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Effect of the specific infant formula mixture of oligosaccharides on local immunity and development of allergic and infectious disease in young children: randomized study

Wpływ żywienia mieszanką mleczną zawierającą oligosacharydy na miejscową odporność i rozwój chorób alergicznych i infekcyjnych u niemowląt: badanie randomizowane

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ABSTRACT

Aim: The aim of our open prospective randomized nutritional intervention study was to evaluate the effect of feeding with a standard infant formula enriched with the specific mixture of oligosaccharides on local digestive immunity system and further development of allergic and infectious diseases in young children. **Material and methods:** Depending on the type of feeding the infants were divided into 3 groups (with random allocation to one of the formula feeding groups): 80 infants who were breastfed, 80 infants consuming the formula supplemented with oligosaccharides, 80 infants fed with a standard formula. **Results:** Breastfed infants had the highest content of Bifidobacteria and Lactobacilli in feces (9.047 ± 1.075 and 7.26 ± 0.65 CFU/g accordingly). In infants fed with formula supplemented with scGOS/lcFOS fecal concentrations of Bifidobacteria and Lactobacilli were similar to those in breastfed infants (8.92 ± 1.011 and 7.22 ± 0.74 CFU/g accordingly). It was found that infants fed with breast milk and supplemented formula had significantly less allergic reactions to food products compared to the babies from the third group (3.92% and 4.84% *vs.* 16.98% accordingly; $p < 0,05$). **Conclusions:** The mixture of prebiotic oligosaccharides (scGOS/lcFOS – 9:1; 8 g/L) has a similar to breast milk positive impact on the factors of local digestive immunity system in formula-fed infants. This effect may reduce the risk of allergic and infectious diseases in children aged up to 18 months of life, compared with babies fed with the standard formula without oligosaccharides.

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Introduction

The influence of breast milk on the development of immunity was known many years ago. Human milk oligosaccharides have influence on the development of immunity and morbidity in infants. The type of diet is one factor that determines the composition of the intestinal microflora of breast-fed infants, which differs from the microflora of bottle-fed infants [1, 2]. In breastfed infants, the intestinal microflora is dominated by Bifidobacteria and Lactobacilli, and this microbial pattern produces beneficial effects on intestinal function and on development of the immune system [2, 3].

Based on the analysis of human milk oligosaccharides (HMO), a prebiotic mixture of 90% short chain galactooligosaccharides and 10% long chain fructo-oligosaccharides (scGOS/lcFOS (9:1; 8 g/L)) has been developed [4, 5].

Studies in preterm [6] and term [2, 7–8] infants have shown that feed supplementation with GOS/FOS produces an intestinal flora similar to that found in breast fed infants. Study showed that the use of this prebiotic oligosaccharide mixture (scGOS/lcFOS) can significant reduction of the total number of infections, respiratory tract infections, fever episodes, and antibiotic prescriptions during the first 2 y of life. The atopic dermatitis (AD), cumulative incidence of other allergy-associated symptoms, like recurrent wheezing and allergic urticaria, was also significantly lower in the sGOS/lcFOS group compared with the placebo group [9].

Our hypothesis was that this mixture of prebiotic oligosaccharides could mimic the immune modulatory function of HMO on local immunity factors, protect mucous membranes of the digestive system, and lead to a reduction in the incidence of allergic and infectious diseases in formula-fed infants. To test this hypothesis, we have planned and conducted an open prospective randomized nutritional intervention study.

Aim of the study

The aim of our study was to evaluate the effect of feeding with a standard infant formula enriched with the specific mixture of oligosaccharides (scGOS/lcFOS; 9:1; 8 g/L) compared to a formula without oligosaccharides and breastfeeding during the first months of life on digestive system local immunity and further development of allergic and infectious diseases in young children.

