Nov 30th, 2017
Zwolle

In vitro and *in vivo* fermentation behaviour of prebiotic carbohydrates

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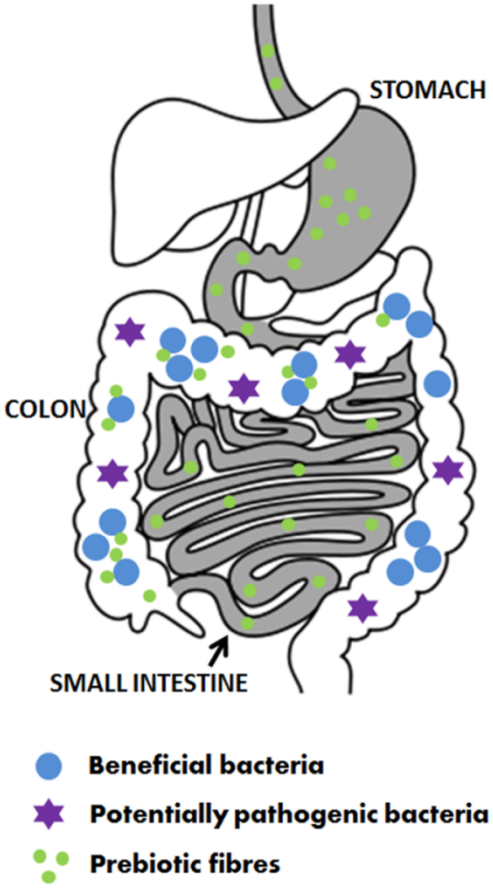
Introducing prebiotic fibres



- Resistant to human GI digestion
- Fermentable by the large intestinal microbiota
- Selectively stimulate beneficial microbes

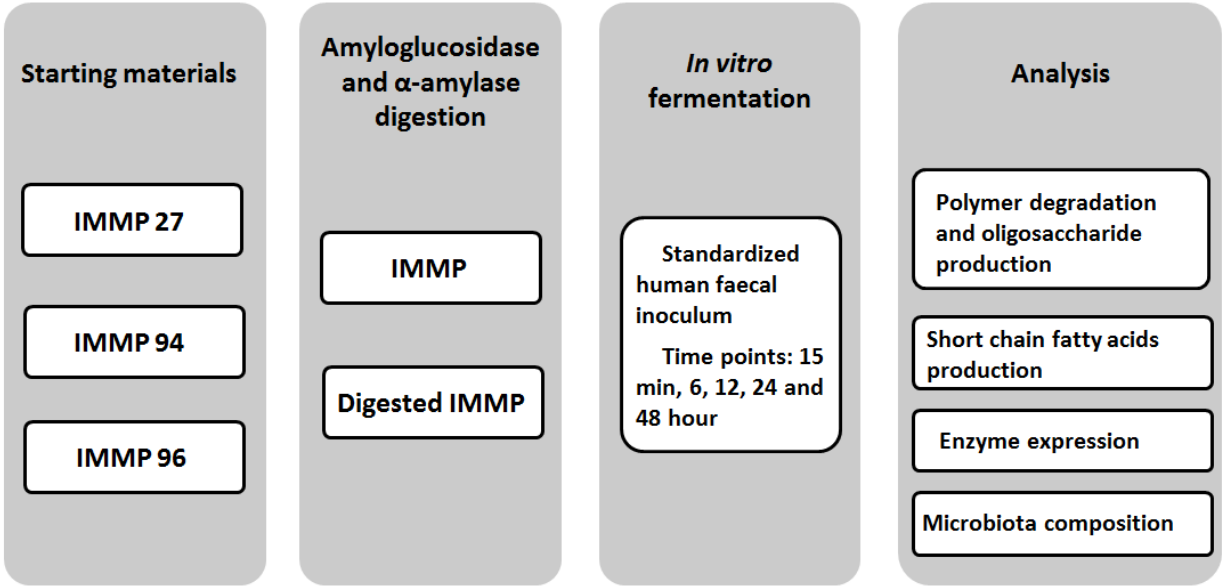
Substances

- Isomalto/malto-polysaccharides (IMMPs)
- Human milk oligosaccharides (HMOs)

IMMP as dietary fibre or prebiotic

(van de Zaal et al Carbohydr Polym 2017 in press)

- Enzyme-modified starch
- α-1,4-glycosidic linkages →α-1,6-glycosidic linkages
- Variable level of α-1,6-linkage levels



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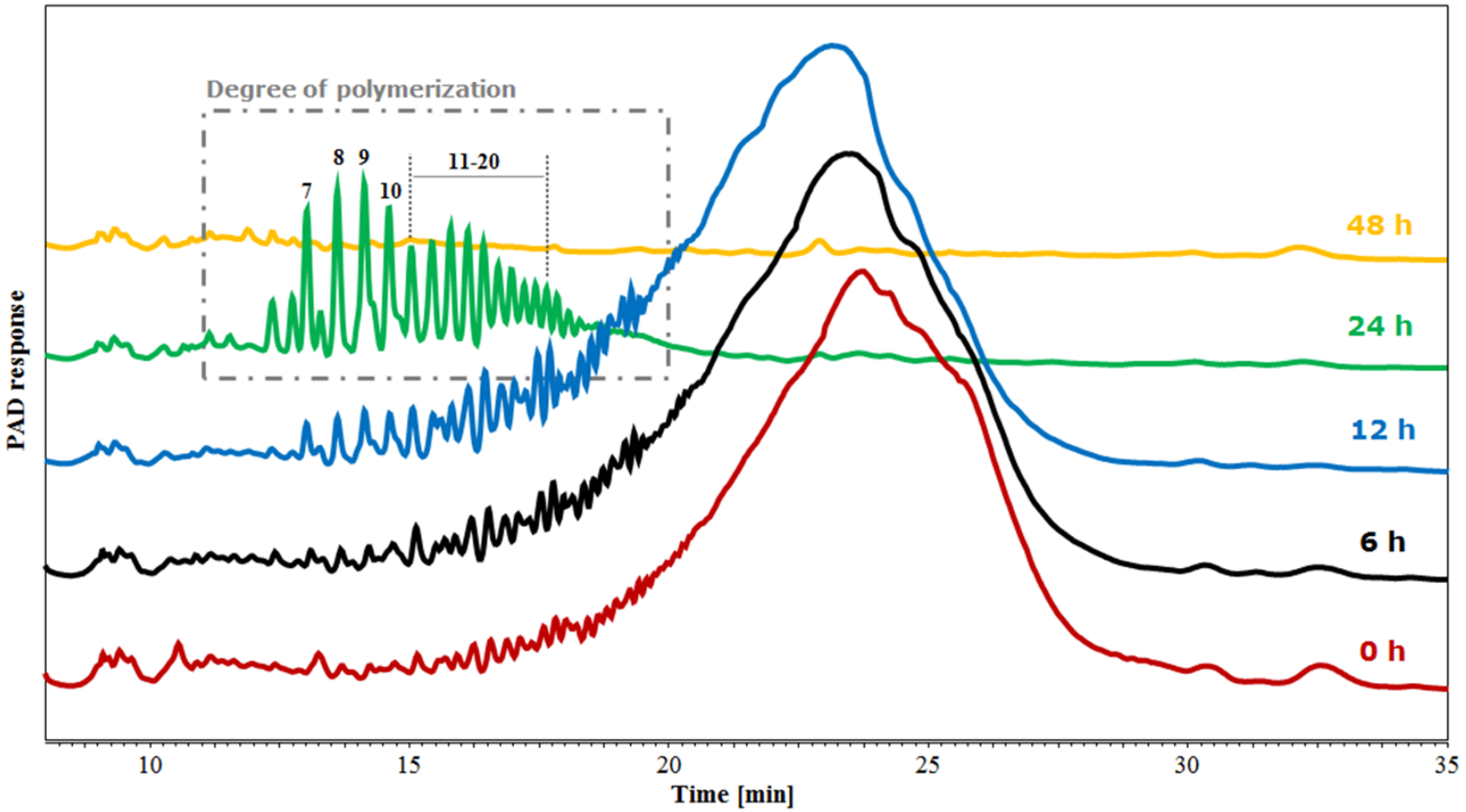
Fermentation of IMMP 96 using human inoculum

