This month’s issue features Holstein breeding, donor milk pasteurization, milk lactose in mammals, and the cheese food matrix.

**The Daddy of All Cows**

- Holstein cattle are the dominant dairy breed worldwide.
- Nearly all Holstein cattle are descendants of just two bulls.
- Introducing more diversity may influence and improve fertility.

Holstein cattle have become the dominant dairy breed worldwide. These black and white cows have become synonymous with dairy in many countries and they produce billions of liters of milk. This has been facilitated by artificial insemination technology that is the basis for herd replacement and genetic improvement programs around the world. With this in mind, Yue et al. [1] conducted a study to examine how much genetic diversity exists in the male chromosomal lineages of modern cattle.

The genome of cattle consists of 30 pairs of chromosomes. As with other species, two of these chromosomes are sex chromosomes, named X and Y. Males have one Y-chromosome and one X-chromosome, whereas females have two X-chromosomes. This means that all male offspring of one bull inherit an identical Y-chromosome from their sire. So if the male ancestral animals have been few in number, the variation in genes that sit on the Y-chromosome is very limited. Considering that the Y-chromosome contains genes that are major contributors to male fertility [2], it becomes a very important factor for management of dairy herds, and for accessing the gene pool that may contribute to selective breeding.

A study by Yue et al. [3] set out to determine Y-chromosome diversity amongst Holstein cattle. The scientists dug into the records and databases for over 60,000 Holsteins in the USA and over 220,000 Holsteins from international sources. These records include pedigree information that could be used to trace the ancestry of dairy cattle up to recent times.

Yue et al. [3] identified the records of bulls from electronic databases held by the National Association of Animal Breeders (USA) beginning in the 1940s, and then physical records that went back beyond those years. The earliest records relating to the original import of Holstein cattle into the USA about 150 years ago. However, there was a significant change in patterns of sire use when artificial insemination was introduced in the 1960s, so animals born in those years were given special attention as founders of modern cattle. They compared these with the pedigrees available on bulls from over 35 countries going back as far as the 1950s.

Next, the study focused on Y-chromosome DNA data that had been determined for 257 modern-day bulls [1]. The Y-chromosome of cattle is particularly rich in repeated regions of identical or similar DNA sequence, referred to as copy number variants (CNV). The 257 bulls were grouped according to the founder animals that gave rise to their pedigree line. The scientists had data from these animals that measured their reproductive capacity or fertility, which had been previously linked to the Y-chromosome CNV.

The research showed that, out of over 62,000 bulls born in the USA between 1950 and 2013, over 99% were the descendants of only two bulls. In other words, all of the bulls from modern times have only one of two Y-chromosomes. This represents an extreme bottleneck in the male line of Holstein dairy cattle. Why would all these progenies arise from only two bulls? Clearly, farmers want to use the best bulls to breed their herds. It’s their way of increasing the efficiency and economic viability of their enterprise. Following the introduction of artificial insemination and a system to deliver semen across the country, it was possible for farmers to choose the very best, and these animals were from the two bulls that were identified. It’s also possible that bulls were removed from the pool due to genes that caused inherited disorders. Yue et al. [3] speculated that the bottleneck could have contributed to declining fertility rates in dairy cattle, which appears to be correlated with the increase in milk production that has been a primary target of improvement strategies. The limitation in Y-chromosomes amongst Holstein bulls may restrict attempts to reverse a decline in male fertility.

How could the limited number of Y-chromosomes be reversed? The simplest answer would be to look outside the USA for bulls with different founders and alternative Y-chromosomes, then import semen.

Unfortunately, when Yue et al. [3] analyzed the pedigrees from over 220,000 Holstein bulls in the international database, they found the same effect and the same founders. The senior scientist in the study, Dr. Liu, has since developed a different strategy. He has
gone back to archived samples of frozen semen and found a bull from a different line to use for breeding [4]. There may be similar archive samples held in repositories around the world. The alternative would be to cross breed using other productive dairy breeds as the source of semen, but the impact of this strategy on herd improvement is likely to be more complex. Modern genomic tools should help to iron out the complexities.


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Alternative Methods to Pasteurize Donor Milk Hold Promise

- Various methods of pasteurization have been proposed for human donor milk other than Holder pasteurization, the current standard.
- Reducing the period of heating during pasteurization appears to kill many pathogens with less damage to the proteins in human milk than HoP delivers.
- High-pressure treatments, ultrasonic processing, and UV irradiation all appear somewhat effective at pasteurizing milk, but in all cases more research is required before they can be widely considered as alternatives.

