MILK COMPOSITION IN THE COW

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Introduction

Milk is traditionally recognized as an excellent source of nutrients and milk proteins constitute up to 20% of the average food protein intake in the United States and Europe. The dairy industry provides a range of high quality traditional dairy products but is also very interested in the development of new generation, dairy-based nutraceutical and functional foods. Milk components, because of their availability, composition and biological activity, offer an excellent medium for exploration.

The great diversity of possible approaches to altering milk composition is a consequence of the complexity of the physiological processes that underpin lactation. In theory, there seems to be no limit to the possibilities for altering milk composition. The potential of the milk-secreting cells (i.e., mammary epithelial cells) is enormous. In practice however, the functional and biological constraints of the mammary epithelium will pose real limitations on the opportunities for altering its secretory composition. Limits to manipulating milk composition will be imposed, for example, by functional characteristics of mammary cells, the finite life span of mammary cells, functional trade-offs between milk components, the requirement for osmotically active secretion, the need to produce carbohydrates in a form suitable for storage, maternal nutritional requirements, and the need to maintain antimicrobial properties that protect the mammary gland. But even in view of these limitations there remains great potential to alter the composition of cow's milk.

There are currently two areas with obvious potential for practical value to influence milk composition; those related to the provision of substrates for milk synthesis (e.g., animal feeding and management, nutrient digestion and utilization, mammary gland function) and those related to the genetics of the cow. The proteins in milk can also be exploited as food ingredients based on their diverse molecular structures, physio-chemical properties, and interactions with other food ingredients. These properties of milk will undoubtedly be extensively exploited in the rapidly expanding world-wide market for nutraceuticals and functional foods. Research interest in exploiting milk proteins as a source of biological and (or) effective ingredients has focused in three main areas: 1) selective isolation of individual protein and peptides to produce better functional or bioactive ingredients, 2) selective hydrolysis and isolation of milk proteins to produce specific peptides, and 3) examination of the biological activities of milk proteins and peptides.

Genetic Approaches to Altering Milk Composition

There are two general strategies by which genetics can be used to alter milk composition. One strategy involves correlating differences in DNA (genes) between cows with specific production traits (e.g., milk composition) and then using the information in breeding programs to increase the number of cows with the more desirable genes, and thereby the frequency of the gene in the dairy population. In essence, conventional breeding has applied this genetic strategy, but without knowledge of the actual genes being selected. New approaches involving marker assisted selection are being developed that promise

more accurate selection for both known genes as well as unknown genes linked to genetic markers.

The other genetic strategy that can contribute new and useful information for modifying milk composition involves identifying and gaining a better understanding of the genes with important roles in determining milk composition. In particular, knowledge of the intricacies of the regulation of the expression of those genes will provide the basis for the development of novel approaches to altering milk composition. A better understanding of the genes and how they interact may also facilitate the development of practical applications of transgenic technology.

Genetic selection and breeding using principles of quantitative genetics

Humans have been using genetic selection to modify the composition of bovine milk since domestication began. Using conventional biometrical selection strategies, the main method of cumulative genetic improvement of economic performance has been by selection on heritable performance traits. Most recently, the dairy cattle industry has realized accelerated genetic progress as a result of centralized record keeping and milk testing programs, widespread use of artificial insemination, and more accurate genetic evaluation using selection index methodology (Hazel et al., 1994). Indeed, rates of genetic improvement for yield of milk, fat, and protein have increased dramatically in recent decades. Though modern dairy breeding programs provide opportunity to alter milk composition, changes are achieved only over the long-term. Future genetic progress in dairy breeding programs is expected and should be enhanced through the use of marker assisted selection strategies for both known and unknown genes. Future progress will continue to depend upon well defined breeding objectives, availability of relevant records, and the effective application of advanced selection index theory.

Exploiting lactation characteristics (genes) of other species

Studies in comparative biology of the over 800 species of mammals are necessary to identify potentially useful characteristics of lactation that can be understood, traced to their genetic basis, and exploited to modify or enhance bovine lactation (for review see Blackburn, 1993; Hayssen, 1993). The extraordinary functional flexibility of the mammary gland is shown in marsupial specializations such as concurrent asynchronous lactation and ontogenetic changes in milk quality. An understanding of how evolution has shaped the mammary gland may provide meaningful insight for humans to alter its function and secretary composition.

Genotyping for unknown (anonymous) genes

Recent advances in molecular genetics are currently being applied to enhance progress in mapping the bovine genome (Barendse et al., 1994; Bishop et al., 1994; Solinas-Toldo et al., 1995) and to locate economically important genes, commonly referred to as quantitative trait loci (QTL). Current mapping techniques use genetic markers called microsatellites to uncover regions of DNA on bovine chromosomes that are informative with respect to a specific measurable phenotypic trait, such as horn development and specific diseases (Charlier et al., 1996; Georges et al., 1993; Georges 1997; Rothschild 1995). Microsatellite markers that are linked to QTL can be used for genotyping. Genotyping is the technique used to identify which form (i.e., allele) of a gene an animal possesses. As with genotyping for known genes, genotype information on anonymous genes (revealed using microsatellite markers) can also be incorporated into breeding

strategies to influence nonproduction or production traits, including characteristics of milk composition (Georges et al., 1995). Disadvantages of the microsatellite marker approach are the requirement for within family analysis of performance data and the confounding need for many animals in order to delineate marker linkages to QTL. Much effort has already been expended on typing with available microsatellite markers and record keeping in attempt to locate QTL for relevant economic traits. The establishment of new and better reference families, ultimately a universal reference family, and more detailed linkage maps will provide the framework for enhancing our ability to uncover linkages to genes controlling economically important traits. International collaboration, to prevent the duplication of research effort, and the use of new information and technologies from the Human Genome Project will also enhance understanding and progress in cattle genetics. Progress will come from emerging molecular technologies like DNA chip technology (Gerhold et al., 1999), which is expected to speed the identification of genetic variation through the use of SNPs (single nucleotide polymorphisms, pronounced “snips”). The identification and use of SNPs present in the bovine genome will enhance the resolution for future linkage mapping studies.

