Effects of Nutrition on Milk Composition: A 25-Year Review of Research Reported in the *Journal of Dairy Science*[1]

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Abstract

A number of major scientific advances have been realized the last 25 years in determining the opportunities and limitations of altering milk composition through nutritional manipulation. Because of the greater sensitivity of milk fat to dietary manipulation than either protein or lactose, nutritional control of milk fat content and fatty acid composition received a great deal of attention. New information emerged linking ruminal production of trans fatty acid isomers with milk fat depression. As a result, research on fatty acid biohydrogenation intensified, yielding new insight on the origin of specific trans fatty acid isomers originating from ruminal biohydrogenation and how these isomers were modified by the action of mammary enzymes. The discovery of conjugated linoleic acid **(CLA)** as a potent anticarcinogen also led to extensive work on enhancing its concentration in milk through nutritional manipulation and discovering the physiological effects of specific CLA isomers. New protected fats were developed in recent years that were designed to resist biohydrogenation and enhance the concentration of unsaturated fatty acids in milk. The nutritional factors receiving the most attention during the last 25 years for their influence on milk protein content were forage to concentrate ratio, the amount and source of dietary protein, and the amount and source of dietary fat. New insights were tested on modes of action whereby fat supplements caused a decline in protein concentration. Changes in milk lactose concentration occur only in extreme and unusual feeding situations, but the basic biology of lactose synthesis and regulation are still being explored using modern molecular techniques. This paper highlights the major advances in controlling milk composition by dietary manipulation and how it impacts the entire animal system from practical feeding studies to basic cellular work on mammary tissue metabolism.

Introduction

The basic driving forces for manipulating the composition of milk are much the same now as they were 25 years ago, and include 1) improving the manufacturing and processing of milk and dairy products, 2) altering the nutritional value of milk to conform to dietary guidelines set forth by governmental agencies, and 3) using milk as a delivery system for nutraceuticals with known benefits to human health. The period from 1980 to 2005 has seen efforts at trying to alter the content or composition of all three components - fat, protein, and lactose. As expected, the greatest changes were made in milk fat and fatty acid composition.

This paper was written with strict adherence to two limitations. First, it is not the intention of this paper to cite the vast scientific literature compiled over the last 25 years relating to manipulation of milk composition. The contributions have been too numerous, and an undertaking such as this would be better suited for a review article in a scientific journal where the merits of each study could be

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evaluated. Instead, this paper will focus on the major advances that have occurred over the last 25 years that are now recognized as significant steps forward in nutritional control of milk composition.

The second limitation was to maintain focus on nutrition. We know that a multitude of factors influence the final composition of milk, including genetics and breed of animal, environment, stage of lactation, parity, and nutrition of the cow. Although all of these factors work in combination with each other to determine the final composition of milk, the focus of this paper is on nutrition of the cow and how it impacts milk fat, protein, and lactose.

With these goals in mind, manipulation of each milk component is discussed separately below with emphasis on the changes desired, the advances in enhancing the absorption and delivery of the desired nutrient to the mammary gland, and utilization of the nutrient by the mammary tissue to achieve the desired objective. Taken collectively, the advances in altering milk composition by dietary manipulation have come from significant contributions of the entire animal system, from practical studies on feeding systems to basic cellular work on mammary tissue metabolism.

Milk Fat

Target

Nutritional control of milk fatty acid profile has received considerable attention over the last 25 years (Mansbridge and Blake, 1997). Whether the goal is to improve manufacturing properties of milk or to enhance the concentration of fatty acids having beneficial health effects in humans, the key objective was usually to increase one or more unsaturated fatty acids in milk. For instance, increasing oleic acid content in milk enhances the plasticity and softness of milk fat, which has interested processors attempting to improve the spreadability of butter. Also, market pressures continued over the last 25 years to find avenues for enhancing the concentration of the "healthy" unsaturated fatty acids in milk. As an example, the Wisconsin Milk Marketing Board in 1988 published recommendations of a Milk Fat Roundtable stating that an "ideal" milk would contain no more than 8% saturated fatty acids, less than 10% polyunsaturated fatty acids, and the remainder (82%) as monounsaturated fatty acids (Berner, 1993). In addition, information emerged about the health effects of unsaturated trans fatty acids produced in the rumen, which led to interest in enhancing their concentration in meat and milk.