In the past few months, SPLASH! has assessed how well Holder pasteurization, or HoP, kills viruses and bacteria, and the extent to which it affects the nutrients, immune proteins, and digestive aids in human milk. Because HoP is widely used by milk banks all over the world, these outcomes are potentially important for a huge number of infants, particularly those born prematurely. In this final article in the series, other methods of pasteurizing milk are brought into focus. Next to HoP, we know relatively little about their performance. Yet various studies show that they hold some promise towards achieving the ideal—a means of reliably preventing germs from proliferating in milk, while also retaining the function of human milk’s proteins.

HoP itself is an eminently simple process. Milk is heated to 62.5°C for half an hour. This method is good at killing pathogens, even extremely dangerous ones such as Ebola. HoP’s weakness is that it damages some immune proteins such as antibodies and certain cytokines, and may alter the digestibility of milk for infants by, for example, reducing the function of an enzyme called bile-salt-stimulated lipase.

The simplest innovation of HoP is to alter the temperature applied, and the period of heating. As per its name, High-Temperature–Short-Time (HTST) pasteurization does exactly this, raising the temperature of donor milk to 72°C for just 15 seconds or so, followed by immediate cooling. According to a review of the evidence published this year [1], HTST, which has been used by the dairy industry since the 1930s, is at least equivalent to HoP when it comes to destroying germs, and outperforms HoP at leaving intact some vitamins and immune proteins, including lactoferrin and a number of cytokines.

A few years ago, Rangmar Goelz and his colleagues at the University Hospital and Children’s Hospital in Tuebingen, Germany, set out to measure how antibodies were impacted by three different heating regimes. Knowing that cytomegalovirus—a virus that becomes reactivated during lactation and is excreted into milk—is destroyed with a mere five seconds of heating to 62.5°C, the team wanted to know the proportion of antibodies that would remain if roughly this or slightly more heat treatment were applied to milk [2]. They found that five seconds of heating to 62°C, 65°C, or 72°C had virtually no effect on a range of antibodies, suggesting that the HTST standard of 72°C for 15 seconds could be reduced if cytomegalovirus were the only concern.

But other germs matter. Hence, a similar study [3] by Charles Czank and a team based at The University of Western Australia, in Crawley, also sought a minimal yet effective temperature treatment—but defined a minimal treatment as what is necessary to inactivate five bacterial species that are among the most common contaminants of human milk: Staphylococcus aureus, Enterobacter cloacae, Bacillus cereus, and Staphylococcus epidermidis. They measured the retention of secretory IgA, lysozyme, and lactoferrin, and report that keeping HoP’s 30-minute pasteurization period while lowering the temperature to 57°C would destroy 99.9% of the...
cells of these species and keep intact at least 90% of the three immune proteins.

Aside from heating, human milk can be pasteurized by applying high pressures, ultraviolet radiation, or ultrasonic processing. Ultrasonic pasteurization is probably the most exotic of these methods since it seeks to create microscopic bubbles, which rapidly collapse and produce shock waves. The shock waves bring associated pressures of 50 MPa as well as a burst of heat of up to 5,000°C in each microscopic bubble’s immediate area. The University of Western Australia team also tested this method [4], and found that the retention rates of lysozyme and other human proteins were generally lower in ultrasonically pasteurized milk than in the lab’s previous 57°C heat-treatment study, and that ultrasonic pasteurization often requires a little bit of heat to be good at killing bacteria—specifically bacteria of the species, Staphylococcus epidermidis and Escherichia coli. Lukas Christen and colleagues in the same lab measured bile-salt-stimulated lipase as opposed to immune proteins and found that more than 90% of this enzyme survived ultrasonic pasteurization as long as the milk’s overall temperature remained below 51.4°C [5]. If the milk got any hotter, bile-salt-stimulated lipase activity rapidly diminished.

Like temperature, pressure causes various changes within a bacterial cell that bode poorly for its survival. High pressures—in the order of 400 to 800 MPa—are proposed as a means of donor milk pasteurization when applied for five to 10 minutes at a time. The studies published so far do not completely agree about whether this method is better than HoP at preserving antibodies [1], but consider as relevant possibilities that it is either superior or the same—implicitly accepting that pressure treatment is highly unlikely to be any worse. Last year a review of high-pressure processing research concluded, more positively, that this method does appear to be better at maintaining human milk proteins than HoP, whilst efficiently inactivating pathogens [6].

