J. Dairy Sci. 109:3898–3913
https://doi.org/10.3168/jds.2025-27192
© 2026, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/).

Growth performance, metabolic, and hematological markers in Fleckvieh dairy calves fed milk replacer or whole milk

Marta Sfulcini,[1] Andrea Minuti,[1]* Giulia Ferronato,[2] Erminio Trevisi,[1] Juan J. Loor,[3] Annalisa Amato,[4] Carmelo Cavallo,[4] Luigi Liotta,[4] and Vincenzo Lopreiato[4]
[1]Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy
[2]Department of Civil Engineering, Architecture, Environment, Land Planning and Mathematics (DICATAM), Università degli Studi di Brescia, 25121, Brescia, Italy
[3]Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801
[4]Department of Veterinary Sciences, Università di Messina, 98168 Messina, Italy

ABSTRACT

Early life is a critical phase for dairy calves, influencing their growth, metabolism, and overall health. This study aimed to evaluate the effects of feeding either whole milk (WM) or milk replacer (MR) on growth performance, metabolic markers, and hematological parameters from birth until weaning at 60 d of age in Fleckvieh dairy. Eighteen heifer calves were assigned to one of 2 dietary treatments: WM (n = 9; DM basis, 29.1% protein, 27.3% fat, and 37.3% lactose; 5.25 Mcal/kg of DM) or MR (n = 9; DM basis, 22.9% protein, 19.1% fat, and 51.73% lactose; 4.76 Mcal/kg DM; Elvor, Maen Roch, France). The MR was prepared at 130 g/L to match solids content in WM. Calves were housed in individual hutches, fed 3 L of milk twice daily until 53 d and then 3 L of milk once daily until weaning (60 d). Calves had ad libitum access to starter and intake was recorded daily. Growth performance was assessed through BW and period-specific ADG (gain per day between successive weighings), whereas metabolic and hematological markers were analyzed through plasma biomarkers and blood cell counts, respectively. Data were subjected to ANOVA using the GLM procedure for single measures and the GLIMMIX procedure for repeated measures. Despite similar total DMI, calves fed WM exhibited an overall greater BW and greater period-specific ADG (0.77 vs. 0.85 kg/d in MR and WM, respectively), and from 21 to 60 d, WM calves were 3.8 kg heavier than MR-fed calves. From the metabolism standpoint, feeding WM resulted in overall greater plasma concentrations of glucose, urea, cholesterol, alkaline phosphatase, and Ca, but lower bilirubin. Specifically, WM calves had greater paraoxonase activity at 35, 45, and 60 d (weaning), greater ceruloplasmin levels at 15, 21, and 28 d, and greater Mg at 60 d of age. Among hematological parameters, WM calves had greater circulating total leukocytes at 21 and 28 d of age due to enhanced neutrophils. Although WM calves consumed milk with greater proportions of fat and protein and lower lactose content, the intake of milk and starter DM and total energy was similar compared with MR calves. However, considering the better growth rate of WM calves (especially in the second week of life), the better balance between fat, protein, and lactose suggests that feeding WM can enhance bioavailability of nutrients. Further research is needed to explore the long-term implications of these feeding strategies on calf health and productivity, particularly in relation to sustainability and economic feasibility.

Key words: early-life nutrition, blood biomarkers, liquid feeding strategy, calf performance

INTRODUCTION

Early life is a critical stage for dairy calves, significantly influencing their development and long-term productivity (Heinrichs and Heinrichs, 2011). During this window, environmental, sanitary, nutritional, and genetic factors interact to shape growth and immune function (Lorenz et al., 2011; Johnson et al., 2018). When growth rates are inadequate, calves may reach weaning underweight, a condition associated with compromised post-weaning development that cannot be entirely corrected through subsequent nutritional intervention (Jasper and Weary, 2002).

Nutrition plays a central role in this early developmental phase, as milk remains the primary source of essential nutrients for calves that have not yet established a functional rumen. Consequently, several feeding strategies are employed during the preweaning period to support

Received July 3, 2025.
Accepted January 5, 2026.
*Corresponding author: andrea.minuti@unicatt.it

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

optimal rumen development, facilitate the transition to solid feed (McGrath, 2016), and reduce disease risk. Among these strategies, the type of liquid feed provided is particularly important. Management practices vary in weaning age, milk allowance, and the choice between whole milk (**WM**) and milk replacers (**MR**; Fischer et al., 2019). In intensive dairy systems, MR is often used instead of WM, and its formulation has historically been driven by cost considerations (Otterby and Linn, 1981), resulting in notable differences in digestibility and nutrient composition.

Typically, MR formulations contain higher lactose but lower fat and protein content in comparison with WM and these compositional differences have been shown to affect growth rates and metabolic markers (Gilbert et al., 2016; Amado et al., 2019). Feeding MR is associated with alterations in circulating metabolites, including nonesterified fatty acids (**NEFA**), IGF-1, glucose, urea, and lipoprotein profiles (Lepczyński et al., 2015; Wilms et al., 2022; Chapelain et al., 2025). Differences among MR formulations have also been reported, with high-lactose MR linked to increased liquid feed intake and enhanced preweaning growth, whereas high-protein MR are associated with improved feed efficiency (Bartlett et al., 2006; Berends et al., 2020; Echeverry-Munera et al., 2021). Higher fat MR (≥23%) may reduce mortality, improve fecal consistency, and decrease the necessity for medical interventions (Urie et al., 2018; Amado et al., 2019; Berends et al., 2020). In addition, higher dietary fat promotes greater fat deposition, whereas adequate levels of metabolizable energy and protein levels are essential to support optimal growth performance (Tikofsky et al., 2001; Hill et al., 2008). Conversely, WM provides a naturally balanced nutrient supply rich in energy and fatty acids that support metabolic activity, immune function, and overall physiological development (Delplanque et al., 2015; Grote et al., 2016). In addition, WM contains several bioactive molecules, including lipids such as sphingomyelin and phytanic acid, which contribute to membrane structure, lipid metabolism, and cellular signaling involved in intestinal maturation and immune modulation (Keenan and Huang, 1972; Chalfant et al., 2004; Zandbergen and Plutzky, 2007; Park, 2009). Moreover, growth factors present at high concentrations in colostrum and at lower levels in milk, including IGF-I, IGF-II, and insulin, may act locally within the gut to support epithelial development and metabolic regulation (Blum and Baumrucker, 2002; Lee et al., 2009).

Despite substantial advances in MR formulation, concerns remain regarding nutrient balance, digestibility, and the metabolic consequences associated with these products (Sweeney et al., 2010; Kertz et al., 2017). Moreover, the considerable variability among MR formulations, particularly in protein, fat, and lactose content, has contributed to inconsistent findings in studies comparing MR with WM. Reported effects on growth, metabolic indicators, and health outcomes vary widely across experiments, making it difficult to draw clear physiological or practical conclusions for calf rearing systems.

Therefore, interest has increased in refining liquid feeding strategy to clarify the underlying physiological mechanisms and to better understand how different liquid feeding strategies affect calf metabolism and overall development. Furthermore, few studies have integrated growth performance with a comprehensive assessment of metabolic and hematological profiles, particularly in dual-purpose breeds such as Fleckvieh calves.

We hypothesized that feeding WM would affect health and biochemical parameters compared with MR adjusted to match the DM content of WM. Accordingly, this study compared metabolic responses and hematological parameters in Fleckvieh dairy calves fed WM or MR from birth to 60 d of age.

MATERIALS AND METHODS

Animal Management and Experimental Design

The experimental protocol was approved by Ethical Animal Care and Use Committee of the Magna Graecia University of Catanzaro (protocol no. 271/2017).

This study was carried out at Fattoria Demetra, a commercial dairy farm (Vibo Valentia, Italy). A total of 18 Fleckvieh heifer calves were enrolled in the trial from November to February, during the winter season and were all born from multiparous cows. Immediately after birth, newborn calves were separated from their dams. According to farm standard procedures, all calves were cleaned and had the navel disinfected with oxytetracycline hydrochloride (Neo Spray Caf Aerosol; Gellini S.p.a., Aprilia, Italy). Calves were weighed and fed 3 L of colostrum by nipple bottle at 2.5 ± 1.5 h after birth. If voluntary colostrum intake did not reach the 3 L, calves were fed with an esophageal feeder to reach the total intake of 3 L of colostrum (Speedy Drencher XL, Agri-Zoo San Marino srl, Domagnano, San Marino). Colostrum was previously collected, pooled to obtain a Brix value of 26%, and subsequently frozen in aliquots of 3 L each at −18°C. Before administration, aliquots were thawed in a water bath at 40°C for 1 h and gently mixed to ensure homogeneity Afterward, calves received 4 feedings of their dam's transition milk (3 L at each feeding) over 2 d (i.e., at 12, 24, 36, and 48 h after birth). All calves were housed in individual pens (2.8 m long × 1.80 m wide) separated by bar fences to allow calves to interact with each other. All pens were straw-bedded with daily refill and completely replaced once weekly.

Table 1. Nutrient composition (% of DM unless otherwise noted) of milk replacer (MR), whole milk (WM), and calf starter

Item	MR[1]	WM[2]	Calf starter[3]
CP, %	22.87	27.41 ± 3.54	17.81
Crude fat, %	19.14	29.33 ± 1.52	2.47
Lactose, %	51.73	37.28 ± 0.95	
Ca, %	0.94		
Na, %	0.52		
P, %	0.73		
Ash, %	7.71		8.77
ME, Mcal/kg of DM	4.76	5.25	2.47
Starch, %			24.38
NDF, %			33.22
ADF, %			23.18
ADL, %			6.85

[1]MR: Elvor, Maen Roch, France. The ingredients, listed in decreasing order of inclusion, were spray-dried skim milk powder, whey, palm vegetable oil, coconut vegetable oil, wheat flour, pea protein, wheat starch, pea flour, and dried *Saccharomyces cerevisiae* yeast. Nutritional additives per kilogram of MR as-such included vitamin A (25,000 IU), vitamin D_3 (5,000 IU), vitamin E as all-rac-α-tocopheryl acetate (125 mg), vitamin K_3 (2 mg), vitamin B_1 (10 mg), vitamin B_2 (8 mg), niacinamide (55 mg), vitamin B_6 (2 mg), vitamin B_{12} (0.07 mg), vitamin C (250 mg), calcium D-pantothenate (40 mg), folic acid (0.6 mg), and choline chloride (300 mg). The formulation also contained DL-methionine (500 mg), L-threonine (980 mg), organic selenium derived from *Saccharomyces cerevisiae* CNCM I-3060 (91 mg), copper from copper sulfate pentahydrate (39 mg), iron from ferrous sulfate monohydrate (331 mg), zinc from zinc sulfate monohydrate (329 mg), manganese from manganese sulfate monohydrate (154 mg), selenium from sodium selenite (0.2 mg), potassium from potassium iodide (2.6 mg), and the technological additive BHT (32 mg).

[2]DM was 12.67%.

[3]Calf starter, Dietovit Excellence, SIVAM Spa, Casalpusterlengo, Lodi, Italy. DM was 87.87%.

