Human Milk and Saliva Synergize to Shape the Infant Oral Microbiome

- An enzyme in human milk interacts with particular compounds in infant saliva to produce hydrogen peroxide and other antibacterial compounds.
- A new *in vitro* study that mimicked the environment of the human infant mouth during breastfeeding found that human milk and saliva mixtures inhibited bacterial growth for up to 24 hours.
- Interactions between human milk and saliva may influence the establishment of the infant oral and gut bacterial communities.

Parents of infants spend a good deal of time wiping up their baby’s drool but probably don’t give a second thought to the important ingredients that drool may contain. Lucky for them, a team of researchers from Australia happily collected and analyzed baby saliva in an effort to identify compounds that may influence the growth of bacterial communities in the infant’s mouth and, subsequently, the rest of their gastrointestinal tract [1, 2].

In 2015, the researchers reported that infant saliva contained nearly ten times the amount of the compounds xanthine and hypoxanthine as adult saliva. Even more interesting, the transition from infant to adult concentrations started at weaning [1]. All signs pointed to a special function for these compounds while the infant was nursing, leading the team to an amazing, and serendipitous, discovery: the interaction of xanthine and hypoxanthine from infant saliva with the enzyme xanthine oxidase (XO) from human milk produces hydrogen peroxide (H2O2) and other antimicrobial compounds [1]. Al-Shehri and colleagues [1] describe it as “a unique biochemical synergism.”

Hydrogen peroxide is a medicine cabinet staple, used on minor scrapes and cuts to prevent infection. It has similar antimicrobial actions inside the infant mouth as well, both on its own and in combination with another milk ingredient, the enzyme lactoperoxidase (LPO) [1, 2]. If enough H2O2 is present, it can activate the LPO system in milk, which in turn produces potent antimicrobial products including reactive oxygen species (e.g., superoxide) and reactive nitrogen species (e.g., peroxynitrite) [1, 2].

Human milk does contain a small amount of hydrogen peroxide on its own (around 27 μM) [1]. But when the researchers mixed human milk with infant saliva, the concentration of H2O2 increased to over 100 μM [1, 2]. Were these higher concentrations enough to activate the LPO system?

In their initial 2015 study, Al-Shehri et al. [1] mixed breast milk with “simulated infant saliva” containing different dilutions of xanthine and hypoxanthine to generate between 18–150 μM H2O2. These mixtures were then added to four different bacterial cultures, two commensal bacteria (aka “the good guys”: *Escherichia coli*, *Lactobacillus plantarum*) and two opportunistic bacteria (the “bad” guys: *Staphylococcus aureus*, *Salmonella* species). The growth of the commensal gut bacteria *E. coli* was unaffected by the saliva-milk mixture, whereas the growth of *L. plantarum*, *Salmonella* species, and *S. aureus* were inhibited in a dose-dependent manner (i.e., more H2O2, more growth inhibition).
These in vitro findings suggested that at higher concentrations, H2O2 does trigger the LPO system. Moreover, when H2O2 acts in combination with the products of the LPO system, it may selectively inhibit the growth of harmful bacteria [1]. The milk-saliva synergy likely plays an important role in establishing the infant oral microbiome, which in turn establishes the bacterial communities that set up residence in the infant’s gut [1].

This conclusion in itself would be worthy of its own SPLASH! feature—but wait, there’s more! The same research team recently published the results of their follow up study [2]. Although still an in vitro model, the 2018 study more accurately replicated the conditions of the infant mouth during breastfeeding; they tested more bacterial species (ten instead of four), they increased the concentration of the bacteria to match that present in an infant’s mouth, and they tested the milk-saliva mixture on combinations of bacteria as opposed to just one species at a time [2].

This time they report the novel finding that bacterial growth (except for methicillin-resistant S. aureus or MRSA) was inhibited immediately upon and for up to 24 hours after exposure to the human milk/saliva mixture, regardless of whether the bacteria were incubated on their own or with other bacterial species [2]. Longer growth inhibition was associated with higher concentrations of H2O2, but importantly these concentrations were still in the micromolar range (for perspective, H2O2 from the medicine cabinet is usually at a concentration of 1 M, nearly one million times more concentrated) [2].

