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Review Article

Nutritional Significance of Designer Milk

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Introduction

With the advancement of science, consumer education, purchasing power and health consciousness, the consumers want diversified, safe and healthy foods. This demand for healthy foods has necessitated the concept of designer milk, egg and other food products. Until recently, emphasis had been on breeding animals that produced more milk. But now attention is to add more value to the milk and studying its health implications. The developments in the field of genetic engineering and biotechnology, resulting in the production of a novel milk called "designer milk". The designer milk is nutritionally enhanced milk tailored to consumer preferences or rich in specific milk components that offers the consumer, greater health benefits, ranging from boosting immunity to relieve diarrhoea.

There has been a decline in livestock product consumption viz. egg and egg products by 50 per cent, liquid milk by 20 per cent, butter by 70 per cent and meat by 11 per cent over the last two decades. Hence to capture the consumer preferences for animal products, it would be beneficial to modify the products in such a way that the health benefits are imporoved with minimum dietary risks. Thus manipulation of milk composition by transgenesis has mainly focused on the use of mammary gland as a bioreactor to derive health benefits as well as to improve the technological properties of milk.

Scope and Benefits

Milk is a major component of the diet in the world. For certain purposes, it would be desirable to have the chance to change the milk composition.

From the dietary and human health point of view:-

- A greater proportion of unsaturated fatty acids in milk fat, would be appreciated
- Possibility to reduce lactose content in milk in order to make it accessible also for persons with expressed lactose intolerance.
- Milk devoid of beta lacto globulin

From technological point of view:-

Alteration of the primary structure of casein helps to improve technological properties of milk. Dairy producers would have the opportunity to choose to:

- Produce high protein milk
- Milk destined for cheese manufacturing that has accelerated curd clotting time, increased yield and more protein recovery.
- Milk containing nutraceuticals.
- Replacement for infant formula.

Transgenic Technology

A transgenic animal has been defined as an animal that is altered by the introduction of recombinant DNA through human intervention. Milk composition can be dramatically altered using this gene transfer. Classical studies have revealed the association between major lacto-protein variants and milk production traits. Introduction of DNA technology in the diary science field has enabled to identify new genetic polymorphism and revealed molecular background of lactoprotien gene expression. Subsequently, several emerging transgenic technologies have focused on the mammary gland. The process involves steps like a DNA construct is designed and built to express the desired protein in animal. The construct is then introduced in to a single cell embryo to allow incorporation of the transgene into animal genome. There are several methods available for this purpose, including retroviral transmission, stem cell transfection and micro injection in to the pronucleus or cytoplasm.

Why use Transgenic technology?

There are various methods of protein production available commercially, including bacterial, fungal and mammalian cell culture systems. However they each suffer to varying degrees from two major limitations. First, many therapeutic proteins have specific configuration that is necessary for activity. They frequently require quite complex post transitional modifications. Bacterial expression systems can not perform most of these modifications. Secondly the cost of building and running modern production facilities for cell culture is extremely high.

In the transgenesis technology, mammary gland is used as bioreactor or as an efficient vat, for the production of specific protein, fat and sugar. Compared to microbial systems or tissue culture, mammary gland of animals have greater regulatory acceptability of transgenes and provide enormous volumetric productivity, since the concentration of endogenous protein is in the order of 4 - 6 per cent and this proteins can be easily recovered without going to complex purification techniques. Moreover, the high production of milk by proven cattle breeds, with capacious and well developed udder system, will reduce the cost of production to a minimum. Producer transgenic animals can be multiplied by embryo technology and emerging methods. Moreover, mammary gland is able to produce post transitional modifications, which is required for stability of proteins.

Designer Milk and its Nutritional Significance

A) Changes in Milk Fat :-

Manipulation of composition of milk fat is possible through dietary manipulations. Studies have demonstrated significant change in the iodine number of milk fat, when the diet was changed from a high to law degree of unsaturation of milk fat [Grummer, 1991].

A “designer cow” called Daisy which can produce semi skimmed (half-fat milk) milk and butter that spreads straight from the refrigerator (soft spreading butter), has been bred by Britain’s Agriculture Developments and Advisory Services (ADAS). Cows fed with fish oil, fish meal or plankton can produce milk rich in Omega - 3 fatty acids, resulting health benefits, including reduced risk of cardiovascular diseases, enhanced development of brain and visual sharpness in infants and improved immunity. Similarly for half fat milk, supplement is dehusked oats and for spreadable butter is rape seed oil. Dietary fat such as corn oil, fed to the cows, in a protected form is found to cause an increased level of conjugated linoleic acid (CLA) in their milk. CLA is found to be one among the most potent anticarcinogens occurring naturally, which can inhibit cancer growth even in extremely low concentration of 0.5 per cent. In addition to feeding supplemental unsaturated fat, unsaturated fatty acid content of milk can be increased by reducing bio-dehydrogenation in the rumen, by reducing the ruminal pH [Latham *et al.*, 1972].