Genotyping for known (candidate) genes

Our present understanding of lactation physiology, including knowledge of the biochemistry of the processes determining milk composition, provides the necessary information to begin genotyping cows for desirable alleles of known genes with the aim of establishing correlations with milk composition. Obvious examples of genes to target include those encoding the major milk proteins (e.g., caseins, β-lactoglobulin), those encoding hormones implicated in regulating lactation and mammary development (e.g., growth hormone and insulin-like growth factor-I), and those encoding enzymes involved in fat metabolism (e.g., fatty acid synthase, acetyl coenzyme A (CoA) carboxylase, lipoprotein lipase, stearoyl CoA desaturase). When correlation between specific alleles of the genes and milk production or composition are established, it will be possible to incorporate the information as selection indices into dairy breeding programs (Falaki et al., 1996; Sabour et al., 1996). As a result of genotyping (marker assisted selection), genetic selection will be more accurate and thus genetic progress will be enhanced.

Routine genotyping would be of great value to the dairy industry. There has been extensive research on milk protein genetic variants (for review see Jakob and Puhan, 1995) including many studies demonstrating that certain phenotypes of κ-casein (κ-CN) and β-lactoglobulin (β-LG) are associated with specific composition and processing properties of milk (Bovenhuis et al., 1992; Lin et al., 1989; Mao et al., 1992; Ng-Kwai-Hang et al., 1990). The research has also revealed which κ-CN and â-LG genetic variants (alleles) are superior for cheese manufacturing. That knowledge prompted European countries (e.g., Austria, Germany, Italy, Switzerland) to begin genotyping dairy sires and dams for milk protein genetic variants. The resulting genotype information has already been incorporated as selection criteria in dairy breeding programs. Initiatives to exploit the genetic variability of milk proteins to enhance, through selection, the properties and cheese yield of the future milk supply represent an effective and simple beginning to a new era in dairy cattle breeding. Similar research on milk protein variants in France led to intensive selection for desirable α_{S1}-casein alleles in goats, whose milk is mainly transformed into cheese (Martin et al., 1995). The modified breeding strategy has already changed allelic frequencies and thereby contributed directly to improving the cheese making quality of milk.

Techniques for genotyping have advanced rapidly. Over ten years ago there was

widespread excitement in animal agriculture about using a technique called restriction fragment length polymorphism (RFLP) analysis for genotyping. The development of RFLP analysis allowed the detection of many molecular differences in known genes, including most of the differences in the major milk protein genes. Although its development represented a major advance, newer molecular methods for gene analysis are now available (Lee et al., 1993; Mercier et al., 1994; Skogen et al., 1994; Timme and Thompson, 1994; Xu and Hall, 1994). The new methods are more versatile and less expensive than RFLP analysis.

One of the main obstacles to widespread application of genotyping in the dairy industry is that it requires a considerable financial investment. The need for an inexpensive method of genotyping many animals prompted our work in this area and led to the development of a simple and reliable genotyping procedure that is based on the use of state of the art molecular techniques (Glimm et al., 1996). The most impressive feature of the procedure is that it is performed in a single tube, which makes it amenable to automation and therefore suited to commercial application.

Molecular genetics applied to provide insight into lactation physiology

New techniques in cell and molecular biology are being used to unravel the details of the complex physiological processes underlying lactation (for review see Lander, 1996). Insight into these processes will in turn lead to novel strategies for altering milk composition as well as modifying other aspects of lactation. Areas of research with the most promise include mammary development and function, digestive physiology, nutrient utilization and metabolism, and mastitis and disease resistance.

Our current understanding of lactation is that gene expression in mammary epithelial cells lies at the heart of the mechanisms controlling milk synthesis and, therefore, milk composition. The genes of a cell carry all the information required to specify the structure and function of the cell. In essence, if a gene in the mammary gland is not turned on (i.e., not expressed), then the protein encoded by the gene will also not be found in the mammary gland. Similarly, if the expression of a certain gene is increased in the mammary gland of cow A compared to cow B, then there will likely be more of the protein (encoded by the gene) in the mammary gland of cow A.

The concept that gene expression is related to milk composition raises two extremely important questions: First, can the genes that control milk composition be found? The simple answer is *yes*. We now have the scientific ability, using molecular technology, to find all of the genes involved in regulating the synthesis of the components in milk. That task may seem impossible considering that about 15,000 genes are expressed simultaneously in each mammary cell. However, the application of new and powerful molecular technologies, such as expression genetics technology (Ausubel et al., 1994) should allow much more rapid progress in the future. The second important question about gene expression and milk composition is: if and when such genes are found, how can that knowledge be applied to benefit the dairy industry? The discovery of genes controlling milk synthesis would certainly represent a landmark advance in dairy cattle breeding. And cost effective genotyping technology can be used to overcome the practical problem of transferring such new and valuable information to the dairy industry.

Confirmation of differential expression of the candidate genes using other molecular techniques is required to avoid false positives. Following confirmation, and even before the identity or function of the gene has been determined, the expression of the gene can

be compared in animals with different production traits, including milk composition and yield. It is expected that desirable alleles of candidate genes can be identified through that process, based on the likelihood of the presence of genetic variability (Grompe, 1993).

It will be necessary to combine the molecular insight generated by differential display and other methods in expression genetics with established quantitative genetic methods in order to develop effective breeding strategies. Finding genes that regulate quantitative traits of economic importance almost surely will allow unforeseen improvements in the efficiency of production of dairy cattle. Differential display technology now allows us to solve many biological questions not previously amenable to research.

