Formulating Dairy Cow Diets for Milk Composition

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Introduction

The composition of milk makes it one of the most nutritious and palatable foods for humans. Milk composition has always been of strong interest to dairy producers because milk pricing has historically been based on milk volume with adjustments for components. Most often, producers are paid on milk volume with adjustments for components or are truly paid on component yield entirely. It is possible to manipulate milk composition by dietary means. However, these alterations can only be achieved within certain biological limits imposed by dairy cow physiology and genetics. Producers should be aware of what nutritional manipulations are most likely to achieve a change in milk components and the cost-benefit associated with these dietary changes. A second very important consideration is to determine whether the desired changes are in milk component yield or percentage because dietary manipulations will often affect milk volume; therefore component yield may change (and revenue) with little or no change in component percentage. Conversely, a lot of effort and cost can be expended to alter milk component percentage, which may appear very impressive but have little or no impact on milk revenue yield.

Milk Secretion

Examination of methods to alter milk composition requires knowledge of the principles involved in milk synthesis. In this manner, feeding systems can be designed to provide the nutrients most likely to elicit a positive response in milk composition. Milk consists of two liquid phases. The aqueous phase consists of minerals, water-soluble vitamins and lactose in solution and proteins in a colloidal suspension (Mepham 1976). The lipid phase consists of fat-soluble vitamins, triglycerides and sterols. The secretory route of fat is distinct from lactose and protein. Milk fat is comprised primarily of triglycerides. The fatty acids (FA) that make up the trlg1ycerides come from the diet (45%), synthesized within the mammary gland (50%) and from body fat tissue (<10%). The dietary FA tend to be longer in chain length. The proportions of FA derived from each source will vary with the diet and the energy status of the animal. Synthesis of milk FA at the mammary gland requires acetic acid and beta-hydroxy-butyrate which are produced during feed fermentation in the rumen. There is an absolute requirement for glucose to produce the glycerol, which is the backbone of the triglyceride in milk fat. Milk fat exists in the form of globules and synthesis of fat is independent of milk volume. It is primarily dependent upon the presence of FA, precursors and hormonal interactions that affect the relative uptake of fat precursors, relative to glucose, from the blood into the mammary gland.

Milk proteins consist of caseins (85%) and whey proteins (15%). Caseins are milk-specific proteins that are the primary protein constituents in cheese formation. Some caseins have specific functions in maintaining milk stability. Other caseins such as beta-casein do not appear to have

any function other than to provide amino acids to the calf. Many of the whey proteins are non-specific to milk as they appear in blood as well. Whey protein content of milk will increase during mastitis because of the increase in the flow of components from the blood to the milk. In general, milk protein synthesis proceeds independently of total milk yield and will be dependent upon the supply of the essential amino acids needed to build the various proteins.

Lactose makes up virtually all of the carbohydrate in milk. As lactose is excreted by the mammary gland, it exhibits an osmotic effect that draws water from the blood. Therefore lactose is the predominant factor affecting milk volume. Because of this, lactose concentration is very constant at about 4.7 to 5.0%. Supply of glucose or glucogenic precursors, such as certain amino acids and propionate (from rumen fermentation) are essential for lactose synthesis. Conditions that limit lactose synthesis will decrease milk volume but not lactose content.

Within a given genetic capacity, milk yield and composition will reflect the external and internal environment of the cow. Variations in milk composition often are the result of variations in the relative secretion rates of the milk constituents, particularly water. The content and composition of milk fat can be altered immediately if there are sudden changes in the supply of energy substrates to the rumen and mammary gland. Milk yield and protein content are more stable and change occurs slowly over a period of days or weeks in response to substrate supply.

Lactose and Other Milk Solids

As stated above, lactose is the main component that generates milk volume. In this regard, it is extremely difficult to alter lactose content of milk. Decreased forage:concentrate ratios have occasionally shown an increase in lactose content, presumably because more propionate is available for glucose synthesis. Certain fat supplements have caused declines in lactose content, perhaps because of their impact on rumen fermentation. However, these responses are variable and extremely small. Likewise, these changes are generally associated with a sub-optimal situation and it is very difficult to get lactose content to exceed the “normal” concentration of about 5% of milk volume. Therefore, with our current level of knowledge, it is unlikely that lactose content can be manipulated appreciably.

Other constituents of the protein free solids not fat (SNF) are mainly minerals and vitamins. The concentrations of these constituents will reflect the nutritional status of the cow but their concentrations are low relative to the other major milk constituents so any changes here will have negligible effect on milk component yield.

Milk Fat Concentration

Historically, milk fat has been the primary component for adjusting the price of milk. It is the component most responsive to dietary change and is able to be manipulated over the widest range. Typically, dairy farmers expend considerable time and money trying to alter milk fat composition.

