

SPLASH! milk science update: November 2014 Issue



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This month's issue features the highlights of the recent IMGC Symposium in Denmark, as well as articles on the memory of lactating rats, dairy for the lactose intolerant, and the initial microbial transfers from mother to offspring.

A Snapshot of the 2014 IMGC Symposium

The Danish city of Aarhus provided the backdrop for this year's 11th International Symposium on Milk Genomics and Human Health. Our hosts at the University of Aarhus provided a windy, but welcoming, setting for an intensive three days of research presentations. Here are some of the main themes and findings:

1) The pre-symposium session set the scene by presenting an update on observational studies on the status of dairy in human nutrition and health. Both human and animal studies have shown strong associations between dairy intake and favorable outcomes in aspects of metabolic health (Carson; Heegaard; Dalsgaard; Holm).

2) Animal genomics in dairy cattle is capitalizing on the developments in genomic selection and shifting focus towards new traits. The Scandinavian experience showed a remarkable 50% increase in genetic gain since the introduction of genomic tools (Stalhammer). We were also treated to an overview of a new EU program to study the genomics and



biology of methane emissions and rumen microbiota using a multi-disciplinary approach (Wallace). QTL were described for milk mineral content in Danish Holstein and Jersey cattle, with high heritabilities for Ca, Zn, K and Mg (Buitenhuis). Traits associated with physical processing and cheese-making properties of milk continue to be a focus for the Danish-Swedish program, with significant progress reported in identifying variation in coagulation properties among breeds and herds (Larson, Poulsen). Finally, a presentation on the use of eigenvectors cautioned dairy scientists to be careful about using correlated traits when performing genetic analysis (Eskildsen).

3) A review of dairy protein quality and sustainability presented some interesting and noteworthy facts and figures. As expected, dairy contributes widely to nutrition, and particularly to providing high quality protein around the world, with a growing impact in new markets. However, the current view is that this is done with significant impact on the environment. Yet, when a food value index is taken into consideration, by including nutrient density as a factor, dairy is a far more sustainable product than otherwise credited (Hooijdonk).

4) The lactation biologists again highlighted the incredible value of model systems. The first ever computer imaging systems analysis of mammary gland architecture, coupled with *in silico* genomic analysis, showed what modern biotechnologies are capable of delivering. Mammary gland architecture dictates milk production capacity in all mammals, including humans, and is fundamental to understanding low-level or failed lactation (Hadsell). Exotic animal models also continue to provide novel milk constituents as new leads for areas as diverse as lung development and powerful anti-microbials (Sharp). They also continue to shed light on drivers of milk production and mammary gland responses (Ramakrishnan, Mobuchon).

5) Infant nutrition, particularly for pre-term infants, was once again a theme at this symposium. Funded by the Danish Research Council, the NEOMUNE program, along with collaborating centers worldwide (especially in China), are taking a global view to identify best practices, and to assess the role of colostrum and milk (Sanglid, Nguyen). Consideration of fundamental physiological factors, including gastrointestinal lipase activity, is critical in defining appropriate formulations for perinatal nutrition (Hellegren). Integrating information from so many centers and across a range of disciplines sparked a lively discussion on the challenges of research translation.

6) The potential for probiotic effects of milk form the basis of an exciting new program that looks at the variation in milk microbes and genetic diversity in mothers from diverse geographical and ethnic backgrounds (McGuire). Their studies,

using advanced tools for dissecting milk components and metabolites, are beginning to unravel additional complexity, but in a context that compares samples based on differences in physiology or pathology in subjects. (Popovic, Spevacek, Beck, Gillespie, Hettinger).

7) Canadian dairy research representatives are always a welcome sight at the symposium. We heard a wonderful description of the integrated resources available to streamline research in the Institute of Nutrition and Functional Foods, from animal sciences to clinical trials. This was exemplified by a detailed description of the impact of dairy as a complex food matrix on health parameters (Turgeon).

8) The endemic health challenges of weight management and metabolic diseases, and the changing dogma on lipids in the context of cardiovascular disease, received a lot of attention at the meeting. The latest meta-analyses show that dairy is not associated with an increase in either metabolic or cardiovascular disease incidence. Moreover, the EpiACT study shows a protective effect for type-2 diabetes (Astrup). The role of protein and milk fat, particularly in the context of a food matrix, are being studied extensively in multi-center programs, like the EU Dairy Health Project, and in laboratory based animal models (Clausen, Amer).

9) A terrific retrospective presented information on the functional effects of milk constituents and the impact of supplemented formulations on infant health. Furthermore, the presentation showed the documentation of the beneficial effects on a range of outcomes, from brain development to *H. pylori* infection (Lonnerdal). Piglet models have become the preferred system for analyzing pre-term and neonatal outcomes. The gold standard for a pig neonatal intensive care unit -and associated analytical tools-- illustrated with a brain and behavior study from Illinois (Radlowski).