At 3 d of age, calves were randomly assigned to one of 2 treatments verifying that there was a similar distribution of BW and total serum protein (**STP**) at 24 h after birth between the 2 treatments: MR (n = 9; BW = 49.75 ± 1.390 kg; STP = 6.5 ± 0.70 g/dL Elvor, Maen Roch, France) or WM (n = 9; BW = 49.81 ± 1.390 kg; STP = 6.3 ± 0.40 g/dL). The chemical compositions of both MR and WM are reported in Table 1.

Three fresh WM samples per week were collected during the trial for a total of 45 samples and immediately analyzed using the Milkoscan FT2 (Foss Electric A/S, 3400 Hillerød, Denmark). Concentration of MR was 130 g/L (as-fed, whereas 125.33 g/L as DM) to get closer to the solid percentage of bovine WM (on average, 12.67%). In fact, the average DM of milk delivered was 752 g/d for MR and 751 g/d for the WM group in the first 53 d of age, and 376 g/d for MR and 375.5 g/d for WM group from 54 d of age to weaning (60 d of age). The MR was prepared by manually mixing the powder with warm water (40°C) using a hand whisk. The WM was collected directly from the bulk tank after milking and warmed to 40°C using an electric heater before feeding. From 3 to 53 d of age, all calves received a total of 6 L/d of WM or MR divided into 2 equal feedings administered at 0800 and 1600 h. At 54 d of age, all calves were stepped down to only one meal of 3 L in the morning and then weaned at 60 d of age. Both MR and WM were administered using open buckets. Refusals of MR or WM were recorded daily.

From 4 d of age, the calves had ad libitum access to a calf starter offered once every morning after MR or WM feeding. The chemical composition of the calf starter is reported in Table 1. The starter was formulated with the following ingredients (descending percentage): corn, dehydrated alfalfa meal, wheat bran, extruded sunflower meal, middling wheat, toasted soybeans, extruded soybean meal, barley, beet pulp, corn cob, molasses, extruded linseeds, calcium carbonate, milk whey powder, yeasts, sodium chloride, sodium phosphate, sodium bicarbonate, and extracts of officinal herbs. As calf starter was bought from an authorized commercial feed mill, the percentage of each ingredient is unknown. The chemical analyses of the calf starter were carried out according to the official AOAC methods (AOAC International, 2000). When calves consumed a substantial portion of the starter feed before the night checks, additional starter was supplied to ensure adequate intake. Daily starter intake was recorded.

Sampling and Analysis

Calves' health status was evaluated daily and feces were scored daily in the morning before milk feeding using a 0 to 3 scale according to the Calf Health Scoring Criteria by School of Veterinary Medicine of the University of Wisconsin–Madison (score 0 being normal; 1 being semi-formed, pasty; 2 being loose, but stays on top of bedding; 3 being water, sifts through bedding). However, calves involved herein did not suffer from any acute health disorders during the entire experimental period. Given the limited number of calves enrolled and the very low frequency of loose but nonpathological feces observed over a short period (2 d), the evaluation of incidence was not considered informative. In fact, only 2 calves in the MR group experienced 2 d with fecal score = 2 (in different periods), and only 1 calf in the WM group showed a comparable event. For this reason, further statistical analysis of incidence was not performed, as it would not add relevant information beyond the descriptive data already reported. Additionally, no vaccination therapy or antibiotic treatment was applied to the enrolled calves. The measurement of the BW of calves was performed at birth, then at 7, 15, 21, 28, 35, 45, and 60 d of age before the morning feeding. Period-specific ADG was calculated based on BW data from 2 consecutive measurements. On d 1, blood samples were collected in clot activator tubes (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ), and total serum protein was measured using a handheld refractom-

eter (PU-ATC temperature compensated; Kernco Instruments Co.) to evaluate passive immunity transfer from colostrum. Blood samples were collected from a jugular vein into lithium-heparin tubes at 1, 7, 15, 21, 28, 35, 45, and 60 d of age (weaning), and into K_3EDTA tubes at all time points except d 1 (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ), before the morning meal. Lithium-heparin tubes were immediately cooled in a water-ice bath and centrifuged within 20 min of collection at 3,500 × *g* for 15 min at 4°C. Plasma was stored at −20°C for further analysis. Plasma biomarkers, except vitamins, were analyzed at 37°C via an automated clinical analyzer (ILAB 650; Instrumental Laboratory, Werfen, Bedford, MA) as reported by Lopreiato et al. (2021) and Morittu et al. (2021). Briefly, metabolites assessed were Ca, P, Mg, Na, K, Zn, glucose, cholesterol, urea, ceruloplasmin, total protein, albumin, globulin, aspartate aminotransferase, γ-glutamyl transferase (**GGT**), alkaline phosphatase, bilirubin, haptoglobin, NEFA, BHB, creatinine, paraoxonase, myeloperoxidase, total reactive oxygen metabolites (**ROM**), and ferric reducing antioxidant power. Plasma IgG (at 24 h after colostrum intake) were assessed with a commercial RID assay (Bovine IgG ID-Ring test, IDBiotech, ImmunoDiffusion Biotechnologies SARL, Issoire, France) according to the manufacturer's instructions.

Blood collected into K_3EDTA tubes was stored in an insulated container at room temperature (15°C–18°C) and analyzed within 2 h of collection using an ADVIA 2120 Hematology System machine (Siemens, Germany). The parameters taken into account in the current study were total leukocyte cell count, leukocyte differential count for neutrophils, eosinophils, basophils, lymphocytes, and monocytes as percentage and absolute number, red blood cell count (**RBC**), hematocrit value (**HCT**), hemoglobin concentration (**HGB**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration (**MCHC**), and red blood cell distribution width (**RDW**). In addition, platelet indices were also analyzed and included platelet count (**PLT**) and mean platelet volume (**MPV**).

Statistical Analysis

The sample size was calculated to achieve a power >0.80 with an $\alpha = 0.05$ using the G*Power package (Faul et al., 2007). The effect size was estimated based on the variance of blood parameters mainly glucose, haptoglobin, cholesterol, and urea. Based on parameters observed in our previous studies (Amato et al., 2023; Sfulcini et al., 2025), the number of 9 animals per group was considered adequate for the main physiological and biochemical endpoints of the study, as it was sufficient to detect biologically meaningful differences. Normal distribution of data was checked using the UNIVARIATE procedure in SAS 9.4 (SAS Institute Inc.). Data with a single measure (STP and IgG concentrations at 24 h after colostrum intake) were subjected to ANOVA (GLM procedure of SAS). Data with multiple observations were analyzed with repeated measures mixed models (GLIMMIX procedure of SAS). Four structures of covariance (compound symmetry, autoregressive order, Toeplitz, or spatial power) were tested and the one with the lowest Akaike information criterion was retained in the model. The models included the fixed effects of the treatment group (**Trt**; WM vs. MR), days of age (day), their interaction (Trt × day), whereas the calf nested within the treatment group was included as the random effect. For plasma, data at 1 d of age (pretreatment) were included as a covariate. Data are presented in tables and figures as LSM. Pairwise comparisons were carried out using the LSD test of SAS. Post hoc comparisons are discussed as significant when $P < 0.05$. Those with values between 0.05 and 0.10 are discussed as tendencies.

RESULTS

Feed Intake and Growth Performance

The effects of feeding MR or WM on growth performance, feed intake, and ME intake are presented in Table 2. Total DMI ($P = 0.82$), both milk and starter feed ($P = 0.81$), were similar between treatments. Furthermore, although total ME consumption did not differ significantly between groups, a tendency toward higher overall ME intake was observed in WM calves ($P = 0.10$). A significant Trt × day interaction was observed for BW ($P < 0.01$). Calves receiving WM were heavier than MR calves at 21, 28, 35, 45, and 60 d ($P < 0.05$; Figure 1A). At d 60, WM calves weighed on average 3.80 kg more than MR calves ($P = 0.02$).

Overall, WM calves have greater period-specific ADG compared with those fed MR ($P = 0.04$). Despite the lack of Trt × day effect ($P = 0.20$), it is noteworthy to highlight that between 7 and 15 d of age, WM calves had an ADG of 0.92 kg/d compared with an ADG of 0.61 in MR calves ($P < 0.01$; Figure 1B).

Plasma Biomarkers

Plasma concentrations of metabolites are presented in Table 3. To verify that calves started from comparable baseline conditions, passive immunity parameters were evaluated at 24 h post-colostrum intake. Regarding passive immunity transfer, no differences were observed between milk treatments for IgG concentrations at 24 h post-colostrum intake (17.78 vs. 17.32 ± 2.73 g/L, MR and WM, respectively; $P = 0.75$). Likewise, plasma total

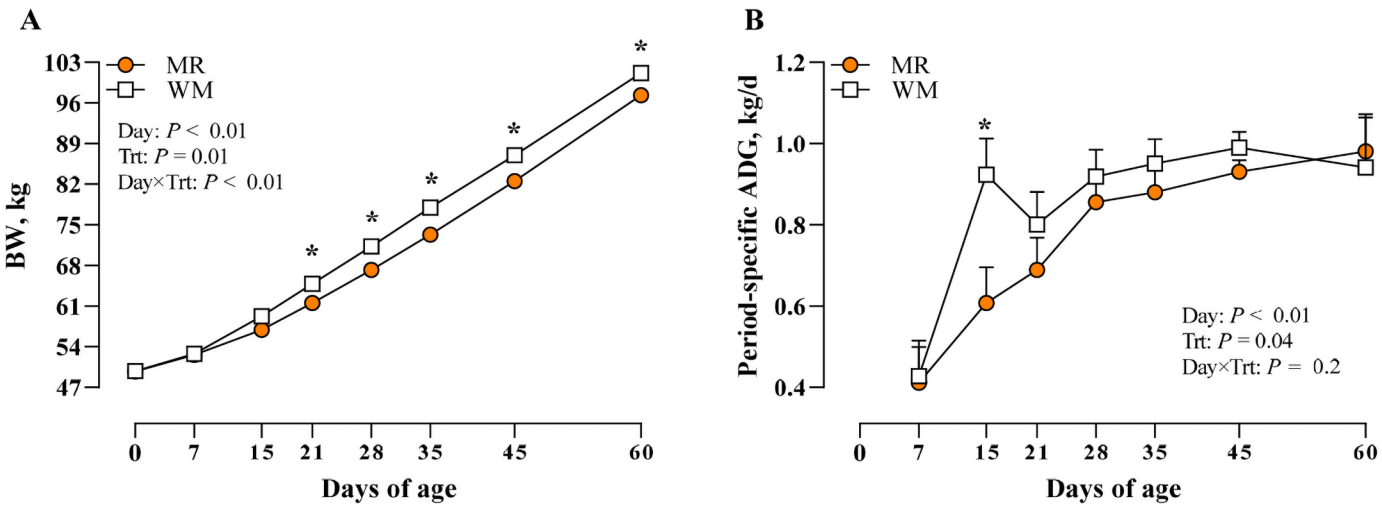

Figure 1. Trend of BW (A) and period-specific ADG (B) in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Period-specific ADG was calculated as the difference in BW between 2 consecutive time points, divided by the number of days between measurements. Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

protein and globulin concentrations were similar between groups (Trt, $P = 0.26$ and 0.31, respectively) and no differences for these parameters were observed at d 1 ($P = 0.25$ and 0.1, respectively). However, a trend for a day × treatment interaction was detected for globulin concentrations ($P = 0.06$). Overall, no differences were detected for plasma GGT (Trt, $P = 0.27$).

