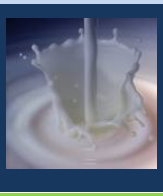
SPLASH! milk science update: March 2013 issue



SPLASH! milk science update

MARCH 2013 issue

In this issue of *SPLASH!*, we discuss the optimization of fermented dairy products for health , the many pathogen-binding forms of milk sIgA, the important role of mice in lactation research, and how early life environment can affect lactation performance . It's a great lineup!

Enjoy!

Fermentation of the Future

- **Dairy products are often fermented to improve their taste or preservation.**
- **Fermentation of dairy products has been associated with beneficial health effects.**
- **Can dairy fermentation be further optimized for superior consumer health?**

Using populations of bacteria or yeasts to change dairy product composition doesn't sound like a wholesome idea, but that is what lies behind the production of cheese, mango lassi and, despite its name, crème fraîche. Some fermented dairy products such as these have been shown to be healthy in ways beyond providing nutrition. Consequentially, food scientists are asking whether the processes that conjure up greater amounts of certain health-promoting ingredients in fermented dairy could be applied more widely and effectively.

But first, of course, they have to figure out what these health-promoting chemicals are and identify the micro-organisms that generate them.

The molecules responsible for these healthy effects perform many different functions. They appear to reduce the odds of cancer, inhibit the growth of nefarious bacterial species, lower blood pressure and prevent blood clots – as well as modulate the immune system, bind useful minerals such as calcium, act like (very much watered down) opium and also as antioxidants. The literature is stuffed with caveats about the requirement for much more data, particularly from human subjects, before firm conclusions about these benefits can be drawn.

But the anecdotal evidence is abundant and suggestive. To run through some examples:

- Molecules with the excellent group title 'sphingolipids', for example, are released from the membranes of milk fat globules during fermentation. In a study of mice with colonic tumors composed of human cancer cells, sphingolipids appeared to cause the cancer cells to die.

- Lactoferrin, itself a fighter of harmful microbes in fresh milk, is broken down by the enzyme pepsin during fermentation, and the result, lactoferricin B, can stop the thrush-causing fungus *Candida albicans* in its path. Another metabolite of lactoferrin, called lactoferricin f(17-30), fights off *Entamoeba histolytica*, the parasite behind amoebic dysentery and thus 70,000 deaths per year.

- A whole bunch of small peptides up to ten amino acids in length inhibit ACE (angiotensin I-converting enzyme), a catalyst that creates the blood vessel constrictor angiotensin II out of its ineffective sister, angiotensin I. By preventing vessel constriction, these little peptides keep high blood pressure in check. They have been found in Gouda, a yellow-colored cheese from the Netherlands, which seems to do good things for rats suffering from hypertension.

- Other peptides that interfere with milk-clotting mechanisms seem to have the same effect on blood. Indeed, κ-casein, a key ingredient for milk clotting, is thought to have evolved from part of fibrinogen, a molecule that plays an analogous role in blood. The upshot is that anti-clotting molecules in milk might reduce the odds of dangerous blood clots in milk drinkers.

The identities of the micro-organisms that generate these medicinal molecules are often known. Lactic acid bacteria are examples of important fermenters. They enrich milk with vitamins and also make small proteins called bacteriocins—antibiotics that work by perforating bacterial cell membranes. One bacteriocin, lacticin 3147, destroys the diarrhea-inducing germ *Clostridium difficile*. Other important fermenters include strains of Lactobacillus, *Propionibacterium*, *Bifidobacterium*, and *Enterococcus*. These fermenters serve double duty synthesizing conjugated linoleic acid, an anti-clotting and anti-cancer agent. (Curiously, aged cheeses contain less of this acid than cheeses with a short ripening period.)

Moreover, bacteria that could have a hand in improved fermentation are being revealed all the time. One such strain is *Lactobacillus helveticus BGRA43* which breaks apart key proteins as it ferments milk such that it imbues the milk with anti-microbial, anti-hypertensive, and immunomodulatory properties.

But in most cases, the enrichment of health-promoting substances was an unintended, and until recently, an unnoticed, side effect of making tasty foodstuffs that last. It isn't always clear whether the chemicals involved survive digestion in the human gut and go on to do good things around the body. This point needs examining before fermentation science can be used to design healthier dairy products in the future.

