



Prewaning nutrient supply improves lactation productivity and reduces the risk of culling in Holstein cows

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ABSTRACT

This study aimed to investigate the effects of preweaning nutrient intake on milk production in the 2 first lactations, risk of culling, and metabolomic profiles of dairy cows fed a restricted (RES) or an elevated (ELE) milk supply preweaning. A total of 86 female Holstein Friesian calves were randomly assigned to either 5.4 Mcal of ME in 8 L of milk replacer (MR) per day (ELE) or 2.7 Mcal of ME in 4 L of MR per day (RES) from d 2 after birth until they were stepped down by 50% from d 42 to d 49 and fully weaned at d 50. Starting from d 50, animals from both treatment groups were fed and managed in the same way, with treatments being blinded, for the entire study duration. At 60 DIM in the first lactation, blood samples were taken for metabolomic analysis. Heifers fed the ELE diet exhibited higher ADG (0.79 vs. 0.49 kg/d) in the preweaning period, leading to higher BW at 70 d of age (94 vs. 85 kg). In the first lactation, cows fed the ELE diet preweaning exhibited higher DMI (+0.7 kg/d), higher milk fat yield (+50 g/d), and higher milk fat content (+0.24%) but lower feed efficiency (−0.04) than cows in the RES group. In the second lactation, fat- and protein-corrected milk yield (+1.8 kg/d), milk fat yield (+115 g/d), milk fat content (+0.22%), and feed efficiency (+0.06) were all higher in ELE cows. Throughout these 2 lactations, ELE cows were leaner, as indicated by a lower BCS. Cows fed the ELE diet preweaning had a higher first-service conception rate during the second lactation than cows fed the RES diet (40.7% vs. 13.0%). Moreover, the likelihood to be culled before third, fourth, or fifth calving was halved when calves were fed ELE compared with RES. Metabolomic profiling revealed distinct metabolomic profiles at 60 DIM in response to preweaning milk supply. The major metabolic pathways affected by preweaning nutrient supply at 60 DIM were

pyrimidine, sphingosine, guanidino, acetamido, and purine metabolism and the tricarboxylic acid cycle. The metabolic profile differences between the groups describe a metabolic configuration that was sustained into adulthood and that may explain the improved productivity and resilience of cows fed a greater milk supply during the preweaning period.

Key words: metabolomics, metabolic programming, preweaning nutrition, milk production, longevity

INTRODUCTION

Several epidemiological studies have demonstrated a strong correlation between the early-life nutritional environment and the risk of cardiovascular disease, diabetes, and metabolic syndrome in adult humans (McMillen and Robinson, 2005). This phenomenon, known as the developmental origins of health and disease, hypothesizes that inadequate early-life nutritional environments induce long-term epigenetic modifications that in turn lead to a higher risk of developing metabolic diseases later in life (Tain and Hsu, 2022). Therefore, poor nutrition early in life induces physiological changes that optimize fitness for environments with low nutrient availability, leading to metabolic diseases when the individuals subsequently encounter abundant nutrition in adulthood (Hayward et al., 2013).

In calves, the link between preweaning nutrition and short-term health is well-recognized. Low nutrient allowances lead to suboptimal growth and impaired immune function (Drackley, 2008; Khan et al., 2011), potentially affecting milk production capacity and reproductive efficiency and increasing the risk of health disorders later in life (van Amburgh et al., 1998; Shamay et al., 2005; Soberon et al., 2012; Curtis et al., 2018). Higher nutrient intake preweaning can improve mammary gland development, as measured on d 54 of life by Soberon and van Amburgh (2017), increase milk production in subsequent lactations (Soberon et al., 2012), and promote greater growth rates that allow for

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Table 1. Composition of the partial mixed ration fed to lactating cows¹

Item	% of DM ($\pm$ SD)
Maize silage	43.6 $\pm$ 5.05
Grass silage	38.0 $\pm$ 8.31
Grass hay	3.6 $\pm$ 1.09
Brewers grain	6.7 $\pm$ 0.87
Wheat grain	4.6 $\pm$ 0.84
Sodium bicarbonate	0.6 $\pm$ 0.11

¹Based on 71 samples.

an earlier age at first calving (Davis Rincker et al., 2011; Heinrichs and Heinrichs, 2011). However, the underlying mechanism linking preweaning nutrition with later reproductive success, future productivity, and metabolic health remains insufficiently understood.

Leal et al. (2021) described that increasing milk allowance preweaning resulted in substantial changes in metabolomic profiles related to energy and protein metabolism and liver function characterized in dairy calves of 49 d of age. Subsequent work by Leal et al. (2025) further investigated those same animals and showed that preweaning nutrient supply can alter insulin sensitivity and metabolic profiles in developing heifers, indicating possible carryover effects that may be relevant to long-term health and performance. Reporting of the effect of this greater preweaning nutrient supply on the productive stages of those animals is pending, particularly their energy metabolism and survival upon lactation cycles and their dairy productivity. This sequential description of metabolomic profiles along development and the final metabolic profiles and productivity of these cows contributes to our understanding of mechanisms explaining the well-documented long-term benefits of improved early-life nutrition of dairy animals.

The present dataset describes the effects of preweaning milk allowance on the productive phase of dairy cows, with a special focus on milk production, survival, and metabolomic profiles, following up on results reported by Leal et al. (2021) and Leal et al. (2025), which provided insights on the metabolic alterations in calves and heifers induced by preweaning nutrient supply. By monitoring the same prospectively randomized cohort of calves until the third lactation, we aim to extend our understanding of the long-term implications of early-life nutrition of dairy cows into adulthood. We hypothesize that the greater preweaning milk allowance will improve energy metabolism in dairy cattle, leading to greater milk production and lactation survival.

MATERIALS AND METHODS

This study was conducted at the Trouw Nutrition Dairy Research Facility (Boxmeer, the Netherlands). The re-

Table 2. Analyzed chemical composition (% of DM $\pm$ SD) of the partial mixed ration and concentrates fed to lactating cows¹

Item	Partial mixed ration ²	High-protein concentrate ³	High-starch concentrate ³
DM	39.9 $\pm$ 3.6	88.0 $\pm$ 0.5	88.1 $\pm$ 0.6
OM	92.7 $\pm$ 0.5	89.9 $\pm$ 0.2	93.4 $\pm$ 0.2
CP	13.9 $\pm$ 0.7	28.6 $\pm$ 2.0	19.2 $\pm$ 0.6
Starch	17.1 $\pm$ 2.7	19.1 $\pm$ 2.1	27.3 $\pm$ 2.6
Sugar	2.4 $\pm$ 0.8	10.4 $\pm$ 0.6	8.1 $\pm$ 1.0
NDF	41.9 $\pm$ 1.7	23.3 $\pm$ 1.2	23.6 $\pm$ 3.4
ADF	22.3 $\pm$ 1.8	11.7 $\pm$ 0.4	12.3 $\pm$ 1.3
ADL	1.8 $\pm$ 0.3	3.4 $\pm$ 0.4	2.2 $\pm$ 0.4
Crude fat	4.0 $\pm$ 0.2	2.9 $\pm$ 0.2	4.9 $\pm$ 0.4

¹Partial mixed ration was supplied ad libitum, high-protein concentrate was supplied at 2.3 kg/cow per day, and high-starch concentrate was supplied based on daily milk yield: primiparous (no concentrate if milk production $\leq$ 24 kg/d; 25 kg/d = 1.5 kg; >25 kg/d = linearly increased by 0.5 kg for every extra kilogram of milk to achieve a maximum concentrate intake of 7.5 kg/d [$\geq$ 37 kg/d]); multiparous (no concentrate if milk production $\leq$ 25 kg/d; 26 kg/d = 0.8 kg; 27 kg/d = 1.5 kg/d; >27 kg/d = linearly increased by 0.5 kg for every extra kilogram of milk to achieve a maximum concentrate intake of 8.5 kg/d [$\geq$ 41 kg/d]).