Materials and methods

Two hundred and forty healthy term newborns were involved into the study on its first stage. Depending on the type of feeding the infants were divided into 3 groups (with random (sealed envelope randomization) allocation to one of the formula feeding groups): the group 1 included 80 infants who were breastfed, the group 2 – 80 infants consuming the formula supplemented with a specific mixture of oligosaccharides, and the group 3 – 80 infants fed with a standard formula (Fig. 1). Enrollment into the second and the third groups took place only if mothers had decided not to breastfeed. Infants were supposed to be breastfed or fed with the allocated formula for at least 2 months.

Babies in the groups did not differ by age at the enrollment, gender, physical and social settings. Participation in the study was voluntary with signing of informed consent by parents. This study was approved by a local Ethics Committee.

Inclusion criteria were:

- Healthy term newborns with birth weight >2500 g appropriate for gestational age.
- Apgar scores >7.
- Uncomplicated early course of neonatal period.
- Impossibility of breastfeeding (for infants randomized into the bottle-feeding groups).
- Informed consent was signed by parents.

Exclusion criteria:

- The minimum possibility of breastfeeding (for infants randomized into the bottle-feeding groups).
- Administration of probiotics and prebiotics before involvement into the study.

Growth parameters (weight, length, head circumference, and BMI) were determined at enrollment, in 2 and at 18 months.

Saliva and fecal samples were taken on the day of inclusion into the study and after 2 months of exclusive feeding with the selected formula or breast milk. Saliva sIgA (sIgA ELISA «Khemo-Medica» Ltd), alpha-defensins HNP1-3 (HNP 1-3 ELISA KIT) and fecal lysozyme (Human LL-37 ELISA TEST KIT) were determined by an ELISA method. Gut microbiota composition was assessed in 2 months after beginning of the study using standard bacteriological methods. Bifidobacteria, Lactobacilli and Candida fungi have been analyzed.

By the end of the second phase of the study, we compared the cumulative incidences of atopic dermatitis (AD), obstructive bronchitis, recurrent wheezing, gastrointestinal and upper respiratory tract infections (URTI) at 18 months depending on type feeding in the first months of life.

AD was diagnosed according to the criteria described by Harrigan and Rabinowitz [10] and Muraro et al. [11]. The diagnosis of AD was confirmed if the following features were detected: pruritus, involvement of the face, skull facial, and/or extensor part of the extremities, and a minimal duration of the symptoms of 4 weeks. Recurrent wheezing was defined as 3 or more physician-diagnosed wheezing episodes [13]. Official medical documents and reports were used.

By the end of the study, the number of children in groups decreased (Fig. 1). The main reasons for dropping out were failure to follow up, poor compliance, change of feeding type, for example, lack of breast milk or replacement of the preselected formula in the bottle-fed groups.

Statistical analysis

Standard methods of descriptive, comparative and categorical analyses were used. If normally distributed continuous

