Research Article **Open Access**

Synbiotic-supplemented organic formula enhances infant gut health: a randomized controlled trial

Xiaoyang Sheng[1], Zhendong Gu[2*], Xuelian Li[2], Jiayi Ni[3]

[1]Department of Developmental Behavioral and Child Healthcare & Department of Medical Psychology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China;[2]Bellamy's Organic Food Trading (Shanghai) Co., Ltd., Shanghai, China;[3]Sprim (China) Consulting Co., Ltd., Shanghai, China.

***Corresponding author:** Zhendong Gu, Bellamy's Organic Food Trading (Shanghai) Co., Ltd, Shanghai, China.

Submission Date: January 14th, 2024, **Acceptance Date**: February 26th, **Publication Date**: February 28th, 2025

Please cite this article as: Sheng X., Gu Z., Li X., Ni J. Synbiotic-supplemented organic formula enhances infant gut health: a randomized controlled trial. *Functional Foods in Health and Disease* 2025; 15(2): 144-161
DOI: https://doi.org/10.31989/ffhd.v15i2.1517

ABSTRACT

Background: Background: Infant gut health is crucial for long-term well-being, with early microbial colonization playing a pivotal role. Organic infant formulas are increasingly sought after, and synbiotics, combining prebiotics and probiotics, have shown promise in promoting beneficial gut microbiota. This randomized controlled trial investigates the impact of a synbiotic-supplemented organic formula on infant gut health.

Objective: This study conducted a dual-center, double-blind, randomized, parallel-controlled trial to evaluate the effects of two cow's milk-based formulas—organic protein milk and organic protein milk with Bifidobacterium animalis subsp. lactis BB-12® and prebiotics DiGenix®—as well as breastfeeding, on gastrointestinal health and growth in infants aged 30 to 120 days.

Methods: Seventy-five healthy full-term infants were enrolled and divided equally into three groups: breastfeeding, organic protein milk formula, and organic protein milk with BB-12® and prebiotics DiGenix® formula. The study included four pediatric follow-ups at 30, 60, 90, and 120 days after birth, before the introduction of complementary feeding.

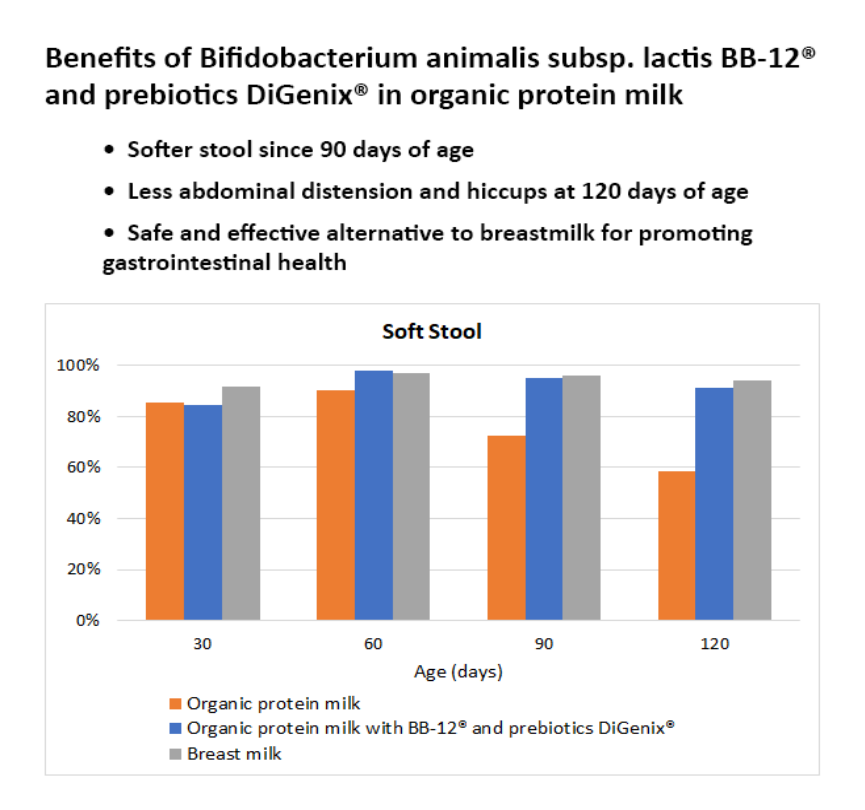
Results: Infants in the organic protein milk with BB-12® and prebiotics DiGenix® and breastfeeding groups had significantly softer stools and higher bowel movement frequencies at 90 and 120 days compared to those in the organic protein milk group, suggesting better gastrointestinal health due to the inclusion of BB-12® in organic protein milk with prebiotics DiGenix®. Growth parameters (weight, length and head circumference) and sleep patterns were similar across

all groups, indicating that all feeding methods provide adequate nutrition and similar sleep quality. However, less gastrointestinal discomfort (bloating and burping) was observed in the organic protein milk with BB-12® and prebiotics DiGenix® and breastfeeding groups, indicating better digestive tolerance. The safety profile was comparable across all groups.

Novelty of the study: This study is among the first randomized controlled trials comparing organic protein milk formula enriched with *Bifidobacterium animalis subsp. lactic* BB-12® and prebiotics DiGenix® to breastfeeding and standard formula in infants. The findings demonstrate significant improvements in gastrointestinal health, including softer stools, increased bowel movement frequency, and reduced digestive discomfort, positioning this formulation as a safe and effective alternative to breastfeeding.

Conclusions: These findings support organic protein milk with *Bifidobacterium animalis subsp. lactis* BB-12® and prebiotics DiGenix® as a safe and effective alternative to breastfeeding for promoting gastrointestinal health. Further research is needed to optimize formula composition and assess long-term health outcomes.

Keywords: Infant Nutrition, Gastrointestinal Health, Cow's Milk-Based Formula, *Bifidobacterium animalis subsp. lactis* BB-12®, Randomized Controlled Trial



Graphical Abstract: Synbiotic-supplemented organic formula enhances infant gut health: a randomized controlled trial.

INTRODUCTION

Breastfeeding is widely regarded as the optimal choice for infant nutrition, offering natural immune protection and comprehensive nutritional support that is crucial for an infant's health and development [1][2]. However, in reality, many mothers are unable to exclusively breastfeed for various reasons and may partially or fully rely on infant formula [3]. In recent years, the development and optimization of infant formulas have become a significant research area, aiming to closely replicate the nutritional composition and functional benefits of breast milk [4,5]. When choosing an infant formula, the primary considerations are natural milk sources and complete nutritional content [6]. A natural milk source ensures that the natural state of nutrients like proteins, lipids, vitamins, and minerals are maintained throughout the production process [7]. Furthermore, nutritional completeness requires that the formula contain all the essential nutrients to support the overall development of infants [8,9]. These demands have driven formula manufacturers to continuously improve their products by enhancing digestibility, absorption efficiency, and immune support for infants.

The establishment and maintenance of a healthy gut microbiota play a crucial role in an infant's early development [10]. Studies have shown that healthy gut microbiota supports the development of the immune system, protects against pathogenic infections, and facilitates normal metabolic functions [11,12]. Consequently, the development of infant formulas has increasingly focused on incorporating functional components that resemble those found in breast milk to mimic and supplement its benefits [13]. Among these strategies, the addition of probiotics is considered a key approach to enhance the functional and health value of infant formulas [14].