With regard to energy metabolism, a significant Trt × day interaction was observed for plasma glucose concentrations ($P < 0.01$), indicating temporal variations between treatments. Specifically, the WM calves had greater glucose at 7, 15, 21, 28, 35, and 60 d compared with MR calves ($P < 0.02$; Figure 2A). Furthermore, a significant Trt × day interaction was detected for plasma urea concentration ($P = 0.03$). Specifically, WM calves had greater urea concentrations than MR calves at 15, 28, and 45 d of age ($P < 0.05$; Figure 2B). A significant Trt × day interaction was detected for plasma creatinine concentrations ($P = 0.01$). Specifically, WM calves showed a marked decrease in creatinine compared with MR calves at 7 d (121.59 vs. 95.33 ± 6.940 μmol/L, MR and WM, respectively; $P < 0.01$; Figure 2C). In both groups, creatinine concentrations declined progressively with increasing age (day, $P < 0.01$).

Concerning liver functionality, a significant Trt × day interaction was observed for plasma cholesterol concentrations ($P = 0.046$). Calves receiving WM showed greater cholesterol concentrations than MR calves at 15, 21, 28, 35, 45, and 60 d ($P < 0.05$; Figure 3A). Paraoxonase activity increased over time (day, $P < 0.01$) and was affected by a significant Trt × day interaction ($P = 0.01$). Specifically, WM calves showed greater paraoxonase

Table 2. Least squares means of growth performance, intake of milk (as DM), starter, total feed, and total ME of Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9)

	Treatment			*P*-value[1]		
Item	MR	WM	SEM[2]	Trt	Day	Trt × day
BW, kg	67.66	70.52	1.193	0.01	<0.01	<0.01
Period-specific ADG, kg/d	0.77	0.85	0.09	0.04	<0.01	0.20
Milk intake, kg of DM/d	0.71	0.73	0.01			
Starter intake, kg of DM/d	0.57	0.59	0.114	0.81	<0.01	0.99
Total DMI, kg/d	1.28	1.31	0.114	0.82	<0.01	0.99
ME[3] intake from milk, Mcal/d	3.48	3.80	0.115			
ME[3] intake from starter, Mcal/d	1.41	1.47	0.283	0.81	<0.01	0.99
Total ME[3] intake, Mcal/d	4.80	5.20	0.283	0.10	<0.01	0.99

[1]*P*-values of the main effects: milk treatment (Trt), days of age (day), and their interaction (Trt × day).

[2]Greatest SEM.

[3]Metabolizable energy of starter feed, milk replacer, and whole milk was calculated based on Drackley (2008) and NASEM (2021).

Table 3. Least squares means of plasma biomarkers at 1, 7, 15, 21, 28, 35, 45, and 60 d of age of Fleckvieh calves fed milk (MR; n = 9) or whole milk (WM; n = 9)

	Treatment			*P*-value[1]		
Biomarker[2]	MR	WM	SEM[3]	Trt	Day	Trt × day
Transfer of passive immunity						
IgG at 24 h, g/L	17.78	17.32	2.73	0.75		
Total protein, g/L	64.38	62.65	2.008	0.26	0.01	0.16
Globulin, g/L	32.55	31.10	1.705	0.31	<0.01	0.06
GGT, U/L	391.82	362.73	129.08	0.27	<0.01	0.98
Energy metabolism						
Glucose, mmol/L	5.58	6.19	0.335	<0.01	<0.01	<0.01
BHB, mmol/L	0.13	0.15	0.022	0.15	<0.01	0.16
NEFA, mmol/L	0.24	0.25	0.056	0.59	<0.01	0.97
Creatinine, µmol/L	112.87	111.61	6.941	0.80	<0.01	0.01
Urea, mmol/L	3.06	3.70	0.446	0.05	<0.01	0.03
Cholesterol, mmol/L	2.79	3.37	0.249	0.01	<0.01	0.05
Liver functionality						
Albumin, g/L	31.68	31.70	0.691	0.97	<0.01	0.63
Paraoxonase, U/mL	53.69	64.57	6.05	0.10	<0.01	0.01
Alkaline phosphatase, U/L	312.37	446.00	64.072	0.01	0.03	0.14
AST, U/L	81.22	72.83	26.523	0.70	<0.01	0.92
Bilirubin, µmol/L	5.33	2.98	1.138	<0.01	<0.01	<0.01
Ceruloplasmin, µmol/L	1.67	1.84	0.214	0.35	<0.01	0.05
Inflammatory response						
Haptoglobin, g/L	0.30	0.25	0.052	0.13	0.91	0.21
Myeloperoxidase, U/L	297.31	290.72	60.483	0.89	0.01	0.91
Mineral						
Ca, mmol/L	2.67	2.78	0.069	<0.01	0.01	0.98
P, mmol/L	3.37	3.19	0.109	0.01	<0.01	0.31
K, mmol/L	5.20	5.04	0.139	0.05	0.07	0.21
Mg, mmol/L	0.89	0.89	0.026	0.83	<0.01	0.02
Na, mmol/L	145.71	145.68	1.097	0.95	0.01	0.48
Cl, mmol/L	104.91	104.18	1.059	0.17	<0.01	0.07
Zn, µmol/L	22.30	17.45	2.283	0.02	<0.01	0.04

[1]*P*-values of the main effects: milk treatment (Trt), days of age (day), and their interaction (Trt × day).

[2]NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase.

[3]Greatest SEM.

activity compared with MR calves at 35, 45, and 60 d of age ($P < 0.01$; Figure 3B). A significant Trt × day interaction was detected for bilirubin concentrations ($P < 0.01$). Although WM calves started with higher bilirubin values at 1 d, they displayed markedly lower concentrations than MR calves between 7 and 45 d of age ($P < 0.01$; Figure 4A). Ceruloplasmin was affected by the interaction of Trt × day effect ($P = 0.05$). Indeed, WM calves had greater ceruloplasmin levels at 15, 21, and 28 d of age compared with MR calves ($P < 0.01$; Figure 4B). Haptoglobin was not affected by Trt, day, or the interaction of Trt × day ($P > 0.10$). A significant Trt × day interaction was observed for plasma Zn concentration ($P = 0.04$). A lower Zn concentration was observed in WM calves than in MR calves at 15, 21, 28, 35, and 45 d of age ($P < 0.05$; Figure 5A). Additionally, alkaline phosphatase activity was overall greater in the WM than MR group (446 U/L and 312.37 ± 64.072 U/L, respectively; $P = 0.01$; Table 3).

Regarding the mineral status of calves, compared with the MR calves, the WM group had overall greater concentration of calcium (2.67 vs. 2.78 ± 0.069 mmol/L, MR and WM, respectively; $P < 0.01$), but lower phosphorus (3.37 vs. 3.19 ± 0.109 mmol/L, MR and WM, respectively; $P = 0.01$, respectively) and lower potassium levels (5.2 vs. 5.04 ± 0.139 mmol/L, MR and WM, respectively; $P = 0.05$). Further, Mg was affected by the interaction of Trt × day ($P = 0.02$), where compared with MR calves, the WM calves had greater concentrations at 60 d (0.91 vs. 0.97 ± 0.026 mmol/L, MR and WM, respectively; $P = 0.05$; Figure 5B).

Hematology Parameters

Hematology parameters are presented in Table 4. The total circulating leukocyte count was affected by the interaction Trt × day ($P = 0.01$; Table 4). At 21 and 28 d of age, compared with MR calves, WM calves had higher total leukocyte counts ($P < 0.01$; Figure 6A). The latter was as consequence of greater circulating neutrophil counts at 21 and 28 d of age in WM than MR calves ($P < 0.01$; Figure 6B) highlighted by the interaction Trt × day (P 0.02). A significant Trt × day interaction was observed

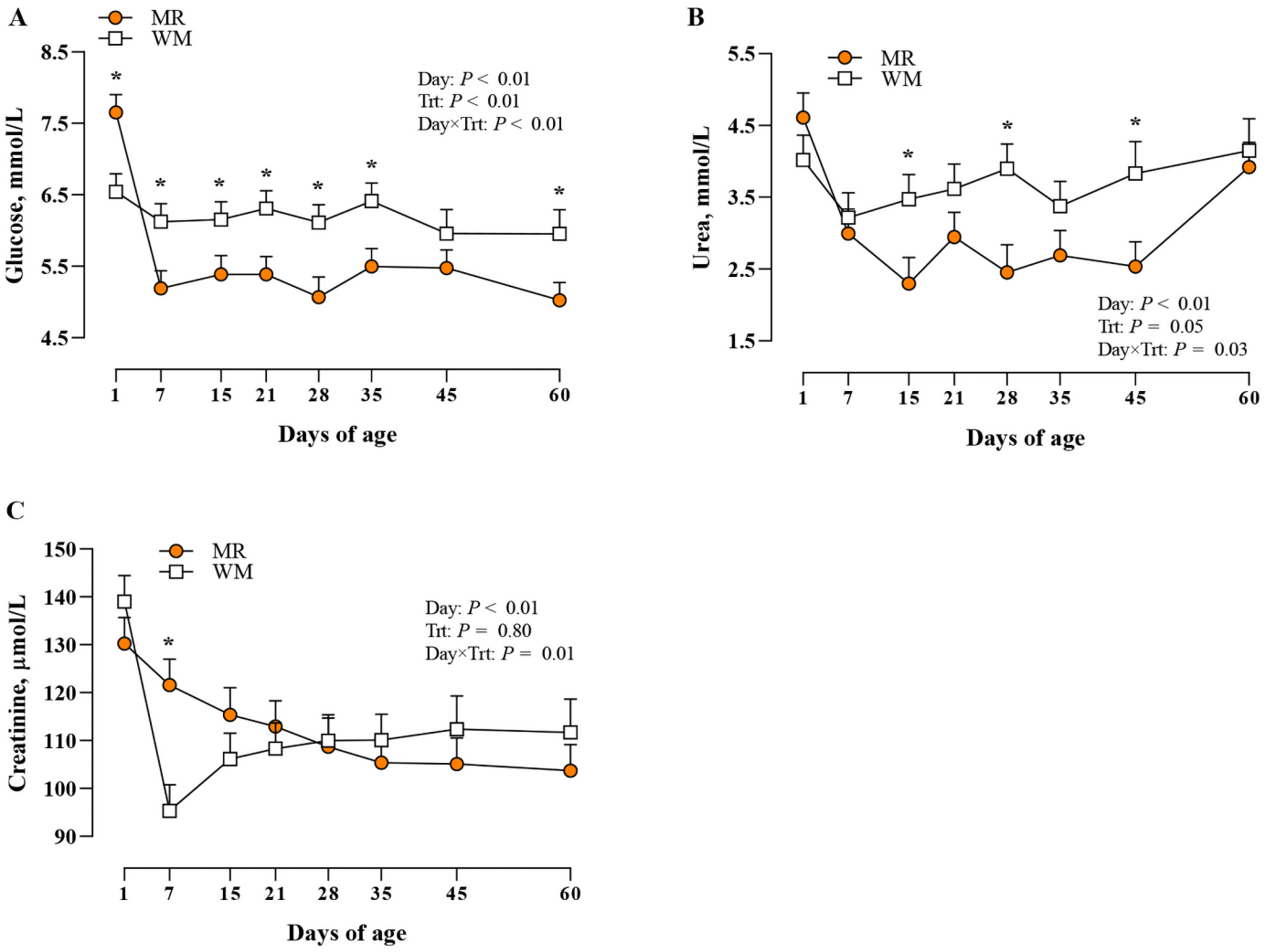

Figure 2. Trend of plasma glucose (A), urea (B), and creatinine (C) concentrations in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

for both neutrophil ($P = 0.04$) and lymphocyte ($P = 0.01$) proportions. At 21 and 28 d, WM calves had greater proportions of neutrophils compared with MR calves ($P < 0.05$; Figure 7A), whereas at the same ages they showed lower proportions of lymphocytes ($P < 0.05$; Figure 7B). In addition, WM calves also had lower lymphocyte proportions at weaning (60 d; $P < 0.01$; Figure 7B).

