, our findings show that postweaning feeding level was the primary factor determining nitrogen retention. Preweaning strategies exerted only indirect effects, likely by shaping compensatory growth responses that manifested after weaning. In addition, fecal nitrogen excretion patterns suggest that differences in ruminal nitrogen use efficiency (Figure 3H and 3I), associated with preweaning ruminal development, may have further influenced overall nitrogen retention. Finally, nitrogen efficiencies below theoretical maximums for this stage (~24.3%; Zanton and Heinrichs, 2008) may reflect the high inclusion of forage (~50%) and a high CP to ME ratio (~75 g CP/Mcal ME), conditions that could divert a portion of the CP toward energy use.

No interaction effects were detected for PD excretion (Table 5). However, a significant Tpost main treatment effect was observed, with calves in the $POST_{700}$ excreting more PD than those in $POST_{500}$ (70 vs. 58 ± 4.15 mmol/d; $P = 0.042$). These values are slightly above the range reported for postweaning calves, which ranged from 37 to 58 mmol/d (Funaba et al., 1997) and 10 to 45 mmol/d during the early postweaning (Terré et al., 2006). Based on Terré et al. (2006), we initially expected differences in PD excretion associated with preweaning MR allowance. However, as discussed earlier, cumulative SF intake in PRE_{20} was higher than in the study by Terré et al. (2006), and no effect of preweaning diet was detected on postweaning solid feed digestibility. These factors