Acetate is the major FA involved in de novo milk fat synthesis. Because of this, it was believed that factors that limited acetate production in the rumen would lead to reduced milk fat synthesis. This made sense because dietary situations that resulted in milk fat depression were generally diets where the rumen acetate:propionate ratio was also low. We now know that milk fat depression is caused by the accumulation in the rumen of long chain trans fatty acids, particularly the trans-10, cis-12 isomer of conjugated linoleic acid (CLA). The t10,c12 CLA is particularly potent as an inhibitor of de novo milk fat synthesis (Moore et. al. 2004).

The unsaturated FA contained in fats are toxic to many rumen bacteria. This is particularly so for FA that are highly unsaturated (such as linolenic acid) and/or have a very long chain length (such as fish oils). Many rumen bacteria are capable of degrading fat in the rumen and partially hydrogenating them to trans FA and CLA. This is done in an attempt at self preservation to make them less toxic to the bacteria. However for complete detoxification, the FA must be completely saturated. Only a very small group of bacteria related to *Butyrovibrio* are capable of the final hydrogenation that removes the trans FA from the rumen (R.J. Wallace, personal communic.). Normally, the cis-9, trans-11 isomer of CLA is the predominant CLA produced in the rumen. However, when the rumen conditions allow certain species of *Propionibacteria* to thrive in the rumen (low pH, rumen available starch), they are responsible for the production of the t10,c12 CLA (N.D. Walker, personal communic.). As long as the *Butyrovibrio* species are present in sufficient numbers to hydrogenate the CLA and trans FA, there will be no milk fat depression. However if the metabolic activity of these critical bacteria are compromised, milk fat depression is likely. These *Butyrovibrio* are highly sensitive to unsaturated FA, particularly fish oils, low rumen pH and ionophores like monensin. If we look at factors that affect the activity of these critical bacteria and their environment, we can easily see which dietary conditions will have the greatest impact on milk fat content. Therefore the critical controlling factors in milk fat synthesis revolve around minimizing the microbial populations responsible for producing the the t10,c12 CLA and maximizing the metabolic activity of microbial populations responsible for converting the CLA and trans fatty acids into saturated fatty acids.

Fiber content in the diet is the most common way that milk fat content is altered. High-fiber diets are associated with stable ruminal pH. Forage is the main route by which fiber is included in dairy cattle diets. Therefore forage:concentrate ratios is a common criterion for adjusting the fiber level in the diet. However, the fiber content cannot be viewed simply as the chemical fiber in the diet, as measured by acid detergent fiber (ADF) or neutral detergent fiber (NDF). The physical effect of fiber on saliva flow, ruminal pH and fermentation patterns have led to the term "effective fiber".

Milk fat content of the diet will remain stable until the acids produced from rapidly fermentable carbohydrates exceeds the cow's ability to neutralize these acids via buffering (saliva) or absorption. As concentrate level increases, the level of milk fat content will decline proportionately unless other efforts are taken to ensure rumen pH does not decrease. With adequate levels of forage in the diet, the type and physical form of the concentrate will have little effect on milk fat content. However, when high-concentrate diets are fed, the concentrate source will have a significant impact on the degree of milk fat depression that occurs. Ground or extensively rolled barley will depress milk fat more than ground or dry rolled corn, with steam-

flaked corn being intermediate. The effect of concentrate on milk fat depression is directly linked to the rate that the concentrate is fermented in the rumen.

Providing sufficient long forage is usually sufficient to maintain fat content when corn-based concentrates are fed. However, when barley-based diets are used, the problem of maintaining milk fat becomes more difficult. Reducing the degree of grain processing with barley can reduce the rate of fermentation and increase milk fat content. However, digestibility may be reduced and the overall effect on milk production has not been determined yet. High-fiber concentrates can be used to reduce the fermentation rate of the concentrate mix. Beet pulp, citrus pulp, dehydrated alfalfa and corn gluten feed are high-fiber feeds that are highly but slowly digestible feeds that can be used to increase milk fat content. Although useful, these feeds are not as effective as fiber from forages in maintaining milk fat content. Therefore, with barley-based concentrates, the value of high quality forage is more important than with corn-based diets. Finely.chopped forages will result in insufficient rumination, thereby decreasing saliva flow and ruminal pH. The ability of forage fiber to maintain milk fat content will be limited if the mean particle length of the forage is inadequate.

Fat supplements are used to enhance the energy density of concentrates without increasing the fermentability of the diet. Fat supplements will affect the amount and type of milk fat present in milk in different ways depending on the source of fat being fed. Inclusion of fats up to about 3% of total diet dry matter will generally increase milk fat content regardless of fat source. Above 3% inclusion rate, saturated fats such as tallow will have a marginal positive effect on milk fat content. Excessive amounts of rumen available unsaturated fats such as canola or soybean oil will have a strong negative effect on milk fat content. This depressing effect on milk fat can be alleviated by protecting the fats from rumen fermentation. Protection of fats can be achieved to varying degrees by feeding whole oilseeds (cottonseeds, soybeans, flaxseed and safflower) or commercial products such as calcium soaps of fatty acids or encapsulated fats. Commercial preparations are more effective than whole oilseeds but are more expensive.

