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Bioactive nutrition: Human milk and milk-derived oligosaccharides

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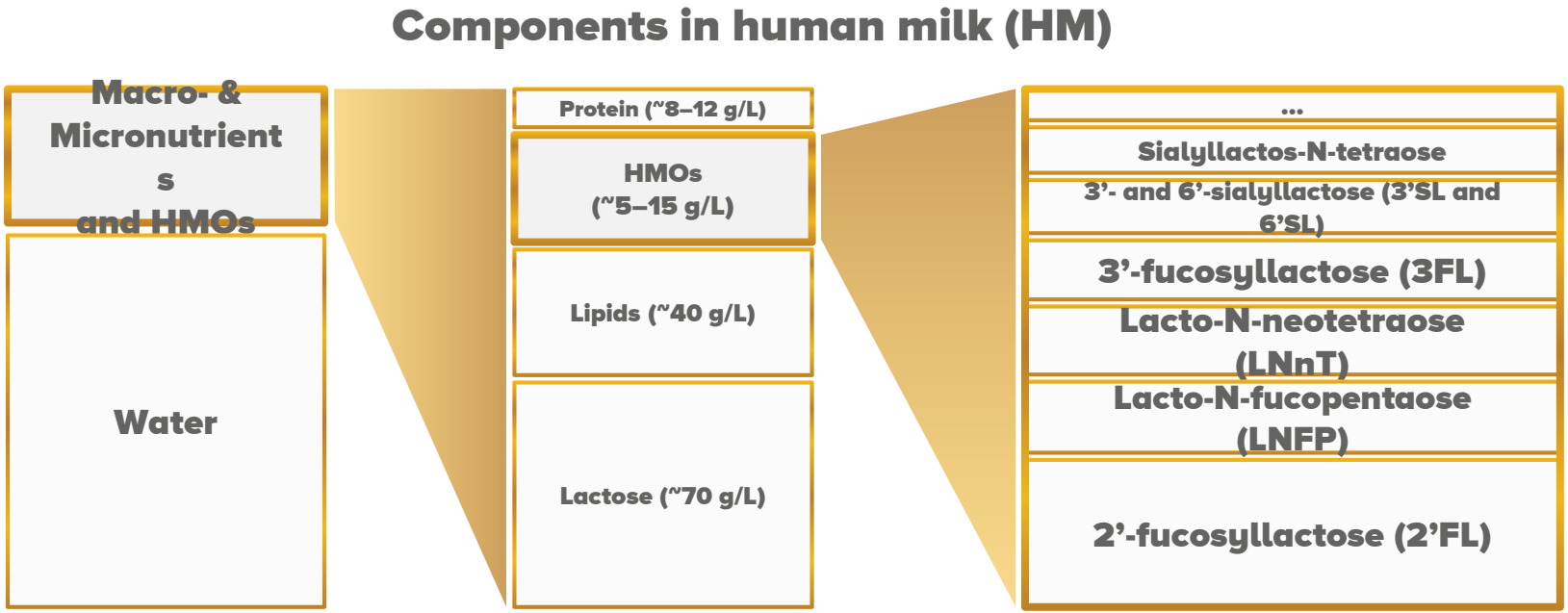
What are human milk and milk-derived oligosaccharides?

Human milk oligosaccharides (HMO)

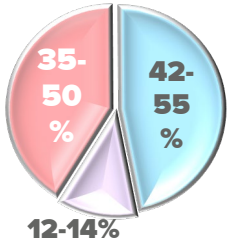
Milk-derived oligosaccharides (MOS)

HMOs are the 3rd largest solid component in human milk



Bode, Glycobiology. 2012; Kunz et al., Ann Rev Nutr. 2000.

Three categories of HMOs with distinctive structures



Non-fucosylated HMOs

Fucosylated HMOs

Sialylated HMOs

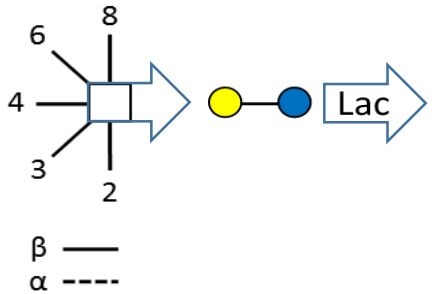
D-Glucose (Glc)

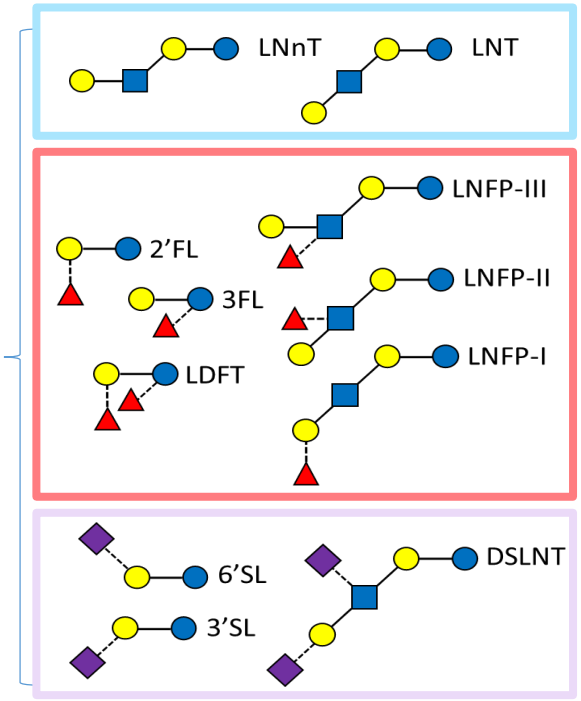
D-Galactose (Gal)