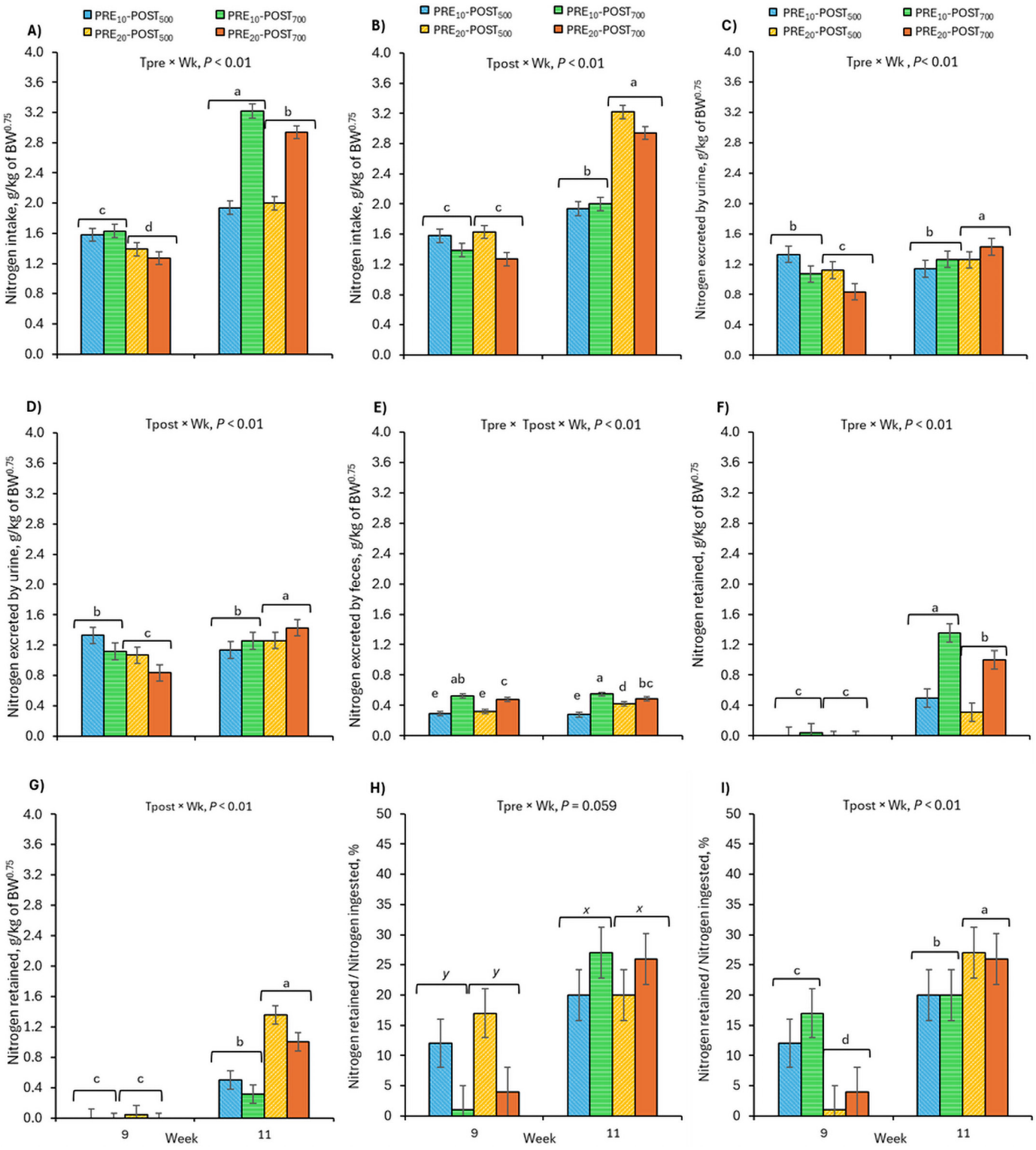

Figure 3. Effects of pre- and postweaning feeding strategies on nitrogen metabolism of Holstein calves at 9 and 11 wk of age. Preweaning calves were fed milk replacer at 10% (PRE_{10}) or 20% (PRE_{20}) of birth BW. Postweaning, calves were fed diets formulated to target an ADG of 500 ($POST_{500}$) or 700 g/d ($POST_{700}$). Panels display LSM ± SEM for the 4 treatment combinations. Depending on the variable, either 2-way (Tpre × Wk or Tpost × Wk) or 3-way (Tpre × Tpost × Wk) interactions were detected, as indicated in each panel. Bars with different letters (a–e; $P < 0.05$, Tukey-adjusted) differ significantly; those with different letters (x and y; $0.05 \leq P < 0.10$) tend to differ.

contributed to the absence of a preweaning treatment effect on PD excretion in our study (Table 5).

No interaction effects were detected for MNY efficiency during the postweaning phase (Table 5). A tendency for a Tpost × wk interaction was observed ($P = 0.082$), but no differences were found between $POST_{700}$ and $POST_{500}$ calves at 9 wk (43.5 vs. 50.6 ± 4.78 g MNY/kg DOMI) or 11 wk (19.5 vs. 27.3 ± 4.79 g MNY/kg DOMI). However, MNY values at 9 wk were unexpectedly high and biologically unrealistic, whereas 11 wk values were within the range reported for young heifers fed forage-based diets (13.6–22.0 g/kg DOMI; Aguerre et al., 2013; Santana et al., 2017; Britos et al., 2018). The atypical 9 wk results likely reflect the transition from liquid to solid feeding, methodological limitations, or both, underscoring the need for further research.

Ruminal Fermentation

In the preweaning phase, PRE_{10} calves tended to have lower average ruminal pH ($P = 0.052$) and lower daily minimum pH than PRE_{20} calves ($P < 0.01$; Table 4), although CV was similar between treatments (9.1% vs. 8.5% ± 1.15%; $P = 0.72$). This tendency reflects the higher SF intake observed in PRE_{10} calves from wk 4 to 8 ($P < 0.01$), which promoted more active ruminal fermentation. These fermentation patterns are also consistent with accelerated rumen epithelial development in PRE_{10} calves, as greater SF intake from 4 to 8 wk likely stimulated VFA production (particularly butyrate), which is known to promote papillae growth and enhance absorptive capacity (Baldwin et al., 2004; Khan et al., 2016; van Niekerk et al., 2021). Such epithelial adaptations may have contributed to the lower average and minimum pH values observed in this treatment. Overall, ruminal pH values were notably low in both treatments (Table 4), considering that pH measured by stomach tube can be 0.3 units higher than that obtained via ruminal cannula in preweaning calves (Terré et al., 2013) and 0.04 to 1.10 pH units higher than rumenocentesis in dairy cows according to the review of Golder and Lean (2024), due to saliva contamination.

Consistent with ruminal pH observed, PRE_{10} calves had greater propionate ($P < 0.01$) and butyrate concentrations ($P < 0.01$), a lower acetate proportion ($P < 0.01$), and a reduced acetate-to-propionate ratio compared with PRE_{20} calves (1.66 vs. 1.95 ± 0.059; $P < 0.01$; Table 4). These results suggest that fibrolytic activity was constrained by the lower ruminal pH and diet characteristics (high NFC and low NDF), leading to a shift toward amylolytic fermentation that promotes greater propionate proportion and lower acetate (van Niekerk et al., 2021).