- *12 h delay of microbial utilization of α-1,6-linked glucose*
- *Full utilization of IMMPs within 48 h*

Analyzed by HPAEC-PAD



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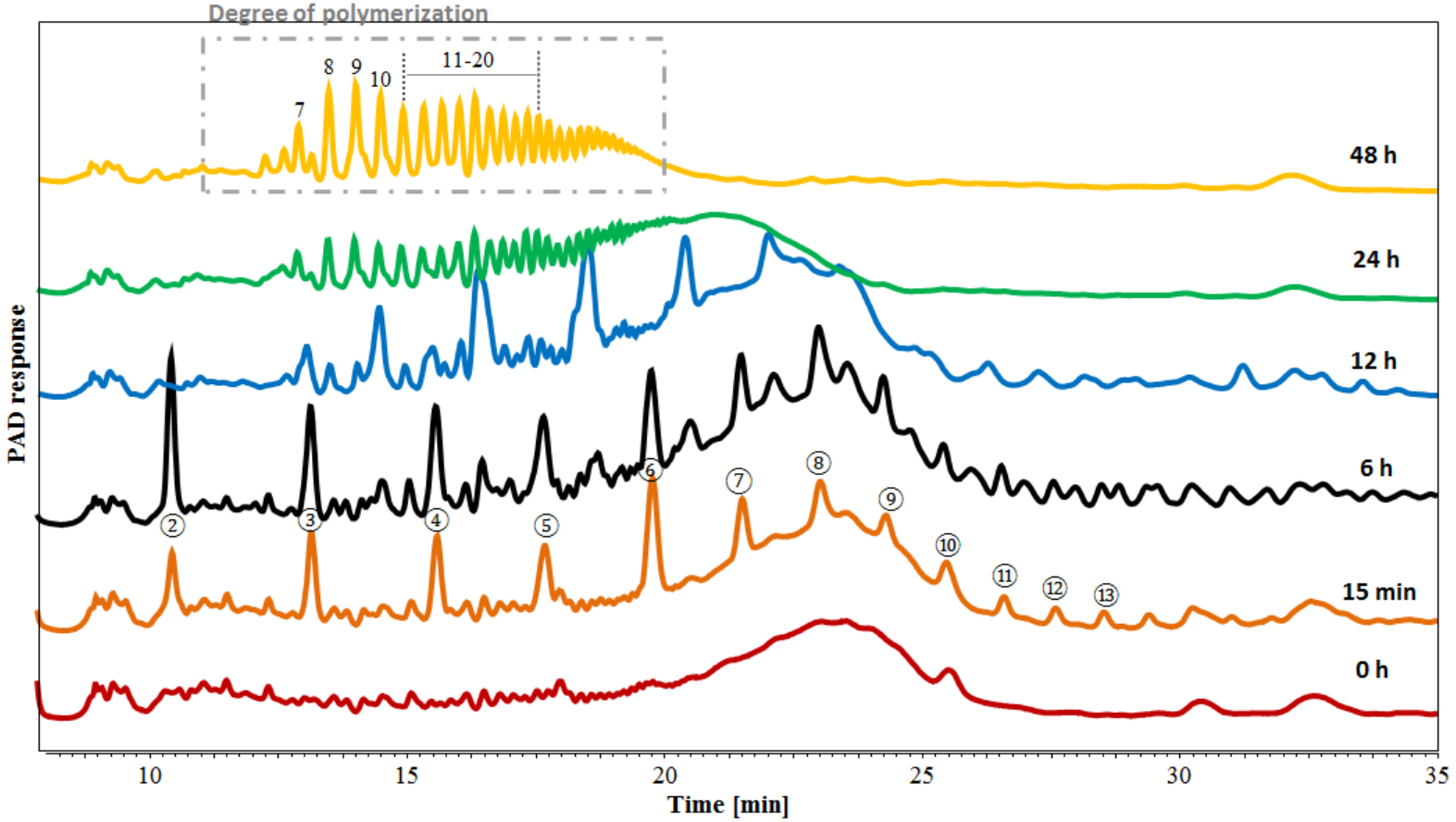
Fermentation of IMMP 27 using human inoculum

- *Direct utilization of α-1,4-linked glucose by microbiota*
- *Presence and utilization of α-1,4-linked glucose postponed the complete utilization of α-1,6 glc chains*

Analyzed by HPAEC-PAD



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In vitro fermentation behavior of IMMPs

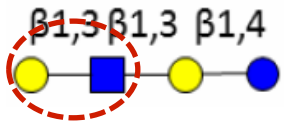
- Isomalto-oligosaccharides were gradually released during *in vitro* fermentation.
- The presence of α-1-4 linked glucose in IMMPs postponed the bacterial utilization of α-1,6-linked glucose.
- Shorter α-1-6 linked glucose chains speed up the fermentation.
- Fermentation of IMMPs promoted the growth of bifidobacteria and lactobacilli.
- IMMPs are slowly-fermentable fibres with prebiotic potential.
- Poster
- *In vivo* study on mice has been performed (collaboration with other SPs).

Fate of HMOs in Infant GI metabolisation

Breast milk

HMOs as substrate

Source of bifidobacteria



- **e.g. LNT contains Lacto-N-biose**

Faeces

First time acquiring bacteria

Deplete

Modulate

Glycosidase

Encode

GI bacteria establishment and growth

- Fucosidase
- Sialidase

- Bifidobacteria
  B. infantis; B. bifidum; B. breve;
- Bacteroides

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Milk and faecal samples

❑ KOALA cohort

- Maastricht University
- 146 pair of mother-infant
- Milk and faecal samples 1 month after birth