A significant Trt × day interaction was detected for MCV ($P = 0.02$) and RDW ($P < 0.01$). From 15 d of age to weaning, WM calves had lower MCV values and greater RDW compared with MR calves ($P < 0.01$; Figures 8A and B). Overall MPV and PLT were lower and greater, respectively, in WM compared with MR calves (Trt, $P = 0.01$ and 0.04; Table 4). In addition, as highlighted by the interaction Trt × day ($P < 0.01$), WM calves had lower HCT proportions at 28 and 35 d of age compared with MR calves ($P < 0.05$; Figure 8C). A significant Trt × day interaction was observed for both MCH ($P < 0.01$) and MCHC ($P < 0.01$). Calves receiving WM had lower MCH from 7 to 28 d ($P < 0.05$; Figure 9A) and lower MCHC from 7 to 21 d of age compared with MR calves ($P < 0.05$; Figure 9B).

DISCUSSION

Optimizing nutritional strategies during early life can enhance calf health and growth, with lasting effects on lactational performance and dairy system sustainability (Leal et al., 2025). In this study we evaluated the effects of feeding either WM or MR on growth performance,

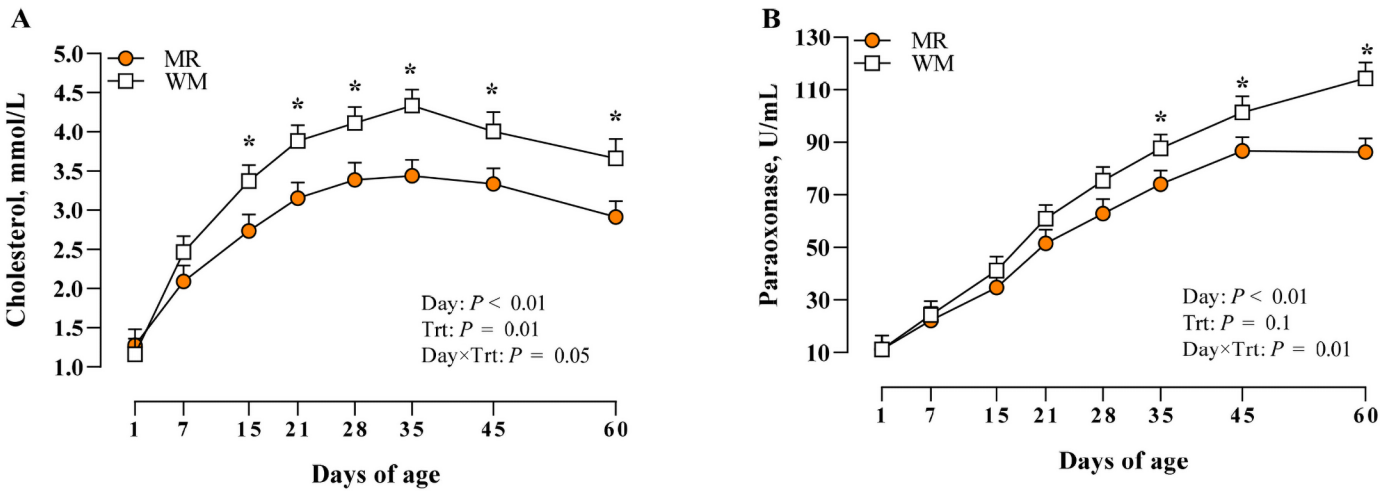

Figure 3. Trend of plasma cholesterol (A) and paraoxonase (B) levels in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

metabolic variables, and hematological indicators from birth to 60 d of age in Fleckvieh dairy calves. The rationale behind this study was to design the MR treatment to closely match the DM content of WM, the traditional liquid feed for calves before MR formulations became widespread in the 1950s; despite MR now accounting for about half of the global market, WM feeding remains common on small farms (Kertz et al., 2017; Chapelain et al., 2025). The MR was adjusted to a volume of 6 L/d, maintaining the same proportion of TS as in WM, which is traditionally fed at this volume from 2 to 3 d of age until 7 to 10 d before weaning, at which point the milk allowance is typically reduced by half.

Effect on Feed Intake and Growth Performance

The similar DMI between the 2 groups was in line with those of Lee et al. (2009) who reported that a similar intake of calf starter by calves fed either WM or MR during the preweaning phase. In our study, calf starter was available ad libitum, but intake did not differ between treatments.

The better growth performance observed in the WM calves supported by BW from d 21 onward, by period-specific ADG from d 7 to 15, aligns with several earlier studies highlighting the nutritional and functional advantages of WM over MR (Murdock et al., 1961; Lee

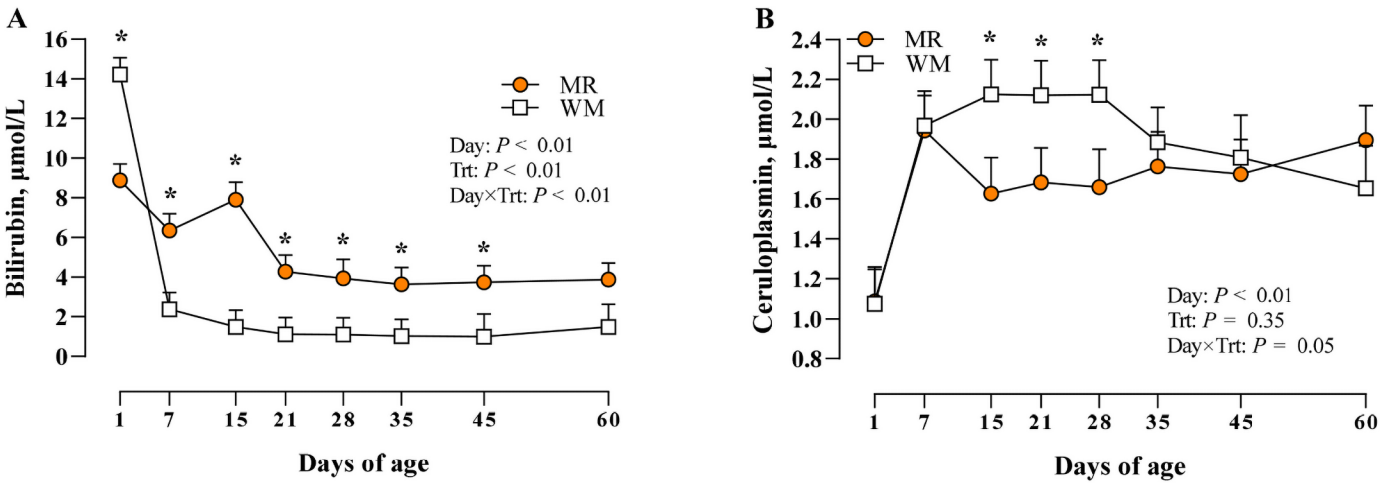

Figure 4. Trend of plasma bilirubin (A) and ceruloplasmin (B) levels in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

Table 4. Least squares means of hematology parameters at 7, 15, 21, 28, 35, 45, and 60 d of age of Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9)

	Treatment			*P*-value[1]		
Parameter[2]	MR	WM	SEM[3]	Trt	Day	Trt × day
Total leukocytes, $\times 10^9$ cells/L	10.11	12.04	1.143	0.14	<0.01	0.01
Neutrophils, $\times 10^9$ cells/L	3.61	4.35	0.731	0.25	<0.01	0.02
Lymphocytes, $\times 10^9$ cells/L	5.50	6.12	0.714	0.33	0.84	0.56
Monocytes, $\times 10^9$ cells/L	0.74	0.83	0.129	0.34	0.24	0.51
Neutrophils, %	33.16	35.50	3.634	0.30	<0.01	0.04
Lymphocytes, %	56.01	51.21	4.174	0.02	0.05	0.01
Monocytes, %	7.33	7.08	1.004	0.69	0.08	0.78
RBC, $\times 10^{12}$ cells/L	9.37	9.44	0.445	0.90	<0.01	0.07
HCT, %	35.58	33.07	1.663	0.22	<0.01	<0.01
HGB, g/dL	10.74	9.01	0.981	0.12	0.76	0.42
MCV, fL	38.12	35.26	0.901	0.03	<0.01	0.02
RDW, %	20.18	22.73	0.733	0.01	<0.01	<0.01
PLT, $\times 10^9$ cells/L	528.10	635.14	53.368	0.04	<0.01	0.30
MPV, fL	6.85	6.18	0.459	0.01	0.63	0.61
MCH, pg	11.56	9.50	0.935	0.08	<0.01	<0.01
MCHC, g/dL	31.60	27.90	2.463	0.08	<0.01	<0.01

[1]*P*-values of the main effects: milk treatment (Trt), days of age (day), and their interaction (Trt × day).