During the preweaning phase, PRE_{10} calves showed lower ruminal NH_3-N concentrations than PRE_{20} calves ($P < 0.01$). This difference may be partly explained by their lower ruminal pH, because proteolytic activity is inhibited at values below 5.5 (Bach et al., 2005). In addition to the effect of pH, the greater starter intake observed in PRE_{10} calves likely stimulated earlier microbial proliferation and fermentative activity, increasing the incorporation of ammonia into bacterial protein and thereby reducing the pool of free ruminal NH_3-N. A more advanced rumen epithelial development in PRE_{10} calves may also have enhanced the absorption of fermentation products, including ammonia, although this mechanism was not reflected in increased urinary N excretion, suggesting that hepatic urea synthesis was not substantially elevated.

Nevertheless, NH_3-N concentrations in both treatments remained above the minimum threshold required for optimal microbial growth (Satter and Slyter, 1974) and were numerically greater than those observed postweaning (Table 5). It is possible that the lower NDF intake in both preweaning treatments, together with the lower ruminal pH (below 5.5 in both treatments; Table 4), limited the development of fibrolytic microbiota, which are the primary microbial group utilizing ruminal NH_3-N.

During the postweaning phase, significant Tpre × Tpost × wk interactions were detected for minimum ruminal pH ($P < 0.01$), total VFA ($P < 0.01$), and butyrate ($P < 0.05$; Figure 4A–4C). At 9 wk, PRE_{20}–$POST_{500}$ calves had lower minimum pH than PRE_{10}–$POST_{700}$, while by 11 wk minimum pH increased in all groups, with PRE_{10}–$POST_{500}$ reaching the highest values (Figure 4A). For total VFA and butyrate, PRE_{10}–$POST_{500}$ calves showed greater concentrations at 9 wk than PRE_{20}–$POST_{500}$ and PRE_{20}–$POST_{700}$, whereas PRE_{20}–$POST_{500}$ had the lowest values at 11 wk (Figure 4). A Tpost × wk interaction was also observed for average pH ($P < 0.01$; Figure 4), values were similar between $POST_{500}$ and $POST_{700}$ at 9 wk but increased by 11 wk, with $POST_{500}$ exceeding $POST_{700}$ ($P < 0.01$). This coincided with a numerical reduction in pH variability (CV: 14.8% to 11.2% ± 0.74%), supporting greater ruminal stabilization during the transition to solid feed (Khan et al., 2016). Significant main effects of Tpre on minimum pH ($P = 0.02$), acetate ($P = 0.03$), propionate ($P < 0.01$), butyrate ($P = 0.047$), and total VFA ($P < 0.01$), together with a Tpre × wk interaction for butyrate proportion ($P = 0.031$) and a tendency for acetate proportion ($P = 0.089$), indicate carryover effects of preweaning feeding strategy, likely mediated by starter intake and cumulative NFC consumption (Table 5). These fermentation patterns suggest that preweaning feeding strategies influenced early ruminal development, consistent with structural

