



## **SPLASH!® milk science update** **January 2020 Issue**



This month's issue features water scarcity, cow milk allergy, and triple-negative breast cancer.

### **Letter from the Editor: SPLASH! in 2020**

Since inception in 2012, we have published an astonishing 93 issues featuring 372 articles on milk science in "SPLASH!® milk science update," the scientific publication of the International Milk Genomics Consortium. 372! That is a lot of different milk topics!

In January of 1995, American cartoonist Gary Larson, then just 44 years old, retired the famous *The Far Side* strip for fear that it would become repetitive. Having personally presided over 372 articles, I have had that fear for *SPLASH!*. The mysteries of milk and lactation run pretty deep and scientists continue to deliver, but it does get more difficult to find 48 milk science topics annually that are truly untouched. In effort to maintain the quality of *SPLASH!* that you have come to enjoy, we will be publishing 6 issues in 2020, still with 4 feature articles per issue for a total of 24 annual topics, and then we'll re-evaluate for 2021. In the intervening months in which *SPLASH!* is not published, you'll still hear from the IMGC as they feature the work of specific scientists and sponsors in a different format.

We are happy to report the return of writers Dr. Anna Petherick, Dr. Sandeep Ravindran, Dr. Lauren Newmark, and Dr. Peter Williamson for 2020. Stay tuned for new hot topics such as dairy and sustainability (starting this issue), applications of artificial intelligence in dairy, and more on milk-oriented microbes.

Thank you for being a *SPLASH!* reader. We have readers from around the world with over 200,000 unique visitors to the website in 2019. Your website clicks provide the "votes" that keep us going and help us learn which topics are reader favorites. Click away, dear reader, and share with your friends!

*Danielle G. Lemay, PhD*

*Founder and Executive Editor, "SPLASH! milk science update"*

### **Droughts, Dairy and Discretionary Foods: Healthy and Environmentally Responsible Diets Can Mean Consuming More Dairy**

- **A recent study calculated the water that is required to produce the food and beverages Australians consume on a daily basis.**
- **The food group that contributes most to water scarcity, given current average consumption levels, is also the least healthy food group, comprising alcohol, and sugary, salty and fatty snacks.**
- **Dairy consumption contributes relatively little to Australia's water scarcity.**
- **The study demonstrates that healthy, environmentally responsible food and beverage consumption habits are possible, by cutting out discretionary foods, and eating more dairy, fruits and vegetables.**

Often, dietary advice is given from singular perspectives. Public health professionals consider nutritional benefits first and foremost. Climate activists, concerned with the carbon footprints of modern lives, frequently lobby for vegetarianism. Few studies have sought to balance these, as well as other potentially competing demands. Yet, the United Nations' Sustainable Development Goals (SDGs) require balance. They aim for both sustainable consumption patterns (Goal 12), and ending all forms of hunger and malnutrition by 2030 (Goal 2).

Unusually, a recent study in the journal *Nutrients* calculates in detail the environmental impact and the

nutritional quality of the diets of more than 9,000 people living (and thus shopping for food and beverages) in Australia [1]. Its conclusions are intriguing for two reasons. First, in a country where water scarcity is of considerable concern, consuming dairy has relatively little water-scarcity impact compared with the impacts of other kinds of food. Indeed, from both a nutritional-quality perspective and an environmentally conscious one, the study's authors propose raising Australians' dairy consumption from 1.46 to 2.5 servings per day. Second, the analysis shows that processed snacks have surprisingly large water-scarcity footprints in addition to their better-known negative health impacts. It is these products that should be the common enemy of advocates of healthy eating and environmental protection.



The analysis, which was conducted by Bradley G. Ridoutt and colleagues at Australia's national science agency—the Commonwealth Scientific and Industrial Research Organisation (CSIRO)—used data from the most recent Australian Health Survey. This survey is conducted by the Australian Bureau of Statistics, and, unlike many dietary surveys, asks participants to recall the food and beverages that they consumed only during the previous 24 hours—as opposed to evaluating their diets in general, which may lead to greater inaccuracies. The survey is immensely detailed, with 5,645 food types classified into 500 minor food groups,

132 sub-major food groups, and 24 major groups. When a participant reports eating apple pie, for example, the ingredients are listed, and apple, sugar, flour and shortening are counted in different food categories.

Ridoutt and his colleagues took these data for more than 9,000 Australians and carefully adjusted it based on how people tend to misremember even what they consumed very recently. Then they worked out, food by food, by the amount swallowed, both the water-scarcity footprint and the nutritional value of every participant's diet. The water scarcity footprint was calculated using three methods, which turned out to produce results that were closely correlated. The research team then compared the diets of different sectors of Australian society, and considered how the nutritional quality of Australians' diets is related to their contribution to the country's water scarcity.