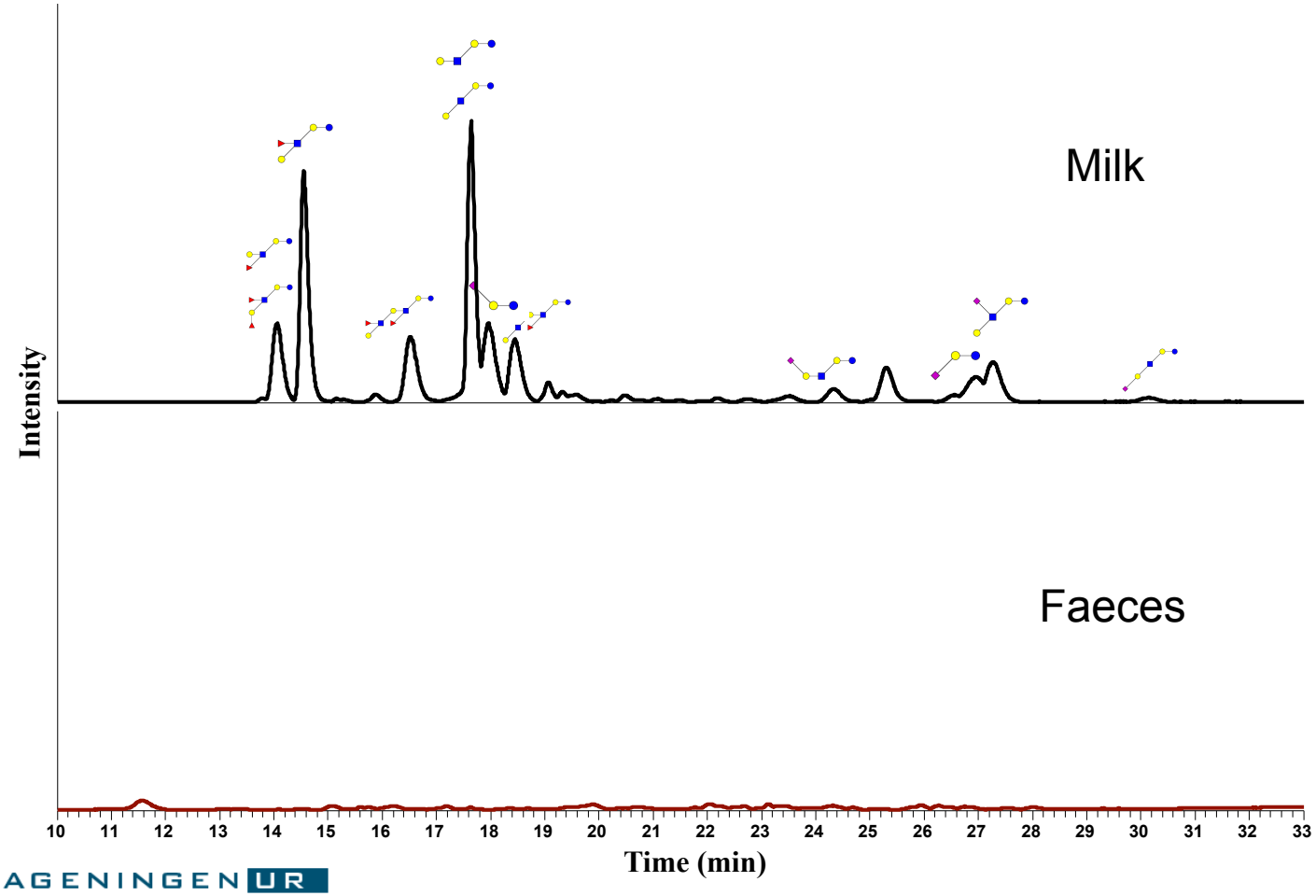
❑ BINGO onderzoek

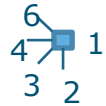
- Radboud University
- 80 pair of mother-infant
- Milk and faecal samples at 3 time points (2wk, 6wk, 12wk)

1 month-old infants showed different consumption patterns

Type A: complete consumption

Analyzed by PGC-UPLC-MS





Indication linkage type

- Glucose
- Galactose
- *N*-acetylglucosamine
- Fucose
- Sialic acid

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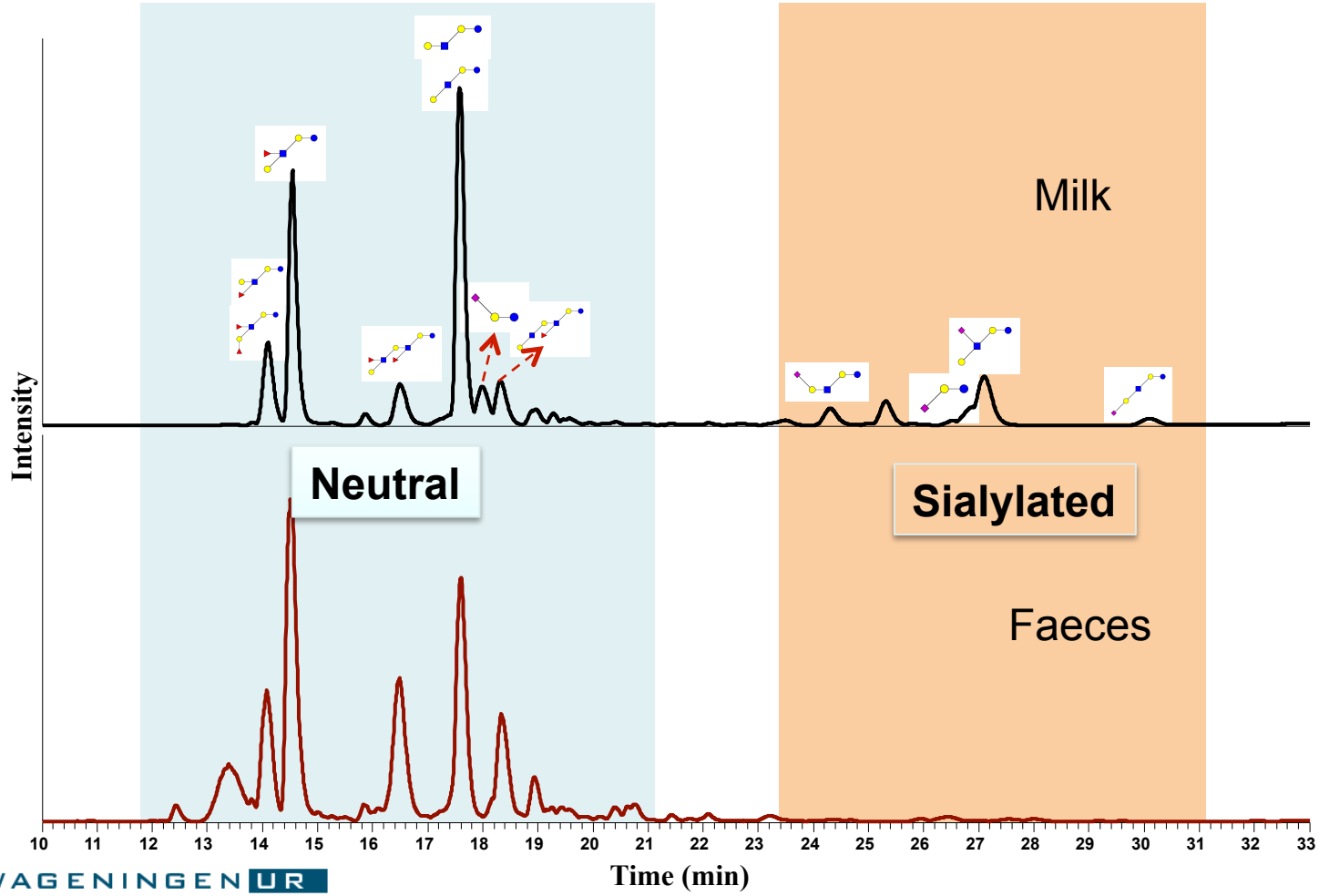
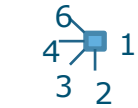
1 month-old infants showed different consumption patterns

Type B: selective consumption

Analyzed by PGC-UPLC-MS





Indication linkage type

- Glucose
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- Fucose
- Sialic acid