Certainly not everything makes it through. But at least two hypertension-attenuating ACE inhibitors do survive the journey through the stomach, past pancreatic proteases and yet more enzymes in the brush border of the intestinal wall. These have three amino acids apiece and have been detected in the aorta of hypertensive rats fed fermented milk. Making the entire journey to the bloodstream isn't always necessary, however, as seems to be the case for proteins called β-casomorphins which act as analgesics rather like morphine (albeit with less potent effects). These do not survive in blood, so their action probably arises from interactions with opioid receptors in the gut. (Ed: If you wish to eat 'happy cheese', β-casomorphin-3 was present in all Edam cheese samples tested in one study.)

But for health-giving molecules that are destroyed by digestion, all is not necessarily lost. In theory, the structures could be modified at the sites where gut enzymes would normally cleave them, enabling them to survive until they can be picked up by the bloodstream. If that strategy doesn't work, the molecules might one day be encased in and protected by microscopic capsules.

And this is where existing knowledge needs much more time to ferment. But the potential is certainly there for a new breed of dairy products designed to do their consumers even more good.

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Chimeric Milk Antibodies Bind More Pathogens

- **Human milk secretory IgA antibodies have a more complex structure than previously thought.**
- **These sIgA antibodies possess multiple sites at which pathogens can bind.**
- **Increased diversity in pathogen recognition reduces the chance that pathogens attach to the infant's gut.**

If antibodies were superheroes, then human milk secretory immunoglobulin A (sIgA) would be "The Avengers", taking on the pathogens no single superhero could stand. Milk sIgA is able to protect infants against a multitude of respiratory and gastrointestinal pathogens, including *E. coli*, salmonella, and pneumonia. But perhaps its greatest known power comes from its unique molecular structure—two IgA molecules held together by a joining chain (J) and a secretory component (s)—allowing sIgA to resist degradation by gastrointestinal enzymes (Figure 1). This makes it one of the few immune factors at the front lines able to bind bacteria and viruses before they attach to the infant's gastrointestinal tract.

New research suggests that there may be a second structural novelty in milk sIgA that gives it an additional edge over invading pathogens. A recent study by Sedykh et al. (2012) found that close to 20% of the sIgA present in human milk might be in chimeric form, or a combination of two different types of sIgA molecules. This finding goes against the current paradigm of immunoglobulin (antibody) structure. All immunoglobulins are composed of two heavy chains (larger polypeptide subunits) and two light chains (smaller polypeptide subunits). The amino acid structure of the heavy chain determines the class of immunoglobulin (i.e., IgA, IgD, IgE, IgG, IgM) and that of the light chain determines whether the antibody is categorized as lambda (λ) or kappa (κ). It has long been thought that the heavy and light chains were identical to one another—a λ-IgA molecule would have two IgA heavy chains and two λ light chains. But Sedykh et al. have found evidence to the contrary; some of the sIgA found in human milk are present as chimeras, composed of both λ and κ light chains (Figure 2).

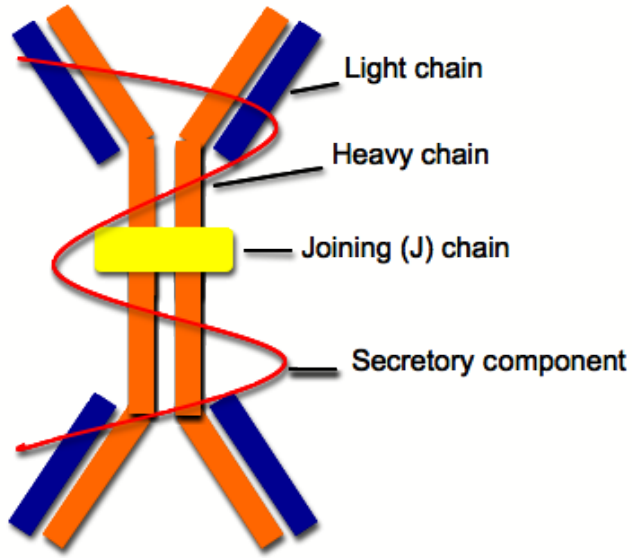

Figure 1 SIgA are dimeric, with two IgA molecules held together by a joining chain. Each IgA molecule is composed of two heavy chains and two light chains. The scretory component helps protect the antibody from being degraded in the infant's gut.

