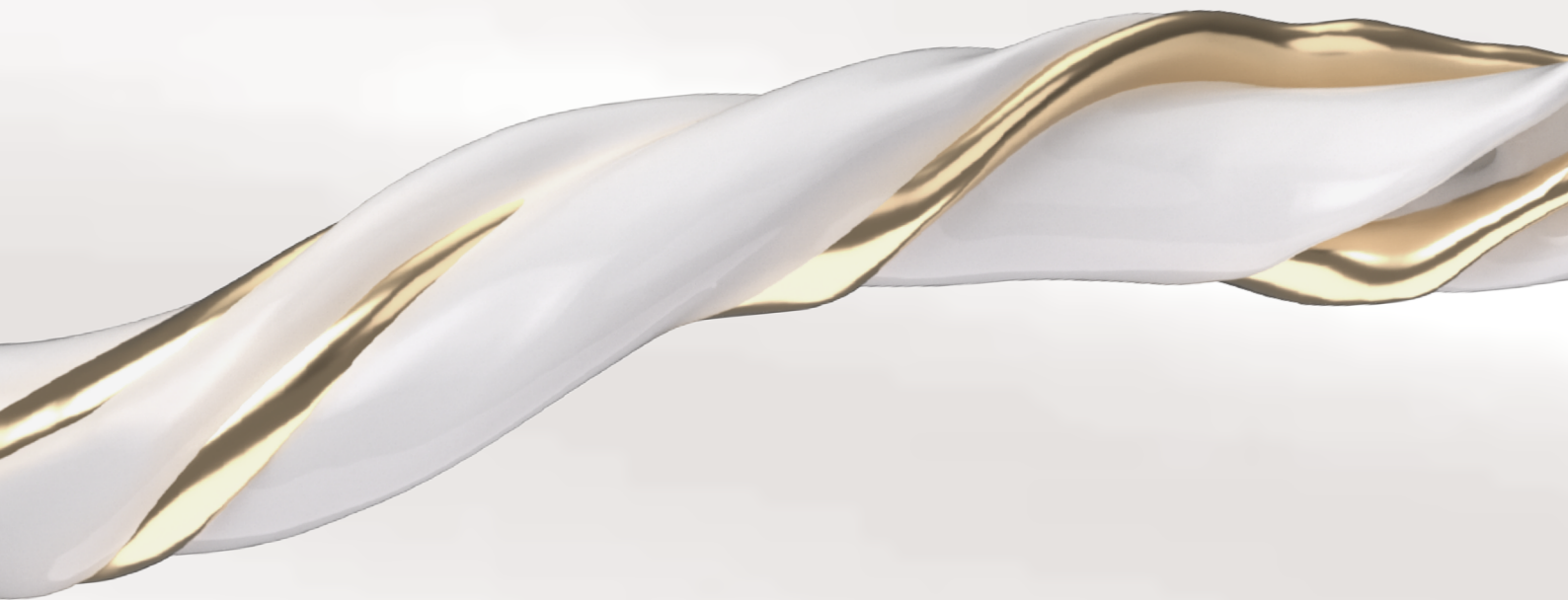


BREAST MILK IS BEST FOR BABIES

Nestlé research on HMOs and probiotics

Publication and main abstracts
from CLARA clinical trial



FOR HEALTHCARE PROFESSIONALS ONLY

* Formula and growing-up milk.

**Research based on a custom survey ran by Harris Interactive amongst Paediatricians recommending the category concluded in 2023 in the top 20 markets for infant formula, follow-up formula and growing-up milk representing 93.7% of sales in 2022 TABS data.

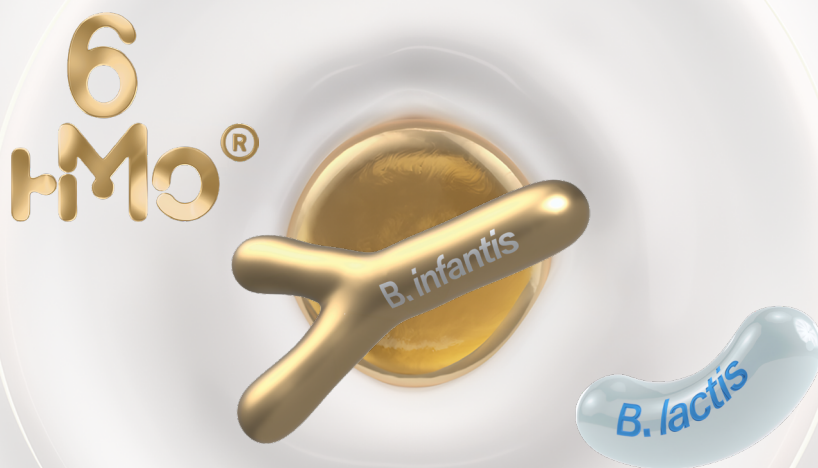
No.1 BRAND RECOMMENDED*
BY PEDIATRICIANS
WORLDWIDE** 

Nestlé: leading the way on early life nutrition research

Nestlé is one of the early pioneers of infant nutrition research and development. Nestlé continues to relentlessly pursue innovations to make sure that infants and children around the world can benefit from the most advanced science-based products.



This booklet provides background on Nestlé research journey on HMOs and main abstracts on the clinical study of formulas with a unique blend of age-adapted 6HMOs, precision probiotic *B. infantis**, plus *B. lactis*.



**B. infantis* LMG11588 is a unique strain due to its ability to utilise our blend of 6 HMOs and is free from antibiotic resistance.

Nestlé: leading the way on HMO research

Why are HMOs important¹?

Human milk oligosaccharides (HMOs) represent the third largest solid component in breast milk. Research suggests HMOs have various functional roles in infant health, including gut, immune and cognition. The absence of HMOs is a major gap in infant formula vs breastmilk and may explain some of the differences in health outcomes between breastfed and formula fed infants.

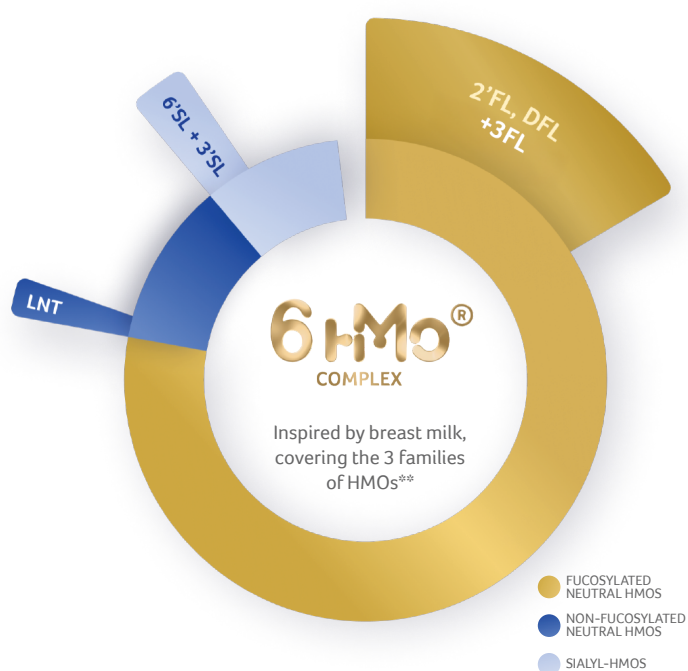


Figure 1. Nestlé 6 HMO complex represents 56% of major HMOs covering all 3 families in breast milk

** Colours represent the 3 families based on the 20 commonly measured HMOs in breast milk. Calculations based on known quantity of HMOs from first 5 publications and unpublished internal data. Open space illustrates that the exact proportions between the families are not known as many HMOs are not quantified. The 6 HMOs in NAN® SUPREMEPRO infant formula cover 56% of the 20 major ones found in human milk (calculations based on stage 1 after human milk).

Key discoveries from Nestlé human milk observational clinical studies²⁻⁵:

- Developed a validated method to quantify >20 major HMOs and confirmed there are 3 main categories
- The concentrations of HMOs vary across the stage of lactation
- The major influencers on composition:
 - Maternal genotype and stage of lactation have the strongest impact
 - Preterm birth influences sialylated HMOs

Specific synbiotic blend⁶⁻⁹:

Synbiotic is “a mixture comprising of live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host”.

HMOs in part exerts their benefits by being metabolized with gut microbiota, of which bifidobacteria is the most predominant in early life.

Not all infant-type bifidobacteria are equally able to use HMOs; consequently, the type present can differently influence infant’s microbial profile and gut environment - a synbiotic effect.