Bifidobacterium, one of the most prevalent beneficial bacteria in the intestines of breastfed infants, plays a critical role in regulating gut microbiota balance, promoting digestive health, and strengthening immune function [15–17]. Among the Bifidobacterium species, *Bifidobacterium animalis subsp. lactis* BB-12® (BB-12®) is one of the most widely used strains [18,19]. Research indicates that BB-12® can rapidly colonize the infant gut and improve gut health through multiple mechanisms. Firstly, BB-12® inhibits the growth of pathogenic bacteria through competitive exclusion, thereby reducing the risk of intestinal infections [20]. It adheres to the surface of intestinal epithelial cells, preventing pathogen attachment, and produces organic protein milk acids such as lactic acid and acetic acid that lower intestinal pH, creating an environment unfavorable for pathogenic bacteria [21]. Secondly, BB-12® stimulates the proliferation of intestinal epithelial cells and enhances the intestinal mucosal barrier function through the generation of its metabolites, such as short-chain fatty acids, thus counteracting potential pathogen invasion. Additionally, BB-12® shows significant benefits in modulating intestinal immune function [22,23]; it can regulate the activity of immune cells like macrophages and T cells, promote the production of anti-inflammatory factors, and thereby reduce excessive inflammatory responses [24–26].

The Nutrition Committee of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition has reviewed and evaluated the addition of probiotics and prebiotics to infant formulas [27,28]. The existing evidence suggests that certain specific probiotic strains may have beneficial effects on infant health [29,30]. However, it also highlighted that different strains may vary in their efficacy in improving gut health and immune function in infants, emphasizing the need for high-quality research to clarify the benefits of probiotic supplementation and further support its use in formula [31].

Therefore, in this study, we aimed to investigate the effects of adding the probiotic BB-12® to infant formula

on gastrointestinal development and stool characteristics in infants. By evaluating the regulatory effects of these specific formulas on the infant digestive system and their potential health benefits, this research seeks to provide a scientific basis for optimizing infant formula and offer more guidance for parents in choosing a suitable feeding option for their infants.

MATERIALS AND METHODS

Study Design: The aim of this study was to investigate the effects of organic protein milk infant formula supplemented with BB-12® on infant tolerance and fecal characteristics. Specifically, the study compared the effects of two bovine milk-based infant formulas and breastfeeding on gastrointestinal development and fecal traits in infants. A secondary objective was to evaluate the impact of the investigational product on digestive comfort, tolerance, and the growth and development of infants. Consequently, the trial was designed as a double-center, double-blind, randomized, parallel-controlled trial. The two centers were located at Qiubin Community Hospital and Nanquan Community Hospital in Jinhua City, Zhejiang Province. The participants were infants aged one month, and the intervention lasted for three months, from one to four months of age. Nutritional feeding included four pediatric follow-up points: before 30 ± 3 days, 60 ± 3 days, 90 ± 3 days, and 120 ± 3 days after birth.

Study Products and Administration: The study included two cow's milk-based infant formulas: Study Product A, Bellamy's Organic protein milk with BB-12® and prebiotics DiGenix® (Bellamy's Organic A), and Study Product B, Bellamy's Organic protein milk (Bellamy's Organic B). The Bellamy's Organic A formula contains 85.2 million CFU of BB-12®, 60 mg of fructo-oligosaccharides (FOS), and 120 mg of galacto-oligosaccharides (GOS) in each scoop (8.8 g) of powder. All study products were provided in their original 800-gram cans without repackaging. However, the original labels were replaced with study-specific labels that included the product description and feeding instructions.

Subject Recruitment: Based on preliminary experimental data and sample size estimates with a significance level of 0.5 and a power of 0.9, 75 healthy, full-term infants were recruited and randomly assigned with equal ratios to three groups: the breastfeeding group, Group A receiving the Bellamy's Organic A product, and Group B receiving the Bellamy's Organic B product. Accounting for a potential 12% dropout rate, the study aimed to ensure that at least 20 subjects in each group (60 in total) complete the study. The recruitment period was from February 3 to July 25, 2023. Recruitment updates were released every two weeks during this period (a total of 10 times).

Inclusion Criteria and Exclusion Criteria: The inclusion criteria are: healthy full-term infants (gestational age 37–42 weeks), aged one month (30 ± 3 days) at the start of the study, normal birth weight (2.5–4 kg), no prior antibiotic treatment, and informed consent from parents or guardians who can comply with the study protocol. Exclusion criteria include: infants with congenital or chronic diseases, complications at birth, allergies or intolerance to cow's milk protein, maternal medication that could affect the infant, inability to comply with follow-up requirements, or receiving special dietary interventions other than breastfeeding.

Comparison of Indexes: First, the basic information of the subjects was compared, including gender, ethnicity, age, anthropometric indicators, birth details, socioeconomic information, and medical history. Second, the primary comparison measures for this study included stool characteristics assessed at 30 days (baseline), 60 days, 90 days, and 120 days of age using the Amsterdam Infant Stool Scale. Additionally, the following indicators were compared at 30 days (baseline), 60 days, 90 days, and 120 days after birth: growth and development

parameters (weight, length, head circumference); gastrointestinal development and tolerance observation scale (anxiety levels: 0 = no anxiety, 1 = a little anxiety, 2 = moderate anxiety, 3 = very anxious, 4 = extremely anxious); crying duration (in hours); gastrointestinal symptoms (0-5 points: 0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = quite severe, 5 = very severe); occurrences of vomiting and nausea; infant sleep observation scale; and infant nutrition and feeding records. Adverse events occurring in both groups of subjects throughout the trial were also compared.

Statistical Analysis: Baseline data were summarized by study group, with means and standard deviations for normally distributed continuous variables, medians (with first and third quartiles) for non-normally distributed variables, and frequencies and percentages for categorical variables. Randomization validity was assessed using the F-test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Efficacy measures at baseline and post-intervention were summarized by group. Comparison between groups was performed using analysis of variance (ANOVA) or analysis of covariance (ANCOVA) for continuous variables, Fisher's exact test for categorical variables, and Kruskal-Wallis test for non-normally distributed variables. Growth and development measurements were adjusted for gender and baseline values in comparisons. Repeated measures logistic regression was applied to compare stool consistency between groups, considering the number of stools per participant. Adverse events, serious adverse events, and withdrawal rates were analyzed to assess product effectiveness. All participants were included in the analysis, and missing data from withdrawals were not replaced. A significance level of 0.05 was used for hypothesis tests, with Bonferroni correction for multiple comparisons. SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

RESULTS

Subject Baseline Characteristics: A total of 75 eligible subjects were enrolled at baseline, with 25 in each of the breastfeeding group, Group A, and Group B. During the trial, 9 subjects withdrew early due to personal reasons: 2 from Group A, 3 from Group B, and 4 from the breastfeeding group, resulting in an overall withdrawal rate of 12%. Ultimately, 66 subjects completed the trial according to the protocol. The subject characteristics, including demographic characteristics, anthropometric measures, allergy history, and socioeconomic status, were comparable among the three groups at baseline (Table 1).