Research then followed to determine the ability of different dietary formulations to reduce milk fat content or enhance the concentration of unsaturated fatty acids. Dietary factors receiving the most attention were the amounts of grain and fat fed to cows. Each of these will be discussed separately, with a greater emphasis on the more researched fat supplements. The control of milk fat and fatty acid composition by fat supplements is complex because the transfer of dietary unsaturated fatty acids to milk can be significantly lessened by several factors including their biohydrogenation by ruminal microorganisms, poor rates of intestinal absorption, and their deposition in adipose tissue rather than in mammary fat. Thus, major advances in using fat supplements to alter milk fatty acid profile included significant work in understanding and controlling fatty acid biohydrogenation by ruminal microorganisms and the uptake and utilization of unsaturated fatty acids by the mammary gland.

Grain feeding

Cereal grains are used liberally in dairy rations in the U. S. because they are a cost-effective source of digestible energy needed for maintaining high levels of milk production. In addition to stimulating milk yield, higher grain intakes can also depress milk fat percentage and alter fatty acid composition. Grain feeding typically reduces the proportions of milk fatty acids having 6 through 16 carbons, and increases the proportion of 18-carbon unsaturated fatty acids. Several theories to explain the cause for the grain-induced milk fat depression were under scrutiny in the early 1980s, but the exact cause was not clear. Two theories receiving most of the attention at the time were: 1) inadequate rumen production of acetate and butyrate to support milk fat synthesis, and 2) propionate from grain stimulates circulating insulin concentration, which redirects metabolites away from mammary tissue. Multiple studies have shown that both theories are unlikely. See Bauman and Griinari (2003) for a recent and thorough account of the theories for milk fat depression.

One of the major breakthroughs on the theories of milk fat depression during the last 25 years was the refocus on trans fatty acids as the causative agent of milk fat depression in dairy cattle. Although trans fatty acids were implicated in milk fat depression many years earlier, it was new studies done in the early 1990's by Dr. Richard Erdman with dairy cattle and mouse studies by Dr. Beverly Teter at The University of Maryland that redirected the attention on trans fatty acids. Studies performed at several locations showed an inverse relationship between trans fatty acids in milk and milk fat content. Several reports indicated substantial increases in milk trans fatty acids without reductions in milk fat content, which raised questions that not all trans fatty acid isomers were associated with milk fat depression. Later, work showed that milk fat depression was more closely associated with the production of trans-10 fatty acid isomers in the rumen than with all trans isomers in general. Grain feeding was shown to enhance the production of the trans-10 fatty acid isomers by ruminal microorganisms. An important study done at Cornell University by Dr. Dale Bauman and colleagues demonstrated severe milk fat depression in cows infused with trans-10, cis-12 CLA, but no depression following infusion of the cis-9, trans-11 CLA isomer. Further work with other conjugated dienes and trienes have failed to find any further inhibitor of milk fat synthesis. Thus, it appears that trans-10, cis-12 CLA is the most likely factor causing milk fat depression.

Fat supplements

Extensive work on feeding fat to dairy cattle occurred over the last 25 years. The emphasis in the early 1980's was on using fat to provide more energy for milk production. During this time period, extensive work was done on developing rumen-inert or bypass fats that minimized digestibility problems that often occurred when feeding unsaturated oils to dairy cows. This led to commercial development of a variety of bypass fats, including calcium salts of fatty acids and products enriched in saturated fatty acids. Analysis of milk fatty acid composition was usually done in the same studies providing a large databank of information on the extent that fat supplements could alter milk fatty acid composition.