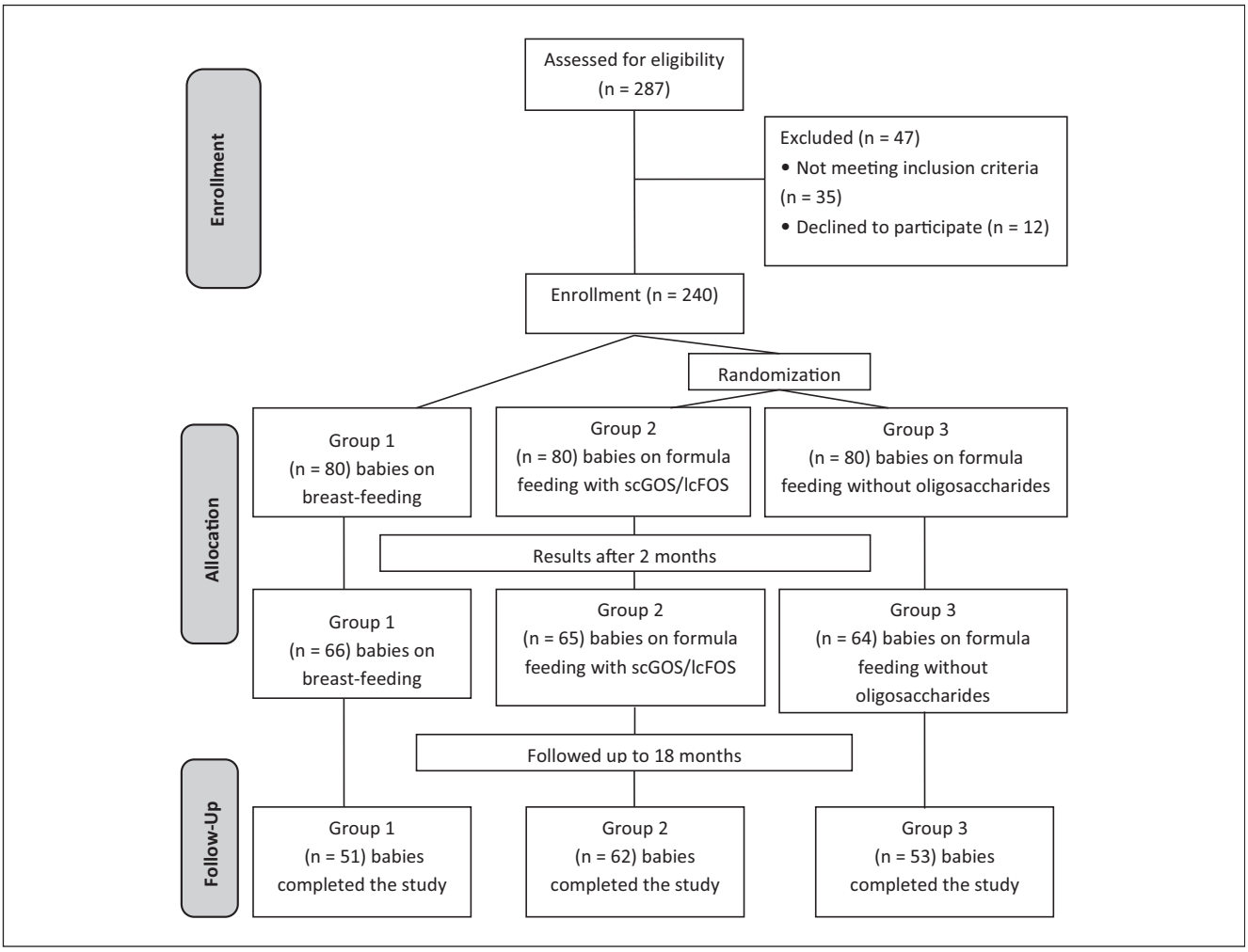

Fig. 1 – Flow chart showing enrollment and disposition of the subjects throughout the study
Ryc. 1 – Plan badania

data are presented as mean ± standard deviation (SD) if not – as median (minimum, maximum). Two-way ANOVA or Kruskal–Wallis ANOVA by ranks and median test were used to compare continuous variables between the three groups. Chi-square or Fisher's exact test were used for comparison of categorical (nominal) variables. All differences between the groups were considered significant if $p < 0.05$. The statistical analysis was conducted with the use of software Statistica 8 (StatSoft Inc., 2008; USA).

Results

The groups were not different in terms of average birth weight, length, head circumference, BMI, Apgar scores, age and growth parameters at enrolment. Feeding with breast milk or specific formula did not produce any reliable effect on growth within the period of observation.

Initial saliva sIgA levels in infants from the all groups were similar but after 2 months a significant difference between two formula feeding groups developed. Saliva concentration of sIgA in infants fed with the formula supplemented with scGOS/lcFOS was rising like in the reference (breastfeeding) group. At the same time no obvious changes were found in infants fed with the formula without scGOS/lcFOS (Fig. 2).

Concentration of lysozyme in feces of infants from the breastfeeding group was high at the inclusion into the study and moderately decreased after 2 months. In infants from the second and third groups, concentrations of fecal lysozyme were significantly lower at the inclusion into the study comparing to the first group. However, after 2 months fecal lysozyme content was significantly higher in infants fed with the formula supplemented with scGOS/lcFOS than in babies fed with the standard formula (Fig. 3).

