This month, we bring you articles on how to measure milk quality, variations in the milk microbiome, the co-evolution of humans and dairy cows, and milk fat and brains.

Enjoy!

The new frontier of milk quality and nutrition

- Due to the complexity of milk, a combination of technologies are used to analyze its nutritional profile.
- Diet and genetics both impact milk quality.

   Lights, camera, action!

If you wandered onto a set filled with cameras, spectrometers, and detectors that enable scientists to see fragments otherwise invisible, you may believe it's a set for an episode of Crime Scene Investigation! In reality, this is the scene of the new frontier of milk analysis.

Because of the importance of milk in both the human diet and the global economy, more and more technology is being used to improve the quality and health attributes of milk. Additionally, technology is used to track what goes into milk in order to satisfy inquiring customers and regulatory agencies, which are becoming more stringent in their standards for food characterization at the molecular level.

Measuring milk quality

Due to the complexity of milk as a matrix, there is no single analytical technique available today capable of completing all aspects of milk analysis. Rather, a combination of old and new tools is being used.

Among them, mass spectrometry, a technique used to identify molecules by measuring their exact mass, has already been widely applied to milk composition analysis. Mass spectrometry was crucial in the past few years for the discovery of new bioactive peptides and glycoproteins in bovine milk; these are now available as commercial products with a variety of added benefits (from lowering your blood pressure, to lowering your cholesterol, to improving your ability to fight bad bacteria).

Additionally, unlike traditional milk quality controls that are unable to detect individual proteins and can be fooled by the addition of exogenous organic molecules, mass spectrometry is capable of unambiguously achieving identification of all milk proteins in a sample. Because of this, mass spectrometry can now be used to detect the presence of undesirable contaminants (e.g., melamine).

The combination of chromatographic techniques, such as the high performance liquid chromatography (HPLC) microchip with mass spectrometry, reduces the time of analysis and obtains even more information by acting as a “filter,” separating molecules before they reach the detector. This technology has gained a lot of attention. It can provide answers to really complicated questions and expand our knowledge about milk by revealing new glycosylated peptides and molecules with identical composition but different structures, and therefore different biological activities.
A recent review published in the Journal of Chromatography A detailed the astonishing increase in the number of papers, over 300 per year, that utilize HPLC-Mass spectrometry for food analysis. A 50-fold increase since 1991 marks it as the fastest growing application for food analysis [1].

**Effect of diet on milk quality**

Although we have the ability to measure levels of nutrients in milk, how do the nutrients get there in the first place? This happens via the diet, thus it is important to keep in mind that milk’s nutrient quality and health properties are strictly related what an animal eats. This piece of information comes, surprisingly, from a recent symposium held in Washington D.C. [2]. Why surprisingly? Because this was not a dairy conference, but a symposium on human milk.

Even though breastfeeding is regarded as the “gold” for nutritional standards (and has been for the past 120 million years!), there is still quite a great deal of research focused on determining the impact of maternal nutritional status on human milk quality and infant wellness. Even in resource-rich environments like the industrialized world, there still may be dietary factors that affect milk nutrient levels. If you pause to think about it, this fact is quite remarkable and should push us to investigate deeper the relationship between what we feed our cows and what is in the milk we drink every day.

**Effect of genes on milk quality**

With all of the precise tools capable of measuring a substance’s molecular make-up, like mass spectrometry, and also our unprecedented capability for sequencing genomes, can we not only answer questions about how diet affects nutrient levels in milk, but also relate the nutrients, and non-nutrients, to an animal’s genetic background?

A fresh approach to answering this question just appeared in a recent issue of Journal of the Science of Food and Agriculture [3]. Ramalho and colleagues describe the importance of preserving and valorizing autochthonous cow breeds to ensure genetic variability and biodiversity. Genetic variability and biodiversity are essential for food production in the future, but also as tools to achieve a new level of milk nutritional quality. For example, the Minhota breed in Portugal was traditionally used for milk production, but because of lower yields compared to the Holstein-Friesian breed, it was abandoned and is now experiencing a decline in the herd. In fact, a few years ago the European Union declared Minhota cows in danger of extinction! New policies focusing on milk quality may help some endangered autochthonous species like the Minhota to actually thrive instead of disappearing altogether from our planet.