10) The presentation on a 20-year R&D journey on osteopontin, described a remarkable story of continual discovery and a tour-de-force in the field (Sorensen). The story highlighted the nature of the commitment required from investors for identifying bioactive components in milk and following through on product development.

The science was punctuated with a splendid sojourn to the beautiful Varna Palaeet for the annual dinner. We were treated to a delicious meal of fine haute cuisine, as well as the classic tones of Schumann, Bach, Rachmaninov ... and the cow song! Great fun!

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Lactating Helps Rats Remember

- **Rats show improved “object-in-place” memory when they start lactating.**
- **The evolutionary rationale put forward for this change is that a lactating rat leaves her offspring unprotected in the nest while she forages, and needs to get back to them quickly.**
- **The effect is brought about by the action of high levels of female sex hormones on several regions of the brain.**

If you were to stop strangers on the street and ask them whether pregnancy and breastfeeding enhances or diminishes memory, some might lean towards an old wives' tale: pregnancy is supposed to bring with it an underperforming or shrunken brain [1]. But why would this make sense from the point of view of evolution? Recently, researchers working on spatial memory in rats have made the point that it doesn't. Instead, they have found that a specific kind of memory, called “object-in-place” memory, actually improves over the course of having and nurturing an infant. Their laboratory rats received a mental boost that may have begun to develop gradually during pregnancy (although it remained hardly noticeable at that point), then became significant when the rats were lactating, and remained even after infants had been weaned.

Why would this be? The working hypothesis of Katherine Tombeau Cost and her team of biologists from New Orleans is that it has to do with foraging under pressure [2]. A lactating rat is under-the-gun to find food quickly and return to protect her nest of vulnerable pups ASAP. On the other hand, a pregnant rat—or one without kids—can take her time. In a world without childcare or a means to lock your front door, it's the equivalent of running to the shops to grab what you need, instead of dawdling in the aisles and browsing the dinner options.

Moreover, “object-in-place” memory—foraging memory, if you like—is quite a complex mental function. The brain has to recognize not merely an object, but also the object's context, which involves the activity of several brain regions, including the hippocampus, the perirhinal cortex, and the medial prefrontal cortex. Thus, improving “object-in-place” memory seems

to require the progressive action of high levels of multiple female sex hormones, and would be as difficult and delicate a task to undo as to affect.



Nor is this kind of spatial memory straightforward to test in the lab. Cost and her colleagues based their experiments on the assumption that rats are sensitive to furniture re-arrangements—that rats tend to spend much longer sniffing around an object that they know has been moved, compared with one that they know has not.

In each “object-in-place” memory test, the team placed their rats in a square box that contained a different object, such as a mug or a bottle, slightly inside each corner. After a while, the rat was returned to its normal home. Then, following either a five or 30-minute break, she was placed back into the same box, but this time two of the four objects had swapped positions. Cost et al. [2] monitored what the rats did next. They repeated this testing procedure before and after the rats became pregnant and at several time points after the animals had given birth.

The researchers also performed the memory test on childless rats for comparison. The five-minute break didn’t appear to toy with any of the rats’ memories: whether pregnant or not, lactating or not, all the rats noticed the internal redesign of the box. But strong differences in the rats’ mental functions became evident in the 30-minute-delay tests. On these occasions, it was only the lactating mothers or the mothers who had recently lactated that appeared to recall the earlier object arrangements.

In the world of the wild rat, the ability to recall where food is currently available cuts foraging time, which could make all the difference to the odds that a predator will snuff out the infants left in the nest. Or, for a more paranoid mother who really, really minimizes time away, a few extra calories might make the difference as to whether she is sufficiently nourished to have another litter down the line. This is shopping IQ revealed, red in tooth and claw.

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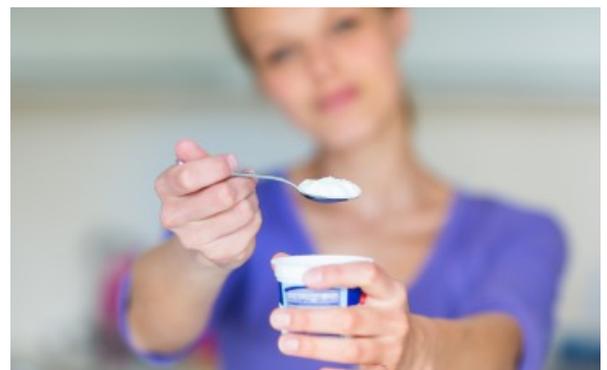
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Dairy for the Lactose Intolerant