Nestlé research on a specific synbiotic blend of age-adapted 6HMOs, precision probiotic *B. infantis** plus *B. lactis*

Demonstrated synergistic effect with a unique combination⁹

Carefully selected for its unique properties, *B. infantis* LMG11588 has the ability to efficiently metabolize the 6HMO complex (2'FL, 3FL, DFL, LNT, 3'SL and 6'SL) with a synergistic effect influencing microbiome metabolites⁹ and is free from antibiotic resistance.

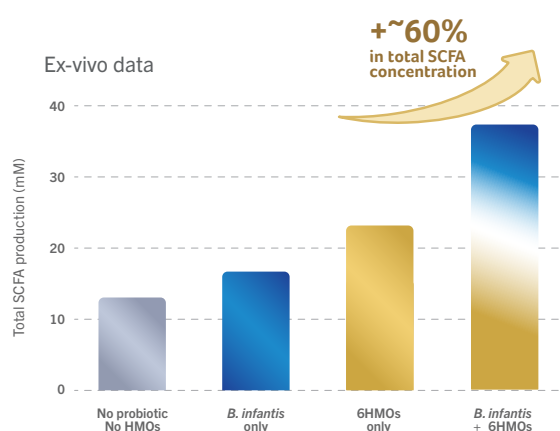
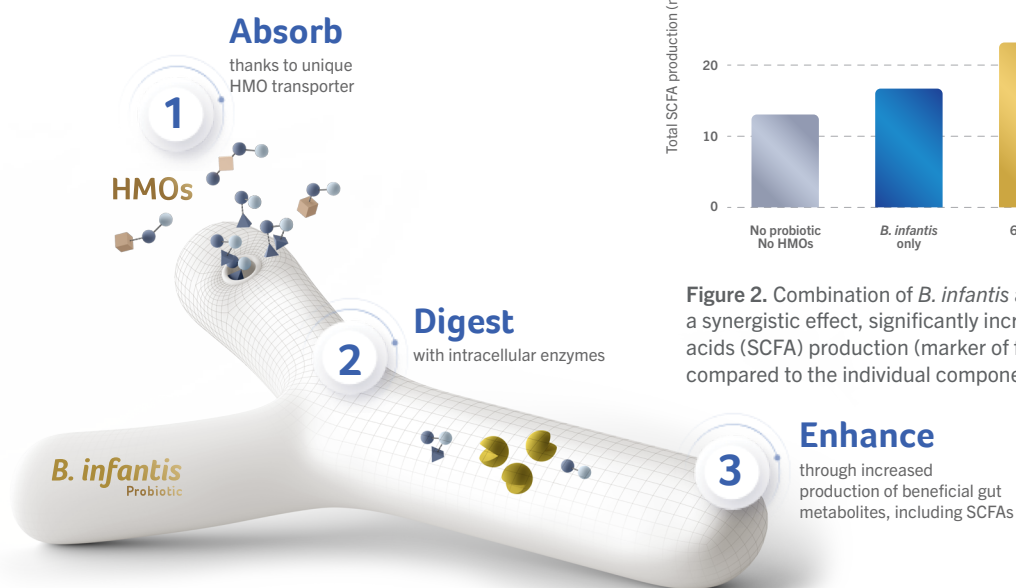


Figure 2. Combination of *B. infantis* and 6-HMOs demonstrated a synergistic effect, significantly increasing short chain fatty acids (SCFA) production (marker of favorable gut environment) compared to the individual components alone.⁹

Figure 1. *B. infantis* can take up specific HMOs from the diverse set of HMOs present in human milk. Once internalized, specific enzymes produced by *B. infantis* digest the internalized HMOs. This results in the formation and release of various metabolites with health-promoting properties for the infant.

*B. infantis**, naturally prevalent in breastfed infants, demonstrated synergistic effect with HMOs pre-clinically that resulted in enhanced gut colonization of beneficial bacteria and immune modulation—warranting clinical validation of these promising benefits. To rigorously

evaluate these benefits, the CLARA clinical trial, testing formulas with a blend of 6HMOs, *B. infantis** plus *B. lactis*, was meticulously designed and successfully completed, following infants all through 15 months of age. The main results will be presented in this booklet.

**B. infantis* LMG11588 is a unique strain due to its ability to utilise our blend of 6 HMOs and is free from antibiotic resistance

¹ Sprenger N, Tytgat HLP, Binia A, et al. J Hum Nutr Diet. 2022;35(2):280-299. ² Samuel TM, Binia A, de Castro CA, et al. Sci Rep. 2019;9:11767.

³ Austin S, De Castro CA, Sprenger N, et al. Nutrients. 2019;11(6):1282 ⁴ Lefebvre et al. Front Nutr. 2020;7 DOI=10.3389/fnut.2020.574459 ⁵ Austin S, Bénet T. Anal Chim Acta. 2018;1010:86-96. ⁶ Swanson K, Gibson G, Hutkins R, et al. Nature. 2020;17:687-701. ⁷ Lordan C, Roche A, Delsing D, et al. Microbiol Mol Biol Rev. 2024;88(1):e00094-23. ⁸ Duboux S, Ngom-Bru C, De Bruyn F, et al. Microorganisms. 2022;10(2):203. ⁹ De Bruyn, F., James, K., Cottenet, G. et al. Commun Biol. 2024;7:943. ¹⁰ Chichlowski . Res. M, et al. Nutrients 2020;12(6):1581. ¹¹ Duar RM, et al. Nutrients 2020;12(11):3247.

Table of contents

SUMMARY OF FULL PUBLICATION 4 MONTHS RESULTS:

Page — 06	Partially hydrolyzed, whey-based infant formula with six human milk oligosaccharides, <i>B. infantis</i> LMG11588, and <i>B. lactis</i> CNCM I-3446 is safe, well tolerated, and improves gut health: a staged analysis of a randomized trial Frontiers in Nutrition 2025
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SUPPORTING ABSTRACTS FOR 4 MONTHS FULL PUBLICATION:

Page — 08	Infant formula with a specific age-adapted synbiotic blend of six human milk oligosaccharides and <i>B. infantis</i> LMG11588 plus <i>B. lactis</i> CNCM I-3446 supports healthy, age-appropriate growth and is well-tolerated: a staged analysis of a double-blind, randomized, controlled trial Abstract presented at Excellence in Pediatrics 2023
Page — 10	Gut microbiota and probiotic establishment in healthy term infants fed an infant formula with a specific age-adapted synbiotic blend of six human milk oligosaccharides and <i>B. infantis</i> LMG11588 plus <i>B. lactis</i> CNCM I-3446: a staged analysis of a double-blind, randomized, controlled trial Abstract presented at Excellence in Pediatrics 2023
Page — 12	Biomarkers of gut maturity, inflammation, and metabolic environment in infants fed formula containing six human milk oligosaccharides, <i>B. infantis</i> LMG11588, and <i>B. lactis</i> CNCM I-3446 Abstract presented at ESPGHAN 2024

OTHER ABSTRACTS:

Page — 14	An age-adapted synbiotic blend of human milk oligosaccharides and <i>B. infantis</i> LMG11588 plus <i>B. lactis</i> may contribute to immune cell homeostasis in formula fed infants Abstract presented at ESPGHAN 2024
Page — 15	Gut microbiota establishment in infants fed formulas with an age-adapted synbiotic blend of six human milk oligosaccharides, <i>B. infantis</i> LMG11588, and <i>B. lactis</i> CNCM I-3446: a staged analysis of a double-blind, randomized trial Abstract presented at American Academy of Pediatrics 2024
Page — 17	Formulas containing six human milk oligosaccharides and two probiotics are safe, well-tolerated, and support adequate growth from birth to 15 months: a double-blind, randomized trial Abstract presented at Nutrition and Growth 2025
Page — 19	Formulas containing six human milk oligosaccharides and probiotics help the age-appropriate development of a specific Bifidobacterium-enriched gut microbiome through age 15 months: a randomized trial Abstract presented at ESPGHAN 2025

Publication Summary:

Partially hydrolyzed, whey-based infant formula with six human milk oligosaccharides, *B. infantis* LMG11588, and *B. lactis* CNCM I-3446 is safe, well tolerated, and improves gut health: a staged analysis of a randomized trial

Jean-Charles Picaud, Janne Marie Moll, Jacob Agerbo Rasmussen, Javier Miranda-Mallea, Olivier Claris, Laura Palomino Fernández, Ignacio Salamanca de la Cueva, Luc Cornette, Philippe Alliet, André Léké, Mireille Castanet, Hugues Piloquet, Virginie de Halleux, Delphine Mitanchez, Yvan Vandenplas, Pierre Maton, Frank Jochum, Dirk Olbertz, Sergio Negre Policarpo, Shaillay Kumar Dogra, Luca Lavalle, Irma Silva Zolezzi, Nicholas P. Hays, Norbert Sprenger

Frontiers in Nutrition 2025;12:1628847

Design & Objective:

Multicenter European trial on healthy formula fed infants randomized to control or test formula with 6HMOs and 2 probiotics. Non-randomized breastfed infants served as reference group. The overall aim was to assess growth, tolerance, and gut microbiome after development (Figure 1).