Untreated vegetable oils high in unsaturated fatty acids have only limited ability to alter milk fatty acid composition. The reason for this was established decades prior to the 1980's and is attributed to the microbial population located mainly in the rumen that transform dietary unsaturated fatty acids. Therefore, delivery of unsaturated fatty acids to mammary tissue is limited even when their dietary concentration is high. The ruminal microorganisms transform unsaturated fatty acids in a process called biohydrogenation (Jenkins, 1993), where microbial enzymes add hydrogen across the carbon:carbon double bonds of the fatty acyl chain, converting the double bond from unsaturated to saturated (Figure 1).

There has been considerable interest over the last 25 years in finding ways to shield dietary unsaturated fatty acids from biohydrogenation in order to enhance their absorption and delivery to the mammary gland (Jenkins, 1998). Figures 2 and 3 show examples of changes in oleic and linoleic acid concentrations in milk fat when various forms of rumen-protected fats were fed to dairy cows. Oleic acid concentration in milk fat varied from 18 to 24% of total fatty acids when control rations containing no added fat were fed to cows. When rumen-protected fats were fed to cows, oleic acid in milk varied from 18 to as much as 48%. The effects of fat source on milk linoleic acid concentration were less dramatic. Linoleic acid concentration in milk normally ranges from 1.5 to as much as 4% when cows are fed control diets with no added fat. Feeding rumen-protected fats increased the upper range of linoleic acid concentration to about 6.5%.

Another significant finding bringing a great deal of attention to biohydrogenation intermediates in milk fat was the discovery that CLA had beneficial effects on human health, most notably cancer-fighting properties. It was the cis-9, trans-11 CLA isomer in particular that received the most attention for its anticarcinogenic properties, which was known to arise from the biohydrogenation of linoleic acid. The recent interest in enhancing biohydrogenation intermediates in milk propagated research to determine the origin and possible enhancement of beneficial fatty acid isomers produced in the rumen.

Many of the advances in nutritional manipulation of milk fat content were made possible by enhancing our basic understanding of the principles of nutrient uptake and utilization by the mammary gland. Many of the advances during the last 25 years were focused on characterizing the regulatory steps in fatty acid synthesis and desaturation. Desaturase activity in the mammary secretory cell converts stearic acid arising from ruminal biohydrogenation to oleic acid that is secreted in milk. Thus, studies have been directed at enhancing activity of delta-9 desaturase in order to increase oleic acid at the expense of saturated fatty acids in milk.

An important discovery within the last few years was the observation that the delta-9 desaturase was the predominant source of the cis-9, trans-11 CLA isomer in milk, which has a number of benefits to human health (including anticarcinogenic properties). Trans-11 arising from biohydrogenation in the rumen is transferred to the mammary tissue and desaturated to cis-9, trans-11 CLA via the delta-9 desaturase. This has shifted attention to manipulating ruminal biohydrogenation to enhance the yield of the trans-11 isomer.

Milk Protein

Target

The nitrogen fractions of milk can be broadly divided into three categories: casein, whey, and nonprotein nitrogen **(NPN)**. Casein comprises the majority of the nitrogen in milk (about 78%), with lesser amounts of whey N (17%) and NPN (5%). In cheese-making, curd structure, curd firmness, and cheese yield are directly related to casein content. The nutritional factors receiving the most attention during the last 25 years for their influence on milk protein content were forage to concentrate ratio, the amount and source of dietary protein, and the amount and source of dietary fat (DePeters and Cant, 1993; Bequette et al., 1998).

Forage to concentrate ratio

In most cases, reducing the proportion of forage in the diet of a cow increases both protein content and yield. Milk protein content can be increased 0.4 percentage units or more if forage proportion in the diet is reduced to 10% or less of the dietary DM. Because a minimum concentration of forage is needed in typical dairy diets (generally 40% or greater) to avoid digestive and metabolic disturbances, reducing the forage to concentrate ratio has not been a practical method of consistently enhancing milk protein content. Another issue has been to determine if forage is the direct cause of milk protein depression, or if it is an indirect effect of decreasing energy intake. Limited research on this question during the last 25 years points to a greater role for energy intake, with fiber content of the ration having little direct influence on milk protein content.

