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Synthetic milk as an early-life functional nutritional intervention modulates rumen microbiota, metabolic pathways and immune function in lambs

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ABSTRACT

Early life is a critical period for metabolic and immune development, during which nutritional inputs may influence physiological maturation beyond basic nutrient supply. This study evaluated a milk-based nutritional formulation as an early-life intervention in lambs. The study further examined associations with microbial fermentation, systemic metabolism, immune responses, growth performance, and maternal physiological responses. Synthetic milk supplementation resulted in body weight gain and average daily gain in lambs, together with elevated circulating growth hormone and insulin-like growth factor-1 levels. Immune indicators, including IgA and IgM, were increased, while pro-inflammatory cytokines were reduced. Supplemented lambs also exhibited higher serum vitamin D_3 concentrations and a tendency toward increased calcium levels. At the microbial level, supplementation altered rumen fermentation profiles, characterized by increased acetate and butyrate concentrations and enhanced activities of microbial-associated digestive enzymes, accompanied by shifts in several fermentation-related bacterial genera. Plasma metabolomic analysis revealed alterations in pathways related to amino acid metabolism, mineral absorption, and protein digestion and absorption. In addition, offspring-directed supplementation accompanied by in maternal body condition, immune indices, and plasma metabolic pathways linked to energy metabolism. Overall, these findings indicate that early-life milk-based nutritional supplementation is associated with coordinated microbial, metabolic, and immune responses in lambs and may influence physiological status within the maternal–offspring unit, supporting further investigation of early-life nutritional strategies in food bioscience.

1. Introduction

Early-life nutrition plays a pivotal role in shaping metabolic programming, immune maturation, and gastrointestinal development, thereby exerting long-term effects on host health and physiological homeostasis (Ratsika et al., 2021; Tremaroli & Bäckhed, 2012). Increasing evidence indicates that nutritional interventions during this period function not only as sources of nutrients but also as bioactive signals that regulate immune responses, gut microbial colonization, and systemic metabolic pathways (Zheng et al., 2014). From a functional food perspective, such nutritional strategies capable of coordinately modulating host–microbiota interactions and metabolic regulation are increasingly recognized as promising approaches to enhance developmental robustness and long-term health outcomes (Pieri et al., 2023).

Milk and milk-derived nutritional formulations represent one of the earliest and most influential dietary exposures in mammals (Ballard & Morrow, 2012; Osman et al., 2021). Beyond providing energy and essential nutrients, milk contains a wide array of bioactive components that participate in the regulation of immune system development, endocrine function, and gastrointestinal physiology (Daniels et al., 2021). In young ruminants, early milk-based nutritional interventions have been demonstrated to promote rumen development, enhance fermentation efficiency, and improve immune competence (Fu et al., 2019). These functional effects are increasingly attributed to the

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modulation of rumen microbial community structure and downstream metabolic signaling pathways, rather than merely increased nutrient intake (Fu et al., 2019; Loor et al., 2016). Accordingly, milk-derived nutritional strategies have attracted growing attention in the field of functional nutrition due to their capacity to regulate physiological development through the microbiota–metabolism–immune axis (Tremaroli & Bäckhed, 2012).

Synthetic milk, formulated to mimic the nutritional and functional properties of natural milk, has been widely applied as a practical nutritional alternative during early life in ruminants. Previous studies have shown that synthetic milk or milk replacers can improve growth performance, antioxidant capacity, gastrointestinal development, and microbial establishment in young ruminants (Berends et al., 2012, 2020; Wei et al., 2023). However, most existing studies have primarily focused on production-related outcomes, whereas the functional mechanisms underlying the regulation of microbial communities, systemic metabolism, and immune responses remain insufficiently elucidated. In particular, under environmentally constrained production conditions such as high-altitude livestock systems, it remains unclear to what extent early milk-based nutritional interventions contribute to coordinated physiological adaptation across microbial, metabolic, and immune levels. Integrative studies linking rumen microbial alterations with host metabolic pathways and immune function are still limited.

In addition, early-life nutritional interventions applied to offspring may exert indirect effects on maternal physiological status, yet this aspect has received relatively little attention. Lactation represents a physiologically demanding state characterized by substantial metabolic burden, during which maternal energy reserves, immune components, and metabolic substrates are continuously mobilized to support milk synthesis (Pawłowski et al., 2019; Rasmussen & Kjolhede, 2004). Previous studies have shown that the intensity and frequency of suckling stimulation are key regulators of milk production and maternal energy allocation, and changes in suckling behavior or litter size can directly influence maternal metabolic load and nutrient utilization efficiency (Hatfield et al., 1995; Loerch et al., 1985). In parallel, the gradual increase in solid feed intake and the implementation of early supplementation in offspring are often accompanied by alterations in suckling patterns, which may reshape the balance between maternal energy partitioning, immune regulation, and body condition maintenance (Valehi et al., 2022). Therefore, changes in offspring feeding behavior or suckling patterns induced by early nutritional supplementation may further influence maternal metabolic load, immune status, and body condition. However, whether and to what extent offspring supplementation can alleviate or modify maternal physiological stress remains unclear. Existing studies evaluating the effects of offspring supplementation on maternal milk production and body condition are still limited and sometimes inconsistent (Lopes et al., 2016; Silva et al., 2017). Accordingly, it remains to be clarified whether early-life functional nutritional interventions can shape maternal–offspring physiological interactions through changes in suckling behavior and metabolic requirements, which provides a key rationale for including maternal responses in the present study.

Gangba sheep, an indigenous breed native to the Qinghai–Tibet Plateau, are adapted to an extreme high-altitude environment characterized by chronic hypoxia, low ambient temperatures, and limited nutritional resources (Zhang et al., 2022). Lambing and early lactation in this breed commonly coincide with periods of forage scarcity and heightened environmental stress (Liu, & TangHuangTangYang, 2025; Zhang et al., 2024). These ecological constraints impose substantial challenges on early growth, immune maturation, and gastrointestinal development. Consequently, Gangba sheep serve as a biologically relevant and sensitive model for evaluating early-life nutritional regulation under extreme environmental conditions. This setting allows for a focused evaluation of early nutritional interventions in relation to host physiology, microbial communities, and maternal–offspring interactions.

Based on this background, we hypothesized that early synthetic milk supplementation may reshape rumen microbial structure and fermentation, thereby influencing systemic metabolism and immune function in lambs. Meanwhile, it may indirectly modulate maternal metabolic load during lactation through changes in offspring feeding and suckling behavior. Therefore, the objective of this study was to investigate the functional effects and underlying mechanisms of synthetic milk as an early-life nutritional intervention in lambs, using Gangba sheep as a high-altitude–adapted animal model. Specifically, we focused on its regulation of rumen microbiota, systemic metabolic profiles, and immune responses, and further explored its potential indirect effects on maternal physiological homeostasis through changes in offspring behavior. By integrating rumen fermentation, microbial profiling, plasma metabolomics, and immune–endocrine indicators, this study aimed to provide mechanistic evidence supporting synthetic milk as a functional nutritional strategy within the maternal–offspring system.

