

## Oligosaccharides Released from Milk Glycoproteins are Selective Growth Substrates for Infant-Associated Bifidobacteria

Sercan Karav

Canakkale Onsekiz Mart University, Turkey

Milk, in addition to nourishing the neonate, provides a range of complex glycans whose construction ensures a specific enrichment of key members of the gut microbiota in the nursing infant, a consortium known as the milk-oriented microbiome. Milk glycoproteins are thought to function similarly, as specific growth substrates for bifidobacteria common to the breast fed infant gut. Recently, a cell wall-associated endo- $\beta$ -*N*-acetylglucosaminidase (EndoBI-1) found in various infant-borne bifidobacteria was shown to remove a range of intact *N*-linked glycans. We hypothesized that these released oligosaccharide structures can serve as a sole source for the selective growth of bifidobacteria. Here, EndoBI-1 was used to release these *N*-glycans from concentrated bovine colostrum at the pilot scale. EndoBI-1-released *N*-glycans supported the rapid growth of *Bifidobacterium longum* subsp. *infantis*, a species that grows well on human milk oligosaccharides, but did not support growth of *Bifidobacterium animalis* subsp. *lactis*, a species which does not. Conversely *Bifidobacterium longum* subsp. *infantis* ATCC 15697 did not grow on the deglycosylated milk protein fraction clearly demonstrating that the glycan portion of milk glycoproteins provides the key substrate for growth. Mass spectrometry-based profiling revealed that *B. longum* subsp. *infantis* consumed 73% of neutral and 92% of sialylated *N*-glycans, while *B. animalis* subsp. *lactis* only degraded 11% of neutral and virtually no (<1%) sialylated *N*-glycans. These results provide mechanistic support that *N*-linked glycoproteins from milk serve as selective substrates for the enrichment of infant-borne bifidobacteria capable of carrying out the initial deglycosylation. Moreover, released *N*-glycans are better growth substrates than the intact milk glycoproteins suggesting that EndoBI-1 cleavage is a key initial step in consumption of glycoproteins. Finally, the variety of *N*-glycans released from bovine milk glycoproteins suggests they may serve as novel prebiotic substrates with selective properties similar to those of human milk oligosaccharides.