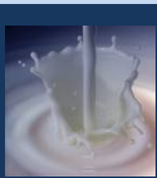
SPLASH! milk science update: July 2012 issue



SPLASH! milk science update
JULY 2012 issue

This month, we bring you new articles about one of the most influential dairy sires in history and how his genome is helping scientists unlock secrets about milk production, the immune properties of proteins found within milk exosomes, the link between the mind and the stomach, and a primer on glycoproteins and their role in milk.

Enjoy!

Fathering 60,000 daughters - he's got a lot to answer!

- **The genetic contribution of a very popular bull was tracked across six generations of dairy cows.**
- **DNA segments containing genes strongly selected by the dairy industry were identified.**
- **In the future, enriched haplotype segments can be used to greatly enhance the accuracy of genetic selection in breeding programs.**

He is legendary, fathering 60,000 daughters, most after his passing to greener pastures. He also sired many sons and grandsons who followed in his immortal footsteps. Walkaway Chief Mark is a name to remember!

Born in 1978, this single Holstein bull is currently responsible for roughly seven percent of the genomes in North American Holstein cows, which now populate many dairy herds throughout the world. He comes from a proud family heritage, which includes his famous father, Pawnee Farm Arlinda Chief, born in 1962. These are "two of the most influential dairy sires in history."

A recent study by Larkin et al.[1] reports the sequencing of the genomes of these two elite dairy sires. Using an innovative approach, the researchers tracked the inheritance of different fragments of the bulls' genomes, or haplotypes, in over one thousand descendents through six generations. The detail imbedded in this DNA detective story has provided unprecedented insight into the elite genetics underlying the productivity of today's dairy industry.

The industry has long exploited genetics to increase milk production. Over the last 50 years, an intense selective breeding process used only the best performing animals to improve milk output and composition. Remarkably, the average milk production per lactation has increased 380% from the 1940s to 2005 in the USA!

The improvement is driven by better management practices, particularly selective breeding employing artificial insemination coupled with extensive individual animal production records. Typically, each sire is ranked based on milk production, milk composition, and fertility traits in thousands of its daughters. Only the elite performing sires are then used in breeding programs.

This practice is called artificial selection as it is driven by humans to enhance desirable production traits in a population of domestic animals. It is no coincidence the first chapter of Darwin's revolutionary book, entitled *The Origin of Species*, focused on heritable changes in domestic animals. As Darwin alluded in his book, artificial selection is an exploitation of natural selection, the process that causes evolutionary change in a population.

Cattle have 29 related pairs of chromosomes and an X or Y chromosome, which together contain the full complement of the genome (or DNA). Small segments, or haplotypes, of DNA within a chromosome come from either the paternal or maternal genome. A haplotype is simply a group of genes inherited together as a segment of DNA from a single parent. The innovative approach taken by the researchers is called 'haplotracking.' It uses small genetic differences in DNA sequence segments to track the inheritance of sire-specific DNA through multiple generations.

Larkin et al. discovered over one million small genetic differences, or single nucleotide polymorphisms (SNPs), in the genomes of Walkaway Chief Mark and Pawnee Farm Arlinda Chief. The SNPs were then used to track haplotype segments characteristic of these elite sires in their descendents over six generations.

Forty-nine haplotype segments were enriched in the descendents, presumably because they harbour genes that contribute to the traits being strongly selected by the industry. From these segments, eleven genes were identified which have roles in milk production, fertility, and disease resistance. Strikingly, some of these genes are already implicated in contributing to economically important dairy production traits. The remaining genes will need to be confirmed in independent analyses.

The scientific strategy used by Larkin et al. exploits the benefits of an artificial and unplanned 'experiment' that has been an integral part of the dairy industry throughout the last half century. It demonstrates how rapidly progress can be made towards the identification of genes directly contributing to complex traits, a notoriously recalcitrant area of genetic research.

The challenge now is to find the exact genetic changes in the identified genes subjected to strong industry selection over the last 50 years and then to understand their biological implications.

Perhaps one of the treasures yet to be revealed in this type of study is the potential to identify regions in the genome implicated in dairy traits that lie between genes. These regions are likely to regulate gene activity from afar and may be key to fully understanding the genetics of complex traits like milk production and milk composition.