N-Acetylglucosamine (GlcNAc)

L-Fucose (Fuc)

N-Acetylneuraminic acid (Neu5Ac)





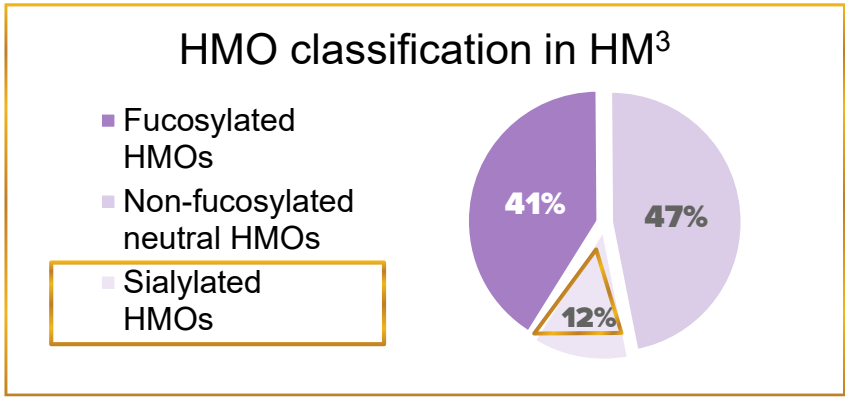
Example of major HMO structures

Austin et al., Nutrients. 2016; Sprenger et al., PLoS ONE. 2017; Bode, Glycobiology. 2012.

MOS are unique from classical prebiotics

- Milk contains oligosaccharides, some of which are identical to those found in HM, however, in lower concentrations[1,2]
- MOS are primarily galacto- oligosaccharides (GOS) that are however unique from common GOS as they contain 3'-sialyllactose (3' SL) and 6'-sialyllactose (6' SL), which are structurally identical to the main types of sialylated oligosaccharides in HM

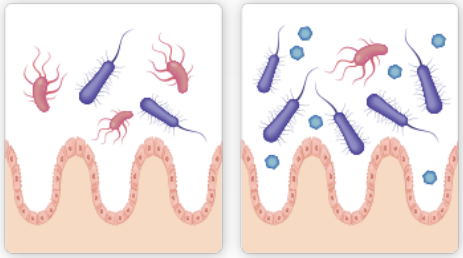
Analyte	MOS	Common GOS
Lactose	✓	✓
Glucose	✓	✓
Galactose	✓	✓
Sialyllactose	✓	**Not present**
Galacto-oligosaccharide (GOS)	✓	✓



1. Gopal & Gill, J Nutr. 2000; 2. Martin-Sosa et al., J Dairy Sci. 2003; 3. Average values adapted from Austin et al., Nutrients. 2016; Sprenger et al., PLoS ONE. 2017; Bode, Glycobiology. 2012.