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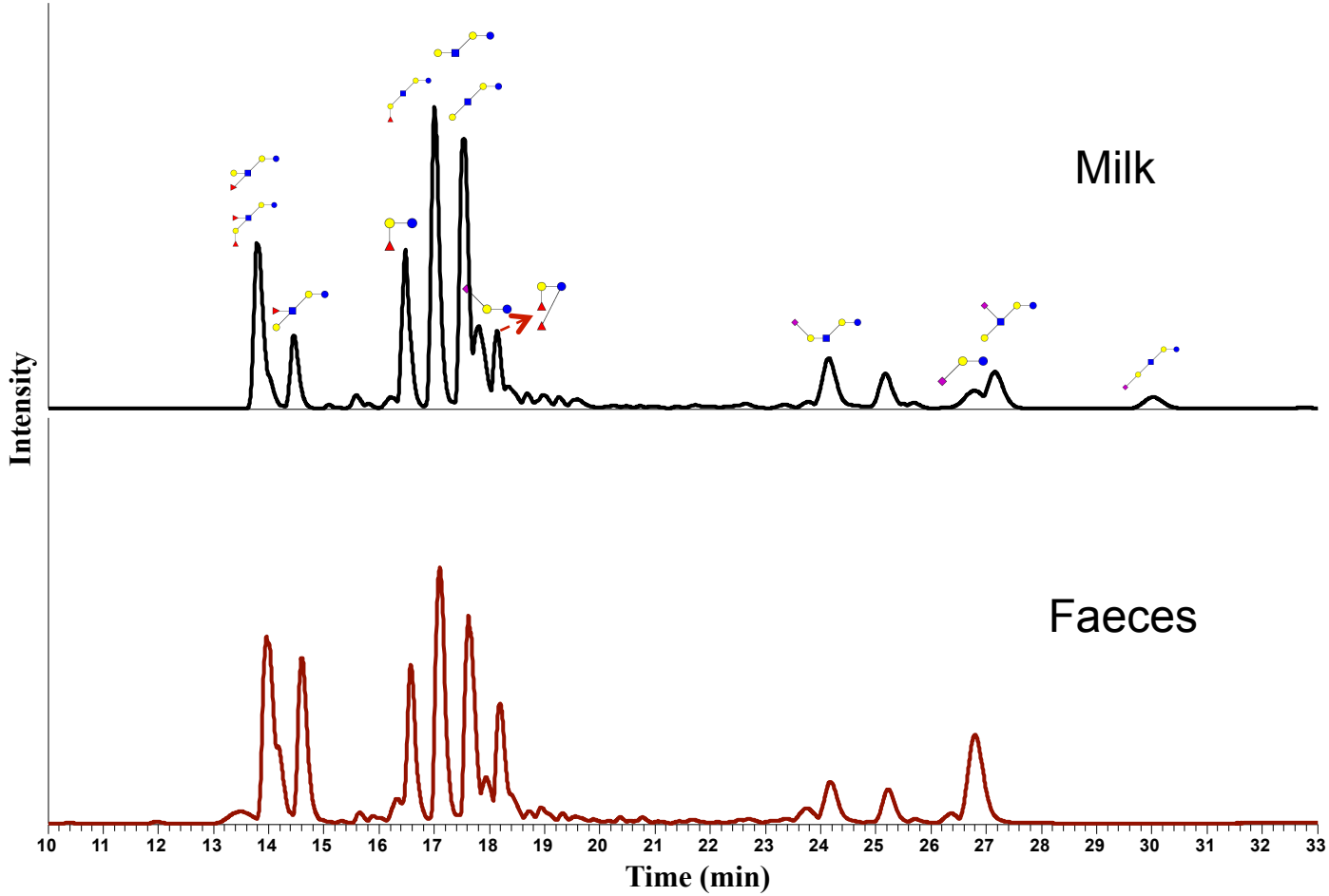
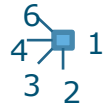
1 month-old infants showed different consumption patterns

Type C: Non-selective and low consumption

Analyzed by PGC-UPLC-MS





Indication linkage type

- Glucose
- Galactose
- *N*-acetylglucosamine
- Fucose
- Sialic acid

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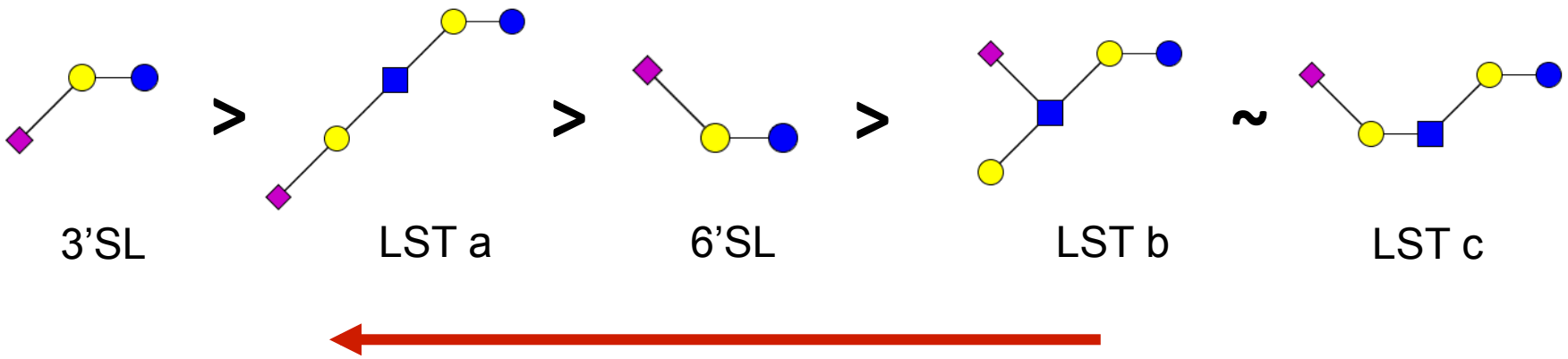
Preference to sialylated HMO isomers

- Linkage type: α 2-3 linked sialic acid is more utilized than α 2-6
- Differences for individual HMO structures



More prefered

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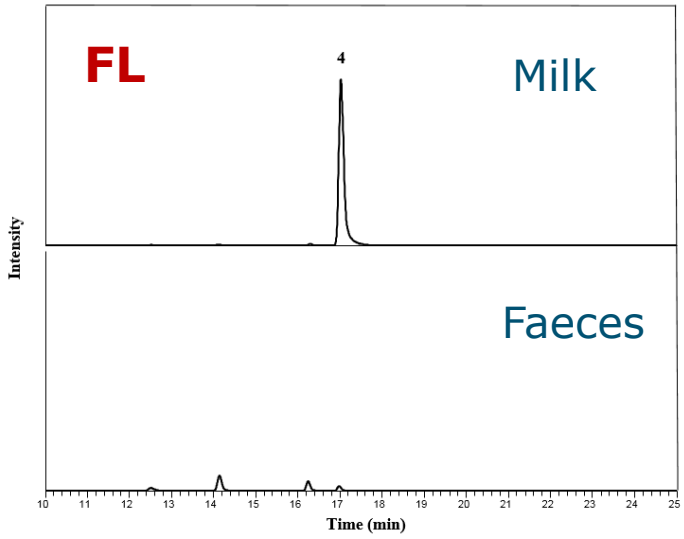
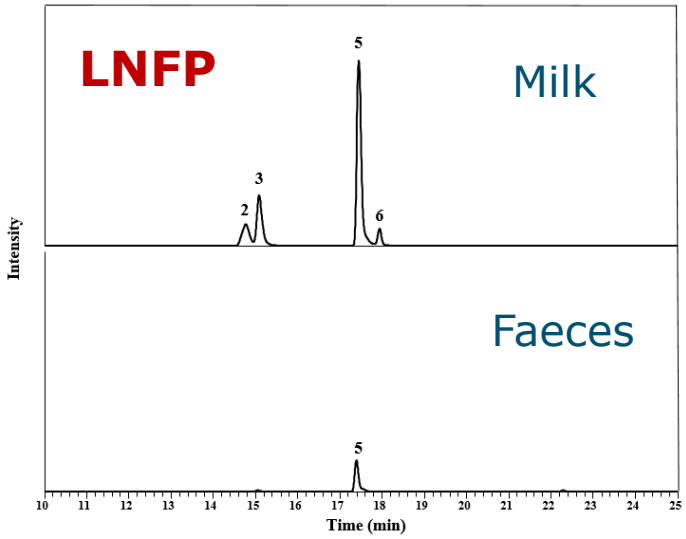
Preference to fucosylated HMOs