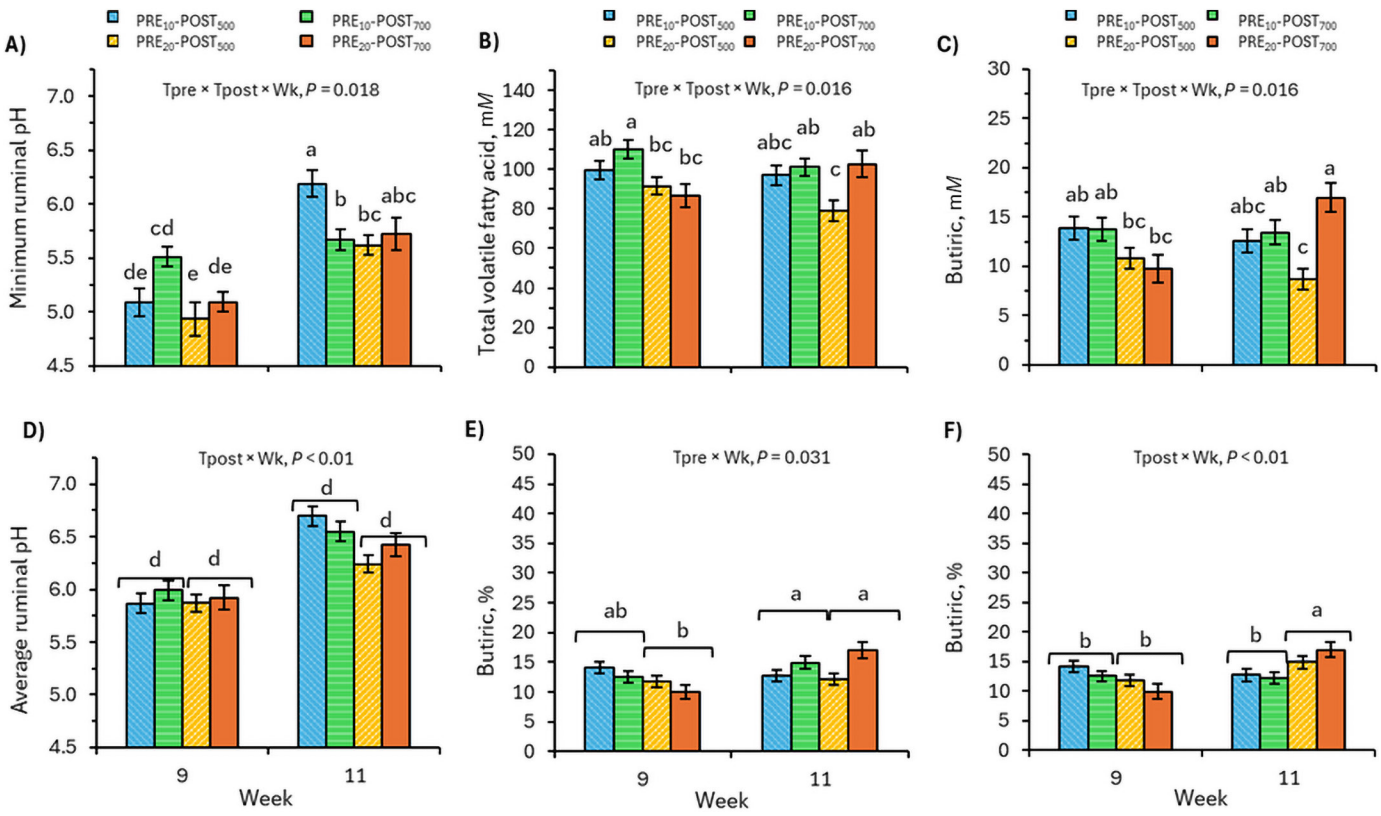

Figure 4. Effects of pre- and postweaning feeding strategies on minimum ruminal pH (A), total VFA concentration (B), butyrate concentration (C), average ruminal pH (D), and butyric proportion (E and F) at 9 and 11 wk. Calves received 10% (PRE_{10}) or 20% (PRE_{20}) of birth BW as milk replacer preweaning; postweaning diets targeted 500 ($POST_{500}$) or 700 g/d ($POST_{700}$). A 3-way interaction (Tpre × Tpost × Wk) was detected in all panels. Bars are means ± SEM for the 4 treatment combinations. Different letters indicate differences among treatment combinations (a–e; $P < 0.05$).

adaptations previously described in young calves (van Niekerk et al., 2021). Although papillae morphology was not evaluated here, the observed VFA profile and minimum pH support this interpretation.

No treatment effects were detected for ruminal NH_3-N, which remained above the 5.0 mg/dL threshold required to support optimal microbial growth (Satter and Slyter, 1974). The elevated ruminal pH observed at 9 and 11 wk may have facilitated greater NH_3-N absorption across the ruminal epithelium, enhanced microbial utilization (Bach et al., 2005), or both. Ruminal lactate was detected both before and after weaning (Tables 4 and 5). Preweaning concentrations were higher in PRE_{20} than in PRE_{10} calves ($P < 0.01$), with no treatment effects postweaning. Similarly, Gelsinger et al. (2020) found detectable lactate in about half of calf samples when evaluating starter feeds designed to induce or mitigate ruminal acidosis, although concentrations were not reported. A recent review by Golder and Lean (2024) underscored the lack of consensus on physiological and pathological ranges: ≤5 to 50 m*M* is often considered normal, >40 to 90 m*M* indicates acidosis, and 50 to 120 m*M* acute lactic acidosis.

Final Remarks

We did not find evidence of interactions between pre- and postweaning feeding strategies on body growth, IGF-1 concentrations, or diet digestibility. However, interactions were detected shortly after weaning for nitrogen metabolism and ruminal fermentation variables. Consistent with previous studies, greater MR supply increased nutrient intake and preweaning growth and generated carryover effects on body growth after weaning. In contrast to some earlier reports, we did not detect negative effects of higher MR allowance on postweaning solid feed digestibility, reinforcing the notion that cumulative NFC intake, rather than MR supply per se, plays a more relevant role. In addition, calves receiving a more restrictive milk replacer allowance exhibited compensatory growth after weaning, tended to retain more nitrogen, and reached similar BW to calves provided with greater MR allowances when offered comparable postweaning diets. Although growth recovery through improved postweaning nutrition is possible, the use of highly restrictive MR feeding strategies (<10% of BW at birth) should be carefully considered from an animal welfare perspective.