Functions of HMO that contribute to immune health

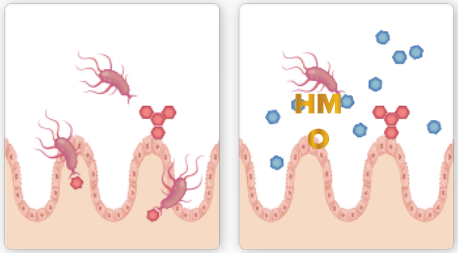
- HMO + HMO



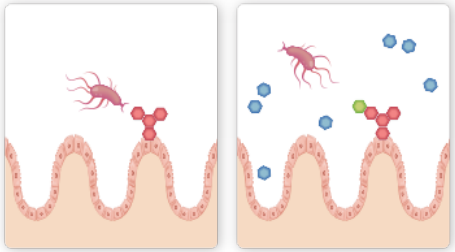
Promote healthy gut microbiota

Prevent bacterial adhesion in the gut

- HMO + HMO



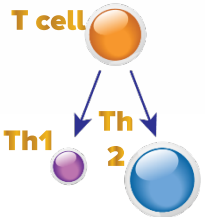
- HMO + HMO



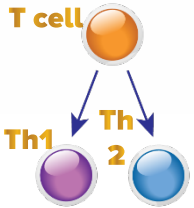
Strengthen gut barrier function

Directly modulate the immune system

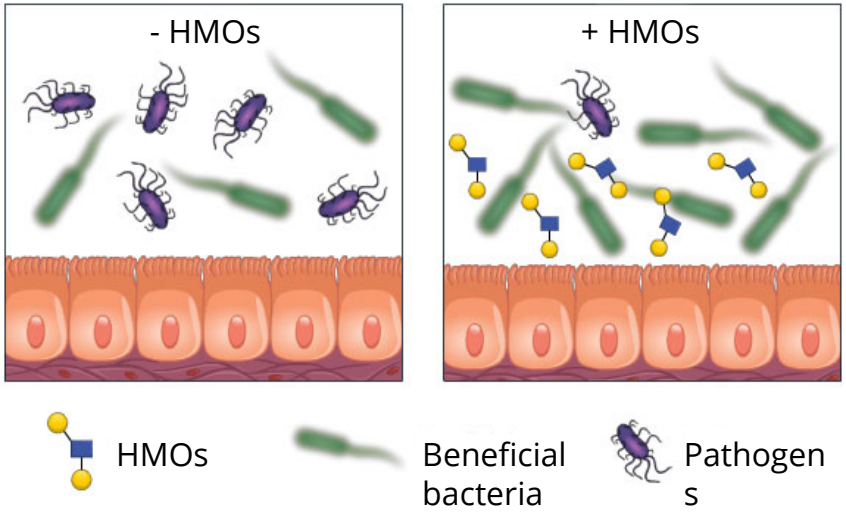
- HMO



+ HMO



HMO promote the growth of beneficial bacteria

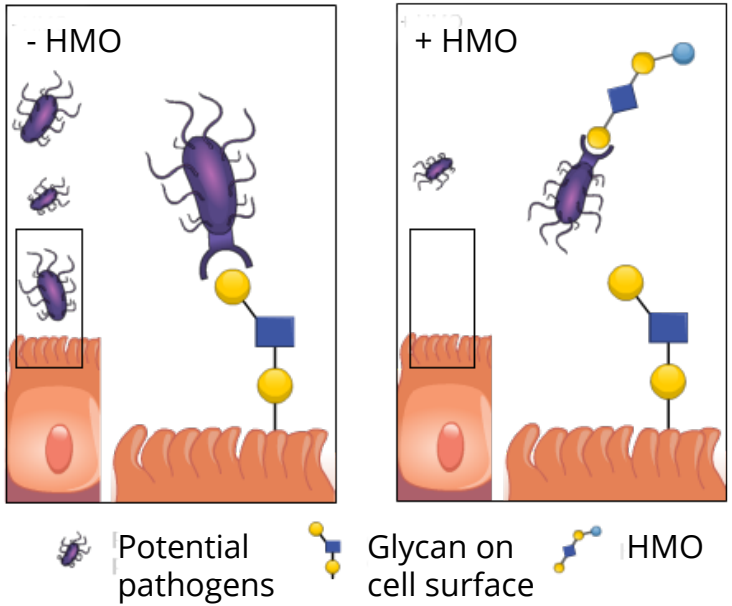


Adapted from Bode, L. 2012.

- Beneficial bacteria, such as bifidobacteria, are the predominant gut microbiota of breastfed infants[1]
- HMOs serve as metabolic substrates for specific beneficial bifidobacteria and provide them with a growth advantage over potential pathogens[2,3,5]
- HMOs do not allow the growth of potentially pathogenic strains of Enterobacteriaceae, Escherichia coli and clostridia[3–5]

*1. Donovan and Comstock, **Ann Nutr Metab**. 2016.; 2. Sela and Mills, **Trends Microbiol**. 2010. 3. Yu et al., **Glycobiology**. 2013.; 4. Hoeflinger et al., **J Agric Food Chem**. 2015. 5. Bode, **Glycobiology**. 2012.*

HMO act as decoy receptors and prevent pathogen adhesion in the gut

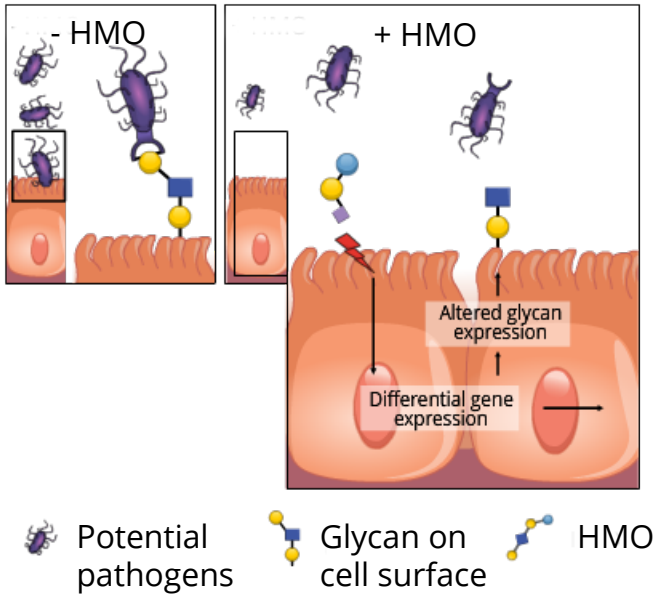


Adapted from Bode, 2012.

- HMOs act as soluble decoy receptors that compete for pathogen binding against the gut mucosa.[1]
- Pathogens need to attach to the mucosal surface to cause infectious disease[1,2]
- HMOs, especially fucosylated HMOs, resemble glycans on the mucosa cell surface to which pathogens adhere[1,2]
- This prevents pathogen adherence to the intestinal wall and reduces their ability to infect the infant[1-4]

1. Bode, Glycobiology. 2012.; 2. Jantscher-Krenn and Bode, Minerva Pediatr. 2012; 3. Angeloni, Glycobiology. 2005; 4. Smilowitz, Annu Rev Nutr. 2014.