Rapidly fermentable dietary carbohydrate has been associated with milk protein content. Several studies utilized a hyperinsulinemic-euglycemic clamp technique to examine raised insulin concentrations without the confounding effects of hypoglycemia. Results demonstrated a modest increase in milk protein unless casein was infused abomasally. When combined, insulin and casein produced substantial increases in milk protein content (10%) and yield (28%). Thus, when rapidly fermentable carbohydrate is fed, greater production of propionate and microbial protein is produced, leading to signals in the cow's body to produce more milk and milk protein.

Amount and source of protein

Unlike forage to concentrate ratio, the effects of amount and source of protein in the diet on milk protein content have been extensively investigated. However, it soon became clear that dramatic changes in either amount or source of protein caused only modest changes in the protein content of milk. The data in Figure 4 show a spread of milk protein from 2.85 to 3.27% as protein content in the diet varied from 15.0 to 19.5% and included a wide variety of protein sources, including rumen-protected amino acids. As pointed out by Dr. Roy Emery at Michigan State University in his 1978 review on feeding for increased milk protein, protein content of milk increases only about 0.02% for each 1% increase in dietary protein (Emergy, 1978).

Low transfer efficiency (25 to 30%) of dietary protein to milk is a major factor accounting for the inability of diet to markedly alter milk protein content. Blood flow through the mammary gland is implicated as a key cause of this poor capture, which is part of the overall process for the coordinated timing of nutrient delivery to the mammary gland. Contrary to this point, studies in cows undergoing a hyperinsulinemic-euglycemic clamp show that both mammary blood flow and amino acid extraction can adjust, leading to enhanced milk protein production. This suggests that the mammary gland has the capacity to alter the uptake of substrates from the arterial supply in response to changes in arterial amino acid concentrations, mammary blood flow, and metabolic activity to improve milk protein production.

Amount and source of fat

As fat supplements were being explored as energy sources for dairy cows, it soon became apparent that feeding additional fat was often accompanied by a decline in milk protein content. As a result, feeding fat had to be limited in markets where milk pricing gave an incentive to protein content. On average, protein content in milk declined 0.03% for each 100 g supplemental fat intake, or about 0.1 to 0.3 percentage units for most typical levels of fat feeding. When fat supplementation reduced milk protein content, the casein fraction declined the most. Fat effects on the whey fraction were inconsistent and NPN generally increased. Because fat supplements increased milk yield when properly fed, total daily production of milk protein remained the same or even increased when fat was fed, despite the decline in protein content.

Several important studies were done during the last 25 years to elucidate the mechanism whereby fat supplements cause this dilution effect, i.e., a greater increase in milk yield than protein yield. One proposal was by Casper and Schingoethe (1989) at the University of South Dakota. They proposed that elevated blood fatty acids from the fat supplement decreased the release of somatotropin, which reduced mammary extraction of amino acids. Work done by Cant et al. (1991) at the University of California led to an alternative proposal. They showed that infusing casein into the abomasum of lactating cows fed 4% yellow grease increased arterial amino acid concentrations but failed to prevent the milk protein depression. In a later study (Cant et al., 1993), they observed a 7% drop in mammary blood flow when cows were fed fat, thus preventing increased removal of critical amino acids as milk synthesis increased. The University of California workers proposed that fat supplements reduced milk protein concentration by reducing blood flow through the mammary gland causing reduced extraction of blood amino acids. In their explanation, milk volume is increased by the higher fatty acids inhibiting mammary de novo fat synthesis, causing a sparing of acetate for oxidation and more glucose available for lactose and milk synthesis.