Production of transgenic cows by microinjection of recombinant DNA into bovine embryos

The genotype of food animals, including cows can now be altered by in vitro microinjection of genetic material (i.e., recombinant DNA) into the pronucleus of the first cell of the developing embryo (zygote) (for review see Ebert and Schindler, 1993; Purcel et al., 1989). After microinjection, the presumably transgenic embryo is transferred to a competent recipient for normal pregnancy. However, the inefficiency of the technique, which requires the maintenance of mostly nontransgenic pregnancies to term, makes it extremely costly. Although advances in methods for early (i.e., before transfer) detection of transgenic embryos may make this approach more feasible (Krisher et al., 1995; Snabes et al., 1994), there is still a need for improvements of practical value. Because of the expense of this approach it has limited use, for example, in the production of high-value recombinant pharmaceutical compounds in the milk of transgenic animals.

Production of chimeric embryos (transgenic animals) using embryonic stem (ES) cell technology and nuclear transfer from cultured cells

An improvement over the technique of pronuclear microinjection of recombinant DNA, followed by inefficient and random insertion of the DNA into the genome, is provided by a repertoire of newer methods for generating transgenic animals, such as embryonic stem (ES) cell technology (Bowen et al., 1994; Wheeler et al., 1995) and nuclear transfer from cultured cells (Campbell et al., 1996). The remarkable ability of ES cells to retain the potential to differentiate into a wide variety of cell types has led to their designation as ES cells. The ES cell technique involves isolating embryonic cells directly from preimplantation embryos and maintaining them in vitro for many generations. So far, the successful application of this procedure has only been reported for mouse cells (for review see Wilmut et al., 1997). The procedure involves injecting the ES cells into a developing embryo (blastocyst) to produce a hybrid or chimeric embryo (i.e., composed of two different genotypes) and, after normal development, a chimeric adult. Desirable DNA (genes) can be integrated into the ES cells and screened before the cells are used to produce chimeric embryos. In this manner, transgenic individuals can be produced. The most promising technique for targeted gene insertion in ES cells is by homologous recombination. Future improvements in gene targeting using homologous recombination may allow for the generation of animals carrying more precise genetic modifications of any gene of interest.

Recent advances in procedures for nuclear transfer using cell lines established in culture offer hope that this strategy may someday become a practical strategy for either enhancing genetic progress or production of high-value pharmaceuticals. As with ES cell technology,

various gene-targeting techniques are used to modify the DNA of the cultured cells before their use as nuclear donors. Thus, continued advances in gene targeting together with progress in ES cell technology or nuclear transfer from cultured cells may ultimately allow the development of small herds that produce milk containing high-value pharmaceuticals or proteins with enhanced nutritional or functional attributes.

A stunning achievement in 1997 was animal cloning from an adult cell, which resulted in the production of a healthy living animal: Dolly the sheep (Wilmut et al., 1997). This accomplishment represented the convergence of advances in several disciplines, including reproductive biology, genetic manipulation, cell culture, and nuclear transfer, in which the nucleus of one cell (i.e., an adult cell) is absorbed into an egg whose own nucleus has been removed. Advances in this technology may some day open new avenues of research, including the direct cloning of transgenic animals that produce valuable proteins in their milk. But at least right now, most work on livestock cloning is still focussed on developing techniques that are based on the use of fetal cells.

Generation of transgenic animals by somatic cell transformation

This technique involves targeting specific somatic cells (e.g., mammary epithelial cells) for transformation with modified genes (recombinant DNA). There are currently several procedures, which are mostly based on gene transfer using retroviral vectors or artificially constructed vesicles (liposomes), for in vitro transformation of various types of somatic cells (for review see Klug and Cummings, 1994). Although in theory the transformation of somatic cells seems superior to germ-line transformation because of its inherent cell type specificity, in practice it is extremely difficult to perform due to the problem of controlling in vivo transformation of only the specific type of somatic cell of interest. Consequently, most current methods for somatic cell transformation are restricted to cell types (e.g., blood cells, immunological cells, liver cells) that can be first isolated, transformed in vitro, and then transferred back into a recipient. However, a few methods for actual in vivo transformation are also being developed, but thus far only for easily accessible cells such as the cells lining the respiratory tract and circulatory system. The accessibility of the mammary epithelium, through the duct system of the gland, together with the commercial interest in using the mammary gland for the production of high-value pharmaceuticals, makes it likely that methods will soon be developed for in vivo transformation of the mammary gland (for review see Schanbacher and Amstutz, 1997). The ability to perform effective, large-scale in vivo transformation of epithelial cells in the bovine mammary gland would be a major advance for the science of altering milk composition. And because only the animal's mammary epithelial cells (i.e., somatic cells) would be transgenic, rather than its germ cells, the animal's offspring would be genetically normal.

Dietary Approaches to Alter Milk Composition

Nutrition offers the most effective means of rapidly altering milk composition. There are many good reasons to exploit the existing potential, especially in view of recent evidence that targeted modification of the fatty acid profile of bovine milk leads to lower plasma cholesterol in humans (Noakes et al., 1996). Even simple changes in the composition of the diet, for example changing the forage to concentrate ratio, can result in changes in milk fat percent in the range of 2.0 to 4.0%.

Increasing the fat content of milk has traditionally been a major focus of dairy cattle breeding programs. With the price incentive for higher fat content, that emphasis was quite

understandable. For many decades it also reflected the market demand for milk fat. More recently however, there has been a significant decline in the demand for milk fat both as a result of substitution of vegetable spreads for butter and a consumer preference for milk with a reduced fat content. About 80% of total milk sales in Canada are in the form of low-fat milk. The U.K. has also had a similar increase in low-fat milk sales. Also significant in recent years is the increasing substitution of 2% fat milk with 1% fat and skim milk.

Some industry analysts believe that per capita consumption of milk fat has bottomed-out, and the modest increases in milk fat consumption observed in the last couple of years provides support for that view. Others are less optimistic and expect that the trend to lower fat intake will continue, resulting in significant surpluses of butter fat in the future unless steps are taken to reduce the concentration of fat in cow's milk. The focus of research should be on understanding the regulation of milk synthesis with an aim to develop technology that allows producers to rapidly respond to changes in consumer demand for milk components.