2. Materials and methods

2.1. Animals, diets and experimental design

A total of 36 healthy ewe–lamb pairs of Gangba sheep were selected. All ewes were multiparous with 2 previous lambing, and had an initial body weight of 20.07 ± 0.81 kg and a body condition score of 2.5 ± 0.23. Lambs were 42–45 days old with an initial body weight of 5.80 ± 0.31 kg. The pairs were randomly assigned to two treatments: a control group (CON, no synthetic milk) and a synthetic milk group (SM). Each group contained 18 pairs with 9 replicates (2 pairs per pen). In the SM group, synthetic milk (purchased from Libaowei (Guangdong) Nutrition Technology Co., Ltd., Guangdong, China) was provided ad libitum at the pen level using lamb-only creep feeders that allowed access to lambs but prevented ewes from consuming the supplement. Synthetic milk intake was recorded on a pen basis, and the pen was considered the experimental unit. In addition, each pen was equipped with stainless-steel feed troughs and water troughs, allowing both ewes and lambs to freely access the basal diet and drinking water. The composition and nutrient levels of the basal diet, as well as the nutritional composition of the synthetic milk, are presented in Table 1. The experiment was conducted

Table 1
Dietary feed composition and nutritional levels of feed and synthetic milk.

Diet composition of feed (%)		Nutritional level of synthesized milk[3]	
Items	Content (%, DM)	Items	Content (Dry matter, %)
Corn	65.0	Crude protein	19.0
Highland barley	10.0	Ether extract	18.0
Soybean meal	15.0	Ash	6.6
Wheat bran	7.0		
Hawthorn powder	1.0		
Premix[a]	1.0		
Salt	0.5		
Limestone	0.5		
Total	100		
Nutritional level[b] (Dry matter, %)			
Crude protein	14.05		
Ether extract	2.90		
Neutral Detergent Fiber	53.85		
Acid Detergent Fiber	38.4		
Ash	7.20		

[a] Premix (content per kilogram): Vitamin A 700,000–1,000,000 IU, Vitamin E 4000 IU, Vitamin K_3 100 mg, Vitamin B_1 100 mg, Vitamin B_2 260 mg, Vitamin B_{12} 1000 μg, Vitamin B_6 100 mg, Vitamin D_3 200,000–500,000 IU, Nicotinic acid 1600 mg, Pantothenic acid 500 mg, Folic acid 50 mg, Cu 0.2-1.5 g, Fe 3-20 g, Zinc 2-12 g, Biotin 10 mg, Manganese 2-14 g, Iodine 10-100 mg, Se 10-50 mg, Cobalt 5-40 mg.
[b -3)] Measured value.

at the Mendecun Village Ranch in Gangba County, Shigatse City, Tibet Autonomous Region, China (88.418°E, 28.262°N; 4700 m altitude).

2.2. Determination of lamb growth performance and Ewe body condition

The trial lasted 49 days, including a 7-day adaptation period and a 42-day formal period. Lamb body weight, height, and body length were measured on Days 1 and 42, and average daily gain (ADG) was calculated based on body weight changes. Ewe body weight and chest circumference were recorded, and body condition was scored according to T/CAAA 078-2022. Daily feed intake of both ewes and lambs was continuously monitored. In addition, ewe and lamb behaviors were analyzed by experienced stockmen using video recordings from six surveillance cameras (Hikvision, Hangzhou, China), including the proportions of time spent eating, standing, and lying, as well as the number of nursing events (MacPherson et al., 2019). Behavioral analysis was conducted by observers who were unaware of treatment allocation (Martínez et al., 2022).

2.3. Sample collection

On Day 42, one ewe–lamb pair was randomly selected from each pen as the experimental unit (n = 9 per treatment). After an overnight fasting period (approximately 12 h), fasting blood samples were collected from the jugular vein using vacuum tubes with or without ethylenediaminetetraacetic acid (EDTA), and plasma and serum were separated. At the same time, rumen fluid was collected from the lambs corresponding to the selected ewe–lamb pairs. After measuring pH, the rumen fluid was filtered through four layers of sterile gauze, temporarily stored on dry ice, and then transferred to a −80 °C freezer for subsequent analysis.

2.4. Determination of serum and rumen fluid indicators

Serum concentrations of immunoglobulin A (IgA, Cat. No. JN82299), immunoglobulin G (IgG, Cat. No. JN82597), immunoglobulin M (IgM, Cat. No. JN20997), interleukin-4 (IL-4, Cat. No. JN21766), interleukin-10 (IL-10, Cat. No. JN83491), interleukin-1β (IL-1β, Cat. No. JN83789), tumor necrosis factor (TNF-α, Cat. No. JN20906), growth hormone (GH, Cat. No. JN83713), insulin-like growth factor-1 (IGF-1, Cat. No. JN81107), insulin-like growth factor binding protein-3 (IGFBP-3, Cat. No. JN81405), growth hormone-releasing hormone (GHRH, Cat. No. JN81703), and growth hormone release-inhibiting hormone (GHIH, Cat. No. JN82001) in lambs and ewes were quantified by enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Jining Biotechnology Co., Ltd., Shanghai, China). All ELISA kits were sheep-specific and validated for ovine samples according to the manufacturer's instructions. These indicators were selected to comprehensively evaluate systemic immune function, inflammatory status, and growth-related endocrine regulation in response to early-life nutritional intervention. Lamb serum calcium (Ca) and phosphorus (P) levels were quantified by inductively coupled plasma mass spectrometry (ICP-MS) (Sun et al., 2021), and vitamin D_3 (VD_3) concentration was analyzed by high-performance liquid chromatography (HPLC) (Wijayabahu et al., 2022). Rumen volatile fatty acids (VFAs) were analyzed by gas chromatography using an Agilent 5977B GC-MS system (Agilent Technologies, Santa Clara, CA, USA), equipped with a flame ionization detector (FID) and a polar capillary column (DB-FFAP, Agilent Technologies; 30 m × 0.25 mm × 0.25 μm). Chromatographic separation was conducted following established protocols for rumen fluid VFA analysis. Individual VFAs including acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were quantified based on the retention times of standards according to previously described methods (Foote, 2022). Ammonia nitrogen (NH_3–N) was measured by UV–visible spectrophotometry (UV-2450; Shimadzu, Tokyo, Japan) with absorbance measured at 620 nm (Cheng et al., 2024; Shen et al., 2023). Activities of pepsin (Cat. No. JN24387), cellulase (Cat. No. JN24574X), neutral xylanase (Cat. No. JN24587), pectinase (Cat. No. JN24452), neutral protease (Cat. No. JN24383X), lipase (Cat. No. JN24761X), and α-amylase (Cat. No. JN24263X) were determined using commercial assay kits (Shanghai Jining Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The lysozyme activity was determined by the turbidimetric method (Louis et al., 2004).

