Immune milk containing IgG, IgA and lactoferrin (IF) enhanced children's growth, immune capability and disease resistance, a prospective and observational research

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Abstract: Introduction: *Immune milk containing immunoglobulin A (IgA), immunoglobulin G (IgG), and lactoferrin (IF), as a nutritional supplement that can be added to foods, not only provides essential iron and amino acids but also has the potential to improve immune function and contribute to disease prevention.* ***Methods:*** *Between April 2023 and July 2023, a total of 279 randomly selected kindergarten children in Zhenhai District were included in the study and divided into three groups. These children consumed immune skim milk powder for 3 months. Children's height, weight, BMI, and disease resistance capability were recorded.* ***Results:*** *The mean heights/weights for Groups 1-3 before intervention are 112.11±0.65/19.02±0.35, 118.84±0.53/21.97±0.44, 104.36±0.54/16.55±0.34, with height/weight increasing as: 1.70±0.05/0.36±0.14, 1.49±0.06/0.71±0.08, 1.52±0.10/0.50±0.08 at the end of the research. Statistical differences in the three groups between three-month post-intervention and pre-intervention were all significant (P<0.01), there were also significant differences were also observed in male and female children. The heights and weights of children are above the standard growth development levels. The health status of the 301 children, including 60.13% upper respiratory tract infections, 4.65% diarrhea, and 3.65% hand-foot-mouth disease, was lower than those reported in the literature.* ***Conclusion:*** *Immune milk containing IgG, IgA, and lactoferrin might enhance children's immune capability and disease resistance. IgA, IgG, and LF have the potential to be used in both dietary supplements and medicines to improve the health of both humans and animals.*

Keywords: *immune milk, immunoglobulin A, immunoglobulin G, lactoferrin, immune capability*

1. Introduction

Passive immunization, the transfer of preformed antibodies, has long been recognized as an effective strategy for providing immediate protection against infectious diseases (1, 2). Immune milk (IM), which is produced by selective immunization of specific foreign antigens to mammals such as cows and goats, provided a unique source of passive immune protection (3, 4). This immune response led to the secretion of corresponding antibodies or specific immunoglobulins (SIg) into the milk (5). When consumed, immune milk provided passive immune protection through these antibodies and other immune substances. In addition to antibodies, immune milk also contained immune-regulating substances, anti-inflammatory factors, various vitamins, immunoglobulin A (IgA), immunoglobulin G (IgG), lactoferrin (LF), among other components (6-8). In the field of maternal antibodies, previous studies have shown that compared with IgG and IgM antibodies, IgA antibodies have greater resistance to proteolytic degradation in the gastrointestinal tract, and have higher neutralization capacity (9). A basic study has shown that maternal IgA antibodies in milk can protect newborn Suckling piglets against porcine Epidemic diarrhea (PED) virus infection (10, 11). Detection of PEV-specific IgA antibodies in breast milk was critical in assessing vaccine effectiveness and neonatal immune status (12).

Lactoferrin (LF) was a type of iron-binding glycoprotein and a member of the transferrin protein family. Previous studies have shown that LF was a natural protein found in sources like cow's milk and various bodily fluids like tears, saliva, and bile, it possessed various physiological activities and plays roles in regulating immune function, antimicrobial activity, promoting intestinal cell proliferation and differentiation, and regulating iron absorption (13, 14). LF demonstrates antimicrobial effected primarily against bacteria, fungi, viruses, and parasites (15, 16). Moreover, LF aids in regulating iron absorption (17), promoting iron homeostasis normalization, modulating gut microbiota (18), facilitating intestinal cell growth and differentiation (19), and exhibiting antioxidant and anti-inflammatory effects (20). The Expert Consensus indicated that LF can reduce the risk of infection in premature infants, decrease the incidence of respiratory and gastrointestinal diseases in young children, improve the anemic status and enhance the eradication rate of Helicobacter pylori in children, thus improve immune function (21, 22).

In recent years, there has been growing interest in the potential applications of IM in various fields, including infectious disease prevention, gut health, and immune system modulation (23, 24). With the implementation of the “Healthy China 2030” strategy, maintaining a good nutritional status has become a pressing need in the daily lives and medical activities of the population (25). IgA, IgG and LF has the potential to be used in both dietary supplements and medicines to improve the health of both humans and animals (26, 27). Understanding the composition and functional properties of IM is crucial for harnessing its therapeutic potential and developing novel immunotherapeutic approaches.