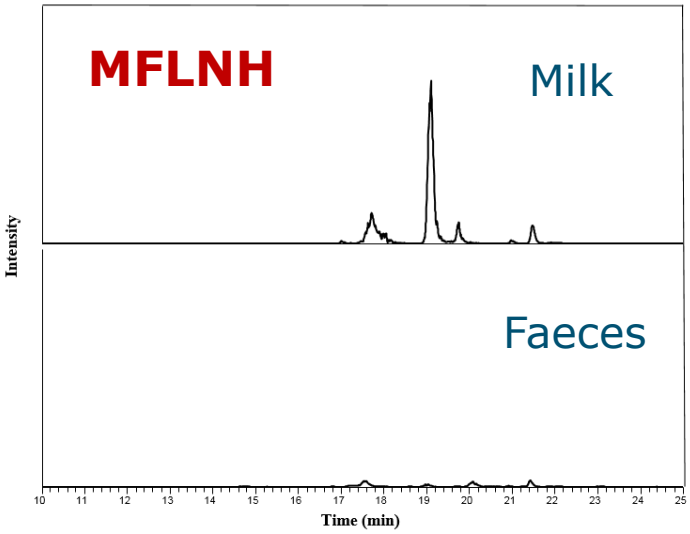
Mono-fucosylated or multi-fucosylated

Analyzed by PGC-UPLC-MS



- Mono-fucosylated HMOs were mostly utilized





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Preference to fucosylated HMOs

Mono-fucosylated or multi-fucosylated

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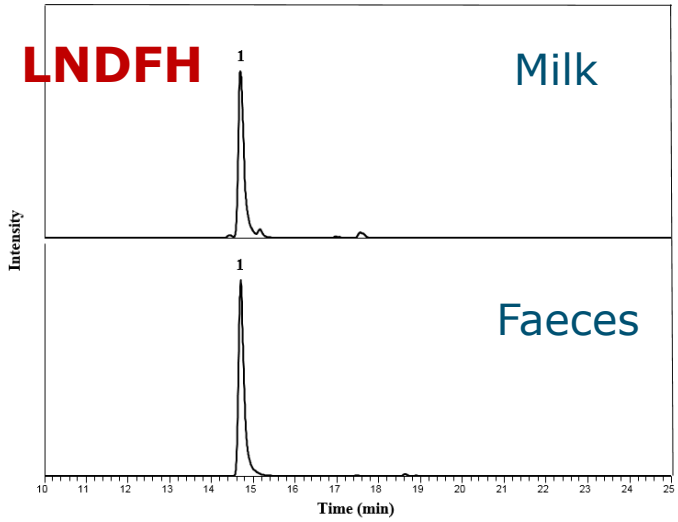
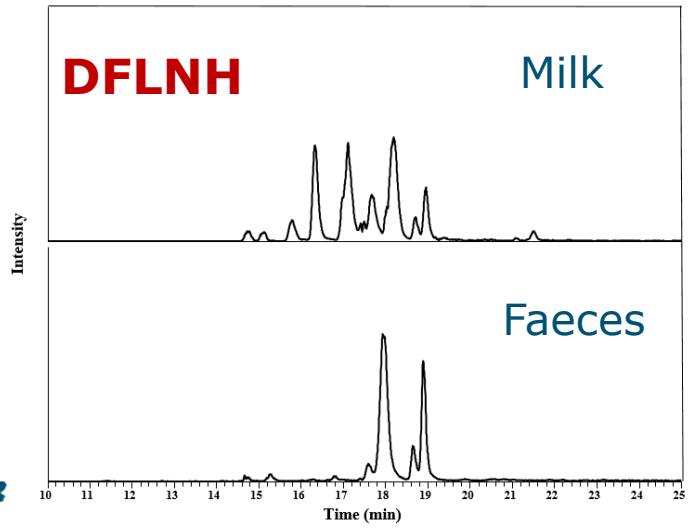
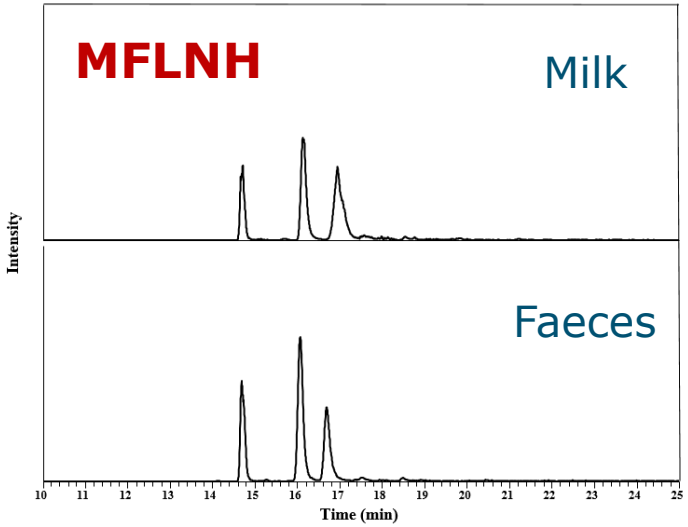


- Di- and tri- fucosylated HMOs were largely retained





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Fermentation of HMOs by 1-mon-old infants

- Huge inter-individual differences were found in the HMO composition among different mother milk samples
- Huge inter-individual differences were found in HMO utilisation in digestive tract of baby
- The consumption patterns can be clustered according to the level and selectivity of utilization of HMOs by infant gut microbiota

- Not easy to relate HMO utilization to milk type, faecal microbiota composition or characteristics recorded for mother and child
- One time point limits interpretation and correlations with essential parameters

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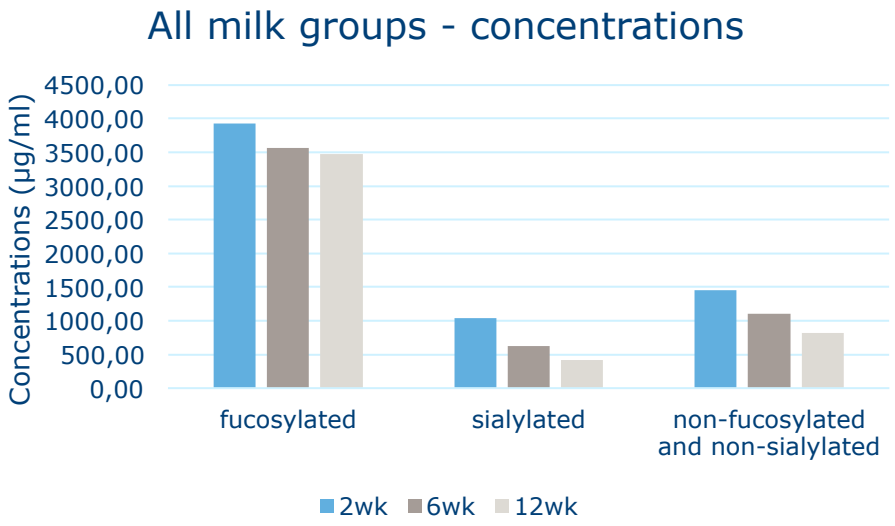
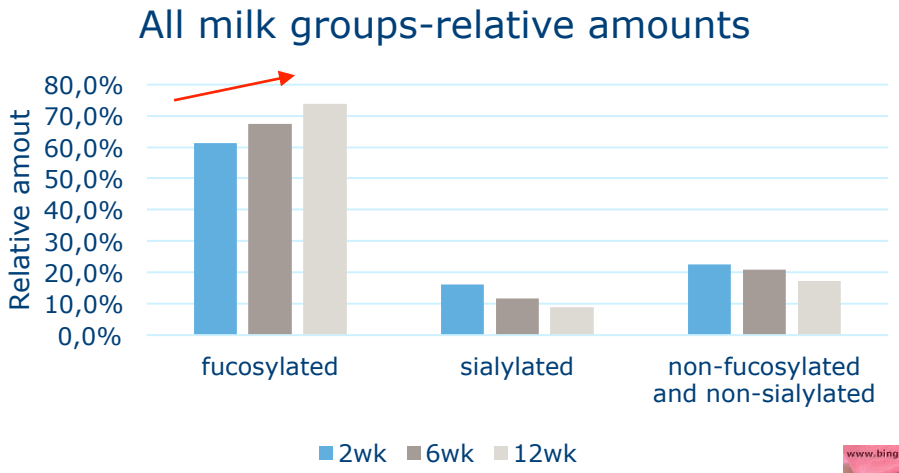
Variation of HMOs in milk over lactation time



- HMO concentration decreased over time
- Fucosylated HMOs increased in relative amounts in time



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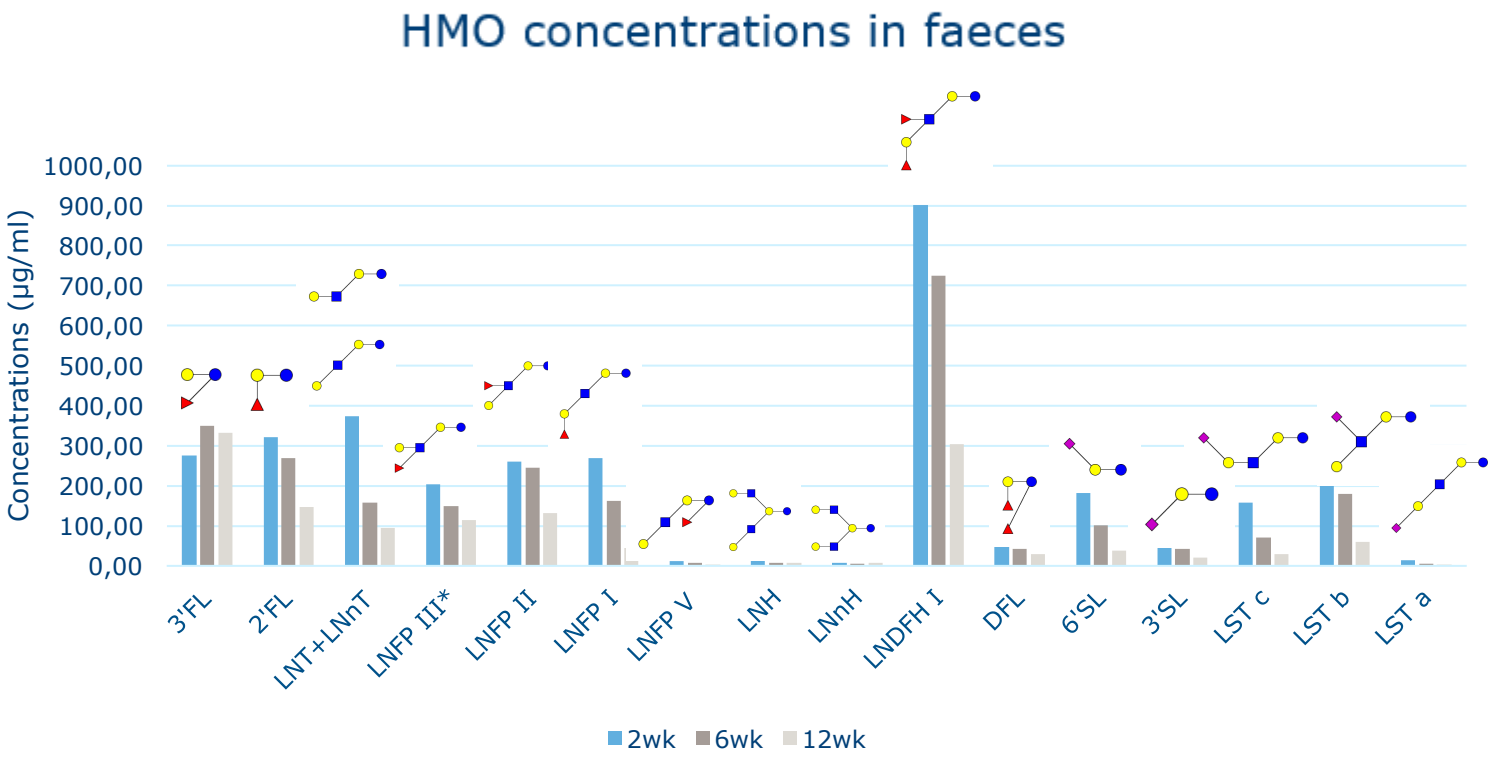
Remaining HMOs in infant faeces



- HMOs decreased over time
- LNDFHI mostly retained

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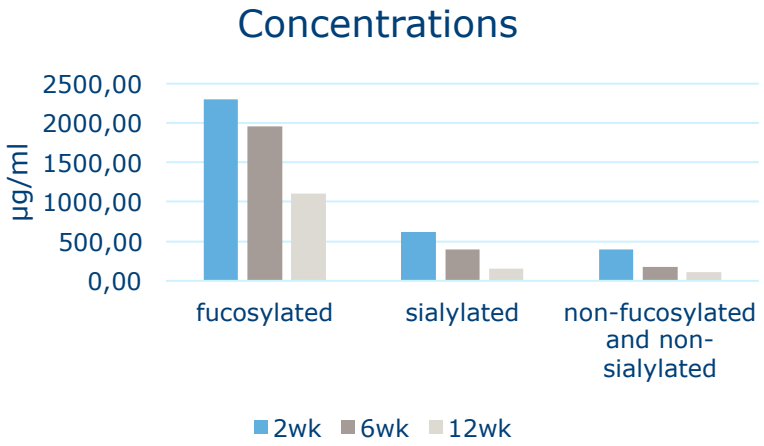
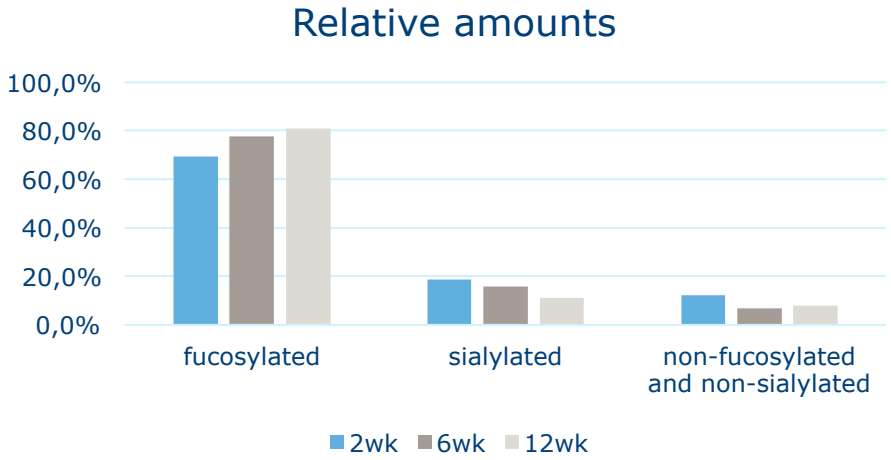
Remaining HMOs in infant faeces



- Trend over time: similar pattern as for milk HMOs



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Effects of external factors on GI microbiota

- **Delivery mode** - First time acquiring bacteria
  - Vaginal delivery: vaginal microbiota, e.g. bifidobacteria;
  - Caesarean section: mother skin or environment.