On average, each Australian's food and beverage consumption requires about 365 liters of water every day. That average conceals considerable variation, however. Unsurprisingly, the water-scarcity footprint was larger for men's diets than for women's diets, as men, being typically larger than women, tend to eat and drink more. But it was also larger for Australians between the ages of 51 and 70 years of age than for those aged 19 to 50 (though the lowest water-scarcity impact group was those over 71 years of age). Among the food habits that contributed to these differences, what the authors term "discretionary foods"—including alcohol, and sugary, salty, fatty snacks—made the biggest difference. Previous research by members of the same research team demonstrated that these foods are responsible for about 30% of dietary greenhouse gas emissions in Australia [2]. This study adds that they contribute 25% of dietary water-scarcity impacts.

Self-evidently, no dietician is likely to encourage people to swallow more of these kinds of foods and more alcohol. However, the category of foods that contributed the second-most to water scarcity was fruit and vegetables, making the balance of considerations—environmental and nutritional—less straightforward for public policymakers. Collectively, Australians' consumption of fruit and vegetables, although less than experts would recommend for health, contributed almost as much to water scarcity as Australians' consumption of discretionary foods. The dairy—and in this study, the meat—components of average diets generated far less water-scarcity impact.

So how should both concerns—worries about droughts and nutrition—be balanced? Ridoutt and his colleagues calculate that if the country were to switch its dietary habits to those recommended by government health experts, the water scarcity footprint of each Australian would rise from 365 to 445 liters of water per day—worsening Australia's water-management problem. But compromise is possible. The researchers identify a group of participants whom they consider to have a high-quality diet from a

nutritional perspective, as well as a low water-scarcity diet.

This group of people eats more fruit and vegetables and more fresh meat and dairy than the average Australian but very little in the way of discretionary food and beverages. They might have a bit of honey on their toast in the morning, a modest square of cake at teatime, and just the one small glass of wine with dinner. They also consume 1.7 times the amount of dairy as the average Australian, which, as it happens, is exactly in line with the ideal diet that nutritionists would recommend. This is possible because dairy has a mediocre impact on water scarcity in Australia. Overall, this diet implies a water-scarcity footprint of 320 liters of water per day, and is much healthier than the average Australian diet.

The study by Ridoutt and his team serves as a demonstration of how dietary advice might be reconsidered in an age where the environmental degradation that results from meeting the nutritional needs of a large and rapidly growing global population is ever more salient. Even though the calculations involved in this study were complex, in some ways the Australian case is simpler than most because more than 90% of the food consumed in the country is also grown there, so supply chains are well documented. Numbers such as those calculated in this paper may help to modify dietary advice, yet, as the authors argue, one of the key insights of their environmental calculations is that major gains can be made in changing the way in which different food products are produced. The same crop grown in a different ecosystem, in greenhouses versus open fields, can have radically different water consumption demands. Those insights, too, should guide public policy.

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## **Nursing Can Provide Long-lasting Protection against Worm Infection in Mice**

- **Transfer of immune molecules from mothers to infants both in utero and via nursing is an important source of protection from disease.**
- **A new study finds that mice can transfer long-lasting immunity against worm infection to their infants via nursing.**
- **The long-lasting effects of maternal immune transfer via nursing was mediated by the transfer of immune cells and not antibodies.**

Newborn babies lack a fully developed immune system, and the transfer of maternal antibodies and other immune molecules to babies via nursing is particularly important for early immune protection (1-4). However, it has so far been unclear whether maternal immune transfer might provide long-lasting immune protection that continues beyond when babies are nursing.

A new study led by Professor William Horsnell of the University of Cape Town and the University of Birmingham finds that mothers' milk can provide long-lasting protection against infection in mice (5). The researchers found that mice infected with a parasitic worm before becoming pregnant were able to confer life-long protection against the worm infection to their infants via nursing.

Infections by various species of parasitic worms, known as helminths, before and during pregnancy are known to have significant consequences for offspring immunity (6-9). "I've been working on helminths for about 15 or 16 years now, and I've been interested in various sorts of contexts of how an infection influences the immune system," says Horsnell.



*Nippostrongylus brasiliensis*.

Immune protection against some helminth species is known to be significantly mediated by antibodies (10,11). "We wanted to have a look and see if we get the same thing here," says Horsnell.