²Based on 71 samples.³Pelleted feed manufactured by ForFarmers (Lochem, the Netherlands). Data from high-protein concentrate are based on 4 samples, and data from high-starch concentrate are based on 69 samples.

sults of earlier phases of this long-term study describing the main metabolic adaptations of neonatal calves (Leal et al., 2021) and heifers (Leal et al., 2025) that were fed 2 levels of milk replacer (**MR**) supply from birth to d 49 of life in a randomized block design have been reported. In this study, 78 Holstein cows were studied from the first until the third calving. All procedures complied with the Dutch Law on Experimental Animals and the ETS123 (council of Europe 1985 and the 86/609/EEC Directive) and were approved by the Animal Care and Use Committee from Utrecht University.

Preweaning and Heifer Rearing Phase

At birth, all calves received 4 L of pasteurized colostrum that was frozen and reheated within 6 h. The 86 female Holstein calves were blocked by sequence of birth and parity of the dams into 2 categories, primiparous or multiparous. Pairs of calves in each block received identical doses of pooled colostrum, resulting in blocks defined by parity of the dam and colostrum quality. Within block, calves were randomly assigned to 1 of 2 treatments, elevated level of nutrient intake (**ELE**) or restricted level of nutrient intake (**RES**). The ELE group received 5.14 Mcal of ME in 8 L of MR per day, whereas the RES group received 2.71 Mcal of ME in 4 L of MR per day. The MR (150 g/L) was fed twice daily through nipple buckets, and the calves had free access to fresh water, calf starter, and wheat straw. The weaning process

Table 3. Effects of preweaning plane of nutrition on first- and second-lactation performance¹

Item	Treatment ²			Missing data, ³ %	P-value ⁴	
	RES	ELE	SEM		TREAT	TREAT × WIM
First lactation (n = 78 cows)						
Total DMI, kg/d	18.6	19.3	0.17	0.1	<0.01	0.73
PMR DMI, ⁵ kg/d	13.7	14.5	0.21	0.1	<0.01	0.76
Concentrate DMI, ⁶ kg/d	4.7	4.6	0.17	0.0	0.94	0.51
Milk yield, kg/d	28.4	28.1	0.42	0.7	0.57	0.41
Fat- and protein-corrected milk, kg/d	29.5	30.0	0.27	7.1	0.21	0.93
Feed efficiency ⁷	1.58	1.54	0.008	8.6	<0.01	0.99
Milk composition						
Fat, %	4.33	4.57	0.043	6.4	<0.01	0.99
Protein, %	3.47	3.49	0.016	4.8	0.27	0.89
Lactose, %	4.69	4.67	0.009	5.8	0.11	0.13
MUN, mg/dL	11.8	11.8	0.13	4.0	0.99	0.26
Fat, g/d	1,214	1,264	9.6	6.4	<0.01	0.93
Protein, g/d	980	979	11.1	4.8	0.96	0.79
Lactose, g/d	1,333	1,317	19.3	5.8	0.56	0.91
BW, kg	595	587	5.2	16.7	0.24	0.98
BCS, 1–5 scale	3.30	3.22	0.02	16.8	<0.01	0.81
Second lactation (n = 61 cows)						
Total DMI, kg/d	22.2	22.9	0.30	2.6	0.16	0.81
PMR DMI, ⁵ kg/d	16.5	17.2	0.32	2.6	0.13	0.82
Concentrate DMI, ⁶ kg/d	5.5	5.5	0.32	2.6	0.96	0.89
Milk yield, kg/d	32.2	32.9	0.62	1.5	0.45	0.22
Fat- and protein-corrected milk, kg/d	33.8	35.6	0.51	10.7	0.03	0.73
Feed efficiency ⁷	1.50	1.56	0.013	11.9	<0.01	0.25
Milk composition						
Fat, %	4.39	4.61	0.043	10.2	<0.01	0.89
Protein, %	3.56	3.55	0.019	9.4	0.61	0.74
Lactose, %	4.58	4.55	0.011	9.3	0.07	0.25
MUN, mg/dL	10.7	11.1	0.19	8.7	0.11	0.18
Fat, g/d	1,387	1,502	20.6	10.2	<0.01	0.89
Protein, g/d	1,140	1,157	14.1	9.4	0.42	0.23
Lactose, g/d	1,492	1,505	29.4	9.3	0.76	0.58
BW, kg	662	649	6.8	18.9	0.22	0.72
BCS, 1–5 scale	3.14	3.05	0.04	5.8	0.10	0.55

¹Weekly records above 40 WIM were discarded to avoid confounding effect with pregnancy and thus potential effect due to different reproduction performance.

²RES = 2.71 Mcal of ME in 4 L of MR per day (150 g/L; 600 g/d of MR powder); ELE = 5.14 Mcal of ME in 8 L of MR per day (150 g/L; 1,200 g/d of MR powder).

³In the first and second lactations, the expected number of weekly records was 2,760 and 1,969, respectively. These values were calculated as the sum of the products between the number of cows at calving and their lactation lengths. In lactations 1 and 2, 63 out of 78 cows (81%) and 41 out of 61 cows (67%), respectively, remained in lactation for the full 40-wk period. Values presented here indicate the number of available data relative to these totals.

⁴TREAT = treatment; WIM = weeks in milk.

⁵PMR = partial mixed ration (fed ad libitum).

⁶Concentrate intake from out-of-parlor feeders.

⁷Feed efficiency = fat- and protein-corrected milk (kg/d)/total DMI (kg/d).

was initiated on d 42, and calves were fully weaned on d 50 of life (Leal et al., 2021). During this phase, calves were housed in individual outdoor hutches (1.07 × 1.60 m) bedded with wheat straw and outfitted with a metal roof attachment to protect the feed and water from precipitation until 70 d of life.

After 70 d of life, heifer calves from both treatment groups were placed in pens of 4 to 6 and managed in the same way, and beyond this point, treatment assignment was kept blind to all of the animal caretakers involved in herd feeding and management. After weaning,

calves were allowed ad libitum access to starter and grower TMR until 4 mo of age, from which time they exclusively received the grower TMR. Heifers became eligible for breeding when they reached 390 kg of BW and underwent artificial insemination (AI) upon signs of estrus. Pregnancy was confirmed by rectal palpation 35 to 45 d after insemination, and heifers were applied up to 3 services to conceive before being eliminated from the study. For lactating cows, a maximum of 6 services was allowed per cow per lactation before the cow was eliminated from the study. As described in the manu-

script reporting the effects of the preweaning dietary treatment on the whole heifer rearing phase (Leal et al., 2025), BW (416 vs. 412 kg, $P = 0.58$) and age (395 vs. 399 d, $P = 0.25$) at first service were not significantly different between ELE and RES. Age at conception was also not significantly different (412 vs. 422 d, $P = 0.19$). Before first calving, 8 animals were excluded from the study (5 in RES and 3 in ELE). Four of these 8 animals had an infectious disease that led to death, 3 did not conceive within the 3 services, and 1 was sold by the farm managers for its high breeding value.

Lactation

A total of 77 heifers reached first calving and consequently initiated lactation. Cows were housed in a freestall barn with a slatted floor. An electronic feed bunk (RIC, Hokofarm Group B. V.) was provided for every 2 cows present. The RIC feed bunks were checked daily to ensure they were functioning properly. The cows were milked twice a day at approximately 0500 and 1700 h in a herringbone milking parlor with 2×6 stands. Additionally, each cow had access to the laying boxes equipped with rubber mattresses and sawdust as bedding material.

All cows were fed the same dry and lactation diets. Cows received the dry cow diet for ~6 wk before expected parturition. The dry diet consisted of (DM basis $\pm$ SD) $39\% \pm 0.5\%$ maize silage, $33\% \pm 0.8\%$ barley straw, $20\% \pm 0.4\%$ pelleted concentrate, and $9\% \pm 0.8\%$ brewers grain. The analyzed chemical composition of the dry cow diet averaged ($\pm$ SD) as follows: $50.3\% \pm 3.1\%$ DM, $93.0\% \pm 0.1\%$ OM, $11.4\% \pm 0.4\%$ CP, $16.6\% \pm 0.9\%$ starch, $51.8\% \pm 1.2\%$ NDF, and $2.6\% \pm 0.2\%$ crude fat. The lactation diet consisted of a lactation partial mixed ration (PMR, Tables 1 and 2) fed ad libitum, a high-protein concentrate restricted to 2.3 kg/cow per day (Table 2), and a high-starch concentrate fed according to milk yield (Table 2). Feed intake was measured individually using the RIC boxes and out-of-parlor concentrate feeders (Fullwood Packo) upon entry into the dry group throughout lactation. Lactating cows had free access to water and PMR, which were mixed and fed daily between ~0900 h and 1000 h in the RIC boxes.

