SPLASH!® milk science update
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This month's issue features the IMGC Symposium, lactoferrin and chemotherapy, and the cow genome.

"Before I Came Here, I Didn't Know That": Highlights From the 15th IMGC Symposium

This year marked the 15th annual IMGC International Symposium, held in downtown Sacramento, California, November 13–15. In spite of noticeable haze outside from the nearby wildfires, researchers, graduate students, and scholars from around the world found community and collaboration inside the beautiful conference center. Twenty-six presentations and 25 posters were complemented by panel discussions, luncheons, and a group dinner and tour of the Golden 1 Center, home of the Sacramento Kings. As in years past, the symposium offered both seasoned and novice researchers a dynamic forum for exploring the innovations and implications of milk science research. Here are some of this year's highlights.

1. In his welcome address, Bruce German recapped the history of the symposium, emphasizing its foundations in cross-connections among research fields. He noted that milk science has always been "a multi-disciplinary challenge" and that the IMGC Symposium itself has been a place where the complexities of that challenge have been explored. A brief tribute to Dr. Gonca Pasin and Gregory "Butch" Dias, Jr. was an opportunity for all to remember two of the symposium's most ardent supporters, and most memorable personalities. Toward the end of the conference, Dr. German pointed out one attendee's comment, "Before I came here, I didn't know that," encapsulated the importance of the symposium as a site of networking among experts across so many different fields.

2. The conference opened with "Hot Topics" that show the breadth of research currently underway, and some of the most exciting discoveries happening around the world. Danielle Lemay presented an overview of *SPLASH!* and its most popular articles in 2018 so far, including camel milk, innate lymphoid cells, and A2 milk. Since its first issue in 2012, *SPLASH!* has published more than 317 articles and is read by more than 3000 subscribers across the globe interested in maternal and neonatal health, integrated dairy production, and multi-omics—all of which were covered in the other "hot topic" talks. In his study of nicotinamide riboside supplementation on NAD and NAD-related co-enzymes, Charles Brenner demonstrated the importance of this component of energy mobilization in lactation. Oral supplementation has shown immediate and longer-term benefits to both offspring and mothers, which suggests a breakthrough in understanding postpartum metabolic processes. John Finley used visual art to help illustrate the Grand Challenge to connect the priorities of the dairy industry with problems and issues in public health and the environment, and he emphasized the current USDA strategy to take an integrated, interdisciplinary approach using complex system analytics to integrate modern dairy management into this scheme. Breast milk composition and underlying maternal genetic variation were the focus of Janet Williams's investigation of data from the multi-center INSPIRE study, which includes samples collected from over 400 women from around the world. This is the first report on genetics from the study and highlighted genetic associations with total milk oligosaccharides, lactose, and protein content.

3. This year's conference covered research in genetics and genomics. A number of presentations highlighted studies of the bovine genome, including current research on milk oligosaccharide profiles (Poulsen), milk fat profiles (Larsen), and phospho-caseins (Visker). Lotte Bach Larsen and colleagues continue their analyses of Danish dairy breeds via the Danish-Swedish milk genomics project. These studies have a common goal of evaluating the potential to use genomic selection to fine tune various properties of milk to improve processing characteristics, particularly for cheese production. Turning to human milk, Ashwantha Kumar Enjapoori and his colleagues examined beta-casomorphin peptides, including BCM-10 and BCM-11, in breast milk. They showed that these bioactive peptides are generated via endogenous milk protease activity in the mammary gland.

4. Application of biotechnology in livestock is undoubtedly one of the most compelling—and controversial—fields of research. Keynote speaker Alison L. Van Eenennaam reviewed some key improvements in food production that have arisen from scientific advances, and presented a rousing expose of the regulatory approval decisions concerning application of the most revolutionary molecular technology of recent times, CRISPR/Cas9 in animals. It seems that any animal with a modification in its genome, whether a null variant or a natural variant, is to be considered a drug by the FDA! Authorities from other jurisdictions around the world have been equally challenged by this advance in technology, and have implemented different but substantially divergent decisions.