In the new study, immune protection against the helminth *Nippostrongylus brasiliensis* was not mediated by antibodies but instead by the transfer of maternal immune cells.

Transferring antibody-containing serum from worm-exposed mothers to the offspring of "naïve" mothers—those that were not infected with the worm—did not significantly protect against worm infection compared with receiving serum from naïve mothers. In addition, the researchers found that protection against worm infection was independent of antibody titers in either the pups or mother, suggesting that transfer of maternally-derived antibody is not required to transfer protection against the worm. "There's the

dogma that the immune influence from the mother via nursing is through passive transfer of antibody," says Horsnell. "That is obviously really important, but there's a lot more going on," he says.

The researchers found that even though maternal immune protection was not mediated by antibodies, it was dependent on maternal B cells, which are the cells that produce antibodies. "We haven't quite answered what they're doing there, but the big thing was that it was the cell transfer that appears to be mediating the long-term effects," says Horsnell.

The maternal transfer of immunity via nursing was also sufficient to protect mice with a genetic immune susceptibility to infection. "One of the things we found that immediately surprised us was that the effects can reverse primary immune deficiency," says Horsnell. "We've got these mice that are inherently susceptible to worm infections and if we nurse them on a mother who's had an infection, it would confer that protection," he says.

"The other thing that surprised us was that the effect was completely driven by nursing, and there was no in utero effect," says Horsnell. When naïve pups of one mouse strain were nursed by worm-exposed mothers of a different mouse strain, the nursing alone was sufficient to significantly protect the pups from worm infection. This experiment also indicated that transferred immune cells from one mouse strain were able to affect the immunity of a different strain. "We also found that it occurred in an allogeneic setting, where the cells get transferred into a setting where they're likely to get rejected, but that's sufficient to still imprint on the offspring's immune system," he says.

It's still unclear how applicable the results would be to humans, but that's something Horsnell is beginning to look at. "That's the killer question, and now that we're starting to do a lot of this work with allogeneic models, that's important because that's a representative system," says Horsnell. "How important it is in humans we don't know, but it is something that we have to consider and we need to think about how we can test it," says Horsnell. "We've just applied with collaborators for funding for a cohort study to look specifically at parasite infections in maternal and offspring cells, and hopefully then we can start to see if what we're seeing does happen in humans," he says.

The results pave the way for many follow-up studies. "It's just one of these papers that's opened up so many questions, and we're trying to see which ones we can answer next," says Horsnell.

Future studies could look at maternal immune transfer after infection with other pathogens. "What this might be suggesting is that everything that we know in the context of direct infection has got a transgenerational potential," says Horsnell. "We also want to see if there's any effect of breastfeeding inherently," he says. "So even if the mother hasn't had a specific immune challenge or an infection, what is the effect of just breastfeeding on the offspring's immunity?"

Horsnell is also looking at how the results could influence maternal vaccination strategies. "Vaccine effects are all framed in the context of passive antibody transfer, and we just don't believe that it's as simple as that anymore," he says. The fact that maternal exposure to an infection before pregnancy can lead to a mother transferring long-term immune benefits to her offspring could potentially have important applications.

"A lot of maternal vaccination strategies are aimed at providing immune protection early in life, but we want to see if that protection can be extended and how we can do that," says Horsnell. "A big focus at the moment is on how we can tweak the maternal vaccination strategy to provide early protection but also maybe see if there's a way that we can target the timing of vaccination to provide the offspring with longer-term protection," he says.

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## Healthy Human Infant Gut Microbes Block Cow Milk Allergy in Mice, Diet, Environment: A Host of Factors Influence Human Milk Fatty Acids

- A new study demonstrates how the types of bacteria living in an infant's gut influence the development of food allergy.
- Feces from human infants with cow milk allergy were enriched with different types of bacteria than feces from healthy infants.
- Germ-free mice bred to have an allergic reaction to the cow milk protein  $\beta$ -lactoglobulin were protected from this allergic response after colonization with fecal samples from healthy human infants.
- Bacteria from the family Lachnospiraceae were enriched in healthy infants and influenced gene expression in mouse intestinal epithelial cells, suggesting they may be protective against cow milk allergy.

Proteins in food often suffer from mistaken identity. Instead of being seen as the innocuous food items they are, immune systems instead take these proteins for harmful invaders and mount a response. To understand why some immune systems are sensitized to cow milk protein whereas others have an inappropriate reaction, researchers are turning to gut bacteria. In animal models and in humans, food allergies have been associated with a lack of diversity in gut bacteria species [1, 2]. And specific research on cow's milk allergy (CMA) suggests that there might be particular species of gut bacteria that can

prevent the development of allergy or allow for complete resolution of CMA in late infancy or early childhood [1, 3].