Milk Lactose

As stated earlier, few studies have detected any significant change in lactose content of milk in cows fed diets in the normal range. Studies using mice have evaluated the impact of low lactose content on milk production. Using gene knockouts of α-lactalbumin, these studies have determined that lactose synthesis requires α-lactalbumin. This may not be advantageous to the dairy industry, as the milk produced was too viscous to be removed by the nursing pups. Therefore, it is likely that postharvest technology will be required to reduce lactose content of milk.

Summary

To the extent that milk pricing is linked to milk components, producers will continue to exploit nutrition of the cow as a means to modify milk composition for maximum economic return. With the complete mapping of the cattle genome not far away, opportunities will be explored to genetically manipulate or develop lines of cows that produce milk with a specific composition. Nutrition will remain an integral part of expressing this modified genetic potential. The greatest opportunities on the horizon for manipulating milk composition will be directed at using milk for delivery of nutraceuticals to enhance human health (Department of Health, 1994; Dixon and Ernst, 2001) and to combat clinical diseases, such as obesity, lactose intolerance, or osteoporosis. Fatty acid profile of milk will continue to receive attention in these areas, as it is a reservoir for many of the unique, and yet unknown, trans isomers of ruminal origin having a wide range of physiological responses. Enhancing specific proteins in milk to enhance human health will also be important, but because milk protein composition is less responsive to diet than fat, postharvest manipulation by processors and food scientists will play a major role.

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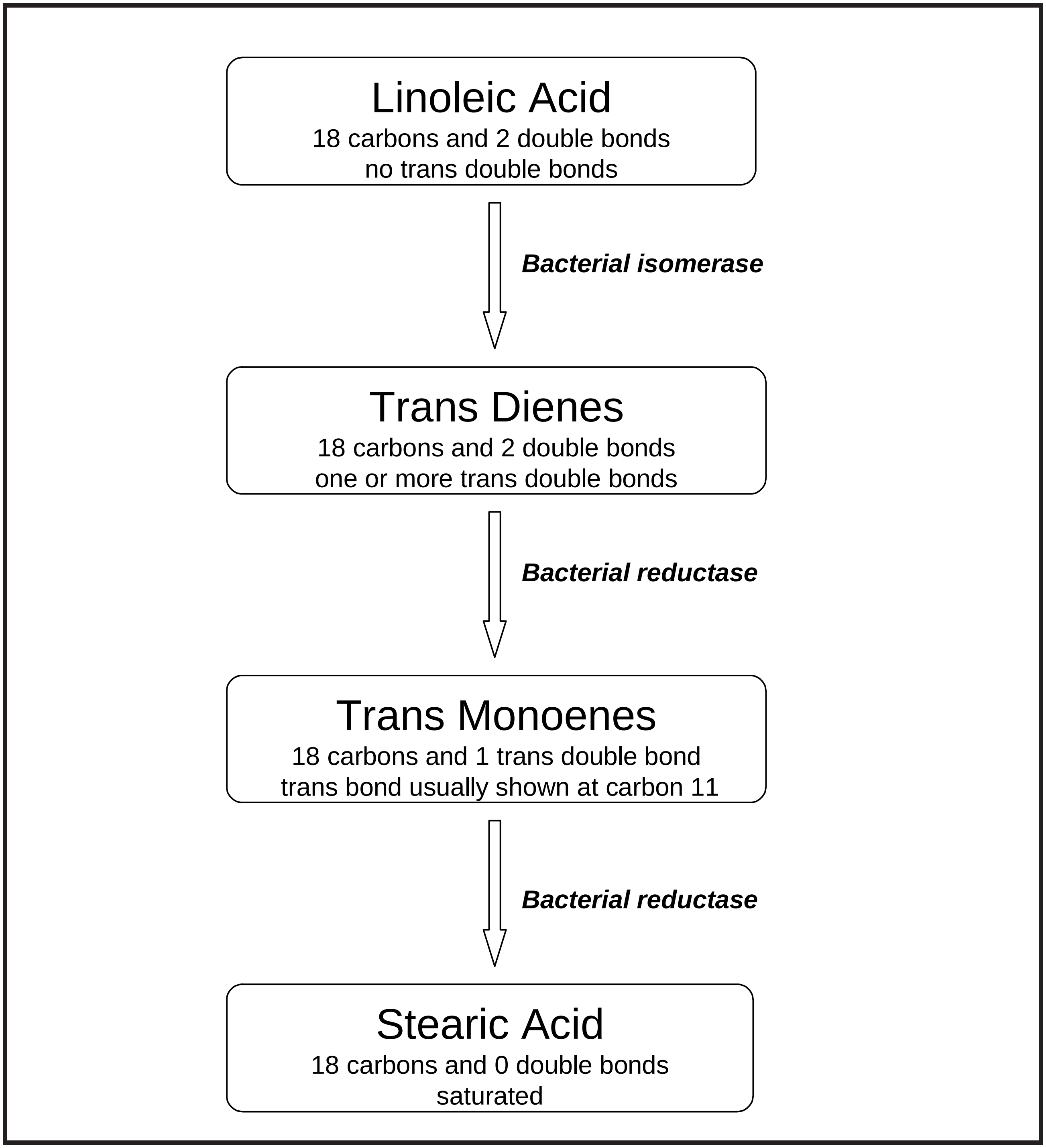