HMO assist gut barrier function

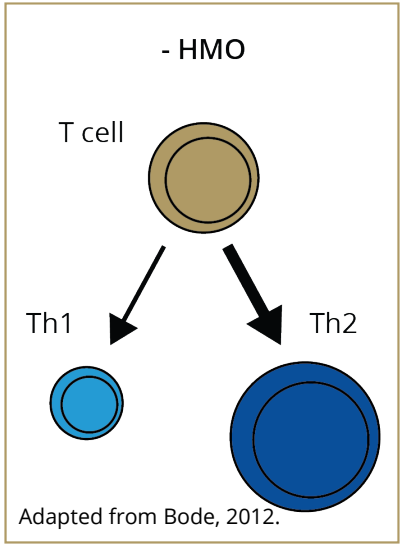


Adapted from Bode, 2012.

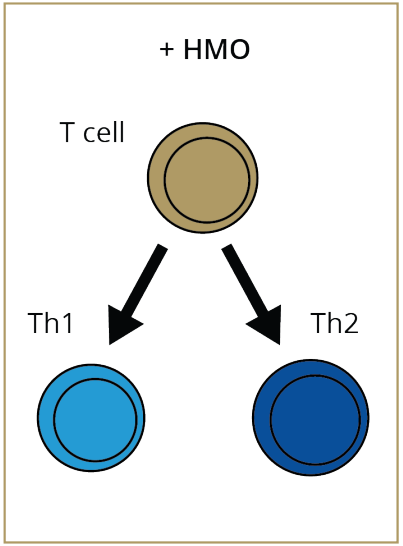
- HMOs can directly interact with intestinal epithelial cells and modulate their glycan expression[1,2]
- Modulating glycan expression acts as alternative mechanism to prevent pathogen attachment to intestinal cells, thereby strengthening gut barrier function[1-3]

Angeloni et al., Glycobiology. 2005.; Bode, Glycobiology. 2012.; Jantscher-Krenn and Bode, Minerva Pediatr. 2012.

HMO directly modulate the immune system



The infant's innate immune system is highly unbalanced towards Th2 response favouring allergic reactions[4,5]



HMOs help promote a shift towards a balanced Th1/Th2 response and drive immune maturation[4,5]

- HMOs can exert microbiota-independent immune effects and directly modulate immune responses by affecting immune cell populations and cytokine secretion[1-3]
- HMOs may either act locally on cells of the gut mucosa-associated lymphoid tissues or on a systemic level[1,2]
- HMOs help lymphocyte maturation and promote a shift towards a balanced Th-cell response[1,4,5]

1. Bode, Glycobiology. 2012.; 2. Donovan and Comstock, Ann Nutr Metab. 2016. 3. Comstock et al., J Nutr. 2017.; 4. Jantscher-Krenn and Bode, Minerva Pediatr. 2012. 5. Eiwegger et al., Pediatr Res. 2004.

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Clinical data to support HMO and MOS

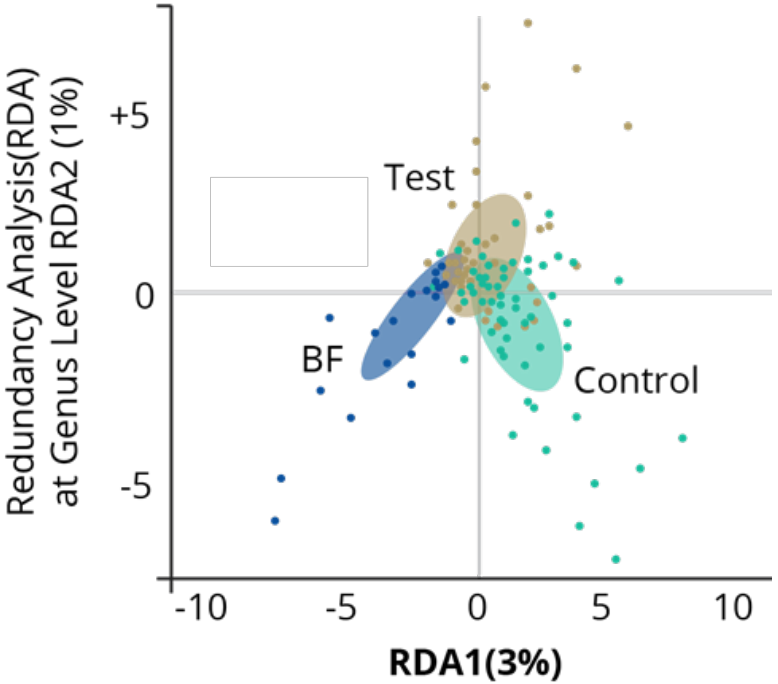
Clinical studies have shown benefits of infant formula supplemented with HMO and MOS

Clinical study with 2'FL (1 g/L) and LNnT (0.5 g/L) supplemented infant formula

Double-blind RCT in healthy infants aged <14 days old, 6-month intervention of formula with additional 6-month follow-up period, including a non-randomized breastfed reference group

2'FL, 2'-fucosyllactose; LNnT, acto-N-neotetraose; RCT, randomized controlled trial.