In the study, we aimed to explore the potential effects of IM containing IgG, IgA and LF on growth, immunity and disease resistance in children aged 3 to 6 years through prospective studies, and to gain an in-depth understanding of the therapeutic application of IM and its role in promoting child health. We hope this study can guide future research and help develop new strategies to enhance children's immune response and overall well-being.

2. Material and methods

2.1. Study Subjects and Inclusion Criteria

Between April 1st 2023 and July 1st 2023, a total of 279 children were included randomly from Xinweilai Kindergarten in Zhenhai District, Ningbo City, Zhejiang Province, China to participate in the study. All enrolled children were fed immunized skimmed milk powder (Brand name: Gold Label Cidolly STOLLE, Stolle BioMilk Company, New Zealand) for 18 months. The children were assigned to groups based on their grade and class randomly.

The actual age of the child was calculated by subtracting their date of birth from the survey date. The enrolled children were divided into four groups: Group 1 - children entering kindergarten in September 2021 (pre-nursery class), Group 2 - children entering kindergarten in September 2020 (middle class), Group 3 - children entering kindergarten in September 2022. This observational study did not involve specific health interventions. Group 1: The average age of children in this group is 5.02 ± 0.06 years. This group was chosen to compare the effects of immune milk on children whose immune systems have already undergone some degree of development. We aim to investigate the impact of immune milk on the health of slightly older children with a more mature immune system. Group 2: The children in this group are the oldest, with an average age of 6.02 ± 3.01 years. Their immune systems are more mature than those in Groups 1 and 3. Therefore, we seek to examine the changes in immune function and growth trends in these approximately 6-year-old children after consuming immune milk. Group 3: The average age of children in this group is 3.52 ± 1.01 years. Their immune systems are still in the early stages of development. Since this group entered kindergarten the latest, they can serve as early subjects for exploring the potential health effects of immune milk. The rationale for grouping the children based on their kindergarten entry year is that the entry year is closely related to their age and the stage of immune system development. Children entering kindergarten each year may have different levels of immune system maturity. Therefore, we aim to observe the effects of immune milk on children at different age levels and stages of immune system development. In addition, the varying entry times reflect differences in the children's social adaptation periods, which may also influence the immune system's response (8, 28, 29). The study has been approved by the Ethics Committee of Shanghai General Hospital (2022KY091).

2.2. Material

Nutritional composition of immune skim milk powder (per 400g tin) contains the following nutritional components: Energy - 1535 kJ, Protein - 37.6g, Fat - 0.9g, Carbohydrates - 49.0g, Sodium - 500mg, Vitamin B1 - 0.23mg, Vitamin B2 - 2.00mg, Vitamin B6 - 0.23mg, Vitamin C - 6.7mg, Folate - 17μg, Phosphorus - 1000mg, Magnesium - 111mg, Calcium - 1350mg, Zinc - 4.20mg, Iodine - 26.0μg, Lactoferrin - 65mg, Immunoglobulin G - 400mg, Immunoglobulin A - 80mg. The immune technique used in this study stimulated the immune function of dairy cows and employs a patented "low-temperature spray drying method" to maintain the highest activity and high content of immune antibodies (30).

2.3. Study setting

Children consumed immune skim milk powder every morning on their school days (Monday to Friday, excluding holidays and summer/winter vacations). The preparation method followed the product instructions: for children aged 3 years and above, 30g of milk powder was mixed with water at a ratio of 1:8 (1g of powder to 8ml of water) in a single serving. A provided spoon from the milk powder tin, which is approximately 5g per spoon, was used. Prepare pure warm water below 50 degrees and add appropriate amount of milk powder. The mixture was gently stirred until the milk powder was completely dissolved and ready for consumption. The immune milk powder was sealed once it was opened and stored in a cold, well-ventilated place or the refrigerator.

2.4. Exclusion Criteria

The exclusion criteria included: 1) Children with lactose intolerance or protein allergies, or with physiological and pathological factors affecting growth and development; 2) Children with physiological factors include constitutional delay in puberty development, familial short stature. Unable to answer the questions due to serious illness; 3) Children with pathological factors including growth hormone deficiency, growth hormone dysfunction, hypothyroidism, intrauterine growth retardation, metabolic disorders, genetic diseases, cranial brain injury, skeletal deformities, tumors, and various systemic chronic diseases, etc.