It should also be noted that postweaning growth targets in this study were modest, and forage inclusion was high, reflecting feeding conditions commonly observed in pasture-based dairy systems. These contextual factors must be considered when extrapolating our findings into more intensive heifer-rearing strategies.

CONCLUSIONS

Our findings partially support the initial hypothesis. No interactions were observed between pre- and postweaning feeding strategies for body growth or diet digestibility. However, significant interactions were detected for nitrogen metabolism and ruminal fermentation shortly after weaning. Increased milk replacer intake enhanced preweaning growth without compromising postweaning solid feed digestibility, reinforcing cumulative starter intake as the primary driver of digestive development. Calves fed restricted milk replacer regimens achieved comparable postweaning BW to those fed higher allowances under similar postweaning strategies, highlighting the occurrence of compensatory growth. Collectively, these results underscore the importance of aligning pre- and postweaning feeding strategies in dairy heifers. The $POST_{700}$ calves showed a postweaning ADG of 745 g/d, which meets the estimated growth rate required to reach ~55% of mature BW by 15 mo of age, whereas $POST_{500}$ calves showed an ADG of 510 g/d.

NOTES

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Nonstandard abbreviations used: dCP = digestibility of CP; dDM = digestibility of DM; dNDF = digestibility of NDF; DOMI = digestible OM intake; EBW = empty BW; EBWg = empty BW gain; HH = hip height; HW = hip width; IPAV = Instituto de Producción Animal de Veterinaria; MEg = ME for growth; MFCP = metabolic fecal CP; MNY = microbial nitrogen yield; MPg = MP for growth; MR = milk replacer; PD = purine derivatives; $POST_{500}$ = early postweaning (from 9 to 21 wk of life) feeding strategies targeting ADG of 500 g/d; $POST_{700}$ = early postweaning (from 9 to 21 wk of life) feeding strategies targeting ADG of 700 g/d; PRE_{10} = preweaning MR allowance 10%; PRE_{20} = preweaning MR allowance 20%; SF = starter feed; tdCP = true digestibility of CP; Tpost = effect of postweaning treatment; Tpre = effect of preweaning treatments; WM = whole milk.

REFERENCES

Adams, R. F., R. L. Jones, and P. L. Conway. 1984. High-performance liquid chromatography of microbial acid metabolites. J. Chromatogr. B Biomed. Sci. Appl. 336:125–137. https://doi.org/10.1016/S0378-4347(00)85136-1.

Adrien, M. L., D. A. Mattiauda, V. Artegoitia, M. Carriquiry, G. Motta, O. Bentancur, and A. Meikle. 2012. Nutritional regulation of body condition score at the initiation of the transition period in primiparous and multiparous dairy cows under grazing conditions: Milk production, resumption of post-partum ovarian cyclicity and metabolic parameters. Animal 6:292–299. https://doi.org/10.1017/S175173111100142X.

Aguerre, M., C. Cajarville, G. V. Kozloski, and J. L. Repetto. 2013. Intake and digestive responses by ruminants fed fresh temperate pasture supplemented with increased levels of sorghum grain: A comparison between cattle and sheep. Anim. Feed Sci. Technol. 186:12–19. https://doi.org/10.1016/j.anifeedsci.2013.08.007.

AOAC International. 1990. Official Methods of Analysis. 15th ed. AOAC International, Arlington, VA.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:E9–E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7.

Balcells, J., J. Guada, J. M. Peiró, and D. S. Parker. 1992. Simultaneous determination of allantoin and oxypurines in biological fluids by high performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 575:153–157. https://doi.org/10.1016/0378-4347(92)80517-T.

Baldwin, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87:E55–E65. https://doi.org/10.3168/jds.S0022-0302(04)70061-2.

Bartlett, K. S., F. K. McKeith, R. A. Molano, M. E. Van Amburgh, M. J. VandeHaar, G. E. Dahl, and J. K. Drackley. 2024. Growth and body composition of dairy calves fed only milk replacer at 3 intakes. J. Dairy Sci. 107:7842–7850. https://doi.org/10.3168/jds.2024-24894.