Infants fed 2'FL + LNnT have microbiota closer to breastfed infants



Gut microbial composition at 3 months of age

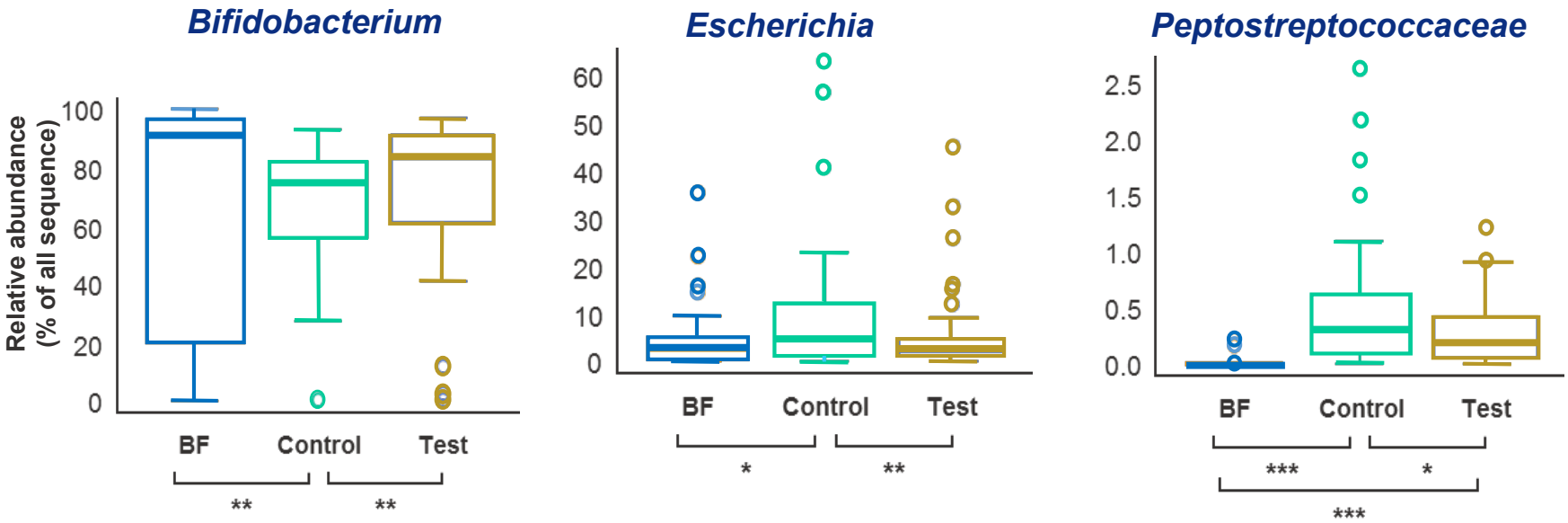
- Breastfed group
- Study formula with 2'FL and LNnT
- Control group

Test versus control group (p<.001)

Berger et al., mBio. 2020

Infant formula with 2'FL and LNnT promotes Bifidobacteria and reduces potentially pathogenic bacteria

Abundance of specific bacteria genera at 3 months of age

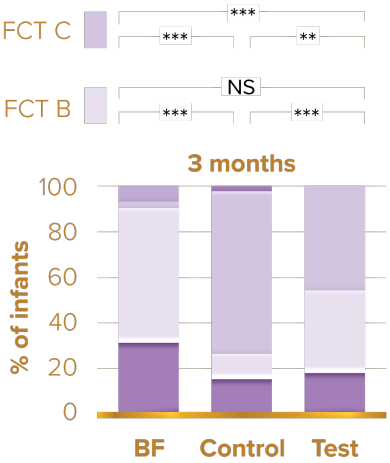


2'FL, 2'fucosyllactose; LNnT, lacto-N-neotetraose; test, infant formula supplemented with 1g/L 2'FL and 0.5 g/L LNnT; BF, breastfed group; *p <0.05; **p <0.01; ***p <0.001.

Berger et al., mBio. 2020

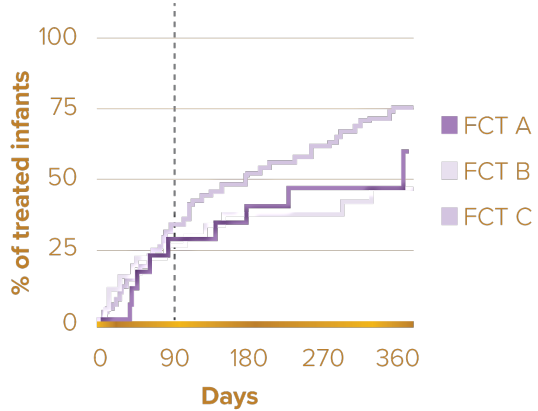
HMOs influence antibiotic use via gut microbiota

Gut microbiota community structure



Distribution of Fecal Community Types (FCT) amongst feeding groups and time points displayed as percentage of infants. Significant differences between feeding groups: *P <0.05; ** P <0.01; *** P <0.001; NS = not significant