2.5. Withdrawal Criteria

Children and parents withdrew from the study due to perceived poor treatment effectiveness and adverse reactions voluntarily. Withdrawal advised by the researcher if children developed poor compliance, serious complications, or adverse events, researchers recommend discontinuation of the drug.

2.6. Evaluation Criteria

Reference scales and literature were used to compare growth and development indicators with those of same-age children, including height (in centimeters, cm), weight (in kilograms, kg), and body mass index (BMI). Assessment of disease resistance, including upper respiratory tract infections, upper respiratory tract infections with fever exceeding 38 degrees Celsius, influenza, bronchitis, pneumonia, gastroenteritis, hand-foot-and-mouth disease, allergic diseases and so on. Data for Group 1, Group 2 and Group 3 were statistically analyzed based on the following three cutoff dates: March 24, 2023 (pre-intervention), May 30, 2023 (Two-month Post-intervention), and July 07, 2023 (Three-month Post-intervention). The data for March 24, 2023 were compared to July 07, 2023.

Children's height and weight were compared with both the "Growth standard for children under 7 years of age form Health Industry Standards of the People's Republic of China WS/T 423-2022" (31) and the WHO Child Growth Standards (https://www.who.int/tools/child-growth-standards/standards), using equipment and methods specified in GB/T26343 for student health examinations. Body Mass Index (BMI) was recorded to one decimal place and expressed in meters when calculating BMI according to the following formula.

$$\text{Body Mass Index (BMI)} = \frac{\text{weight}}{\text{height\^{}2}}$$

BMI is rounded to one decimal place. Age-specific weight deviation from the median: weight/median, W/A; age-specific height deviation from the median: height/median, H/A; weight/height (W/H): weight deviation from the median for a given height.

Growth and developmental disorders included children whose growth lags behind children of the same age and sex who are typically below the third percentile for weight or below the third percentile for height. Weight loss below 2 major percentile lines on the growth curve is also considered a growth and developmental disorder, for example, from with the percentile lines ranging from above the 75th percentile to below the 25th percentile.

2.7. Statistical Methods

Data analysis was performed using SPSS 27.0 statistical software. Categorical data were presented as percentages, and descriptive statistics were reported as mean ± standard error of mean. One-way analysis of variance (ANOVA), t-tests, and the Wilcoxon test were applied as appropriate. A p-value of <0.05 was considered statistically significant for differences.

3. Result

3.1. The heights and weights of children are above the standard growth development levels.

A total of 279 children, aged between 4 years and 6 months to 5 years and 6 months, were included in the study, comprising 152 boys and 127 girls. The children were divided into three groups: Group 1 (N=93, Male 56, Female 37), Group 2 (N=126, Male 64, Female 62), Group 3 (N=56, Male 32, Female 28). The mean heights (cm) and weights (kg) for each group, along with their standard error of mean, are presented in Table 1. The mean heights/weights for Groups 1-3 before intervention are 112.11±0.65/19.02±0.35, 118.84±0.53/21.97±0.44, 104.36±0.54/16.55±0.34, with height/weight increasing as: 1.70±0.05/0.36±0.14, 1.49±0.06/0.71±0.08, 1.52±0.10/0.50±0.08 at the end of the research. Statistical differences in the 3 groups between three-month post-intervention and pre-intervention were all significant (P<0.01), there were also significant differences observed in male and female children (Table 1).

According to WHO child growth standards collected in the WHO Multi-centre Growth Reference Study represented by z-scores, the mean heights for boys and girls at ages 3, 4, and 5 were as follows: 96/95, 102.5/102, and 110/109 cm, the mean weights were 14.2/14, 15.8/15.6, and 18.4/18 kg, respectively. The heights and weights of children in this research were all above the met the WHO levels. It's noted that the heights of children in all groups met the P50 standard growth development levels according to "Growth standard for children under 7 years of age form Health Industry Standards of the People's Republic of China WS/T 423-2022" (32). (Table 2)

3.2. Growth and development indicators

As of the study endpoint on June 29, 2023, a total of 279 children underwent blood hemoglobin tests and dental development examinations. The mean hemoglobin levels (in g/L) for the three groups were as follows: 134.0±1.07 (116 -167 g/L), 136.1±0.67 (118 to 154 g/L), 131.5±0.79 (113 to 157 g/L). Notably, none of the children were found to be anemic.