Figure 1. Study design: A multicenter European trial in which healthy formula-fed infants aged ≤ 14 days were randomized to control formula (partially hydrolysed 100% whey-based formula) or test formula (same formula supplemented with a blend of 6 HMOs, *B. infantis** and *B. lactis*: NAN® SUPREMEPRO SINERGITY™)

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Continuation of abstract >**Endpoint:**

Weight gain velocity through age 4 months, as well as abundance of *Bifidobacteria*, immune and gut health markers, gastrointestinal (GI) tolerance, and adverse events (AEs) through age 15 months.

Key Messages:

Supplementation with a specific age-appropriate synbiotic:

- Promoted, healthy age-appropriate growth and was safe:
 - Weight gain velocity plus z scores for weight, length, head circumference and BMI that are non-inferior to those of the control group.
 - Physician-reported adverse events (AEs) were similar between all 3 groups.
- Modulate microbiota and gut environment:
 - Significantly increases abundance of beneficial bifidobacteria, as well as the supplemented *Bifidobacterium longum* subsp. *infantis* LMG 11588 and *B. lactis*; decreases abundance of potential pathogenic *C. difficile* and fecal pH.
- Enhance gut barrier maturation and support normal gastrointestinal (GI) tolerance:
 - Promote gut health marker that moved closer to those of the breastfed reference group.
 - Resulted in parent-reported GI tolerance and stool patterns comparable to those observed in the control group.

Conclusion:

Infant formula supplemented with a synbiotic blend support healthy, age-appropriate growth, good GI tolerance, and increased the abundance of beneficial bifidobacteria through 4 months of age.

Weight gain velocity through age 4 months, as well as abundance of *Bifidobacteria*, immune and gut health markers, gastrointestinal (GI) tolerance, and adverse events (AEs) through age 15 months.

Infant formula with a specific age-adapted synbiotic blend of six human milk oligosaccharides and *B. infantis* LMG11588 plus *B. lactis* CNCM I-3446 supports healthy, age-appropriate growth and is well-tolerated: a staged analysis of a double-blind, randomized, controlled trial

Miranda, Javier; Claris, Olivier; Gil-Campos, Mercedes; Salamanca, Ignacio; Cornette, Luc; Alliet, Philippe; Léké, André; Castanet, Mireille; Piloquet, Hugues; de Halleux, Virginie; Mitanchez, Delphine; Vandenplas, Yvan; Maton, Pierre; Jochum, Frank; Olbertz, Dirk; Negre, Sergio; Lahiry, Anirban; Silva Zolezzi, Irma; Hays, Nicholas P.18; Sprenger, Norbert; Picaud, Jean-Charles

Abstract presented at Excellence Pediatrics 2023

Background:

Formulas supplemented with human milk oligosaccharides (HMOs) and probiotics may promote multiple health benefits, especially immune-related ones in formula-fed infants. This ongoing trial assesses growth, gastrointestinal (GI) tolerance, and gut microbiome outcomes in infants fed with formulas supplemented with 6 HMOs and two probiotics from birth to age 15 months (mo). Here, we report staged endpoint analysis results for all infants up to age 4.

Methods:

In a multicenter European trial (Clinical trials.gov: NCT04962594), formula-fed infants (FFI) aged ≤ 14 days (d) were randomised to control formula (CF, n=117, partially hydrolysed 100% whey-based formula) or experimental formula (EF, n=119, same formula

supplemented with 1.77g/L HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. lactis* [CNCM I-3446], and *B. infantis* [LMG11588]). Non-randomized breastfed infants (BF, n=82) served as a reference group. The primary outcome was weight gain velocity (WGV) in EF vs. CF, measured as mean daily WG from enrollment to age 4mo, with a non-inferiority margin of -3g/d . The secondary objective was to demonstrate increased *Bifidobacterium* abundance in EF vs. CF at age 3 (reported separately). Additional endpoints in this report include anthropometric measures, stooling patterns (3-D parent diary), GI tolerance (validated Infant Gastrointestinal Symptom Questionnaire [IGSQ, score range 13-65]), and serious adverse events through 4mo.

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Results:

WGV from enrollment to age 4mo (Table) was non-inferior in EF vs. CF-fed infants. In the per-protocol set, the estimated mean difference (95% confidence interval) in WGV between EF (n=72; mean±SD, 29.0±5.4g/d) and CF (n=77; 27.8±5.1g/d) fed infants was 1.50g/d (−0.15, 3.15; p-value for non-inferiority<0.001); WGV in BF infants was similar (n=51; 26.9±5.7g/d). Throughout this study, interval EF and CF groups had similar weight-for-age, length-for-age, head circumference-for-age, and weight-for-length z-scores and all mean±95%CI z-scores were tracked closely (±~0.5 SD) with the WHO median. EF and CF groups had similar soft stooling patterns and IGSQ scores at all time points. Mean IGSQ scores at 4 were low (≤22.7) in all groups, indicating good GI tolerance. Parent-reported and physician-confirmed SAEs were similar in EF (18 events in 18 infants) and CF (17 events in 15 infants); no SAEs were considered to be related to the study formulas.

Conclusions:

A partially hydrolysed infant formula with a specific synbiotic blend of 6 HMOs and *B. infantis* plus *B. lactis* supports healthy, age-appropriate growth is safe and well-tolerated through 4 months of age. Future analyses through 15 months of age will provide additional evidence on the impact of EF feeding on these endpoints, as well as fecal and blood biomarkers of immune development.

Disclosure:

A Lahiry, I Silva Zolezzi, NP Hays and N Sprenger are employees of Société des Produits Nestlé SA, the sponsor

Table: Difference in weight gain velocity (g/day) from enrolment to age 4 months

Population	Groups	Weight gain velocity, g/day, mean (SD)	Difference between groups ¹		
			Estimate	95% CI	Non-inferiority p-value
Full analysis set	CF (n=98)	27.9 (5.3)			
	EF (n=95)	29.5 (5.9)	1.59	0.07, 3.11	<0.001
Per protocol set	CF (n=77)	27.8 (5.1)			
	EF (n=72)	29.0 (5.4)	1.50	-0.15, 3.15	<0.001

¹ From an ANCOVA model with baseline weight, sex, mode of delivery and centre as covariates. CF, control group; EF, experimental group.

Gut microbiota and probiotic establishment in healthy term infants fed an infant formula with a specific age-adapted synbiotic blend of six human milk oligosaccharides and *B. infantis* LMG11588 plus *B. lactis* CNCM I-3446: a staged analysis of a double-blind, randomized, controlled trial

Jean-Charles Picaud, Janne Marie Moll, Javier Miranda, Olivier Claris, Mercedes Gil-Campos, Ignacio Salamanca, Luc Cornette, Philippe Alliet, André Léké, Mireille Castanet, Hugues Piloquet, Virginie de Halleux, Delphine Mitanchez, Yvan Vandenplas, Pierre Maton, Frank Jochum, Dirk Olbertz, Sergio Negre, Anirban Lahiry, Paula Rodríguez-García, Irma Silva Zolezzi, Nicholas P. Hays, Norbert Sprenger

Abstract presented at Excellence Pediatrics 2023

Background:

Human milk oligosaccharides (HMOs) are non-digestible fibers driving gut microbiome development in early life and promoting multiple health benefits. *B. longum* subsp. *infantis* (*B. infantis*) is an HMO-utilizing infant-type *Bifidobacterium* species dominating early life gut microbiome. It is increasingly recognized as important for age-appropriate gut health and immune development, and globally, a progressive loss of HMO-utilizing bifidobacteria is observed. This ongoing trial aims to assess growth and gastrointestinal tolerance (reported separately) as well as gut microbiome modulating effects in infants fed with formulas supplemented with 6 HMOs and two probiotics from birth to age 15 months (mo). The key secondary objective being higher *Bifidobacterium* abundance in experimental formula (EF) vs. control formula (CF) fed infants at age 3 mo. Here, we report staged endpoint analysis results for all infants up to age 3 mo.