5. Day two of the program began with a presentation from Steven Frese and Jennifer Smilowitz on the impact of probiotic supplementation of a naturally-occurring *Bifidobacterium longum* subspecies *infantis* on gut microbial colonization in infants (the IMPRINT study). Children fed the probiotic showed correction of dysbiosis in developing gut microbiota, including down regulation of antibiotic-resistant species. Analysis of stools from these children showed changes in metabolite profiles compared with children in the study who did not receive the supplement, indicating improvements in digestion, and they also found evidence of increased human milk oligosaccharide-derived energy mobilization and reduced intestinal mucin degradation. Importantly, the general well-being of the supplemented children was improved, with better consistency and a reduced frequency of stools. Diana Taft reported on her findings of gut microbiota in diverse samples collected from sites in USA, Europe, Gambia, and Bangladesh. The study profiled multiple complex microbial patterns, but Diana was particularly focused on prevalence of *Bifidobacterium longum* subspecies *infantis.* Using classical measures of transmission, she found that prevalence of this microbe may be dependent on breastfeeding duration. The morning session was concluded by a presentation from PhD student Alok Kumar from Hyderabad. Following from his supervisors own studies as a student in Australia, he presented on anti-microbial bioactivity of milk from the platypus, a representative of the most ancient mammals, the monotremes. Analysis of the physical properties of the milk-derived protein known as EchAMP, a member of the cathelcidin family, showed that the bioactivity of the protein is likely to arise from a helical conformation within the protein structure.

6. This year's meeting included a first ever session on milk microbiomes. Shelley McGuire revealed milk microbiome differences and core players across 10 human populations. Mary Kable described changes in the cow's milk microbiome during milk transport, processing, and storage. The talks were followed by a rousing panel discussion.

7. Milk glycans were once again represented at the conference. Carlito Lebrilla surprised the audience by revealing the glycan composition of a lot more than just milk, profiling hundreds of whole foods. Ishita Shah presented new data on milk oligosaccharides, and how they keep gut cells together and alter the response to pathogens.

8. Presentations on the final day of the conference highlighted milk lipids. In her overview of milk fat globule membranes, Sophie Gallier discussed the results of her team's study of MFGM-supplemented formula and its

effects on cognition in infants up to 12 months of age. Student Travel Award recipient Lauren Brink furthered this consideration with her research on the different variations of MFGM components in commercial infant formulas. Fellow Student Travel Award recipient Bartijn Pieters next presented the exciting results of his work on the treatment of osteoarthritis patients with milk-derived extracellular vesicles, which promises preliminary therapeutic potential.

9. The final three presentations all focused on digestion of human milk, with Dave Dallas and Veronique Demers-Mathieu discussing their lab's work on proteins and bioactive peptides. Looking at preterm and term infants, they continue to study the differences in biopeptides released, focusing on immunoglobulin survival during digestion. Didier Dupont presented an overview of some of the compositional and structural differences between human milk and commercial infant formula, using ten years' of data to show how better formulas might be produced.

Next fall's 2019 conference will be in Aarhus, Denmark. See you all then!

Contributed by
Professor Peter Williamson
Associate Professor, Physiology and Genomics
University of Sydney, Australia

Dr. Katie Rodger
Managing Editor
SPLASH!® milk science update

Lactoferrin Makes Food Taste Better for Patients Undergoing Chemotherapy

- **Excess iron and low levels of certain proteins in the saliva of people undergoing chemotherapy is why these individuals often experience a persistent metallic taste.**
- **A new study finds that giving people tablets of a milk protein, lactoferrin, reduces the sensory abnormality of metallic taste.**
- **By tracking the chemical contents of participants' saliva, the study authors showed that sensory improvements were reflected in shifts in levels of iron and various proteins.**

Iron is central to survival. As part of haemoglobin, it carries oxygen around the body. But excess levels have been linked to the development of cancer and to various sensory disorders [1]. When a cancer patient embarks on a course of chemotherapy, he often develops a persistent metallic taste in his mouth that is thought to be caused by iron in his saliva. Realizing this, a team of researchers from Virginia Tech decided to test whether giving chemotherapy patients a substance that mops up free iron, which is naturally present in human tears, milk, bile and saliva, might bring back the patients' normal sense of taste [2]. Although their study is small, they report remarkable success.