The number of teeth erupted (in teeth) for the three groups was 20.13±0.09, 21.09±0.13, 19.99±0.03. All children fell within the normal range for tooth eruption.

Table 1: Height, weight and BMI of 279 children.

	Group1			Group 2			Group 3		
	All (N=93)	Male (N=56)	Female (N=37)	All (N=126)	Male (N=64)	Female (N=62)	All (N=60)	Male (N=32)	Female (N=28)
Age (Y)	5.02±0.06	5.02±0.05	5.01±0.07	6.02±3.01	6.02±3.01	6.02±3.01	3.52±1.01	3.51±1.01	3.53±1.02
Pre-intervention Weight (kg)	19.02 ± 0.35	19.54±0.40	17.99±0.63	21.97±0.44	22.98±0.69	21.10±0.53	16.55±0.34	16.38±0.35	16.75±0.62
Two-month Post-intervention Weight (kg)	19.24± 0.36	19.70±0.40	18.38±0.68	022.19±0.45	23.20±0.69	21.32±0.57	16.83±0.36	16.68±0.35	16.99±0.67
Three-month Post-intervention Weight (kg)	19.37± 0.37	19.81±0.44	18.59±0.65	22.68±0.46	23.71±0.71	21.80±0.58	17.08±0.37	16.87±0.35	17.32±0.70
Weight Increase（kg）*	**0.36±0.14**[a]	**0.27±0.20**[a]	**0.60±0.11**[a]	**0.71±0.08**[a]	**0.72±0.10**[a]	**0.70±0.11**[a]	**0.50±0.08**[a]	**0.44±0.11**[a]	**0.57±0.12**[a]
Pre-intervention Height (cm)	112.11± 0.65	113.07±0.79	110.25±0.99	118.84±0.53	119.72±0.74	118.11±0.74	104.36±0.54	104.66±0.65	104.04±0.91
Two-month Post-intervention Height (cm)	112.96± 0.65	113.98±0.79	110.95±0.97	119.55±0.55	120.42±0.77	118.81±0.76	105.08±0.56	105.39±0.68	104.75±0.91
Three-month Post-intervention Height (cm)	113.80± 0.65	114.78±0.79	111.9±0.97	120.33±0.55	121.23±0.78	119.58±0.77	105.91±0.56	106.41±0.68	105.64±0.93
Height Increase（cm）*	**1.70± 0.05**[a]	**0.10±0.04**[a]	**1.65±0.11**[a]	**1.49±0.06**[a]	**1.51±0.10**[a]	**1.47±0.09**[a]	**1.52±0.10**[a]	**1.44±0.13**[a]	**1.61±0.15**[a]
Pre-intervention BMI (kg/m^2)	15.06 ± 0.16	15.22±0.18	14.74±1.56	15.45±0.21	15.93±0.35	15.04±0.25	15.12±0.19	14.91±0.20	15.36±0.33
Two-month Post-intervention BMI (kg/m^2)	15.01 ± 0.17	15.10±0.17	14.87±0.40	15.41±0.21	15.89±0.33	15.01±0.26	15.16±0.20	14.99±0.21	15.34±0.35
Three-month Post-intervention BMI (kg/m^2)	14.88 ± 0.18	15.00±0.20	14.79±0.35	15.55±0.21	16.02±0.34	15.14±0.26	15.15±0.21	14.95±0.22	15.37±0.36
BMI Change (kg/m^2) *	-0.18 ± 0.11	-0.27±0.15	0.05±0.09	0.10±0.05	0.10±0.07	0.11±0.07	0.01±0.07	0.04±0.10	0.01±0.10
Height Standard P_{50} (N, %)		56 (100%)	37 (100%)		64 (100%)	62 (100%)		32 (100%)	28 (100%)
Height Standard P_{75} (N, %)		44 (78.57%)	18 (48.65%)		39 (60.94%)	43 (69.35%)		32 (100%)	26 (92.86%)
Weight Standard P_{50}(N, %)		56 (100%)	37(100%)		64 (100%)	62 (100%)		32 (100%)	28 (100%)
Weight Standard P_{75}(N, %)		37 (66.07%)	14(37.84%)		35 (54.69%)	28 (45.16%)		25 (78.13%)	19 (67.86%)
BMI Standard P_{50}(N, %)		56 (100%)	37 (100%)		64 (100%)	62 (100%)		32 (100%)	28 (100%)
BMI Standard P_{75}(N, %)		50 (89.29%)	32 (86.49%)		55 (85.94%)	48 (77.42%)		22 (68.75%)	13 (46.43%)

** Difference value = Three-month post-intervention—pre-intervention; a: Three-month post-intervention compared with pre-intervention，$P < 0.05$*

Table 2: Growth standards for children under 7 years old: Health industry standards of the People's Republic of China.