Methods:

In a multicenter European trial (Clinical trials.gov: NCT04962594), healthy infants aged ≤ 14 days were randomized to CF (n=117, partially hydrolyzed 100% whey-based formula), or EF (n=119, same formula supplemented with 1.77g/L HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. lactis* [CNCM I-3446], and *B. infantis* [LMG11588]). Non-randomized breastfed infants (BF, n=82) were enrolled as reference. Fecal samples collected at baseline and at age 3 mo were analyzed for microbiota via shotgun metagenomics. The full analysis set (FAS) sample size for microbiota analyses was n=111 (CF), n=105 (EF), and n=72 (BF). Microbiome differences between the randomized groups EF and CF were assessed analyzing differential abundance using a compositionality-bias-corrected mixed linear model adjusting for baseline age and study site, as well as differential prevalence using a Fisher's exact test.

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Results:

At baseline, *Bifidobacterium* abundance was similar between EF and CF fed infants. At age 3mo, EF fed infants had a significantly higher *Bifidobacterium* abundance compared to CF fed infants (Figure). Notably, both at baseline and at age 3mo BF infants had a bimodal distribution with high and low *Bifidobacterium* abundance; low abundance may indicate a lack of HMO utilization capacity. In the CF group, many infants also showed very low abundance at both timepoints, while all EF fed infants had a higher bifidobacteria abundance at 3 mo. Similar group differences were observed for infant-type bifidobacteria (*B. longum* subsp. *longum*, *B. infantis*, *B. breve*, *B. bifidum*, and *B. scardovii*). Both *B. infantis* and *B. lactis* were significantly higher in EF compared to CF groups, showing a prevalence of 100% and 97%, respectively. *B. infantis* LMG51188 strain tracking showed very high probiotic colonization in EF (~75% of infants). Pathogen analysis via virulence factors revealed very low prevalence (<10/288 for *Escherichia coli* and *Sarcina perfringens* (formerly known as *Clostridium perfringens*);

0 for *Campylobacter jejuni*). Only toxigenic *Clostridiodes difficile* showed a high prevalence and abundance at age 3 mo in the CF group, with both being significantly lower in the EF group.

Conclusion:

An infant formula with a specific synbiotic blend of 6 HMOs and *B. infantis* plus *B. lactis* supported the expansion of beneficial bifidobacteria, especially infant-type species in all infants. The reduced load and prevalence of toxigenic *C. difficile* indicates improved gut health via reduction of a risk factor for gastrointestinal infection.

Disclosure:

A Lahiry, I Silva Zolezzi, NP Hays and N Sprenger are employees of Société des Produits Nestlé (SPN) SA, the sponsor of the trial. JM Moll and P Rodríguez-García are employees of Clinical Microbiomics and collaborated under a research contract with SPN.

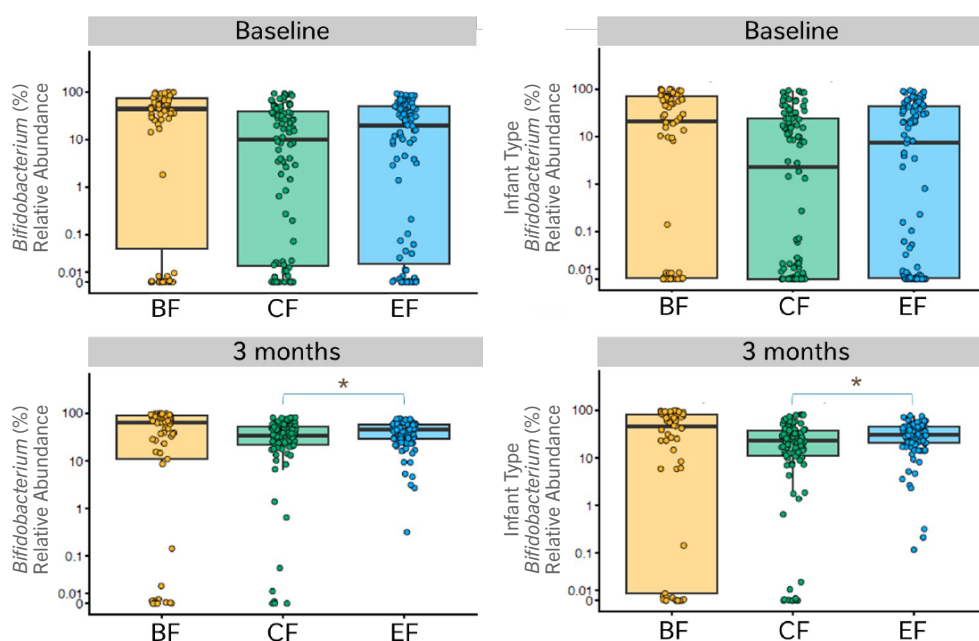


Figure. *Bifidobacterium* (total and infant-type) abundance at baseline and at age 3 months (abundance shown on a pseudo-log scale). In the FAS, EF group had significantly increased *Bifidobacterium* abundance compared to CF group at age 3 months (* $p < 0.05$; total *Bifidobacterium* abundance (median (interquartile range [IQR])): EF: 44.7% (30.8%); CF: 34.1% (29.3%), infant-type *Bifidobacterium* abundance: EF: 31.4% (24.5%); CF: 22.6% (27.0%)). Boxplots display the lower and upper quartiles (upper and lower box edges, respectively), the median (horizontal line inside box), and the extreme of the data observations within 1.5*IQR (whiskers).

Biomarkers of gut maturity, inflammation, and metabolic environment in infants fed formula containing six human milk oligosaccharides, *B. infantis* LMG11588, and *B. lactis* CNCM I-3446

Javier Miranda, Olivier Claris, Mercedes Gil-Campos, Ignacio Salamanca, Luc Cornette, Philippe Alliet, André Léké, Mireille Castanet, Hugues Piloquet, Virginie de Halleux, Delphine Mitanchez, Yvan Vandenplas, Pierre Maton, Frank Jochum, Dirk Olbertz, Sergio Negre, Anirban Lahiry, Irma Silva Zolezzi, Nicholas P. Hays, Norbert Sprenger, Jean-Charles Picaud

Abstract presented at ESPGHAN 2024

Objectives:

Formulas supplemented with human milk oligosaccharides (HMOs) and probiotics may promote favorable changes in biomarkers of gut maturity, inflammation, and metabolic environment in infants. Here, we report secondary, staged analysis results (baseline and age 3 months [mo]) from an ongoing trial of infants fed formulas supplemented with 6 HMOs and two probiotics from birth to 15mo.

Methods:

In a multicenter European trial (Clinical trials.gov: NCT04962594), formula-fed infants aged ≤ 14 days were randomized to control (CF, partially hydrolyzed 100% whey-based; n=117) or experimental formula (EF, same formula plus 1.77g/L HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. lactis* [CNCM I-3446; 1×10^6 CFU/g], and *B. infantis* [LMG11588; 5×10^5 CFU/g]; n=119). Non-randomized breastfed infants (BF; n=82) were a reference group. Stool samples were analyzed for biomarkers of intestinal immune response (total secretory immunoglobulin A [sIgA]), inflammation (calprotectin), and maturity (alpha-1 antitrypsin [AAT]), plus markers of

gut metabolic environment (pH and short chain fatty acids [SFCA]). ANCOVA models were corrected for baseline value, age, and site; a propensity score was included to allow comparison with the non-randomized BF group.

Results:

No significant differences in sIgA or calprotectin (mg/kg dry stool) were observed (Figure); higher sIgA values in BF are likely due to breast milk intake. AAT (mg/g dry stool) in EF was significantly lower than CF ($p=0.015$; Figure), while not different than BF. Mean $\pm$ SD fecal pH in EF (6.7 ± 1.1) was lower than CF (7.0 ± 0.8 ; $p=0.032$), while both groups were higher than BF (4.9 ± 1.3 ; $p<0.0001$). EF and CF had lower acetate (% total SFCA) than BF (both $p<0.033$); propionate was higher in EF vs. BF ($p=0.005$).

Conclusions:

Partially hydrolyzed infant formula with a synbiotic blend of 6 HMOs and two probiotics positively supports gut maturity and lowers pH at age 3mo. Analyses at later timepoints will provide evidence of the impact of this formula on additional health-related outcomes.