[2]RBC = red blood cells; HCT = hematocrit; HGB = hemoglobin; MCV = mean corpuscular volume; RDW = red blood cell distribution width; PLT = platelet count; MPV = mean platelet volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[3]Greatest SEM.

et al., 2009; Moallem et al., 2010). However, results across studies are not consistent: Some have reported advantages for MR feeding during the preweaning phase (Hill et al., 2009; Chapelain et al., 2025), whereas others found no significant overall differences between MR and WM (Sharpe and Heins, 2021). Interestingly, in our study, although the nutrient composition of the 2 liquid diets differed, with the WM group receiving slightly greater energy levels, the divergence in ADG emerged early (d 7–15), leading to different BW between groups and then remained stable over time. Based on these results, it is evident that the 2 groups began to diverge at 15 d of age, with calves receiving WM showing higher ADG than MR calves. This advantage persisted through 28 d and then continued with the same trend up to 60 d of age. The key point is that the difference established at 15 d allowed the WM calves to maintain their advantage during the subsequent period. If the growth differences were solely due to nutritional content, a progressively widening gap in BW would be expected as the trial ad-

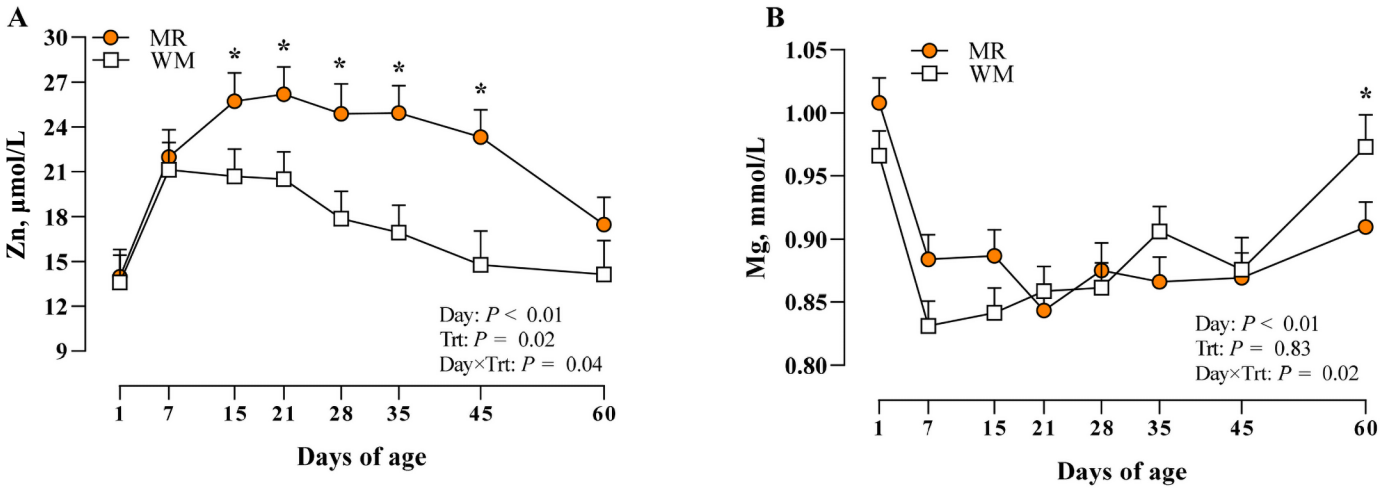

Figure 5. Trend of plasma mineral Zn (A) and Mg (B) concentrations in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

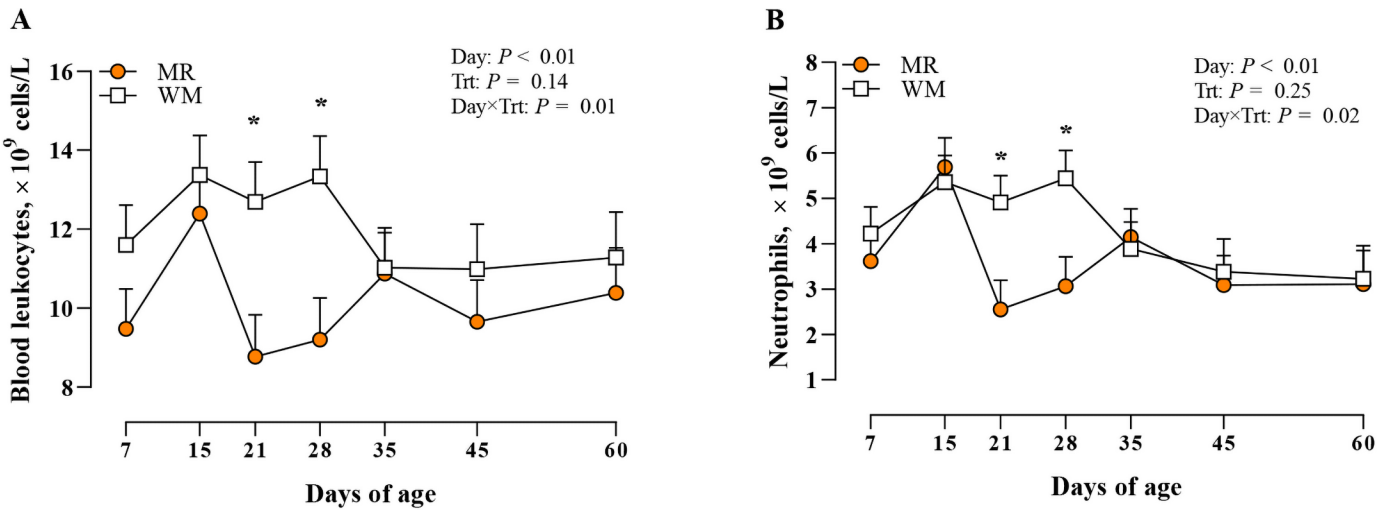

Figure 6. Trend of blood circulating total leukocytes (A) and neutrophil (B) cells in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

vanced. Instead, the persistence of a constant difference in BW suggests that the effects of the feeding treatment were mostly exerted during a specific window in early life. This supports the hypothesis that other mechanisms, potentially related to early metabolic programming or gastrointestinal development, may have contributed to the observed differences in growth, especially when calves must to overcome the critical transition phase between the end of passive immunity and the onset of acquired immunity.

The differences in growth performance outcomes may be attributed not only to the different nutrient composition of the liquid diets, but also to non-nutritional bioactive components such as enzymes and hormones. The WM contains peptides that provide essential nutrients, immunological protection, and biologically active compounds with antioxidant, anticarcinogenic, and therapeutic properties (Séverin and Wenshui, 2005; Dhar et al., 2024). In this context, in our study, the MR (with approximately only 50% skim milk powder) contained lower casein levels compared with WM. This lower casein content may have affected chymosin secretion and abomasal clotting, leading to faster nutrient flow and altered digestibility, in contrast to the slower flow and

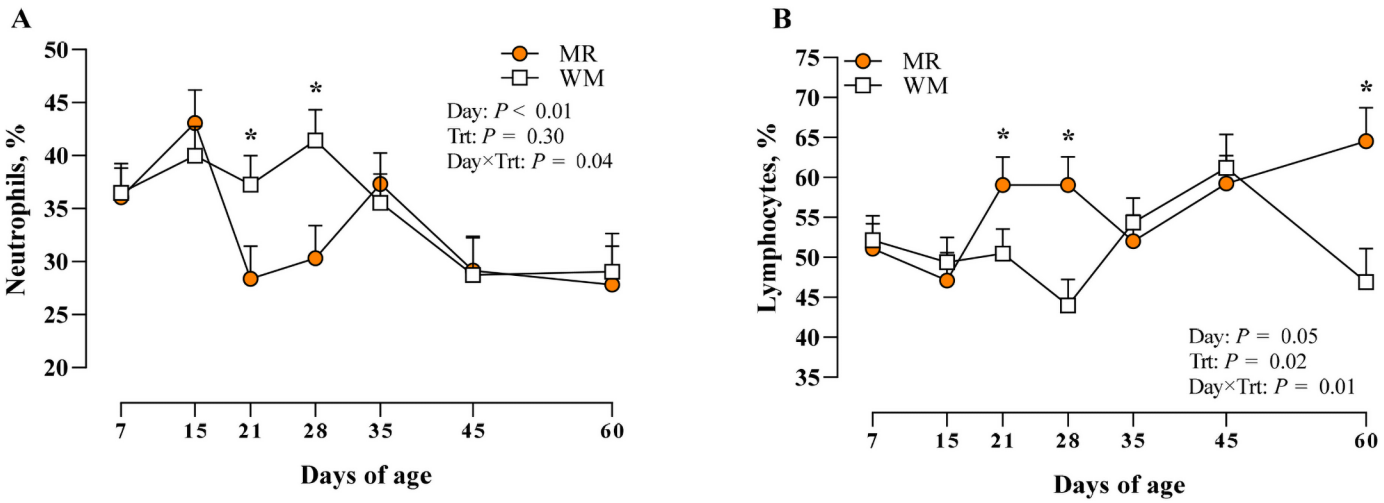

Figure 7. Trend of blood percentage of circulating neutrophil (A) and lymphocyte (B) cells in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

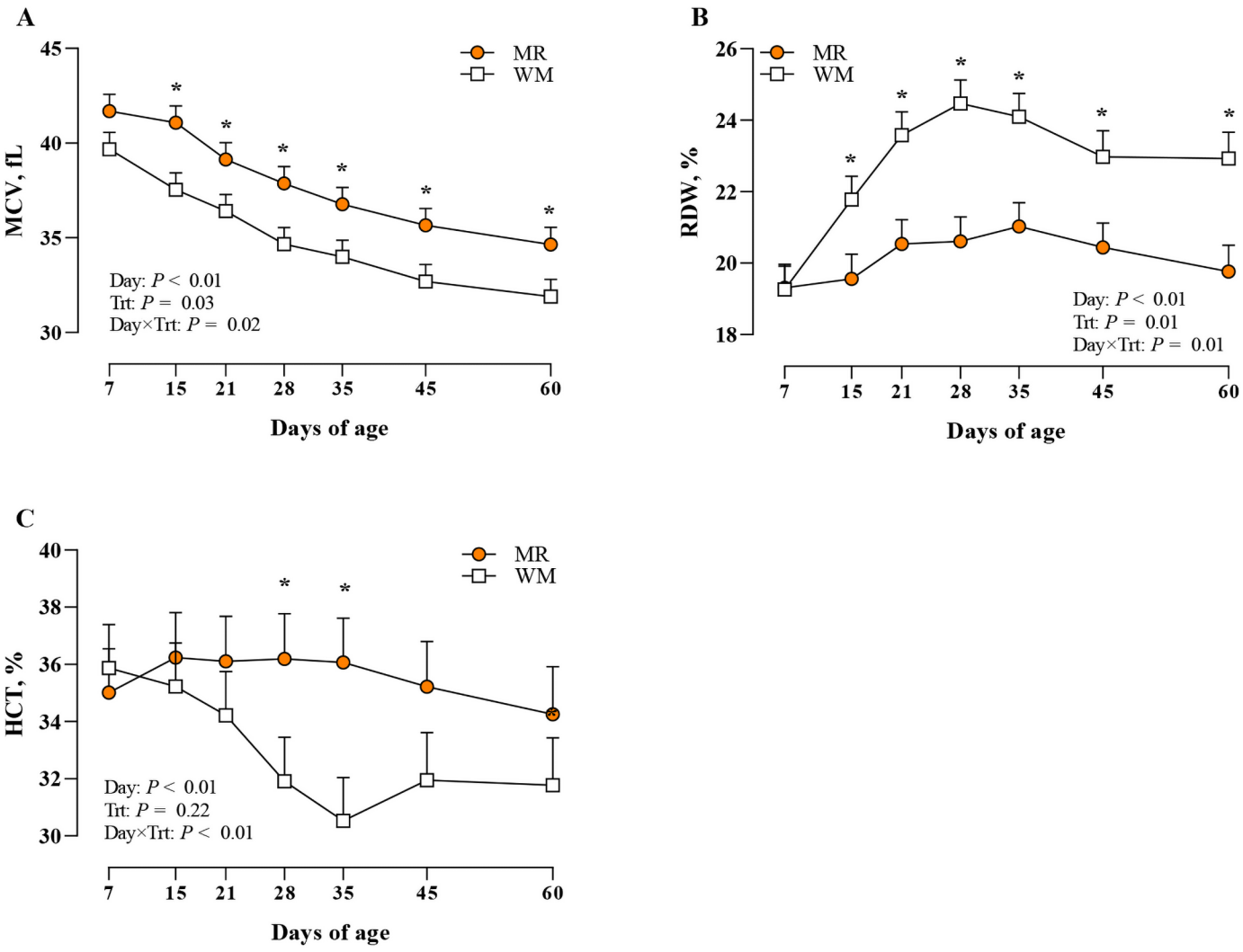

Figure 8. Trend of mean corpuscular volume (MCV; A), red blood cell distribution width (RDW; B), and hematocrit (HCT; C) in Fleckvieh calves fed milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

improved feed efficiency typically observed with WM (Garnot et al., 1977; Caugant et al., 1992; Lammers et al., 1998; Wilms et al., 2024). In the first weeks of life, calves have limited digestive enzymatic activity and benefit from clotting in the abomasum for optimal protein digestion. After 3 wk, they can digest both clotting and nonclotting MR similarly, which often include whey and soy proteins (Longenbach and Heinrichs, 1998; Grobler, 2008). In addition, the higher fat content of WM may also have contributed to the slower abomasal emptying observed, as dietary fat is known to stimulate cholecystokinin release and delay gastric transit (Bell and McLeay, 1978; Welboren et al., 2021).