Taken together, the two studies [1, 2] demonstrate that the H2O2 generated from human milk–infant saliva interactions, in combination with the other oxidative radicals produced from activation of milk LPO, regulate the growth of bacterial communities, or microbiota, of the infant mouth. With each nursing event, there is the potential for immediate and prolonged antimicrobial effects, which may be critical in keeping pathogenic organisms from colonizing the infant gut. [2].

But what if there isn’t a “nursing event”? Formula-fed infants do not receive the XO enzyme and therefore do not make enough H2O2 to activate the LPO system. As predicted, there are significant differences in oral microbial communities between formula- and human milk-fed infants [1, 2]. But not all infants receiving human milk receive the XO enzyme, either. Heating and freezing damage the enzyme, meaning that infants drinking pasteurized human milk or milk that has been previously frozen—which is often the case with pumping—will have missed out on the antimicrobial effects of H2O2 and LPO as well. It is not yet known how these infants’ oral microbiomes may differ, if at all, from those receiving fresh milk via breast or bottle. Such a comparison has the potential to tease out how important H2O2 is, among the many ingredients in infant drool, in regulating the types and quantities of microorganisms in the infant mouth and gut.


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Cow Milk Phospholipids Can Improve Cognitive Performance under Stressful Conditions

- Stress influences cognitive performance, and phospholipids have been shown to reduce certain stress responses.
- A new study investigated the stress-buffering effects of milk-based phospholipids on cognitive performance and response to stress.
- The study found that dietary intake of phospholipids from cow milk improved reaction time on certain tasks among perfectionist men.

Stress affects us in many ways, including well-studied impacts on cognition [1,2]. “We know stress, in certain situations, can negatively impact some domains of cognitive performance,” says Dr. Neil Boyle
A new study by Boyle and his colleagues examined whether intake of certain kinds of fats, known as phospholipids, might protect against the detrimental effects of stress on cognitive performance [3]. Phospholipids play important structural and functional roles in our brain and nervous system, and phospholipids from cow and soy milk have been shown to have stress-buffering effects [4-8].

“The interest stemmed from early evidence of the capacity of phospholipids to mediate the cortisol response to stress and exercise,” says Boyle. In humans, cortisol is a primary moderator of the acute effects of stress on cognitive function [9,10]. Stress often affects cognitive performance only when it significantly elevates cortisol levels [11,12].

“Therefore, we were interested to see if phospholipids could reduce the cortisol response to induced stress and subsequently offer some protective effects on cognitive performance undertaken under the context of the stressor,” says Boyle. “A psychosocial stressor was used as this type of stressor is the most activating of the cortisol stress response, and it is also the type of stressor commonly experienced in everyday life,” he says.

The researchers decided to examine the effects of phospholipid supplementation on the cognitive performance of individuals that were particularly vulnerable to stress, and focused on a population of “perfectionist” men. Perfectionism—which includes excessive standards, self-criticism, and a need for order—has been associated with an increased fear of failure and an increased responsivity to cortisol [13,14].

“We selected perfectionists as the negative effects of stress and cortisol on cognitive performance are often seen in individuals that demonstrate the highest cortisol response to stress provocation,” says Boyle. “Perfectionist tendencies have previously been associated with this tendency to high cortisol responsiveness,” he says.

Designing appropriate experiments to accurately assess the effects of phospholipids was a challenge. “Changes in cognitive performance as a result of dietary interventions tend to be quite small and can therefore be lost or washed out by differences between individuals,” says Boyle. “Therefore, it is often beneficial to examine changes in performance after an intervention within the same person,” he says.