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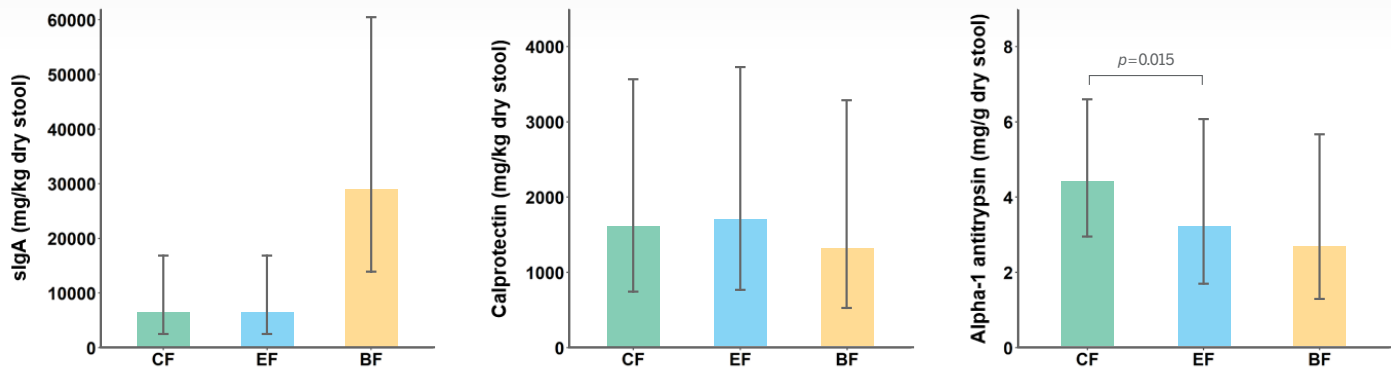


Figure. Geometric mean and geometric standard deviation values of fecal biomarkers of intestinal immune response (total secretory immunoglobulin A [sIgA]), gut inflammation (calprotectin), and gut maturity (alpha-1 antitrypsin) at 3 months of age. CF, control formula group; EF, experimental formula group; BF, breastfed reference group. P-values from ANCOVA models with baseline value, age, site, and propensity score as covariates. P-values were adjusted for multiplicity using Benjamini-Hochberg correction.

Disclosure:

Anirban Lahiry, Irma Silva Zolezzi, Nicholas P. Hays and Norbert Sprenger are employees of Société des Produits Nestlé S.A., the sponsor of the trial.

An age-adapted synbiotic blend of human milk oligosaccharides and *B. infantis* LMG11588 plus *B. lactis* may contribute to immune cell homeostasis in formula-fed infants

Jean-Charles Picaud, Karine Vidal, Janne Marie Moll, Clara study group, Anirban Lahiry, Irma Silva Zolezzi, Nicholas P. Hays, Norbert Sprenger, Mario Noti

Abstract presented at ESPGHAN 2024

Objectives:

Decreased levels of human milk oligosaccharides (HMO)-utilizing bifidobacteria have been associated with inflammation and immune dysregulation in early life and may increase the risk of chronic inflammatory disorders including allergies. In an ongoing study, we examined systemic immune cell profiles in formula-fed infants supplemented with an age-adapted synbiotic.

Methods:

In a multicenter European trial (NCT04962594), healthy infants aged ≤ 14 days were randomly assigned to either a control formula group (CF; $n=117$, partially hydrolyzed 100% whey-based formula) or an experimental formula group (EF; $n=119$, CF supplemented with 1.77g/L of 6 HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. lactis* CNCM I-3446, and *B. infantis* LMG11588. In a sub-cohort, blood samples were collected from CF ($n=39$), EF ($n=35$) and a reference group of breast-fed infants (BF, $n=16$) at 4 months of age. Peripheral blood mononuclear cells (PBMCs) were isolated and immune cell frequencies analyzed by ChipCytometry. Spearman correlation matrices were calculated for immune cell-network analyses.

Results:

At 4 months of age, T-helper (Th) 2 cells were significantly decreased in EF compared to CF ($p=0.033$), following a comparable pattern in BF. Although the relative abundance of Th1 and Th17 cells was comparable among all feeding

groups, cell-network analyses identified different modules of co-regulated immune cell populations in EF compared to CF infants. In EF, but not CF, Th1 cells positively correlated with Th2, Th17, regulatory T cells and antibody producing CD38+ plasmablasts suggesting a balanced cross regulation among these populations (Figure 1).

Conclusions:

An age-adapted synbiotic blend composed of 6 HMOs, *B. infantis* plus *B. lactis* led to a decrease in systemic Th2 cells associated with a balanced cross-regulation of T-helper cell subsets, potentially contributing to immune homeostasis in formula fed infants. Future analyses up to 15 months of age may provide additional evidence on the impact of this blend on infant health.

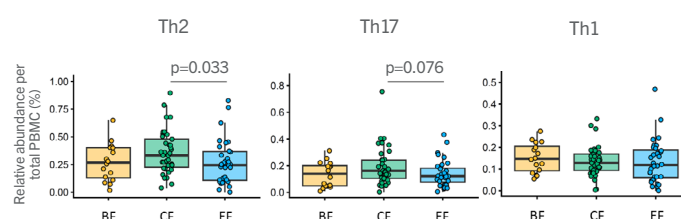


Figure 1. Relative abundance of T-helper type (Th) 2, Th17 and Th1 cells in peripheral blood mononuclear cells (PBMCs) of control formula (CF, $n=39$) and experimental formula (EF, $n=35$) fed infants at 4 months of age. Breastfed infants ($n=16$) served as reference control. Individual data are plotted with mean as bar and standard deviation. Non-parametric t-test was used to compare EF versus CF.

Gut microbiota establishment in infants fed formulas with an age-adapted synbiotic blend of six human milk oligosaccharides, *B. infantis* LMG11588, and *B. lactis* CNCM I-3446: a staged analysis of a double-blind, randomized trial

Jean-Charles Picaud, Janne Marie Moll, Javier Miranda-Mallea, Olivier Claris, Laura Palomino Fernández, Ignacio Salamanca de la Cueva, Luc Cornette, Philippe Alliet, André Léké, Mireille Castanet, Hugues Piloque, Virginie de Halleux, Delphine Mitanchez, Yvan Vandenplas, Pierre Maton, Frank Jochum, Dirk Olbertz, Sergio Negre Policarpo, Nicholas P. Hays, Norbert Sprenger

Abstract presented at American Academy of Pediatrics 2024

Objectives:

Human milk oligosaccharides (HMOs) drive gut microbiome development in early life via microbes able to metabolize specific HMOs. *B. longum subsp. infantis* (*B. infantis*) can do so and is increasingly recognized to contribute to early postnatal immune development. Globally, a progressive loss of HMO-metabolizing bifidobacteria is observed. In this ongoing trial we assessed gut microbiome modulating effects in infants fed formulas supplemented with 6 HMOs and two probiotics from birth to age 15 months (mo). Here, we report staged endpoint analysis results for all infants up to age 6mo.

Methods:

In a multicenter European trial (Clinical trials.gov: NCT04962594), formula-fed infants aged ≤ 14 days were

randomized to control ($n=117$, partially hydrolyzed 100% whey-based formula), or test formula ($n=119$, same formula supplemented with 1.77g/L HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. lactis* [CNCM I-3446; 1×10^6 CFU/g], and *B. infantis* [LMG11588; 5×10^5 CFU/g]). Non-randomized breastfed infants ($n=82$) were enrolled as reference. Complementary diet was allowed from 4mo. Fecal samples collected at baseline, 3 and 6mo were analyzed for microbiota via shotgun metagenomics. Microbiome differences between control and test formula groups were assessed analyzing differential abundance using a compositionality-bias-corrected mixed linear model adjusting for baseline age and study site, as well as differential prevalence using a binomial generalized linear regression model.