Almost all components of milk are subject to manipulation, however, the potential for change varies according to the component. In general, fat percent and the fatty acid composition of milk fat are most amenable to change, whereas lactose is least amenable and protein is intermediate. Changes in milk composition are not always obvious. For example, total protein concentration could remain constant but significant changes could occur in the ratio of casein to non-protein nitrogen. Similarly, substantial changes could occur in the fatty acid composition of milk fat without alterations in milk fat percent.

Influence of diet on protein in milk

Milk protein concentration and composition are influenced by many factors, but the magnitude of changes are less than those observed for milk fat content and composition. The low efficiency (25 to 30%) of conversion of dietary nitrogen into milk protein may be partly responsible for the small and often inconsistent response to protein supplementation (Bequette et al., 1998). Our lack of knowledge about how dietary protein and amino acids influence the composition and yield of protein in milk make it difficult to formulate diets that are biologically efficient and thus economical. We need to identify and characterize the regulatory processes underlying amino acid and protein metabolism during lactation. Knowledge of those processes should open

the door for us to improve the ability of the mammary gland to compete for and convert amino acids into milk protein.

There have been many studies involving protein or amino acid supplementation of dairy cattle (for review see Bequette et al., 1998). In studies where increases in milk protein yield have been observed, the increases have generally been discouragingly small. Bequette et al. (1998) have conducted a series of intragastric and intraveinous amino acid infusion studies with an aim to delineate whether tissue partitioning, pattern, amount, or mammary uptake of amino acids limit milk protein synthesis. This group has also studied other factors that may influence amino acid and protein metabolism, such as stage of lactation and the level of dietary protein.

The compilation of data from this series of experiments on amino acid digestion and protein metabolism in the lactating dairy cow reveals several apparent interactions. Briefly, experiments to identify the relative importance of essential versus non-essential amino

acid supply to the mammary gland, using cows on a low protein diet (140 g of CP/kg of DM), reveal that there is no additional advantage to increasing the posthepatic supply of non-essential amino acids. Thus, they apparently are not limiting to milk protein synthesis. On the other hand, the results of other amino acid infusion experiments demonstrated that there is likely an interaction between total and essential amino acid metabolism in the liver, which may ensure adequate essential amino acids for milk protein synthesis.

The response to amino acid supply changes with advancing lactation, which probably reflects alterations in tissue sensitivity to hormones and other metabolic regulators. The crude protein content of the basal diet also seems to have an influence on the utilization of amino acids for synthesis of milk proteins. Another finding from amino acid infusion work is that there is a larger increase in milk protein content with intravenous compared to gastrointestinal infusion. But milk protein output and recovery are almost identical regardless of route of administration.

It has been shown that mature cows have lower milk protein percentages yet higher protein yields when compared to heifers (Boila et al., 1993). And increasing the level of dietary protein from deficient to more adequate levels (10 vs. 13 and 16% dietary crude protein) results in a linear increase in milk protein percent (Burgess and Nicholson, 1984). Decreasing the rate and extent of digestion of dietary protein in the rumen has been shown to increase milk protein percent, although a greater positive effect is seen on milk yield and milk protein yield (Robinson et al., 1992). Alterations in rate of degradation of dietary protein do not always increase milk protein percent, but sometimes increase non-protein nitrogen content at the expense of casein and (or) whey protein (Casper and Schingoethe, 1989; Khorasani et al., 1994; Robinson et al., 1991). Protein has also been infused; for example, ruminal infusion of casein has been shown to increase milk protein percent (Hurtaud et al., 1993). Ruminal infusion of butyrate also results in an increase in milk protein percent (Huhtanen et al. 1993), whereas propionate infusion is without effect (Hurtaud et al., 1993). Increasing the level of concentrate (greater than 50% of dietary dry matter) in the diet generally results in higher milk protein yield (Ingalls et al., 1980; Vinet et al., 1980). Similarly, the source of starch (barley vs. corn) can influence characteristics of the protein fractions in milk (Khorasani et al. 1994).

Dietary supplementation with lipid usually has a negative effect on milk protein percent, but not all additions cause changes in milk protein yield (Boila et al. 1993; Robinson and Burgess 1990; Grummer et al. 1993; Drackley and Elliot 1993; Kim et al. 1993, Khorasani et al. 1991). Total daily production of milk protein may remain constant, or increase, in situations where milk yield is increased by fat supplementation. The effect of source of forage or grain is small, yet occasionally differences due to forage source (Khorasani et al., 1993), and grain source (Khorasani et al., 1994) do occur. Although dietary-induced changes in milk fat content may have a relatively small effect on milk protein concentration, they can have a substantial impact on the protein to fat ratio and thus the relative yields of protein and fat.

Influence of diet on fat content and composition in milk

The ruminant digestive system allows for dietary manipulation of milk fat content. The rumen is essentially a large fermentation vat, which provides an excellent environment for the growth of microorganisms that utilize the animal’s feed as a substrate. Ruminal bacteria and protozoa will vary in number and type depending on the type and amount of forages and concentrates fed, which in turn influence fermentation end products.

Microorganisms convert much of the dietary carbohydrate to volatile fatty acids (VFA), which are absorbed into the blood stream and become the primary source of energy for the cow. The VFA also serve as important building blocks for milk fat, as well as lactose. The VFA having the greatest impact on milk fat synthesis are acetic and propionic acids. High fiber diets promote greater production of acetate, whereas low fiber diets promote increased production of propionate. Acetate serves as one of the primary precursors for milk fat synthesis and propionate serves as a substrate for various metabolic functions, such as lactose synthesis via glucose formation and energy for whole body metabolism.

Milk fat is comprised primarily of triglycerides (95 to 98% of total lipids) with phospholipids, cholesterol, free fatty acids, and monoglycerides making up the remainder (Palmquist and Beaulieu, 1993). Milk fatty acids are either derived from dietary sources, and transported to the mammary gland, or synthesized *de novo* by

mammary epithelial cells and then esterified in the epithelial cells before secretion into milk.