Benito and Carlos [2006] conducted a study on the effect of Omega 3 fatty acid enriched milk on the cardiovascular risk factors linked to metabolic syndrome. The enriched milk, contained Omega-3 fatty acids EPA (0.003 g/100 ml) and DHA (0.034 g/100 ml), oleic acid (1.14 g/100 ml), folic acid (30 ùg/100 ml), and vitamin E (1.5 mg/ 100 ml). After three months of supplementation, the researchers reported a significant reduction of 13 per cent in triglyceride levels, six per cent in total cholesterol levels, 7.5 per cent in LDL-cholesterol, and 5.7 per cent in alipoprotein B in the test group but not the control group. Omega-3 enrichment of dairy products is on the increase in Europe with 20 new launches of enriched milk and 19 omega-3 enriched yoghurts hitting European shelves in 2005.

Identification of Genetic markers associated with differences in composition and structure of milk fat help the researchers, to selectively breed the cows, which naturally produce low milk fat. These genetic researches are based on desaturase gene to select animals that will produce safer, healthier and more marketable milk fat. eg:- stearoyl coA desaturase appear to have influence on concentration of conjugated linolenic acid in the milk fat and a reduction in the enzyme Acetyl coA carboxylase, will lead to a dramatic reduction in the fat content of milk and reduce the energy required by the animal to produce milk.

B] Changes in Milk Protein

Mammalian milk contains six major proteins, which are classified into two groups, the casein consisting of alpha S1, alpha S2, ß and k casein and the whey proteins viz. alpha lactoglobulin and ß lactoglobulin. Specific alteration in the properties of milk proteins or the addition of heterologous proteins into the milk can add variety to the dairy products in terms of chemical composition and lead to development of desired dairy products. Several potential changes made are listed in the table 1.

Table1. Potential changes in milk through genetic engineering

Potential modifications	Changes in the milk
Increase in casein content	Increased protein, better manufacturing properties
Engineered casein	Better manufacturing properties
Remove beta lacto globulin	Better manufacturing properties
Remove fat	Easier to produce low fat milk products, decrease the butter surplus, increase solid content.
Produce beta galactosidase, lactose, produce antibodies of pathogens	Safe food, mastitis prevention

I. Increasing k- casein content :

In the mammary gland, k- casein is associated with prevention of calcium induced precipitation of alpha S1, S2 & beta casein. A higher k- casein content, was found to be associated with smaller micelle size and heat stability of milk was markedly improved by addition of k- casien.

2. Modification of ß casein in milk:-

An increase in ß casein in milk proteins can increase the firmness of the curd by 50 per cent. ß casein after the removal of their phosphorus moiety when added to artificial micelles can increase clotting time and decrease effusion of whey. These attributes are extremely valuable during manufacture of different cheese varieties. Another modification that has been made to ß casein is the deletion of plasmin cleavage site causing prevention of bitter flavour in cheese due to plasmin cleavage.

3. Increasing the alpha S1 and alpha S2 casein content :-

A higher content of alpha S2 casein, which contains 10-13 phosphoserine and two cystein residues in the bovine species would obviously increase the nutritional value of casein, which is deficient in sulphur containing amino acids; it also increase micelle stability and helps to improve the cheese yield.

4. Producing milk devoid of ß lactoglobulin :-

Milk is an allergic trigger in a significant fraction of infants and ß lactoglobulin, which is not found in human milk is believed to be one of the main culprits. Elimination of this protein from cow's milk

Table. 2 Transgenic mammary expression of proteins

Protein expressed	Produced in the milk
Ovine ß lactoglobulin	Mice
Bovine alpha lactalbumin	Mice
Bovine ß -casein	Mice
Bovine alpha-S1 casein	Mice
Human clotting factor IX	Mice, sheep
Human alpha-1 antitrypsin	Mice, sheep
Human tissue plasminogen activator	Mice, goat
Human lactoferrin	Mice
Human urokinase	Mice
Ovine trophoblast interferon	Mice
Human protein C	Mice, pig.

is unlikely to have any detrimental effects, on either to cow or to man and might overcome many of the major allergic problems associated with cow's milk. The use of ribosome anti ß lactoglobulin m RNA is found to be helpful to prevent synthesis of ß lactoglobulin in bovine species. Examples of genes introduced in to the transgenic animals with resultant production of different types of protein in milk are listed in table 2.