Measurements and Chemical Analysis

Milk yields were recorded automatically at each milking during the first 2 lactations. Milk was sampled on Tuesday and Thursday mornings and Monday and Wednesday evenings. Samples were collected in tubes containing sodium azide and bronopol as preservatives and stored at 4°C. Morning samples and evening samples were pooled by weighing each individual sample proportionally to yield per milking, and the results were com-

bined to calculate a weekly average milk composition value and a weekly average milk yield. Samples were analyzed for fat, CP, lactose, and urea (Qlip, Deventer, the Netherlands) using a Foss MilkoScan FT6000 (Foss; ISO, 2013). The fat- and protein-corrected milk (FPCM) yield (corrected to 4.0% fat and 3.3% protein; kg/d) was calculated as milk yield (kg/d) $\times$ $[0.377 + 0.116 \times \text{fat} (\%) + 0.06 \times \text{protein} (\%)]$.

After milking, the cows were weighed, and their BCS was automatically recorded using an automated BCS system (DeLaval, Tumba, Sweden). Fertility data such as DIM at first AI, first-service conception rate, number of inseminations per pregnancy, and age at first, second, third, fourth, and fifth calving were also collected.

All chemical analysis of feedstuffs was performed by Eurofins Agro (Wageningen, the Netherlands) using near-infrared spectroscopy. Their analytical methods are accredited by Raad voor Accreditatie, the national accreditation body of the Netherlands.

Metabolomic Analysis

Serum metabolomic analysis was performed on samples from 25 complete blocks of 50 cows (27 ELE and 23 RES). Blood samples were collected via jugular venipuncture into 10-mL potassium EDTA Vacutainers (Becton Dickinson) between 1000 and 1200 h at 60 DIM during the first lactation. Cows were sampled at this stage of lactation to assess their metabolic status during peak milk production. All blood samples were centrifuged immediately after collection at $2,800 \times g$ for 30 min at room temperature, and 1.5 mL of serum was pipetted into 4 two-milliliter cryotubes and stored at -80°C until processed, as described by Evans et al. (2009), at Metabolon Inc. (Durham, NC).

One of the aliquots was used for metabolomic analysis. Before the initial extraction step, recovery standards were included as a quality control (QC) measure. Methanol was used to extract samples to precipitate protein, DNA, and RNA, release small molecules that were bound to protein or trapped in the precipitated protein matrix, and recover diverse metabolites. The centrifuged extract was separated into 5 fractions: 2 for analysis using separate reversed-phase (RP) ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods with electrospray ionization (ESI) in positive-ion mode, one for analysis using RP/UPLC-MS/MS with ESI in negative-ion mode, one for analysis using hydrophilic interaction chromatography UPLC-MS/MS with ESI in negative-ion mode, and one to serve as a backup. Three types of controls were used. First, a pooled matrix sample was created by combining small volumes of each experimental sample. This sample served as a technical replicate throughout the dataset. Second, extracted water

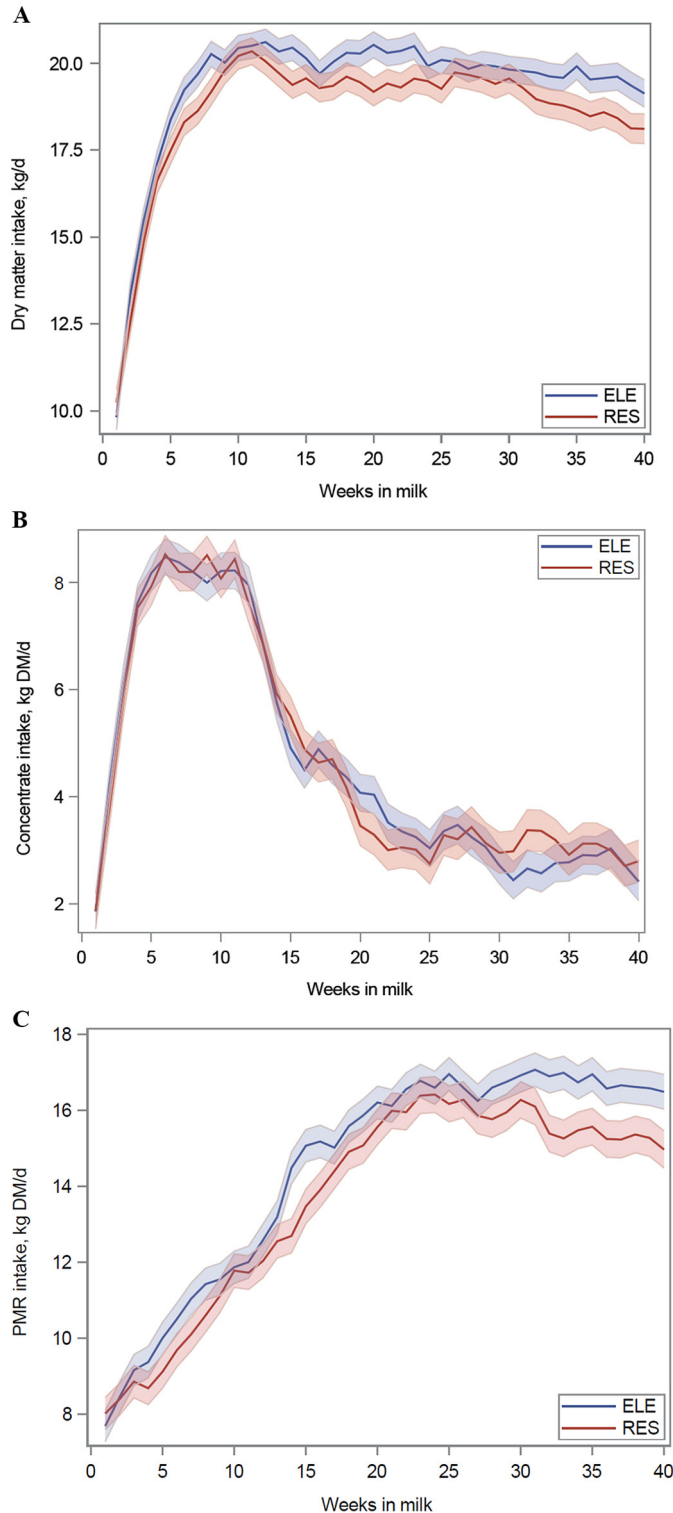


Figure 1. Dry matter intake (kg DM per day, A), concentrate intake from the out-of-parlor feeder (kg DM per day, B), and partial mixed ration (PMR) intake from the roughage feeders (kg DM per day, C) expressed in weeks relative to calving for primiparous cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Continuous lines are LSM, and shaded areas represent the SE.