Why alter the fatty acid composition of milk? One reason is that bovine milk fat has been criticized because it contains a less desirable balance of fatty acids than vegetable or fish oils. In particular milk fat contains a substantial concentration of C14:0 and C16:0 and relatively low concentrations of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Although the scientific data on the relationship between saturated fat and cardiovascular and other diseases is equivocal, there is an increasing body of scientific data to support the view that an acceptable fatty acid profile does not require extreme changes in the fatty acid composition of milk. Many fatty acids such as C18:1 and C18:0 which were previously targeted as undesirable are now considered neutral or positive from a human health perspective. Indeed C18:0 is as effective as C18:1 in reducing plasma cholesterol (Jensen et al., 1991). The high concentration of C16:0 and C14:0 in milk continues to be a concern to many in the medical community. Thus, increasing C18:0 and C18:1 fatty acids at the expense of C14:0 and C16:0 is considered desirable from a human health perspective. It also offers the additional benefit of a softer butter arising from the change in the fatty acid profile of milk fat.

Influence of diet on protein to fat ratio in milk

Any reduction in one of the major milk solids provides an opportunity for the cow to divert those nutrients into other milk components. For example, although dietary induced changes in milk fat content may have a relatively small effect on milk protein concentration, they can have a substantial impact on protein to fat ratio and thus relative yields of protein and fat.

Increasing the proportion of grain in the diet results in greater propionate production, which in turn leads to increased odd chain and branched chain fatty acids plus enhanced gluconeogenesis. As the proportion of concentrate is increased, milk fat percent declines with the effect being particularly pronounced as the concentrate exceeds 50 to 60% of dietary dry matter (Ingalls et al., 1980; Steacy et al., 1983; Vinet et al., 1980). In two recent experiments we evaluated the effect of concentrate level (50 vs. 75% of dietary DM) and stage of lactation on milk composition.

In early lactation milk fat content declined from 2.84 to 2.37 as the concentrate level in the

diet was increased from 50 to 75% of dietary dry matter. Protein concentration also increased but protein yield was unaffected as milk yield tended to be lower in cows fed the high concentrate diet. Milk protein to fat ratio ranged from 1.09 to 1.45. Interestingly, the effect of concentrate level was most pronounced in late lactation with milk fat content declining from 4.21 to 2.91% and protein to fat ratio increasing from 0.81 to 1.22. Reduced milk fat percent has also been attributed to lower ruminal production of fat precursors (acetate and β-OH-butyrate) and an inhibitory effect of methylmalonyl CoA (produced from propionic acid) on fatty acid synthesis in the mammary gland. However, recent data implicate trans fatty acid, produced in the rumen as an intermediate of lipid biohydrogenation, as the causative agent in milk fat depression (Griinari et al., 1998).

Ruminal digestion of lipid

Lipids present in forages and grains are generally triacylglycerols, galactosylacylglycerols, and phospholipids with C18:2 and C18:3 being the principal fatty acids. Lipolysis of fatty acids must occur before biohydrogenation because the microbial saturase enzymes require free carboxyl groups as a substrate (Palmquist and Jenkins, 1980). Short and medium chain fatty acids can be metabolised to VFA, absorbed across the rumen wall, or pass to the intestine. The process of microbial saturation of fatty acids (biohydrogenation) is a multi step process requiring several species of bacteria and protozoa (Byers and Schelling, 1988).

Biohydrogenation of C18:2 and C18:3 involves an isomerization reaction that converts the cis-12 double bond to a trans -11 isomer followed by reduction to trans -11 C18:1 and ultimately to C18:0, which is the principal product of microbial hydrogenation of C18:1, C18:2, and C18:3 fatty acids (Jenkins, 1993). Hydrogenation of the Ä11 trans double bond is the rate limiting step in biohydrogenation of fatty acids (Palmquist and Jenkins, 1980). Some trans isomers produced in the rumen escape further biohydrogenation and ultimately are incorporated into storage lipids and milk fat (Wu et al., 1991; Griinari et al., 1998). Microbial synthesis of branched and odd-numbered chain fatty acids (e.g., C15:0) also occurs in the rumen and these fatty acids are present in carcass and milk lipids. Branched chain fatty acids arise from the substitution of isobutyrate, isovalerate, and 2-methylbutyrate for acetate in the microbial synthesis of fatty acids. Similarly, odd chain fatty acids are derived from microbial use of the odd-chain fatty acids, propionate and valerate, as precursors for fatty acid synthesis.

Intestinal absorption of fatty acids

Stearic acid is the principal fatty acid reaching the intestine, reflecting the ruminal saturation of dietary oleic, linoleic, and linolenic acids. Saturated fatty acids reaching the duodenum such as C18:0 are subject, in part, to desaturation by intestinal and mammary desaturase activity. As a result, the C18:0 to C18:1 ratio is lower in milk than in intestinal digesta, which reflects the mechanism used by the ruminant to preserve the fluidity of milk fat. The jejunum is the primary site of long chain fatty acid absorption but absorption can also occur in the duodenum and ileum. Fatty acids reaching the small intestine interact with bile and pancreatic acids to form micelles, which are absorbed into the intestinal cells where they are reesterified. Chylomicrons, formed by the addition of triglycerides to apolipoprotein B48, exit the cell and enter the lymphatic system (Byers and Schelling, 1988). Fatty acids whose chain length is 14 carbons or less can be absorbed directly without the need to form chylomicrons. These fatty acids are transported to the liver where

they are oxidized. Chylomicrons are transported to the liver and peripheral tissues where triglycerides are released through the action of lipoprotein lipase (Christie et al., 1986).