The lowest level of saliva α-1-3 defensin concentration we identified in infants was from the breastfeeding group. Defensins' concentrations in babies fed with the formula supplemented with scGOS/lcFOS were similar to the values in the breastfeeding group and significantly different from the values of infants fed with the standard formula. The increased level of saliva α-1-3 defensins produced by neutrophils in infants from the third group may indirectly indicate formation of pathological bacterial gut colonization

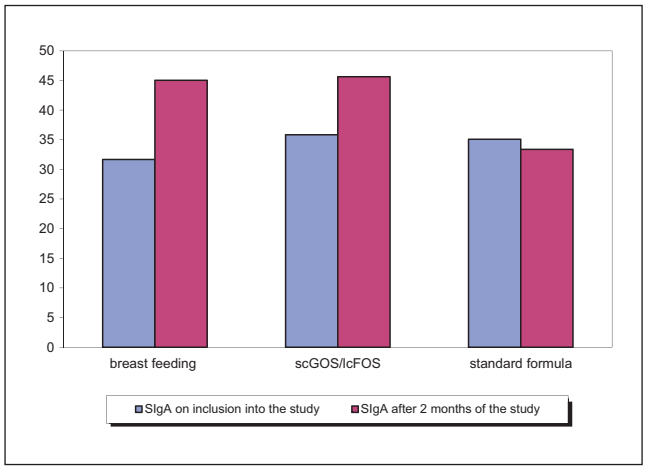

Fig. 2 – The level of sIgA (mcg/ml) in saliva depending on the type of feeding at the inclusion into the study and after 2 months (*$p < 0.05$)

Ryc. 2 – Poziom sIgA (mcg/ml) w ślinie zależnie od rodzaju żywienia po włączeniu do badania i po okresie 2 miesięcy (*$p < 0,05$)

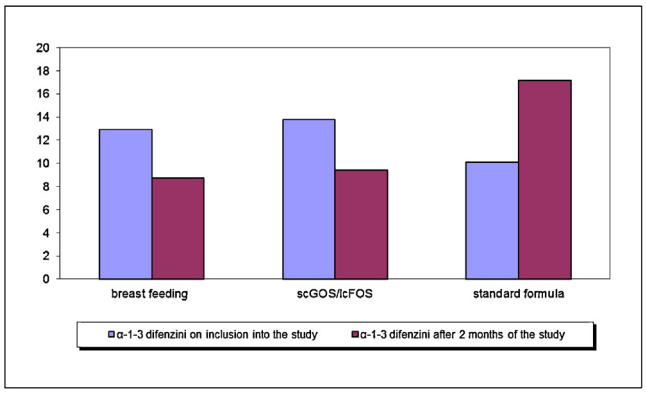

Fig. 4 – Saliva concentrations of α-1-3 defensins (pg/ml) in infants depending on the type of feeding at the inclusion into the study and after 2 months (*$p < 0.01$)

Ryc. 4 – Stężenie α-1-3 defensyny (pg/ml) w ślinie niemowląt w zależności od rodzaju żywienia- na początku badań i po okresie 2 miesięcy (*$p < 0,01$)

and as a result – protective distress of immune reactions (Fig. 4).

Analyzing quantitative features of gut microbiocenosis we determined that breastfed infants had the highest content of bifidobacteria and lactobacilli in feces (9.047 ± 1.075 and 7.26 ± 0.65 CFU/g accordingly). In infants fed with formula supplemented with scGOS/lcFOS fecal concentrations of bifidobacteria and lactobacilli were similar to those in breastfed infants (8.92 ± 1.011 and 7.22 ± 0.74 CFU/g accordingly). In infants fed with the standard formula without oligosaccharides concentrations of bifidobacteria and lactobacilli in feces were significantly lower (7.81 ± 0.83 and 6.81 ± 0.93 CFU/g accordingly; $p < 0.05$ for the both comparisons) (Table I).