The fourth pasteurization method currently under broad consideration is ultraviolet radiation. Although UV light is a well-known disinfectant, milk is annoyingly opaque for UV to be a straightforward option—as it is for water—and so UV radiation can only penetrate milk’s outer surface. Stirring constantly during the pasteurization process gets around this problem, however. As with high-pressure pasteurization, few studies have assessed UV pasteurization’s affect on a wide range of pathogens and immunoproteins. But those that are published are generally positive. In two separate studies, Christen et al. saw a decrease in five species of bacteria and retention of bile-salt-stimulated lipase after UV-irradiation [7], and less bacterial growth after UV-processing than after HoP-processing when samples were afterward left at 37°C for a few hours [8]. However, another study by the lab at the University of Western Australia reported that UV irradiation failed to completely halt cytomegalovirus gene transcription, cautioning that the method requires fuller evaluation [9].

For the moment, HoP is the rule for donor milk. In many ways that seems reasonable; HoP’s effectiveness against germs is in tune with the cautionary tone of the Hippocratic Oath. Yet, the evidence suggests that shorter heat treatments and some other methods probably also avoid doing harm, meanwhile allowing donor milk to do even more good. For them to be rolled out among milk banks, however, the evidence that they kill all manner of pathogens needs to be bolstered with more tests on more pathogens. If that is established, infant health stands to gain.


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Milk Lactose From A to Zebra

- Lactose is the primary carbohydrate in most mammal milks, but its concentration varies considerably across species.
- Milk lactose concentration is intimately tied to a species’ life history and ecological niche.
- Species that fast during lactation, including bears and whales, produce milks with very little lactose, whereas species that lactate for prolonged periods of time, like humans, produce milk with higher lactose concentration.

If you have a hard time digesting lactose from cow’s milk, you may want to avoid drinking monkey milk. Rhesus macaque monkeys produce milk with 8% lactose, almost twice the amount found in cow’s milk. With a barely detectable quantity of lactose, a better option for intolerant individuals would be milk from grey seals (that is, if you can get past that it is also 70% fishy-tasting fat and probably not the most appetizing choice for your morning bowl of cereal).

Understanding why rhesus monkeys make such high-sugar milk, grey seals make such low-sugar milk, and why other mammals, like cows, are somewhere in between requires consideration of both what the offspring needs to grow and develop and what the mother needs to maximize her reproductive success. Far from just a story about sugar, understanding lactose variation in milk is a story of mammalian adaptation and diversity.

Unique and Complex

Lactose is a double sugar, or disaccharide, composed of one glucose molecule and one galactose molecule. Although there are other disaccharides in nature, the glucose-galactose combination is unique to milk. Indeed, the synthesis of lactose depends upon the presence and activity of another unique milk ingredient, the protein alpha-lactalbumin [1]. Before alpha-lactalbumin becomes a bioactive whey protein in milk, it functions as an enzyme responsible for binding glucose and galactose inside mammary cells. Because only mammary cells express the alpha-lactalbumin gene, only mammary cells are able to make lactose.

Lactose synthesis seems like a complicated process—why don’t the mammary glands simply pass on the monosaccharides glucose and galactose in milk instead? One reason may be because complex molecules offer an advantage over single molecules [2]. Osmotic concentration is a measure of how many molecules are in a solution; one lactose molecule has half the osmotic concentration of two monosaccharides. Because lactose exerts less osmotic pressure per unit mass than glucose, more carbohydrates can be transferred in milk while maintaining the same ion concentration (aka isosmotic) as the mother’s plasma [1].

The fact that lactose is a unique sugar offers another advantage to mammals. Bacteria love to dine on sugars, and most carbohydrate macronutrients in nature contain only glucose. Only bacteria that evolved the ability to digest lactose would be able to colonize milk (either in the mammary gland or the digestive tract of the infant) [2]. Thus, the novelty of lactose may have offered a protective effect, for both mothers and infants, against a large number of microbes in the environment [2].

The First Milk-Makers

Lactation is an evolutionarily old adaptation. Mammals that lay eggs (monotremes, such as the platypus), mammals with pouches (marsupials, like the koala), and mammals with placentas (eutherians, like humans) all lactate but diverged more than 100 million years ago. This suggests lactation may be even older and have its origins from a pre-mammal ancestor over 150 million years ago [1].