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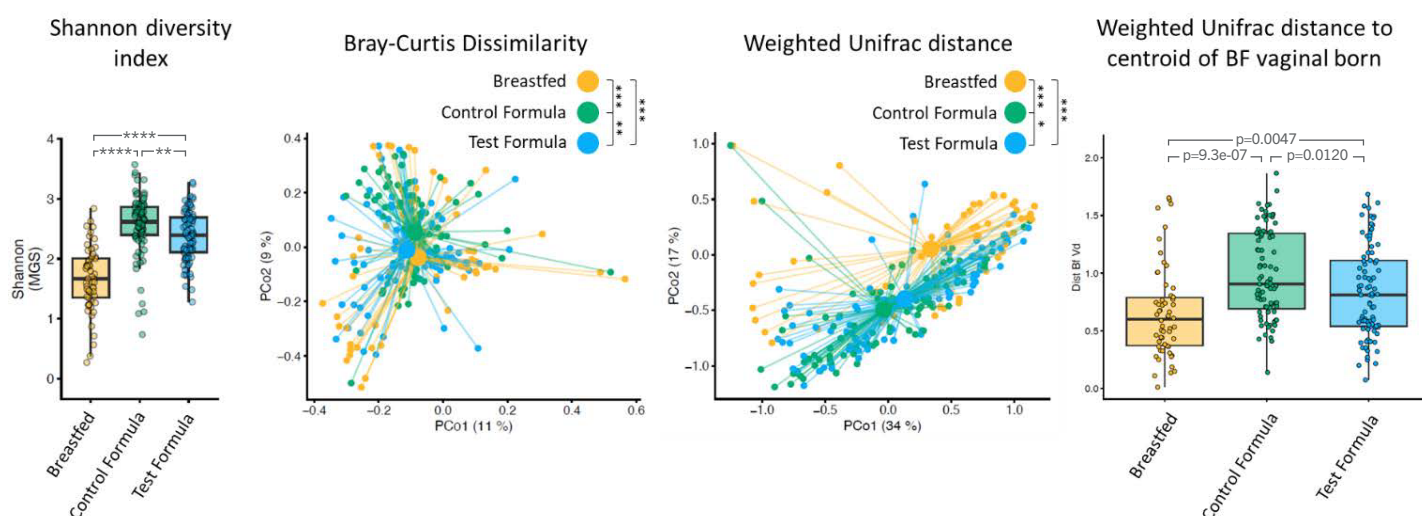


Figure 1. Intra-individual (Shannon at species [MGS]-level) and inter-individual (Bray-Curtis dissimilarity, Weighted Unifrac distance) diversity measures at 6 months of age by feeding group (full analysis set). Weighted Unifrac distance to breastfed vaginally born infants illustrates the closeness of the randomized feeding groups to the reference. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

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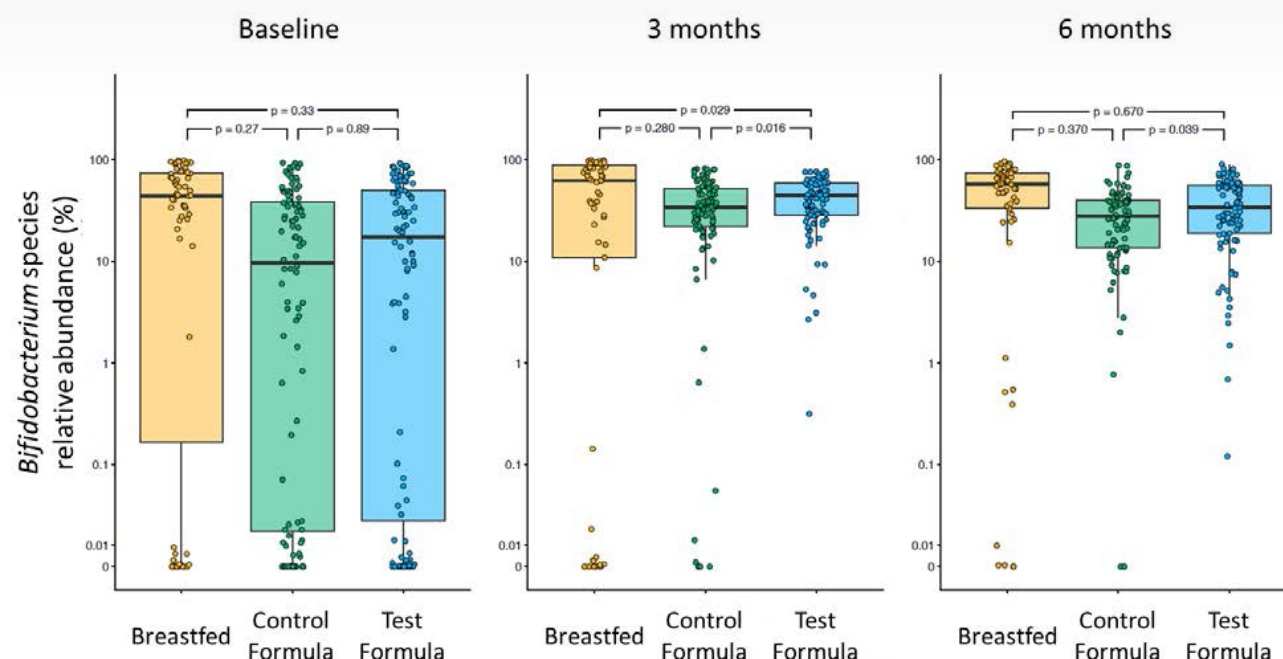


Figure 2. Relative *Bifidobacterium* species abundance shown per infant and feeding group (full analysis set) on a pseudo-log scale. Statistics are assessed using a cross-sectional, bias-corrected mixed effects model that corrects for baseline age (fixed effect) and study site (random effect).

Results:

At baseline, diversity measures were similar between test and control groups, while at 6mo both intra- and interindividual diversity measures showed significant differences with test formula-fed infants being closer to breastfed (**Figure 1**). At 3 and 6mo, test formula-fed infants had a significantly higher abundance of all (**Figure 2**) and infant-type *Bifidobacterium* species (*B. longum* subsp. *longum*, *B. infantis*, *B. breve*, *B. bifidum*, and *B. scardovii*) compared to control formula-fed infants and closer to breastfed.

Ruminococcus gnavus, a mucolytic species associated with inflammatory conditions, was significantly higher in control formula-fed infants at both 3 and 6mo compared to test formula and breastfed infants. *B. infantis* and *B. lactis* were significantly higher in test formula-fed infants compared to controls at 3 and 6mo. and strain tracking of *B. infantis* revealed high prevalence of the provided LMG11588 strain in the test formula group and no replacement of autochthonous strains. Pathogen analysis via virulence

factors revealed primarily the presence of toxigenic *Clostridioides difficile* with a high prevalence and abundance at age 3 and 6mo in the control formula group and significantly lower prevalence in test formula-fed and breastfed infants.

Conclusions:

An infant formula with a specific synbiotic blend of 6 HMOs and *B. infantis* plus *B. lactis* supported the expansion of beneficial HMO metabolizing bifidobacteria to 6mo, especially infant-type species in all infants. The lower presence of mucolytic *R. gnavus* and toxigenic *C. difficile* in test formula-fed infants indicates improved gut ecology via reduction of risk factors.

Disclosure:

NP Hays and N Sprenger are employees of Société des Produits Nestlé (SPN) SA, the sponsor of the trial. JM Moll is an employee of Clinical Microbiomics and collaborated under a research contract with SPN.

Formulas containing six human milk oligosaccharides and two probiotics are safe, well-tolerated, and support adequate growth from birth to 15 months: a double-blind, randomized trial (ID 581)

Miranda-Mallea J., Claris O., Fernández L.P., Salamanca De La Cueva I., Cornette L., Alliet P., Léké A., Castanet M., Piloquet H., De Halleux V., Mitanchez D., Vandenplas Y., Maton P., Jochum F., Olbertz D., Policarpo S.N., Lavallo L., Zolezzi I.S., Hays N., Sprenger N., Picaud J

Abstract presented at American Nutrition and Growth 2025

Background & Objectives:

Formulas supplemented with human milk oligosaccharides (HMOs) and probiotics may promote health-related benefits in formula-fed infants. Here we report growth and gastrointestinal (GI) tolerance in infants fed formulas supplemented with 6 HMOs and two probiotics from birth to age 15 months (m).

Methods:

In a multicenter European trial (Clinicaltrials.gov: NCT04962594), formula-fed infants aged 14 days were randomized to control formula (n=117, partially hydrolyzed 100% whey-based formula) or test formula (n=119, same formula supplemented with HMOs [2'FL, DFL, 3-FL, LNT, 3'SL, 6'SL], *B. infantis* [LMG11588; 5x10⁵ CFU/g], and *B. lactis* [CNCM I-3446; 1x10⁶ CFU/g]). Non-randomized breastfed infants (n=82) served as a reference group. Anthropometry, stool consistency, GI tolerance (measured by validated index score), and adverse events (AEs) were assessed through 15m.