C. Changes In Milk Sugar:-
Reduction of lactose in milk:-

Transgenic low lactose milk production could offer a more balanced approach for managing lactose intolerance than current post harvest or lactose replacement products. An attempt had been done, in transgenic mice, by Jost and colleagues in 1999 by introducing a gene, which induce the production of enzyme, phlorizin hydrolase (lactase), in mammary gland and in these mice, it is found that lactose content of the milk is reduced by 50 per cent. This enzyme is normally present in small intestine, whose absence causes, lactose intolerance. Previously attempts to generate low lactose milk in vivo have targeted, gene knock out or ribosome approaches to reduce the activity of lactose synthetase binary complex. Now, it is technically feasible to produce transgenic livestock carrying this transgene, with similar or better expression levels.

D. Human Like Milk :-

A human like milk, enriched with human lysozyme and lactoferrin might enhance defence against gastrointestinal infections and promote iron transport in the digestive tract. A construct comprising human lysozyme cDNA driven by the bovine alpha S1 casein promoter yielded a high level of lysozyme in mice [Maga *et al*, 1994]. Obtention of a transgenic bull carrying a human lactoferrin construct was also reported [Krimpenfort *et al*, 1991]. Milk with human lipase helps to increase the digestibility of lipids in infants. The aforementioned studies drive towards humanization of non human mammal milk.

E. Increasing Total Solids, Reducing Lactose and Water Content of Milk:-

Animals that produce milk with 33 per cent more total solids and 17 per cent less lactose than normal animals have been generated by trasgene. In transgenic mice, the activity of enzyme lactose synthatase with in the mammary gland is decreased by 35 per cent. Due to decrease in lactose synthatase activity, less lactose is being produced, less water is being transferred in to milk, causing a reduction in the milk volume. Using the technology, it is possible to get milk from dairy cow, that contains 6.5 per cent protein, 7 per cent fat, 2.5 per cent lactose and 50 per cent less water. It would have a number of economic and processing benefits viz. 50 per cent reduction in the shipping cost of milk and since the cow would be producing one half her normal volume of milk, there would be less stress on the cow and on her udder. On manufacturing side, after removal of fat, skim milk having twice protein content of the normal milk could be produced. This type of milk, would also make it easier to produce low lactose or lactose free "hard dairy products". There is decrease incidence of mastitis as less lactose is available for organisms.

F. Milk with Human Therapeutic Proteins:-

Manipulation of milk composition by transgenesis has mainly focused on the use of mammary gland as bioreactor to produce pharmaceuticals, primarily for economic reason. Biomolecules of pharmaceutical and medical importance have recently been expressed in the milk, by tagging the genes of interest to the promoters, derived from genes encoding milk proteins.

Transgenic animals can secrete needed proteins such as blood clotting factors needed by human hemophilia sufferers, in their milk. Genes encoding useful human proteins such as alpha 1- antitrypsin ATT, have been inserted in to sheep that express in their milk. A serine protease, called plasmin, occurring naturally in milk, will limit the proteolysis of casein, there by decrease the curd yield and can produce organoleptic defects and gelation of UHT milk. By transgenesis, it is possible to induce, the expression of gene, for production of specific inhibitor of either plasmin or plasminogen activator helps to overcome this problem. It is possible to produce goat milk with recombinant human anti-thrombin III - an anticoagulant seen in human blood. Rabbit and sheep milk with alpha

1- antitrypsin, fibrinogen, and lipase to treat pancreatic insufficiency in digesting dietary lipids has also been produced. Insulin and growth hormone have also been obtained from the milk of transgenic cows, sheep or goats. The major advantage of transgenic technology is that proteins can be produced at a low cost. Milk through transgenesis for treatment of diseases such as phenylketonuria, hereditary emphysema, and cystic fibrosis has also done.

G. Specific Antibodies and Resistance to Infection

It has also been shown that specific antibodies can be produced in transgenic animals. It might be possible to produce antibodies in the mammary gland, that are capable of preventing mastitis infection or antibodies that aid in preventing human diseases. Milk with antibodies against Salmonella, Listeria and other human pathogens have been produced. Economic production of Malaria vaccine from goat milk has been tried by GTC Biotherapeutics

Status of Designer Milk and its Future Prospects

Recent universal concerns over genetically modified foods would tend to suggest that any company, attempting to mass market, such products is going to run in to serious problems, for sometime. It will be difficult to give assurance that transgenic animals only contains, the desired genetic alteration, because, it is quite possible that, additional random genetic modifications are introduced in to a genome, during the process of selection for desired alteration. Controversy will inevitably surround all biotechnological manipulations aimed at increasing milk production or altering milk composition. Ultimate acceptability will depend on four factors

- Animal welfare
- Demonstrable safety of product
- Enhanced properties of the product and
- Increased profitability, as compared with conventional practice.

Various ethical, legal and social aspects of biotechnological research need to be addressed through public education to bring about greater understanding of the issues involved and to find classical solutions for the existing issues. In the foreseeable future, herds producing many different types of milk will presumably be available allowing consumers and industrials to choose the milk best adapted to their needs !!!

Conclusion

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 α-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.

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