Table 1. Subject baseline characteristics

Indicators	Group A (n=25)	Group B (n=25)	Breast milk group (n=20)	Overall P-value	A vs. B P-value
Demographic data					
Sex (%):				0.878	/
Male	11 (44.00)	13 (52.00)	13 (52.00)		
Female	14 (56.00)	12 (48.00)	12 (48.00)		
Han nationality (%)	25 (100.00)	25 (100.00)	25 (100.00)	NA	/
Mother Age (years)	31.28±1.46	31.40±0.96	31.44±1.16	0.888	/
Gestational age (weeks)	39.26±0.77	39.13±0.69	39.15±0.72	0.782	/
Mode of delivery (%):				0.024	0.387
Natural labor	17 (68.00)	13 (52.00)	22 (88.00)		
Caesarean section	8(32.00)	12 (48.00)	3 (12.00)		

Indicators	Group A (n=25)	Group B (n=25)	Breast milk group (n=20)	Overall P-value	A vs. B P-value
Mother's childbearing age (years)	30.68±3.59	31.16±4.21	29.72±3.36	0.387	/
Marital status of mother (yes, %):	25 (100.00)	25 (100.00)	25 (100.00)	NA	
Number of live births (%) [a]	0 (0, 1)	1 (0, 1)	1 (0, 1)	0.085	0.222
Breastfeeding after birth (%)	25 (100.00)	24 (96.00)	25 (100.00)	1.000	/
Breastfeeding duration (days) [a]	30 (15, 32)	31 (12, 32)	32 (31, 32)	0.085	0.776
Exclusive breastfeeding during the period (%)	0 (0.00)	1 (4.00)	25 (100.00)	<0.0001	1.000
Postnatal formula feeding (%)	25 (100.00)	25 (100.00)	0 (0.00)	<0.0001	1.000
Milk feeding duration (days) [a]	31 (30, 32)	31 (30, 32)	0 (0, 0)	<0.0001	0.858
Pure milk powder feeding during the period (%)	2 (8.00)	4 (16.00)	0 (0.00)	0.155	/
Family history of allergies					
Family Asthma or allergies (yes, %)	0 (0.00)	0 (0.00)	0 (0.00)	NA	
Resident Smokers (yes, %)	13 (52.00)	14 (56.00)	9 (36.00)	0.347	/
Exposed to smoking (yes, %)	2 (8.00)	4 (16.00)	2 (8.00)	0.718	/
Socioeconomic information					
Number of people living in the same household (person) [a]	5 (4, 5)	5 (4, 5)	5 (4, 5)	0.507	/
Living area of the house				0.171	/
Less than 60 m^2	1 (4.00)	0 (0.00)	1 (4.00)		
60-90 m^2	7 (28.00)	4 (16.00)	11 (44.00)		
90-120 m^2	9 (36.00)	12 (48.00)	10 (40.00)		
More than 120 m^2	8 (32.00)	9 (36.00)	3 (12.00)		
Mother's education level				0.772	/
Graduated from primary school	0 (0.00)	0 (0.00)	0 (0.00)		
Junior high school graduate	0 (0.00)	2 (8.00)	1 (4.00)		
Graduated from high school/college	15 (60.00)	15 (60.00)	15 (60.00)		
Bachelor's degree	8 (32.00)	7 (28.00)	9 (36.00)		
Master’s degree and above	2 (8.00)	1 (4.00)	0 (0.00)		
Father's education level				0.472	/
Primary school	0 (0.00)	0 (0.00)	0 (0.00)		
Junior high school	0 (0.00)	0 (0.00)	1 (4.00)		
High school/College	14 (56.00)	17 (68.00)	17 (68.00)		
Bachelor's degree	11 (44.00)	7 (28.00)	7 (28.00)		
Master’s degree and above	0 (0.00)	1 (4.00)	0 (0.00)		
Mother employed (yes, %)	16 (64.00)	13 (52.00)	18 (72.00)	0.380	/
Father employed. (yes, %)	25 (100.00)	25 (100.00)	25 (100.00)	NA	
Average monthly household income (RMB)				0.775	/
Less than 3000	0 (0.00)	0 (0.00)	0 (0.00)		
3000-5999	0 (0.00)	1 (4.00)	0 (0.00)		
6000-8000	4 (16.00)	6 (24.00)	6 (24.00)		
More than 8000	21 (8400)	18 (72.00)	19 (76.00)		

Group A was fed with organic protein milk with *Bifidobacterium animalis subsp. lactis* BB-12® and prebiotics DiGenix®; Group B was fed with organic protein milk. Unless otherwise stated, data presented are mean ± standard deviation or frequency (%). Between-group comparison was performed using one-way analysis of variance for continuous variables and using Fisher exact test for categorical variables.

[a] Data presented are median (1st quartile, 3rd quartile). Between-group comparison was performed using Kruskal-Wallis test.

Defecation of Subjects: The Amsterdam Infant Stool Scale was used to compare stool characteristics, and the statistical results showed no significant differences in 24-hour stool frequency, stool volume, stool hardness and color between Group A and Group B at baseline (Table 2). However, the frequency of defecation in both organic milk groups was significantly lower than that in the breastfeeding group (all $p < 0.0001$), while the volume per defecation was significantly higher ($p = 0.009$ for Group A and $p = 0.001$ for Group B) compared to that in the breastfeeding group. No significant differences were observed in other defecation indices among the three groups. During the intervention, the defecation frequency in both organic milk groups was significantly lower than that in the breastfeeding group at 60 days of age ($p = 0.0003$ and $p < 0.0001$, respectively) and at 90 days of age ($p = 0.014$ and $p < 0.0001$, respectively). At 120 days of age, the defecation frequencies of Group A and the breastfeeding group were comparable and were both significantly higher than that of Group B ($p = 0.014$ and $p = 0.001$, respectively). At 90 and 120 days of age, fecal hardness in Group A and the breastfeeding group was significantly different from Group B ($p = 0.014$ and $p = 0.009$ at 90 days; $p = 0.009$ and $p = 0.005$ at 120 days), with Group B having lower fecal water content (harder stools). No significant differences in stool volume or stool color were observed among the three groups from 60 to 120 days of age.

Table 2. Defecation within 24 hours (Amsterdam Infant Stool Scale)

Indicators	Age (days)		Group A	Group B	Breast milk group	P-value		
						A vs. B	A vs. Breast milk	B vs. Breast milk
Number of bowel movements, times	30		2.56±1.33	2.48±1.42	4.76±1.09	0.827	<0.0001*	<0.0001*
	60		2.08±0.88	1.78±1.04	3.09±0.79	0.262	0.0003*	<0.0001*
	90		1.71±0.62	1.32±0.72	2.23±0.75	0.062	0.014*	<0.0001*
	120		1.48±0.15	1.09±0.53	1.62±0.50	0.014*	0.366	0.001*
Number of stool samples	30		64	62	119	/	/	/
	60		50	41	71	/	/	/
	90		41	29	49	/	/	/
	120		34	24	35	/	/	/
Stool texture	30	Watery	10 (15.63)	8 (12.90)	10 (8.40)	0.606	0.387	0.664
		Soft	54 (84.38)	53 (85.48)	109 (91.60)			
		Shaped	0 (0.00)	1 (1.61)	0 (0.00)			
		Hard	0 (0.00)	0 (0.00)	0 (0.00)			
	60	Watery	1 (2.00)	2 (4.88)	2 (2.82)	0.735	0.857	0.680
		Soft	42 (98.00)	37 (90.24)	69 (97.18)			
		Shaped	0 (0.00)	2 (4.88)	0 (0.00)			
		Hard	0 (0.00)	0 (0.00)	0 (0.00)			
	90	Watery	0 (0.00)	0 (0.00)	0 (0.00)	0.014*	0.844	0.009*
		Soft	39 (95.12)	21 (72.41)	47 (95.92)			
		Shaped	2 (4.88)	7 (24.14)	2 (4.08)			
		Hard	0 (0.00)	1 (3.45)	0 (0.00)			
	120	Watery	0 (0.00)	0 (0.00)	0 (0.00)	0.009*	0.675	0.005*
		Soft	31 (91.18)	14 (58.33)	33 (94.29)			
		Shaped	3 (8.82)	8 (33.33)	2 (5.71)			
		Hard	0 (0.00)	2 (8.33)	0 (0.00)			