But traditional laboratory experiments of stress usually result in a habituation of the participants’ cortisol responses with repeat exposure to stress. “We spent a lot of time modifying existing stressors to reduce habituation in response so we could examine cognitive performance after a stressor before and after phospholipid intake in the same individuals,” says Boyle.

The researchers examined the effects of six weeks of daily intake of either a drink containing cow milk-derived phospholipids or a placebo drink that did not contain phospholipids on 54 perfectionist men. They measured the participants’ stress responses to an acute psychosocial stressor, and also their subsequent cognitive performance.

The researchers found that phospholipid intake improved post-stress reaction time performance on an attention-switching task, in which participants had to rapidly switch between multiple tasks. Supplementation with phospholipid did not significantly reduce salivary cortisol responses to stress. “We did not demonstrate an effect on cortisol response, but an effect on subjective arousal rating and a small
impact upon reaction time,” says Boyle.

Working memory performance was unaffected by phospholipid supplementation, suggesting that the benefits of phospholipid intake may be specific to certain cognitive domains. Phospholipid intake also increased subjective levels of energy and arousal during peak stress exposure, which may have increased participants’ stress-coping potential. The researchers suggest that subjective stress-buffering effects of phospholipid intake may explain the improved cognitive performance in the absence of attenuated cortisol response.

“The findings were not sufficient in isolation to make dietary recommendations, which would require replication and more numerous and larger studies,” says Boyle. “However, the small reaction time effects found may be suggestive of some benefit in contexts in which small performance improvements are beneficial,” he says.

Future studies will be needed to better understand the mechanisms by which phospholipids affect cortisol responses to stress. Larger follow-up studies could also look at the effects of phospholipids in specific populations.

“I suspect any positive effects of phospholipids are more likely to be evidenced in samples that are more commonly vulnerable to cognitive deficits or the negative effects of stress,” says Boyle. “For example, an intervention aimed at protecting or improving cognitive performance may be more likely to have an effect in an elderly sample compared to a young sample,” he says.

Phospholipids may also be particularly helpful in the context of sports. “I consider sports performance, particularly sports endurance, as relevant future areas of research,” says Boyle. “Early evidence suggests phospholipids may offer performance and recovery improvements in this context,” he says.


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Genetic Editing Eliminates Dairy Cattle Horns

- Horns on dairy cattle can injure their handlers and other cattle.
- Physical dehorning of cattle is widely practiced, but producers, animal rights activists, and the public want a more acceptable and long-term alternative.
- Genetic editing technology can permanently eliminate horns from dairy cattle while potentially maintaining their hard-won elite dairy production genetics.

Next time you are running with the bulls in Pamplona you may have a moment of vivid, but very brief, clarity and think “If only the bulls were Polled.” In a significant breakthrough, scientists used genetic editing technology to produce hornless dairy cattle (Polled cattle) thereby potentially eliminating a controversial animal welfare issue, the physical dehorning of dairy cattle, while likely retaining their elite dairy production genetics [1-3].

History of Horns

Cattle have horns for good reasons. In the past, they came in very handy for defense against predators and, at the end of the day, predators were often left hurt, hungry, and humiliated. Horns on males also made a big statement in the social group of the herd. They signified health and vitality to females, and a threat to their competitive peers also striving to find females for mating. But times change.

Over the last 10,000 years, humans selectively bred cattle to produce more meat or milk. The raw ingredient of this process is genetic (DNA) variation in the cattle population, i.e., the genetic differences that make individuals different from each other. Consequently, today’s cattle are unlike their undomesticated ancestors, both in form and function as the former were bred to be highly specialized for the dairy or beef industries. Even over the last 60 years, scientists calculated that global livestock productivity has remarkably increased by 20–30% largely due to selective breeding [4]. In the dairy industry, this means more milk from each cow. But horns are still present in many cattle breeds, particularly dairy breeds, because in the past they were a low priority in most breeding programs [1]. However, in the modern dairy production system, horns threaten injuries to handlers and other cattle; the everyday competition between cattle for feed, water, and shade is intense. Horns are clearly an animal welfare issue in this production system. Consequently, about 80–90% of dairy cows have their horns or horn buds removed each year in the U.S.A, usually when the animals are very young [1, 5, 6]. Similar rates also occur in many other countries.