2.5. Rumen microbial diversity analysis

Genomic DNA from lamb rumen fluid was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) and verified by 1% agarose gel electrophoresis. Subsequently, the high-variable region V3-V4 of the bacterial 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Xie, & CidanCisangCiwangLiuWuCidengChilieKangZhuBasang, 2025). Amplicons were sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using paired-end sequencing (2 × 300 bp, PE300). Raw reads were quality-filtered and clustered into operational taxonomic units (OTUs) at 97% similarity using the UPARSE (v7.0) pipeline implemented on the Majorbio platform, with chimeric sequences removed. Taxonomic classification was assigned using the Ribosomal Database Project (RDP) Classifier (v2.11) against the SILVA 16S rRNA reference database with a confidence threshold of 0.7. Alpha diversity indices were calculated using Mothur (v1.30.1). Beta diversity was assessed using Bray–Curtis distances and visualized by principal coordinate analysis (PCoA) in QIIME 2. Differentially abundant taxa were identified using linear discriminant analysis effect size (LEfSe) with $P < 0.05$ and an LDA score threshold of 2.0.

2.6. Metabolomic analysis

As described, 100 μL of each sample was mixed with 400 μL of extraction solution (acetonitrile:methanol = 1:1, v/v) containing 0.02 mg/mL L-2-chlorophenylalanine as an internal standard (Xie et al., 2025). After nitrogen drying and re-dissolution, the supernatant was subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS). Quality control (QC) samples were prepared in the same way and injected regularly to monitor system stability. Metabolomic analysis was performed using a Thermo UHPLC–Exploris 240 system (Thermo Fisher Scientific, Bremen, Germany) equipped with an ACQUITY HSS T3 column. Raw data were processed using Progenesis QI software for peak alignment and metabolite identification. Data preprocessing included missing-value filtering (80% rule), minimum-value imputation, total-sum normalization, removal of variables with QC relative standard deviation (RSD) > 30%, and $\log_{10}$ transformation. Multivariate analyses, including principal component analysis (PCA) and orthogonal partial least squares–discriminant analysis (OPLS-DA), were conducted in R (v4.2.2) using the ropls package with 7-fold cross-validation. Differential metabolites were selected based on variable importance in projection (VIP) > 1 and $P < 0.05$, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed in Python.

2.7. Quantification of key differential metabolites by LC–MS/MS

To further validate the untargeted metabolomic results, five representative metabolites were selected from the key pathways for targeted analysis. Serum concentrations of methionine, glycine, valine, glutamic acid (lambs), and pantothenic acid (ewes) were quantified a targeted ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method. Specifically, after thawing at 4 °C, 100 μL of serum was mixed with 300 μL of precooled acetonitrile containing stable isotope-labeled internal standards for protein precipitation, followed by vortexing and centrifugation at 14,000×*g* for 15 min at

4 °C. The resulting supernatant was filtered through a 0.22-μm membrane and injected into a triple-quadrupole mass spectrometer (QTRAP 5500, AB Sciex, Framingham, MA, USA) equipped with a hydrophilic interaction liquid chromatography (HILIC) column for analysis. Quantification was performed in positive multiple reaction monitoring mode, and metabolite concentrations were calculated using matrix-matched calibration curves ($R^2 \geq 0.99$). QC samples covering multiple concentration levels were analyzed throughout the analytical sequence to monitor method performance and to confirm that precision and accuracy remained within acceptable limits for bioanalytical validation.

2.8. Statistical analysis

Data were analyzed using Student's t-test with treatment as the fixed effect in JMP software (version 10; SAS Institute Inc., Cary, NC). Statistical significance was declared at $P \leq 0.05$, while values of $0.05 < P \leq 0.10$ were interpreted as indicative of a tendency. Spearman correlation analysis was performed to evaluate the linear associations between microbial relative abundances and differential ruminal metabolites using GraphPad Prism (version 9.5; GraphPad Software, San Diego, CA, USA). Correlation coefficients were calculated, and statistical significance was determined at $P < 0.05$.

3. Results

3.1. Effects of synthetic milk supplementation on growth performance

Compared with the CON group, synthetic milk supplementation resulted in higher body weight and ADG in lambs ($P < 0.05$; Table 2). Lamb height showed an increasing tendency ($P = 0.10$), and leg girth was greater in the supplemented group ($P = 0.05$).

3.2. Effects of synthetic milk supplementation on serum immune and hormone profiles

Synthetic milk supplementation significantly improved the levels of IgA and IgM in the serum of lambs ($P < 0.05$, Table 3). Serum IL-1β concentration was significantly reduced ($P < 0.05$), whereas TNF-α exhibited a decreasing trend ($P = 0.06$). Additionally, synthetic milk supplementation significantly increased serum concentrations of GH, IGF-1 and VD_3 ($P < 0.05$), and there was a tendency to increase the level of serum calcium ($P = 0.07$).

3.3. Effects of synthetic milk supplementation on rumen fermentation characteristics and digestive enzyme activities

Supplementation with synthetic milk significantly increased acetate, butyrate, and total VFA concentrations, as well as the acetate-to-propionate ratio and the molar proportions of butyrate and isovalerate in the rumen fluid of lambs ($P < 0.05$; Table 4). In addition, NH_3–N concentration and isobutyrate tended to increase ($P = 0.06$ and 0.07, respectively), whereas the molar proportion of propionate tended to decrease ($P = 0.07$). Moreover, synthetic milk supplementation significantly enhanced the activities of cellulase, lysozyme, and pectinase ($P < 0.05$), with a tendency for increased pepsin activity ($P = 0.09$).

3.4. Effects of synthetic milk supplementation on rumen microbiota composition

Eighteen rumen fluid samples from lambs were collected using a

Table 2
Effect of supplementary feeding of synthetic milk on the growth performance of lambs.

Items	Treatment[a]		SEM	*P*-value
	CON	SM		
Initial body weight, kg	5.81	5.79	0.39	0.9366
Final body weight, kg	7.53	8.65	0.25	0.0029
Initial body height, cm	40.61	40.22	0.89	0.7599
Final Body height, cm	43.53	45.24	0.71	0.0985
Initial body length, cm	41.17	40.12	0.97	0.4472
Final Body length, cm	45.24	46.12	0.84	0.4636
Initial leg girth, cm	5.48	5.49	0.13	0.9289
Final leg girth, cm	5.96	6.29	0.11	0.0504
Average daily gain, g/d	41.67	68.52	4.65	0.0003
Average Daily Intake of Synthesized milk for lamb, mL/d	–	30.70		

[a] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

Table 3
Effect of supplementary feeding of synthetic milk to lambs on serum immunity and hormones of lambs.

Items	Treatment[a]		SEM	*P*-value
	CON	SM		
Immunoglobulin A, g/L	10.96	13.26	0.42	0.0014
Immunoglobulin G, g/L	11.59	10.76	0.62	0.3596
Immunoglobulin M, g/L	3.40	3.81	0.07	0.0013
Interleukin 1β, pg/mL	54.71	34.21	3.35	0.0005
Interleukin 4, pg/mL	89.10	75.94	7.65	0.2382
Interleukin 10, pg/mL	103.64	96.12	6.34	0.4136
Tumor necrosis factor, ng/L	648.63	520.67	45.56	0.0639
Growth Hormone, ng/L	12.81	15.36	0.74	0.0279
Insulin-like growth factor-1, ng/L	168.75	260.09	18.71	<0.0001
Insulin-like growth factor-binding protein-3, pg/mL	73.85	78.52	8.42	0.6999
Growth hormone-releasing hormone, pg/mL	19.10	21.04	1.98	0.4991
Growth hormone-inhibiting hormone, pg/mL	187.18	205.08	11.89	0.3030
Serum calcium, μg/mL	90.76	94.54	1.55	0.0745
Serum phosphorus, μg/mL	61.03	63.62	2.02	0.3786
Vitamin D_3, μg/mL	0.14	0.21	0.01	0.0041

[a] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

Table 4
Effect of supplementary feeding of synthetic milk to lambs on rumen fermentation parameters of lambs.

Items	Treatment[a]		SEM	*P*-value
	CON	SM		
pH	6.30	6.53	0.17	0.3528
NH_3-N, mg/g	19.13	27.79	2.89	0.0600
Acetate, mmol/L	27.87	36.46	1.31	0.0035
Propionate, mmol/L	15.20	16.78	1.41	0.4597
Isobutyrate, mmol/L	0.59	0.74	0.05	0.0705
Butyrate, mmol/L	4.87	8.93	0.79	0.010
Isovalerate, mmol/L	0.73	1.74	0.42	0.1426
Valerate, mmol/L	1.66	2.05	0.42	0.5411
Total VFA (mmol/L)	50.92	66.70	4.51	0.0416
Acetate-to-propionate ratio	1.83	2.17	0.19	0.0483
Acetate, %	54.73	54.66	6.24	0.8416
Propionate, %	29.85	25.16	2.21	0.0729
Isobutyrate, %	1.16	1.11	0.14	0.4661
Butyrate, %	9.56	13.39	0.92	0.0217
Isovalerate, %	1.43	2.61	0.11	0.0418
Valerate, %	3.26	3.07	0.54	0.1681
Pepsin, U/mL	127.85	210.67	30.73	0.0858
Cellulase, μg/min/mL	150.23	262.32	17.46	0.0011
Lysozyme, μg/mL	4.59	6.81	0.45	0.0402
Neutral xylanase, U/100L	11.49	11.10	0.37	0.4719
Pectinase, U/L	2.04	2.63	0.13	0.0106
Neutral protease, nmol/min/mL	4829.79	5219.98	295.59	0.3726
Lipase, U/L	2.85	2.65	0.61	0.8222
α-Amylase, U/L	0.59	0.63	0.05	0.5871

[a] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

rumen fluid sampler, with an average sequence length of 415 base pairs (bp). Alpha diversity analysis indicated that the abundance of different species and the sequencing data were reasonable and stable (Fig. 1A–D). Beta diversity analysis based on PCoA showed that supplementary feeding of synthetic milk to lambs altered the rumen microbial community structure of lambs (Fig. 1E). At the phylum level, there was no significant difference in microbiota between the two treatment groups (Fig. 1F). And supplementary feeding of synthetic milk to lambs significantly increased the relative abundances of *NK4A214_group*, Christensenellaceae_*R-7_group*, *norank_f_Eubacterium_coprostanoligenes_group*, and Prevotellaceae_*NK3B31_group* in the rumen microbiota of lambs and reduced the relative abundance of *Dialister* at the genus level ($P < 0.05$, Fig. 1G–H). In addition, 46 lamb rumen microbiota with differential abundances were identified through LEfSe cladogram and LEfSe bar (Fig. 2A–B). Among them, there were 15 biomarkers in the CON group, while 31 biomarkers in the lambs of the SM group. Spearman correlation analysis revealed significant associations between ruminal fermentation parameters, digestive enzyme activities, and specific bacterial taxa (Fig. 3). NH_3-N concentration was negatively correlated with *Dialister* (R = −0.62, $P = 0.033$), whereas acetate showed a positive correlation with *Dialister* (R = 0.65, $P = 0.022$). Isobutyrate was positively associated with Rikenellaceae_*RC9_gut_group* (R = 0.61, $P = 0.036$), and butyrate was positively correlated with Lachnospiraceae_*NK3A20_group* (R = 0.62, $P = 0.033$). Pepsin activity exhibited positive correlations with *Clostridia_UCG-014* (R = 0.59, $P = 0.042$) and *NK4A214_group* (R = 0.64, $P = 0.024$). In addition, cellulase activity was strongly and positively correlated with *NK4A214_group* (R = 0.82, $P = 0.001$) and Christensenellaceae_*R-7_group* (R = 0.64, $P = 0.026$), but negatively correlated with *Dialister* (R = −0.83, $P = 0.001$).

3.5. Effects of synthetic milk supplementation on plasma metabolomic profiles

Using LC-MS/MS technology, we analyzed the plasma of ewes and lambs in both the CON and SM groups, identifying a total of 2978 metabolites. PCA showed clear separation between the SM and CON groups (Fig. 4A). In the plasma of lambs in the SM group, 99 metabolites were up-regulated ($P < 0.05$, VIP >1, Fold change (FC) = 1) and 35 metabolites were down-regulated (Fig. 4B). Notably, pathways related to mineral absorption and protein digestion and absorption were enriched (Fig. 4C).

3.6. Effects of synthetic milk supplementation on Ewe body condition, immune status, and plasma metabolomics

Supplementary feeding of lambs with synthetic milk did not significantly affect the body weight of the ewes (Table 5). However, it resulted in improvements in chest circumference ($P < 0.05$) and body condition score ($P = 0.09$) of the ewes. It also significantly increased the serum levels of IgA and IgM in ewes ($P < 0.05$, Table 6) while decreasing IL-10 levels ($P < 0.05$). Additionally, there was a tendency for IgG levels to increase ($P = 0.07$) and for TNFα levels to decrease ($P = 0.06$). Interestingly, the average daily feed intake of ewes and lambs was significantly increased in SM group ($P < 0.05$, Table 7). In addition, synthetic

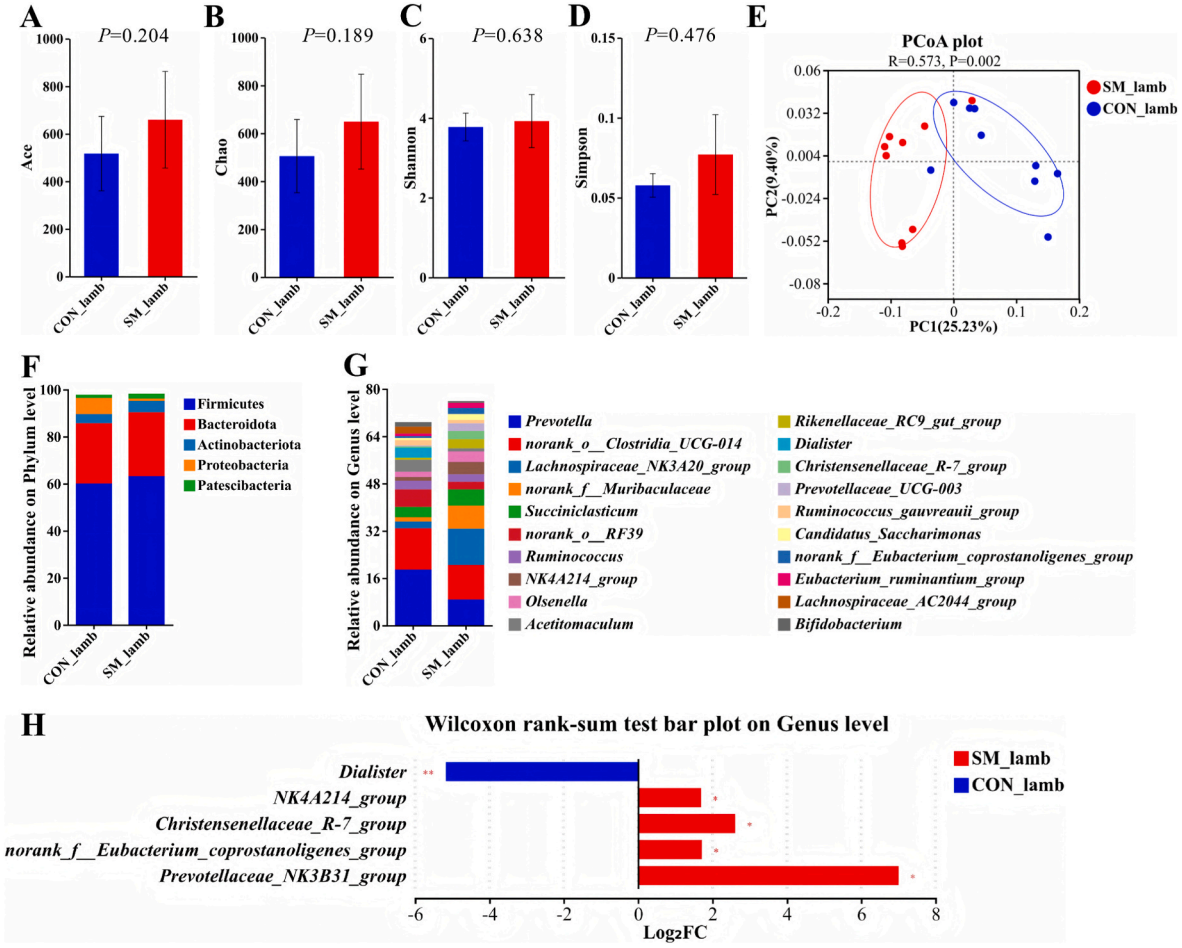

Fig. 1. Effects of supplementary feeding with synthetic milk on the rumen microbiota of lambs. (A) Ace index; (B) *Chao1* index; (C) Shannon index; (D) Simpson index; (E) PCoA chart; (F) Phylum level bar chart; (G) Genus level bar chart; (H) Differential microbes at the genus level. CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9. *$P < 0.05$, **$P < 0.01$.

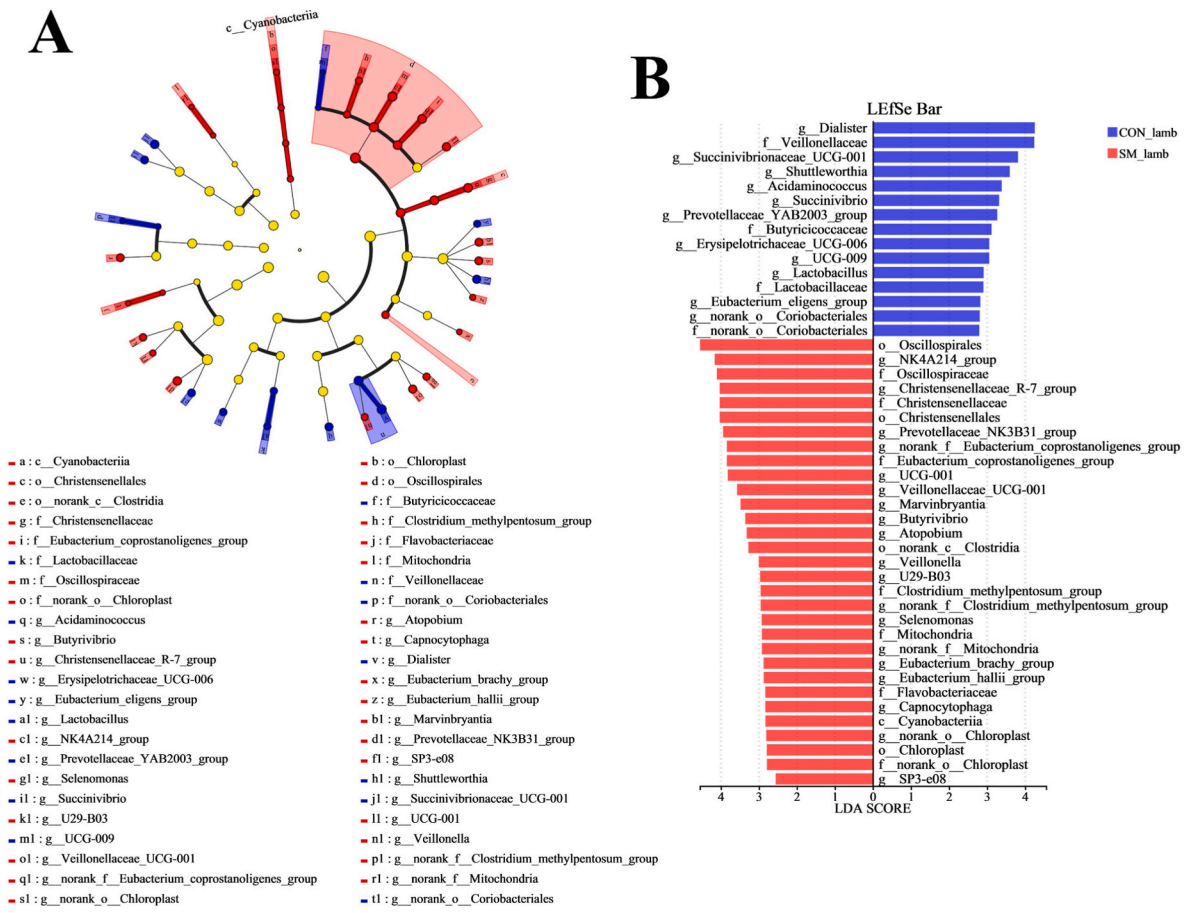

Fig. 2. LEfSe multi-level species differential discrimination analysis chart. (A) LEfSe cladogram; (B) LEfSe Bar. CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

milk supplementation significantly reduced the frequency of suckling events ($P < 0.05$). The PCA plot showed separation between the ewes in the SM group and those in the CON group (Fig. 4D). And the volcano plot revealed that 54 metabolites were up-regulated and 48 were down-regulated in the plasma of ewes in the SM group (Fig. 4E). Topological analysis and enrichment statistics further indicated changes in the Pantothenate and coenzyme A (CoA) biosynthesis pathway (impact value > 0.10, $P < 0.05$, Fig. 4F). The metabolite reaction network diagram constructed based on the KEGG pathway further confirmed the central role of C00864 (i.e., pantothenic acid) in this pathway (Fig. 4G).