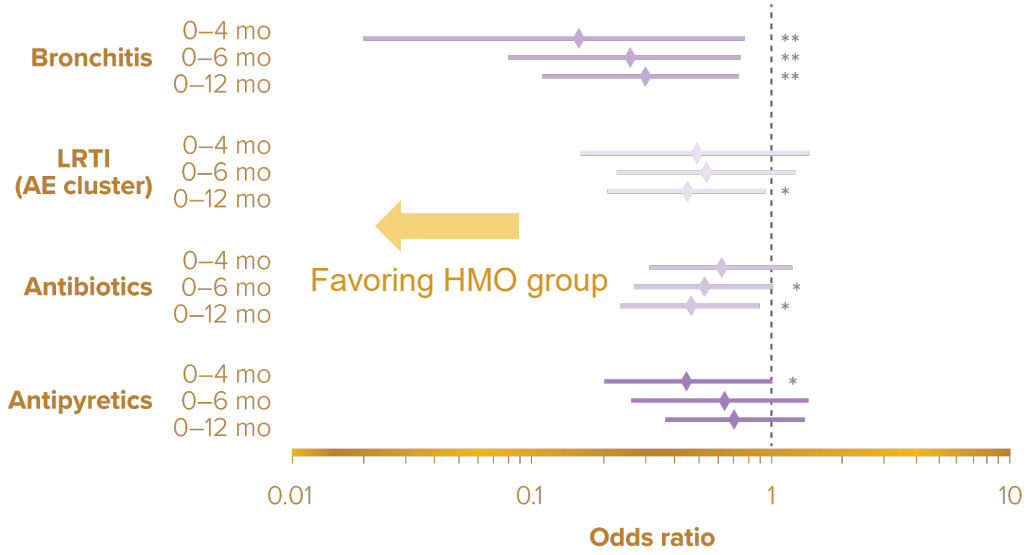
Infants with Fecal Community Type (FCT) C had a 2x increased likelihood of antibiotic use during the first year compared to FCT B (P = 0.02)



Kaplan-Meier plot of time to first antibiotics use by FCT (A, B, and C); BF infants were not included in the FCT groups; x-axis = time (days), y-axis = part of infants in each FCT.

Berger et al., mBio. 2020

Fewer reports of respiratory illness and medical use in infants consuming formula with 2'FL and LNnt



Odds ratios with 95% CIs; analyzed using Fisher's exact tests; *P<0.05; **P<0.01; AE, adverse event; LRTI, lower respiratory tract infection.

Infants receiving HMO had fewer reports of:

- bronchitis through 4, 6, and 12 months
- lower respiratory tract infection (AE cluster) through 12 months
- antibiotics use through 6 and 12 months
- antipyretics use through 4 months

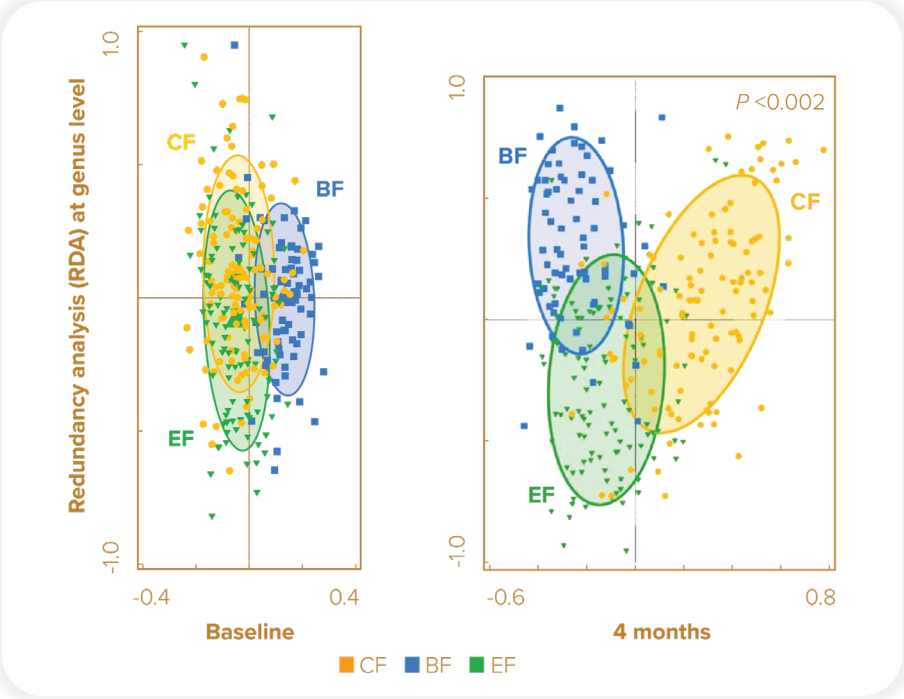
Berger et al., mBio. 2020

Clinical study with MOS (7.2 g/L) supplemented infant formula

Double-blind RCT in healthy infants aged ~3 weeks old, 4-month intervention of formula, including a non-randomized breastfed reference group

RCT, randomized controlled trial.