The potential benefit of this and related studies is the more efficient identification of new elite performing bulls, based on the identified enriched haplotype segments and genes, long before sire-based progeny testing. This has potential to accelerate the introduction of new genetic gain into a population and reduce industry costs. The information may also indirectly provide a quantitative measure of the extent of inbreeding, thereby flagging management intervention to maintain appropriate levels of genetic diversity within a herd. This is a desirable long-term aim.

The DNA from Walkaway Chief Mark and his father, Pawnee Farm Arlinda Chief, have provided a lot of answers. Their legacy is a group of specific DNA segments that is likely to dominate the dairy industry into the future.

Larkin DM, Daetwyler HD, Hernandez AG, Wright CL, Hetrick LA, Boucek L, Bachman SL et al. (2012) Whole-genome resequencing of two elite sires for the dtection of haplotypes under selection in dairy cattle*. Proc Natl Acad Sci USA*.109: 475-486.

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Milk vesicles uncovered

- **Over 2100 proteins located within exosomes in Holstein cow milk were identified.**
- **Many of these proteins likely contribute to immune system functioning in both the mother and calf.**
- **Due to the immune properties of these exosomal proteins, they may provide a new avenue for cancer treatment or other beneficial therapies.**

As dairy animals go, Holstein cows are record breakers. They produce more milk than any other breed or species, enough to fill a large tank truck over the course of their lifetimes. But the details of what's in their milk isn't entirely clear. Among the mystery ingredients are those that reside within exosomes, tiny membrane-bound packages that tote around proteins from their host cell. Now Timothy Reinhardt and colleagues working for the Agricultural Research Service of the US Department of Agriculture, in Ames, Iowa, have analysed the protein contents of these vesicles. The list of molecules they report offers intriguing suggestions about what exosomes do and why human milk has them, too.

Take, for example, the diversity of exosome proteins. Were exosomes merely a byproduct of the process of milk manufacture, they might have the same complement of proteins occurring in roughly the same proportions as can be found in milk fat globule membranes. That would seem likely if they were merely smaller or misformed milk fat globules. And at first blush it seems to be the case: the four most abundant exosome proteins also occur in globules. But they are relatively much less common in exosomes, composing just 0.4-1.2% of the exosome proteome, compared to 15-28% of that of milk fat globule membranes. So exosomes appear to be a second pathway of cell membrane secretion into milk.

In all, Reinhardt and his team identified 2,107 different proteins during their analysis of exosomes. Among them are molecules involved in cell-to-cell communication in the immune system and molecules that aid in transporting white blood cells into the blood stream. Other proteins within the exosomes are involved in a different kind of intracellular communication, which again suggests active biological roles for exosomes.

What are the exact functions of exosomes? This is the next step for this area of research. One idea is exosomes act as an anti-infective booster in milk, possibly reducing the incidence of mastitis as well as benefiting the calf.

This theory is backed up by other analyses. In human milk as well as cow's milk, exosomes have been shown to harbour miRNAs that can both transfer to immune cells and package and present antigens to immune cells--hints that they might modulate immune cell function.

Potentially, then, exosomes could be purified and used as drugs. So far, two Phase 1 clinical trials using human exosomes (from dendritic cells) to treat cancer have reported encouraging results. The idea is that protein complexes in exosomes called MHC class I complexes might prime T-cells to kill tumours. And now, following Reinhardt et al.'s paper, maybe a few more labs will be spurred on to look for potential therapies for cancer in humble Holstein milk. Species differences may play a complicating factor, but after all, it does contain MHC class I complexes.

Reinhardt TA, Lippolis JD, Nonnecke BJ, Sacco RE. (2012) Bovine milk exosome proteome. J Proteomics. 75:1486-1492.

Escudier B, Dorval T, Chaput N, Andre F, Caby MP, Novault S, Flament C et al. (2005) Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial, J Transl Med 3:10-22.

Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N. (2005) A phrase I study of dexosomes immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 3:9-16.