The substance in question is called lactoferrin. It features frequently in *SPLASH!®* due its versatile anti-microbial properties, and therefore its importance in protecting newborn babies from pathogens. Lactoferrin has several means of destroying bacteria, among them sequestering free iron that bacteria need to keep growing, and using its iron-attracting capabilities in breaking up bacterial cell membranes [3]. In short, if the human body needs to mop up iron, lactoferrin is its go-to molecule.

The team at Virginia Tech had previously studied the emergence of a metallic taste in healthy people. Six years ago, Andrea Dietrich and Susan Duncan were co-authors on a paper that described having healthy participants rinse their mouths with an iron-rich solution. Once the metallic taste was established in this way, the investigators tested substances like the anti-oxidants vitamin C and E, an ion-binding molecule called EDTA (ethylene-diaminetetraacetic acid), as well as lactoferrin, to find out which was best at quenching the flavor [4]. The results clearly showed that only lactoferrin did the job completely. So the next step was obvious: to test whether it worked in cancer patients undergoing chemotherapy.

Their latest paper, published this fall, reports changes in the salivary composition and metallic taste experienced by 19 individuals with cancer, and 12 healthy subjects with normal taste perception and of similar age to the cancer patients. The cancer patients involved had diverse forms of the disease—colorectal, breast, brain, pancreatic, lymphoma and myeloma cancers—but all had found that chemotherapy threw off their sense of taste and turned it metallic. Not only did the researchers set out to measure changes in the participants' sensory perceptions, but also to profile the minerals and proteins in each participant's saliva before and after the lactoferrin treatment.

The study results were much as hoped. First, taste assessments that were scored as abnormal were found for individuals with high concentrations of iron in their saliva, and who also had unusual levels of various salivary immune proteins—for example, low levels of salivary alpha-amylase, and of proteins known as PIP (or prolactin-inducible protein) and zinc-alpha-2-glycoprotein. The treatment—taking three lactoferrin tablets per day for a month—significantly lowered the sensory abnormality scores of the cancer patients. Curiously, the improvements continued for another 30 days, even though the treatment stopped. These patterns were reflected in the changes recorded for chemical constituents of saliva. Iron levels reduced in response to lactoferrin supplementation, and also kept reducing for an extra month. PIP and zinc-alpha-2-glycoprotein saw an uptick in their levels at 30 days, and salivary alpha-amylase also appeared to have been upregulated. Generally, however, changes in the levels of relevant proteins did not persist beyond the month of treatment.

The researchers who ran the study believe that several mechanisms explain their findings. The first and most obvious is that lactoferrin in saliva was binding free iron that was more common in the saliva of people undergoing chemotherapy. But the results showing changes in levels of salivary proteins also suggest that the treatment reduced the metallic taste in these individuals' mouths by triggering shifts in mucosal immunity—in other words, by stimulating the production of anti-inflammatory and anti-bacterial proteins. It is furthermore possible that lactoferrin could have relieved the metallic taste by helping in some way with the repair of neurodegeneration—although this is was not demonstrated by the experiment.

The number of cancer patients in this study is small, but the outcome is very promising and in line with earlier research. Moreover, the healthy subjects did not experience a hike in their salivary lactoferrin levels during supplementation, presumably because their livers were breaking down excess levels of it. This research suggests a straightforward and fairly low-cost way of improving the quality of life for many, many people having a rough time. Indeed, there are thought to be about two million people in the United States alone who experience abnormal taste and smell due to chemotherapy treatment [2]—and many more millions worldwide.