To further investigate these potential protective microbes, a team of researchers from the University of Chicago colonized germ-free mice (that is, mice that were bred to be free of any microorganisms) with human fecal material from four healthy infants and four infants with known allergy to  $\beta$ -lactoglobulin (BLG), a whey protein in cow milk and common allergen [4]. All of the infants were formula-fed, and mice were provided identical formula as their human donors. Prior to colonization with human fecal material, all germ-free mice were sensitized to BLG so that each animal's immune systems created anti-BLG immune factors. When exposed to cow milk, all mice exhibited an

anaphylactic response (drop in core body temperature) and a BLG-specific immune response (immunoglobulins directed specifically at BLG). After colonization with human gut microbes, the CMA-colonized mice produced significantly higher BLG-specific immune factors than their healthy counterparts. Moreover, all of the mice receiving healthy infant microbiomes were protected from an anaphylactic response [4].

The team then replicated their experiment with fecal material from four healthy breast-fed infants and four breast-fed infants with CMA to exclude an influence of formula feeding on gut microbiome development. Once again, mice that received their microbiome from healthy infants were protected from anaphylactic response to the milk protein BLG, but those receiving CMA fecal samples exhibited a significantly greater drop in their core body temperature [4].

Having established an association between the composition of the microbiome and CMA allergy, the team sought to establish causation—were there particular types of bacteria present in healthy infants that protected against the pathological immune response? To answer this question, they turned to the contents of the infants' diapers.

A microbial analysis on stool samples from the eight formula-fed fecal donors identified 58 bacteria types (or operational taxonomic units, OTUs) that had significantly different concentrations between the healthy and CMA infants [4]. The 34 OTUs that were more abundant in the healthy donors were grouped as potentially protective, whereas the 24 that were more abundant in CMA infants were grouped as potentially non-protective. The variable determined to have the largest effect on separating the two groups—that is, the OTU that was enriched in healthy infants and healthy colonized mice compared with their CMA counterparts—was a member of the Lachnospiraceae bacteria family, in the Clostridia class [4]. This was an exciting result, as previous studies had suggested a protective role of Lachnospiraceae species in the development of food allergies.

Not to take away from fecal analysis, but here is where their research gets really interesting. Knowing that food allergies begin in the gut, the team isolated intestinal epithelial cells from the lower portion (ileum) of the small intestines of mice colonized with both healthy and CMA fecal samples. They sequenced the RNA in each cell to determine which genes the cell expressed, and to quantify that gene expression (e.g., was that cell making a lot of that particular gene relative to other cells?). Cells from healthy mice expressed different genes than did cells from CMA mice, and the integration of the OTU data with gene expression data pinpointed precisely which microbes were responsible for the genetic difference: nine OTUs were significantly correlated with an increase in gene expression in the intestines of healthy or CMA colonized mice, five of these were OTUs that were grouped as potentially protective, and of these five, three were part of the family Lachnospiraceae [4].

Multiple lines of evidence now pointed to Lachnospiraceae species, particularly *Anaerostipes caccae*, as potentially protective against CMA. Lachnospiraceae bacteria are believed to maintain homeostasis in the intestines by influencing the production of immunomodulatory metabolites and through induction of

regulatory T cells. Both of these actions are critical for maintaining immune tolerance to harmless antigens such as food proteins. Altering a microbiome might be slightly more complicated than taking a Lachnospiraceae chewable gummy, but microbiome-based therapies offer promise for food allergy treatment, and possibly even prevention.

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## Why Breastfeeding Protects against the Most Dangerous Type of Breast Cancer

- **It has been known for some time that women who breastfeed have lower rates of breast cancer, and in particular, triple-negative breast cancer.**
- **New research shows that one reason for this is that a milk protein called alpha-casein confers protection against cancer initiation.**
- **Alpha-casein lowers triple-negative breast cancer risk by reducing levels of an upstream gene regulator called HIF-1alpha in breast cancer stem cells and in “activated fibroblasts.”**

For some time it has been known that women who have their first pregnancy in their twenties, who have many children, and who breastfeed for extended periods have a lower risk of developing breast cancer than other women. It has also been well-established that the link between breastfeeding and lower risk is strongest for triple-negative breast cancer, a particularly dangerous form of the disease. Until recently, however, science has been unable to explain why. In a series of experiments, researchers at the University of Manchester and the University of Edinburgh, in the UK, have now demonstrated that the production of a milk protein called alpha-casein confers protection in human cells. It does this by downregulating signals that induce and maintain a population of tumor cells called breast cancer stem cells [1]. The work suggests a new avenue for developing small molecule drugs.