Lactose may not have been on the ingredient list in the first milks—at least not on its own. Monotremes and marsupials are the oldest mammal lineages and have oligosaccharides (carbohydrates made of 3–10 monosaccharides) as their primary carbohydrates [1-3]. Their milks are not completely lactose free—there is usually a lactose molecule at the end of the sugar chain—but they lack any free lactose molecules [2]. Coupled with this observation is the small amount of alpha-lactalbumin made by monotremes. Urashima [3] have argued that this was the ancestral condition: the first milks contained oligosaccharides and most likely lacked any free lactose. Over time, mammary glands began to make more alpha-lactalbumin, which in turn increased the production of lactose. Finally, lactose became an important energy source for some marsupials and eutherian infants, and was produced separately from oligosaccharide chains [3].

Many mammalian milks have little to no lactose [2], indicating that, despite being an ancient milk ingredient, it is not required for mammalian development. Take, for example, bears, which astonishingly give birth and lactate while hibernating (and by extension, while fasting). Bear mothers cannot replenish resources in the den, and milk production must, therefore, balance the nutritional needs of the bear cubs with the survival of the mother. The mother’s main concern is conserving glucose, to fuel her brain, and water [4]. Both of these go hand-in-hand with lactose. Lactose creates an osmotic gradient and draws water into the cells of the mammary gland; the higher the concentration of lactose, the more water (and thus, the more dilute the milk) [2]. High-fat, low-sugar milks are the solution. Fat, unlike carbohydrates, can be stored for a rainy (or milky) day. And if you are a large mammal, like a bear, you can store enough fat to provide energy for your growing cubs during hibernation [4].

Whales and seals may not hibernate, but they do lactate while fasting, and not surprisingly also produce high-fat, low-sugar (and water) milks [2, 4]. Their large body size allows them to store large amounts of fat, allowing for some remarkable adaptations. The
blue whale gains nearly 100,000 pounds of blubber during pregnancy to maintain lactation for roughly six months [5]. The hooded seal only lactates for four days, but in this time transfers approximately 60 pounds of fat to her offspring [2, 4].

Some Like It Sweet

For fasting bears, whales, and seals, the production of high-sugar milks (which are synonymous with high water and low fat) would quickly deplete maternal glucose and water stores, and fail to support infant growth and development. For primates (a group that includes humans, apes, monkeys, lemurs, lorises, and tarsiers), the opposite scenario is true. Primates nurse for longer than would be predicted for mammals of their body size. To put it in perspective, a 12,000-pound elephant nurses for roughly the same amount of time as a 40-pound chimpanzee. The production of high-fat, low-sugar milk among primates would quickly deplete maternal resources [6]. Moreover, most primate species nurse on demand. Frequent nursing stimulates lactose synthesis in the mammary gland, which subsequently increases milk’s water content [6].

The relationship between lactose and water also is clearly demonstrated when looking at milks of mammals, such as zebras, that live in arid environments [6]. Zebra milk is nearly 90% water, and the milk lactose concentration is similar to that of milk from primates (around 7%) [2]. In zebras, selection favored increased lactose synthesis and extremely dilute milks to prevent dehydration in infants with high water turnover; in primates, selection favored the production of low-energy milks to support an extended infancy period [2, 6]. This is an exquisite example of convergent evolution; two different evolutionary lineages converged on the same solution (high sugar milks) to solve different problems.

Sugar Babies

Lactose is probably best known as the sugar that causes intestinal discomfort in the majority of humans. But taking a step back and looking at lactose across mammals puts it in a much more positive light. Many adult humans may be lactose intolerant, but human infants, like all primate infants, are highly dependent on lactose for successful growth and development. Indeed, much of the diversity we see in mammals’ life history patterns and ecological niches is only possible because of this unique and complex sugar.

Cheese and Butter Have Different Effects on LDL Cholesterol

- A new study finds that consuming butter induces a significantly greater increase in LDL cholesterol compared with cheese, and the effects are particularly pronounced in participants with high baseline LDL cholesterol.
- The results suggest that the cheese food matrix helps attenuate some of the cardiometabolic effects of consuming saturated fats.
- The new study adds to the evidence that the effects of dietary saturated fats on cardiovascular disease risk may depend on the food source.