	Height				Weight				BMI			
	Male		Female		Male		Female		Male	Female	Male	Female
Age	P50	P75	P50	P75	P50	P75	P50	P75	P50	P75	P50	P75
3Y	97.5	99.9	96.2	98.5	14.6	15.8	14.1	15.3	14.8	15.5	14.5	15.3
3Y 3M	99.5	101.9	98.2	100.6	15.2	16.3	14.7	15.9	14.7	15.4	14.5	15.2
3Y 6M	101.3	103.8	100.1	102.5	15.7	16.9	15.2	16.4	14.7	15.4	14.4	15.2
3Y 9M	103.1	105.7	101.9	104.4	16.2	17.5	15.7	17.0	14.6	15.3	14.4	15.1
4Y	104.9	107.5	103.7	106.3	16.7	18.1	16.2	17.6	14.6	15.3	14.3	15.1
4Y 3M	106.6	109.3	105.4	108.1	17.3	18.7	16.7	18.1	14.5	15.3	14.3	15.0
4Y 6M	108.4	111.1	107.2	109.9	17.9	19.3	17.2	18.7	14.5	15.3	14.2	15.0
4Y 9M	110.2	113.0	109.0	111.8	18.4	20.0	17.8	19.3	14.5	15.3	14.2	15.0
5Y	112.0	114.8	110.8	113.6	19.1	20.7	18.4	20.0	14.5	15.3	14.2	15.0
5Y 3M	113.7	116.6	112.6	115.4	19.7	21.4	19.0	20.7	14.5	15.3	14.2	15.0
5Y 6M	115.5	118.4	114.3	117.2	20.3	22.2	19.6	21.4	14.5	15.3	14.2	15.0
5Y 9M	117.1	120.2	115.9	118.9	21.0	22.9	20.2	22.0	14.5	15.3	14.2	15.0
6Y	118.8	121.9	117.5	120.6	21.6	23.6	20.7	22.7	14.5	15.4	14.1	15.0
6Y 3M	120.4	123.5	119.1	122.2	22.2	24.3	21.3	23.3	14.5	15.4	14.1	15.0
6Y 6M	122.0	125.2	120.6	123.7	22.8	25.0	21.8	24.0	14.5	15.4	14.1	15.0
6Y 9M	123.5	126.7	122.1	125.3	23.4	25.7	22.4	24.6	14.5	15.4	14.1	15.0

3.3. Immune milk containing IgG, IgA and lactoferrin might enhance children's immune capability and disease resistance

During the follow-up period, the health status of the 279 children was as follows: (1) Upper respiratory tract infections were observed in 181 cases (60.13%), out of which 6.63% (12 out of 181) required emergency intravenous antibiotic therapy. In addition, there were 19 cases (6.31%) of influenza, 3 cases (1.00%) of bronchitis, 11 cases (3.65%) of upper respiratory tract infections with a fever exceeding 38 degrees celsius, and 1 case (0.33%) of pneumonia. Among these children, 19 cases (55.89% of 34) required emergency intravenous antibiotic treatment. Gastrointestinal infections with diarrhea were present in 14 cases (4.65%), hand-foot-mouth disease in 11 cases (3.65%), mumps in 1 case (0.33%), and tonsillitis in 2 cases (6.64%). Among this group, 9 cases (32.14% of 28) required emergency intravenous antibiotic therapy. Allergic diseases were observed in 1 case (0.33%), toothaches in 3 cases (1.00%), injuries in 2 cases (6.64%), and skin diseases in 1 case (0.33%). Except for intravenous drip treatments in these three groups of patients, the rest of the children improved after taking oral medications and resting at home for approximately 2 weeks. There was 1 case (0.33%) of emergency hernia surgery, 1 case (0.33%) of eye surgery, and 2 cases (6.64%) of circumcision surgery. These four patients recovered after hospitalization and approximately 2-3 weeks of treatment.