Triple-negative breast cancer gets its name from the absence of three types of receptor on the surface of its tumor cells. The disease is in fact a group of cancers that share this characteristic. Because of the lack of these receptors—specifically, receptors for the hormones estrogen and progesterone, and HER2 receptors—there are no targets for common hormone therapies and drugs like Herceptin, making triple-negative breast cancer especially hard for doctors to treat.

Triple-negative breast cancers contain a small group of cells that are the core of the problem. These stem cells populate other cells that form the tumor, and are associated with metastasis. The development of stem cell characteristics is

in turn promoted by another kind of cell that surrounds the breast cancer: “activated” fibroblasts. These are normal fibroblasts (i.e., connective tissue cells) that produce the proteins “alpha-smooth muscle actin” and “fibroblast activation protein-alpha.” When fibroblasts around a breast cancer are activated, they secrete a lot of interleukin-6, a signalling molecule that can encourage normal cells to behave like stem cells [2].

To make the point that alpha-casein influences the development of triple-negative breast cancer, the research team, led by Kirsten Garner of the University of Manchester, first observed that cancer cells make an unusually low level of this protein. With data on alpha-casein gene expression from 250 samples of different triple-negative breast cancers, they demonstrated that the lower the expression levels, the greater the odds of patients surviving without relapse. Then, to probe the mechanism underlying this pattern, the researchers set up populations of triple-negative cancer cells that were all induced to start producing unusually large amounts of alpha-casein. They tested whether this affected the activity of an enzyme—*aldehyde dehydrogenase*—that is associated with poor outcomes in breast cancer. The results showed that large amounts of alpha-casein indeed cut the enzyme's activity, compared with cells that produced normal amounts. This finding suggested that alpha-casein works by affecting cancer *stem* cells.

The link between alpha-casein and stem cells established, the next task was to explain it. To do this, the team then created populations of breast cancer cells in cultures, and used viruses to insert genes into them that would facilitate the measurement of levels of something called HIF-1alpha, which regulates the expression of many genes. Fibroblasts make large amounts of HIF-1alpha when they become activated, and as such, lots of HIF-1alpha is understood by oncologists to be a sign that these cells have developed into a cancer-promoting environment.

When these populations of breast cancer cells were cultured for 72 hours in alpha-casein—effectively, soaked in it—the fibroblasts made very little HIF-1alpha. Up to 72 hours, the effect proved to be dose-dependent: the more hours of incubation in alpha-casein, the less HIF-1alpha the fibroblasts made. By contrast, alpha-casein incubation did not affect levels of other gene-regulating molecules that the team also tested. In the paper they write, “we note that fibroblasts are not exposed to milk proteins in intact homeostatic mammary tissue but envisage that in the context of mammary remodelling during lactation, weaning and involution, the fibroblasts will be exposed to milk that builds up in the ducts and leaks into the tumour microenvironment.”

The fact that HIF-1alpha levels varied according to the presence of alpha-casein does not necessarily mean that the two kinds of molecule directly interact. Instead, alpha-casein might affect something that goes on to affect the levels of HIF-1alpha. Through further experiments, Garner and her colleagues demonstrated that powerful gene regulatory factors called STAT1 and STAT3 are crucial in regulating HIF-1alpha levels. When alpha-casein affects STAT1, this leads to reductions in HIF-1alpha in activated fibroblasts. Conversely, when it affects STAT3, it lowers HIF-1alpha levels in breast cancer stem cells, indicating that the cells have reduced activity. In this way, alpha-casein reduces triple-negative breast cancer risk via two mechanisms. These findings suggest that drugs that target STAT1 and STAT3 might help to fight this aggressive form of breast cancer.

This work helps explain a medical mystery. In short, the more time women spend breastfeeding, the longer that cells of their breast tissue are likely to be exposed to alpha-casein, and this implies the longer that the initiation of triple-negative breast cancer is inhibited by alpha-casein's effect on STAT1 and STAT3. Most importantly, getting to grips with the details of the mechanisms by which lactation protects against this disease is good news for women everywhere, whether or not they have breastfed.

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## Sponsor Feature: California Dairy Research Foundation



California Dairy Research Foundation (CDRF) promotes sustainable dairy farming practices and is co-hosting the California Dairy Sustainability Summit in Sacramento this March. [Read more!](#)

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