MOS shifts microbiome closer to that of breastfed infants

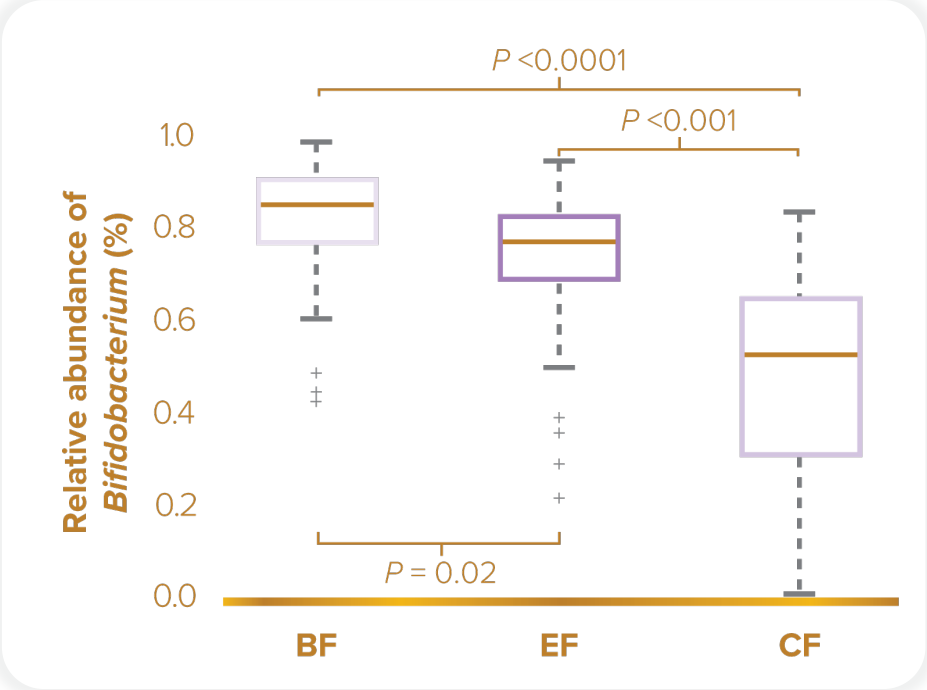


Global microbiome compositions at 4 months of age

- **EF is closer to BF**; CF infants were separated from both BF and EF
- Separation between BF and MOS contributed to the skin commensals *Propionibacterium* and *Staphylococcus*; probably reflecting skin contact rather than direct impact of dietary components

Estorninos et al., manuscript in preparation

MOS increases bifidobacteria

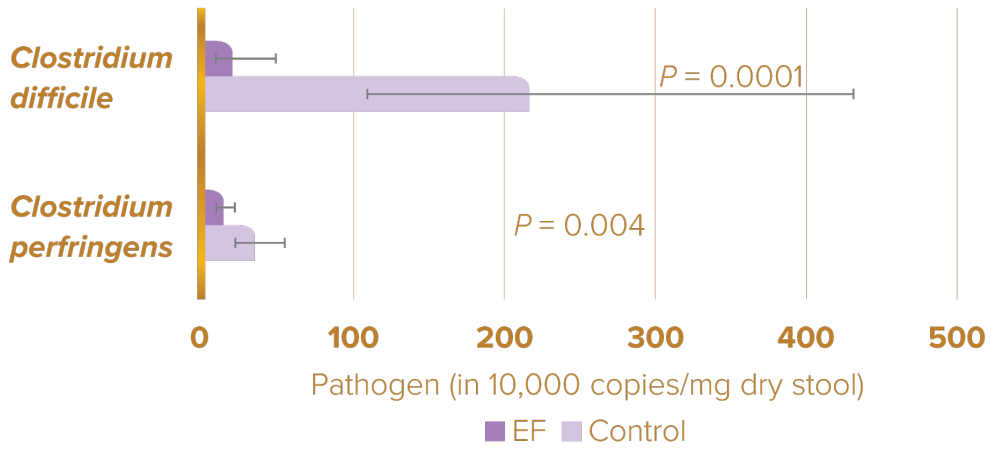


Relative abundance of bifidobacterial at 4 months of age

- Bifidobacterium abundance was ~50% higher in EF infants compared with CF infants and **closer to that in BF infants**.

Estorninos et al., manuscript in preparation

MOS decreases pathogenic bacteria



Clostridium difficile presence; odds ratio [95% CI] =0.42 [0.18, 0.97], P=0.001
Clostridium perfringens presence; odds ratio [95% CI] =0.42 [0.18, 0.97], P=0.001

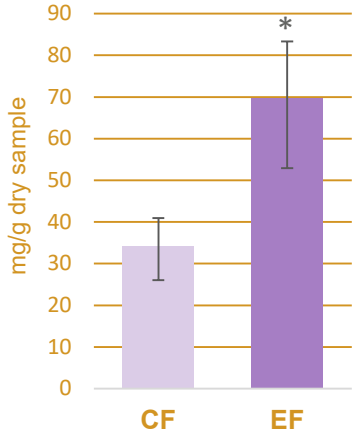
Diarrheal pathogens at 4 months of age

- Infants fed MOS had lower risk of *Clostridium difficile* and *perfringens* presence than control group.
- Both are opportunistic pathogens that can cause diarrhea in infants and children. Their reduction decreases the risk for developing diarrheal illness.