- **Lactose intolerant people are usually able to consume live, unflavored yogurt without experiencing the symptoms that typically accompany their consumption of milk.**
- **Helpful bacteria in yogurt are to thank for this, as well as the particular buffering capacity and viscosity of yogurt.**
- **Eating other foods as part of the same meal and even the choice of the species of bacteria used in the yogurt-making process do not appear to make yogurt digestion harder for lactose-intolerant people.**

Ever watched a lactose intolerant friend shovel yogurt into their mouth, and wondered, in anticipated horror, at what the outcome may be? Strange as it may seem, fully lactose intolerant people tend to have little problem digesting yogurt, even though its lactose content is approximately equal to milk’s. There is no need to check for the nearest toilet. The explanation for this apparent puzzle lies with the bacteria in yogurt. Each millilitre of the stuff contains about 100 million bacterial cells—and each of these acts like a microscopic



supplement, a capsule or pill if you like, of the enzyme that lactose intolerant people cannot make: lactase.

Scientists have suspected that this might be going on in the bellies of lactose intolerant people since the 1970s, but it has taken many years to gather the proof. The original hints came from experiments on rats that found that the bacteria in live yogurt stayed alive for a surprisingly long three hours after the animals had swallowed them [1]. In the 1980s, the research progressed to measuring the amount of hydrogen in the breath of human subjects. In theory, a measure of how flammable someone's burps would be can reveal the activities of colonic microorganisms after a potentially lactose-intolerant person has consumed lactose. The higher the levels of hydrogen in someone's breath, the less able they are to metabolize this sugar—hence it remains intact all the way along their digestive tract until the colon, where hydrogen-producing microorganisms metabolize it instead. In 1984, researchers at the University of Minnesota reported that the breath of lactose-intolerant folks contained just a third of the levels of hydrogen after they ate yogurt than was the case after they consumed an identical amount of lactose from milk [2].

So, what exactly is going on? The answer is manifold. As previously mentioned, live yogurt provides a community (of bacteria) to which a lactose intolerant yogurt lover unknowingly outsources the parts of digestion that they are ill-equipped to handle on their own. There are two important facets of the process of yogurt digestion that make this possible. The first keeps the bacteria in yogurt alive as they pass through the protein-busting, acidic soup of the stomach. Yogurt achieves this because it is a natural buffer. Tests have shown that yogurt needs about thrice as much acid to shift its pH from 4.1 to 2.0 as milk does—which explains the strangely long life of the bacteria in those rats in the 1970s.

Second, yogurt is slower than milk at moving through the gut; the longer transit time is probably due to milk's runnier consistency. The yogurt bacteria therefore have more time to work their magic on lactose once they've dodged the bullet that is the stomach's gastric juices. At this point in their gastrointestinal journey, they meet bile, a substance that has been found to increase the activity of these bacteria's lactase enzymes, perhaps by making their cells more permeable to lactose [3]. All in all, the bacteria in yogurt then get the job of lactose digestion done before the microorganisms that give lactose intolerant people a high score on the hydrogen breath test do: yogurt bacteria operate almost entirely in the small intestine, not in the colon. The result is the absence of unpleasant symptoms.

With the basic science more or less understood, recently researchers have wondered about the effects of flavored yogurts, eating yogurt in combination with other kinds of food, and the relative efficiencies of different species of yogurt bacteria at digesting lactose on behalf of their human consumers.

Unfortunately for the lactose intolerant who prefer the taste of flavored yogurt, it doesn't do as well as unflavored yogurt on the hydrogen breath test. In one study, flavored yogurt scored 77 parts per million of hydrogen, placing it between unflavored yogurt (at 37 ppm/h) and milk (185 ppm/h) [4]. For some reason, a greater proportion of the lactose in flavored yogurt survives to the colon than in the case of unflavored yogurt. Why this would happen remains unclear. Theories include the dilution of yogurt by flavoring components (like fruit), some kind of osmotic influence of added sugar in the stomach, and potential, later, inhibitory effects of glucose. Similarly, the mechanistic picture is rather unclear when it comes to eating yogurt concurrently with other sorts of food. Might this undo the buffering effects of yogurt in the stomach? Apparently not, at least not in the case of 10 accompanying meals tested by 22 people, under the guidance of a different team at the University of Minnesota [5]. They focused on some typical breakfasts, such as toast and peanut butter. But even when the subjects ingested pretty acidic foods with their yogurt, like orange juice, banana (surprisingly, pH 4.5) and fruit jelly on their toast, the lactase-digesting effects of yogurt remained intact; if anything, they improved.