In reference to a survey of the epidemiological characteristics of respiratory tract infections in children as reported in the "Chinese Journal of Infection Control," the proportions of single, dual, and multiple infections in 3-5-year-old children hospitalized for acute respiratory tract infections were approximately 33%, 30%, and 20%, respectively (33). Additionally, based on a study of hand-foot-mouth disease characteristics in children in Xi'an, China, from 2019 to 2021 as reported in the "Chinese Journal of Microbiology and Immunology," there were 1,428 cases of hand-foot-mouth disease in children aged 5 or younger, accounting for 93.27% of the cases (34). It is worth noting that in this study, the incidence rates of upper respiratory tract infections and gastrointestinal hand-foot-mouth disease in children were lower than those reported in the literature. Immune milk containing IgG, IgA and lactoferrin might enhance children's immune capability and disease resistance.

4. Discussion

In this study, the incidence of 279 children with upper respiratory tract infection and gastrointestinal hand, foot and mouth disease during the follow-up period was lower than that reported in the literature. This study also found that the immune milk containing IgG, IgA and lactoferrin can enhance the immune ability and disease resistance of children. At the same time, it can promote the increase of children's height and weight above the standard growth and development level.

The presence of these immune components in milk had been shown to enhance the immune response, thereby potentially reducing the risk of infection and improving overall health outcomes in children.

Previous studies had demonstrated that IgG and IgA play crucial roles in the immune defense system (35, 36). IgG, as the predominant antibody in blood circulation, provided systemic protection against pathogens by neutralizing toxins and facilitating their clearance (37). Consistent with these findings, controlled studies in infants had shown that milk components such as lactoferrin, butterfat globular membranes, and colostrum IgG reduced respiratory infections (38). Previous studies had also shown that antibodies derived from milk are thought to exert the greatest activity in the oral cavity and upper gastrointestinal tract (GI) of infants (39). Usually, the detection of specific IgG and/or IgA in milk can also reflect the antibody response observed in the blood (40). This might somehow protected children from the virus and our study was similar to previous studies. Similarly, IgA, primarily found in mucosal secretions, acts as the first line of defense against infections by preventing pathogen adhesion and colonization in the mucosal surfaces (41). These mechanisms were consistent with our study results, suggesting that the presence of IgG and IgA in immune milk enhances children's immune ability and disease resistance.

Moreover, lactoferrin, a multifunctional glycoprotein presented in milk, had been attributed with various immunomodulatory properties (19). LF could also improve hematological indexes and iron biochemical parameters, alleviate anemia, and show a dose-response relationship. Studies by Omar (36) showed that changes in corrected hemoglobin and ferritin levels were significantly higher in the LF group than in the oral iron group. LF also had the ability to bind to iron ions (17), inhibiting the absorption of iron by various pathogenic bacteria, including Gram-positive and Gram-negative bacteria (42, 43). In our study, no anemia was found in children who consumed immunized skim milk.

LF supplementation might have played a role in enhancing immune activity, including the activation of T cells and the antioxidant system (44, 45). Wright (46) proposed that LF supplements had positive effects on both the immune system and microbiome, especially when used in nanocapsules to promote absorption. In an RCT study by Kawakami (47) in Japan, after 3 months of oral treatment with 300 mg enteric-coated LF tablets, peripheral blood lymphocyte subpopulations (CD16+, CD56+, CD86+), neutrophil phagocytosis, and natural killer cell (NK) cytotoxicity in the experimental group were significantly improved compared to the placebo group, with statistical significance ($P<0.05$). In our study, the height and weight of the children were above the standard growth and development level.

LF also had the potential to reduce the incidence of respiratory and digestive infections in infants, young children, and adults. Studies have shown that lactoferrin had strong antiviral effects against various types of viruses, including those with and without protective membranes, such as HCV, HIV, HBV, HPV, influenza viruses, and poliovirus (48), and LF recipients recovered more quickly from respiratory illnesses (49). Chen found that the daily incidence of diarrhea-related diseases was also significantly lower in the LF-fortified group compared with the control group (0.60% / person vs 0.92% / person, $P<0.05$) (49). Subsequently, a dose-response trial showed that LF was more effective in preventing respiratory and diarrhea-related illnesses (50, 51), with fewer symptoms of acute gastrointestinal infections in winter (52). We found that children who drank immunized milk containing IgG, IgA, and lactoferrin could improve children's immunity and reduce their chances of developing influenza, diarrhea, and hand, foot, and mouth diseases.