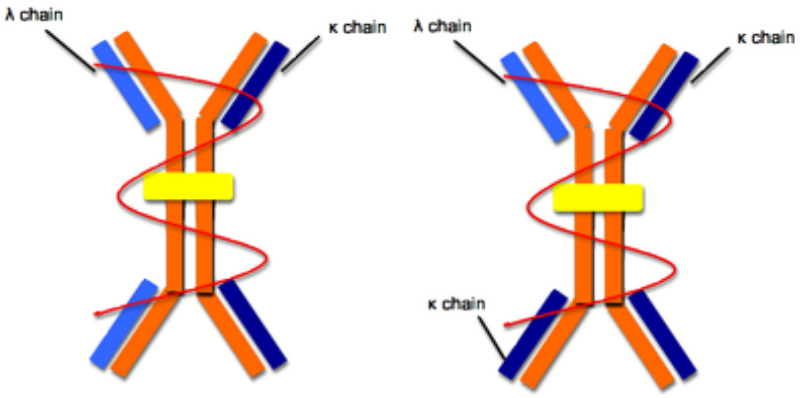

Figure 2 An example of a chimeric milk sIgA molecule. Instead of identical light chains, sIgA chimeras have both λ and κ light chains.

The authors are not precisely sure when or how this half-molecule exchange of light chains occurs but emphasize that this is not an anomaly—17 (± 4)% of all milk sIgA collected from five mothers during early lactation (weeks 1 - 3) were λ-κ-sIgAs. They also call attention to the fact that these chimeras are not dysfunctional antibodies but rather can be more efficacious in binding antigens. Instead of having two identical binding sites, sIgA with both λ and κ chains are able to bind a greater diversity of antigens. Human milk may be unique among mammals in its high concentration of sIgA, but in the battle against pathogens, the best strategy isn't always having more soldiers. An increased likelihood of being able to bind, and subsequently neutralize, antigens in the infant's mucosal surfaces could provide a huge advantage to human infants.

The study focused on early milk samples that have been demonstrated to have significantly higher concentrations of sIgA than those of mature milk. It would be interesting to see if the concentration of chimeras decreases as lactation progresses as well. This could suggest an emphasis on diversification of antigen recognition during the time at which the infant's immune system is most naïve.

Human infants are not unique in being born without a fully functional immune system, and as such, chimeric sIgA (or chimeric forms of other immunoglobulins) could be integral to infant immune defense in multiple mammalian species. However, it has been suggested that humans may be unique in having a greater concentration and greater diversity of immune factors, (e.g., oligosaccharides, Warren et al., 2001). Comparative studies on the light chain molecular structure of sIgA could provide additional data to evaluate this developing hypothesis.

There are still many important issues for immunologists to tackle, including identifying when sIgA molecules exchange λ and κ chains and the chemical signals that prompt them to do so. Despite this, researchers interested in the role of milk sIgA in infant immunity should not ignore the importance of the discovery of sIgA chimeras. Future studies should consider both antibody concentration as well as antibody molecular structure —it is intriguing to consider that both have been under selection to increase human infant immune response.

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From Mice and Cows and Kangaroos to Dairy Industry Value

- **The complexities of lactation performance may be unraveled by genomics.**
- **Genomics gives traditional genetics much greater resolution and accuracy.**
- **Functional genomics deals with how genes make physiological sense.**
- **Integration of the two adds much greater power to understanding lactation.**
- **There are potential benefits across the dairy value chain from using these approaches.**

With the development of genomic tools for dairy cows, what value do studies of lactation genomics in mice and other animals hold for dairy innovation? A recent study reported in the January 2013 issue of *Physiological Genomics* (1) is the latest in a series of studies of lactation in mice that have involved scientists affiliated with the IMGC (2-7). This latest study focused on lactation performance, a correlate of milk output.