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Moods and foods

- **Foods turn on "reward circuitry" in our brains.**
- **Compared to soy milk or water, drinking cow's milk produced better moods in individuals.**
- **Ingestion of high-fat milk reduces stress responses in rat pups.**
- **The effects of human and cow's milk on neurobiology is an emerging research topic.**

Food is awesome. Most of us enjoy eating food. That's because when we ingest food --particularly foods rich in sugars, fats, and salt--the dopaminergic "reward" circuitry in the brain is activated. This triggers feelings of pleasure. In an ancestral environment, when such foods were rare, the pleasurable sensation would stimulate the adaptive response of gorging. Lately, this adaptive response tends to get us into trouble, for those of us "lucky" enough to live in the modern obesogenic environment (Power and Schulkin, 2009) because what was once rare is now plentiful. These adaptive signals from our gut to our brain can influence our emotions and moods in complex ways. Nutritious, delicious, or suspicious, our meals are mental.

Unfortunately, there are substantial gaps in our understanding of the relationship between food and the mind. Most investigations of the mind's response to meals have looked at conditioned response (think Pavlov's dog), marketing to shape consumer behavior, how particular dishes evoke memories (Grandma's apple pie), and cross-cultural food preferences. All of these areas of research have to do with social or conditioned learning about food. But knowing that 2+2=4 (something learned) is different than understanding why our minds are adapted to learn about math…or food. Food taps into social, cultural, emotional, and unconscious mental states, providing compelling evidence that our mind is on our gut, and our gut is in our mind.

Recently Geier and colleagues explored the relationship between food and mood. Their investigation of milk on mental well-being was conducted in sixty adult subjects in Germany. Subjects drank cow's milk and soy milk (water was a referent), and then responded on a Lickert scale how cold-hot, light-heavy, at ease-unease, alert-tired, invigorated-weakened, and comforted-melancholic they felt. In general, the researchers found that drinking cow's milk produced better moods in individuals than did drinking soy milk or water. It's not surprising that milk beat out water-calories excite our mind more than no calories. Similarly we could expect that the subject pool was more familiar with cow's milk than with soy milk. Therefore, the familiarity of cow's milk may have contributed to the better moods reported in response to drinking cow's milk.

Geier et al.'s study was pursued as a first step in developing a psychological test to understand the effects of food on mental well-being by demonstrating that consumption of particular foods produces detectable differences in mood states. But the pathways underlying that relationship are in need of greater research effort, particularly in understanding their establishment during early development. Compellingly, it has been documented that ingestion of high-fat milk reduces stress responses in rat pups (Trottier et al., 1998). Similarly, the neural circuitry of appetite is established in part by foods consumed during early development (Bouret & Simerly, 2006), and food preferences can be learned via breast-milk (Harris, 2008). Taken together, these findings reveal that our food selections, and the foods we feed our babies, may have important implications for psychological well-being.

Bouret SG & Simerly RB. (2006) Developmental programming of hypothalamic feeding circuits. *Clin Genet*. 70: 295–301.

Geier U, Hermann I, Mittag K, Buchecker K. (2012) First steps in the development of a psychological test on the effect of food on mental well-being. *J Sci Food Agric*. Early View.

Harris G. (2008) Development of taste and food preferences in children. *Curr Opin Clin Nutr Metab Care*. 11: 315–319.
Power ML & Schulkin J. (2009) *The evolution of obesity*. Baltimore: The Johns Hopkins University Press.

Trottier G, Koski KG, Brun T, Toufexis DJ, Richard D, Walker CD. (1998) Increased fat intake during lactation modifies hypothalamic-pituitary-adrenal responsiveness in developing rat pups: a possible role for leptin. *Endocrinology*. 139: 3704-3711.