Biosynthesis of milk fatty acids

In contrast to nonruminants, the liver of ruminants has a very limited capacity for fatty acid synthesis due to the extremely low acetyl CoA carboxylase (ACC) activity, the enzyme which catalyses the rate limiting step in *de novo* synthesis of fatty acids. The two primary sites of fatty acid synthesis in the bovine are adipose tissue and the mammary gland. Fatty acid synthesis in these tissues is under hormonal control, which influences both nutrient delivery and the activities of ACC and fatty acid synthase (FAS; Ponce-Castaneda et al., 1991). At parturition, fatty acid synthesis increases in the mammary gland at the expense of adipose tissue. That change is due to increased expression of the ACC and FAS genes in the mammary gland in response to the requirement for milk fat synthesis for lactation (Ponce-Castaneda, et al., 1991).

Milk fatty acids are derived in part from dietary long-chain fatty acids, microbial synthesis of fatty acids, or body stores of fat, with the remainder being synthesized from short-chain fatty acids (primarily acetate and â-0H butyrate) arising from microbial digestion of carbohydrate in the rumen (Chilliard, 1993; Jenkins, 1993; Wu et al., 1991). Microbial synthesis of branched and odd number fatty acids in the rumen plus intermediates of ruminal biohydrogenation of polyunsaturated fatty acids (e.g., trans -11 C18:1) contribute to the diversity of fatty acids in milk fat.

Fatty acid synthesis in the mammary gland follows a similar pathway to that in adipose tissue except that the mammary gland does not have elongase activity and thus cannot produce fatty acids greater than 16 carbons. The first committed step in fatty acid synthesis is the conversion of acetyl-CoA to malonyl CoA, which is catalysed by ACC. Chain elongation (up to C16) involves the addition of two carbon units, requiring ATP and FAS. The mammary gland also has stearoyl CoA desaturase activity that permits conversion of stearic acid to oleic acid. About 50% of milk fatty acids are synthesised in the mammary gland, 40 to 45% are of dietary origin, and the remainder arises from adipose tissue (Palmquist and Jenkins, 1980). Long chain fatty acids are primarily of dietary origin and are transported to the mammary gland in chylomicrons or very low density lipoproteins (VLDL). Medium chain fatty acids can be synthesised in the mammary gland or be of dietary origin. Lipoproteins reaching the mammary

gland are hydrolysed in the capillary epithelium by lipoprotein lipase and become available for incorporation into milk fat.

Dietary supplementation with unsaturated long-chain fatty acids

The balance between mammary *de novo* synthesis of short and medium chain fatty acids and dietary and microbial long-chain fatty acids presented to the mammary gland can be substantially altered by manipulating the diet of the animal. The trend toward more widespread supplementation of dairy cattle diets with fat provides an opportunity to alter milk composition by judicious selection of fat sources with a desirable fatty acid profile. However, the extensive biohydrogenation of unsaturated fatty acids in the rumen poses a challenge to efforts aimed at increasing the concentration of polyunsaturated fatty acids in milk. Increasing the monounsaturated fat content of milk is much easier because the

mammary gland can convert stearic acid to oleic acid using the enzyme stearoyl CoA desaturase. In essence, the unsaturated fatty acids must be fed in a form that resists biohydrogenation in the rumen.

In the absence of ruminal biohydrogenation, feeding unsaturated fatty acids causes substantial shifts in the fatty acid composition of milk fat. The extent to which milk fatty acid composition is amenable to alteration by the feeding of oilseeds is illustrated by the results obtained by Australian workers in the 1970's (McDonald and Scott, 1977). Cows fed protected lipid supplements containing linseed oil and safflower oil produced milk containing greater than 30% of C18:2 and 20% of C18:3. In contrast, milk from control cows contained about 2% and 1%, respectively of those fatty acids. From the data, we can conclude that the ability of the mammary gland to secrete those fatty acids in milk is not a limiting factor in feeding strategies designed to alter milk composition. In practise, the upper limits to level of unsaturated fatty acids are dictated by the effect of the fatty acids on the processing quality of milk and milk products, plus the availability of lipid supplements that are adequately "protected" from biohydrogenation in the rumen.

We have conducted a number of studies in our laboratory to examine the efficacy of various oil sources as a means of increasing the unsaturated fatty-acid content of milk. In one of those studies we examined the influence of Jet-sploded Whole Canola Seed (JWCS) on milk composition. The Jet-sploding process involves the application of dry heat and the process can be controlled to achieve the desired internal seed temperature. Inclusion of JWCS in the diet caused a substantial reduction in $C_{16:0}$ and increased concentrations of C18 fatty acids. Total C18 fatty acids increased from 30.8 to 51.7. The effect of feeding JWCS on the ratio of C16 to total C18 was particularly marked, with the ratio from 1.15 for cows fed the control diet to 0.32 at the highest dietary level of JWCS inclusion. The effect of JWCS on the concentration of C18:2 and C18:3 in milk was relatively small, indicating that extensive biohydrogenation of those fatty acids occurred in the rumen. Although the changes in C18:2 and C18:3 were minor or absent, the substantial reduction in C16:0 and elevated concentrations of C18:1 are interpreted to indicate that supplementing the cows diet with this lipid source improved the nutritional quality of milk for humans.

Based on data in the literature, milk and milk products of acceptable quality, containing elevated levels of C18:2 and C18:3, can be produced. However, such products may have a shorter shelf life and require modifications to standard procedures for manufacture of butter and cheese (McDonald and Scott 1977), especially when unsaturation of milk fat is substantially increased. Dietary lipid supplementation, targeted at decreasing the ratio of C16:0 to C18:1 or C16:0 to total C18:0 without markedly increasing C18:2 and C18:3 will help minimize effects on the processing quality of milk.

Milk as a Source of Functional Ingredients and Nutraceuticals

Although the primary role of the components in milk is to contribute to neonatal nutrition, evidence is accumulating that supports other physiological roles such as modulators of digestive and metabolic processes. Another exciting, new direction for the dairy industry is in research to identify and characterize milk components with nutraceutical and functional properties that are associated with human health.