The capacity of dairy cows to produce milk varies between cattle breeds and, to a lesser degree, within a breed. Geneticists have been studying and analyzing this variation for many decades and have developed selection procedures to capture the desired traits in more members of dairy herds. This has delivered considerable on-farm productivity increases for milk supply, but it relied on estimating the breeding value of a bull from a lengthy and costly system coupled with widespread herd recording for the important production characteristics or traits.

The genomics era has now introduced DNA-based methods that provide greater accuracy in genotyping and the capability for more rapid selection of desirable traits using molecular breeding values (8). These developments also provide a way to incorporate more complexity in measurements that reflect characteristics that affect the physiology of dairy cows. The key to capitalizing on the power of this technology is to have the appropriate measurements, which can take considerable time when measuring multiple generations of dairy cow lactation performance. This is where laboratory studies with mice can contribute.

So why choose mice? Although not identical, the physiology of the mammary gland is similar between species. The study published last month confirms that there are core physiological events that are reflected in the gene expression patterns of mice, and these are similar to cattle and other species during the lactation cycle. The advantage of studying mice is that they can produce multiple litters in a short space of time and hence have multiple lactations to study. Therefore, we also have the capacity to make lots of relevant measurements for analysis (7). This can help to develop selection procedures that have positive effects on dairy cow health and economic traits yet avoid unwanted deleterious effects, like reduced fertility.

The genetic makeup of an animal can account for variation in traits in two ways: by affecting the structure of a functional protein or by affecting the amount of a protein that is produced. Structural variants that have a major effect have been identified in dairy cows, e.g., in the protein DGAT1 which influences milk fat. The impact of changing levels of gene expression, and thereby the proteins that they produce, is more subtle and is usually the result of multiple changes. It is also influenced by other events, like nutrition and energy use. We can measure these using genomic tools that capture a snapshot of how much a gene is turned on at any one time or place in the body.

There may be thousands of changes occurring from one stage to another, and there is a high level of interconnectivity between the events. However, the events are not random; they are controlled in a coordinated manner. One way to think of it is that they group into patterns, and the change in patterns occurs in waves. An example of how these patterns emerge and change during lactation in mice is shown in the published paper. Interpretation of these patterns by comparing groups of studies like these is emerging, and the results may be assembled into biological networks. Ultimately, this will capture the most robust patterns that explain the complexity of the lactation system, and it can be reduced to key components. The advantage of having simple key measures of a complex system is that it will allow the development of real-time measures that can be incorporated into improving efficient dairy herd management systems.

Consumer health and well-being are key drivers of product innovation for dairy. Sophisticated and real-time measures of milk production could capture valued milk components or assist in split herd management for specified milk composition. Mice can be used as a model here, too. Mouse milk composition varies with the type or strain of mouse, and the way in which it is managed. The impact of this diversity on growth and development characteristics of baby mice can be correlated and followed for the lifetime of the animals.

So, what about the kangaroo? They represent both an extreme example of diversity in milk production and lactation biology, and they have the capacity to demonstrate how varied milk proteins can protect the newborn and affect developing tissues (9-11). Along with other species, they demonstrate the potential of milk, and they shed light on what may be possible with dairy. They also help to define those essential biological elements of any lactation system and so contribute to the development of a simplified system for applications in dairy cow management.

Taken together, the use of mouse laboratory studies and the exploration of diversity in lactation between species will provide a valuable resource for dairy innovation into the future.

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How Much Milk Does a Cow Produce? Depends on Early Life Conditions

- **Organisms have limited energy and resources that must be allocated toward growth, maintenance, and reproduction.**
- **How a mother allocates her energy during lactation can affect her offspring later in their lives.**
- **The mechanisms by which mammary gland function is impacted by early life trade-offs are not clear.**

Maternal nutritional conditions during pregnancy are known to have substantial impacts on infant development. This was most clearly demonstrated by research into the outcomes of infants from the Dutch Hunger Winter of 1944. Because determination and differentiation of cell lines occur during embryonic development, nutritional conditions and other environmental insults early during pregnancy can substantially alter offspring phenotype, including behavior and general health. For example, the Hunger Winter produced different results depending on whether the mother's nutrition was most interrupted during the first, second, or third trimester, or during lactation. Research into fetal programming and developmental origins of health and disease has identified that early life nutritional conditions affect numerous physiological systems and organ structures, putting individuals at later risk for kidney, liver, heart, pancreatic, neurobiological, endocrine, and reproductive dysfunction (Schug et al., 2012). Simply put, our early life experiences leave their mark.