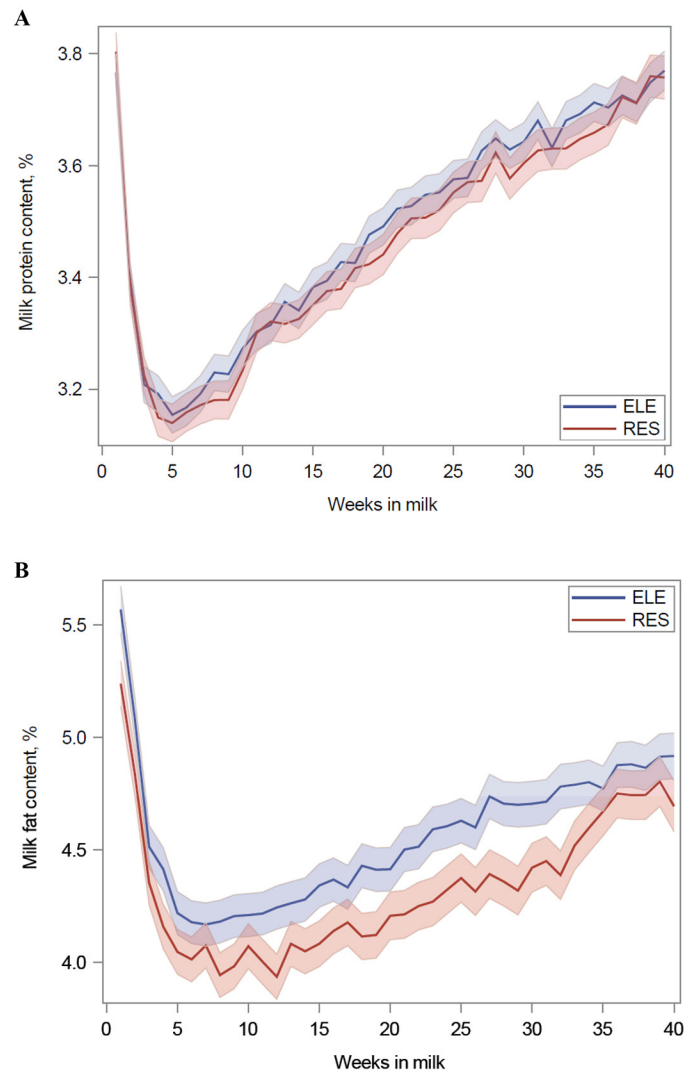


Figure 2. Milk protein content (%; A) and milk fat content (%; B) expressed in weeks relative to calving for primiparous cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Continuous lines are LSM, and shaded areas represent the SE.

samples were used as process blanks. Finally, a cocktail of QC standards was spiked into each analyzed sample to monitor instrument performance and facilitate chromatographic alignment. To determine instrument variability, the median relative standard deviation (RSD) was calculated for the standards added to each sample before injection into the mass spectrometers. Typically, the median RSD was 4% to 6% when there were at least 30 samples. To assess the variability of the entire process, we calculated the median RSD for all endogenous metabolites (i.e., noninstrument standards) found in 100% of the combined matrix samples. During the platform

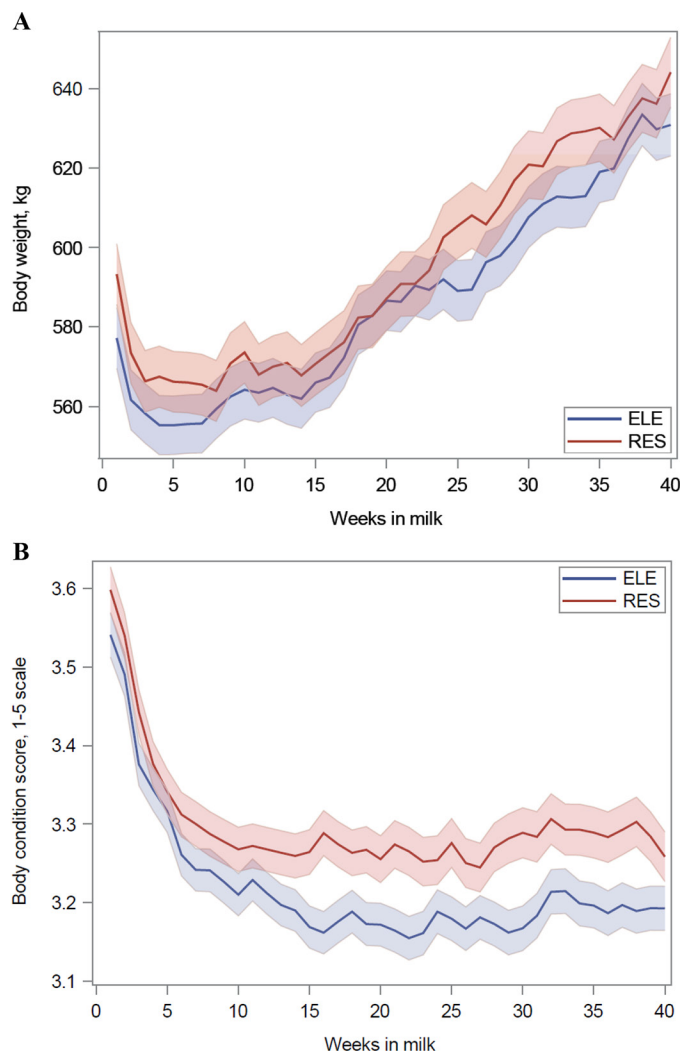


Figure 3. Body weight (kg, A) and BCS (1 to 5 scale, B) expressed in weeks relative to calving for primiparous cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Continuous lines are LSM, and shaded areas represent the SE.

run, we randomly distributed the experimental samples and evenly placed QC samples throughout the injections.

The analytical methods employed a Waters ACQUITY UPLC system and a Thermo Scientific Q-Exactive high-resolution/accurate mass spectrometer. These were connected to a heated ESI source and Orbitrap mass analyzer operating at a resolution of 35,000 mass. The MS analysis involved alternating between MS and data-dependent MSⁿ scans and utilizing dynamic exclusion. Although the scan range varied slightly between methods, it covered the mass-to-charge ratio (m/z) range of 70 to 1,000 m/z .

We used Metabolon's hardware and software (Metabolon, Morrisville, NC) to extract raw data, identify peaks, and perform QC processing. To identify the compounds, we relied on Metabolon's library of over 3,300 commer-

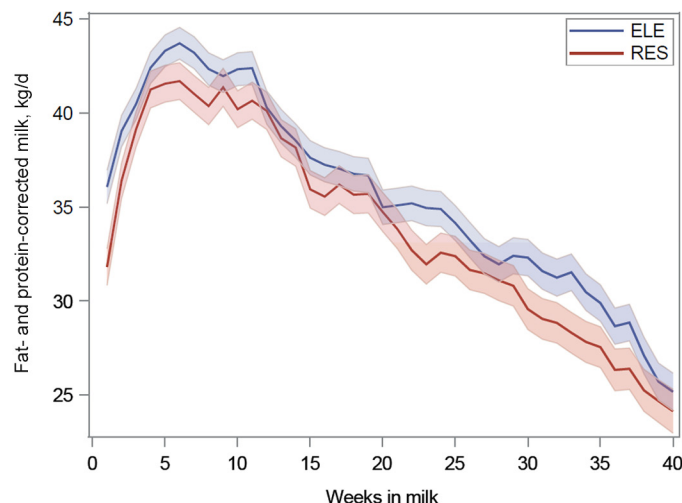


Figure 4. Fat- and protein-corrected milk yield (kg/d) expressed in weeks relative to calving for second-lactation cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Continuous lines are LSM, and shaded areas represent the SE.

cially available purified standard compounds, each with a specific retention-to-time index (**RI**), (m/z), and chromatographic profile (including MS/MS spectral data). We quantified the peaks using the area-under-the-curve method, and each compound was corrected in run-day blocks by aligning the medians to equal one (1.00) and normalizing each data point proportionately. Quality control and curation processes were designed to ensure accurate and consistent identification of true chemical entities and to remove those representing system artifacts, misassignments, and background noise.

Calculations and Statistics

For individual cows, all data were averaged per week. Dry matter intake, milk yield, FCPM, milk component yield and contents, BW, and BCS were analyzed with the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The model accounted for the fixed effects of treatment (ELE vs. RES), weeks in milk (**WIM**, 1 to 40), their 2-way interaction, and the random effect of block ($n = 43$). Weeks in milk entered the model as the repeated term, and an autoregressive type 1 covariance structure was used. Differences between treatments at different WIM were determined using the LSMEANS SLICE statement.