The poorer growth performance often observed in MR-fed calves has been attributed to heat-damaged protein sources (Lynch et al., 1978), limited AA availability (Kanjanapruthipong, 1998) and protein denaturation during processing steps such as homogenization and drying, which can disrupt fat globule membranes and damage bioactive components, as indicated by the lower nitrogen index of whey proteins (Wilms et al., 2022). In addition, the inclusion of plant proteins in MR, such as soy and wheat protein concentrates, may limit the supply of indispensable AA and reduce apparent protein digestibility (Montagne et al., 2001, 2003; Lee et al., 2009). As demonstrated in several studies, the preweaning ADG has the capacity to influence the phenotypic expression of milk yield (Soberon et al., 2012; Soberon and Van Amburgh, 2013; Leal et al., 2025). Therefore, improving the quality and digestibility of MR protein sources remains essential not only for supporting early growth but also for optimizing long-term productivity.

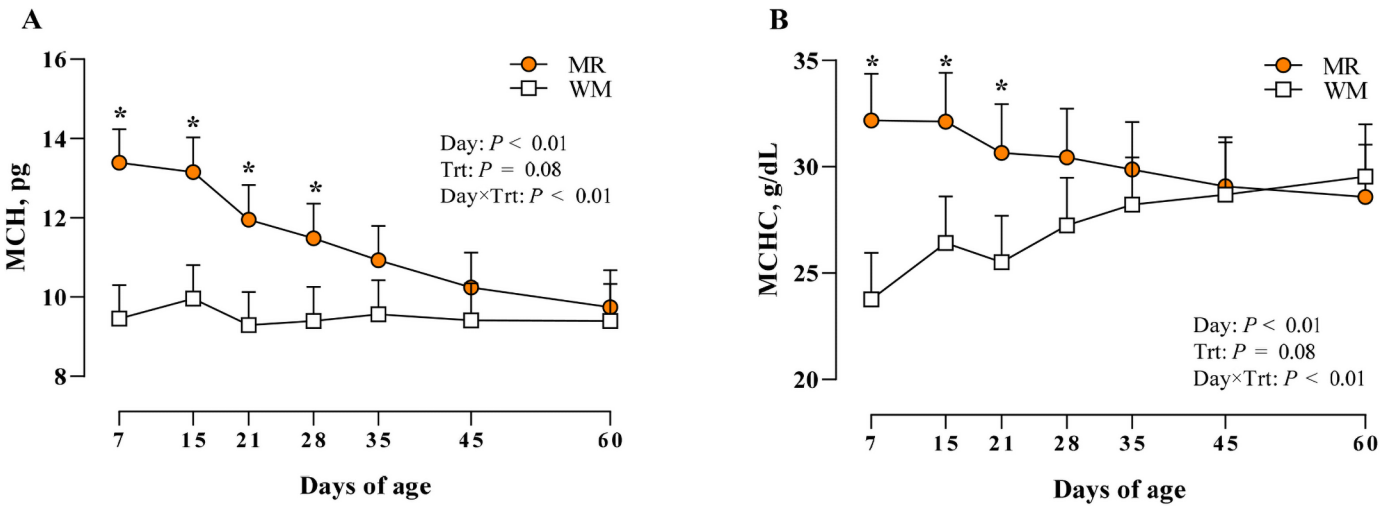

Figure 9. Trend of mean corpuscular hemoglobin (MCH; A) and mean corpuscular hemoglobin concentration (MCHC; B) in Fleckvieh calves milk replacer (MR; n = 9) or whole milk (WM; n = 9). Data are presented as LSM and error bars represent SEM. *Differences between groups within each time point ($P \leq 0.05$) are denoted with an asterisk. Trt = treatment.

Effect on Hematological and Immune-Related Parameters

As passive immunity acquired through colostrum wanes, the calf's immune system experiences a period of vulnerability. Many components of the immune system, including complement activity and the function of neutrophils, macrophages, and B cells, remain immature until at least 2 to 4 wk of age and continue to develop until puberty (Reber et al., 2006; Chase et al., 2008). As indicated by the IgG concentrations measured 24 h after birth, both groups exhibited comparable levels of passive immunity transfer. Given this similar initial status of passive immunity, any subsequent differences in immune parameters are likely attributable to postnatal factors, such as diet composition and nutrient bioavailability. At 21 and 28 d, the WM-fed calves exhibited leukocytosis, characterized by an increase in neutrophil cell count that lead to a lower percentage in lymphocytes. The increase of the latter could be supported by the stimulation effects played by milk proteins (α-, β-, and κ-casein fractions and whey proteins; Bogucki et al., 2023). As passive immunity provided by colostrum decreases, the animal's immune system undergoes stress (Chase et al., 2008). From 7 to 15 d, WM-fed calves also had a greater period-specific ADG, which could be linked to the enhanced bioavailability (digestion and utilization) of nutrients (protein and energy) along with other known (minerals, vitamins, enzymes, and hormones) and unknown growth factors (Blum and Baumrucker, 2002; Lee et al., 2009). This nutritional advantage likely helped WM calves better cope with the physiological stress associated with the decline of passive immunity, contributing the observed differences in growth performance during this critical window.

Effect on Metabolism

Plasma glucose concentrations are often positively correlated with ADG (Chandler et al., 1968; Kitchenham et al., 1975; Lammers et al., 1998). Glucose, produced endogenously through gluconeogenesis or derived from the hydrolysis of lactose in milk (Pantophlet, 2018), represents a key energy source for neonatal calves. As previously discussed, the dynamics of clot formation can influence gastric emptying; accordingly, the passage rate of lactose-containing liquid into the duodenum is also affected, potentially affecting nutrient absorption (Radostits and Bell, 1970). In casein-rich diets such as WM, the liquid phase leaves the abomasum rapidly, resulting in an earlier glucose appearance in plasma, whereas the curd formed by casein and fat is digested more slowly, sustaining nutrient release over time (Wilms et al., 2024). This combination of an early peak followed by a prolonged absorption phase may explain why WM-fed calves in our study still had higher glucose concentrations before the morning meal. Insulin dynamics also contribute to these patterns, as MR-fed calves, because of their higher lactose intake, typically show greater postprandial glucose and insulin peaks, followed by a faster return to baseline (Wilms et al., 2024). Further studies with detailed postprandial sampling are warranted to confirm this interpretation.

Urea is generated when AA are broken down in the body and is affected by the diet (Wilms et al., 2022). In this regard, the greater plasma urea in WM calves at 15,

28, and 45 d was likely the result of higher protein intake in that group.

Regarding liver activity, the greater concentration of alkaline phosphatase (**ALP**) in the WM group contrasts previous reports (Wang et al., 2022; Chapelain et al., 2025). This enzyme serves as a marker of bone proliferation, as an indicator of liver function (Sato et al., 2005), and its elevated level in WM calves may have been associated with the higher growth rate observed in these calves. However, a more plausible explanation is that the differences in ALP levels were not due to variations in liver function or greater growth, but rather to the uptake of ALP from the unpasteurized WM fed to WM calves.

Paraoxonase, an acute-phase protein (**APP**; Feingold et al., 1998), has a strong association with lipid metabolism (Turk et al., 2004). Its levels increased over time, reflecting the immaturity of the liver at birth, physiological maturation, and immune stimulation (Inanami et al., 1999; Orro et al., 2008). The higher concentrations observed in the WM group at 35, 45, and 60 d may be partly explained by a greater fat intake, as paraoxonase is associated with high-density lipoproteins and plays a key role in lipid transport and antioxidant defense (Bionaz et al., 2007). However, the continued increase in paraoxonase levels from d 45 to 60, despite a concurrent decrease in cholesterol levels, may also suggest a more efficient hepatic development in WM calves, indicating that the rise in paraoxonase is not solely due to dietary fat intake. Among other APP, variations in haptoglobin were detected at 7 and 60 d, with the MR group displaying greater levels. From 15 to 28 d, the WM group had greater ceruloplasmin concentrations. These findings align with Chapelain et al. (2025), with all values remaining within the healthy range (Chapelain et al., 2025). However, further studies are needed to determine whether the elevated ceruloplasmin levels are due to increased absorption from WM or to improved hepatic development. Notably, ceruloplasmin should not be interpreted as an inflammatory marker in this context, as the available data do not support this role in calves (especially considering that other markers such as haptoglobin, bilirubin, and ROM weaken the inflammatory interpretation). The greater bilirubin concentration in the MR group could be attributed to a greater turnover of hemoglobin, whereby globin is recycled into AA and the heme portion is degraded to bilirubin, ultimately resulting in increased bilirubin production compared with WM-fed calves (Chapelain et al., 2025).

The progressive increase in blood cholesterol levels over time, particularly in calves fed WM, align with findings reported in the literature (Hammon and Blum, 1998; Lepczyński et al., 2015; Chapelain et al., 2025), suggesting a potential correlation with increased fat intake. The greater plasma cholesterol concentrations in WM calves from 15 to 60 d likely reflect greater dietary fat intake and may support increased bile acid synthesis, given that cholesterol is the primary precursor. This could enhance lipid digestion. Simultaneously, the lower plasma bilirubin concentrations observed in the same group may indicate more efficient hepatic uptake and biliary excretion of bilirubin, rather than reduced hemoglobin turnover or hepatic dysfunction.

The greater values of MCV, CHCM, MCH, MCHC, hematocrit (%), and lymphocyte (%) observed in calves fed MR may have reflected physiological adaptations to differences in the nutritional composition and bioavailability of key micronutrients. The MCV decrease reflected the replacement of RBC containing fetal hemoglobin with smaller RBC containing hemoglobin (Jain, 1986; Brun-Hansen et al., 2006). Although WM had the lowest MCV, it was compensated by the greater RBC number to maintain a normal HGB. The higher MCV in MR calves suggests an expansion in erythrocyte size, which may be indicative of altered erythropoiesis, potentially driven by differences in iron intake between MR and WM diets (Chapelain et al., 2025). Differences in erythrocyte parameters should also be interpreted considering that MR are largely supplemented with iron to historically prevent anemia, which results in greater HGB and HCT (Chapelain et al., 2025). The elevated CHCM, MCH, and MCHC values in MR-fed calves further support the hypothesis that there was a greater intracellular HGB, which may have been influenced by variations in plasma volume, hydration status, or erythrocyte turnover (Sarma, 1990). However, the lower hematocrit observed in MR-fed calves compared with those receiving WM does not suggest hemoconcentration. Indeed, HCT is positively associated with dehydration in calves (Westhoff et al., 2024), indicating that MR-fed calves in our study were likely less affected by dehydration.