Interestingly, the authors of this paper made a head-to-head comparison between the milk from the Minhota and Holstein-Friesian breeds on the matter of lipids profile and composition (not in relation to the yield since we already know that Holstein would win!). The “competition” was fair as all animals were raised in the same region and received analogous feeding.

Quite surprisingly, the Minhota had a healthier ratio of long-chain polyunsaturated fatty acids (the so-called PUFA), omega-3, and omega-6. The Holstein-Friesian milk contained a ratio of omega-3 to omega-6 that was disproportionately high in omega-6 (already typical of the Western countries’ diets). The milk from Minhota showed a more balanced ratio, approaching what is considered to be the ideal, healthy ratio. There is considerable evidence that greater intake of omega-3 polyunsaturated fatty acids is associated with a lower risk of stroke both in women and men. This healthier lipid profile, along with other features reported in the study, suggest Minhota’s milk may offer additional health advantages to its drinkers.

**Wrap**

We have now reached a new frontier of milk analysis. This unprecedented ability to measure milk’s nutritional quality in such detail, combined with the ability to gain genomic information about the animals, will lead to the production of niche dairy products designed to enhance human health.


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Breast milk contains bacteria; that much is known. Some studies (although not, alas, the Human Microbiome Project) have even characterized the bacterial community found in milk. But how does the composition of such a community vary among women? And how might it change over the course of lactation?

Raul Cabrera-Rubio, of the University of Valencia in Spain, and his colleagues have set about answering these two questions using the milk of 18 women, sampled within two days, at both one month and six months after they gave birth. The study also looked at how mom's weight and mode of delivery affected the bacteria. Therefore, half of the 18 women chosen for the study had cesarean deliveries and half had vaginal births.

The results were curious. Perhaps more expectedly, the researchers report that overweight moms have less diverse bacterial communities throughout lactation than moms of normal weight. In particular, larger mothers' colostrums tend to be rich in *Lactobacillus*, and, later on, their milk has lots of *Staphylococcus* and less *Bifidobacterium* compared to the milk of moms with healthy body masses. Meals rich in staph and lower in bifs probably aren't ideal for a baby.

But less expected, the important distinction between modes of delivery was not between vaginal births and cesareans in general. Instead, moms who gave birth naturally had milk bacterial communities similar to women who had non-elective cesareans. It was the elective cesareans that were the outliers. Moreover, these bacterial differences remained in the milk even six months after birth.

Why? Cabrera-Rubio and colleagues suggest physiological stress signals -- hormones, basically -- associated with labour may have a greater influence over microbial transmission from the mom's body to her breast milk than the physical process of vaginal birth.

That is an intriguing idea, but one they put forward without many hypothetical details. (Although they do suggest the hormones may alter the mom's gut permeability.) And, also, it's an idea that is postulated with only a small sample size behind it. (Six moms in their study had elective cesareans, and three had non-elective cesareans.)

To find out if their findings are representative, researchers first need a better understanding of how microbes get into breast milk. Several ideas exist. Mom's gut microbes might move around in her circulatory system, or in her lymphoid system. The hop from intestines to the mammary gland may be entirely passive, or could involve selection of certain taxa. Additionally, bacteria in an infant's mouth might colonise the milk ducts over time.

Not enough is known about these possibilities. But the infant-oral route, at least, receives some backing from Cabrera-Rubio's study. It finds that a mother's breast milk produced six months after birth contains more of the typical inhabitants of an infant's mouth than milk produced just one month after birth.

Now we know more of the story: bacterial communities in breast milk differ between women and by lactation stage. But how this happens and why it might be adaptive are still open questions.