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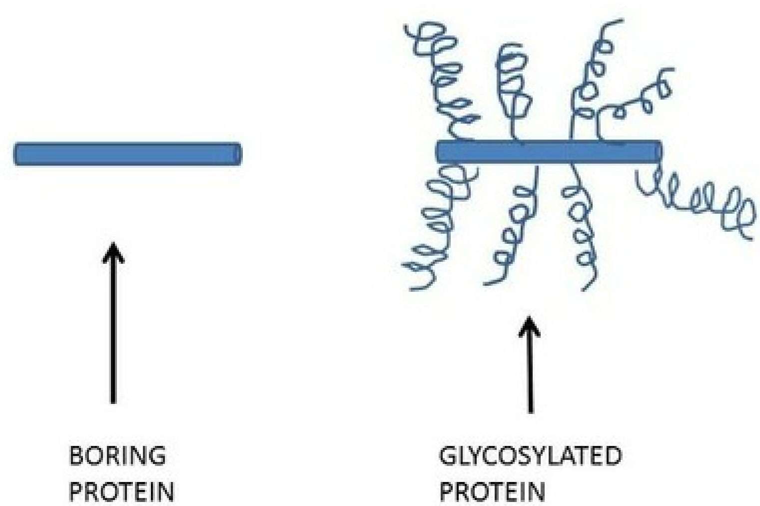
Glyco-what?

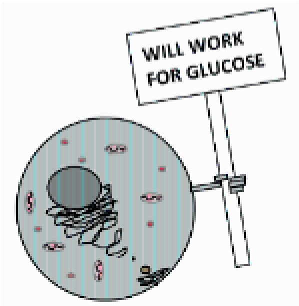
When is a milk protein not just a protein? Well, most of the time. Most proteins in milk are actually "glycoproteins"—protein and carbohydrates linked together. In fact, most proteins in general are glycosylated, meaning that carbohydrates are attached to them.

Some of us tend to ignore this fact because glycosylated proteins are more difficult to study than the same protein without carbohydrates.

Why do carbohydrates and proteins ever link together? Protein and carbohydrate molecules each have many possible biological functions; together, the number of things they can do multiplies.

One of the major functions of glycosylations on proteins is to facilitate communication. Think of a glycosylated protein (or "glycoprotein") as a person holding a bunch of different signs. The person is acting as the protein backbone and each sign as a set of sugar molecules. Cells, like people, need to communicate with each other, and these sugar molecules are the language of communication.





If the strings of sugar molecules were actually signs, imagine what they would say. "*WILL WORK FOR GLUCOSE.*" Seriously, though, some might say, "*I'm old, time to retire*" or "*I'm on your team*." You can imagine that "*I kill old cells*" would be searching for "*I'm old*" and that "*I kill foreigner invaders*" would be highly stimulated by "*I'm a foreign invader.*"

Another example of a function of glycosylations can be illustrated by mucins. Mucins, some of which are secreted into milk, are heavily glycosylated proteins. When mucins are secreted by the digestive tract, they form a gel-like layer to protect the intestine cells from the stuff being digested and the chemical soup that's doing the digesting. Basically, mucins keep our guts from digesting themselves! The plethora of glycosylations on a mucin's protein backbone is nature's way of saying Keep Out.

In addition to enabling communication, sugar molecules on a protein can also assist with cell-cell adhesion. When "*I am an immune cell*" is swimming through the blood stream, cells near a site of inflammation will do more than just display the glycoprotein sign, "*Help! Our neighbor is infected!*" They also display sticky glycoprotein "adhesion molecules" to slow down the infected cell killer and direct it to the cell that needs to be killed. Like hailing a cab for a friend, the neighboring cells stick out their carbohydrate side chains to hail the passing immune cells.

So what are glycoproteins doing in milk? Some proteins in milk are probably glycosylated for the same reason that many extracellular proteins are glycosylated: the sugar molecules help the protein keep a certain shape necessary for its function or to keep the protein from dropping like a rock out of the solution. It's difficult to move or digest "rocks."

Glycosylations on the really cool glycoproteins do a lot more. In "The many faces of lactoferrin," Ross Tellam eloquently described the latest research on the glycosylations of lactoferrin. If there was a gang of cool glycoproteins, lactoferrin would be leader of the gang. It is heavily glycosylated with many different sugar complexes, each of which target specific pathogens for binding and neutralization. If lactoferrin binds the pathogen, it can't then invade and infect gut cells. Instead of having a key to binding one or two pathogens, lactoferrin is like a locksmith; it has keys to bind an enormous variety of pathogens. Lactoferrin is the Swiss Army knife of locksmiths. The lactoferrin content of bovine milk alone should be worth more than the price of fluid milk today.

After the analytical chemists painstakingly dissect the structures of glycoproteins in milk, biologists still need to figure out what they do. It's time to decipher the writing on the signs.

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