Saturated fatty acids (SFAs) have long been considered detrimental to cardiovascular health, with dietary guidelines advocating for a restriction of dietary SFAs to reduce the risk of cardiovascular disease (CVD) [1]. However, increased consumption of SFAs may not always be associated with increased CVD risk, and the effects of SFAs on CVD risk may instead depend on the food source [2-5].

A new randomized control trial conducted by Professor Benoit Lamarche of Université Laval compared the cardiometabolic effects...
of consuming saturated fats in butter compared with consuming saturated fats in cheese [6]. “The basis of this project was the controversy about the effects of saturated fats on cardiovascular disease,” says Didier Brassard, a graduate student in Lamarche’s laboratory and the first author of the new study. “So the idea was to compare two different food sources of saturated fats, which in our project was butter compared with cheese, as well as to compare that with other nutrients,” he says. In addition to cheese and butter, the researchers also gave participants diets rich in monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and carbohydrates.

Brassard and his colleagues compared the effects of these five diets on a number of cardiometabolic risk factors, including blood lipid concentrations and blood pressure. “One of the most interesting findings of this paper was that butter induces a significantly greater increase in LDL cholesterol than cheese,” says Brassard. “So saturated fat in cheese and saturated fat in butter do not behave the same when they are digested,” he says. “A new thing was that the effects occurred especially among the people who had a greater concentration of LDL cholesterol to start with,” says Brassard.

HDL cholesterol concentrations were similar after the cheese and butter diets, although they were significantly higher than after the carbohydrate diet. There was no significant difference between the effects of the diets on inflammation markers, blood pressure, and insulin-glucose homeostasis.

The differences in the effects of butter and cheese are consistent with previous studies. “Other randomized control trials in the past have shown that saturated fats in butter and cheese behave differently when they are eaten in equal amounts,” says Brassard. In addition, a meta-analysis showed that for a similar intake of saturated fats and ratio of SFAs to PUFAs, the consumption of hard cheese significantly reduced LDL cholesterol and HDL cholesterol compared with butter [5].

The researchers hypothesized that the cheese food matrix was responsible for the attenuation of the cardiometabolic effects associated with the consumption of SFAs. Previous studies suggested that the beneficial effects of certain dairy products on CVD risk appear to be mediated by the dairy matrix [7]. “There are also randomized control trials that supported the idea that the food source and the food matrix may influence the effect of saturated fats on health,” says Brassard [4].

The researchers tried to estimate the effect of the cheese matrix on cholesterol. “The whole idea was just to look at how much saturated fat was in the diet, and based on previously published equations, compare the predicted effect on LDL versus what was observed,” says Brassard [8]. “What was predicted and the observed concentration were different,” he says. “What we can say is that there are nutrients in cheese that are reducing the overall effect of saturated fats so that they do not induce as much change in blood lipids compared with butter,” says Brassard.

“What we show in this paper is that the food matrix matters, as different foods with the same nutrients can have different effects on cardiometabolic risk factors, mainly blood lipids and LDL cholesterol,” says Brassard. “So it’s important to look beyond nutrients and think in terms of foods,” he says.

It’s still unclear what components of the food matrix may be responsible for influencing CVD risk. “This is pretty elusive at the moment,” says Brassard. Researchers have hypothesized that the effects of the cheese matrix may be mediated by calcium, or minerals, or the presence of bacteria. “There are a couple of hypotheses, but nothing is clear at the moment,” says Brassard. A recent study by Lamarche’s lab showed that the cheese matrix modulates the effects of dairy fat on lipid metabolism [9]. “They showed that the cheese matrix influences digestion,” says Brassard. “So this is another possibility for why the cheese matrix induces a different response than the butter matrix,” he says.

Lamarche, Brassard and their colleagues plan to continue studying the effects of the food matrix. Another study of theirs looks at the effects of the food matrix on cholesterol efflux capacity, a marker of HDL functionality [9]. “We observed a food matrix effect, as butter induces a greater change in cholesterol efflux capacity than cheese,” says Brassard. “That study also supports the notion that the food matrix influences lipid metabolism,” he says.

These studies add to the growing evidence that the food source of saturated fats is important in assessing their health impacts. “From a public health perspective, it’s important to look beyond nutrients and think in terms of food, because different foods have different effects, and this is another example of that,” says Brassard.


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