- **Feeding type** – Growth of bacteria
  - Breast-feeding exclusively;
  - Infant formula introduced

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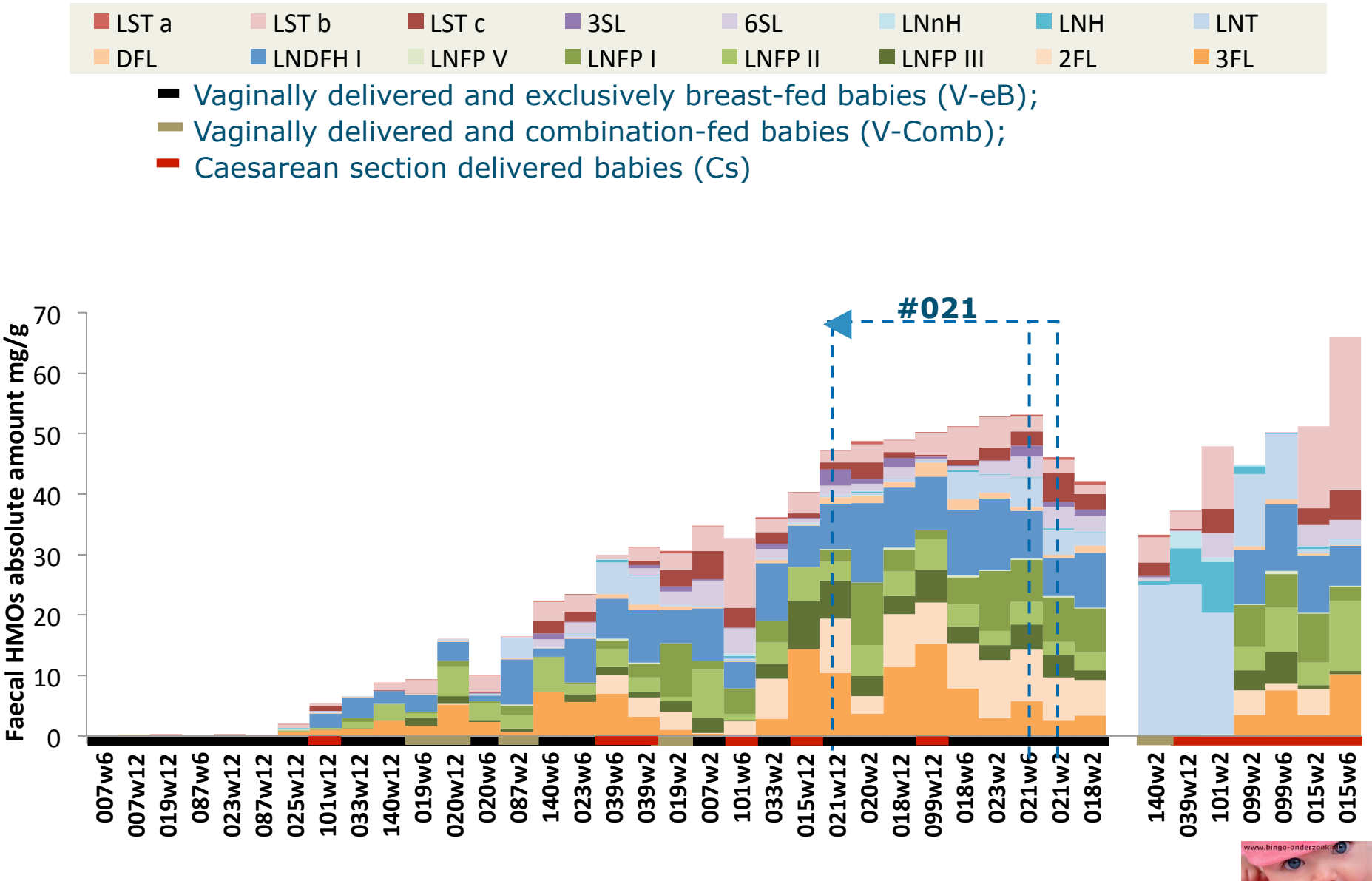
Faeces: HMO digestion patterns (Secretor) classified by multi-stages and similarity



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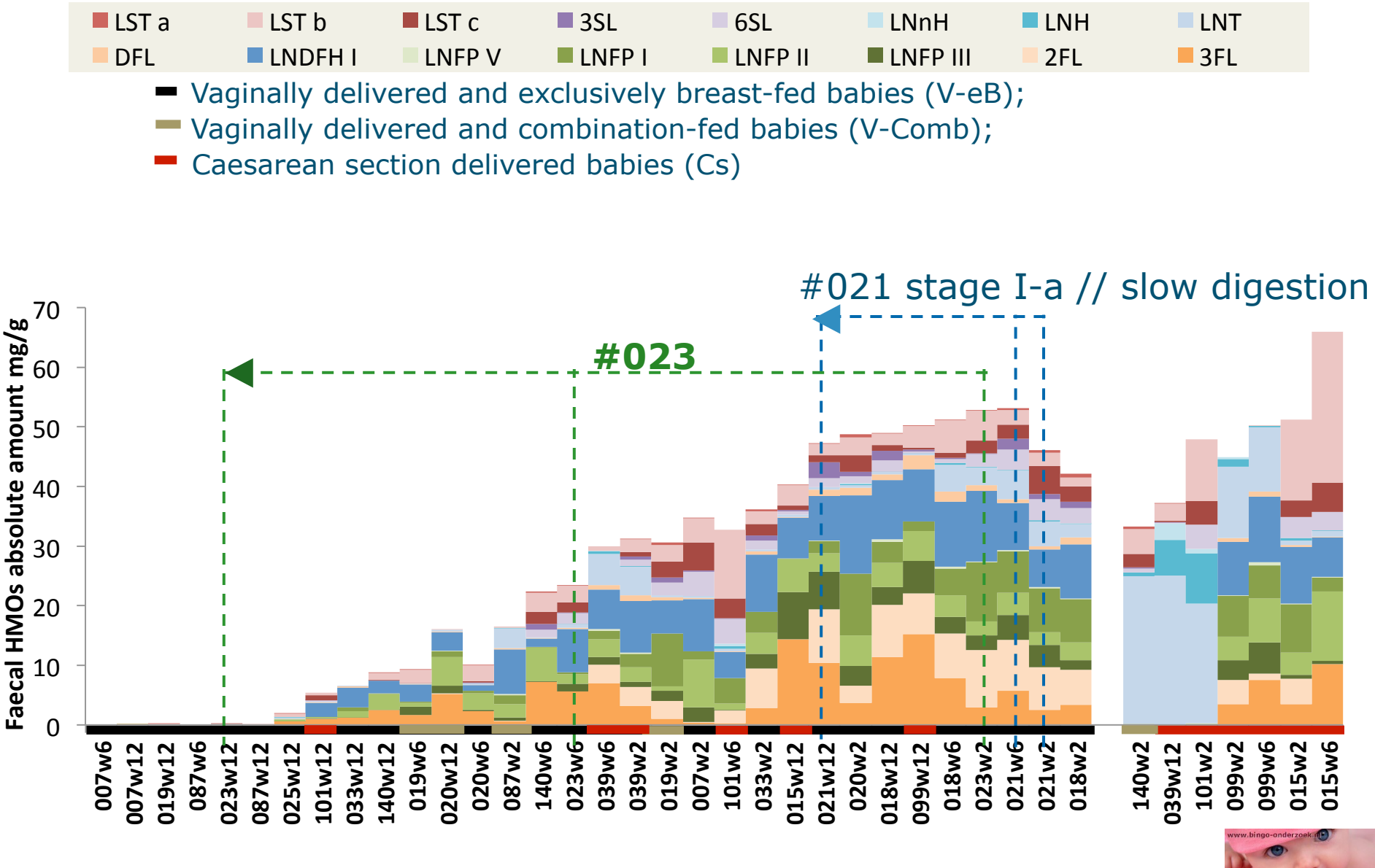
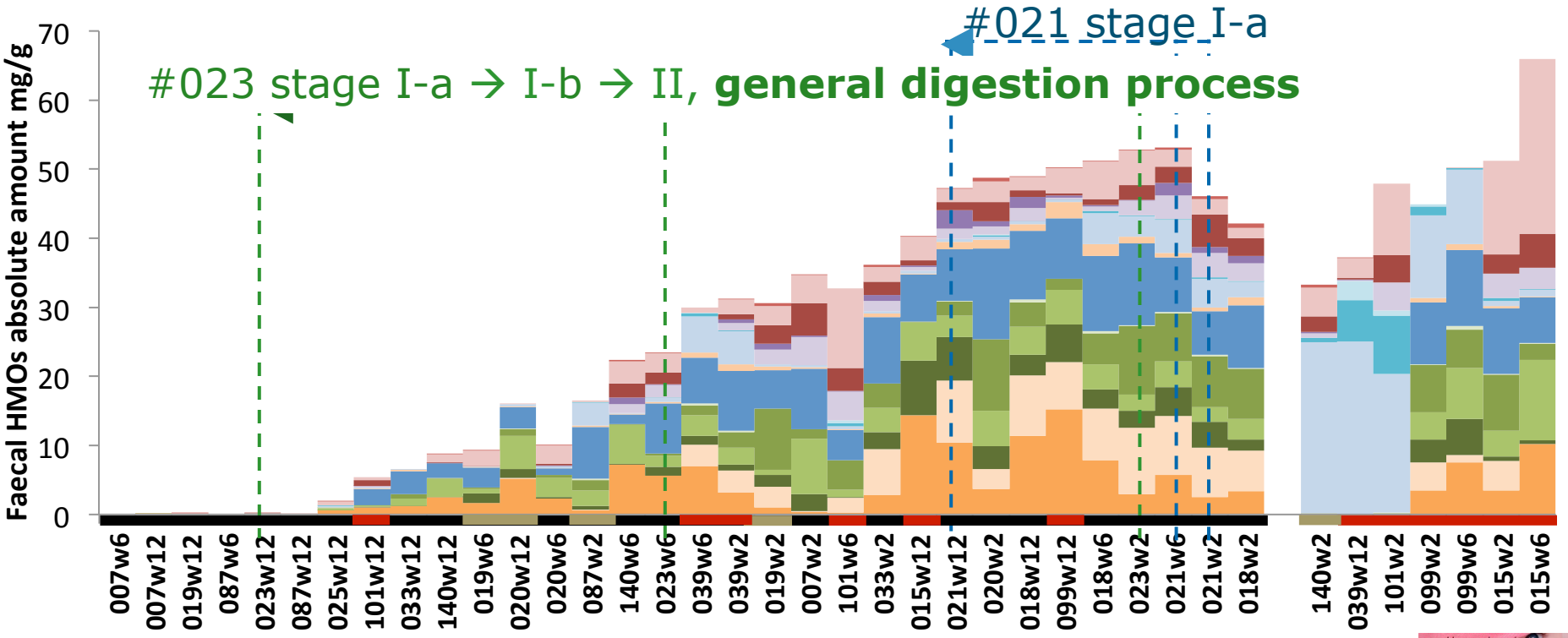


Faeces: HMO digestion patterns (Secretor) classified by multi-stages and similarity

LST a | LST b | LST c | 3SL | 6SL | LNnH | LNH | LNT
DFL | LNDFH I | LNFP V | LNFP I | LNFP II | LNFP III | 2FL | 3FL

- Vaginally delivered and exclusively breast-fed babies (V-eB);
- Vaginally delivered and combination-fed babies (V-Comb);
- Caesarean section delivered babies (Cs)



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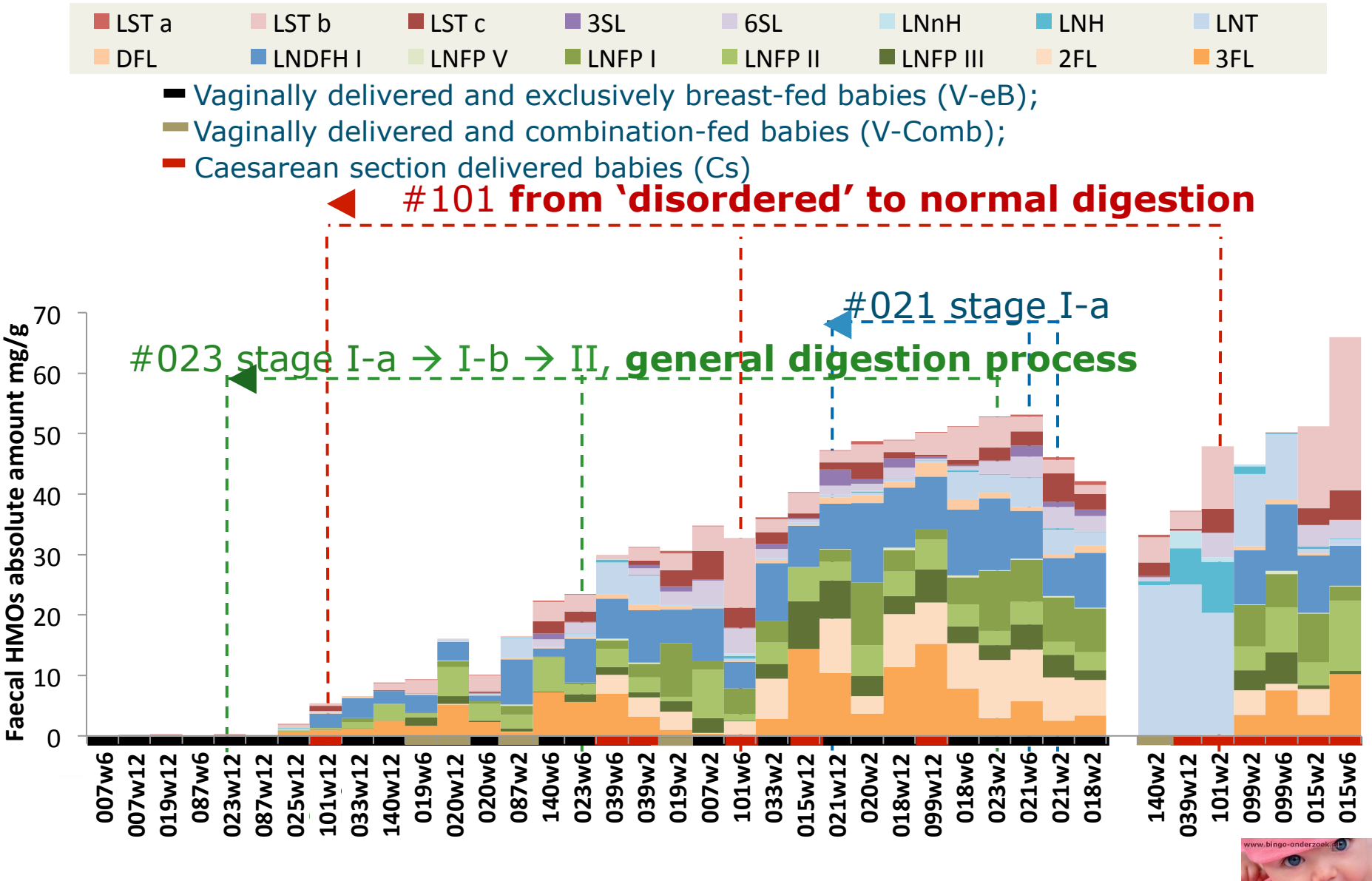

Fermentation of HMOs over lactation time

Milk

- Concentrations of breast milk HMOs decreased during lactation
- Fucosylated HMOs showed an increase in relative amount

Faeces

- The concentrations of faecal HMOs showed similar decreases as milk HMOs
- LNDFH I remained in the highest concentration in infant faeces till later time points
- External factors (feeding type, delivery mode) affect the HMO digestion mainly in the early time points

General conclusions

- Methods are present to evaluate the prebiotic potential of individual fibers by *in vitro* and *in vivo* fermentation.
- High-throughput analytical methods (PGC-UPLC-MS, HPAEC and NMR) are optimized to determine HMO profiles of mother milk and infant faecal samples. The problem of under-estimation of 3'-Fucosyllactose has been solved.
- The involvement of large sample number of both mother milk and infant faeces, as well as different time points over lactation time, lead to more comprehensive observations.
- The correlations between HMO fermentation profiles and microbiota data are studied by collaboration with Klaudyna from SP4.

CARBOHYDRATE COMPETENCE CENTER

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