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Contributed by
Anna Petherick
Professional Science Writer & Editor
www.annapetherick.com

Family Trio Sings for Genomic Supper

- **Genome sequence variations used in conjunction with selective breeding programs for agricultural animals, like dairy cows, can increase industry productivity.**
- **High-quality reference genome sequences are the key to exploitation of information present in genomes.**
- **A new method for assembling a genome sequence using the trio of mom, dad, and an offspring greatly reduces errors.**
- **The new method identified errors in a "reference" cow genome sequence and will lead to its improvement.**

Solving a giant crossword puzzle and completely sequencing a genome have a lot in common, including despair and satisfaction. The puzzle just requires the assembly of all components into the one correct pattern. The first 90% is fast and furious. One's confidence grows as the unique solution becomes tantalizingly close. Satisfaction seemingly guaranteed. But then, the last 10% rears its ugly head and frustratingly devours time and confidence. "*I can't get no satisfaction*"—the plaintive words of Mick Jagger mercilessly resonant. The stark realization is depressing. Most of the puzzle is correct, but there must be an error somewhere. But it's hard to go back. The inevitable outcome is to accept something that is mainly correct and move on—"y*ou can't always get what you want*." However, all is not lost. Koren and nine colleagues [1] recently developed a very smart solution for completing the genomic puzzle with much lower error rates. They used a genomic trio of mom, dad, and one offspring for maximal effect and then tested their method in three species. The results were impressive, particularly for the cow.

What is a Genome Sequence?

A genome is the complete set of genetic material (DNA) present in a cell. All life has DNA or its first cousin RNA, which is present in a few viruses. The genome contains the genetic blueprint for the form and function of life. That's a big call! DNA is a long double helical molecule present in the nucleus of a cell. Amazingly, there is about two meters of highly compacted DNA present in the nucleus of each mammalian cell, which is only six one–thousandths of a millimetre in diameter [2]. There is one long molecule of DNA associated with each chromosome. The sequence of a genome is the ordered sequence of the building blocks of DNA, called nucleotides, in all chromosomes. A mammalian genome contains about three billion nucleotides. Long stretches of these nucleotides code for the tens of thousands of genes and their regulatory regions. The nucleotide code for genes is read and ultimately converted into biological action by molecular machinery present within the cell. (Surprisingly, only about 8% of the genome seems functional [3]. The big mystery is the role of the remaining 92%.)

What's All the Fuss About a Genome Sequence?

A genome sequence contains four massive books of information. First, it contains the detailed blueprint for how a fertilized egg develops into a complex multicellular organism, like a cow. The patterns of gene

activities in cells underpin the amazing process of development eventually leading to an array of very different and precisely positioned cell types in an individual. Second, inscribed in DNA is a detailed history book recording past events. It describes the evolution of a species, population migrations over large periods of time, and monumental battles with diseases and changing environments. Like all history books, it was written by the victors, i.e. the survivors who successfully reproduced and passed on their DNA to future generations. However, about 99% of all species that ever lived are extinct [4], but their ghostly genetic footprints lie everywhere in the genomes of modern-day species [5]. Third, the individual variations in the genome sequence provide a catalog of Who's Who at the Zoo for today's population. Nowhere is this better highlighted than for the solving of crimes using forensic DNA or the ability to trace food from the plate back to pasture to guarantee food safety. Perhaps one the most important applications is the use of genetic variations in agricultural production animals and plants for the accelerated selective breeding of individuals with the most desirable production traits. For example, genetic variants could be used to select for bulls, even when immature, that are likely in the future to sire cows producing more milk for less feed. Fourth, the genome contains a medical textbook that has recorded the results of gigantic natural experiments occurring throughout the ages. Scientists repeatably note that the most variable regions of the genome often involve immune defense genes [5, 6]. The variations in these genes were enriched in the population by past battles with disease and today they can provide clues for solutions to present day diseases [5, 7, 8]. Genetic variations can also point to previously unsuspected genes affecting health [7, 8]. The key to deciphering and exploiting the information present in these books is a high-quality genome sequence, often called a "reference" genome sequence.

A Genome Sequence is the Ultimate Extreme Puzzle

Sequencing a "complete" genome for a complex life form, like a mammal, is not for the faint-hearted, even 14 years after the human genome was "completed" [9]. The word "completed" is a technical term that refers to a very low DNA sequence error rate and no omitted genomic regions. "Completion" is a tall order. Scientific consortia have "completed" the genome sequences for only about five complex life forms (human, mouse, fruit fly, a worm, and a yeast) [10]. As of 2017, Lewin and colleagues [10] noted that there were about 2,500 genome sequences for complex life forms, but only about 25 were higher quality "reference" sequences. A good "reference" genome sequence is fundamentally important for understanding the unique biology of a species and for the identification of genetic variations in their population that can be exploited for a wide range of purposes, including gene discovery and DNA variation-assisted selective breeding of animals and plant species used in agriculture.