And what about the different species of bacteria that can be employed to ferment milk in the yogurt-making process, and give the final product that tangy, yogurt-y taste? So far, the research that has been done indicates rather minimal differences between the most widely used species, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. In some tests, the amount of bacteria has been shown to matter; when the population is cut from 100 million to 10 million per milliliter, its lactase-digesting assistance is rendered pointless [6].

So, dairy-adoring people who struggle to drink milk without an embarrassing or uncomfortable incident are fine to gobble yogurt, with whatever they like and, ideally, of the unflavored variety. Most people without northern European genetic ancestry fall into that group. Ultimately, lactose intolerance comes down to the genetics, or more specifically to the promoter (on/off switch) region of the gene that encodes the lactose-digesting enzyme, lactase—which everybody has, because all humans are powered exclusively by breast milk or its modern replacements early in life. There are various indications that the deactivation of this promoter that happens after the toddler years of lactose-intolerant people can be gently undone by gradual and continual exposure to lactose. Indeed, yogurt appears to be ideal for this biochemical training. The tolerance of people who initially struggle to digest lactose has been found to improve when they regularly eat some yogurt. Only a little yogurt, once per day for 15 days, is enough to make a difference on the hydrogen breath test [7].

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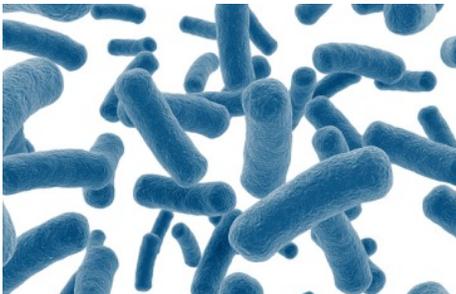
Microbial Transfer from Mother to Offspring

- **Embryos have first contact with microbes via the placenta.**
- **Birth is the baby's second point of contact with the microbial world.**
- **Postnatally, the transfer of bacteria from the mother to the infant continues via breastfeeding.**
- **Breast milk contains a complex and dynamic microbiome, which colonizes the infant's gut, offering a unique avenue of environment-specific development of the immune system and the gastrointestinal tract.**
- **In addition to their significance for the newborn, breast milk microbiota reflect those of the lactating breast, and its health status.**

Until recently, it was thought that the maternal reproductive system is sterile, and that a baby's first contact with bacteria was during birth while working its way through the birth canal. This long-standing dogma has been challenged by studies demonstrating that almost all tissues in the body are full of germs.

Aagaard and colleagues (1) specifically assessed the placenta. Amazingly, they reported the presence of a unique microbiome, including nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria phyla, resembling to some extent that of our oral cavity. This now unmistakably shows that the baby's first contact with bacteria is not at birth, but while still in utero.

A few years ago, Rodriguez and colleagues demonstrated by testing the meconium of healthy babies that bacteria are transferred to the fetus from the mother (7). Previous studies on the presence of bacterial strains in umbilical cord blood and amniotic fluid also showed that the embryo is clearly not sterile. Additionally, the known differences between the vaginal microbiome and that of newborn infants further reinforce the fact that the birthing process is not the initial point of inoculation.



Babies start developing their own microbiome early on during embryonic life upon contact with the placenta. This process can be influenced by a number of factors, such as preterm birth or maternal infection during pregnancy. Then, a second major microbial contact occurs during birth, followed by contact with bacteria from breast milk.

In recent years it is becoming apparent that in the normal lactating breast there is a healthy bacterial flora, which then is transmitted into the breast milk. Of the genera known to be present in human milk, the most abundant are *Streptococcus*, *Staphylococcus*, *Serratia*, and *Corynebacterium*. However, the milk microbiome is rather complex and dynamic, and at the same time unique to an individual mother and her infant. This microbial balance can change during a breast infection, such as mastitis, although it is not clear whether the symptoms are the result or the cause of this change in the microbial balance of the breast, and thus of the breast milk. What is certain is that these changes in the breast milk microbiome during a breast infection reflect changes in the lactating breast, and may therefore provide diagnostic opportunities.

What do the bacteria in milk do once ingested by the baby? Rodriguez and colleagues provided evidence that these breast milk bacteria colonize the infant's gut, and consequently, it appears that they have a functional significance in breast milk (7). The uniqueness of the breast milk microbiome of a dyad demonstrates that the mother transmits her microbes to her baby in a personalized way, facilitating the development of the baby's immune system and gut health in an environment-specific manner.

In addition to microbes, immunomodulatory components, such as activated immune cells and bioactive factors, are transmitted from the mother to her baby during breastfeeding, further enabling maximal protection of the newborn as well as optimal development of its immune system. What is more, these breast milk components are dynamic and rapidly respond to the offspring's changing needs as well as to maternal factors. As lactation research progresses, more secrets of breast milk physiology will be revealed, with potentially enormous health benefits and implications for both mothers and their infants.

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Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium.