



SPLASH!® milk science update **November 2019 Issue**



This month's issue features milk fat and chili peppers, ancient baby bottles, cheese allergies, and the dairy calf resistome.

Skim Milk Beats Hot Chili's Burn Just Like the Full-Fat Option

- **Chili peppers contain capsaicin that interacts with some fat-loving receptors, causing chili sauce to produce a burning sensation in the mouth.**
- **Milk contains tiny fat droplets that effectively block capsaicin's interaction with these receptors.**
- **Researchers have shown that fat-free milk reduces capsaicin burn, suggesting that milk's protein content may contribute to the interaction.**

Every so often on *The Jimmy Fallon Show*, celebrities attempt an interview whilst eating chicken wings that are doused in chili sauce. With every next chicken wing the sauce gets hotter, and the celebrity responses become less coherent. There is always a glass of milk on the table. It is there, presumably, because the show's producers know that milk has been shown to be especially useful at extinguishing the sensation of burning in the mouth. Indeed, SPLASH! [previously reported](#) on milk's great powers as a palate cleaner. In a recent issue of the journal *Physiology and Behaviour*, Alissa Nolden of Penn State University and her colleagues put milk to the test again [1]. They evaluate how well both fat-free and full-fat milk fare against other drinks. Their result that full-fat milk beats alternatives to milk was unsurprising. But the fact that fat-free milk also performed well suggests that milk's ability to reduce the burning sensations left by chili peppers may result, in part, from its protein components, rather than merely its fat content.



The substance that makes chilis hot is capsaicin. It is a non-polar molecule, and for this reason, liquids that contain fat have been thought to be the best candidates to bathe one's mouth in when suffering from a chili-scorched mouth. Thus, experiments have pitted olive oil against ice cream and milk, to test which of these fat-containing substances is considered by participants to be most effective at quelling capsaicin-induced discomfort. This previous research has shown that fat content on its own isn't everything. Milk outperforms olive oil, which has much higher fat content because, it is thought, its tiny fat droplets are dispersed in the form of an emulsion. This means that they are easily able to flow around the mouth and block the non-polar-molecule-attracting receptors (called transient receptor potential vanilloid-1) that surround the taste buds, to which capsaicin binds [2].

The recently published research by Nolden and her team instead tests milk with two different levels of fat content against several common beverages. The experimental set-up required the 72 participants to drink a small amount—just 30 ml—of spicy tomato juice (specifically, "Master of Mixes 5 Bloody Mary Mix"). Immediately after swallowing it, they had to rate the strength of the burning sensation that they experienced on a scale of from 0 to 100, with 100 indicating "the strongest imaginable sensation of any kind." Then they quickly consumed a test beverage that was provided in a 40-ml aliquot, and they continually re-evaluated the strength of the burn in their mouth every 10 seconds for the next two minutes.

Between each two-minute evaluation process, the participants took a short break to let any remaining oral flames die down. That done, they moved on to testing the next beverage among those up for evaluation. The full list was composed of water, full-sugar cola, cherry Kool-Aid reconstituted from dry mix, seltzer water, skim milk, whole milk, and a brand of non-alcoholic beer called O'Doul's. To ensure that neither the

temporary building up of tolerance to chili, nor a crescendo of unrelenting burn affected the results over the course of the experiment, the order of the test beverages was different for those taking part.

To analyze the results, the researchers adjusted each participant's score according to their individual sensitivity to chili, so that records of the degree of burn abatement could be compared on the same scale. Relative to suffering the effects of capsaicin with nothing to drink, all of the beverages significantly reduced the burning sensation. The beverages that were most effective 10 seconds after swallowing the hot Bloody Mary mix were Cherry Kool-Aid, alcohol-free beer, full-fat and fat-free milk, with little to distinguish them. However, over the course of the two minutes of monitoring, both milks started to overtake and clearly outstrip the other beverages in the chili-burn-quenching stakes. At the end of the test period, both full-fat and fat-free milk were approximately equally effective. Sugary beverages—Kool-Aid, beer, and cola—formed a group that performed clearly worse than milk but also clearly better than water.

These findings are intriguing because they suggest that mechanisms involving sugar and protein may reduce capsaicin's effects, in addition to the better-known mechanism that involves fats blocking the capsaicin receptor (TrpV1). The details of these additional mechanisms are unclear. Other researchers have proposed that sucrose has an analgesic effect via the opioid system [3]. That is, it reduces the feeling of pain because it encourages neurons in the brain to release neurotransmitters such as endorphin, and these activate opioid receptors there.

The evidence that proteins might also work against chili-burn comes from the milk results. Skim milk contains roughly the same level of protein as whole milk, while virtually lacking the fat component. The fact that the two milks performed equally well in the experiment suggests that a mechanism involving protein may even be more effective than one involving fat. Nobody is sure why protein might have this effect, but one suggestion is that casein—milk's most common protein—might solubilize or emulsify capsaicin, and in doing so stop much of it from reaching receptors because it is stuck in solution. The research to back up that idea, however, is yet to be done. Nonetheless, it does suggest there may be wisdom in chewing the chicken wing and drinking a glass of milk at the same time as one consumes chili sauce.

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Residue of Ruminant Milk Identified in Prehistoric Baby Bottles

- Archaeologists have found ceramic vessels believed to be infant feeding vessels in prehistoric sites as old as 8,000 years across Europe.
- For the first time, a team of European archaeologists and geochemists were able to perform organic residue analysis on three such vessels to understand what types of foods they may have contained.
- Three different chemical analyses indicate the vessels were used for consuming cow, sheep, and/or goat milk.
- Ruminant milk may have been an important supplemental or weaning food for infants and young children in agricultural populations.

It has been quite an amazing year for milk-related anthropology research. First came a study [1] in the fall of 2018 on [barium levels in the molar](#) of a 250,000 year old Neanderthal fossil that demonstrated the child

was weaned between two and three years of age, similar to the age of weaning in modern human populations. Using the same methods on even more ancient teeth, a study [2] published this summer found that australopithecines living 2 million years ago likely weaned one to two years later than modern humans. Then in September, an analysis [3] of plaque on several 6,000-year-old human teeth from Great Britain provided the oldest direct evidence of human consumption of cow, sheep, and goat milk. And to end the year comes a study [4] that combines the topics of weaning and dairy agriculture—organic residue analysis on 3,000-year-old ceramic artifacts suspected of being baby bottles found fatty acids unique to ruminant milk fats, demonstrating cow or sheep or goat milks were used as weaning foods for infants and young children after the advent of agriculture.

Archaeologists have long suspected that certain ceramic vessels they unearthed functioned in the same way as modern day baby bottles, owing to their unique shape—that includes a spout or straw-like projection through which a liquid could be poured or suckled—and the fact that they are commonly found in graves of young children [4]. Presumably, that liquid was animal milk, due to the widespread use of animal milks as weaning foods across modern day populations, and because the vessels' archaeological appearance and increase in prevalence map well onto our understanding of the origins and development of dairy agriculture. The earliest such vessels come from a 7,500-year-old Neolithic site in Germany and they become more prevalent in settlements and graves across central European sites associated with Bronze Age (ca. 5,000 years ago) and Iron Age (ca. 3,000 years ago) prehistoric populations [4].



Putting these suspicions and presumptions to the test, however, has proven difficult. Vessels with small openings limit sample collection for organic residue analysis, the method of choice for trying to reconstruct ancient diets from ceramic artifacts. But a team of European archaeologists and geochemists was recently granted access to three vessels with a more open, bowl form that were found in two Iron Age graves (ca. 2,800–2,500 years ago) and one Bronze age grave (ca. 3,200–2,800 years ago) from southern Germany [4]. Vessel 1 was found in a grave at the feet of a young child (0–6 years of age) and resembles a small bowl with a spout. Vessel 2 was found near the right hip of a one-year-old child and is shaped like a pipe. Vessel 3 was broken but was investigated because of its provenience in a cremation burial of a 1–2-year-old

child [4].

The high lipid content pulled from the surfaces suggested the vessels were used in sustained processing or consumption of food or liquids high in fat. But the presence of fatty acids with 12 and 14 carbons, which are rare in prehistoric ceramics, pointed specifically to ruminant milk fat (cow, sheep, or goat milk, but not human) [4]. This finding was corroborated by the results from two other chemical analyses: stable carbon isotope ($\delta^{13}\text{C}$) analysis of fatty acids and high-temperature gas chromatography-mass spectrometry to identify specific triacylglycerols (TAGS). Specifically, the $\delta^{13}\text{C}$ values of lipid residues from all three vessels plotted in the ruminant milk fat region while the TAGS identified from vessel 2 were diagnostic of ruminant fats (TAGS from vessels 1 and 3 were not detectable, suggesting they had been degraded while buried) [4].

Three independent lipid analyses identified ruminant milk fat residue on bottle-like ceramic vessels pulled from child graves. The study team believes this constitutes unequivocal evidence that these vessels were primarily used for feeding ruminant milk to infants and young children, most likely during the mixed feeding period (mother's milk and other foods) and during weaning [4].

There is one additional piece of evidence that suggests it was infants and children, as opposed to sick or elderly members of the populations, consuming ruminant milk from these vessels. The oldest baby bottle-like vessel yet discovered is nearly 8,000 years old, which predates the spread of the lactase persistence (LP) gene (allowing for lactose tolerance) in Europe by at least 3,000 years [3]. However, infants and

young children do not need a LP gene in order to digest ruminant milk as lactase is produced by all humans through infancy and early childhood (after all, human milk has nearly twice the lactose as cow milk). Neolithic (and even some Bronze Age) adults may not have been able to consume substantial amounts of ruminant milk without digestive issues, but it appears that they recognized the same was not true for infants and young children.

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Harnessing Cheese Microbes to Reduce an Allergy-like Reaction to Cheese

- **Histamine can be present at high concentrations in fermented foods and can lead to an allergy-like reaction known as histamine intoxication.**
- **A new study analyzes bacteria present on long-ripened cheese and identifies pathways involved in histamine degradation.**
- **The results suggest that these bacteria and their pathways could be harnessed to reduce histamine levels in cheese and other fermented foods.**

Cheese has been a part of human diet for thousands of years. Its production relies on the complex interplay between many different microbes, which contribute to the flavor, texture, and aroma of cheese during the ripening process.



This is particularly true of long-ripened cheeses, which can spend months on the shelf being acted upon by bacteria and fungi. "In long-ripened cheeses, you produce your cheese wheels and store them in your ripening cellar for the desired amount of time, and then you have the formation of a biofilm on the cheese rind, which is very important for the aroma and flavor production," says Dr. Stephan Schmitz-Esser of Iowa State University.

However, long-ripened cheeses are also a common source of biogenic amines such as histamine (1-3). Histamine can influence the mammalian immune, cardiovascular and gastrointestinal systems, and can be produced by bacteria during food fermentation (1,4,5). "Histamine formation in fermented foods is a huge issue because there are these intolerances associated with histamine, because it's such a potent chemical that can do so many things," says Schmitz-Esser.

Eating high levels of histamine can lead to an allergy-like reaction known as histamine intoxication, resulting in asthma, rashes, swelling, hives and diarrhea (6,7). One way to prevent histamine intoxication is to reduce its formation or degrade it in foods, and researchers have been interested in finding bacteria that can break down histamine. "There's a big interest in the potential to reduce histamine content in food," says Schmitz-Esser.

In a new study, Schmitz-Esser and his colleagues analyzed the bacteria present in Vorarlberger Bergkäse

(VB), a long-ripened artisanal hard cheese, and identified histamine-degrading bacteria as well as genes and pathways involved in histamine degradation (8). "Ultimately, we could utilize the bacteria to reduce histamine content during the ripening process so that producers would be able to produce cheeses that are ripened normally but with reduced histamine content," says Schmitz-Esser. "The big breakthrough here is that we can now identify which genes specifically are responsible and whether these genes are present in other bacteria as well," he says.

In previous work, Schmitz-Esser characterized the microbial community present on VB (9). During VB's ripening time, which can last anywhere from 3 to 18 months, bacteria and fungi form a rind on the cheese. One of the major types of bacteria identified in these rinds is called *Brevibacterium*, and it is also used for ripening many other cheeses (10,11). Schmitz-Esser was able to isolate and sequence various *Brevibacterium* strains from this cheese.

In the new study, Schmitz-Esser characterized the abundance of *Brevibacterium* on VB cheese rinds, and analyzed the contribution of these bacteria to the cheese ripening process. He found that *Brevibacterium* were abundant on VB rinds throughout the 160-day ripening period. The study also found that important cheese-ripening enzymes were conserved among cheese-associated *Brevibacterium*. "There seem to be a couple of really conserved elements and others that are less conserved," says Schmitz-Esser.

When Schmitz-Esser analyzed three cheese-associated strains of *Brevibacterium* in more detail, he found that their genomes contained a potential metabolic pathway responsible for histamine degradation. "We kind of stumbled upon the histamine degradation capability, because we got genome sequences and then we found histamine-related genes and then we did tests that showed that these bacteria can indeed degrade histamine," he says. "The histamine degradation pathway is not present in all of the strains that we have analyzed, so this seems to be strain-specific."

The fact that this histamine degradation pathway is present in certain *Brevibacterium* strains suggests that these strains could be used to create foods with reduced histamine. "*Brevibacterium* are very common cheese rind organisms, so we know they are abundant and would be very well suited to be used during cheese ripening," says Schmitz-Esser. "Ultimately, we could imagine using these specific strains for ripening to produce texture, flavor, and aroma, but at the same time they would also reduce the histamine content in the cheeses," he says. "That might be useful for the producers to sell cheeses that are histamine-reduced and target people that may be avoiding cheese or other fermented foods because of their histamine intolerance," says Schmitz-Esser.

One of the *Brevibacterium* strains that Schmitz-Esser analyzed also included a small circular piece of DNA, known as a plasmid, which he suggests might contain genes that help the bacteria adapt to life on cheese rinds. "The potentially interesting aspect of that for the future is that if you have plasmids, this is one of the prerequisites to develop tools to potentially genetically modify those strains," says Schmitz-Esser. "This may be relevant in the future from a biotechnological perspective," he says.

Schmitz-Esser is continuing to analyze the genes and pathways he discovered and their role in histamine degradation. "One thing that we are interested in is trying to find more bacteria from different types of cheeses that have these capabilities and to analyze their genomes to see whether we find the same set of genes," he says.

The overarching goal is to analyze these pathways in more detail to better understand the cheese ripening process and the role of cheese-ripening microbes. "There's definitely a need to understand these systems better," says Schmitz-Esser. "For many bacteria and fungi on the cheese rinds, we don't know very much about how specifically they contribute to cheese ripening," he says. "We know they are there and they may be abundant, which implies they may be important, but there's still a lot left to learn," says Schmitz-Esser.

Understanding these microbes and their role could have important economic and food safety implications. "If we understand these systems better, such as which microbes are there and how they contribute to

different parts of cheese ripening, we might be able to potentially reduce ripening times or maybe identify protective cultures that could suppress foodborne pathogens or things like that,” says Schmitz-Esser. “These are the kinds of directions we want to go in the future.”

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Fighting the Resistome

- **Antibiotic-resistant microbes threaten the health of humans and livestock.**
- **Animals and humans host a natural reservoir of microbial antibiotic resistance genes (the resistome).**
- **The resistome of dairy calves is large, diverse, and highly dynamic.**
- **Early life diet in calves could repress the resistome and thereby decrease the risk of spreading of microbes with resistance to antibiotics.**

We are incredibly lucky. We live at a time when antibiotics work their magic saving people from infections. Only a few generations ago, infections reigned supreme and struck down some people in most families. It had always been that way, but memory quickly fades. Modern society assumes that the effectiveness of antibiotics is here to stay—it's a monument to human ingenuity. However, the continuing emergence of antibiotic-resistant microbes and the lack of new antibiotics in the developmental cupboard are looming threats to human health [1, 2], and a stark reminder that today's respite from infection could easily be temporary.



Scientists conclude that the key to maintaining an arsenal of effective antibiotics is to understand how microbial resistance to antibiotics develops and spreads so that effective containment measures can be put in place [1, 3, 4]. Antibiotic-resistant microbes naturally arise. They circulate in complex microbial ecosystems and are prevalent in areas where antibiotics are used, especially within humans, food animals, and their immediate environments [1, 3–5]. Therefore, the management of microbial antibiotic resistance requires focus on containment measures in all areas where the resistance arises.

Cattle harbor antibiotic-resistant microbes [3–8]. However, scientists have shown that these microbes are not found in food products derived from cattle due to the stringent and multilayered hygiene practices

used in abattoirs and the dairy industry [3]. They also agree that there is great difficulty identifying and tracking all antibiotic-resistant microbes in cattle and their local environment, and assessing the risk that these microbes could transfer antibiotic resistance into microbes that cause infections in humans. An exciting new scientific approach, exemplified in dairy calves, generated detailed and very useful information that may help contain the spread of antibiotic-resistant microbes in livestock [4].

Tracking Antibiotic-Resistant Microbes

A recent publication in the prestigious scientific journal *Nature Communications* reports results from an investigation that tracked all microbial antibiotic resistance genes (the resistome) in fecal material from newborn dairy calves until they were ten weeks old [4]. The resistome reveals the potential for the development of antibiotic resistance in a community of microbes [3]. The microbes in fecal material likely mirror the composition of the complex microbial ecosystem in the calf's digestive tract. The investigators' results were comprehensive, surprising, and suggested simple practical ways to minimize the spread of antibiotic-resistant microbes in livestock. The eight investigators were based at the University of California Davis and the USDA. Jinxin Liu was the first author on the publication, and David Mills was leader of the multidisciplinary research team.

Microbes Killing Microbes

The microbial arms race has been going on since the dawn of life. Some microbes naturally produce antibiotics that do not affect themselves but kill other types of microbes. The microbes producing antibiotics increase their survival chances and reproductive success at the expense of other microbes. In defense, some microbes develop countermeasures to specific antibiotics. The latter microbes acquire new genes, which produce proteins that neutralize the effects of specific antibiotics and allow these microbes to prosper even in the presence of an antibiotic. This ancient ability of microbes to become resistant to antibiotics is a major problem today. The antibiotics used in humans and animals to treat infections are related to naturally occurring antibiotics and therefore are susceptible to antibiotic resistance mechanisms in microbes. During the last 80 years, many microbes that cause infection in humans rapidly became resistant to antibiotics; some are resistant to multiple antibiotics [1, 2, 9]. The warning siren is blaring.

Microbes Swap DNA

Microbes can quickly spread antibiotic resistance genes into larger populations of microbes. They pass on these genes to their descendants during normal reproduction (vertical transfer). However, scientists have repeatedly discovered that some microbes also use a much faster and promiscuous method of dispersal of antibiotic-resistant genes. These microbes contain an additional piece of DNA containing one or more antibiotic-resistant genes, which is easily transferred into very different microbes (horizontal transfer) [6, 10]. Swapping pieces of DNA is part of life for most microbes. The heightened risk with horizontal transfer is that the non-infectious antibiotic-resistant microbes can act as reservoirs for antibiotic resistance genes and opportunistically transfer these genes into microbes that cause disease. Worse, some microbes acquire resistance to multiple antibiotics through this mechanism [6].

Surprises in the Fecal Resistome

Liu and colleagues collected fecal samples from newborn dairy calves until weaning at 10 weeks of age [4]. They initially identified the range of microbial families and their abundances in each sample. The diet of the calves changed from colostrum just after birth, to milk with progressively increased amounts of a plant-based "calf starter" until weaning. The colostrum was bottle-fed to the calves only on their first day. The dietary transition was associated with large and surprisingly rapid changes in fecal microbial populations. Presumably, this was a reflection of the changes in the microbial ecosystem in the calf intestinal tract. There was also a much greater diversity of microbial species with increasing calf age as the microbial species transitioned from ones specialized in the digestion of colostrum and milk to those that digested cellulose in the plant feed. Liu and colleagues presented strong evidence that specific microbes present in the colostrum helped to "seed" the microbial population detected in fecal material at an early age. The implication is that a calf may get much more than just food from its dam.

The investigators then focused attention on the microbial resistome in the fecal samples [4]. They obtained huge quantities of DNA sequence information from the massive number and diversity of microbes present in the samples. By comparison of the DNA sequences with a database of DNA sequences coding for known microbial antibiotic resistance genes, Liu and colleagues identified a resistome consisting of over 300 genes that potentially conferred resistance to 17 classes of antibiotics. The resistome was large and complex. Over 50% of the antimicrobial resistance genes identified when the calves were two days of age likely came from microbes containing multiple resistance genes. Hence, some microbes could potentially have resistance to multiple classes of antibiotics. However, the investigators noted that the abundance of the latter microbes decreased as the calves aged, as did the entire resistome. The investigators predicted that the resistome came from 75 bacterial families, although just a few microbial families carrying the antibiotic resistance genes were overwhelmingly dominant in terms of microbial population numbers [4]. The resistome, like the microbial populations, changed dramatically with age, although the changes were more extensive than simply a reflection of the changes in the microbial populations.

The investigators determined the relationship between the age-related changes in the resistome and the corresponding changes in microbial genes that produced specific proteins involved in feed digestion [4]. They concluded that the decrease in the resistome with age was associated with the increasing numbers of microbes that digest plant polysaccharides, which, coincidentally, contained few antibiotic resistance genes. Thus, diet can modify the resistome. Liu and colleagues also identified about 70 antibiotic resistance genes on small pieces of DNA with potential capability for horizontal transfer between different types of microbes. These genes potentially conferred resistance to 10 classes of antibiotics and came from a wide diversity of fecal microbial species. Perhaps these genes will be high priorities in the future for the monitoring of antibiotic-resistant microbes.

Implications

Liu and collaborators demonstrated that pre-weaned dairy calves “*serve as a reservoir*” for antibiotic resistance genes [4]. Their research highlighted a new way of detecting and tracking all antibiotic resistance genes in highly dynamic and complex microbial ecosystems. The investigators suggested that the dairy industry should better control the collection and handling of colostrum to reduce the introduction of antibiotic-resistant microbes to newborn calves. Liu and colleagues concluded that exposing calves to prebiotic foods or probiotic bacteria very early in life may displace microbes carrying antibiotic resistance genes and “*reduce the likelihood of further environmental spread.*” Vigilance and simple changes to industry practices could help contain the livestock microbial resistome and ensure the continuing availability of effective antibiotics for use in humans and animals.

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