Koren and colleagues explain that scientists have struggled with the technical challenges required to improve the quality of "reference" genome sequences [1]. They note that the general strategy used for sequencing a genome is to produce millions of very small overlapping DNA sequences from one individual and then virtually assemble these sequences into fewer but longer sequences. The process, in theory, can lead to a complete genome sequence. However, the assembly process is confounded by three difficulties. First, some parts of the genome are recalcitrant to DNA sequencing, like a spoilt child determined not to cooperate. These regions can be conquered, but they require personalized attention, additional time, and more finance. Second, other sequences have near identical copies present at thousands of different places in the genome. Hence, it is hard to know exactly where a specific repeat sequence goes in the genomic puzzle—it's a little like trying to complete the picture puzzle entitled "polar bear in a snowy winter's day." Mastering repeats also requires additional tailored and expensive approaches. Third, genetic variation confounds the virtual assembly of short DNA sequences into a genomic sequence. The latter is where Koren and nine colleagues made huge progress [1].

Mom and Dad Improve Their Offspring's Genome Sequence

Variety is the spice of life and so it is in genetics. Each human individual is about 99.9% identical to others in the population, but the 0.1% difference, often numbering millions of variations in the genome, is what makes a person genetically unique. Koren and colleagues explain that genetic variation is also rampant even within the individual, who is an amalgamation of the variations inherited from their parents [1]. These genetic differences in an individual make genome sequence assembly difficult. A human has 46 chromosomes consisting of 22 chromosomal pairs and a pair of sex chromosomes XY or XX. One chromosome of a matching chromosomal pair in the offspring comes from mom and one from dad. Hence, the offspring contains genetic variations that may be different at the same relative position in a matched pair of chromosomes—a little like spelling differences for the same English and American word. In production animals, one way to decrease the problem of genetic variation is to sequence the genome of a partially inbred animal (produced from related parents), which has markedly fewer genetic variations than an outbred animal. Inbreeding cannot be used for sequencing many species due to a variety of practical reasons, and for humans, it is a major ethical issue. Moreover, the inbred individual is not representative of the wider population both in terms of its genetic variation and biology. Hence, its relevance to the form and function of other individuals is questionable. This is particularly true for some production animals that are the result of crosses between parents from very different breeds or breeding lines.

Koren and colleagues inspirationally embraced genetic variation as a tool to improve "reference" genome sequences [1]. Unlike other strategies designed to address the same issue, their method becomes more efficient for assembling a genome sequence when there is greater genetic variation within an individual. Their approach is very smart. They first obtained a large number of short DNA sequences from each parent. They then took longer DNA sequences obtained from the offspring and matched these to the short DNA sequences from each parent. This strategy generated two "bins" of the long offspring DNA sequences, one containing offspring DNA sequences that came from mom and the other from dad. Using these "binned" DNA sequences, Koren and colleagues independently assembled each of the parent-specific offspring genomes (called haplotype-resolved genomes). For a human, these two genomes represented the DNA contributions from the 23 chromosomes present in the parental egg or sperm. The investigators then fully reconstructed the offspring genome by combining these two parent-specific genomes, which together represented the DNA in all 23 pairs of the offspring chromosomes.

New Method Shines

A new method has to jump the daunting bar of independent validation and versatility. Many fall heavily at this final hurdle. Koren and colleagues tested their method using three very different species; a small plant called Arabidopsis, a human, and the offspring from a parental cross between two cattle subspecies represented by the Brahman and Angus cattle breeds [1]. The method worked very well in all three circumstances. Perhaps the most striking results were obtained with the cattle cross where 99% of the offspring-assembled genome could be directly attributed to a specific parent. Measures of the quality and accuracy of the parental contribution to the cow offspring genome sequence were very high. The investigators then compared each parental contribution to the offspring genome with a decade old cow "reference" genome sequence. Surprisingly, the investigators's comparisons revealed 3,178 instances where small parts of the old genome assembly were probably back to front. The crossword puzzle had errors. The investigators confirmed this problem using additional and independent analyses. The old cow "reference" genome sequence was not perfect, but it was very useful. Like an aging car, it is about to be replaced by a shiny new model.