Physical Dehorning of Cattle

Veterinarians and livestock producers report that physical dehorning of cattle is painful (although analgesics are now widely used), expensive, associated with the ever-present risk of infection, and typically dehorning causes a small production set back [7, 8]. There is public disquiet about the dehorning procedure, and it has raised the ire of animal rights activists. Consequently, physical dehorning is an animal welfare issue even though the primary purpose of dehorning is to improve animal (and human) welfare. It’s a Catch-22. Dairy producers and animal rights activists agree that there is an urgent need for an alternative solution to this tricky animal welfare issue. Now there is one—genetically edited dairy cattle that have no horns! It’s an amazing tale that reveals how innovative science can solve a fundamental practical problem in the livestock industry and also challenge government regulatory authorities.

Alison Van Eenennaam is part of the vanguard of scientists using gene editing technology in livestock, and she is an advocate for government regulatory acceptance of the use of the technology in livestock animals. She argues that the FDA should regulate animal products made by the technology rather than the technology itself [2]. Van Eenennaam is based at the University of California at Davis where she is a
Cooperative Extension biotechnology specialist. She is hard to track down. Her broad vision of translating scientific advances into practical solutions for use in agriculture is in big demand. I fleetingly caught up with Van Eenennaam by email and phone as she rapidly transited airports on different continents and eventually arrived back at UC Davis.

Van Eenennaam has published extensively in scientific journals, including several timely discussion articles describing the potential of gene editing technology and its challenges for regulatory approval for use in livestock. She specializes in crossing the boundaries between academia, applied livestock production systems, the biotechnology industry, and government regulation. That's an impressive skill. Nowhere are all of these issues better illustrated than in the production of hornless dairy cattle [1, 2]. In this research project, Van Eenennaam worked closely with scientists from Recombinetics, an innovative biotechnology company based in Minnesota that specializes in gene editing technology applications in agriculturally important animals.

The Polled Gene

Van Eenennaam explains that some cattle breeds, like the Angus beef breed, are hornless due to a natural genetic variation or allele that arose in the distant past [1]. Many scientists have shown that this allele, called Polled, prevents horn growth when an individual inherits one or two copies of the Polled allele from its parents, i.e., the Polled allele is dominant to the usual Horn allele, at one precise region in the cow genome [2]. The genome is the complete DNA sequence of an individual and it includes all genes. Unfortunately, dairy cattle generally do not carry the Polled allele and hence these animals have horns [1, 2].

For some beef breeds, there has been a history of selective breeding for the absence of horns that has resulted in an increased prevalence of Polled cattle in these populations. This is the tried and tested method used to enrich for a desirable trait in a cattle population. However, Van Eenennaam and colleagues point out that selective breeding is a slow process, usually taking many generations before the trait is at high frequency in the population [1, 3]. They also noted that “conventional breeding methods to decrease the incidence of the Horned allele (i.e., increase the incidence of the Polled allele in the dairy cattle population) will increase inbreeding and slow genetic improvement” for important dairy production traits [3]. Van Eenennaam and colleagues emphasized that dairy producers historically selected animals “with the highest genetic potential for milk production, health, structural soundness, and fertility.” This hard-won genetic merit is like gold in the bank. Thus, Van Eenennaam and colleagues argue that there is a risk that conventional selective breeding for the absence of horns in dairy cattle could now compromise this important genetic heritage.

If only there was a simple and efficient way to directly transfer the small and natural Polled allele from beef cattle to dairy cattle. This process could quickly produce dairy cattle without horns and importantly without the loss of their elite dairy genetics. Van Eenennaam’s scientific team working with scientists from Recombinetics have done just that [1].