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How dairying shaped the human genome

- Most adult mammals are unable to digest lactose.
- Genetic mutations enabling adult humans to digest lactose originated in at least three different populations worldwide.
- Those capable of digesting lactose greatly benefited from this ability, providing them with an evolutionary advantage over those without the genetic mutation.
- Now more than a third of the world’s adult human population is able to digest lactose.

Something amazing happened about 10,000 years ago - humans developed organized agriculture, profoundly influencing the emergence of civilizations in many regions of the world. The adaptations of humans long ago to this agricultural revolution, particularly a change in diet, have left signatures in the human genome that today can be read like a chapter in a history book. The most clear and most revealing example of changes in the human genome relates to dairying.

This DNA detective story extraordinaire not only tells us about the past, but helps us understand the present. The first tantalizing details were published in 2002, although this last year has seen an explosion of revelations as scientists exploit the power of DNA sequence information to unravel the history of humans and their genetic adaptations to the agricultural revolution.

Livestock, food, and milk

The domestication of livestock species by humans provided a reliable, energy-dense food source, meat. Livestock could be herded and moved to better pastures when required, and importantly, they could be grown on land unsuitable for other forms of agriculture. Then, over 7,500 years ago, independent mutations arose in the DNA of a few isolated humans allowing them, as adults, to fully exploit the huge calorific advantage of a second livestock food, milk. In addition to energy-rich carbohydrates and fatty acids, milk also contains rich sources of calcium and protein, both essential for the good health of humans. The genetic legacy of these few ancient humans is prevalent in many populations today.

Lactase persistence

Lactose is the most abundant sugar in milk. Young mammals, before weaning, have the ability to digest lactose in their small intestine. The enzyme responsible for digesting lactose is called lactase-phlorizin, or lactase for short. It converts lactose into glucose and galactose - two simple sugars readily absorbed from the small intestine and used as primary energy sources to power the rapid growth of young of animals.

Most adult mammals cannot digest lactose because, after weaning, they lose the ability to produce lactase in their small intestine. This is also true for about 65% of humans today; their adult level of lactase is about ten percent of the level in a child. These adult individuals are 'lactose intolerant' and drinking large quantities of milk results in diarrhoea, abdominal bloating, flatulence, cramps, and nausea. In the large intestine of such an individual, the osmotic effects of undigested lactose cause diarrhoea, while bacterial fermentation of lactose produces gases that contribute to the other ailments.

At the dawn of agriculture, some humans acquired, by chance, new and highly specific mutations in their genomes that enabled them to digest lactose as adults. Different mutations appeared in three different geographical regions of the world; all the mutations caused the continual production of lactase in the small intestine throughout the life of an individual. The ability of adults to digest lactose is called lactase persistence. These populations were already using domesticated animals, mainly for meat, clothes, and draught power, and now they had a major survival advantage: a new source of energy-dense food from livestock more valuable than the animals’ meat. This nutritional asset enabled these groups to prosper and produce more children. Succeeding generations inherited the beneficial mutation, which became more frequent in these populations and eventually emerged as a characteristic genetic signature of the population.

As a result of modern genetics technologies, the precise locations of these mutations in the human genome have been exquisitely defined. Each mutation represents a change in a single unit of information in the human genome, which has a total of about three billion units of genetic information. The most famous of these mutations (technically known as -
13,910*T) lies some distance from the lactase gene, but is located in a critical region normally responsible for the suppression of lactase production after weaning. The mutation destroys this regulatory role and the lactase gene remains fully active throughout life.

The genetic evidence for the link between mutation and ability to drink milk as an adult is also strongly supported by new pottery analyses that show ancient populations living 7,000 years ago, and known to carry the lactase persistence mutation, also stored milk in significant quantities, presumably for consumption by the adult community. This did not occur in populations that did not carry the mutation, even though they herded livestock.