3.7. Key differential metabolites

For lambs, synthetic milk supplementation significantly increased the serum concentrations of methionine, glycine, valine, and glutamic acid, whereas it decreased the level of pantothenic acid in the serum of ewes (Table S1).

4. Discussion

Early life represents a critical window for metabolic programming and immune maturation. During this period, dietary components act not only as nutrient sources but also as bioactive modulators shaping host physiology (Mullaney et al., 2022; Ratsika et al., 2021). In high-altitude environments such as the Qinghai–Tibet Plateau, this developmental window is likely subjected to additional physiological constraints, including chronic hypoxia, low ambient temperatures, and limited nutrient availability, which may further sensitize early-life responses to nutritional interventions. The present study demonstrates that early-life supplementation with synthetic milk can be regarded as a targeted nutritional intervention, leading to coordinated improvements in growth-related endocrine responses, immune status, and gastrointestinal metabolic capacity in lambs.

Supplementation with synthetic milk significantly increased body weight gain and ADG in lambs, accompanied by elevated serum concentrations of GH and IGF-1. These findings suggest that early milk-derived nutritional provision may enhance activity of the somatotropic axis, thereby providing stronger endocrine support for somatic growth and development. Previous studies have reported that artificial rearing or intensified milk supplementation elevates IGF-1 levels in young ruminants and is closely associated with improved growth performance (Gerbert et al., 2017; Ghaffari et al., 2021). In the context of Gangba sheep, which are raised under high-altitude conditions characterized by limited maternal milk yield and increased energetic demands, the observed enhancement of the GH–IGF-1 axis may represent a compensatory endocrine adaptation facilitating early growth under environmental stress.

In parallel, improvements in growth-related phenotypes in the present study were accompanied by synchronous modulation of immune indices, including increased IgA and IgM concentrations and reduced pro-inflammatory cytokines IL-1β and TNF-α. This pattern is consistent with previous findings showing that early nutritional interventions can improve immune homeostasis and attenuate inflammatory responses in calves (Reyer et al., 2024; Wilms et al., 2025). Given that high-altitude hypoxia is often associated with low-grade systemic inflammation and immune dysregulation, the immune-stabilizing effects observed here may be particularly relevant for lambs raised on the Qinghai–Tibet Plateau. The simultaneous improvement in growth performance and

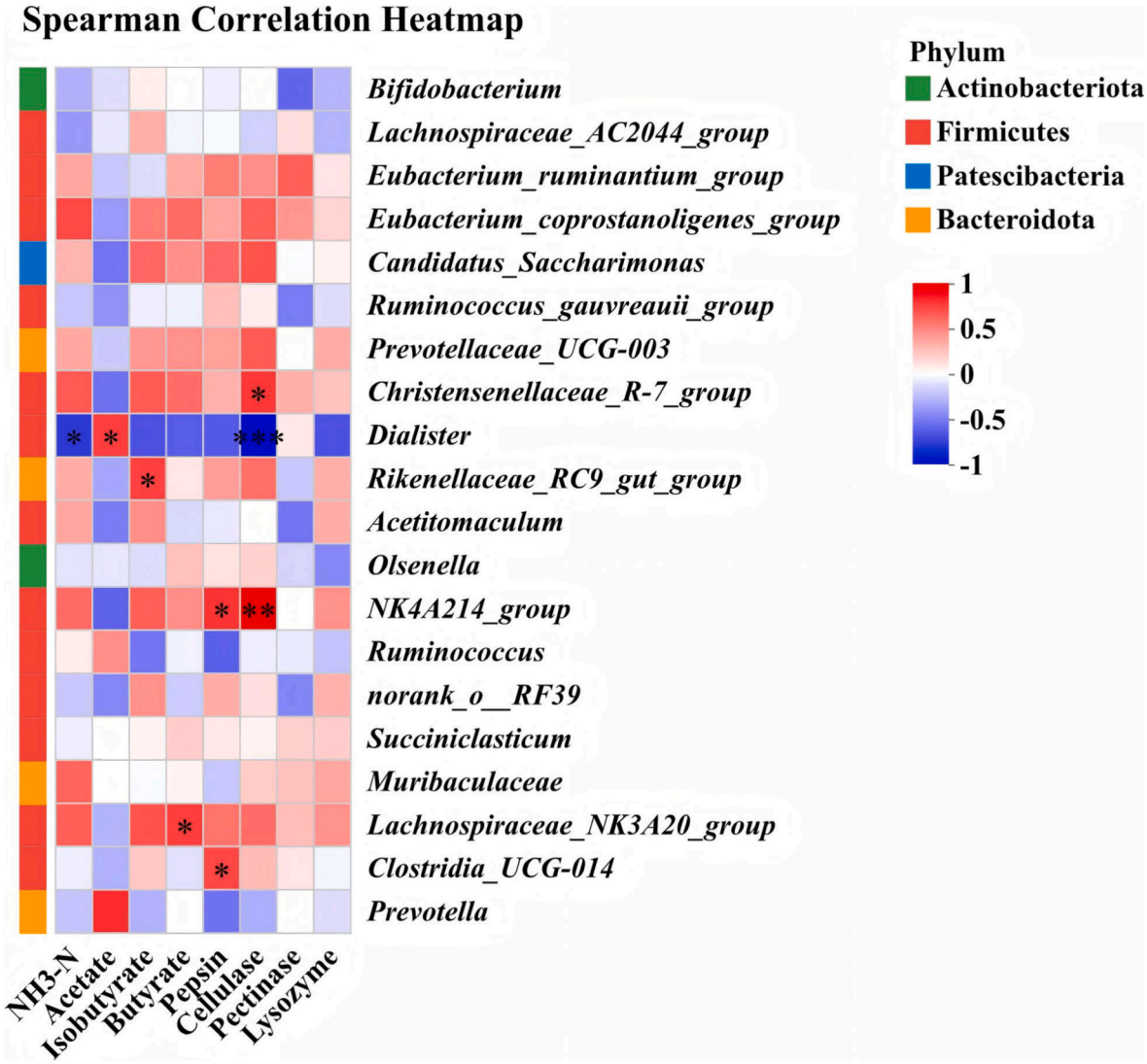

Fig. 3. Microbiota-Rumen metabolites Spearman correlation heatmap, n = 9. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

immune indices observed in this study suggests that synthetic milk supplementation may support early development in lambs beyond simply accelerating growth rate. Rather than acting on a single physiological endpoint, this nutritional intervention appears to be associated with a more stable developmental state during early life. Such a pattern is consistent with the concept of functional nutrition, which emphasizes the role of early dietary inputs in shaping physiological function and health trajectories through interactions between endocrine and immune systems (Ratsika et al., 2021; Tremaroli & Bäckhed, 2012). In addition, serum vitamin D_3 concentrations were higher in supplemented lambs, accompanied by a tendency toward increased serum calcium levels, indicating a potential improvement in micronutrient status. This finding may be particularly relevant under high-altitude conditions, where reduced ultraviolet exposure, limited dietary mineral intake, and increased skeletal demands may predispose animals to suboptimal vitamin D and calcium status. Given the established roles of vitamin D and minerals in immune regulation and skeletal development (Wilkens et al., 2017; Willems et al., 2013), these changes may provide further support for physiological development during early life.