Genetic Editing Technology Dehorns Cattle

Van Eenennaam and her group are pioneers in the application of a new genetic editing technology in livestock, which potentially has many broad applications in agriculture [9]. For the hornless trait in cattle, Van Eenennaam explained that the genome editing technology is a safe and relatively fast way of transferring a small and naturally occurring genetic variant from one breed of cattle into another breed [1, 2]. It’s like using scissors to cut out a single letter or a word from a very large book and then inserting, at the same place, a different letter or word, copied from another edition of the same book. Apart from the intended word or letter change, the book is left as if new. The story is the same except for one small change. Van Eenennaam suggests that gene editing technology can be used to simply speed up what humans have been doing for thousands of years using the very slow process of selective breeding for desirable traits.
There are two versions of the gene editing technology. TALEN is the older version, and CRISPA is the sleek new version. They are a little like smartphones; the old one works, but the new one has many additional desirable features. Van Eenennaam’s commercial colleagues used the TALEN technology to produce hornless cattle. Importantly, the new CRISPA technology leaves no DNA “footprint” of the technology in the genome; the only change is at the intended targeted region. In contrast, some forms of the TALEN technology leave behind a tiny DNA “footprint” adjacent to the targeted change in the DNA. This technological difference could be important from a strict regulatory perspective, but currently, the broad brush of regulation treats both identically.

The Van Eenennaam team’s spectacular success using the TALEN technology was demonstrated by the birth of two hornless male cattle in 2015, in reproductive crosses where the offspring normally should be horned. These bulls then sired six hornless calves born in 2017 [1]. Van Eenennaam says that these animals look like normal healthy calves [4]. She reluctantly concedes, however, that the FDA requires highly detailed assessments of the health and safety of these animals and multiple generations of their offspring as a food source for humans [2, 4]. This could take quite a while.

Implications

The greatest challenge to using genetically edited hornless cattle in the dairy industry is FDA approval, which oddly classifies these cattle in the same category as a new animal “drug” [4]. Van Eenennaam encapsulates this issue in a discussion paper entitled “Regulate genome-edited products, not the genome editing itself” [2]. The title is particularly relevant for the hornless dairy cattle, as the source of the Polled allele was a beef breed of cattle consumed by humans for a thousand years and the Polled genetic variation was transferred into a breed of dairy cattle whose milk was consumed by humans for hundreds of years. What is perhaps missed in the regulatory maze is that history is an excellent judge of food safety. Van Eenennaam and colleagues may have recently ruffled a few regulatory feathers by presenting a well-reasoned case outlining why the relevant FDA regulation was “not fit for purpose” [4]. Sometimes the status quo needs a nudge.

Van Eenennaam emphasized that the gene editing technology introducing the Polled genetic variation into the genome of dairy cattle will improve animal welfare by eliminating the need for physical dehorning, and the technology used did not change the genome except at one small and precise target region where there was already a natural genetic variation in cattle populations. Although the regulatory status of these cattle is certainly vague, their future value in the dairy production system seems clear.

Scientists argue that there is potential for enormous advantages in livestock agriculture by using gene editing technology to produce “fitter, healthier, and more productive farm animals” [9]. It is still early days, but these animals may be needed to solve some of tomorrow’s major agricultural production challenges. Future editions of SPLASH! will highlight other examples of how gene editing could improve animal welfare while helping to feed the world.

Declaration: Dr Van Eenennaam works in conjunction with a private company, Recombinetics, and the author (RT) has no relevant commercial interests.

No Causal Link between Breastfeeding and Metabolic Health

- Observational studies have suggested that breastfeeding improves maternal metabolic health in the long run.
- A randomized controlled trial called PROBIT that assigned some new mothers to an intervention to promote prolonged and exclusive breastfeeding found instead that breastfeeding had no effect on mothers’ later blood pressure or body fat levels.
- Recent research from a study called HUNT considered women’s metabolic health indicators before they become pregnant and found that they were far more influential predictors of long-term metabolic health than breastfeeding.