We have also found a higher concentration of Candida fungi in feces of infants from the third group in comparison with the other babies (3.97 [0; 7.2] CFU/g *vs.* 3.65 [0; 5.73] CFU/g and 3.82 [0; 6.4] CFU/g accordingly in the first and second groups; $p > 0.05$).

Our results suggest that in infants fed with the formula supplemented with a specific mixture of oligosaccharides (scGOS/lcFOS – 9:1; 8 g/L), the features of intestinal microbiocenosis development are similar to those seen in breastfed babies. It is very important at this age because appropriate intestinal microbiocenosis formation can influence children's health in the future.

Parents of 195 infants agreed to participate in the second (follow-up) stage of the study. 166 children (51 in the breastfeeding group, 62 in the scGOS/lcFOS group and 53 in the formula without oligosaccharides group) completed the 18 months follow-up period (Fig. 1).

Babies were breastfed or received predefined formula for 9.73 ± 3.54; 8.19 ± 3.45 and 8.22 ± 2.99 months accordingly by groups ($p > 0.05$). We did not find significant differences between the groups in terms of age at introduction of cow's milk and the first solid foods (Table II).

At the age of 18 months, we analyzed the incidence of gastrointestinal and upper respiratory tract infections (URTI). Infants fed with breast milk and with the formula supplemented with scGOS/lcFOS had similar morbidity (0.27 ± 0.07 *vs.* 0.28 ± 0.05 episodes/child/18 months of gastrointestinal infections and 2.82 ± 0.96 *vs.* 2.81 ± 0.51 episodes/child/18 months of URTI accordingly; $p > 0.05$). At the same time the incidence of gastrointestinal and URT infections in the second group was significantly lower than in infants fed with the formula without scGOS/lcFOS (0.28 ± 0.05 *vs.* 0.78 ± 0.12 episodes/child/18 months and 2.81 ± 0.51 *vs.* 5.78 ± 0.97 episodes/child/18 months accordingly; $p < 0.001$) (Fig. 5).

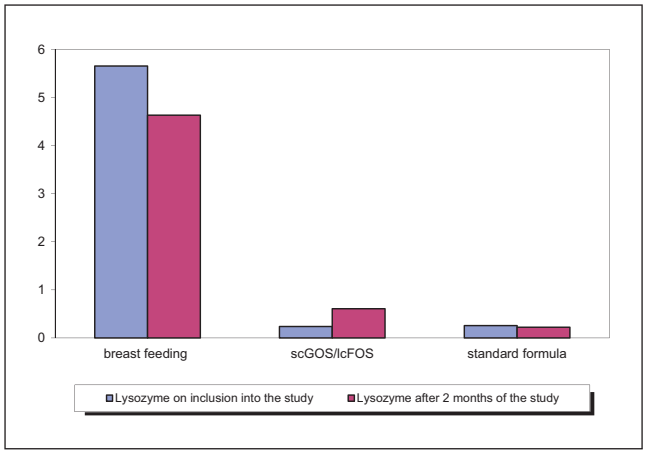

Fig. 3 – Concentration of lysozyme in feces (mcg/ml) in infants depending on the type of feeding at the inclusion into the study and after 2 months (*$p < 0.05$)

Ryc. 3 – Stężenie lyzozymu w kale(mcg/ml) u niemowląt zależnie od rodzaju żywienia po włączeniu do badania i po okresie 2 miesięcy (*$p < 0,05$)

Table I – Comparison of fecal *Bifidobacteria* and *Lactobacilli* content in infants depending on the type of feeding after 2 months of study
Tabela I – Porównanie ilości bifidobakterii i Lactobacilli w kale niemowląt w zależności od rodzaju karmienia po 2 miesiącach