Other factors within the mother and the environment beyond nutrition may affect offspring development because of trade-offs. Life-history theory, an area within evolutionary biology, is predicated on the fact that organisms have limited energy and resources that must be allocated toward several biological "imperatives"- maintenance, development, and reproduction (Stearns, 1992). Maintenance is basically keeping your body alive–such as thermoregulation and immune responses. Growing (i.e., adding mass) and development (i.e., skeletal ossification) also require energy and resources. And producing that most valuable currency of natural selection–BABIES–does not come cheap.

Because you can only spend, or burn, a calorie once, individuals face trade-offs among maintenance, growth, and reproduction. Under famine conditions children become stunted, reducing allocation of resources to gaining stature (Gørgensa et al., 2012). Female Olympic athletes often have reduced fertility due to altered ovarian function (DeSouza et al., 2010). Why cycle if body fat reserves are too low to sustain pregnancy or lactation? And once reproductive, females face trade-offs between current and future reproduction. Allocating too much energy to the current offspring can deplete mothers and delay future reproduction while females recover. Natural selection favors adaptations that allow females to maximize their lifetime reproductive success–the total number of offspring produced over their reproductive careers. Underlying mechanisms in female reproductive physiology are seemingly sensitive to nutritional intake and body condition, and through these mechanisms fertility, pregnancy, and lactation are regulated and resources allocated to the developing offspring.

In some mammalian species, females can produce more offspring over their lifetime if they can sustain overlapping pregnancy and lactation. This is actually a characteristic feature of some marsupials (Tyndale-Biscoe and Renfree, 1987). While simultaneous pregnancy and lactation can increase reproductive output, it also sets the stage for competition for maternal resources between milk synthesis for the infant and nutrient transfer via the placenta to the fetus.

In December, González-Recio and colleagues reported that overlapping pregnancy and lactation produced epigenetic consequences for the fetus that manifested in adulthood. They used a sample of >40,000 Holsteins, and admirably controlled for other genetic and environmental factors. Heifers that were gestated by a mother who was also lactating produced significantly less milk and died at younger ages! Of course, just like when signing a contract, it's important to read the fine print. These heifers produced ~52 kg less milk per lactation, but since the average production per annum for a Holstein is on average ~10,000 kg of milk, translated that is ~0.005% less milk. They also died only 16 days earlier than heifers that were gestated by a female who was not lactating. However, these were detectable effects that suggest early embryonic and fetal development *IS* sensitive to any reductions in resource allocation. Moreover, from a biological perspective, we would expect this effect to be greater in wild-living mammals that aren't fed or provided with any veterinary care.

Along similar lines, Soberon and colleagues showed that among calves reared on milk replacer, trade-offs between maintenance and growth reduced milk yield in adulthood (2012). Standard rations of milk replacer were provided to calves, but calves born during the winter had to burn more energy for thermoregulation. Less energy was available for growth and they did indeed grow significantly more slowly. Interestingly, for every additional kg of average daily gain in pre-weaning, body mass resulted in 850-1100 kg more milk on their first lactation (effect size varied between commercial and educational herds). This was directly linked to the amount of milk replacer the calves got above their maintenance needs. Thermoregulation wasn't the only maintenance cost some calves had to pay. Getting sick in early life also impacted future production–calves that received antibiotics went on to produce ~500 kg less milk on their first lactation.

The precise underlying mechanisms by which mammary gland function is impacted by early life trade-offs (either by the dam or the calf) are not yet clear. However, the effects are likely through epigenetic modification of gene expression (see Ross Tellam's column from May 2012). Important questions remain about the length of critical windows in which developing organisms are sensitive to environmental influences and the possibility for reversing or mediating early life programming. Until we better understand the proximate pathways, these phenomenological results still provide valuable insights. Dairy scientists may be able to further improve milk production by shaping animal husbandry practices to optimize early life development. And most importantly, these results illustrate the value of theoretical and evolutionary perspectives for understanding lactation biology (Hinde and German 2012).

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