Figure 1. Major steps in the biohydrogenation of linoleic acid by ruminal microorganisms. Depending on conditions in the rumen, various proportions of stearic acid and trans intermediates are produced from linoleic acid. The trans diene intermediates usually include various conjugated isomers or conjugated linoleic acid.

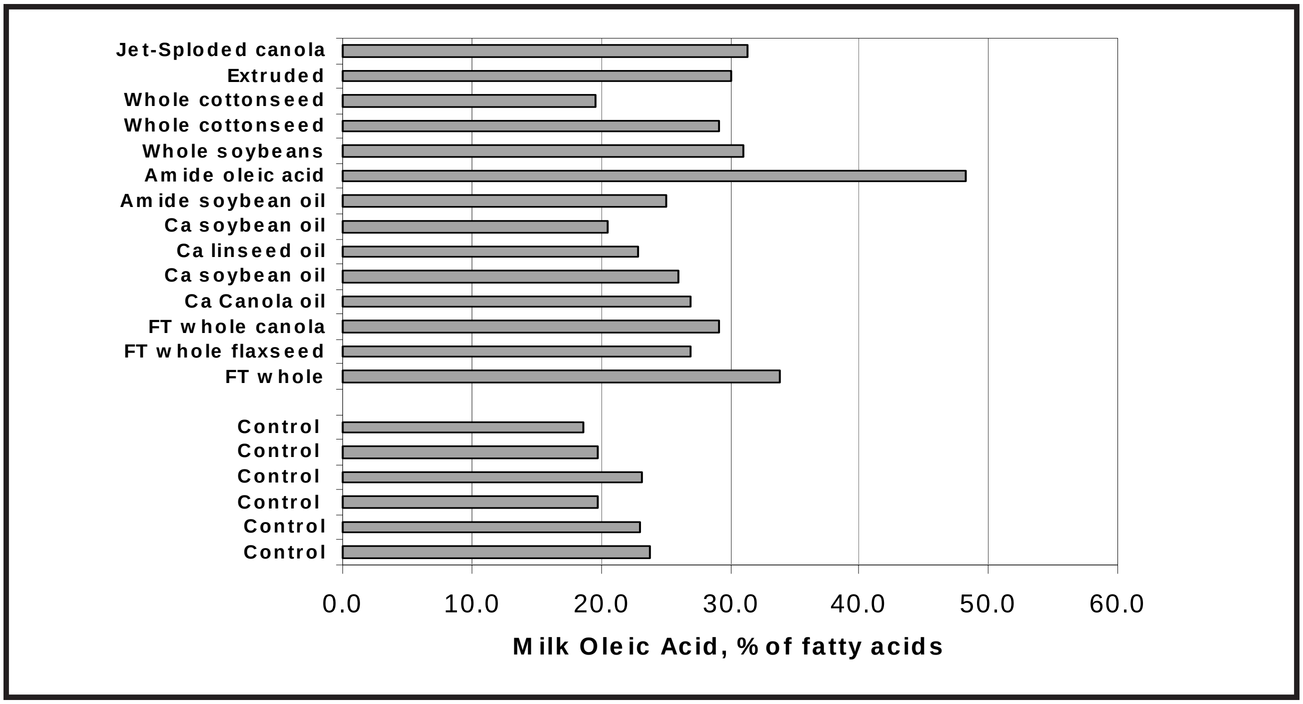

Figure 2. Samples of data from published studies showing the extent that oleic acid concentration in milk varies when lactating cows are fed control diets with no added fat or diets containing various sources of rumen-protected