	Group 1 (breast feeding)	Group 2 (formula with scGOS/lcFOS)	Group 3 (standard formula)	*p*
Bifidobacteria, CFU/g*	9.047 (1.075)	8.92 (1.011)	7.81 (0.83)	<0.05
Lactobacilli, CFU/g*	7.26 (0.65)	7.22 (0.74)	6.81 (0.93)	<0.05

*mean, SD in brackets
*średnia, SD (odchylenie standardowe) w nawiasie

Table II – Infants' age at introduction of unmodified cow's milk and solid foods by the groups
Tabela II – Wiek niemowlęcia w chwili wprowadzenia niemodyfikowanego krowiego mleka i stałego pokarmu w grupach

	Group 1 (breast feeding)	Group 2 (formula with scGOS/lcFOS)	Group 3 (standard formula)	*p*
n	51	62	53	
The term of unmodified cow's milk introduction (months)*	9.30 (3.68)	8.40 (3.58)	8.17 (3.31)	>0.05
The term of solid foods introduction (months)*	5.2 (1.4)	4.68 (0.89)	4.65 (1.19)	>0.05

*mean, SD in brackets
*średnia, SD (odchylenie standardowe) w nawiasie

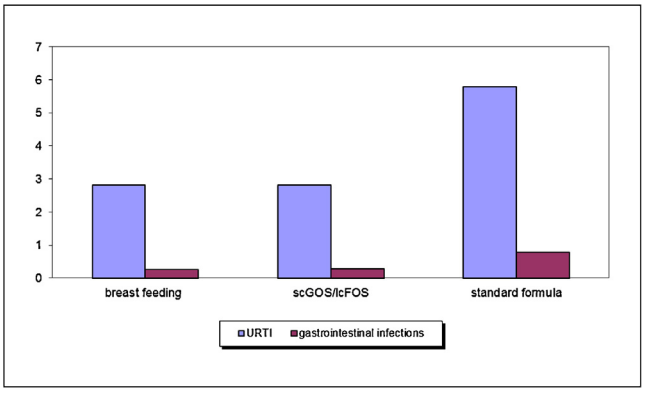

Fig. 5 – Cumulative incidence of gastrointestinal and upper respiratory tract infections (URTI) at the age of 18 months
Ryc. 5 – Czestość występowania ostrych infekcji jelitowych i infekcji górnych dróg oddechowych u dzieci w wieku 18 miesięcy

It was found that infants fed with breast milk and supplemented formula had significantly less allergic reactions to food products compared to the babies from the third group (3.92% and 4.84% *vs.* 16.98% accordingly; $p < 0.05$).

Allergic reactions to cow's milk protein were observed significantly rarer in children who were breastfed or received supplemented formula in comparison with the third group (1.96% and 3.23% *vs.* 15.09% accordingly; $p < 0.01$). The incidence of atopic dermatitis (AD), most commonly seen in allergic infants, was also the highest in babies fed with the standard formula without oligosaccharides (16.98% *vs.* 3.92% and 4.84% accordingly in the first and the second groups; $p < 0.05$).

There was a similar situation with the respiratory system allergic symptoms such as recurrent wheezing and with gastro-intestinal symptoms of food allergy (Table III).

In summary our data suggest that feeding with infant formula supplemented of with the prebiotic oligosaccharide mixture (scGOS/lcFOS) during the first 6 months can protect

Table III – Comparative frequency of allergic reactions during the first 18 months of life depending on the type of feeding
Tabela III – Porównanie częstości występowania reakcji alergicznych u dzieci w ciągu pierwszych 18 miesięcy życie w zależności od rodzaju karmienia

	Group 1 *n* = 51	Group 2 *n* = 62	Group 3 *n* = 53	*p*
Allergic reactions to food*	3.92 (2)	4.84 (3)	16.98 (9)	<0.05
Allergic reactions to caw's milk protein*	1.96 (1)	3.23 (2)	15.09 (8)	<0.05
AD*	3.92 (2)	4.84 (3)	16.98 (9)	<0.05
Respiratory system allergic symptoms*	1.96 (1)	4.84 (3)	13.21 (7)	<0.05
Gastro-intestinal symptoms of food allergy*	1.96 (1)	3.23 (2)	13.21 (7)	<0.05

*percentage, number of children in brackets
*procenty, liczba dzieci w nawiasie

infants and toddlers from infections and allergic reactions during the first 18 months of life producing the effect similar to the effect of breast milk.