Proteins

The major milk proteins, caseins, are a source of nutritional proteins but their specific bioactivities have not been fully elucidated. However, due to their interaction with calcium phosphate to form colloidal calcium phosphate micelles, they may act as a carrier of calcium and phosphate, thereby increasing the bioavailability of these minerals (Yamauchi, 1992). Of the whey proteins, "-lactalbumin is the B subunit of the enzyme lactose synthetase, which promotes the selective transfer of galactose to glucose during lactose biosynthesis. The role of $-lactoglobulin is thought to be related to the binding of retinoid and hydrophobic molecules such as fatty acids because of its structural homology to plasma retinol binding protein (Papiz, et al, 1986; MacLeod, et al., 1995ab). Immunoglobulins in bovine milk provide passive immunity function. Lactoferrin, on the other hand, promotes iron absorption due to its iron binding capability (Yamauchi, 1992). Lactoferrin is also reported to possess antimicrobial effect (Yamauchi, 1992).

Bioactive peptides derived from milk proteins

The "white mining" of milk proteins has led to the discovery of a vast array of peptides with different biological activities. Some of the reported activities of these peptides include: opioid agonistic and antagonistic activities, immunostimulation, mineral carriers, antithrombotic effect, and anti-hypertensive capability (Yamauchi, 1992; Meisel et al., 1990; Fiat et al., 1993).

Milk protein derived anti-hypertensive biopeptides - ACE (Angiotensin-I Converting Enzyme) inhibitors

The number one factor that affects arteriosclerosis is high blood pressure (hypertension). There are many factors that affect hypertension but in the regulation of blood pressure and the pathophysiology of hypertension, angiotensin I-converting enzyme (EC 3.4.15.1) plays an important role in increasing blood pressure. Rennin, a proteinase, catalyses hydrolysis of angiotensinogen to angiotensin I, a decapeptide which is physiologically inactive. However, angiotensin I is converted to angiotensin II by angiotensin I-converting enzyme (ACE), which cleaves a dipeptide at the carboxyl end of angiotensin I. The octapeptide angiotensin II is the most physiologically active component of this rennin-angiotensin system. Inhibitors of ACE were first obtained from the venom of snakes and recently, several ACE nhibitors were identified and isolated from food materials such as casein, sour milk, fish muscle, dried bonito, tryptic hydrolysate casein, and from milk fermented by *L. helveticus* or *L. delbrueckii* subsp. *Bulgaricus (*Maruyama et al., 1987; Yamauchi, 1992; Nakamura et al., 1995; Nakamura et al., 1996).

The goal of this new research area is to develop innovative and technologically derived bioactive peptides that will possess inhibitory activity against angiotensin I-converting enzyme (ACE) and (or) peptides with anti-hypertensive properties that can reduce blood pressure. These peptides may possess significant commercial value as biologically active dietary components prepared from enzymatically hydrolysed milk protein fractions. At present, many inhibitory peptides against ACE are derived by chemical synthesis. Replacement of synthetic peptides by peptides derived from milk proteins should be advantages from the public perspective.

A new product opportunity: Glycomacropeptide from ê-casein

The enzymatic hydrolysis of rennet or chymosin splits ê-casein into two peptide fragments at the ^{105}Phe - ^{106} Met bond. The first peptide fragment, f1-105, is the hydrophobic para-ê-casein which co-precipitates with other casein components in the presence of calcium ions. The second peptide fragment, f106-169, is the hydrophilic C-terminal fragment called glycomacropeptide (GMP) and remains in solution together with whey proteins. Several biological functions of GMP have been reported (Chu, et al. 1996ab): 1) Bifidus growth promoting factor, 2) antigastric activity in dogs, 3) antithrombotic activity in guinea-pigs, 4) depression of platelet aggregation, 5) inhibition of binding of oral actinomyces and streptococci to polystyrene surfaces, 6) inhibition of cholera toxin binding to its receptor, and 7) growth inhibition of lactic acid bacteria, and *Staphylococcus aureus*.

We isolated casein GMP from sodium caseinate hydrolysate solution by ultrafiltration and high purity was achieved by cation exchange chromatography (Chu et al., 1996ab). The absence of phenylalanine in ê-casein GMP may lead to the development of a therapeutic formula for the treatment of phenylketonuria (PKU). At present, there are two kinds of commercial products available for PKU diet therapy: 1) formula based on the mixture of pure amino acids and 2) protein hydrolysates from which the phenylalanine has been removed by activated carbon, ion exchange resin, and other methods. However, it is reported that the patients with PKU dislike these therapeutic formulas because of their bad taste and odours. It is possible to isolate GMP from casein using a combination of enzymatic hydrolysis (renneting) and ultrafiltration for PKU diets.

Dairy ingredients as potential nutraceuticals: â-lactoglobulin.

To date, four genetic variants of â-lactoglobulin, A, B, C, D have been identified with A and B being the most common in North America. The conformation and physico-chemical properties of â-lactoglobulin have been studied extensively and the amino acids sequences in â-lactoglobulin family showed that 33 out of the 55 amino acids residues are homologous in all members of the â-lactoglobulin family and these residues provide the characteristic biological and functional properties (Phillips et al., 1992; Papiz et. al., 1986). The â-barrel structure of â-lactoglobulin shows remarkable resemblance to retinol-binding protein in human plasma (Papiz et al., 1986). There is also other evidence supporting a role for â-lactoglobulin as a transport protein: 1) it has conformational homology with retinol-binding protein (Papiz, et al., 1986), 2) it forms a strong binding complex with retinol (MacLeod et al., 1995abc), 3) it is relatively stable under acidic conditions, (Papiz et al., 1986) and 4) it resists peptic hydrolysis (MacLeod et al., 1995abc). Together with the resistance of â-lactoglobulin to pepsin hydrolysis and its stability under very acidic conditions, the presence of a vitamin A precursor receptor in the small intestine of human neonates has led to the suggestion that â-lactoglobulin is a transport protein for this group of compounds (MacLeod, et al. 1995 abc; MacLeod, et al. 1996). We have developed technology to selectively isolate â-lactoglobulin from whey using biospecific subunit exchange affinity chromatography (BSEAC) (MacLeod et al., 1995ab). This process may result in the commercial manufacture of â-lactoglobulin-reduced whey protein concentrates as well as a native pure â-lactoglobulin fraction. Both products may have specific functional and nutraceutical applications.

