Designer milk – An imminent milestone in dairy biotechnology

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Nutritional and genetic interventions to alter the milk composition for specific health and/or processing opportunities are gaining importance in dairy biotechnology. Altered fatty acid and amino acid profiles, more protein, less lactose and absence of β-lactoglobulin are some challenges of 'designing' milk for human health benefits. Alteration of primary structure of casein and lipid profile, increased protein recovery, milk containing nutraceuticals and replacement for infant formula are some of the processing advantages envisaged. Final acceptability of the newly designed products will depend on animal welfare, safety and enhanced health properties of the products and increased profitability vis-à-vis conventional practices.

RECENT evidences of epidemiological linkages between diet and chronic diseases have prompted the search for new clinical insights into the relationship between food and the onset (or prevention) of disease. In this context, the extra-nutritional therapeutic attributes of milk and milk products are no longer debated. Advances in biotechnology and genetic engineering have hinted at possibilities that were hitherto not fathomed in the field of dairying. It is now firmly established that a new generation of value-added products can be harvested from milk and milk products. While until recently, emphasis has been on breeding large animals to produce more milk, the attention is now tuned to adding more value to milk and studying its health implications. Milk composition can be altered by nutritional management or through the exploitation of naturally occurring genetic variation among cattle. By a thorough understanding of the biochemistry, genetic traits and changes in the cow's diet that affect milk synthesis and composition, ways and means to manipulate milk composition to suit specific needs can be found. By combining the two approaches of nutritional and genetic interventions, researchers are now hoping to develop 'designer milk' tailored to consumer preferences or rich in specific milk components that have implications in health as well as processing. This article exposes the readers to the potential that exists in altering the milk composition or 'designing' milk by nutritional and genetic approaches so as to achieve specific health and/or processing opportunities.

Opportunities of 'designing' milk

Milk is an ingredient that is consumed globally. The current interests, processing- and research-wise are the healthful and therapeutic aspects of milk and milk products. To realize the full potential of these advantages, it would be desirable to have the opportunity to alter milk composition in several ways. For diet and human health measures, the actions that would be beneficial are: (a) generate a greater proportion of unsaturated fatty acids (USFA) in milk fat, (b) reduce lactose content in milk in order to cater to persons suffering from lactose intolerance and (c) remove β-lactoglobulin (β-lg) from milk. From a technological point of view, there exist vast opportunities in: (a) alteration of primary structure of casein to improve technological properties of milk, (b) production of high-protein milk, (c) engineering milk meant for cheese manufacturing that leads to accelerated curd clotting time, (d) increased yield and/or more protein recovery, (e) milk containing nutraceuticals and (f) replacement for infant formula.

Modifications in protein

One of the major products of the mammary glands being protein, exciting research and technology opportunities extend the frontiers for better protein supplementation. Improved amino acid profile by the addition of L-taurine, L-leucine and L-phenylalanine offers additional nutritional benefits.

Transgenic cows secreting elevated levels of β-(8–20%) and κ-caseins (twofold) have been produced by genetic engineering[1]. β-casein, which is the most abundant milk protein, is involved in binding calcium phosphate and thus controlling milk calcium levels. Higher κ-casein content in milk is linked to smaller micelles, better heat stability, and improved cheese-making properties. In the transgenic animals engineered by Brophy's group, the total milk protein increased by 13–20% and total milk casein by 17–35% compared to non-transgenic control cows. This has obviously, a positive influence on the cheese yield and also

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on the casein and milk protein concentrate industry. Edible casein is used in vitamin tablets, instant drinks and infant formulas, whereas acid casein is used for paper coatings, cosmetics, button making, paints and textile fabrics[2].

Caseins, particularly the β-, α_{s1}- and α_{s2}-caseins, being easily digestible are quite sensitive to plasmin, a serine protease occurring naturally in milk along with plasminogen. Plasmin activity leads to limited proteolysis in milk. This offers a dual disadvantage of decreasing the curd yield in cheese and inducing organoleptic defects and gelation of ultra high temperature-treated milk. Milk augmented with specific inhibitors of either plasmin or plaminogen activator would therefore be a boon to the process industry[3].

A2 Milk™ from commercial dairy herds is being marketed in New Zealand and Australia at a small premium over regular or A1 milk. A2 Corporation scientists claim that as A2 Milk™ has only negligible amounts of the A1 β-casein in it, the perceived risks associated with the consumption of this type of casein (such as autism or Asperger's syndrome, child diabetes, schizophrenia and coronary heart disease) are effectively removed[4]. They further maintain that A2 β-casein was the original β-casein gene, whereas subsequent genetic mutation generated A1 β-casein. Hence, all milk produced by cattle thousands of years ago, before the large-scale domestication of cows, was A2 Milk™ (www.a2corporation.com). The animals producing A2 Milk™ are not genetically modified. It is achieved by selectively milking only those cows that naturally produce milk without A1.

Modifications in fat

The 'ideal' milk fat for human health would contain < 10% poly unsaturated fatty acids (PUFA), < 8% saturated fatty acids (SFA), and > 82% monosaturated fatty acids (MUFA)[5]. Although it may not be possible to achieve this 'ideal' milk fat composition, manipulation of composition of milk fat is possible through altering the feeding practices for dairy cows and also through genetic interventions.

Decreasing the level of saturation in milk fat

Feeding of unsaturated fats in an encapsulated or protected form results in a prompt rise in the degree of unsaturation of the serum lipids, tissue fat and milk fat[6]. Feeding highly unsaturated oils (e.g. soybean oil) caused depression in milk fat, but increased the proportion of USFA to SFA in milk (www.extension.iastate.edu). A study at the University of Alberta[7] revealed that feeding canola oil in the encapsulated form (to protect it from biohydrogenation by the rumen microorganisms) led to higher increases in linoleic (18 : 2) and linolenic (18 : 3) acids than while feeding unprotected oil seeds. As the melting point of milk fat containing USFA is more, the spreadability of butter made from this milk improved tremendously. An Australian study involving the feeding of a special blend of canola and soybean meal in the protected form resulted in doubling in the spreadability of butter[8]. When taken out of a fridge at 5°C, the butter was nearly as spreadable as margarine, without losing its special eating qualities. Clinical trials revealed that consumption of dairy products made from this milk led to decrease in LDL levels in the blood of the consumers.

Studies at the University of California (Davis) are focused on the desaturase gene to produce milk with decreased levels of SFA[9]. Efforts are underway to determine if genetic differences among breeds and individual animals are translated into ratios of SFA and USFA.

Increasing conjugated linoleic acid levels in milk fat

Dairy products are rich in conjugated linoleic acid (CLA), a product synthesized in the rumen during the biohydrogenation of linoleic acid. Table 1 lists the CLA content in selected dairy products. Research has shown that it is possible to influence the extent of ruminal biohydrogenation and the concentration of CLA absorbed and incorporated into milk fat. A diet rich in linoleic acid led to increasing the CLA levels in milk fat twofold[10]. Incorporating CLA along with soy oil in the diet of cows increased the CLA levels, simultaneously decreasing the SFA in milk fat[11]. Furthermore, milk from a grass-fed cow can have five times as much CLA as milk from a grain-fed animal (J. Robinson, 2003, www.eatwild.com).

Table 1. CLA content in selected dairy products

Dairy product	Total CLA (mg/g fat)
Whole cow milk	5.5
Condensed milk	7.0
Ice cream	3.6
Butter	4.7
Cultured buttermilk	5.4
Sour cream	4.6
Yoghurt	
Plain	4.8
Low fat	4.4
Non-fat	1.7
Frozen	2.8
Cheese	
Brick	7.1
Muenster	6.6
Sharp Cheddar	3.6
Colby	6.1
Mozzarella	4.9
American processed	5.0
Romano	2.9
Parmesan	3.0
Cottage	4.5
Ricotta	5.6

Source: www.nationaldairycouncil.org

Table 2. Health benefits of CLA as evidenced in selected animal models

Animal model	Condition monitored	Dosage/treatment	Response
Rats	Chemically induced mammary tumour	0.05–0.5% CLA in diet	Dose-dependent decrease in mammary tumour
	Chemically induced colon cancer	0.5% CLA in diet (administered by gavage)	Inhibition of formation of aberrant crypts in the colon
	Pregnancy and lactation	0.25 or 0.5% CLA in diet	Improvement in postnatal growth and feed efficiency of pups
Mice	Chemically induced stomach tumours	0.8 ml CLA in olive oil, (administered by gavage)	CLA-treated mice developed 50% as many tumours
	Chemically induced skin tumours	1.5% CLA in diet	Decrease in tumours with 1% dietary CLA
Rabbits	Cholesterol, blood vessels	0.5 g CLA/rabbit/day for 12 weeks	Decrease in total and LDL cholesterol and in atherosclerotic plaques in the aorta
Chicks, rats and mice	Body mass and weight	0.5% CLA in diet	Decrease in body fat, lean body mass and carcass water
Chicks and rats injected with endotoxin	Weight loss	0.5% CLA in diet	Decrease in post-injection weight loss

Source: Beaulieu and Drackley[10].

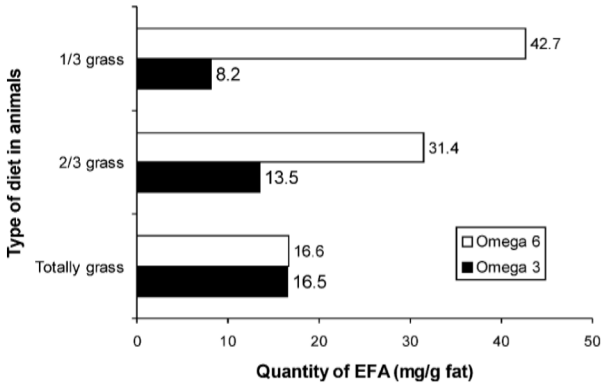

Figure 1. Effect of grass feeding on EFA content in milk. Source: Dhiman *et al.*[33].

CLAs reportedly suppress carcinogens, inhibiting proliferation of leukaemia and cancers of the colon, prostate, ovary and breast. They are the only natural fatty acids accepted by the National Academy of Sciences, USA as exhibiting consistent antitumour properties at levels as low as 0.25–1.0% of total fats[12]. The other reported beneficial health effects of CLA as supported by biomedical studies with animal models are antiatherogenic effect, altered nutrient partitioning and lipid metabolism, antidiabetic action (type II diabetes), immunity enhancement and improved bone mineralization[13]. Table 2 summarizes some of the health benefits of CLA as evidenced in selected animal models.

Animal variation is also a major source of differences in the milk fat content of CLA. Bauman and Perfield[14] discovered that the 9,11 isomer of CLA in milk fat is synthesized by the cow and not rumen bacteria as had earlier been reported. Synthesis involves a mammary enzyme, delta-9 desaturase, which acts on a trans-fatty (vaccenic) acid produced by rumen bacteria. Several genetic factors that regulate the expression of the delta-9 desaturase gene have been identified.

Omega fatty acids

Scientific research indicates that PUFA content in modern diets (nearly 30% of calories) is far too high. It is suggested that our PUFA intake should not be much greater than 4% of the caloric total, in approximate proportions of 2% ω-3 linolenic acid and 2% ω-6 linoleic acid (S. Fallon and M. G. Enig, 2000, www.aspartame.ca). Milk from pastured cows contains an ideal ratio of essential fatty acids (EFAs). It is evident from Figure 1 that replacing grass in the diet with grains or other supplements increases the proportion of ω-6 to ω-3 fatty acids. Too much ω-6 in the diet creates an imbalance that can disrupt the production of prostaglandins leading to increased tendency to form blood clots, inflammation, high blood pressure, irritation of the digestive tract, depressed immune function, sterility, cell proliferation, cancer and weight gain. On the other hand, deficiency in ω-3 is associated with asthma, heart disease and learning deficiencies. There are reports that roughly equal amounts of these two fats in the diet will result in lower risk of cancer, cardiovascular disease, autoimmune disorders, allergies, obesity, diabetes, dementia and some mental disorders (www.flax.com/newlibrary/ESSENT.html).

Reducing fat content in milk

As a variation to altering the fat composition, Wall *et al.*[15] suggested that modifying the cow's genetic makeup to enable it to produce milk with 2% fat would reduce the cost of feed per kg milk by 22%. In changing the fat composition, targetting enzymes that influence the synthesis of fat is important. As an example, reduction of acetyl CoA carboxylase that regulates the rate of fat synthesis within the mammary gland would translate to a drastic reduction in the fat content of milk and reduce the energy required by the animal to produce milk[16].

Type of fatty acids vs product quality

The type of fatty acids present in milk fat can influence the flavour and physical properties of dairy products. There are reports that butter produced from cows fed with high oleic sunflower seeds and regular sunflower seeds were equal or superior in flavour to the control butter[17]. The experimental butter was softer, more unsaturated and exhibited acceptable flavour, manufacturing, and storage characteristics. Extruded soybean and sunflower diets yielded Cheddar cheese that had higher concentration of USFA while maintaining flavour, manufacturing and storage characteristics similar to that of control cheese[18]. It is also beneficial from a safety point of view, as the accumulation of fatty acids, namely C12, C14, C18 : 1 and C18 : 2 enhanced the safety of cheese against *Listeria monocytogenes* and *Salmonella typhimurium*[19].

Modifications in lactose

Lactose, the milk sugar cannot be transported to the blood stream directly whereas the monosaccharides glucose and galactose which result from the enzymatic hydrolysis of lactose, can. For many human beings, the level of the hydrolysing enzyme lactase or β-galctosidase (β-gal) declines early in life to the point of virtual absence in adulthood. When such individuals ingest milk or milk products, the lactose remains undigested and mal-absorbed in the gut, where it causes retention of water by its osmotic action. This water retention coupled with the bacterial production of large volumes of carbon dioxide leads to intestinal upset and dehydration[20]. As milk is a major component in the human diet, lactose intolerance limits the use of valuable nutritional source for many people. In addition, since milk can provide much of the required calcium for maintaining bone health, lactose intolerance can also be associated with osteopaenia in later life – an issue of increasing importance in old people[21].

The consequences of intolerance can be limited by dietary changes such as avoidance of dairy products or through the use of β-gal-replacement (pre-harvest) or hydrolysed low-lactose (post-harvest) products. Each of these management strategies requires dietary supplementation and varies in its efficiency. Not only is there an associated economic cost, but such strategies also do not adequately satisfy the world's nutritional needs.

Pre-harvest methods of lactose reduction

α-lactalbumin (α-LA) is one of the major milk proteins present in almost all mammalian milk. It interacts with β-1,4-UDP-galactosyltransferase (UDP-gal) to modify substrate specificity of this enzyme, virtually creating a unique binding site for glucose and leading to the synthesis of lactose[22]. The pre-harvest methodologies of reducing lactose involve either the removal of α-LA and gene 'knock-out' methodologies or introducing the lactase enzyme into milk via mammary gland-specific expression. Although these successful approaches provide valuable tools to address milk physiology, they reduce the overall sugar content of milk, resulting in highly viscous milk. Studies on mice have revealed that reduction of lactose via α-LA deletion was inappropriate because it impaired milk volume regulation. The milk of such mice was highly viscous with high protein (88%) and fat (60%), no α-LA and no lactose[23]. Knocking out the UDP-gal gene in mice also produced milk with no lactose, but high viscosity[22].

An alternative to produce low-lactose milk is overexpression of β-gal in milk. However, the monosachharides produced within the formed milk increase the osmotic pressure within the alveolar lumen, thereby drawing more water and resulting in further dilution of other milk components[24].

Jost *et al.*[25] explained an *in vivo* technique for low-lactose milk production. They generated transgenic mice that selectively produced a biologically active β-gal in their milk. In these transgenic mice, the lactose content of the milk is at least halved, even though the β-gal expression levels were relatively low. The authors claim that it is likely that at least twofold greater levels of lactose-reduction could be achieved. In contrast to the previous studies by Bremel *et al.*[24] and Karatzas and Turner[23], these experiments led to reduction in the lactose content while retaining most of the monosaccharide content of the milk. In addition, transgene expression did not affect the milk protein levels, thus helping maintain a balanced nutrient supply as reflected in the similar growth curve reared on transgenic or control milk. It is likely that transgenic low-lactose milk production could offer a more balanced approach to managing lactose intolerance than post-harvest or lactose-replacement products. It is also technically feasible to produce transgenic livestock carrying this transgene. It is likely that similar or better expression levels could be achieved.

Humanization of bovine milk

It is said that mother's breast milk is the ultimate designer food for babies. However, due to varying reasons, a number of infants are fed formulas based on bovine milk. The composition of these formulas could be greatly improved to suit the needs of the infant by incorporation of ingredients that resemble those of human milk, thereby 'humanizing' the bovine milk.

Lactoferrin (LF), the iron-binding protein has antimicrobial properties and may also mediate some effects of inflammation and have a role in regulating various components of the immune system. Its level in human milk is about 1 g/l (in human colostrum about 7 g/l). As the levels of LF in cow's milk are only about one-tenth that in human milk, this has caught the attention of those involved in designing human milk replacement formulas. Pharming, NV (Leiden, The Netherlands) developed the first transgenic bull in

the late 1980s and a line of transgenic cows to produce several proteins, including human LF (hLF)[26].

Human milk contains 0.4 g/l of lysozyme (LZ), an enzyme that provides it with antibacterial activity. Active human lysozyme (hLZ) has been produced in the milk of transgenic mice at concentrations of 0.78 g/l (ref. 27). On the processing front, the expression of LZ in milk results in the reduction of rennet clotting time and greater gel strength in the clot. A double transgenic cow that co-expresses both hLF and hLZ in milk may also reduce the incidence of intra-mammary infection or mastitis.

Yet another application of transgenic technology could be to produce the human lipase, which is stimulated by bile salt in the milk of bovines. The lipase thus produced could be used as a constituent of formulas to increase the digestibility of lipids, especially in premature infants who have low β-gal activity[28]. From a study involving African-Americans between the ages 12 and 40 years, Johnson *et al.*[29] concluded that the cause of milk intolerance in as many as one-third of the subjects claiming symptoms after ingestion of a moderate amounts of milk was not its lactose content.

Cow milk allergenicity in children is often caused by the presence of β-lg, which is absent in human milk. Elimination of this protein by knocking out β-lg gene from cow's milk is unlikely to have any detrimental effects on either cow or human formula and might actually overcome many of the major allergy problems associated with cow's milk. Further, as milk protein allergenicity studies demonstrate that all food proteins are potential allergens and that allergenic structures are widely spread throughout the protein molecule, milk is a good model in the search for means of characterizing allergenic structures in food[30]. Therefore, while developing strategies for the identification and evaluation of potential allergenicity in novel foods, many of the technological practices used in the assessment of milk protein allergenicity can be adapted.

Milk with human therapeutic proteins

Industrial interest has focused on the production of high value, low volume therapeutic proteins in the milk of domestic animals. In this context, several human proteins have already been expressed with success. GTC Biotherapeutics (Framingham, MA) uses both goats and cows to produce more than 60 therapeutic proteins, including plasma proteins, monoclonal antibodies and vaccines. One product that is in late stages of testing is recombinant human antithrombin III (produced in goat milk), an anti-coagulant protein found in blood[26]. GTC is also working on a project to develop a malaria vaccine from goat milk. It is understood that a litre of goat milk can contain up to 9 g of the transgenic protein and that eight goats can produce enough vaccine to inoculate 20 million people. The cost to produce a transgenic protein in goat milk can thus be 3 to 30 times cheaper than the current method using mammalian cell culture. PPL Therapeutics (Edinburgh, UK and Blacksburg, VA) is working with rabbits and sheep to produce α-1-antitrypsin, fibrinogen, and a lipase to treat pancreatic insufficiency in digesting dietary lipids. Products such as insulin and growth hormone have also been obtained from the milk of transgenic cows, sheep or goats (E. T. Margawati, 2003, www.actionscience.org). The major advantage of transgenic technology is that proteins can be produced at a low cost. Economic comparison of production costs of human tissue plasminogen activator (htPA) through bacterial fermentation, mammalian cell culture and cow transgenic technology estimates the cost/g of htPA to be 20,000, 10,000 and 10 US dollars respectively[23]. Transgenic animals can also secrete proteins such as blood clotting factors needed by human haemophilia-sufferers in their milk[31].

Miscellaneous advantages

The use of molecular biology to reduce the presence of pathogenic organisms in milk is a potentially advantageous prospect. It might be possible to produce specific antibodies in the mammary gland that are capable of preventing mastitis infection or those that aid in preventing human diseases. Thus, one can foresee antibodies against salmonella, lysteria or other pathogens that will produce safer milk products. While recombinant immunoglobulins have been expressed in mammalian transgenic milk (J. V. Gaviilondo and J. W. Larrick, 2002, www.alai.cigb.edu.cu), a calf with a gene that promotes the growth of red cells in humans has been produced by transgenesis (www.publicscan.fi). Research is also underway to manufacture milk through transgenesis for treatment of diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis (E. T. Margawati, 2003, www.actionscience.org).

In an interesting combination of sericulture and dairying, goats that produce spider silk in milk have been engineered. When spider genes were introduced into the cells of lactating goats, they secreted silk in tiny strands along with their milk. These polymer strands could be woven into threads after extracting them from the milk and used for applications such as military uniforms, medical microsutures, and tennis racket strings[32].

The future

Mice that produce milk with 33% more total solids (40–50% TS) and 17% less lactose than normal control mice have been generated by transgene. As the increase in the TS is associated with a decrease in total milk volume, the same amount of total milk fat and protein is being produced in a lesser total milk volume. If this technology could be propagated in dairy animals, milk that contains 6.5% protein, 7% fat, 2.5% lactose and 50% less water is not an improbable accomplishment. The advantages would be (a) direct economic benefit in terms of 50% reduction in the cost of shipping milk, (b) less stress on the cow and on

her udder, since the cow would be producing one-half her normal volume of milk, (c) skim milk with twice protein content and half the lactose content of normal milk, (d) easier to produce low lactose or lactose-free dairy products, (e) better product yields due to concentration, (f) reduction in total whey output because of low milk volume and lactose content and (g) decrease in mastitis as less lactose available for organisms.

Despite all these promising prospects, there is a tendency among human beings to resist change, especially those that trouble their feeling and instincts. As all changes that arise as a consequence of biological research would fall into these categories, there is bound to be tremendous resistance to topics such as transgenics. The future of biotechnologically derived foods is, therefore, at crossroads even after two decades of positive results. Hi-tech milk processing may be more acceptable to consumers than transgenesis for altering milk composition. Controversy will inevitably tint all biotechnological manipulations aimed at increasing milk production or altering milk composition. Ultimate acceptability will depend on the four key factors of animal welfare, demonstrable safety of the product, enhanced health properties of the product and increased profitability compared with conventional practices. Various ethical, legal and social aspects of biotechnological research need to be addressed before we would see designer transgenic herds similar to the organic herds that thrive in the current economic and social climate.

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