Implications

Koren and colleagues' new method can very efficiently dissect out the parental contributions to an offspring's genome [1]. Importantly, the new genome sequence assembly method will markedly improve "reference" genome sequences for a range of species and will pay handsome dividends for all agricultural production animals. The investigators noted that the method will better sample the genetic variation derived from each parent. Their method will potentially greatly improve the accuracy of selective breeding in agricultural animal populations, guided by DNA sequence variations, to enhance desirable production traits and improve industry productivity. This aspect is particularly relevant where the commercial livestock population going to market is the result of a cross between parents from very different genetic backgrounds, e.g. crosses between different breeds of cattle. In this case, a commercially relevant cattle trait in the offspring may result from contributions from different genetic variations in the parents. The investigators also implied that their method is useful for characterizing specific immune-related regions of the human genome that normally are difficult to correctly sequence due to large-scale variations in the human population and within an individual. Some of these genomic regions are particularly important as they define the compatibility of individuals for organ transplantation. The accurate identification of parental contribution to the offspring genome will also aid the discovery of specific gene variants that affect the health of humans and animals. These genes may provide clues aiding the discovery of new ways to improve health. The trio of mom, dad, and an offspring, together, more easily solve the genomic puzzle. Sometimes, for puzzles, the process is just as important as the outcomes.

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Contributed by
Dr. Ross Tellam (AM)
Research Scientist
Brisbane, Australia

Looks Can Be Deceiving: Similar Gut Bacteria Have Different Functions in Breast-Fed and Formula-Fed Infants

- **Because of the demonstrated health benefits of human milk oligosaccharides to the development of the infant gut microbiome, some infant formulas include commercial oligosaccharides, including galacto-oligosaccharides.**
- **A new study reports that although infants fed formula with commercial oligosaccharides had similar types of gut bacterial communities as breast-fed infants, their bacteria had distinct metabolic functions related to other ingredients in formula and breast milk.**
- **The health consequences of these differences are not yet known, but researchers identified several metabolic functions involved with preventing infection that were unique to breast-fed microbiomes.**

Infant formula manufacturers are faced with an extremely difficult task: they must transform cow or plant-based milks into a liquid that mimics human milk. This mimicry involves more than just copying human milk's ingredient list, however. Formula must also match human milk in performance, an especially difficult endeavor when considering many components are highly complex and specific to human milk.

Such is the case with human milk oligosaccharides (HMOs), carbohydrates with a prebiotic function. Because of their demonstrated benefits to the development of the infant's gut bacterial communities (or microbiome), many formulas add commercial oligosaccharides. On paper, these commercial oligosaccharides seem to be perfect stand-ins for the real thing—they are similar to HMOs in molecular structure and are known to promote the growth of breastfed-like bacterial communities. But a new study [1] demonstrates that even though the microbiomes look similar, gut bacteria from formula- and breast-fed infants follow different sets of genetic instructions.

Copycats

If the goal of formula is to copy human milk ingredients, it's no surprise that oligosaccharides top the list. They are the third most plentiful component of human milk and are highly diverse, with over 200 different varieties (that is, different structural configurations) discovered so far [2, 3]. The quantity and diversity of HMOs is unique across mammals, with cow milk containing only trace amounts and only 40% of the types found in human milk.

Unlike the milk carbohydrate lactose, HMOs are not digested by the infant and are not used for infant nutrition. Instead, HMOs act as prebiotics that feed the bacteria in the infant gut. In doing so, they beneficially influence the composition and function of the infant gut microbiome [1-3]. Additionally, they limit infections and illness by keeping dangerous pathogens from adhering to cells in the infant gut [3]. This is not just important for infant health; recent research suggests that the types and quantities of bacteria that make up the infant gut microbiome also influence the composition of the adult microbiome [4-6].