fat. Rumen-protected fat sources included whole oilseeds, amides of fatty acids, calcium **(Ca)** salts of fatty acids, and formaldehyde-treated **(FT)** fats.

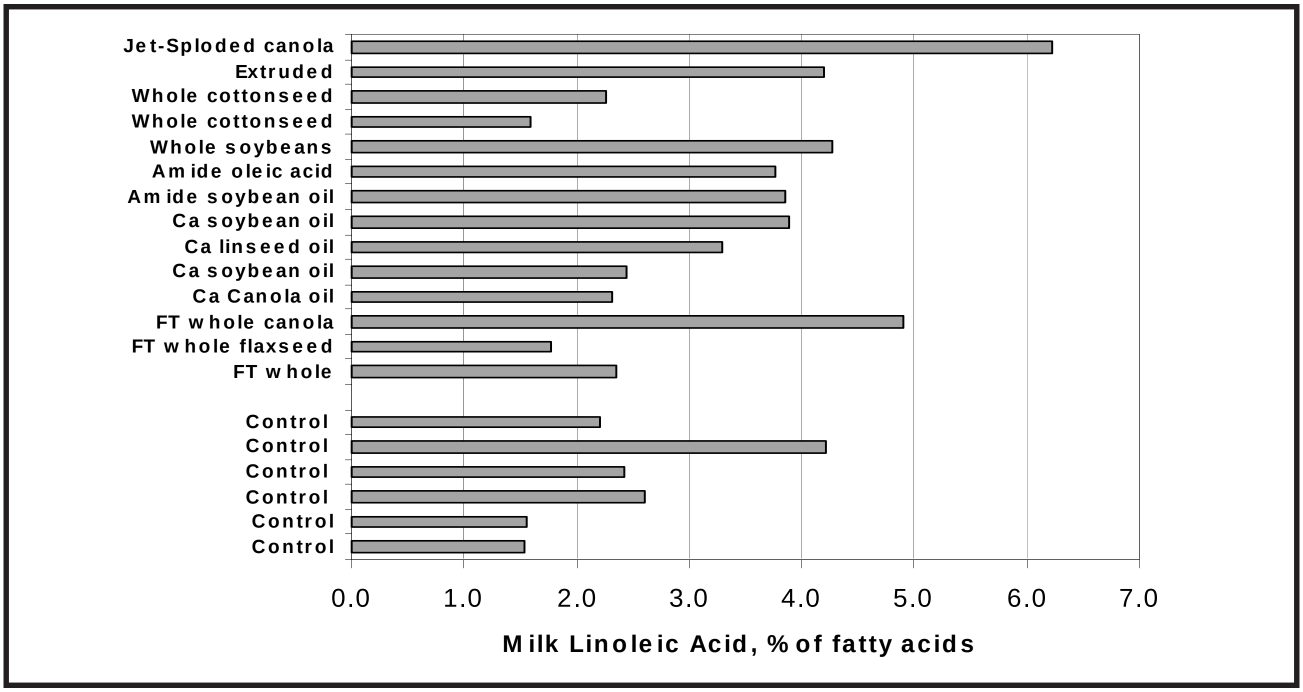

Figure 3. Samples of data from published studies showing the extent that linoleic acid concentration in milk varies when lactating cows are fed control diets with no added fat or diets containing various sources of rumen-protected fat. Rumen-protected fat sources included whole oilseeds, amides of fatty acids, calcium **(Ca)** salts of fatty acids, and formaldehyde-treated **(FT)** fats.