First-service conception rate, conception rate, and survival to next lactation were analyzed by logistic regression using the GENMOD procedure in SAS (version 9.4, SAS Institute) with a binomial distribution and the logit link function. Additionally, the odds of remaining in the

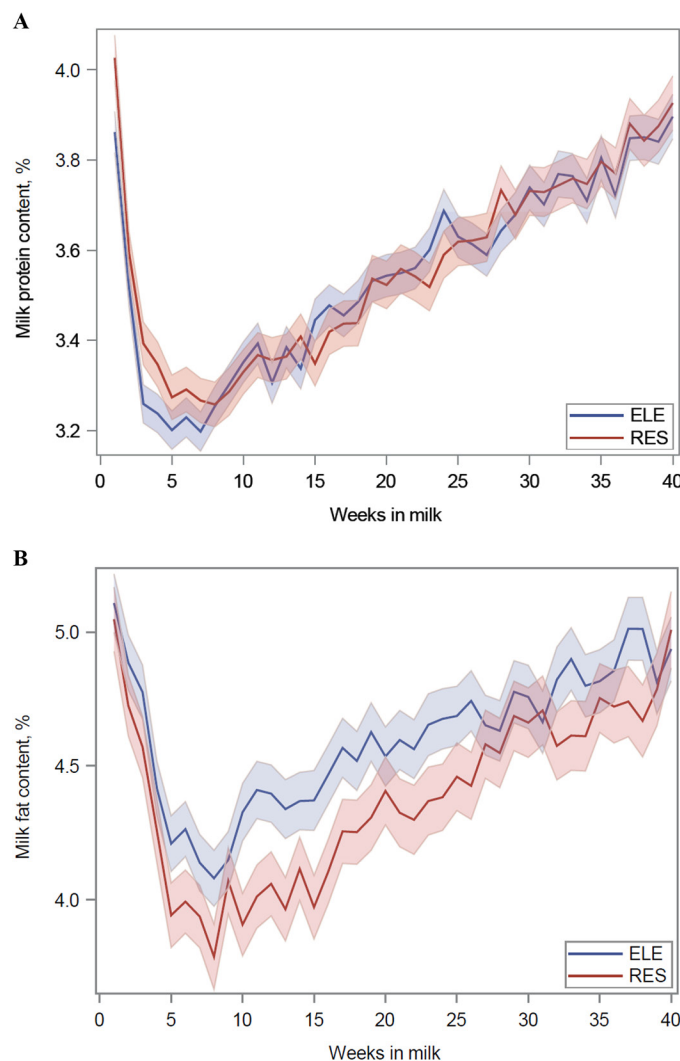


Figure 5. Milk protein content (%; A) and milk fat content (%; B) expressed in weeks relative to calving for second-lactation cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Continuous lines are LSM, and shaded areas represent the SE.

herd were analyzed with multivariable survival analysis using Cox's proportional hazards regression with the PHREG procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC). In this analysis, the event considered was culling. As some cows were still alive at the end of the study period, these cows were censored. Days in milk at first service and at conception, calving interval, and age at each calving were analyzed using the PROC MIXED procedure in SAS (version 9.4, SAS Institute) to evaluate the fixed effect of dietary treatment and random effect of block. The probability of conception with fewer inseminations was analyzed by ordinal logistic regression using the GLIMMIX procedure in SAS (version 9.4, SAS

Table 4. Effects of preweaning plane of nutrition on reproduction performance of first- and second-lactation cows ($\pm$ SE)

Item	Treatment ¹		<i>P</i> -value
	RES	ELE	
First lactation (n = 78 cows)			
Cows, no.	38	40	
First-service conception rate, %	40.0	47.4	0.53
Conception rate, %	73.7	82.5	0.35
DIM at first service, d	70 ± 4.6	70 ± 4.3	0.96
DIM at conception, d	111 ± 15.1	109 ± 13.1	0.93
Calving interval, d	388 ± 15.3	389 ± 13.3	0.97
Second lactation (n = 61 cows)			
Cows, no.	28	33	
First-service conception rate, %	13.0	40.7	0.03
Conception rate, %	57.1	69.7	0.31
DIM at first service, d	67 ± 4.2	62 ± 3.6	0.46
DIM at conception, d	91 ± 18.3	104 ± 13.4	0.63
Calving interval, d	365 ± 19.1	386 ± 14.0	0.48

¹RES = 2.71 Mcal of ME in 4 L of MR per day (150 g/L; 600 g/d of MR powder); ELE = 5.14 Mcal of ME in 8 L of MR per day (150 g/L; 1,200 g/d of MR powder).

Institute Inc., Cary, NC) with a multinomial distribution. The random effect of block was included in the model.

The biochemical profiles of serum samples from 50 cows were analyzed using Welch's 2-sample *t*-tests on natural log-transformed data in ArrayStudio (OmicSoft ArraySuite 10.0, Qiagen N. V.). Enrichment and self-contained pathway testing were performed in the statistical computing software R (version 3.5.0). Enrichment was tested using a hypergeometric test, and for the self-contained test, a Fisher statistic test with a *P*-value determined from a permutation test was performed (Mitchell, 2015).

Significance was declared when $P < 0.05$, and trends were declared when $0.05 \leq P < 0.10$.

RESULTS

As previously described by Leal et al. (2021), heifers fed the ELE diet exhibited higher ADG in the preweaning period (ADG before weaning was initiated was 0.79 vs. 0.49 kg/d for ELE and RES, respectively, $P < 0.01$), leading to higher BW at 70 d of age (94 vs. 85 kg, $P < 0.01$). We also reported that this preweaning nutritional intervention had sustained effects on metabolism, as shown by lower glucose-stimulated insulin blood concentrations for ELE compared with RES at d 370 of life, indicating a potential improvement in insulin sensitivity (Leal et al., 2025). The current paper focuses on the effects of preweaning MR supply on cow performance during lactation in terms of milk production, reproduction, and health (as approximated by probability of culling). Readers are referred to previous papers (Leal

Table 5. Effects of preweaning plane of nutrition on survival rate and age at calvings ($\pm$ SE)

Item	Treatment ¹		<i>P</i> -value
	RES	ELE	
Total cows enrolled, no.	43	43	—
Survival from birth to first calving			
Cows at calving, no.	38	40	—
Percent of total, %	88.4	93.0	0.46
Age at first calving, d	699 $\pm$ 8.8	685 $\pm$ 8.4	0.26
Survival to second calving			
Cows at calving, no.	28	33	—
Cows reaching first calving, %	73.7	82.5	0.35
Percent of total cows enrolled, %	65.1	76.7	0.23
Age at second calving, d	1,083 $\pm$ 17.5	1,070 $\pm$ 15.2	0.60
Survival to third calving			
Cows at calving, no.	16	23	—
Cows reaching second calving, %	57.1	69.7	0.31
Percent of total cows enrolled, %	37.2	53.5	0.13
Age at third calving, d	1,421 $\pm$ 25.6	1,474 $\pm$ 18.7	0.21
Survival to fourth calving			
Cows at calving, no.	10	18	—
Cows reaching third calving, %	62.5	78.3	0.28
Percent of total cows enrolled, %	23.3	41.9	0.06
Age at third calving, d	1,913 $\pm$ 15.4	1,832 $\pm$ 9.1	0.07
Survival to fifth calving			
Cows at calving, no.	6	11	—
Cows reaching fourth calving, %	60.0	61.1	0.95
Percent of total cows enrolled, %	14.0	25.6	0.17
Age at third calving, d	2,204 $\pm$ 24.8	2,128 $\pm$ 14.6	0.16

¹RES = 2.71 Mcal of ME in 4 L of MR per day (150 g/L; 600 g/d of MR powder); ELE = 5.14 Mcal of ME in 8 L of MR per day (150 g/L; 1,200 g/d of MR powder).

et al., 2021, 2025) for more details on the effects of the dietary treatment during the preweaning period and during the heifer rearing period.

Animal Performance

Performance parameters of cows from both treatment groups in their first and second lactations are displayed in Table 3. In the first lactation, ELE cows consumed more DM than RES cows, at 19.3 kg/d and 18.6 kg/d, respectively ($P < 0.01$, Figure 1A). As concentrate intake from the out-of-parlor feeders did not differ between groups ($P = 0.94$, Figure 1B), the increase in DMI was mainly driven by an increase in voluntary consumption of the PMR ($P < 0.01$, Figure 1C). Although average daily milk yield was similar between treatments ($P = 0.57$), the ELE cows produced more milk fat (+50 g/d, $P < 0.01$, Figure 2). This greater milk fat yield was associated with greater milk fat content, at 4.57% for ELE cows compared with 4.33% for RES cows ($P < 0.01$, Figure 2B). Protein content did not vary between the treatment groups ($P = 0.27$, Figure 2A). Body weight was comparable in both treatment groups, whereas BCS was lower in ELE cows (3.22 vs. 3.30, $P < 0.01$, Figure 3). Feed efficiency, expressed as the ratio of FPCM to DMI, was lower in ELE cows than RES cows (1.54 vs. 1.58, $P < 0.01$).