Ideally, formula manufacturers would just add HMOs to their products, but HMO structural complexity and sheer diversity make them extremely difficult to extract or synthesize. In lieu of the real thing, commercial oligosaccharides—specifically galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS)— have been added to formula since that late 1990s [3,7]. These carbohydrates are not as complex as HMOs but have been found to promote the growth of bacterial microflora similar to that of breastfed infants (i.e., *Bifidobacterium*) [1-3, 7]. As a result, infants fed formula supplemented with GOS and FOS have microbiomes that look more similar to breast-fed infants than those fed formula without added oligosaccharides [1]. While this is undoubtedly a major breakthrough in infant nutrition [7], it appears that GOS and FOS may not be enough to fully impersonate human milk.

The Gut Reaction

The real test of the ability of GOS and FOS to fill the role of HMOs in formula is to determine whether the gut bacteria in formula-fed infants are doing the same things as their colleagues in breast-fed infant guts. This requires not only identifying which types of bacteria are present in the gut, but also the types of amino acids and biomolecules, such as vitamins, those bacteria have the potential to synthesize.

A new study [1] from a team of researchers from Washington University in St Louis presents just such a functional comparison. The team tested the hypothesis that even gut microbiomes that appear similar will have drastically distinct functions based on specific components in the infant diet. The community of bacteria that lives in the infant's gut will also appear in infant stool, allowing for a less invasive way to collect gut bacterial DNA. Genomes from 402 fecal samples representing 30 sets of twins between 0 and 8 months of age were matched with parent-provided data on diet; the sample included breast-fed infants, formula-fed infants (including those consuming formula supplemented with GOS/FOS), and infants that received both breast milk and formula.

As predicted, diet corresponded to the (potential) metabolic function of the infant's gut microbiome. Infants that received the majority of their nutrition (>50%) from breast milk had microbiomes made up of more bacteria capable of synthesizing branched-chain amino acids, as well as the amino acids methionine, cysteine, arginine, and theorinine [1]. These amino acids are found at lower concentrations in breast milk compared with formula. Conversely, there were fewer bacteria that synthesized amino acids that are plentiful in breast milk, namely histidine and trytophan [1].

A nearly reverse pattern was identified in fecal samples from formula-fed infants; they had fewer gut bacteria with the genetic potential to make methionine, cysteine, and arginine (which formula has a lot of) and more capable of making histidine and tryptophan (which formula has very little of).

Remarkably, it appears that the genetic functions of the gut bacteria correspond to infant nutritional requirements; gut microbes pick up the slack and synthesize amino acids that are lacking in breast milk (or formula). The researchers call this milk-microbiome complementarity, and believe it is an adaptation to infant protein balance [1].

Performance Evaluation

So how much of an impact did commercially added oligosaccharides make on microbiome function? Although the study found that infants fed formula with GOS and FOS had breast-fed like microbiomes (i.e., enriched with Bifidobacteriaceae), they did not match breast-fed infants in amino acid synthesis [1]. Specifically, infants with greater exposure to GOS and FOS had more bacteria able to synthesize branched-chain amino acids and theorinine (like breast-fed infants), but also had fewer bacteria able to synthesize cysteine and arginine (the opposite of breast-fed infants) [1].

The team only measured potential function of the bacterial DNA rather than which amino acids were actually transcribed, thus this study cannot speak to the health consequences of these different metabolic pathways. However, they speculate that the genetic instructions that are more plentiful in breast-fed microbiomes may relate to the improved immune function associated with human milk. For example, arginine and cysteine (enriched only in breast-fed infant microbiomes) may be involved in preventing infection [1].

Thus, although GOS- and FOS-enriched formulas do mimic human milk in composition, they can't claim to match it in performance. But studies like this provide critical stage notes for formula manufacturers to improve their product's performance. Even small changes in microbiome function in early infancy can have profound impacts on individual health years (and even decades) later [4-6].

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Contributed by
Dr. Lauren Milligan Newmark
Researcher, Science Writer

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