Results:

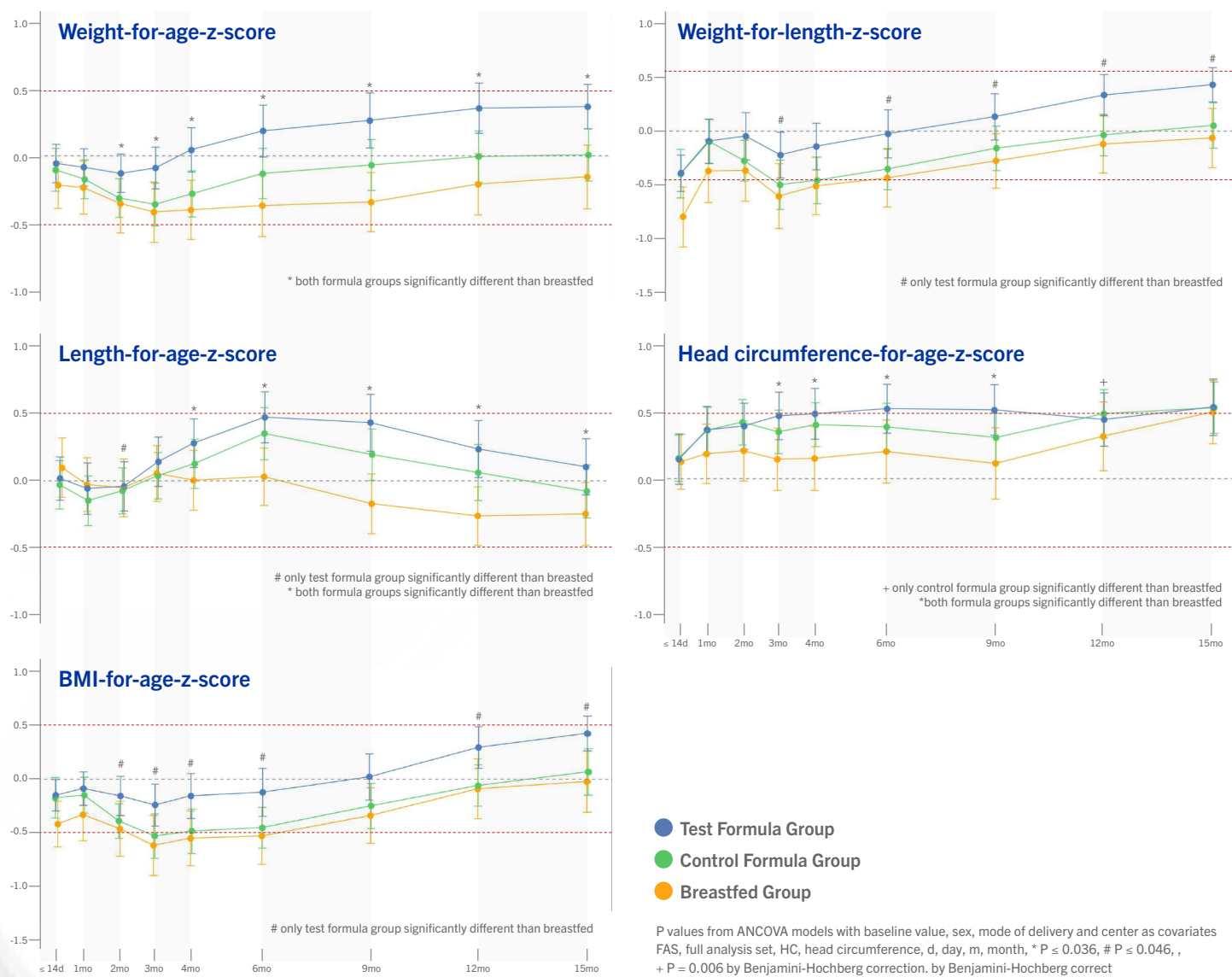
There were no significant differences between test and control formula groups in anthropometric z-scores at any time point; values in breastfed infants were significantly lower than in formula-fed groups for most scores at most time points (Figure). There were no significant differences in stool consistency between test and control formula groups at any time; formula-fed and breastfed infants had similar stool consistency from 9 to 15m. For both formula-fed groups, GI symptom index scores were comparable to those observed in breastfed infants through 12m and significantly lower at 15m, indicating sustained good GI tolerance. AE incidence through 15m was similar in both formula-fed groups.

Conclusions:

An infant formula supplemented with 6 HMOs and two probiotics supports age-appropriate growth through 15m and is well tolerated.

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Figure. Mean $\pm$ 95% CI anthropometric z-scores from enrolment to age 15 months (FAS)

Formulas containing six human milk oligosaccharides and probiotics help the age-appropriate development of a specific *Bifidobacterium*-enriched gut microbiome through age 15 months: a randomized trial

Jean-Charles Picaud, Janne Marie Moll, Jacob Agerbo Rasmussen, Javier Miranda-Mallea, Olivier Claris, Laura Palomino Fernández, Ignacio Salamanca de la Cueva, Luc Cornette, Philippe Alliet, André Léké, Mireille Castanet, Hugues Piloquet, Virginie de Halleux, Delphine Mitanchez, Yvan Vandenplas, Pierre Maton, Frank Jochum, Dirk Olbertz, Sergio Negre Policarpo, Shaillay Kumar Dogra, Luca Lavalle, Irma Silva Zolezzi, Nicholas P. Hays, Norbert Sprenger

Abstract presented at ESPGHAN 2025

Objectives:

Formulas supplemented with human milk oligosaccharides (HMOs) and probiotics may promote immune-related benefits in formula-fed infants through supporting an age-appropriate gut microbiome development. We assessed gut microbiome development in infants fed formulas supplemented with 6 manufactured HMOs and two probiotics from birth to age 15 months (m).

Methods:

In a multicenter European trial (Clinicaltrials.gov: NCT04962594), formula-fed infants aged ≤ 14 days were randomized to control formula (n=117, partially hydrolyzed 100% whey-based formula) or test formula (n=119, same formula supplemented with HMOs [2'FL, DFL, 3FL, LNT, 3'SL, 6'SL] at 1.77g/L until 6m, 0.87g/L from 6-12m, and 0.75g/L from 12-15m, and *B. infantis* [LMG11588; 5×10^5 CFU/g], and *B. lactis* [CNCM I-3446; 1×10^6 CFU/g]). Non-randomized breastfed infants (n=82) served as reference group. Fecal samples were analyzed for microbiome and key metabolites.

Results:

The following global microbiome indices showed differences between infants fed test or control formulas: 1) intra- (at 3m) and inter- (at 3 and 6m) individual diversity; 2) interindividual bifidobacteria diversity (3, 6 and 15m); and 3) microbiome maturation trajectory (with fewer infants off the trajectory in the test formula group). Butyrate and related microbial metabolic capacity were significantly lower in test versus control (3 and 6m; $p < 0.05$) reaching similar levels thereafter following a more pronounced increase (6 to 12m) as seen in breastfed infants. At the level of taxa, relative abundance of infant-type bifidobacteria were higher through 6m in infants fed test versus those fed control (Figure 1, Panel A); *B. infantis* and *B. lactis* were also higher through 15m. At the strain level, infants fed the test formula had higher *B. infantis* LMG11588 (supplemented in the formula), as well as other autochthonous strains through 15m (Figure 1, Panel B).

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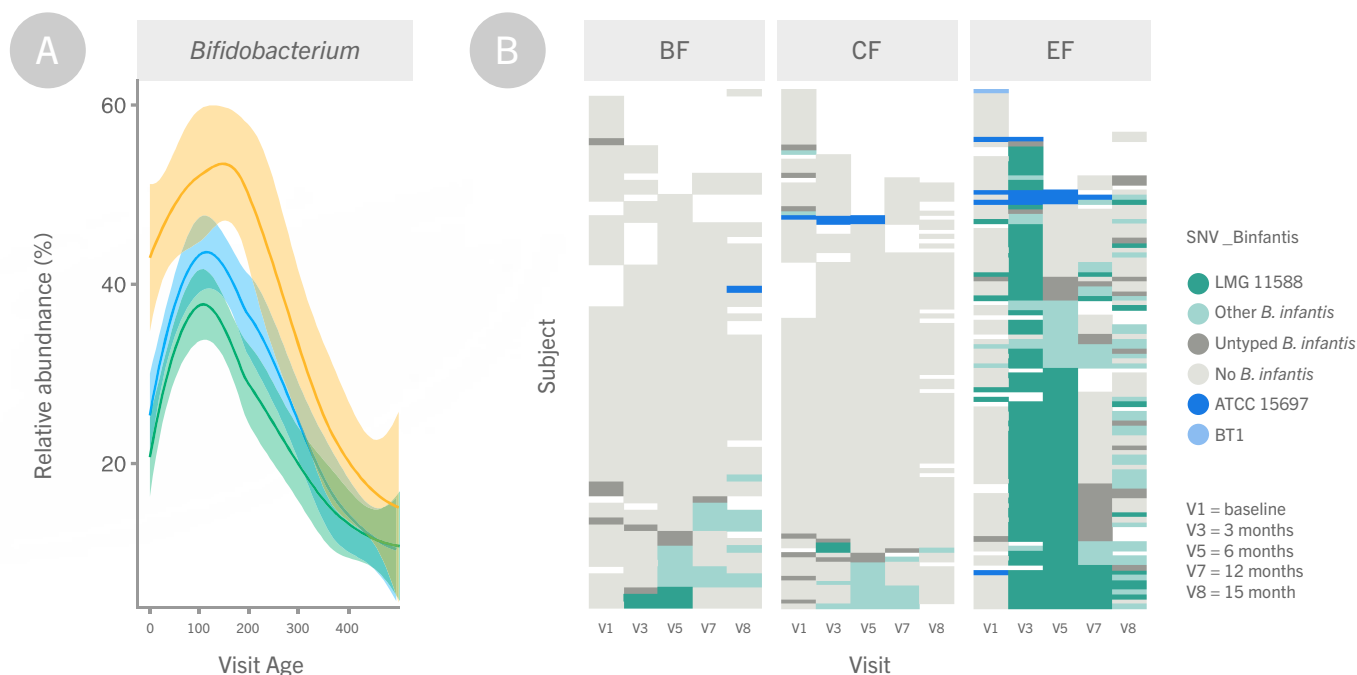
Conclusions:

Infant formulas supplemented with a synbiotic blend support the development of an age-appropriate gut microbiome with specific, beneficial bifidobacteria through age 15m. This may contribute to overall health and immune development.