During the second lactation, the numerical increase in DMI for the ELE group (+0.7 kg/d) persisted; however, the difference was no longer statistically significant ($P = 0.16$). Fat- and protein-corrected milk was significantly increased for ELE by 1.8 kg/d ($P < 0.01$, Figure 4), and in contrast to the first lactation, ELE cows had a greater feed efficiency than RES cows (1.56 vs. 1.50; $P < 0.01$). The increase in FPCM was mainly explained by an increase in milk fat yield (+115 g/d, $P < 0.01$), with milk fat content also being higher for ELE cows (4.61% vs. 4.39%, $P < 0.01$, Figure 5B). Milk lactose content tended to be lower for ELE cows (4.55% vs. 4.58%, $P = 0.07$), whereas milk protein content was not significantly different between the 2 groups ($P = 0.61$, Figure 5A). Cows fed ELE remained leaner during the second lactation, as indicated by a tendency toward a lower BCS (3.05 vs. 3.14, $P = 0.10$).

Fertility and Probability of Culling

Results for the first-service conception rate in the first and second lactations are shown in Table 4. During the first lactation, first-service conception rate (47.4% vs. 40.0%, $P = 0.53$) and DIM at first service (70 $\pm$ 4.3 for ELE vs. 70 $\pm$ 4.6 for RES) were not significantly different between ELE and RES cows. From the 33 cows that

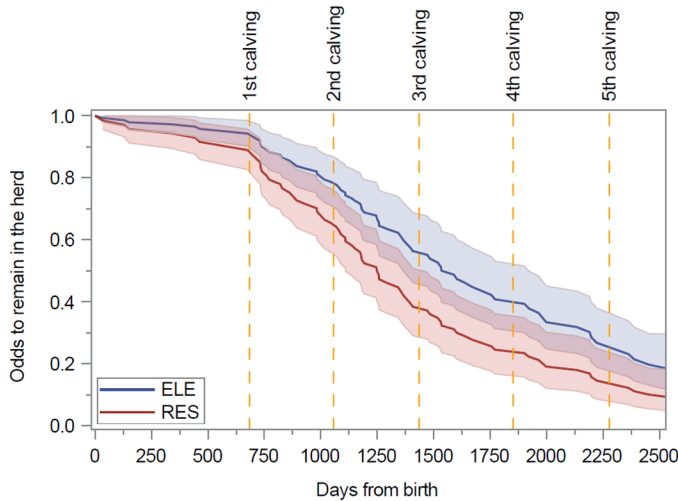


Figure 6. Probability that cows will remain in the herd, and thus avoid culling, in relation to days from birth for cows fed an enhanced (blue line; ELE) or restricted (red line; RES) amount of nutrient intake preweaning. Hazard ratios (HR) were 0.46 (95% CI = 0.09–2.45, $P = 0.36$), 0.40 (95% CI = 0.15–1.07, $P = 0.07$), 0.49 (95% CI = 0.24–1.00, $P = 0.05$), 0.44 (95% CI = 0.22–0.86, $P = 0.02$), and 0.46 (95% CI = 0.24–0.87, $P = 0.02$) for surviving until first, second, third, fourth, and fifth calving, respectively. Vertical dotted lines represent median ages at calving. Continuous lines are LSM, and shaded areas represent the SE.

had a second calving in the ELE group, 18 conceived at the first insemination, 5 at the second insemination, and 4 at the third insemination, and 6 needed between 4 and 6 inseminations to conceive. For the 28 RES cows, 14 conceived at the first insemination, 6 at the second insemination, and 2 at the third insemination, and 6 needed between 4 and 6 inseminations to conceive. The odds ratio for conceiving with fewer inseminations in the ELE group compared with the RES group 1.16 (0.34–3.93, $P = 0.80$).

In the second lactation, at similar DIM at first service (62 ± 3.6 for ELE vs. 67 ± 4.2 for RES), ELE cows had a higher first-service conception rate than RES cows (40.7% vs. 13.0%; $P = 0.03$). Among the 23 ELE cows that had a third calving, 11 conceived at the first insemination, 6 at the second insemination, and 4 at the third insemination, and 2 needed 4 inseminations to conceive. Among the 16 RES cows, 3 conceived at the first insemination, 9 at the second insemination, and 2 at the third insemination, and 2 needed 4 inseminations to conceive. The odds ratio for conceiving with fewer inseminations in the ELE group compared with the RES group was 2.48 (0.52–11.93, $P = 0.25$).

Survival rates and related metrics are outlined in Table 5, and the odds for remaining in the herd from birth to fifth calving are presented in Figure 6. Using logistic regression, none of the survival rates were significant, despite meaningful numerical differences at second

(76.7% vs. 65.1%, $P = 0.23$), third (53.5% vs. 37.2%, $P = 0.13$), fourth (41.9% vs. 23.3%, $P = 0.06$), and fifth calving (25.6% vs. 14.0%, $P = 0.17$). However, using Cox's proportional hazard regression, hazard ratios (HR) were 0.46 (95% CI = 0.09–2.45, $P = 0.36$), 0.40 (95% CI = 0.15–1.07, $P = 0.07$), 0.49 (95% CI = 0.24–1.00, $P = 0.05$), 0.44 (95% CI = 0.22–0.86, $P = 0.02$), and 0.46 (95% CI = 0.24–0.87; $P = 0.02$) for surviving until the first, second, third, fourth, and fifth calving, respectively. This highlights that the likelihood of being culled before the third, fourth, or fifth calving is halved when calves are fed ELE as compared with RES. Ages at first, second, third, fourth, and fifth calving were not significantly different between ELE and RES (Table 5).

Among all cows culled until fifth calving, the 5 main reasons were mastitis (10 RES, 8 ELE), fertility (8 RES, 9 ELE), infectious diseases that led to death or euthanasia (6 RES, 6 ELE), lameness (3 RES, 2 ELE), and udder morphology (3 RES, 1 ELE). Another 3 cows (1 RES, 2 ELE) were culled to comply with the P quota being enforced in the Netherlands, 1 RES cow was sold before first calving for her high breeding value, 1 RES cow was culled due to low milk yield, and another ELE cow was sent to slaughter due to an infectious disease. Unfortunately, reasons for culling remain unknown for 7 cows (4 RES, 3 ELE).

Metabolomic Profiles

Blood metabolites at 60 DIM during the first lactation associated with preweaning nutritional treatments are detailed in Table 6. Several AA and related compounds were lower in ELE cows than RES cows, including 1-methyl-4-imidazoleacetate, 1-methylguanidine, 1-ribosyl-imidazoleacetate, 4-guanidinobutanoate, creatinine, N-formylmethionine, N-methylphenylalanine, and S-1-pyrroline-5-carboxylate. In terms of lipid metabolites, ELE cows had higher levels of sphingosine, sphingosine 1-phosphate, 7- α -hydroxy-3-oxo-4-cholestenoate, and propionylglycine than RES cows. The carbohydrate mannose was also identified to be higher in the ELE group. Conversely, our analysis found that bile acids deoxycholate and ursodeoxycholate were lower in the ELE cows. It is noteworthy that the nucleotides 2'-deoxycytidine, 3-ureidopropionate, 5-methyl-2'-deoxycytidine, 5-methylcytidine, allantoic acid, allantoin, N₁-methylinosine, and N₂,N₂-dimethylguanosine presented lower concentrations in the ELE cows. Several peptides and xenobiotics, such as gamma-glutamylthreonine, prolylglycine, ¹H-quinolin-2-one, erythritol, lanthionine, and pyrrolidine, were also decreased in the ELE treatment group.

To further elucidate the effects of preweaning nutrient supply on the biochemical signatures at 60 DIM, enrichment and self-contained pathway testing was performed.