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.

Britos, A., J. L. Repetto, and C. Cajarville. 2018. Does it make a difference supplementing pasture silage with starchy concentrates or soyhulls on intake, digestion and rumen environment? Livest. Sci. 218:85–91. https://doi.org/10.1016/j.livsci.2018.09.021.

Broderick, G. A., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen–protected methionine in lactating dairy cows. J. Dairy Sci. 92:2719–2728. https://doi.org/10.3168/jds.2008-1277.

Brown, E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, J. W. Forrest, R. M. Akers, R. E. Pearson, and M. W. Nielsen. 2005b. Effect of increasing energy and protein intake on mammary development in heifer calves. J. Dairy Sci. 88:595–603. https://doi.org/10.3168/jds.S0022-0302(05)72723-5.

Brown, E. G., M. J. VandeHaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, D. H. Keisler, and M. W. Nielsen. 2005a. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. J. Dairy Sci. 88:585–594. https://doi.org/10.3168/jds.S0022-0302(05)72722-3.

Bruinjé, T. C., J. P. Rosadiuk, F. Moslemipur, H. Sauerwein, M. A. Steele, and D. J. Ambrose. 2021. Differing planes of pre- and postweaning phase nutrition in Holstein heifers: II. Effects on circulating leptin, luteinizing hormone, and age at puberty. J. Dairy Sci. 104:1153–1163. https://doi.org/10.3168/jds.2020-18810.

Chapman, C. E., P. S. Erickson, J. D. Quigley, T. M. Hill, H. G. Bateman II, F. X. Suarez-Mena, and R. L. Schlotterbeck. 2016. Effect of milk replacer program on calf performance and digestion of nutrients with age of the dairy calf. J. Dairy Sci. 99:2740–2747. https://doi.org/10.3168/jds.2015-10372.

Chen, X. B., and M. J. Gomes. 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details. Rowett Research Institute, Aberdeen, UK.

Choi, Y. J., I. K. Han, J. H. Woo, H. J. Lee, K. Jang, K. H. Myung, and Y. S. Kim. 1997. Compensatory growth in dairy heifers: The effect of a compensatory growth pattern on growth rate and lactation performance. J. Dairy Sci. 80:519–524. https://doi.org/10.3168/jds.S0022-0302(97)75965-4.

Costa, M., A. Bussoni, R. Mello, M. Santoro, D. Rodríguez, and F. Landa. 2010. Campos de recría en el Uruguay: gestión de los recursos y formas contractuales. Agrociencia (Montev.) 14:66–76. https://doi.org/10.31285/AGRO.14.628.

Davis Rincker, L. E., M. J. VandeHaar, C. A. Wolf, J. S. Liesman, L. T. Chapin, and M. S. Weber Nielsen. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. J. Dairy Sci. 94:3554–3567. https://doi.org/10.3168/jds.2010-3923.

De La Quintana, E., A. Mendoza, C. Cajarville, O. Bentancur, and J. L. Repetto. 2018. Post-weaning feeding levels on feeding behavior, growth and development in Holstein dairy heifers. Cienc. Rural 48:e20161083. https://doi.org/10.1590/0103-8478cr20161083.

Deelen, S. M., T. L. Ollivett, D. M. Haines, and K. E. Leslie. 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97:3838–3844. https://doi.org/10.3168/jds.2014-7939.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey, and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105–kilogram body weight. J. Dairy Sci. 84:830–842. https://doi.org/10.3168/jds.S0022-0302(01)74541-9.

Drackley, J. K. 2008. Calf nutrition from birth to breeding. Vet. Clin. North Am. Food Anim. Pract. 24:55–86. https://doi.org/10.1016/j.cvfa.2008.01.001.

Funaba, M., K. Kagiyama, T. Iriki, and M. Abe. 1997. Duodenal flow of microbial nitrogen estimated from urinary excretion of purine derivatives in calves after early weaning. J. Anim. Sci. 75:1965–1973. https://doi.org/10.2527/1997.7571965x.

Gelsinger, S. L., W. K. Coblentz, G. I. Zanton, R. K. Ogden, and M. S. Akins. 2020. Physiological effects of starter–induced ruminal acidosis in calves before, during, and after weaning. J. Dairy Sci. 103:2762–2772. https://doi.org/10.3168/jds.2019-17494.