Modern science has recreated one of the mutations in cells grown in culture and in genetically modified mice. This mutation allows for the maintenance of lactase gene activity in both cases, thus unequivocally proving the mutation causes lactase persistence.

**Historical tracking of the migration of human populations**

The lactase persistence mutations are genetic signatures that also define the geographical origins of migrating human populations in history. Indeed, one can track the movement of human populations by measuring the frequency of the mutations in today’s populations.

Lactase persistence is particularly prevalent in northwestern European populations. The frequency of the mutation in northwestern Europe decreases as you move south and east, reaching near zero frequency east of the Indian subcontinent. The current distribution of the mutation reflects past population migrations. Other mutations causing lactase persistence arose in a few pastoralist, milk-drinking populations in sub-Saharan Africa and the arid lands of Arabia, the Sahara, and eastern Sudan. These independent genetic events highlight the strong selective advantage of lactase persistence in populations where dairying was also commonly practised.

**Additional survival advantages associated with lactase persistence**

The mutations causing lactase persistence may have provided other survival advantages, although there are still scientific controversies in these areas. Northwestern European populations have a low supply of vitamin D due to limited sunlight. Lack of vitamin D can lead to poor calcium absorption and the bone disease, rickets. One of the best sources of calcium is milk, and moreover, the ability to digest lactose is also associated with enhanced calcium absorption. Hence, lactase persistence in this European population could also be important for promoting healthy bone growth, a key health trait. In some African populations, lactase persistence may also be associated with survival advantage in cholera endemic regions. These additional advantages of lactase persistence may have accelerated the increase in frequency of these mutations in human populations.

**Evolution in action**

Lactase persistence is an example of evolution in action. Random genetic variation within a few individuals conferred an increased survival advantage in communities that herded livestock and were able to drink milk as adults. The individuals carrying the mutation prospered from the benefits of drinking milk, reproduced more efficiently, and their descendents eventually dominated the population. Thus, the trait of lactase persistence became a characteristic of the population as it adapted to the benefits of drinking milk.

One wonders how many other clues regarding past cultural and agricultural practices are waiting to be discovered by the next group of forensic scientists interested in unravelling the mystery of migrating human populations and their genetic adaptations to changing diets.

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When fat is fabulous: Milk and infant neurodevelopment

- Fats in milk are important for myelination of neurons in infants.
- Infant genotype, gender, and gestation length affect uptake of milk fat.
- Sphingomyelin is enriched in breast milk.
- More sphingomyelin = more neurodevelopment (with caveats)

Fat is back, baby! After a pretty extensive smear campaign, fats are now recognized necessities for a healthy, balanced, adult diet. But for infants, fats have always been an essential constituent in mother’s milk and formula. Previous columns have addressed proteins and hormones in milk and their potential effects on infant development. However, the lion’s share of research effort in the topic of early nutrition has been dedicated to fatty acids and cognition. This is because fatty acids, made from phospholipids and triglycerides, are critical structural components of the brain.

Fatty acids and IQ

Last fall, Isaacs and colleagues in the UK reported on an experimental study in which 107 preterm infants were randomly assigned to either a standard formula diet or a fatty acid-enhanced formula diet. This study was initiated before long-chain polyunsaturated fatty acids were typically included in commercially available formulas. Interestingly, there were NO DIFFERENCES between the two formula groups when cognition was assessed 10 years later (e.g., the fatty acid enhanced formula was not associated with better cognitive performance). However, baby girls fed the fatty acid-enhanced formula DID have improved cognitive performance compared to baby girls fed “regular” formula, it was only for boys that there was no effect.