Conjugated linoleic acid (CLA) as a natural dairy food component with newly recognized biological activity

Because of consumer concerns over the role of dietary fat, particularly saturated fat and

cholesterol, in fitness and health, milk fat consumption has declined steadily throughout the Western World over the last decade. Though during the last two years butter sales have increased, milk fat prices are still relatively low (Jimerez-Flores, 1996). The challenge to the dairy industry is to provide viable and competitive markets for milk fat-derived value-added products.

Conjugated linoleic acid (CLA), which refers to a mixture of octadecadienoic acid with conjugated double bonds, is a natural food component with newly recognized biological activity. It exists in various isomeric forms: c-9, t-11; t-10, c-12; t-9, t-11; t-10, t-12; c-9, c-11; c-10, c-12 and c-10, t-12. Several researchers have reported anticarcinogenic properties of CLA in animal models. Ha et al., (1990, 1989) showed that synthetic CLA inhibited the development of mouse epidermal and mouse forestomach cancer. Conjugated linoleic acid suppresses the development of rat mammary tumors (Ip et al., 1991). Physiological levels of CLA were shown to be cytostatic and cytotoxic to human malignant melanoma, and colorectal and breast cancer cells in vitro (Schultz et al., 1992). The hypocholesterolemic and anti-atherogenic effects of CLA in hamsters and rabbits were demonstrated by Nicolosi et al. (1993) and Lee et al. (1994). Conjugated linoleic acid is certainly a multifunctional milk-fat derived nutraceutical with a strong potential market place. Further research into the anticarcinogenic efficiency of CLA, manipulating CLA levels in dairy products by dietary and processing means, and developing CLA-fortified food products will likely lead to a new generation of designer foods.

Probiotics, prebiotics, and synbiotics in functional foods

Probiotics are defined as foods which contain live bacteria that exert beneficial effects for the host by improving the microbiological balance in the intestine (Fuller, et al., 1997; Gibson, et al., 1995). In most industrial practices, production of functional foods containing probiotics is initiated by commercially available pure cultures of GRAS (generally regarded as safe) status. It is estimated that about 30% of the food supply is based on fermented products such as fermented vegetables, cereal, meats, and dairy products. The most commonly used probiotics are lactic acid excretors like lactobacilli and bifidobacteria, which are usually added to products or used in fermentation.

The health benefits reported for probiotics include: a) re-establishment of balanced intestinal microflora, b) improving colonization resistance and or prevention of diarrhoea, c) systemic reduction of serum cholesterol, d) reduction of fecal enzymes,

potential mutagens that may induce tumors, e) improved calcium absorption, and f) synthesis of vitamins and predigestion of proteins.

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacteria in the colon that have the potential to improve host health (Fuller, et al., 1997; Gibson, et al., 1995). Food ingredients classified as prebiotics must not be hydrolyzed or absorbed in the upper gastrointestinal tract. Because of the survivability and colonization difficulties that abound with probiotics, the prebiotic approach offers an attractive alternative. Many oligo- and poly-saccharides occur naturally and meet the criteria of prebiotics (Tanaka et al, 1998; Yaeshima, 1996; Playne et al., 1996). The possible prebiotic effect of these oligosaccharides is that they may selectively stimulate the growth of bifidobacteria (Gibson, et al., 1995; Tanaka et al., 1998; Yaeshima, T., 1996). Bifidobacteria have a number of

health promoting properties: a) improved intestinal environment, b) inhibition of pathogenic bacteria, c) immunomodulation, d) synthesis of B vitamins, e) lowered blood ammonia and cholesterol, and f) inhibition of
tumor formation. The expanding group of food products with bifidobacteria is currently the strongest marketing point for prebiotic oligosaccharides.

Synbiotics are mixtures of probiotics and prebiotics (Fuller, et al., 1997; Gibson, et al., 1995). This mixture would benefit the host by improving both survival and implementation of the selected microbial supplements. The synbiotic approach offers a further alternative in which the probiotic would be introduced in conjunction with a specific prebiotic. As a result, the growth of the live bacteria is expected to be enhanced.

Concluding Remarks

Matching production of milk components to demand improves biological efficiency and ultimately enhances the overall profitability of the dairy industry. Consumer demand for the major components of milk has changed considerably over the past two decades. To align production with demand successfully, the dairy industry must improve its ability to respond rapidly to changes in consumption patterns. Though we continue to increase our understanding of the biological control of milk protein, fat, and lactose synthesis, the dairy industry is well placed to exploit the potential to alter milk composition. A more complete understanding of lactation should emerge as dairy scientists apply new molecular methods such as expression genetics and DNA chip technology to uncover the details of the biological processes determining milk composition. As new genes and genetic markers are discovered and applied, there will also be improvements in the accuracy of selection in dairy breeding programs. The potential to use nutrition, as a tool to alter the composition of milk, especially the milk fatty acid profile and milk fat percent has not yet been fully exploited. Recent evidence that targeted modification of the fatty acid profile of bovine milk leads to lower plasma cholesterol in humans reveals the practical potential that exists. The opportunity is now for the dairy industry to exploit the existing potential to alter the composition of milk for the benefit of the producer, processor, and consumer.

Continued research on nutraceutical and functional ingredients in milk should lead to the development of a broad range of high added-value products. As new components are discovered, or new uses identified, there will be a concomitant demand for milk with composition suitable to isolation or concentration of the components to levels that are of practical benefit. The ability to meet such future demands for changes in milk composition will, as with our current ability to meet existing demand for changes, be limited not only by the state of knowledge of lactation biology, but also by the same biological constraints for proper mammary function that limit our current ability to alter milk composition. So beyond the challenge to prove the benefit or function of these milk components is also the need to develop cost effective methods for their extraction and purification.

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