Indicators	Age (days)		Group A	Group B	Breast milk group	P-value		
						A vs. B	A vs. Breast milk	B vs. Breast milk
The amount of defecation	30	A little	10 (15.63)	8 (12.90)	38 (31.93)	0.614	0.009*	0.001*
		<25%	24 (37.50)	22 (35.48)	49 (41.18)			
		25-50%	16 (25.00)	15 (24.19)	32 (26.89)			
		>50%	14 (21.88)	17 (27.42)	0 (0.00)			
	60	A little	1 (2.00)	0 (0.00)	4 (5.63)	0.822	0.067	0.123
		<25%	14 (28.00)	12 (29.27)	26 (36.62)			
		25-50%	28 (56.00)	24 (58.54)	37 (52.11)			
		>50%	7 (14.00)	5 (12.20)	4 (5.63)			
	90	A little	0 (0.00)	0 (0.00)	1 (2.04)	0.884	0.114	0.173
		<25%	6 (14.63)	4 (13.79)	9 (18.37)			
		25-50%	22 (53.66)	15 (51.72)	29 (59.18)			
		>50%	13 (31.71)	10 (34.48)	10 (20.41)			
	120	A little	0 (0.00)	0 (0.00)	1 (2.86)	0.474	0.633	0.278
		<25%	1 (2.86)	3 (12.50)	5 (14.29)			
		25-50%	24 (68.57)	10 (41.67)	17 (48.57)			
		>50%	10 (28.57)	11 (45.83)	12 (34.29)			
Stool color	30	Yellow	25 (39.06)	19 (30.65)	43 (36.13)	0.378	0.421	0.988
		Orange	34 (53.13)	34 (54.84)	57 (47.90)			
		Green	5 (7.81)	7 (11.29)	19 (15.97)			
		Brown	0 (0.00)	2 (3.23)	0 (0.00)			
		Meconium	0 (0.00)	0 (0.00)	0 (0.00)			
		Pottery clay	0 (0.00)	0 (0.00)	0 (0.00)			
	60	Yellow	22 (44.00)	13 (31.71)	33 (46.48)	0.476	0.762	0.287
		Orange	25 (50.00)	27 (65.85)	35 (49.30)			
		Green	3 (6.00)	1 (2.44)	3 (4.23)			
		Brown	0 (0.00)	0 (0.00)	0 (0.00)			
		Meconium	0 (0.00)	0 (0.00)	0 (0.00)			
		Pottery clay	0 (0.00)	0 (0.00)	0 (0.00)			
	90	Yellow	12 (29.27)	8 (27.59)	17 (34.69)	0.577	0.956	0.638
		Orange	26 (63.41)	16 (55.17)	25 (51.02)			
		Green	3 (7.32)	5 (17.24)	7 (14.29)			
		Brown	0 (0.00)	0 (0.00)	0 (0.00)			
		Meconium	0 (0.00)	0 (0.00)	0 (0.00)			
		Pottery clay	0 (0.00)	0 (0.00)	0 (0.00)			
	120	Yellow	2 (5.88)	2 (8.33)	5 (14.29)	0.763	0.999	0.814
		Orange	28 (82.35)	20 (83.33)	23 (65.71)			
		Green	4 (11.76)	2 (8.33)	7 (20.00)			
		Brown	0 (0.00)	0 (0.00)	0 (0.00)			
		Meconium	0 (0.00)	0 (0.00)	0 (0.00)			
		Pottery clay	0 (0.00)	0 (0.00)	0 (0.00)			

Group A was fed with organic protein milk with *Bifidobacterium animalis subsp. lactis* BB-12® and prebiotics DiGenix®; Group B was fed with organic protein milk. Data presented are mean ± standard deviation or frequency (%). Between-group comparison was performed using analysis of variance for continuous variables and using repeated-measures logistic regression for stool characteristics. Pair-wise group comparisons were adjusted using Bonferroni method. *: $p<0.05/3$

Growth and Development of Subjects: At baseline and throughout the trial intervention, there were no significant between-group differences in anthropometric measures or related WHO growth and development Z-scores among the three groups at any of the visits (Table 3).

Table 3. Anthropometric measurements

Indicators	Age (days)	Group A	Group B	Breast milk group	P-value		
					A vs. B	A vs. Breast milk	B vs. breast milk
Age (days)	30	31.28±1.46	31.40±0.96	31.44±1.16	0.727	0.641	0.907
	60	61.54±1.10	60.96±1.33	61.26±1.01	0.087	0.408	0.375
	90	91.54±1.06	90.82±1.65	91.45±1.34	0.077	0.829	0.126
	120	122.04±1.11	121.45±1.50	121.76±1.09	0.119	0.458	0.423
Weight, g	30	4598.80±496.83	4628.00±465.94	4494.80±362.03	0.912	0.339	0.285
	60	5737.50±630.95	5739.13±583.67	5560.00±565.59	0.982	0.478	0.467
	90	6695.83±762.42	6695.45±698.62	6459.09±676.59	0.901	0.361	0.438
	120	7326.09±825.29	7314.55±805.86	7061.90±720.75	0.768	0.283	0.436
Length, cm	30	54.38±1.48	54.60±1.83	54.41±1.41	0.719	0.936	0.659
	60	58.85±1.91	58.83±2.41	58.33±2.03	0.531	0.130	0.374
	90	62.22±2.30	62.31±2.29	61.59±2.16	0.484	0.136	0.434
	120	64.74±2.35	64.77±2.32	64.21±2.31	0.448	0.213	0.623
Body mass index, kg/m ^{2}	30	15.51±1.12	15.49±0.94	15.16±0.65	0.896	0.166	0.208
	60	16.52±1.10	16.56±0.94	16.30±0.80	0.654	0.963	0.627
	90	17.24±0.99	17.20±1.04	16.98±0.83	0.945	0.580	0.539
	120	17.43±1.08	17.40±1.26	17.08±0.86	0.948	0.380	0.417
Head circumference, cm	30	37.30±1.28	37.20±1.01	37.31±0.56	0.577	0.882	0.682
	60	39.03±1.01	38.82±1.08	38.89±0.70	0.860	0.438	0.553
	90	40.54±1.22	40.41±1.26	40.18±0.97	0.978	0.167	0.184
	120	41.62±1.29	41.43±1.29	41.24±1.03	0.773	0.165	0.274
WHO weight-age Z score	30	0.39±0.82	0.40±0.79	0.19±0.53	0.910	0.370	0.312
	60	0.52±0.87	0.54±0.89	0.23±0.66	0.935	0.257	0.229
	90	0.73±0.98	0.72±0.93	0.40±0.67	0.972	0.241	0.237
	120	0.72±0.97	0.68±0.99	0.39±0.65	0.909	0.251	0.304
WHO length-age Z score	30	0.07±0.74	0.13±0.96	0.03±0.61	0.743	0.902	0.651
	60	0.54±0.97	0.54±1.25	0.23±0.88	0.977	0.355	0.345
	90	0.79±1.09	0.83±1.19	0.43±0.85	0.882	0.280	0.229
	120	0.86±1.11	0.84±1.21	0.57±0.84	0.984	0.400	0.415
WHO weight-length Z score	30	0.48±0.73	0.44±0.72	0.23±0.48	0.849	0.206	0.281
	60	0.23±0.66	0.29±0.80	0.15±0.53	0.770	0.707	0.510
	90	0.32±0.62	0.30±0.65	0.19±0.48	0.914	0.501	0.579
	120	0.32±0.71	0.29±0.79	0.10±0.55	0.884	0.316	0.394
WHO body mass index-age Z score	30	0.50±0.80	0.46±0.70	0.24±0.47	0.892	0.187	0.235
	60	0.31±0.72	0.33±0.69	0.14±0.49	0.894	0.397	0.332
	90	0.39±0.69	0.37±0.69	0.21±0.48	0.929	0.381	0.439

Indicators	Age (days)	Group A	Group B	Breast milk group	P-value		
					A vs. B	A vs. Breast milk	B vs. breast milk
WHO head circumference-age Z score	120	0.33±0.72	0.29±0.81	0.10±0.54	0.881	0.299	0.376
	30	0.32±1.05	0.17±0.80	0.27±0.59	0.564	0.850	0.697
	60	0.29±0.83	0.12±0.89	0.10±0.69	0.479	0.515	0.957
	90	0.45±0.98	0.33±1.04	0.09±0.79	0.697	0.228	0.420
	120	0.46±1.01	0.26±1.03	0.08±0.83	0.545	0.237	0.558

Group A was fed with organic protein milk with *Bifidobacterium animalis subsp. lactis* BB-12® and prebiotics DiGenix®; Group B was fed with organic protein milk. Data presented are mean ± standard deviation. Between-group comparison was performed using analysis of covariance, adjusted for sex. Models for post-intervention data also adjusted for baseline measurements. Pair-wise group comparisons were adjusted using the Bonferroni method. WHO, World Health Organization.

Tolerance and Gastrointestinal Symptoms of Subjects: There were no significant differences in tolerance and gastrointestinal symptom scores among the three groups at baseline (Table 4). Abdominal bloating severity was significantly lower in Group A and the breastfeeding group than in Group B at 120 days of age during the trial intervention (p = 0.015 and 0.010, respectively). Hiccup severity was significantly lower in the breastfeeding group than in Group B at 60, 90, and 120 days of age (p = 0.014, 0.007, and 0.007, respectively), and was also significantly lower in Group A than in Group B at 120 days of age (p = 0.012). The severity of flatulence in the breastfeeding group was significantly lower than that in Group B at 90 and 120 days of age (p = 0.006 and 0.008, respectively). There were no significant differences in tolerance and gastrointestinal symptom scores between Group A and the breastfeeding group during the trial. Except for the above indicators, there were no significant differences in the tolerance and other gastrointestinal symptom scores among the three groups during the intervention period. Sleep patterns during the study period were also comparable among the three groups (Supplemental Table S1). The two formula feeding groups had similar daily formula intake amount at each visit during the study (Supplemental Table S2).

Table 4. Tolerability and gastrointestinal symptom scores

Indicators	Age (days)	Group A	Group B	Breast milk group	P-value		
					A vs. B	A vs. Breast milk	B vs. breast milk
Tolerance (last 24 hours)							
Anxious	30	1 (0, 1)	1 (0, 1)	1 (0, 1)	0.881	0.691	0.815
	60	1 (0, 1)	1 (0, 1)	1 (0, 1)	0.760	1.000	0.751
	90	0 (0, 1)	1 (0, 1)	1 (0, 1)	0.697	0.797	0.875
	120	1 (0, 1)	0 (0, 1)	0 (0, 1)	0.960	0.790	0.786
Crying duration, hours	30	1 (1, 2)	1 (1, 2)	1 (1, 2)	0.489	0.918	0.607
	60	1 (1, 2)	1 (1, 2)	1 (1, 2)	0.831	1.000	0.820
	90	1 (0.5, 1)	1 (1, 1)	1 (0, 1)	0.950	0.682	0.585
	120	1 (1, 1)	1 (1, 1)	1 (0, 1)	0.838	0.869	0.738
Gastrointestinal symptoms (last 7 days)							
The severity of	30	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.954	0.795	0.767

Indicators	Age (days)	Group A	Group B	Breast milk group	P-value		
					A vs. B	A vs. Breast milk	B vs. breast milk
abdominal distension	60	0 (0, 1)	0 (0, 2)	0 (0, 1)	0.199	0.633	0.099
	90	0 (0, 0.5)	0.5 (0, 1)	0 (0, 1)	0.086	1.000	0.068
	120	0 (0, 0)	0.5 (0, 1)	0 (0, 0)	0.015*	0.781	0.010*
The severity of hiccups	30	0 (0, 1)	0 (0, 1)	0 (0, 0)	0.990	0.761	0.751
	60	0 (0, 0.5)	1 (0, 1)	0 (0, 0)	0.065	0.512	0.014*
	90	0 (0, 0)	1 (0, 1)	0 (0, 0)	0.022	0.548	0.007*
	120	0 (0, 0)	0 (0, 1)	0 (0, 0)	0.012*	0.716	0.007*
Severity of flatulence	30	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.784	0.568	0.399
	60	0 (0, 1)	0 (0, 1)	0 (0, 0)	0.276	0.444	0.097
	90	0 (0, 0)	0 (0, 1)	0 (0, 0)	0.025	0.451	0.006*
	120	0 (0, 0)	0 (0, 1)	0 (0, 0)	0.028	0.383	0.008*
Diarrhea severity	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.977	0.317	0.317
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.307	0.307	1.000
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
Constipation severity	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.317	1.000	0.317
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
Severity of colic (cramp)	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.317	1.000	0.317
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	0.307	0.317
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
Severity of diaper dermatitis (diaper rash)	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.959	0.338	0.296
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.836	0.662	0.548
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.910	0.807	0.733
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.279	0.501	0.678
Severity of dorsal arch	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
Vomiting times, times	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.668	1.000	0.668
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.356	0.720	0.590
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.928	0.626	0.573
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.927	0.172	0.162
Number of nausea, times	30	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	60	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.530	0.328	0.153
	90	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000
	120	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.000	1.000	1.000

Group A was fed with organic protein milk with Bifidobacterium animalis subsp. lactis BB-12® and prebiotics DiGenix®; Group B was fed with organic protein milk. Data presented are median (1st quartile, 3rd quartile). Between-group comparison was performed using Kruskal Wallis test. Pair-wise group comparisons were adjusted using the Bonferroni method. *: $p<0.05/3$

Adverse Events: The occurrence of adverse events during the trial were summarized by event type and group (Table 5).

There were no significant between-group differences in the incidence of gastrointestinal or overall adverse events.

All adverse events were unrelated to the investigational products, and no serious adverse events occurred during the trial.

Table 5. Adverse events by study group

Adverse events code	Name of the adverse event	Group A (n=25)	Group B (n=25)	Breast milk group (n=25)	Overall P-value
Gastrointestinal tract					
GI001	Vomit	2	3	3	/
GI004	Flatulence	6	10	1	/
GI004	Diarrhea	1	1	1	/
Number of gastrointestinal adverse events		9	14	5	/
Number of gastrointestinal adverse events (%)		8 (32.00)	12 (48.00)	5 (20.00)	0.126
Musculoskeletal					
MS003	Trauma	1	0	0	/
Respiratory system					
RESP001	Cold/Respiratory Viral Infections	1	4	0	/
Body system					
BODY022	Have a fever	1	1	0	/
Skin					
SK001	Diaper rash	6	3	6	/
SK003	Dry skin	0	0	1	/
SK004	Eczema	0	0	1	/
SK0033	Abscess	0	1	0	/
Other					
UG017	Hydrocele	0	0	1	/
Total number of adverse events		**18**	**23**	**14**	**/**
Total number of adverse events (%)		**14 (56.00)**	**16 (64.00)**	**10 (40.00)**	**0.271**

Group A was fed with organic protein milk with Bifidobacterium animalis subsp. lactis BB-12® and prebiotics DiGenix®; Group B was fed with organic protein milk. Data presented are frequency (%). Between-group comparison was performed using Fisher exact test.