Demonstrating cause and effect can be a tricky business. In some areas of medicine, where double-blind prospective trials are commonplace, it is less of a challenge. By comparison, in the field of public health, researchers often have to gather information as best they can—clues about human motivations, traces of behaviors, and diseases—and then do their best to identify the links. Scientists studying whether mothers who breastfeed have better long-term metabolic health than mothers who do not breastfeed have come up against these problems. Recent work has focused sharply on isolating the causal pattern, and has found that breastfeeding itself does not affect long-term maternal metabolic health [1].

To understand the subtleties of the argument, it helps to consider a brief history of research findings in the field. On the one hand, observational studies have suggested a connection between breastfeeding and maternal metabolic health thereafter. [6]. For example, scientists have repeatedly found that women who breastfeed are at lower risk of developing type-2 diabetes [2-5], and that breastfeeding appears to help women lose weight gained during pregnancy among mothers who breastfed their first child for a year or more, compared with mothers who breastfed their first child for six months or less [7]. However, a large study called PROBIT (Promotion of Breastfeeding Intervention Trial) did not find a link [8].

There are first-principles reasons to view the PROBIT results as particularly reliable. It was a randomized controlled trial, and so all of the known, unmeasurable, and even unknown influences on women’s long-term metabolic health should logically have been the same among those who breastfed and those who did not. This means that these influences should not have affected the results—as is theoretically possible in observational studies, which rely on statisticians consciously factoring in what else might have led to changes in mothers’ metabolic health. In the PROBIT study, the researchers compared the blood pressures and body fat levels 11.5 years after giving birth, of over 6,000 women who were encouraged to breastfeed, and just under 6,000 women who were encouraged to care for their infants as usual. Of those who were encouraged to breastfeed, 44.5% did in fact exclusively breastfed for at least three months, while 7.1% exclusively breastfed for this amount of time among those who were encouraged to provide normal care. As previously mentioned, the study found no significant difference.

The most recent research claims to explain the contradictory results. It is based on a large population cohort study in Nord-Trøndelag county in Norway, which lasted from 1987 to 2008 (the study is called the Nord-Trøndelag Health Study, or HUNT). Over this period, several rounds of questionnaires asked enrolled women about their pregnancies, breastfeeding duration, and other kinds of health data, which were also
assessed in clinical examinations. Because the study period was so long, and the same individuals completed multiple questionnaires, the research team could focus on the women who had never given birth when the first round of data was collected, and who then went on to have their first child during the study period. This enabled the research team to peek into women’s health status before they gave birth for the first time, rather than solely consider links between breastfeeding and metabolic health later in life.

The team, led by Nils-Halvdan Morken of the University of Bergen, and Eszter Vanky of the Norwegian University of Science and Technology, Trondheim, also in Norway, analyzed data for 1,403 women. Taking lots of indicators into account—such as body mass index, blood pressure, cholesterol levels and abdominal obesity—they found that the women who started out with the best health tended to breastfeed the longest, and then went on to have the best long-term metabolic health. Conversely, the women who had the worst health before they became pregnant breastfed for the least amount of time, and then ended up with the worst health later in life. Taken together, the data suggest that breastfeeding has little causal influence. Instead, pre-pregnancy metabolic health was the main influence on cholesterol levels, serum glucose, BMI and so on, as women approached their autumnal years.

The finding that women with poor metabolic health struggle to breastfeed for very long is unsurprising. Other scientists have reported this, and proposed a hormonal mechanisms to explain the conclusion [9]. Fatty tissue is understood to accumulate the hormone progesterone, which among other things triggers the onset of milk production. One study has found that obese women produce less prolactin, which is needed for ongoing milk production in response to an infant suckling [10].

How does the new study help women? For one thing, it underscores the importance of taking care of one’s metabolic health throughout life, including during one’s youthful years. That breastfeeding had little influence in this study may seem reassuring to some; however, there are so many well-established benefits for breastfed infants over formula-fed infants that no decision about whether or not to breastfeed should be made based on the HUNT results.


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