Discussion

This study showed that GOS/FOS supplementation influenced intestinal microbiota and could positively modulate infant's immune system development and reduce some allergic and infectious morbidity in infants and toddlers aged up to 18 months.

In a double-blind randomized placebo-controlled study, infants received a hypoallergenic whey formula with either 8 g/l GOS/FOS in a 9:1 ratio or 8 g/l maltodextrine (placebo) for 6 months. At 3 months of age, children were vaccinated with Hexavac against a.o. diphtheria, tetanus, polio (DTP). At 6 months of age, plasma samples were collected from 84 infants (verum group $n = 41$, placebo group $n = 43$). Levels of total immunoglobulins (Ig) and of cow's milk protein (CMP-) and DTP-specific Ig were measured. GOS/FOS supplementation led to a significant reduction in the plasma level of total IgE, IgG1, IgG2 and IgG3, whereas no effect on IgG4 was observed. Concentration of CMP-specific IgG1 was significantly decreased. DTP-specific immunoglobulin levels were not affected. This study showed that GOS/FOS supplementation induced a beneficial antibody profile. GOS/FOS reduced the total immunoglobulin response and modulated the immune response toward CMP, while leaving the response to vaccination intact. This suggests that oral GOS/FOS supplementation is a safe method to restrain the atopic march [12].

The reduced total immunoglobulin levels of the various isotypes, especially IgE, may be associated with the reduced incidence of AD in the GOS/FOS supplemented group [10]. This contrasts the study of Kalliomäki et al. [13] who showed that reduction of the frequency of AD by *Lactobacillus rhamnosus* GG supplementation was not accompanied by changes in total or specific IgE levels. This may suggest that the prebiotic mixture of GOS/FOS has a stronger immunomodulatory potential than this specific probiotic strain.

Moro reported a significant reduction in infant eczema (RR 0.42, 95% CI 0.21, 0.84) up to six months age in infants receiving a mixture of fructo- and galacto-oligosaccharides [10].

In a prospective, randomized, double-blind, placebo-controlled design, healthy term infants with a parental history of atopy were fed either a prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula with extensively hydrolyzed cow milk whey protein during the first 6 months of life. Following this intervention period, blind follow-up continued until two years of life. During this period, infants in the scGOS/lcFOS group had significantly lower incidence of allergic manifestations. Cumulative incidences for AD, recurrent wheezing, and allergic urticaria were higher in the placebo group, (27.9, 20.6, and 10.3%, respectively) than in the intervention group (13.6, 7.6, and 1.5%) ($p < 0.05$). Infants in the scGOS/lcFOS group had fewer episodes of physician-diagnosed overall and upper respiratory tract infections ($p < 0.01$), fever episodes ($p < 0.00001$), and fewer antibiotic prescriptions ($p < 0.05$). Early dietary intervention with oligosaccharide prebiotics had a protective effect against both allergic manifestations and infections. The observed dual protection lasting beyond the intervention period suggests that an immune modulating effect through the intestinal flora modification may be the principal mechanism of action [11].

Conclusions

The mixture of prebiotic oligosaccharides (scGOS/lcFOS – 9:1; 8 g/L) has a similar to breast milk positive impact on the factors of local protection of mucous membranes such as lysozyme, α-defensins 1-3, sIgA, intestinal microbiota in formula-fed infants. This effect may reduce the risk of allergic and infectious diseases in children aged up to 18 months of life, compared with babies fed with the standard formula without oligosaccharides.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

None declared.

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The study was carried out according to scientific theme of Pediatrics Department of Lviv National Medical University, state registration number № 0108U101130.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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