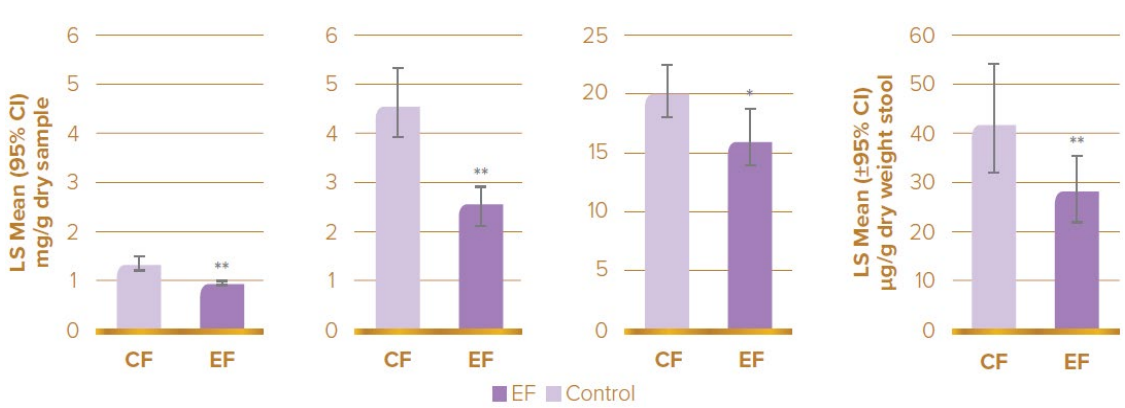
Estorninos et al., manuscript in preparation

MOS improve GI immune and health markers

Secretory immunoglobulin A (sIgA)



α-1 antitrypsin | **Calprotectin** | **Elastase** | **Myeloperoxidase**

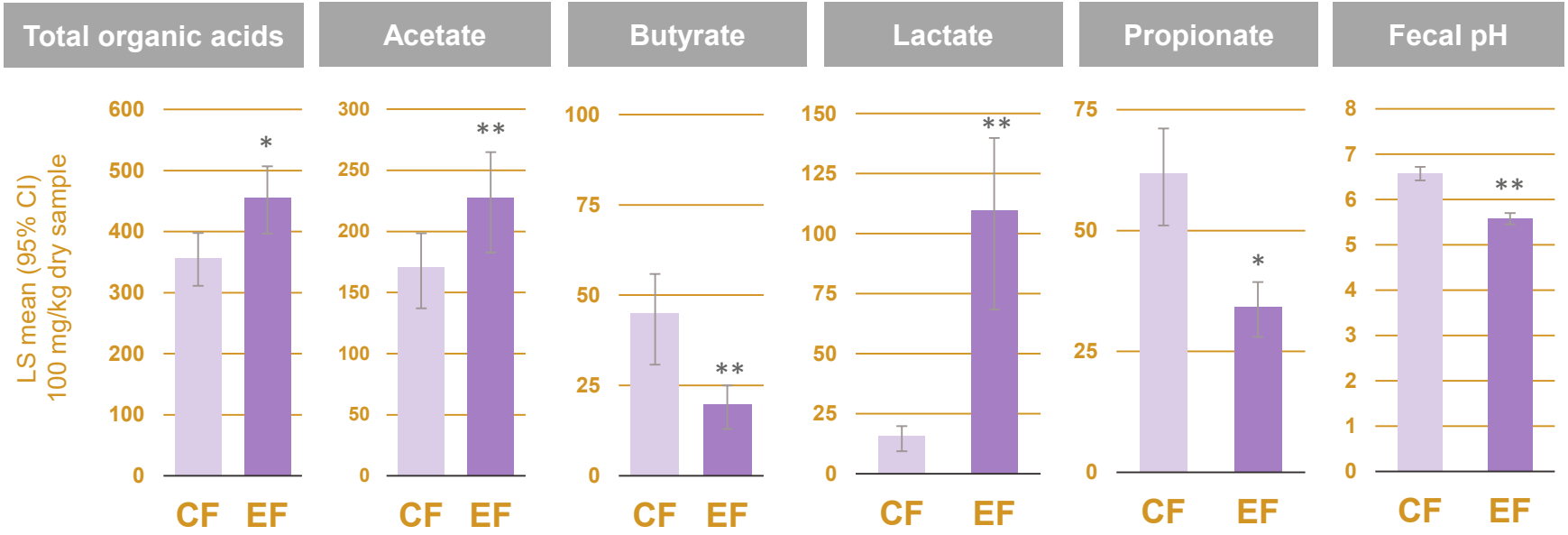


P <0.01; **P <0.0001; graphs show 4 months data as LS means with SD as error bars

- Higher sIgA and improved markers of GI barrier integrity/permeability and inflammation suggest MOS have a positive effect on mucosal immunity and gut maturation.

Estorninos et al., manuscript in preparation; GI, gastrointestinal.

MOS influences fecal microbiota metabolism



*Significantly different EF vs. CF: *p<0.05; ** p<0.0001;* ***graphs show 4 months data as LS means with SD as error bars***

- Short chain fatty acid profile reflects the difference in bacterial composition of the colon between EF and CF (higher levels of lactate, lower fecal pH).

Estorninos et al., manuscript in preparation

Summary of clinical trial results

- **HMO* and MOS promote a gut microbiome closer to breastfed infants including an increase in bifidobacteria.**
- **HMO* and MOS decrease the abundance of potentially and opportunistic pathogenic bacteria in the microbiome, respectively.**
- **HMO* significantly reduced illness (LRTI and bronchitis) and antibiotic use through 12 months of age.**
- **MOS enhances intestinal immunity and GI barrier integrity/permeability.**

* Refers to the combination of 2'FL and LNnT; GI, gastrointestinal; LRTI, lower respiratory tract infection.

Thank you for your attention