Disclosure:

S.K. Dogra, L. Lavalle, I.S. Zolezzi, N.P. Hays, and N. Sprenger are employees of Société des Produits Nestlé S.A. (SPN), the sponsor of this trial. J.M. Moll and J.A. Rasmussen are employees of Cmbio, with whom SPN had a service agreement. The remaining co-authors have no relevant financial or non-financial competing interests to declare.

Figure 1. Relative abundance and presence at strain level per infant and feeding group through 15m



Loess fit of the relative *Bifidobacterium* abundance (Panel A) and presence of *B. infantis* strains by feeding group (Panel B)

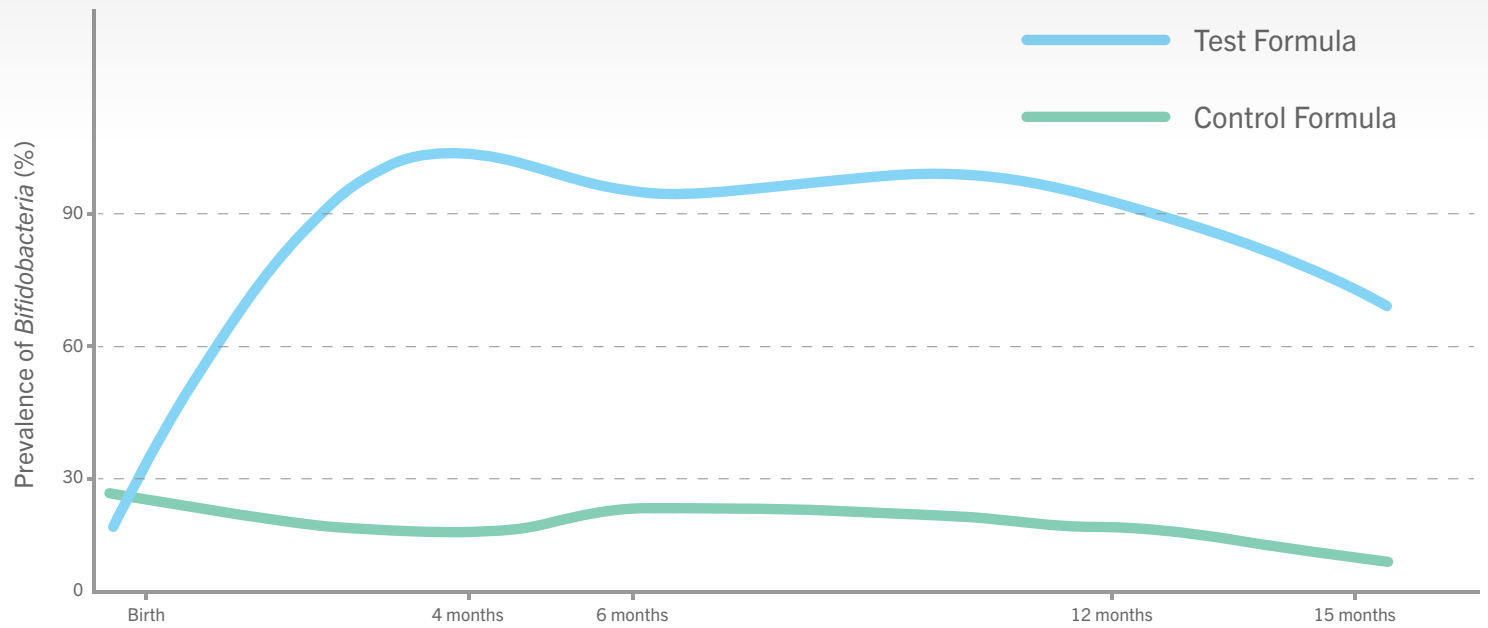
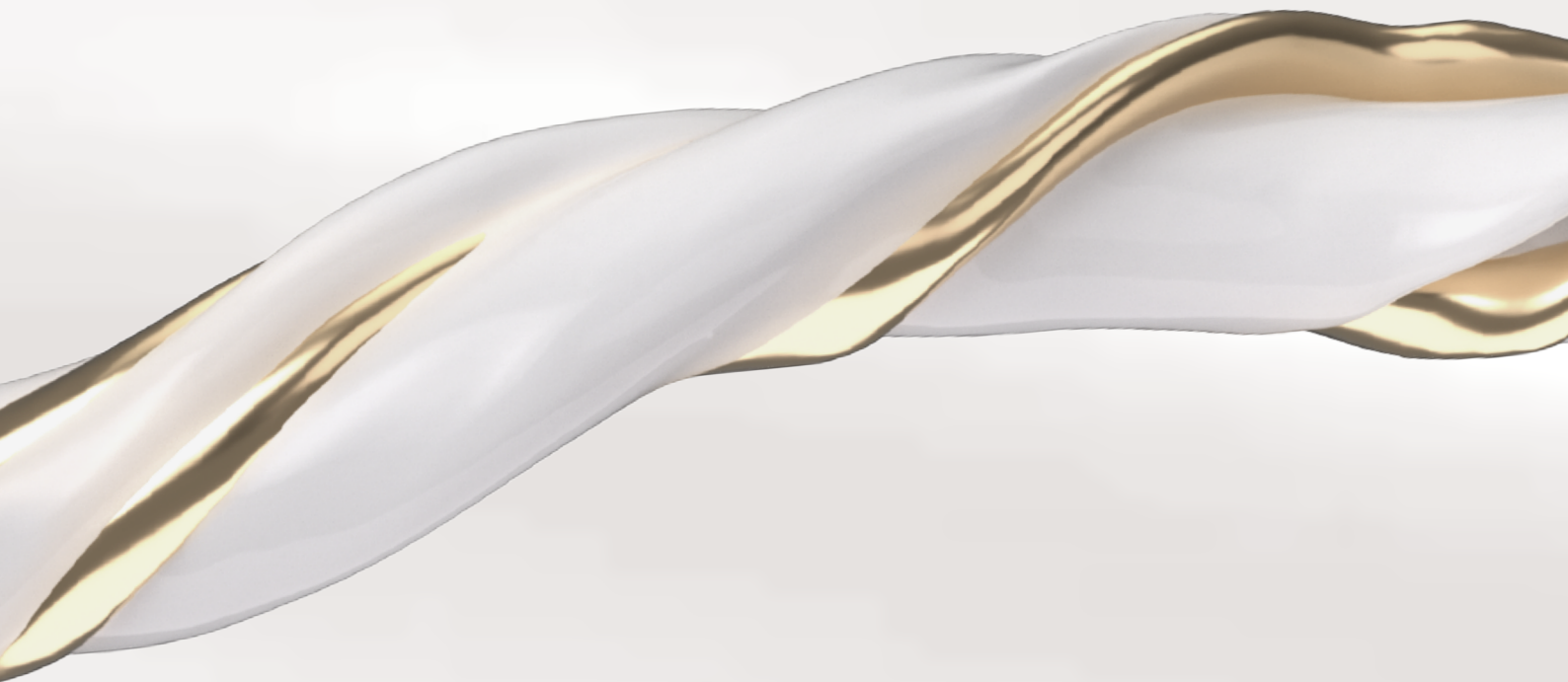


Figure 2. Prevalence of *B. infantis* and *B. lactis* in infants within each group (Adapted from the abstract) demonstrated a higher prevalence of these beneficial bacteria that was sustained throughout toddlerhood, up to 15 months

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