One important thing to remember, though, is that it’s not just what is in the milk, but how the infant assimilates and metabolizes those bioactive constituents. And depending on infant gender, whether the infant was term or preterm, and even infant genotype can influence how ingested milk is processed. For example, scientists have shown an infant’s utilization of fatty acids is as important as the presence of fatty acids in milk (Caspi et al., 2007). Using a sample of >1000 subjects in New Zealand, Caspi et al. showed that the effects of breastfeeding on IQ are mediated by genetic variation in fatty acid metabolism. Their candidate gene, FADS2, is instrumental in fatty acid metabolic pathways, and the two alleles C and G can seemingly influence those pathways. Infants were either CC or GG homozygous, or CG heterozygous. On their own, these genotypes were not associated with IQ. However, individuals who were breastfed AND carried the C allele (either CC or CG) had a ~6 points higher IQ than individuals with the C allele who were formula-fed (before the inclusion of DHA in formula). In contrast, GG individuals showed no differences in IQ whether or not they were breast- or formula-fed. Importantly, this study controlled for numerous factors, such as maternal IQ, fetal growth, and socioeconomic status. But this study was retrospective and just compared formula-feeding vs. breast-feeding history with genotype- the actual fatty acids in milk were not investigated.
More to the story: Phospholipids

A recent pilot study conducted by colleagues in Japan suggests that formula fortified with a particular phospholipid may confer some benefits for infant neurodevelopment and function. Sphingomyelin is a phospholipid, and in rats, contributes to myelination of the brain. In our brains, neurons send “messages” to one another through electrical signals. These signals travel down a long cable coming from the neuron, known as an axon. When axons are wrapped in a sheath of lipids, called myelin, the signal travels even more quickly between neurons. It’s sort of like highway tunnels that allow us to bypass cities without distractions. Importantly, in humans, much of our myelination occurs after we’re born, so fats in milk, and particularly sphingomyelin, are really important during this critical post-natal period of neurodevelopment.

In their pilot study, Tanaka et al. (2012) supplemented breast milk with formula. Of the phospholipids in the experimental formula, 20% were sphingomyelin while in the control formula, only 13% of the phospholipids were sphingomyelin. Twelve preterm infants were randomly assigned to the experimental group and twelve were assigned to the control group. Infants who received the sphingomyelin-fortified milk had slightly higher levels of sphingomyelin in their blood and performed slightly better on some cognitive tests than the control group. There were a number of constraints on interpreting the data: a small sample size, many outcome measures, many statistical tests, and no ability to control for breast-milk consumption. Despite these constraints, the general pattern suggests that greater consumption of sphingomyelin has the potential to improve neurodevelopment.

The really interesting thing is that sphingomyelin makes up MUCH more than 20% of phospholipids in human breast milk. In 1984, Bitman and colleagues investigated sphingomyelin in the milk of US women who delivered at term (37+ weeks, N=6), 1-2 weeks preterm (31-36 weeks, N=28), and very preterm (26-30 weeks, N=18). Specifically, he investigated the sphingomyelin concentration in colostrum, transitional, and mature milk. At all time points, among all groups, sphingomyelin was >30% of the phospholipid content and was usually closer to 40%. More recently, Wang and colleagues (2000) demonstrated that among Japanese women delivering full-term infants, sphingomyelin was 31% of the phospholipid content. Both research teams also reported some variation among mothers around the mean values, as would be expected.

A complex system

The key take-home message from the growing body of literature is that one main research imperative is to comprehensively investigate what constituents are in breast milk and their concentrations among mothers and populations as well as across lactation (Neville et al., 2012). Randomized trials that fortify formula one by one with different constituents thought to be bioactive in the newborn are going to constrain our understanding of breast milk (and therefore slow down improvements to formula). This is because few, if any, milk constituents exert their bioactivity in a vacuum from other constituents. Those complex interactions are going to be overlooked, compromised, or misattributed by a reductionist approach.

If I wanted to understand how a plane flies, I wouldn’t add rivets one by one to secure the wing to the plane, awaiting that magic rivet that makes flight possible. No one rivet is responsible for securing the plane wing, just as no single constituent in milk determines neurodevelopment and cognition. It’s an exquisitely, magnificently complex system.


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