In addition to systemic responses, synthetic milk supplementation was associated with pronounced changes in rumen fermentation characteristics and microbial functional activity. VFAs, the primary products of rumen microbial fermentation, serve not only as energy substrates but also participate in epithelial development and immune-related signaling processes (Costanzo et al., 2021; Wang et al., 2022). In the present study, supplemented lambs exhibited higher ruminal acetate and butyrate concentrations, while isobutyrate and ammonia nitrogen levels showed increasing trends. These findings are indicative of enhanced fermentative activity and microbial nitrogen utilization, which may reflect improved substrate availability and functional capacity of the rumen microbiota. In high-altitude production systems, where forage quality and intake are often constrained, such improvements in rumen fermentation efficiency may contribute substantially to supporting early-life energy supply. The observed increase in total VFA concentration indicates enhanced microbial fermentation capacity and energy supply in the rumen. Furthermore, the higher acetate-to-propionate ratio, together with increased cellulase activity, suggests enhanced fiber fermentation and improved cellulolytic microbial activity. Collectively, these changes reflect a more mature and functionally efficient rumen fermentation pattern in supplemented lambs. Among individual VFAs, butyrate is particularly relevant due to its recognized role in maintaining epithelial integrity, supporting immune tolerance, and contributing to metabolic homeostasis (Costanzo et al., 2021). Therefore, the increase in ruminal butyrate concentration observed here may represent a key metabolic link between microbial activity and host physiological responses. Such changes could contribute to rumen

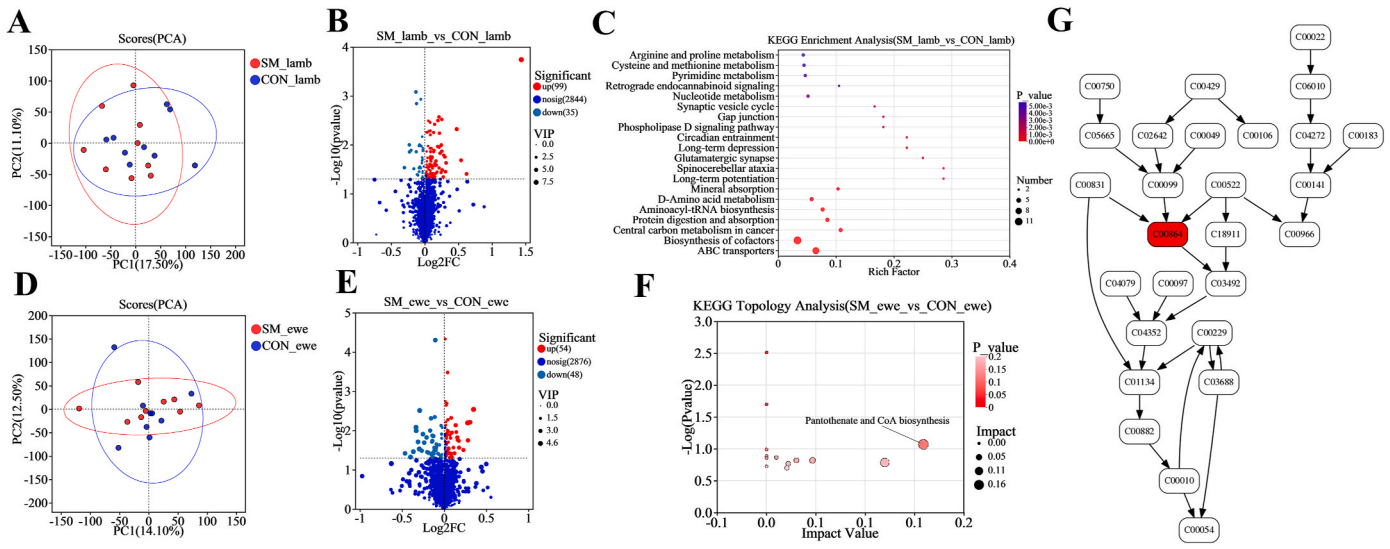

Fig. 4. Effects of supplementary feeding with synthetic milk on the plasma metabolome of lambs and ewes. (A) PCA analysis diagram of lambs; (B) Difference statistics and volcano plot of lambs; (C) KEGG enrichment analysis plot of lambs. (D) PCA analysis diagram of ewes; (E) Difference statistics and volcano plot of ewes; (F) KEGG topology analysis of ewes; (G) Metabolite reaction network diagram of KEGG pathway. CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

Table 5
Effect of synthetic milk supplementary feeding for lambs on the body condition of ewes.

Items	Treatment[a]		SEM	*P*-value
	CON	SM		
Initial body weight, kg	20.98	21.42	0.58	0.5827
Final body weight, kg	21.76	23.33	0.90	0.2258
Initial chest circumference, cm	74.93	75.83	1.03	0.5421
Final chest circumference, cm	73.44	76.28	0.92	0.0368
Initial body condition score, cm	2.44	2.58	0.11	0.3673
Final body condition score, cm	2.39	3.01	0.11	0.0860

[a] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

Table 6
Effect of supplementary feeding of synthetic milk to lambs on serum immunity of ewes.

Items	Treatment		SEM	*P*-value
	CON	SM		
Immunoglobulin A, g/L	16.87	19.94	0.97	0.0398
Immunoglobulin G, g/L	13.50	15.50	0.73	0.0696
Immunoglobulin M, g/L	3.92	4.72	0.24	0.0316
Interleukin 1β, pg/mL	123.74	109.37	9.09	0.2803
Interleukin 4, pg/mL	86.06	99.17	4.02	0.0009
Interleukin 10, pg/mL	181.52	145.21	5.84	0.0005
Tumor necrosis factor, ng/L	855.01	797.40	18.32	0.0631

[1] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

epithelial development and barrier function during early adaptation to solid feed under environmental stress.

Consistent with the observed shifts in fermentation profiles, synthetic milk supplementation was also associated with increased activities of several microbial-associated digestive enzymes, including cellulase, pectinase, and lysozyme. Rather than representing isolated changes, these enzyme responses collectively suggest an overall enhancement of rumen microbial digestive capacity. Such enhancement may be particularly advantageous for Gangba lambs, which are exposed to solid feeds at an early age under nutritionally challenging plateau

Table 7
Effect of supplementary feeding of synthetic milk to lambs on the average daily feed intake and behaviors of ewes and lambs.

Items	Treatment		SEM	*P*-value
	CON	SM		
Average daily feed intake, g/d	704.34	777.78	25.32	0.0171
Ewe behavior				
Feed intake, %	19.35	18.94	1.21	0.7994
Standing, %	32.14	29.52	6.39	0.8848
Lying down, %	59.43	60.18	5.54	0.5927
lamb behavior				
Feed intake, %	8.31	7.98	0.54	0.2487
Standing, %	21.54	23.97	1.98	0.1584
Lying down, %	63.48	61.97	2.65	0.6534
Number of lactations	3.27	2.61	0.19	0.0357

[1] CON: control group (lamb without synthesized milk), SM: synthesized milk group (lamb with synthesized milk), n = 9.

conditions. Interestingly, the increase in lysozyme activity may indicate improved innate immune defense at the gastrointestinal interface (Wang et al., 2025; Zhu et al., 2021), potentially contributing to greater resilience against microbial challenges during early development.