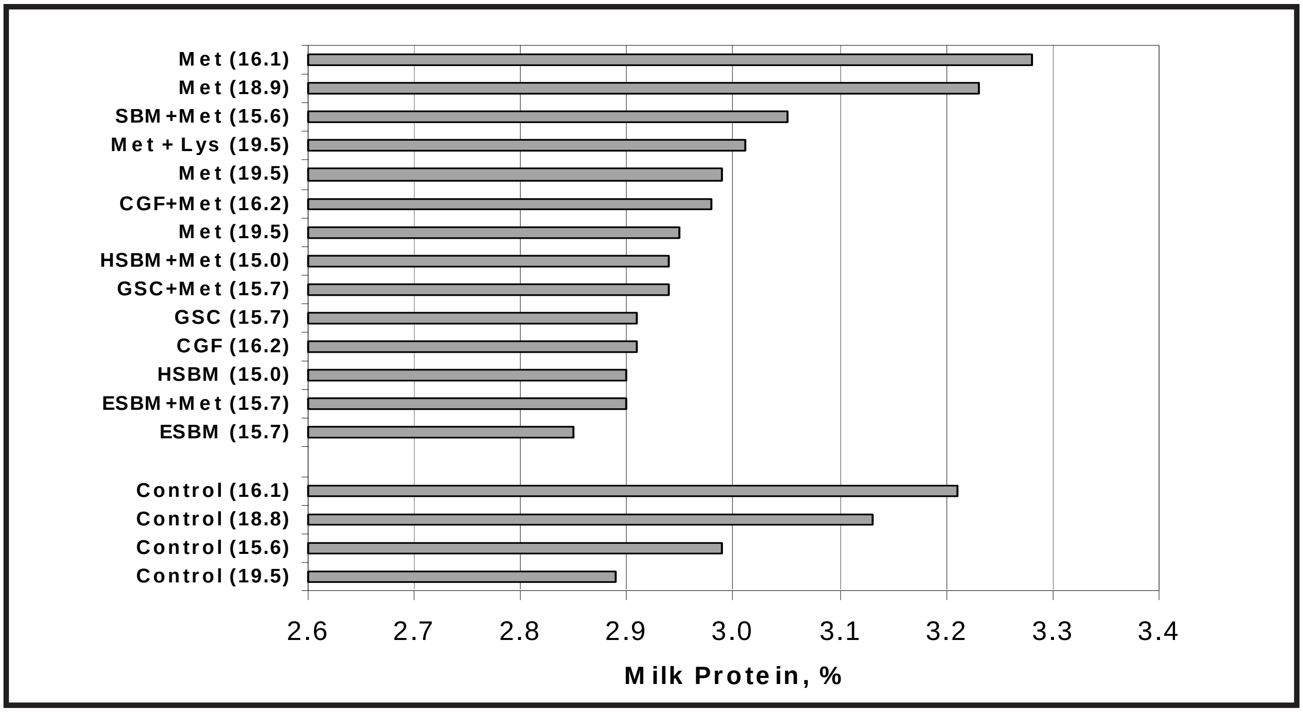

Figure 4. Samples of data from published studies showing the extent that milk protein percentage varies with amount and type of dietary protein. Dietary protein percentage is shown in parenthesis following source of protein (CGF = corn gluten feed, ESBM = extruded soybean meal, GSC = ground shelled corn, HSBM = heated soybean meal, Lys = rumen protected lysine, Met = rumen protected methionine, and SBM = soybean meal .