CONCLUSIONS

The present study examined the impact of feeding WM versus MR from birth to 60 d of age on the growth performance of Fleckvieh calves. Despite no alteration in DMI, the results showed that feeding WM significantly improved growth performance, as evidenced by greater ADG from 7 to 15 d leading to greater BW compared with calves fed MR. This pattern likely reflects differences in nutrient bioavailability and utilization between WM and MR, which may have influenced the metabolic profile and immune-related markers, especially after passive immunity waned. Furthermore, the WM group also exhibited enhanced immune function markers, indicated by leukocytes and increased neutrophil counts. Metabolically, WM-fed calves had greater glucose and cholesterol levels, reflecting greater energy and fat intake, together

with markers reflecting greater hepatic efficiency. Conversely, MR-fed calves exhibited alterations in hematological parameters, most likely reflecting greater iron content typically present in MR. Further research is needed to explore the long-term implications of WM feeding on calf development, especially in relation to subsequent lactation outcomes.

NOTES

The study was funded by the Romeo ed Enrica Invernizzi Foundation (Milan, Italy) and supported by the Doctoral School on the Agro-Food System (Agrisystem) of the Università Cattolica del Sacro Cuore (Piacenza, Italy). The experimental protocol was approved by Ethical Animal Care and Use Committee of the Magna Graecia University of Catanzaro (protocol no. 271/2017). The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ALP = alkaline phosphatase; APP = acute-phase protein; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; HCT = hematocrit value; HGB = hemoglobin concentration; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; MPO = myeloperoxidase; MR = milk replacer; NEFA = nonesterified fatty acids; PLT = platelet count; PON = paraoxonase; RBC = red blood cell count; RDW = red blood cell distribution width; ROM = reactive oxygen metabolites; STP = total serum protein; Trt = treatment; WM = whole milk.

REFERENCES

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

Amato, A., A. Minuti, L. Liotta, L. Cattaneo, M. Sfulcini, E. Trevisi, and V. Lopreiato. 2023. Whole cottonseed inclusion in starter feeds improves performance, inflammometabolic profile, and rumination behavior in Holstein dairy calves. JDS Commun. 4:422–427. https://doi.org/10.3168/jdsc.2022-0368.

AOAC International. 2000. Official Methods of Analysis. AOAC International, Rockville, MD.

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.

Bell, F. R., and L. M. McLeay. 1978. The effect of duodenal infusion of milk, casein, lactose and fat on gastric emptying and acid secretion in the milk-fed calf. J. Physiol. 282:51–57. https://doi.org/10.1113/jphysiol.1978.sp012447.

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

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90:1740–1750. https://doi.org/10.3168/jds.2006-445.

Blum, J. W., and C. R. Baumrucker. 2002. Colostral and milk insulin-like growth factors and related substances: Mammary gland and neonatal (intestinal and systemic) targets. Domest. Anim. Endocrinol. 23:101–110. https://doi.org/10.1016/S0739-7240(02)00149-2.

Bogucki, M., M. Bogusławska-Tryk, M. Stanek, I. Kuropatwí Nska, and J. Bujko. 2023. Effect of milk replacer on calf rearing performance and hematological and biochemical blood indices. Acta Sci. Pol. Zootech. 22:33–40. https://doi.org/10.21005/asp.2023.22.1.04.

Brun-Hansen, H. C., A. H. Kampen, and A. Lund. 2006. Hematologic values in calves during the first 6 months of life. Vet. Clin. Pathol. 35:182–187. https://doi.org/10.1111/j.1939-165X.2006.tb00111.x.

Caugant, I., H. V. Petit, R. Charbonneau, L. Saovie, R. Toullec, S. Thirouin, and M. Yvon. 1992. In vivo and in vitro gastric emptying of protein fractions of milk replacers containing whey proteins. J. Dairy Sci. 75:847–856. https://doi.org/10.3168/jds.S0022-0302(92)77824-2.

Chalfant, C. E., Z. Szulc, P. Roddy, A. Bielawska, and Y. A. Hannun. 2004. The structural requirements for ceramide activation of serine-threonine protein phosphatases. J. Lipid Res. 45:496–506. https://doi.org/10.1194/jlr.M300347-JLR200.

Chandler, P. T., R. D. McCarthy, and E. M. Kesler. 1968. Effect of dietary lipid and protein on serum proteins, lipids and glucose in the blood of dairy calves. J. Nutr. 95:461–468. https://doi.org/10.1093/jn/95.3.461.

Chapelain, T., J. N. Wilms, J. Martín-Tereso, L. N. Leal, and J. B. Daniel. 2025. Performance, nutrient digestibilities, and metabolic profiles of Holstein calves fed whole milk or milk replacer at 2 feeding levels. J. Dairy Sci. 108:2422–2444. https://doi.org/10.3168/jds.2024-25556.

Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. North Am. Food Anim. Pract. 24:87–104. https://doi.org/10.1016/j.cvfa.2007.11.001.

Delplanque, B., R. Gibson, B. Koletzko, A. Lapillonne, and B. Strandvik. 2015. Lipid quality in infant nutrition. J. Pediatr. Gastroenterol. Nutr. 61:8–17. https://doi.org/10.1097/MPG.0000000000000818.

Dhar, H., S. Verma, S. Dogra, S. Katoch, R. Vij, G. Singh, and M. Sharma. 2024. Functional attributes of bioactive peptides of bovine milk origin and application of in silico approaches for peptide prediction and functional annotations. Crit. Rev. Food Sci. Nutr. 64:9432–9454. https://doi.org/10.1080/10408398.2023.2212803.

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.

Echeverry-Munera, J., L. N. Leal, J. N. Wilms, H. Berends, J. H. C. Costa, M. Steele, and J. Martín-Tereso. 2021. Effect of partial exchange of lactose with fat in milk replacer on ad libitum feed intake and performance in dairy calves. J. Dairy Sci. 104:5432–5444. https://doi.org/10.3168/jds.2020-19485.

Faul, F., E. Erdfelder, A.-G. Lang, and A. Buchner. 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39:175–191. https://doi.org/10.3758/BF03193146.

Feingold, K. R., R. A. Memon, A. H. Moser, and C. Grunfeld. 1998. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis 139:307–315. https://doi.org/10.1016/S0021-9150(98)00084-7.

Fischer, A. J., C. Villot, J. K. van Niekerk, T. T. Yohe, D. L. Renaud, and M. A. Steele. 2019. Invited Review: Nutritional regulation of gut function in dairy calves: From colostrum to weaning. Appl. Anim. Sci. 35:498–510. https://doi.org/10.15232/aas.2019-01887.

Garnot, P., R. Toullec, J. L. Thapon, P. Martin, M. T. Hoang, C. M. Mathieu, and B. R. Dumas. 1977. Influence of age, dietary protein and weaning on calf abomasal enzymic secretion. J. Dairy Res. 44:9–23. https://doi.org/10.1017/S0022029900019890.

Gilbert, M. S., A. J. Pantophlet, J. J. G. C. van den Borne, W. H. Hendriks, H. A. Schols, and W. J. J. Gerrits. 2016. Effects of replacing lactose from milk replacer by glucose, fructose, or glycerol on en-