Gelsinger, S. L., A. J. Heinrichs, and C. M. Jones. 2016. A meta–analysis of the effects of preweaned calf nutrition and growth on first–lactation performance. J. Dairy Sci. 99:6206–6214. https://doi.org/10.3168/jds.2015-10744.

Golder, H. M., and I. J. Lean. 2024. Invited review: Ruminal acidosis and its definition—A critical review. J. Dairy Sci. 107:10066–10098. https://doi.org/10.3168/jds.2024-24817.

Haisan, J., M. Oba, D. J. Ambrose, and M. A. Steele. 2018. Short communication: The effects of offering a high or low plane of milk preweaning on insulin-like growth factor and insulin-like growth factor binding proteins in dairy heifer calves. J. Dairy Sci. 101:11441–11446. https://doi.org/10.3168/jds.2017-14339.

Hill, T. M., H. G. Bateman II, J. M. Aldrich, and R. L. Schlotterbeck. 2010. Effect of milk replacer program on digestion of nutrients in dairy calves. J. Dairy Sci. 93:1105–1115. https://doi.org/10.3168/jds.2009-2458.

Hill, T. M., H. G. Bateman II, J. D. Quigley III, J. M. Aldrich, R. L. Schlotterbeck, and A. J. Heinrichs. 2013. Review: New information on the protein requirements and diet formulation for dairy calves and heifers since the Dairy NRC 2001. Prof. Anim. Sci. 29:199–207. https://doi.org/10.15232/S1080-7446(15)30225-4.

Hötzel, M. J., C. Longo, L. F. Balcão, C. S. Cardoso, and J. H. C. Costa. 2014. A survey of management practices that influence performance and welfare of dairy calves reared in southern Brazil. PLoS One 9:e114995. https://doi.org/10.1371/journal.pone.0114995.

Hu, W., T. M. Hill, T. S. Dennis, F. X. Suarez-Mena, K. M. Aragona, J. D. Quigley, and R. L. Schlotterbeck. 2020. Effects of milk replacer feeding rates on growth performance of Holstein dairy calves to 4 months of age, evaluated via a meta-analytical approach. J. Dairy Sci. 103:2217–2232. https://doi.org/10.3168/jds.2019-17206.

Jahn, E., and P. T. Chandler. 1976. Performance and nutrient requirements of calves fed varying percentages of protein and fiber. J. Anim. Sci. 42:724–735. https://doi.org/10.2527/jas1976.423724x.

Kaps, M., and W. R. Lamberson, ed. 2017. Biostatistics for Animal Science. 3rd ed. CABI, Wallingford, UK.

Khan, M. A., A. Bach, D. M. Weary, and M. A. G. von Keyserlingk. 2016. Invited review: Transitioning from milk to solid feed in dairy heifers. J. Dairy Sci. 99:885–902. https://doi.org/10.3168/jds.2015-9975.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi. 2007. Pre-and post-weaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:876–885. https://doi.org/10.3168/jds.S0022-0302(07)71571-0.

Kiezebrink, D. J., A. M. Edwards, T. C. Wright, J. P. Cant, and V. R. Osborne. 2015. Effect of enhanced whole–milk feeding in calves on subsequent first–lactation performance. J. Dairy Sci. 98:349–356. https://doi.org/10.3168/jds.2014-7959.

Lascano, G. J., and A. J. Heinrichs. 2011. Effects of feeding different levels of dietary fiber through the addition of corn stover on nutrient utilization of dairy heifers precision-fed high and low concentrate diets. J. Dairy Sci. 94:3025–3036. https://doi.org/10.3168/jds.2010-3831.

Marcondes, M. I., and A. L. Silva. 2021. Determination of energy and protein requirements of preweaned dairy calves: A multistudy approach. J. Dairy Sci. 104:11553–11566. https://doi.org/10.3168/jds.2021-20272.

Mertens, D. R. 2003. Challenges in measuring insoluble dietary fiber. J. Anim. Sci. 81:3233–3249. https://doi.org/10.2527/2003.81123233x.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. The National Academies Press, Washington, DC.

Ockenden, E. M., V. M. Russo, B. J. Leury, K. Giri, and W. J. Wales. 2025. Early life nutrition and its effects on the developing heifer: Growth, nutritive intakes, and metabolism. J. Dairy Sci. 108:3515–3528. https://doi.org/10.3168/jds.2024-25546.