Table 6. Metabolites associated with preweaning nutritional treatments¹

Class ²	Metabolite	PubChem ID	Fold change	P-value
AA	1-Methyl-4-imidazoleacetate	75810	0.87 ^a	0.04*
AA	1-Methylguanidine	10111	0.78 ^a	0.03*
AA	1-Ribosyl-imidazoleacetate	5117448	0.83 ^a	<0.01*
AA	2,3-Dihydroxy-2-methylbutyrate	301941	0.84	0.07
AA	4-Guanidinobutanoate	500	0.89 ^a	0.03*
AA	5-Aminovalerate	138	0.81	0.06
AA	Citrulline	9750	0.94	0.07
AA	Creatinine	588	0.95 ^a	0.03*
AA	Cysteinylglycine disulfide	22833544	0.94	0.09
AA	Hydroxyasparagine	97663	0.93	0.08
AA	N ₆ ,N ₆ ,N ₆ -trimethyllysine	440120	0.95	0.05
AA	N-Acetyl-isoptreanine	22833544	0.83	0.06
AA	N-Formylmethionine	439750	0.79 ^a	<0.01*
AA	N-Methylphenylalanine	4657542	0.74 ^a	0.03*
AA	Picolinate	1018	0.81	0.05
AA	Pipecolate	849	1.22 ^a	<0.01*
AA	Pyroglutamine	134508	0.91	0.07
AA	S-1-pyrroline-5-carboxylate	1196	0.89 ^a	0.04*
Carbohydrate	Mannose	18950	1.09 ^a	0.04*
Cofactors and vitamins	α-Tocopherol	14985	0.91	0.08
Energy	2-Methylcitrate/homocitrate	439459	0.81 ^a	<0.01*
Energy	Citrate	311	0.87 ^a	0.03*
Energy	Succinylcarnitine (C4-DC)	71464481	0.82 ^a	<0.01*
Lipid	1-(1-Enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)	9547058	1.18 ^a	0.02*
Lipid	1,2-Dipalmitoyl-GPC (16:0/16:0)	452110	1.09	0.10
Lipid	13-HODE + 9-HODE	43013	1.37	0.05
Lipid	2-Palmitoyl-GPC (16:0)	15061532	1.29	0.06
Lipid	3-Hydroxybutyrylcarnitine	53481617	2.44	0.06
Lipid	3-Methylglutarate/2-methylglutarate	—	0.84	0.07
Lipid	7-α-Hydroxy-3-oxo-4-cholestenoate (7-Hoca)	3081085	1.25 ^a	0.04*
Lipid	7-Ketodeoxycholate	188292	0.57 ^a	0.05*
Lipid	Behenoylcarnitine (C22)	71464577	1.38	0.06
Lipid	Caproate (6:0)	8892	0.83	0.06
Lipid	Chenodeoxycholate	10133	0.63	0.08
Lipid	Cholate	221493	0.62 ^a	0.01*
Lipid	Choline	305	1.08	0.10
Lipid	Deoxycholate	222528	0.65 ^a	0.01*
Lipid	Propionylglycine	98681	0.71 ^a	<0.01*
Lipid	Sphingomyelin (d18:2/18:1)	52931155	1.44	0.09
Lipid	Sphingosine	5353955	1.32 ^a	0.02*
Lipid	Sphingosine 1-phosphate	5283560	1.19 ^a	0.01*
Lipid	Ursocholate	20849089	0.61 ^a	0.02*
Lipid	Valerate (5:0)	7991	0.51 ^a	0.02*
Nucleotide	2'-Deoxycytidine	13711	0.81 ^a	<0.01*
Nucleotide	2'-O-Methyluridine	102212	0.88	0.07
Nucleotide	3-Ureidopropionate	111	0.9 ^a	0.02*
Nucleotide	5,6-Dihydrouridine	94312	0.89	0.05
Nucleotide	5-Methyl-2'-deoxycytidine	440055	0.88 ^a	<0.01*
Nucleotide	5-Methylcytidine	92918	0.9 ^a	0.02*
Nucleotide	Allantoic acid	203	0.88 ^a	0.04*
Nucleotide	Allantoin	204	0.89 ^a	0.01*
Nucleotide	Cytosine	597	0.82	0.10
Nucleotide	Dihydroorotate	648	0.79	0.07
Nucleotide	N ₁ -Methylinosine	65095	0.78 ^a	<0.01*
Nucleotide	N ₂ ,N ₂ -Dimethylguanosine	92919	0.7 ^a	0.03*
Peptide	Gamma-glutamylthreonine	76078708	0.87 ^a	0.03*
Peptide	Leucylglycine	79070	0.74	0.06
Peptide	Prolylglycine	6426709	0.77 ^a	<0.01*
Xenobiotics	¹ H-Quinolin-2-one	6038	0.84 ^a	0.05*
Xenobiotics	2,8-Quinolinediol sulfate	166594805	0.76	0.07
Xenobiotics	3-Hydroxyhippurate	450268	0.84	0.05
Xenobiotics	3-Methylcatechol sulfate	134817859	0.79	0.08
Xenobiotics	Erythritol	222285	0.85 ^a	0.03*
Xenobiotics	Lanthionine	98504	0.81 ^a	0.03*
Xenobiotics	Pyrraline	122228	0.72 ^a	0.04*

^aFold change (FC) > 1.0 indicates greater relative concentration for ELE, and FC < 1.0 indicates lower relative concentration for ELE in a pairwise comparison between treatment groups (RES vs. ELE; n = 25 heifers per treatment).

¹RES = 2.71 Mcal of ME in 4 L of MR per day (150 g/L; 600 g/d of MR powder); ELE = 5.14 Mcal of ME in 8 L of MR per day (150 g/L; 1,200 g/d of MR powder).

²Metabolites having a significant FC are listed according to metabolite classes.

*False discovery rate-corrected *P* < 0.05, *t*-test.

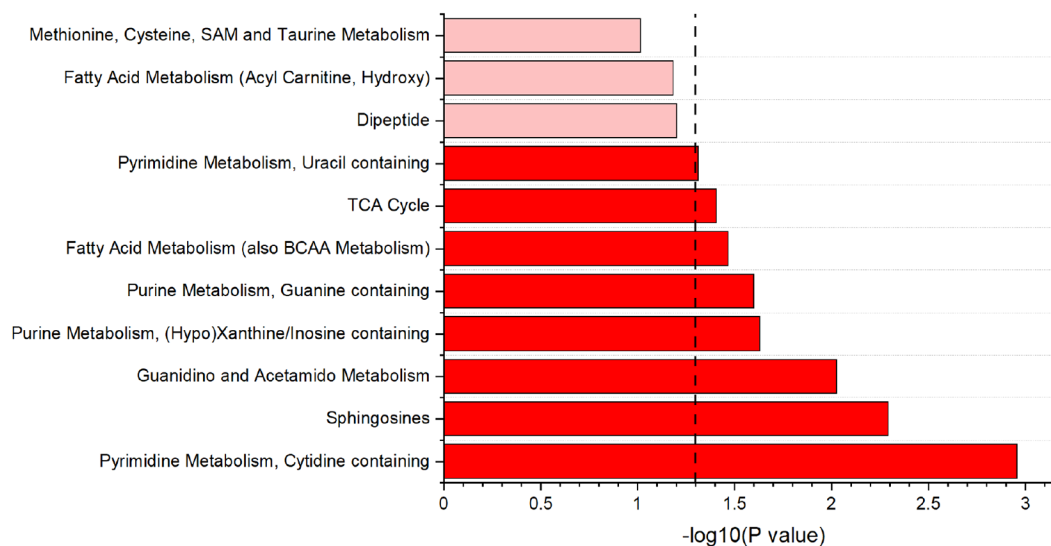


Figure 7. Visualization of meaningful metabolic pathways evidenced by enrichment analysis based on the metabolites detected at 60 DIM in the serum of dairy cows ($n = 50$) fed different nutrient intakes preweaning. Metabolic pathways shown in darker red had $P < 0.05$, whereas those in lighter red had $P < 0.10$.

As depicted in Figure 7, 6 main biological processes were predicted to be significantly affected by the 2 treatments: pyrimidine metabolism (including cytidine and uracil), sphingosine metabolism, guanidino and acetamido metabolism, purine metabolism (comprising hypoxanthine, xanthine, inosine, and guanine), fatty acid metabolism (including branched chain AA metabolism), and the tricarboxylic acid (TCA) cycle.