Alterations in rumen microbial composition further support the functional implications of synthetic milk supplementation. While microbial community structure at the phylum level remained relatively stable, distinct shifts were observed at the genus level. Specifically, the relative abundances of *NK4A214_group*, Christensenellaceae_*R-7_group*, *norank_f_Eubacterium_coprostanoligenes_group*, and Prevotellaceae_*NK3 B31_group* were increased, whereas *Dialister* abundance was reduced. Previous studies have linked these enriched taxa to carbohydrate fermentation, VFA production, and improved metabolic efficiency (Cerdó et al., 2019; Chen et al., 2024). The enrichment of these taxa may reflect a microbiota-level adaptive response facilitating efficient energy extraction under high-altitude nutritional constraints. Spearman correlation analysis further demonstrated significant associations between specific bacterial genera and fermentation metabolites (acetate, isobutyrate, and ammonia nitrogen) as well as cellulase activity. These coordinated microbial–functional linkages suggest that synthetic milk supplementation promotes the establishment of a rumen microbiota with enhanced fermentative efficiency and fiber-degrading capacity.

Moreover, several of the enriched genera have been associated with anti-inflammatory effects and metabolic health in previous studies, whereas *Dialister* has often been linked to microbial dysbiosis and inflammatory conditions (Pedrogo et al., 2018; Pramanick et al., 2021). Together, these microbial shifts suggest that synthetic milk supplementation may contribute to immune homeostasis through microbiota-mediated mechanisms.

Plasma metabolomic analysis provided direct evidence for systemic metabolic modulation induced by early-life synthetic milk supplementation. In lambs, differential metabolite analysis and pathway enrichment indicated significant alterations in amino acid metabolism, mineral absorption, and protein digestion and absorption pathways. Elevated concentrations of methionine, glycine, valine, and glutamic acid suggest enhanced availability of substrates involved in protein synthesis, immune regulation, and energy metabolism (Gao et al., 2024; Liu et al., 2025; Yu et al., 2021). Under high-altitude conditions, where increased basal metabolic demands and hypoxia-related stress may elevate amino acid requirements, such metabolic adjustments may be particularly beneficial for maintaining growth and immune competence. Increasing evidence indicates that amino acids function not only as structural nutrients but also as key metabolic regulators of immune cell activity and endocrine signaling (Yao et al., 2021). Glycine, in particular, has been reported to exert immunomodulatory effects and to participate in growth-related signaling pathways (Muñoz et al., 2023). The increased abundance of these amino acids may therefore contribute to the observed improvements in IgA, IgM, and IGF-1 levels, further supporting the coordinated regulation of a microbiota–metabolism–immune axis. Consistently, these metabolic alterations closely aligned with changes in rumen microbial composition and fermentation characteristics, suggesting that rumen microbiota may act as an upstream driver of systemic metabolic responses. Such cross-level integration among microbial, metabolic, and immune processes represents a defining feature of functional nutritional interventions.

An important yet relatively underexplored aspect of early nutritional interventions is their indirect influence on maternal physiology. In the present study, synthetic milk supplementation of lambs was associated with improved body condition scores and immune indices in ewes without markedly affecting maternal body weight. Behavioral observations revealed a reduced nursing frequency in supplemented groups, suggesting a potential alteration in energy allocation between ewes and lambs. Interestingly, the average daily feed intake at the ewe–lamb pair level was also higher in the supplemented group. Because both ewes and lambs had free access to the feed, this increase likely reflects enhanced overall feeding activity and earlier exposure of lambs to solid feed rather than changes in maternal intake alone. Such shifts in feeding behavior may have facilitated rumen functional stimulation and potentially contributed to the improved fermentation characteristics and microbial activity observed in the supplemented lambs. These findings indicate that offspring-directed nutritional supplementation may alleviate maternal metabolic burden during lactation; however, this interpretation still requires further validation. This hypothesis is partially supported by plasma metabolomic data from ewes, which revealed significant alterations in the pantothenate and coenzyme A biosynthesis pathway. Pantothenic acid plays a central role in energy metabolism and anabolic processes, including milk synthesis, and changes in this pathway may reflect modifications in metabolic pressure associated with lactation (Costanzo et al., 2021; Ragaller et al., 2010). However, given the complexity of maternal metabolic regulation, these metabolic changes cannot be directly interpreted as evidence of reduced milk production or alleviated metabolic burden and require further experimental validation. Similarly, the observed increase in maternal immunoglobulin levels and attenuation of pro-inflammatory tendencies may indicate an improved immune status; however, a direct causal relationship between offspring supplementation and maternal immune modulation remains speculative and warrants further investigation.

Collectively, synthetic milk supplementation was closely associated with coordinated changes in rumen microbial structure, fermentation-derived metabolic profiles, and systemic metabolic and immune states in lambs, suggesting its potential involvement in the coordinated development of physiological functions during early life. Importantly, when interpreted within the context of high-altitude adaptation, these findings highlight the potential of early nutritional interventions to buffer environmental constraints and support physiological resilience in both offspring and dams. This study provides systems-level physiological and metabolomic evidence that advances understanding of how early nutritional interventions may modulate host–microbiota–metabolism interactions and offers a foundation for further investigation of nutrition-based strategies in food bioscience.

5. Conclusion

Early-life supplementation with synthetic milk was associated with consistent changes in metabolic and immune indicators in lambs, together with shifts in rumen microbial composition and fermentation-related metabolic profiles. These responses coincided with improved growth performance during a critical developmental period, suggesting that milk-based nutritional input may contribute to physiological maturation beyond basic nutrient supply. Importantly, nutritional supplementation directed at offspring was also accompanied by measurable changes in maternal body condition and metabolic status, as reflected by alterations in circulating metabolites and immune-related indices. This observation indicates that early-life nutritional strategies may influence energy allocation and physiological balance at the level of the maternal–offspring unit, rather than acting solely on the developing animal. Taken together, the present findings provide integrative physiological and metabolomic observations supporting a role of early milk-based nutritional formulations in shaping host metabolic and microbial responses. While further studies are required to clarify the underlying molecular mechanisms, this work offers a bioscientific reference for the development and evaluation of early-life nutritional interventions within the scope of food bioscience.

CRediT authorship contribution statement

Yining Xie: Writing – original draft, Validation, Project administration. **Xiaokang Jing:** Methodology, Data curation. **Junhong Wang:** Investigation, Data curation, Formal analysis. **Zhaohan Zhan:** Software, Methodology. **Bao Yi:** Writing – review & editing, Supervision. **Liang Chen:** Writing – review & editing, Funding acquisition. **Hongfu Zhang:** Resources, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2026.108582.

Data availability

Data will be made available on request.

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