Quigley, J. D., W. Hu, J. R. Knapp, T. S. Dennis, F. X. Suarez-Mena, and T. M. Hill. 2019. Estimates of calf starter energy affected by consumption of nutrients. 2. Effect of changing digestion on energy

content in calf starters. J. Dairy Sci. 102:2242–2253. https://doi.org/10.3168/jds.2018-15354.
Rosadiuk, J. P., T. C. Bruinjé, F. Moslemipur, A. J. Fischer-Tlustos, D. L. Renaud, D. J. Ambrose, and M. A. Steele. 2021. Differing planes of pre- and post-weaning phase nutrition in Holstein heifers: I. Effects on feed intake, growth efficiency, and metabolic and development indicators. J. Dairy Sci. 104:1136–1152. https://doi.org/10.3168/jds.2020-18809.
Santana, A., C. Cajarville, A. Mendoza, and J. L. Repetto. 2017. Combination of legume-based herbage and total mixed ration (TMR) maintains intake and nutrient utilization of TMR and improves nitrogen utilization of herbage in heifers. Animal 11:616–624. https://doi.org/10.1017/S1751731116001956.
Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32:199–208. https://doi.org/10.1079/bjn19740073.
Schild, C. O., R. D. Caffarena, A. Gil, J. Sánchez, F. Riet-Correa, and F. Giannitti. 2020. A survey of management practices that influence calf welfare and an estimation of the annual calf mortality risk in pastured dairy herds in Uruguay. J. Dairy Sci. 103:9418–9429. https://doi.org/10.3168/jds.2020-18177.
Silva, A. L., M. I. Marcondes, E. Detmann, F. S. Machado, S. C. Valadares Filho, A. S. Trece, and J. Dijkstra. 2015. Effects of raw milk and starter feed on intake and body composition of Holstein × Gyr male calves up to 64 days of age. J. Dairy Sci. 98:2641–2649. https://doi.org/10.3168/jds.2014-8833.
Terré, M., M. Devant, and A. Bach. 2006. Performance and nitrogen metabolism of calves fed conventionally or following an enhanced–growth feeding program during the preweaning period. Livest. Sci. 105:109–119. https://doi.org/10.1016/j.livsci.2006.05.001.
Terré, M., M. Devant, and A. Bach. 2007. Effect of level of milk replacer fed to Holstein calves on performance during the preweaning period and starter digestibility at weaning. Livest. Sci. 110:82–88. https://doi.org/10.1016/j.livsci.2006.10.001.
Terré, M., L. Castells, F. Fàbregas, and A. Bach. 2013. Short communication: Comparison of pH, volatile fatty acids, and microbiome of rumen samples from preweaned calves obtained via cannula or stomach tube. J. Dairy Sci. 96:5290–5294. https://doi.org/10.3168/jds.2012-5921.
Terré, M., C. Tejero, and A. Bach. 2009. Long-term effects on heifer performance of an enhanced-growth feeding programme applied during the preweaning period. J. Dairy Res. 76:331–339. https://doi.org/10.1017/S0022029909004142.
van Niekerk, J. K., A. J. Fischer-Tlustos, L. L. Deikun, J. D. Quigley, T. S. Dennis, F. X. Suarez-Mena, T. M. Hill, R. L. Schlotterbeck, L. L. Guan, and M. A. Steele. 2020. Effect of amount of milk replacer fed and the processing of corn in starter on growth performance, nutrient digestibility, and rumen and fecal fibrolytic bacteria of dairy calves. J. Dairy Sci. 103:2186–2199. https://doi.org/10.3168/jds.2019-17372.
van Niekerk, J. K., M. Middeldorp, L. L. Guan, and M. A. Steele. 2021. Preweaning to postweaning rumen papillae structural growth, ruminal fermentation characteristics, and acute-phase proteins in calves. J. Dairy Sci. 104:3632–3645. https://doi.org/10.3168/jds.2020-19003.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Wang, K., U. Arshad, and M. Niu. 2025. Invited review: Enhancing quality of dairy cattle research through adequate power analysis. J. Dairy Sci. 108:8981–9003. https://doi.org/10.3168/jds.2024-25793.
Weatherburn, M. W. 1967. Phenol–hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971–974. https://doi.org/10.1021/ac60252a045.
Williams, P. E. V., and A. I. Frost. 1992. Feeding the young ruminant. BSAP Occas. Publ. 15:109–118. https://doi.org/10.1017/S0263967X00004134.
Zanton, G. I., and A. J. Heinrichs. 2008. Analysis of nitrogen utilization and excretion in growing dairy cattle. J. Dairy Sci. 91:1519–1533. https://doi.org/10.3168/jds.2007-0624.

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