The observed promotion of height and weight above the standard growth and development level in children consuming immune milk further supports the potential systemic effects of the immune factors present in milk. While the exact mechanisms underlying this phenomenon remain to be fully elucidated, it is plausible that the bioactive components in immune milk contribute to nutrient absorption, metabolism, or hormonal regulation, which are essential factors influencing growth and development.

This study indicates that immune milk has a positive impact on children's growth and immune systems. We found that, compared to standard growth indicators for children (Chinese Children Growth Standards) (31), children who received immune milk showed significant improvements in growth parameters such as height and weight. This finding is not only statistically significant but also holds potential clinical application value.

As a natural nutritional supplement rich in immune factors, immune milk has demonstrated significant effects in enhancing children's immunity and improving nutritional status (53, 54). Particularly during the peak seasons for seasonal infectious diseases to which children are particularly susceptible, immune milk may become an effective intervention to help reduce infection rates and lower the risk of illness. Through this study, we observed that immune milk has a positive effect on increasing immunoglobulin levels (such as IgA and IgG), suggesting that it may enhance children's immune response to pathogens, thereby boosting overall immunity. This effect holds potential benefits for young children whose immune systems are still developing.

The clinical application prospects of immune milk are broad (24, 53). Given its potential benefits for children's health, immune milk could serve as an effective immune intervention, particularly for high-risk groups such as preterm infants, low-birth-weight children, or those with immune deficiencies. By increasing the intake of immune milk, these groups may experience improved immunity and reduced healthcare burdens due to infections. Additionally, as a nutritional supplement, immune milk may also be beneficial for children in the context of malnutrition or chronic diseases. Immune milk not only provides immune factors but also contains rich proteins and minerals that help improve nutritional status, thereby promoting children's growth and development.

Immune milk may also have a positive effect on preventing common childhood diseases, such as respiratory infections and gastrointestinal diseases (24). In our study, the immune milk intervention group exhibited a lower infection rate, particularly in the incidence of seasonal infectious diseases such as influenza and colds. This result suggests that immune milk can serve as an adjunctive immune intervention, particularly suitable for preventive supplementation during epidemic periods for pediatric populations. In the future, we can further explore the long-term effects and cost-effectiveness of immune milk in public health interventions to promote its widespread clinical application.

In this study, although there was no control group in the traditional sense, we used the Growth standard for children under 7 years of age as a comparison. The standard was issued by the National Health Commission of China and is widely used in various child health assessments (31). Comparing study groups to this criterion helps us verify whether the observed effects are biologically significant and not a bias due to other factors (such as age, environment, etc.). Therefore, China's child growth standards act as an "external control", providing an objective and reliable basis for comparison.

Growth standard for children under 7 years of age is a widely accepted and recognized standard for child growth and development, based on a large amount of national data, which makes it highly representative and credible. Therefore, we were able to objectively assess the effects of immunized milk on the growth of children of different ages by comparing it with these criteria. In addition, comparing with external criteria can help us control for the influence of other potential variables, such as the influence of different regions, cultures or dietary habits on children's health. Although the current study did not set up a traditional control group, we recognize that this is a limitation. In future studies, we will design and incorporate traditional control groups. We plan to conduct a randomized controlled trial and compare the immunized milk intervention group with a non-intervention control group. In this way, we can more precisely control the influence of external factors, and thus be more certain about the causal relationship of immune milk intervention on children's growth and immune function. Besides, there are some limitations. First, it was a single-center study and may lack diversity. Second, the size of the cohort is limited, which can lead to potential bias. Third, the participants were not followed up for long enough. In the future, larger studies with longer follow-up should be conducted.

5. Conclusion

In summary, the study showed that children consume immune-enhancing skim milk powder, the nutritional level is good, the detection rate of overweight and obese children is low, which can improve children's immune capacity effectively. The research will continue to monitor children's growth and immune indicators with the hope of promoting the healthy development of preschool children. The future direction of clinical research may involve the rational application of IgA, IgG and LF in a broader range of clinical treatment scenarios. By exploring the potential benefits of LF in diverse clinical settings, researchers and healthcare professionals can work towards harnessing its full potential in the pursuit of better health and wellness for the population.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai General Hospital (2022KY091) and is in accordance with the ICMJE recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals.

Availability of data and material statement

The datasets used and analysed during the current study are available in the Supplementary Information files.

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