ergy partitioning in veal calves. J. Dairy Sci. 99:1121–1132. https://doi.org/10.3168/jds.2015-10062.
Grobler, S. M. 2008. Growth performance of Holstein calves fed milk or milk replacer with or without calf starter. MS thesis. Animal Nutrition, University of Pretoria, Pretoria, South Africa.
Grote, V., E. Verduci, S. Scaglioni, F. Vecchi, G. Contarini, M. Giovannini, B. Koletzko, and C. Agostoni. 2016. Breast milk composition and infant nutrient intakes during the first 12 months of life. Eur. J. Clin. Nutr. 70:250–256. https://doi.org/10.1038/ejcn.2015.162.
Hammon, H. M., and J. W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J. Nutr. 128:624–632. https://doi.org/10.1093/jn/128.3.624.
Heinrichs, A. J., and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. J. Dairy Sci. 94:336–341. https://doi.org/10.3168/jds.2010-3170.
Hill, S. R., K. F. Knowlton, K. M. Daniels, R. E. James, R. E. Pearson, A. V. Capuco, and R. M. Akers. 2008. Effects of milk replacer composition on growth, body composition, and nutrient excretion in preweaned Holstein heifers. J. Dairy Sci. 91:3145–3155. https://doi.org/10.3168/jds.2007-0860.
Hill, T. M., H. G. Bateman II, J. M. Aldrich, and R. L. Schlotterbeck. 2009. Effect of consistency of nutrient intake from milk and milk replacer on dairy calf performance. Prof. Anim. Sci. 25:85–92. https://doi.org/10.15232/S1080-7446(15)30679-3.
Inanami, O., A. Shiga, K. Okada, R. Sato, Y. I. Miyake, and M. Kuwabara. 1999. Lipid peroxides and antioxidants in serum of neonatal calves. Am. J. Vet. Res. 60:452–457. https://doi.org/10.2460/ajvr.1999.60.04.452.
Jain, N. 1986. Schalm's Veterinary Hematology. Lea & Febiger, Philadelphia, PA.
Jasper, J., and D. M. Weary. 2002. Effects of ad libitum milk intake on dairy calves. J. Dairy Sci. 85:3054–3058. https://doi.org/10.3168/jds.S0022-0302(02)74391-9.
Johnson, K. F., N. Chancellor, C. C. Burn, and D. C. Wathes. 2018. Analysis of pre-weaning feeding policies and other risk factors influencing growth rates in calves on 11 commercial dairy farms. Animal 12:1413–1423. https://doi.org/10.1017/S1751731117003160.
Kanjanapruthipong, J. 1998. Supplementation of milk replacers containing soy protein with threonine, methionine, and lysine in the diets of calves. J. Dairy Sci. 81:2912–2915. https://doi.org/10.3168/jds.S0022-0302(98)75852-7.
Keenan, T. W., and C. M. Huang. 1972. Membranes of mammary gland. VI. Lipid and protein composition of Golgi apparatus and rough endoplasmic reticulum from bovine mammary gland. J. Dairy Sci. 55:1586–1596. https://doi.org/10.3168/jds.S0022-0302(72)85725-4.
Kertz, A. F., T. M. Hill, J. D. Quigley III, A. J. Heinrichs, J. G. Linn, and J. K. Drackley. 2017. A 100-Year Review: Calf nutrition and management. J. Dairy Sci. 100:10151–10172. https://doi.org/10.3168/jds.2017-13062.
Kitchenham, B. A., G. J. Rowlands, R. Manston, and S. M. Dew. 1975. The blood composition of dairy calves reared under conventional and rapid-growth systems. Br. Vet. J. 131:436–446. https://doi.org/10.1016/S0007-1935(17)35239-9.
Lammers, B. P., A. J. Heinrichs, and A. Aydin. 1998. The effect of whey protein concentrate or dried skim milk in milk replacer on calf performance and blood metabolites. J. Dairy Sci. 81:1940–1945. https://doi.org/10.3168/jds.S0022-0302(98)75767-4.
Leal, L. N., J. B. Daniel, J. Doelman, B. R. Keppler, M. A. Steele, and J. Martín-Tereso. 2025. Preweaning nutrient supply improves lactation productivity and reduces the risk of culling in Holstein cows. J. Dairy Sci. 108:5875–5888. https://doi.org/10.3168/jds.2024-26102.
Lee, H. J., M. A. Khan, W. S. Lee, S. H. Yang, S. B. Kim, K. S. Ki, H. S. Kim, J. K. Ha, and Y. J. Choi. 2009. Influence of equalizing the gross composition of milk replacer to that of whole milk on the performance of Holstein calves. J. Anim. Sci. 87:1129–1137. https://doi.org/10.2527/jas.2008-1110.
Lepczyński, A., A. Herosimczyk, M. Ożgo, and W. F. Skrzypczak. 2015. Feeding milk replacer instead of whole milk affects blood plasma proteome and lipid profile in preruminant calves. Pol. J. Vet. Sci. 18:91–99. https://doi.org/10.1515/pjvs-2015-0012.
Longenbach, J. I., and A. J. Heinrichs. 1998. A review of the importance and physiological role of curd formation in the abomasum of young calves. Anim. Feed Sci. Technol. 73:85–97. https://doi.org/10.1016/S0377-8401(98)00130-8.
Lopreiato, V., M. H. Ghaffari, L. Cattaneo, G. Ferronato, A. S. Alharthi, F. Piccioli-Cappelli, J. J. Loor, E. Trevisi, and A. Minuti. 2021. Suitability of rumination time during the first week after calving for detecting metabolic status and lactation performance in Simmental dairy cows: A cluster-analytic approach. Ital. J. Anim. Sci. 20:1909–1923. https://doi.org/10.1080/1828051X.2021.1963862.
Lorenz, I., J. Fagan, and S. J. More. 2011. Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. Ir. Vet. J. 64:9. https://doi.org/10.1186/2046-0481-64-9.
Lynch, G. P., T. L. Pike, and J. Bond. 1978. Nutritional responses of calves fed milk or a milk replacer. J. Dairy Sci. 61:212–220. https://doi.org/10.3168/jds.S0022-0302(78)83580-2.
McGrath, J. J. 2016. Accelerated pre-weaning growth rates in dairy calves: Do antioxidants have a place? Anim. Prod. Sci. 56:1275–1284. https://doi.org/10.1071/AN15310.
Moallem, U., D. Werner, H. Lehrer, M. Zachut, L. Livshitz, S. Yakoby, and A. Shamay. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. J. Dairy Sci. 93:2639–2650. https://doi.org/10.3168/jds.2009-3007.
Montagne, L., I. Crévieu-Gabriel, R. Toullec, and J. P. Lallès. 2003. Influence of dietary protein level and source on the course of protein digestion along the small intestine of the veal calf. J. Dairy Sci. 86:934–943. https://doi.org/10.3168/jds.S0022-0302(03)73676-5.
Montagne, L., R. Toullec, and J. P. Lallès. 2001. Intestinal digestion of dietary and endogenous proteins along the small intestine of calves fed soybean or potato. J. Anim. Sci. 79:2719. https://doi.org/10.2527/2001.79102719x.
Morittu, V. M., A. Minuti, A. Spina, M. V. Riboni, F. Piccioli-Cappelli, E. Trevisi, D. Britti, and V. Lopreiato. 2021. Age-related metabolic changes of pre-weaned Simmental calves fed whole bulk milk and ad libitum calf starter. Res. Vet. Sci. 135:237–243. https://doi.org/10.1016/j.rvsc.2019.10.003.
Murdock, F. R., A. S. Hodgson, and T. H. Blosser. 1961. Milk replacers for dairy calves. I. A comparison of an all milk by-product replacer and limited whole milk with and without chlortetracycline. J. Dairy Sci. 44:1711–1716. https://doi.org/10.3168/jds.S0022-0302(61)89944-X.
NASEM. (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. National Academies Press, Washington, DC.
Orro, T., S. Jacobsen, J. P. LePage, T. Niewold, S. Alasuutari, and T. Soveri. 2008. Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. Vet. J. 176:182–187. https://doi.org/10.1016/j.tvjl.2007.02.010.
Otterby, D. E., and J. G. Linn. 1981. Advances in nutrition and management of calves and heifers. J. Dairy Sci. 64:1365–1377. https://doi.org/10.3168/jds.S0022-0302(81)82709-9.
Pantophlet, A. J. 2018. Glucose homeostasis and insulin resistance in veal calves: Studies on the effects of age, nutritional modulations and the applicability of metabolic profiling techniques. PhD thesis. Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University Medical Centre Groningen, Groningen, the Netherlands. https://research.rug.nl/en/publications/glucose-homeostasis-and-insulin-resistance-in-veal-calves-studies.
Park, Y. W. 2009. Overview of bioactive components in milk and dairy products. Pages 1–12 in Bioactive Components in Milk and Dairy Products. Y. W. Park, ed. Wiley Blackwell.
Radostits, O. M., and J. M. Bell. 1970. Nutrition of the pre-ruminant dairy calf with special reference to the digestion and absorption of nutrients: A review. Can. J. Anim. Sci. 50:405–452. https://doi.org/10.4141/cjas70-063.
Reber, A. J., A. Lockwood, A. R. Hippen, and D. J. Hurley. 2006. Colostrum induced phenotypic and trafficking changes in maternal

mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. Vet. Immunol. Immunopathol. 109:139–150. https://doi.org/10.1016/j.vetimm.2005.08.014.
Sarma, P. R. 1990. Red cell indices. Chapter 152 in Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. H. K. Walker, W. D. Hall, and J. W. Hurst, ed. Butterworths, Boston, MA.
Sato, J., M. Kanata, J. Yasuda, R. Sato, K. Okada, Y. Seimiya, and Y. Naito. 2005. Changes of serum alkaline phosphatase activity in dry and lactational cows. J. Vet. Med. Sci. 67:813–815. https://doi.org/10.1292/jvms.67.813.
Séverin, S., and X. Wenshui. 2005. Milk biologically active components as nutraceuticals. Crit. Rev. Food Sci. Nutr. 45:645–656. https://doi.org/10.1080/10408690490911756.
Sfulcini, M., V. Lopreiato, F. Piccioli-Cappelli, V. Patrone, M. Bisaschi, I. Yoon, A. M. Zontini, M. Barbato, L. Cattaneo, I. Archetti, E. Trevisi, and A. Minuti. 2025. *Saccharomyces cerevisiae* fermentation-derived postbiotics supplementation to dairy calves: Effects on growth, metabolism, immune status and preliminary first lactation outcomes. Animals (Basel) 15:2728. https://doi.org/10.3390/ani15182728.
Sharpe, K. T., and B. J. Heins. 2021. Growth, health, and economics of dairy calves fed organic milk replacer or organic whole milk in an automated feeding system. JDS Commun. 2:319–323. https://doi.org/10.3168/jdsc.2021-0084.
Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. J. Dairy Sci. 95:783–793. https://doi.org/10.3168/jds.2011-4391.
Soberon, F., and M. E. Van Amburgh. 2013. Lactation Biology Symposium: The effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: A meta-analysis of current data. J. Anim. Sci. 91:706–712. https://doi.org/10.2527/jas.2012-5834.
Sweeney, B. C., J. Rushen, D. M. Weary, and A. M. de Passillé. 2010. Duration of weaning, starter intake, and weight gain of dairy calves fed large amounts of milk. J. Dairy Sci. 93:148–152. https://doi.org/10.3168/jds.2009-2427.
Tikofsky, J. N., M. E. Van Amburgh, and D. A. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim. Sci. 79:2260–2267. https://doi.org/10.2527/2001.7992260x.
Turk, R., D. Juretic, D. Geres, N. Turk, B. Rekic, V. Simeon-Rudolf, and A. Svetina. 2004. Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Res. Vet. Sci. 76:57–61. https://doi.org/10.1016/j.rvsc.2003.08.001.
Urie, N. J., J. E. Lombard, C. B. Shivley, C. A. Kopral, A. E. Adams, T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244. https://doi.org/10.3168/jds.2017-14019.
Wang, S., Y. C. Pai, and T. Bhatt. 2022. Kinematic synergies in overground slip recovery outcomes: Distinct strategies or a single strategy? Gait Posture 95:270–276. https://doi.org/10.1016/j.gaitpost.2021.01.025.
Welboren, A. C., B. Hatew, O. López-Campos, J. P. Cant, L. N. Leal, J. Martín-Tereso, and M. A. Steele. 2021. Effects of energy source in milk replacer on glucose metabolism of neonatal dairy calves. J. Dairy Sci. 104:5009–5020. https://doi.org/10.3168/jds.2020-19405.
Westhoff, T. A., R. A. Molano, M. E. Van Amburgh, and S. Mann. 2024. Hemoconcentration differs in neonatal dairy calves: Considerations for assessment of transfer of passive immunity. JDS Commun. 5:713–718. https://doi.org/10.3168/jdsc.2024-0570.
Wilms, J. N., M. H. Ghaffari, P. S. Darani, M. Jansen, H. Sauerwein, M. A. Steele, J. Martín-Tereso, and L. N. Leal. 2024. Postprandial metabolism and gut permeability in calves fed milk replacer with different macronutrient profiles or a whole milk powder. J. Dairy Sci. 107:184–201. https://doi.org/10.3168/jds.2023-23368.
Wilms, J. N., M. H. Ghaffari, M. A. Steele, H. Sauerwein, J. Martín-Tereso, and L. N. Leal. 2022. Macronutrient profile in milk replacer or a whole milk powder modulates growth performance, feeding behavior, and blood metabolites in ad libitum-fed calves. J. Dairy Sci. 105:6670–6692. https://doi.org/10.3168/jds.2022-21870.
Zandbergen, F., and J. Plutzky. 2007. PPARα in atherosclerosis and inflammation. Biochim. Biophys. Acta 1771:972–982. https://doi.org/10.1016/j.bbalip.2007.04.021.

ORCIDS

Marta Sfulcini, https://orcid.org/0000-0002-3689-1883
Andrea Minuti, https://orcid.org/0000-0002-0617-6571
Giulia Ferronato, https://orcid.org/0000-0001-9153-8540
Erminio Trevisi, https://orcid.org/0000-0003-1644-1911
Juan J. Loor, https://orcid.org/0000-0003-1586-4365
Annalisa Amato, https://orcid.org/0009-0009-0591-4366
Carmelo Cavallo, https://orcid.org/0009-0005-8971-1777
Luigi Liotta, https://orcid.org/0000-0002-3242-1817
Vincenzo Lopreiato https://orcid.org/0000-0001-6965-7340