DISCUSSION

Earlier reports on these animals described the growth and metabolic differences in calves fed different amounts of MR preweaning and found that a higher MR allowance improved growth rates and altered energy, protein, and liver metabolism compared with a lower MR allowance (Leal et al., 2021). Building on these findings, Leal et al. (2025) followed the same cohort of calves and assessed the sustained effects of preweaning nutrition on growth, reproductive performance, and glucose metabolism in the early postweaning and heifer growing phase. Although the growth advantage was not apparent at 330 d of age, indications of altered insulin sensitivity were present, including lower plasma insulin concentrations after a glucose tolerance test in ELE cows and altered metabolomic profiles persisting into the heifer growing phase. Therefore, the objective of the current study was to follow these same heifers and explore the consequences of the contrasting preweaning MR supply level on lactation performance. The results showed that ELE cows had higher DMI, milk fat yield, and milk fat content during

first lactation and higher FPCM, milk fat yield, and milk fat content during second lactation than RES cows. Notably, ELE cows had also a higher first-service conception rate in second lactation and a lower probability to leaving the herd from birth to third calving.

Our findings align with previous research demonstrating that a higher preweaning ADG has a positive effect on milk production later in life (Shamay et al., 2005; Moallem et al., 2010; Soberon et al., 2012). Nonetheless, the increase in FPCM in ELE cows during second lactation was primarily driven by the higher milk fat yield. That could be attributed to changes in lipid metabolism that remained until lactation caused by preweaning nutrient intake (Leal et al., 2021).

The consistently lower BCS observed in ELE cows during their first and second lactations, despite the similar BW to RES cows, suggests that preweaning nutrition may have long-term effects on body composition and energy partitioning. The latter is supported by changes in feed efficiency, being negative in first lactation and positive in second lactation. Overall lower BCS throughout the first 2 lactations and higher feed efficiency in second lactation agree with results reported by Roche et al. (2009), which indicated that cows with better nutritional status in early lactation might prioritize milk production, leading to increased mobilization of body reserves and a subsequent decline in BCS.

The effects of preweaning nutrition on the long-term reproductive performance of dairy cows has been a subject of increasing interest in recent years (Bruinje et al., 2019, 2021). Nevertheless, there are limited data on the

effect of preweaning nutrient intake on the reproductive performance of lactating cows. In the current study, ELE and RES cows had similar first-service conception rates (47.4% vs. 40.0%) and DIM at first service (70 ± 4.3 for ELE vs. 70 ± 4.6 for RES) during first lactation. This is consistent with our previous findings in the same animals during the heifer growing phase, where no differences were observed in age at first service, first-service conception rate, age at conception, or number of services per conception between the ELE and RES heifers (Leal et al., 2025). Interestingly, cows fed an ELE nutrient intake preweaning had a higher second-lactation conception rate at first insemination (40.7% vs. 13.0%) than RES cows. The fertility results of ELE cows align well with the first insemination pregnancy rates reported by Ma et al. (2022) for multiparous cows inseminated after a 50-d voluntary waiting period. The mechanism by which preweaning nutrient intake could influence reproductive efficiency in adulthood is probably multifaceted and likely involves interplay among metabolic, endocrine, and epigenetic factors (Sánchez-Garrido et al., 2022). For instance, early-life nutrition has been found to influence the development of key organs, such as the liver and pancreas, which are important regulators of reproductive hormones and energy homeostasis (Soberon and van Amburgh, 2017), and on the gene expression profiles of adipose tissue (Leal et al., 2018). Accordingly, an increase in preweaning milk allowance (10 L vs. 5 L of whole milk per day) was found to positively affect prepubertal follicular growth, serum leptin concentrations, and LH pulse characteristics (Bruinje et al., 2019, 2021).

Our survival analysis revealed that the risk of culling from birth to first calving was similar between treatments, whereas from birth to second calving, ELE cows had a numerically lower risk of being culled than RES cows (HR = 0.40), and from birth to third, fourth, or fifth calving, the risk of being culled was significantly lower in ELE cows than in RES cows (HR $\leq$ 0.49). As pointed out by De Vries and Marcondes (2020), following an analysis of 11,985 dairy herds in the United States, low conception rates are a major factor influencing culling decisions. Thus, the reduced fertility of RES cows in second lactation could have increased culling, which partially explains their lower survival rates from birth to third calving. However, before fifth calving, a similar number of RES and ELE cows left the herd due to fertility reasons (8 vs. 9). In the current study, culling decisions were made by farm managers who were blinded to the dietary treatments and instructed to follow their usual farm management practices. As a result, culling decisions encompassed both voluntary and involuntary culling. However, the frequency of voluntary culling, the subjective component of culling, was moderate, accounting for only 5 out of 69 culling events. Therefore,

poor fertility and health, intrinsic factors of the cows, were the primary reasons for herd exits. Thus, this data may be interpreted as indirect evidence that preweaning nutrition might also affect the immune competence of cows, ultimately affecting their health and resilience. In a recent study, Ockenden et al. (2023a, 2023b) found that calves fed a higher volume of milk (8 L/d) exhibited better growth, immune competence, and metabolic characteristics than calves fed low volumes of milk (4 L/d) preweaning. When accessing these same calves at 12 mo of age, the authors found that the immunological advantages were maintained, which seem to indicate an imprinting of the immune system. Nevertheless, further studies are needed to improve our understanding of the effect of preweaning nutrition on immunity, as, in contrast to Ockenden et al. (2023a), other studies have reported a lower adaptive response (Foote et al., 2007) and reduced neutrophil response (Obeidat et al., 2013) during the preweaning phase for calves fed an elevated plane of nutrition. However, when Obeidat et al. (2013) evaluated the neutrophil response during the immediate postweaning period or the grower phase, the response was not different between calves fed a high or low plane of nutrition preweaning. This seems to suggest that the timing of the measurement is very important.

The metabolomic analysis at 60 DIM in first lactation revealed different metabolic profiles between ELE and RES cows. This is consistent with the findings in the preweaning period (Leal et al., 2021) and growing heifers (Leal et al., 2025). The analysis revealed that metabolites associated with AA, carbohydrate, lipid, and nucleotide metabolism diverged in ELE and RES cows. Moreover, the pathway enrichment analysis indicated that the TCA cycle, fatty acid metabolism, purine metabolism, guanidino and acetamido metabolism, sphingosine metabolism, and pyrimidine metabolism were the main metabolic pathways affected by different nutrient intake levels preweaning. These metabolic alterations suggest that preweaning nutrient intake has long lasting effects on metabolic pathways that have important implications for both productive performance and probability of remaining in the herd.

This study demonstrates that optimizing preweaning nutrition plays a key role in improving the lifetime productivity of dairy cows and may be a powerful management tool that dairy farmers can use to improve the sustainability of dairy farming. When the average daily productivity in the first and second lactation is multiplied by the number of days cows were in lactation (up to 280 d per lactation) and expressed relative to the total number of animals enrolled at the start of the study (43 animals per treatment), an average of 13,436 kg of FPCM was produced per ELE cow within the first 280 d of the 2 first lactations. When expressed relative to the total possible

lactation days (560 d; 2 lactations $\times$ 280 d), the average daily FPCM for ELE cows was 24.0 kg/d. This is substantially higher than the equivalent productivity for RES cows, which produced 11,089 kg of FPCM during the same period (i.e., 19.8 kg/d).

CONCLUSIONS

This study confirmed the importance of early-life nutrition in the lifetime productivity of Holstein dairy cows. Increasing the milk allowance from 600 to 1,200 g of milk powder daily for the first 49 d of life improved milk performance and reduced the risk of culling. Primiparous cows were leaner and had greater voluntary feed consumption and higher milk fat yield. The benefits of this nutritional intervention extended into the second lactation, resulting in higher milk fat yield and improved feed efficiency. Beyond improved milk productivity, the risk of culling before the third calving was halved in cows that received higher nutrient intake during the first days of life. This study demonstrates how early-life nutrition serves as a powerful lever for improving the sustainability of milk production.

NOTES

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Nonstandard abbreviations used: AI = artificial insemination; ELE = elevated preweaning milk sup-

ply; ESI = electrospray ionization; FPCM = fat- and protein-corrected milk; HR = hazard ratio; m/z = mass-to-charge ratio; MR = milk replacer; PMR = partial mixed ration; QC = quality control; RES = restricted preweaning milk supply; RI = retention-to-time index; RP/UPLC-MS/MS = reversed-phase ultra-performance liquid chromatography-tandem mass spectrometry; RSD = relative standard deviation; TCA = tricarboxylic acid; WIM = weeks in milk.

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