DISCUSSION

This study evaluated the effects of two cow's milk-based infant formulas and breastfeeding on the gastrointestinal development, stool characteristics, digestive comfort, tolerance, and growth of infants aged 30 to 120 days. The results of the study suggested that organic protein milk with BB-12® and prebiotics DiGenix® formula performed better than organic protein milk formula in supporting infant growth and development, improving gastrointestinal tolerance, and enhancing stool characteristics, showing similarities to the effects of breastfeeding in these areas [32]. These findings provide strong scientific evidence for the optimization of infant formula development and further support the strategy of incorporating probiotics in infant formulas to promote gastrointestinal health in non-breastfed infants.

Besides nutrients, breast milk contains bioactive compounds that support the growth and development of infants. Bioactive compounds evoke a bioactive impact on the human body, ideally to promote health [33]. When added to infant formulas, these compounds can enhance the functional and health value of the formulas and improve their simulation of breast milk. Our study found that infants in the organic protein milk with BB-12® and prebiotics DiGenix® group and the breastfeeding group had significantly softer stools at 90 and 120 days of age compared to those in the organic protein milk group. This suggests that feeding with the organic protein milk with BB-12® and prebiotics DiGenix® formula is similar to breastfeeding in maintaining stool softness, which could be attributed to the probiotics included in its formulation. The BB-12® probiotic has been demonstrated to improve gut microbiota composition [34], promote stool softening, and enhance bowel regularity [35]. Conversely, infants in the organic protein milk group had firmer stools, which may indicate a relative disadvantage in promoting intestinal health and stool elimination [36].

The synergistic effects of prebiotics and probiotics in infant nutrition are a key area of emerging research [37,38]. Prebiotics such as GOS and FOS are non-digestible fibers that serve as a food source for beneficial gut bacteria, promoting their growth and activity [39]. When combined with probiotics like BB-12®, GOS and FOS enhance the survival and colonization of these beneficial microbes in the gut, leading to improved gastrointestinal health and immune function [40]. Specifically, BB-12® benefits from the selective fermentation of GOS and FOS, which produces short-chain fatty acids to lower intestinal pH and inhibit the growth of harmful bacteria [41]. This symbiotic relationship between prebiotics and probiotics helps to mimic the effects of breast milk, supporting a balanced infant gut microbiota, improving stool consistency, and reducing gastrointestinal discomfort, making it highly beneficial for non-breastfed infants.

Further analysis also showed that infants in the organic protein milk with BB-12® and prebiotics DiGenix® and the breastfeeding groups had significantly higher bowel movement frequencies at 120 days compared to those in the organic protein milk group. Increased bowel frequency is generally considered a positive indicator of gastrointestinal health, especially in infancy. Therefore, these results suggest that the addition of BB-12® and prebiotics DiGenix® in infant formula facilitates the simulation of the beneficial gastrointestinal effects of breastfeeding.

The results of the study indicated that there were no significant differences in supporting infant growth and development among the three feeding methods. This finding is crucial as it demonstrates that both formulas can provide adequate nutrition to meet the normal growth needs of infants. While the effects of formula feeding and breastfeeding on growth are comparable, the importance of stool characteristics and gut health for long-term infant development should not be overlooked. The advantage of the organic protein milk with BB-12® and prebiotics DiGenix® in promoting gut health could make it a superior choice for infant formula.

Infants in the organic protein milk with BB-12® and prebiotics DiGenix® and breastfeeding groups experienced significantly less bloating and burping at 120 days of age than those in the organic protein milk group, suggesting a greater advantage in gastrointestinal tolerance for the former two groups. Additionally, breastfed infants exhibited significantly less burping between 60 and 120 days and less gastrointestinal bloating between 90 and 120 days compared to those in the organic protein milk group. This data clearly supports the effectiveness of the organic protein milk with BB-12® and prebiotics DiGenix® in reducing gastrointestinal discomfort, likely due to its unique formulation.

However, the clinical significance of these mild symptoms over the long term requires further exploration.

There were no significant differences among the feeding groups in terms of night sleep duration, day sleep duration, number of night awakenings, or duration of night awakenings. This indicates that different feeding methods have no obvious impact on infant sleep patterns, suggesting that sleep quality is balanced across feeding groups when other factors (such as gastrointestinal symptoms) are similar. This finding may be of particular relevance to mothers choosing formula feeding, which has comparable performance with breastfeeding in this regard [42].

Moreover, the incidence of adverse events did not significantly differ among groups, and no serious adverse events related to the trial products were reported. These findings provide solid safety data for the two infant formulas used in this study, supporting their use as safe alternatives to breastfeeding [43]. For infants who cannot be breastfed, organic protein milk with BB-12® and prebiotics DiGenix® may represent a safer and more effective choice. Overall, feeding with organic protein milk with BB-12® and prebiotics DiGenix® demonstrated similarities to breastfeeding in gastrointestinal tolerance and stool characteristics and even outperformed organic protein milk in certain gastrointestinal health parameters [44,45]. This suggests that its formula design has successfully emulated some of the beneficial effects of breast milk on infant health [46]. However, the study also showed no significant differences in growth and sleep quality among the three feeding methods, indicating that in terms of growth metrics, the performance of these formulas is very close to that of breast milk.

Functional foods are “natural or processed foods that contain effective and non-toxic amounts of bioactive compounds, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms” [47]. As a manufactured food designed for closely replicating human breast milk, infant formulas with the supplementation of bioactive compounds such as probiotics and prebiotics have demonstrated their potential as functional foods. This research provides novel insights into the role of synbiotics in infant nutrition, being among the first clinical trials to evaluate organic protein milk-based formula supplemented with *Bifidobacterium animalis subsp. lactis* BB-12® and prebiotics DiGenix®. Unlike conventional cow milk formulas, this formulation closely mimics the gastrointestinal benefits of breastfeeding, as evidenced by softer stools, higher bowel movement frequency, and reduced bloating and burping. The study further confirms this synbiotic-enhanced formula’s safety and nutritional adequacy, offering a scientifically validated alternative for infants who cannot be breastfed. These findings highlight the potential of targeted synbiotic formulations in improving digestive health, warranting further research on their long-term benefits and optimization in infant nutrition.

Despite the promising findings, several limitations need to be considered. The study duration was limited to 120 days, which may not fully capture the long-term effects of the formulas on infant development. Further research with longer follow-up periods is necessary to assess sustained benefits [26]. Additionally, the sample size was relatively small, and the findings may not be generalizable to all populations. A larger, more diverse sample would provide a clearer understanding of the formula’s impact across different groups. While no significant differences were found in sleep patterns, the study did not explore other potential influences on infant behavior and development. Future studies should investigate the broader effects of these feeding methods on cognitive and behavioral outcomes. Moreover, external factors, such as environmental or genetic

influences, were not fully controlled for and could affect gastrointestinal health. Further studies are needed to account for these variables. Finally, more in-depth research into the specific components of the formula and their physiological mechanisms is necessary to optimize the nutritional and functional properties of infant formulas and further improve infant health [48]. Through more refined analyses and broader studies, we can better understand the role of infant formula and its comparability to breast milk, thereby better guiding clinical practice and maternal and infant feeding choices.

CONCLUSIONS

While the findings of this study provide strong evidence supporting the efficacy and safety of the organic protein milk with BB-12® and prebiotics DiGenix® formula, further research is warranted to explore its long-term effects, its impact across diverse populations, and its broader implications on overall infant health and development.

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; BB-12®, Bifidobacterium animalis subsp. Lactis BB-12®; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; SD, standard deviation; WHO, World Health Organization.

Acknowledgement: BB-12® is a registered trademark of Chr. Hansen A/S, a subsidiary of the Novonesis Group. DiGeniX® is a trademark of Bellamy's Organic Food Trading (Shanghai) Co., Ltd. Their support has been instrumental in enabling the successful completion of this research. The authors would like to express their sincere gratitude to Sprim China for their excellent work in coordinating the study.

Conflict of Interest Statement: Zhendong Gu and Xuelian Li are employees of Bellamy's Organic Food Trading (Shanghai) Co., Ltd., which provided funding for this study. Other authors declare no conflicts of interest regarding the publication of this article.

Funding Statement: This study was funded by Bellamy's Organic Food Trading (Shanghai) Co., Ltd.

Ethics Statement: This study was approved by the Shanghai Nutrition Society Ethics Committee on December 23, 2022, with the approval number Ying Shen [2022] No.03, and it was registered on January 24, 2023, with the registration number NCT05695300.

Author Contributions

Xiaoyang Sheng: conceptualization, methodology, writing and reviewing. Zhendong Gu: conceptualization, writing and reviewing. Xuelian Li: conceptualization, writing and reviewing. Jiayi Ni: data management and analysis, writing and reviewing. All authors read and approved the final version of the manuscript.

REFERENCES

1. Martin CR, Ling PR, Blackburn GL. Review of infant feeding: Key features of breast milk and infant formula. *Nutrients*. 2016;8(5). DOI: https://doi.org/10.3390/nu8050279
2. Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. *The Lancet*. 2016;387(10017):475-490. DOI: https://doi.org/10.1016/S0140-6736(15)01024-7
3. Brown A, Raynor P, Lee M. Healthcare professionals' and mothers' perceptions of factors that influence decisions to breastfeed or formula feed infants: A comparative study. *J Adv Nurs*. 2011;67(9):1993-2003. DOI: https://doi.org/10.1111/j.1365-2648.2011.05647.x
4. Lönnerdal B. Infant formula and infant nutrition: Bioactive proteins of human milk and implications for composition of infant formulas. *American Journal of Clinical Nutrition*. 2014;99(3). DOI: https://doi.org/10.3945/ajcn.113.071993
5. Koletzko B, Bhutta ZA, Cai W, Cruchet S, El Guindi M, Fuchs GJ. Compositional requirements of follow-up formula for

use in infancy: Recommendations of an international expert group coordinated by the early nutrition academy. *Annals of Nutrition and Metabolism*. 2013;62(1):44-54. DOI: https://doi.org/10.1159/000345906

6. Du N, Dimaggio DM, Porto AF. Nutrition Content of Young Child Formulas. *J Pediatr Gastroenterol Nutr*. 2023;76(4):512-516. DOI: https://doi.org/10.1097/MPG.0000000000003712
7. Setianingsih T, Hussain N. Analysis of the completeness of specific nutritional interventions as an effort to prevent stunting: an observational study. *Padjadjaran Journal of Dentistry*. 2023;35(3):237. DOI: https://doi.org/10.24198/pjd.vol35no3.50724
8. Ballard O, Morrow AL. Human Milk Composition. Nutrients and Bioactive Factors. *Pediatr Clin North Am*. 2013;60(1):49-74. DOI: https://doi.org/10.1016/j.pcl.2012.10.002
9. Castillo N, McCoy M. Associations Between Infant Formula Exposure, Housing Instability and Postneonatal Mortality Among Participants in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC). *Matern Child Health J*. Published online 2024. DOI: https://doi.org/10.1007/s10995-024-03981-9
10. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J. The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews*. 2017;81(4): e00036-17. DOI: https://doi.org/10.1128/mmbr.00036-17
11. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157(1):121-141. DOI: https://doi.org/10.1016/j.cell.2014.03.011
12. Chuang YF, Fan KC, Su YY, Chen MH, Chen YT, Chen YJ. Precision probiotics supplement strategy in aging population based on gut microbiome composition. *Briefings in Bioinformatics*. 2024;25(4): bbae351. DOI: https://doi.org/10.1093/bib/bbae351
13. Lönnerdal B. Infant formula and infant nutrition: Bioactive proteins of human milk and implications for composition of infant formulas. *American Journal of Clinical Nutrition*. 2014;99(3). DOI: https://doi.org/10.3945/ajcn.113.071993
14. Lin Y, Xie Z, Li Z, Zhang S, Chen F, Wang B. The microbiota-gut-brain axis: A crucial immunomodulatory pathway for *Bifidobacterium animalis* subsp. *lactis*' resilience against LPS treatment in neonatal rats. *International Journal of Biological Macromolecules*. 2024; 266:131255. DOI: https://doi.org/10.1016/j.ijbiomac.2024.131255
15. Lin Y, Xie Z, Li Z, Zhang H, Zheng B, Chen S, et al. The microbiota-gut-brain axis: A crucial immunomodulatory pathway for *Bifidobacterium animalis* subsp. *lactis*' resilience against LPS treatment in neonatal rats. *Int J Biol Macromol.* 2024;266. DOI: https://doi.org/10.1016/j.ijbiomac.2024.131255
16. Turroni F, Milani C, Duranti S, Mahony J, van Sinderen D, Ventura M. *Bifidobacteria* and the infant gut: An example of co-evolution and natural selection. *Cell Mol Life Sci.* 2018;75(1):103-118. DOI: https://doi.org/10.1007/s00018-017-2672-0
17. Cai R, Zhang J, Song Y, Liu X, Xu H. Research Progress on the Degradation of Human Milk Oligosaccharides (HMOs) by Bifidobacteria. *Nutrients*. 2025;17(3). DOI: https://doi.org/10.3390/nu17030519
18. Szajewska H, Chmielewska A. Growth of infants fed formula supplemented with Bifidobacterium lactis Bb12 or Lactobacillus GG: A systematic review of randomized controlled trials. *BMC Pediatr*. 2013;13(1). DOI: https://doi.org/10.1186/1471-2431-13-185
19. Li Y, Arai S, Kato K, Tsuji NM, Kiyono H, Kawamura YI, et al. The potential immunomodulatory effect of *Bifidobacterium longum* subsp. *longum* BB536 on healthy adults through plasmacytoid dendritic cell activation in the peripheral blood. *Nutrients.* 2024;16(1). DOI: https://doi.org/10.3390/nu16010042
20. Taipale T, Pienihäkkinen K, Isolauri E, Larsen C, Brockmann E, Alanen P, Jokela J, Söderling E. et al. Bifidobacterium animalis subsp. lactis BB-12 in reducing the risk of infections in infancy. *British Journal of Nutrition*. 2011;105(3):409-416. DOI: https://doi.org/10.1017/S0007114510003685
21. Collado MC, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. *Lett Appl Microbiol*. 2007;45(4):454-460. DOI: https://doi.org/10.1111/j.1472-765X.2007.02212.x
22. Yan F, Polk DB. Probiotics and immune health. *Curr Opin Gastroenterol*. 2011;27(6):496-501. DOI: https://doi.org/10.1097/MOG.0b013e32834baa4d
23. Castro-Herrera VM, Fisk HL, Wootton M, Lown M, Owen-Jones E, Lau M, et al. Combination of the Probiotics *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis*, BB-12 Has Limited Effect on Biomarkers of Immunity and Inflammation in Older People Resident in Care Homes: Results From the Probiotics to Reduce Infections iN CarE home reSidentS Randomized, Controlled Trial. *Front Immunol*. 2021; 12:643321. DOI: https://doi.org/10.3389/fimmu.2021.643321.
24. Splichal I, Donovan SM, Kindlová Z, Stranak Z, Neužil Bunešová V, Sinkora M, et al. Release of HMGB1 and Toll-

like Receptors 2, 4, and 9 Signaling Are Modulated by Bifidobacterium *animalis* subsp. *lactis* BB-12 and *Salmonella* Typhimurium in a Gnotobiotic Piglet Model of Preterm Infants. *Int J Mol Sci.* 2023;24(3):2329. DOI: https://doi.org/10.3390/ijms24032329&#8203

25. Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy*. 2007;37(4):498-505. DOI: https://doi.org/10.1111/j.1365-2222.2006.02629.x
26. Yang J, Qu H, Liu Q, Wang Y, Cao J, Jiang F, et al. Global Insights and Key Trends in Gut Microbiota Research for Premature Infants: A Bibliometric and Visualization Study. *J Multidiscip Healthc.* 2024; 17:4611-4626. DOI: https://doi.org/10.2147/JMDH.S483332
27. Van Den Akker CHP, van Goudoever JB, Shamir R, Domellöf M, Embleton ND, Hojsak I, et al. Probiotics and preterm infants: A position paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr.* 2020;70(5):664-680. DOI: https://doi.org/10.1097/MPG.0000000000002655
28. Natale F, Fusco C, Limatola M, Cante L, Alfieri R, Mollo N, et al. Gut Microbiome modulation in patients treated with an enriched nutraceutical composition: A multi target strategy. *Functional Foods in Health and Disease.* 2024;14(12):883-897. DOI: https://doi.org/10.31989/ffhd.v14i12.1437
29. Thomas DW, Greer FR, American Academy of Pediatrics Committee on Nutrition, American Academy of Pediatrics Section on Gastroenterology, Hepatology, and Nutrition. Clinical report—Probiotics and prebiotics in pediatrics. Pediatrics. 2010;126(6):1217–1231. DOI: https://doi.org/10.1542/peds.2010-2548
30. Madtohsoh M, Detarun P, Wichienchot S. Development Sangyod rice protein-based formula for cow milk allergic infant and its gut microbiota modulation. *Functional Foods in Health and Disease*. 2022;12(7):380-392. DOI: https://doi.org/10.31989/ffhd.v12i7.943
31. Van Den Akker CHP, Van Goudoever JB, Shamir R, Domellöf M, Guellec I, Lapillonne A, et al. Probiotics and preterm infants: A position paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. *J Pediatr Gastroenterol Nutr.* 2020;70(5):664-680. DOI: https://doi.org/10.1097/MPG.0000000000002655
32. Yang Y, Wang Z, Bai J, Qiao H. Prebiotic Peptide Synthesis: How Did Longest Peptide Appear? *J Mol Evol*. Published online 2025. DOI: https://doi.org/10.1007/s00239-025-10237-9
33. Martirosyan D, Lampert T, Lee M. A comprehensive review on the role of food bioactive compounds in functional food science. *Functional Food Science - Online ISSN: 2767-3146*. 2022;2(3):64-78. DOI: https://doi.org/10.31989/FFS.V2I3.906
34. Bock PM, Martins AF, Schaan BD. Understanding how pre- and probiotics affect the gut microbiome and metabolic health. *Am J Physiol Endocrinol Metab*. 2024;2024(1):E89-E102. DOI: https://doi.org/10.1152/ajpendo.00054.2024
35. Holscher HD, Czerkies LA, Cekola P, Joseph LJ, Ledesma KA, Sanoshy KD, et al. Bifidobacterium lactis Bb12 enhances intestinal antibody response in formula-fed infants: A randomized, double-blind, controlled trial. *J Parenter Enteral Nutr*. 2012;36(1 SUPPL.). DOI: https://doi.org/10.1177/0148607111430817
36. Moreira TG, Cox LM, Da Silva P, Mangani D, De Oliveira MG, Escobar G, et al. Dietary protein modulates intestinal dendritic cells to establish mucosal homeostasis. *Mucosal Immunol.* 2024. DOI: https://doi.org/10.1016/j.mucimm.2024.06.006
37. Salminen S, Stahl B, Vinderola G, Szajewska H. Infant formula supplemented with biotics: Current knowledge and future perspectives. *Nutrients*. 2020;12(7):1-20. DOI: https://doi.org/10.3390/nu12071952
38. Mulinari Turin de Oliveira N, Schiebel CS, Sauruk da Silva K, de Mello Braga LLV, Bach C, Maria-Ferreira D. Efficacy of Dietary Supplementation in the Relief of Inflammatory Bowel Disease: A Systematic Review of Animal Studies. *Nutr Rev*. Published online 2025. DOI: https://doi.org/10.1093/nutrit/nuae224
39. Zhu P, Savova MV, Kindt A, Wopereis H, Belzer C, Harms AC, et al. Exploring the Fecal Metabolome in Infants With Cow's Milk Allergy: The Distinct Impacts of Cow's Milk Protein Tolerance Acquisition and of Synbiotic Supplementation. *Mol Nutr Food Res.* 2025;69(1):e202400583. DOI: https://doi.org/10.1002/mnfr.202400583
40. Shehata AA, Yalçın S, Latorre JD, Basiouni S, Attia YA, Abd El-Wahab A, et al. Probiotics, Prebiotics, and Phytogenic

Substances for Optimizing Gut Health in Poultry. *Microorganisms.* 2022;10(2):395.
DOI: https://doi.org/10.3390/microorganisms10020395

41. You S, Ma Y, Yan B, Pei W, Wu Q, Ding C, Huang C. The promotion mechanism of prebiotics for probiotics: A review. *Front Nutr*. 2022; 9:1000517.
DOI: https://doi.org/10.3389/fnut.2022.1000517
42. Sáez-Fuertes L, Kapravelou G, Grases-Pintó B, Bernabeu M, Knipping K, et al. Maternal Synbiotic Supplementation with B. breve M-16V and scGOS/lcFOS Shape Offspring Immune Development and Gut Microbiota at the End of Suckling. *Nutrients.* 2024;16(12):1890.
DOI: https://doi.org/10.3390/nu16121890
43. Sáez-Fuertes L, Kapravelou G, Grases-Pintó B, Bernabeu M, Knipping K, Garssen J, et al. Early-Life Supplementation Enhances Gastrointestinal Immunity and Microbiota in Young Rats. *Foods.* 2024;13(13):2058.
DOI: https://doi.org/10.3390/foods13132058
44. Gramenzi A, Clerico L, Belà B, Di Leonardo M, Fusaro I, Pignataro G. Modulation of Canine Gut Microbiota by Prebiotic and Probiotic Supplements: A Long-Term In Vitro Study Using a Novel Colonic Fermentation Model. *Animals*. 2024;14(22). DOI: https://doi.org/10.3390/ani14223342
45. Chen X, Shi S, Sun C, Li S. A Study of the Relationship between Inflammatory Immune Function and Intestinal Flora in Adolescent Patients with First-Episode Depression. *Actas Esp Psiquiatr*. 2024;52(1):1-9.
46. Luo Y, Li J, Wu S, Li Y, He F, Cheng R. Oral supplementation with Bifidobacterium longum ssp. infantis and 2'-fucosyllactose revives gut microbiota perturbation and intestinal and immune developmental delay following early-life antibiotic challenge in BALB/c mice. *J Dairy Sci*. 2025;108(1):101-118.
DOI: https://doi.org/10.3168/jds.2024-24912
47. Martirosyan D, Lampert T, Ekblad M. Classification and regulation of functional food proposed by the Functional Food Center. *Functional Food Science - Online ISSN: 2767-3146*. 2022;2(2):25-46.
DOI: https://doi.org/10.31989/FFS.V2I2.890
48. Wang Y, Rui B, Ze X, Liu Y, Yu D, Liu Y, et al. Sialic acid-based probiotic intervention in lactating mothers improves the neonatal gut microbiota and immune responses by regulating sialylated milk oligosaccharide synthesis via the gut–breast axis. Gut Microbes. 2024;16(1):2334967.
DOI: https://doi.org/10.1080/19490976.2024.2334967