Increasing the frequency of feeding during the day should result in greater milk fat content. Smaller, more frequent meals will reduce fluctuations in ruminal fermentation, and increase average ruminal pH. These factors should lead to increased milk fat content. Despite this, changes in milk fat content due to more frequent feeding have been variable. Part of this lack of response may be due to com-based concentrates. The effect of frequent feeding may be more effective with barley-based diets.

Mineral buffers are an effective means of compensating for diets that are fermented too rapidly. Sodium and potassium bicarbonates and magnesium oxide, but not calcium carbonate, will help to maintain ruminal pH. In this manner, buffers will help elevate depressed milk fat content but will not elevate fat content above "normal" levels.

Milk Protein Content

Milk protein content can be manipulated by dietary means but the magnitude of the response will be far less than that observed for milk fat content. The natural variation in protein content is

less than for fat content. Interest in factors affecting protein content has been relatively recent compared to milk fat content.

Underfeeding energy can cause a severe decline (up to 0.3 percentage units) in milk protein content. Feeding energy above requirements will cause a smaller increase in milk protein content. Emery (1978) reported an increase of 0.015 % unit/ Mcal NEI (net energy of lactation) intake. Part of this effect of energy intake has been attributed to forage:concentrate ratio. As the fiber content and proportion of forage in the diet increase, milk protein content will decline, although results have been quite variable.

The type of grain used in the concentrate will influence milk protein content. When barley and corn are compared, the results generally favor corn-based diets. Increasing the proportion of concentrate in the diet will increase milk protein content for corn diets but not for barley diets. The reasons for these observations are unclear but appear to be related to the fermentation rate of the concentrate, absorption of glucose from "bypass" starch and interactions with insulin in the cow. Although the mechanisms and practical feeding strategies have not been worked out, it would appear that feeding high levels of concentrates that are resistant to rapid fermentation in the rumen may have a positive effect on milk protein concentration. Of course, feeding rumen resistant starch will decrease the amount of microbial protein produced in the rumen and this can lead to less metabolizable protein and lower milk protein content.

The feeding regime that most consistently negatively affects milk protein content is fat supplementation. Regardless of the form or source, supplemental fat tends to depress milk protein content. The reason for this depression has not been determined but appears to be related to the fat content of the diet, regardless of the energy intake. The magnitude of the decline has been determined to be 0.04 % units/ % supplemental fat (Sporndly 1986). Whether the effect of this negative effect is from decreased rumen microbial protein synthesis or is under hormonal control is unclear.

Severe protein undernutrition will cause large decreases in milk protein content. However, feeding additional protein above the cow's requirement will not change protein content. If supplemental protein is relatively undegradable in the rumen, milk protein yield will increase due to increased milk volume, but milk protein content may not be unaffected

Various methods have been used to increase the supply of protein and AA to the small intestine, including feeding proteins with high values for RUP, and chemical or physical treatments which increase the RUP value of a feed. In recent years, productive diets for ruminants have been supplemented with various sources of RUP. Of the more common sources, fishmeal, blood meal, dried distillers grains and corn gluten meal have been used. Based on amino acid profiles and rumen degradability, corn and its by-products (e.g. corn gluten meal) are relatively good sources of leucine but are low in lysine. Fishmeal is a good source of methionine but soybean meal is not. Blood meal is a good source of lysine but is low in methionine. Methionine is likely to be limiting when legume or animal proteins are the main source of RUP and often appears as deficient in many dairy diets.

Heat treatment has been used to decrease ruminal degradation of proteins and amino acids. Heating causes carbonyl groups of sugars to combine with free amino groups of proteins in the Maillard reaction. Amino acids also form peptide links with asparagine and glutamine (Belitz and Grosch, 1987). The resulting peptide linkages from heating are more resistant to enzymatic hydrolysis. Oil seed protein sources are the most economical to treat with heat. For example, Benchaar et al. (1993) reported that feeding extruded lupins increased the flow of AA to the duodenum of cows by 34% and increased apparent absorption of AA in the small intestine by 58%. Roasting and extrusion have also been extremely popular methods to increase the RUP content of soybeans. Some precautions must be taken when heat-treating proteins, as excessive heat can cause essential amino acids such as lysine, methionine, and cystine to be extensively damaged.

Increasing the amount of rumen RUP has not always increased the amount or changed the quality of AA reaching the small intestine. In many instances microbial protein production has decreased when RUP increased, probably because of a reduction in diet fermentability. This caused an increase in RUP but a decrease in microbial protein, resulting in no net change in total AA flow to the small intestine. No single feed source of RUP provides a balance of essential AA that matches the essential AA profile of milk. In addition, many feeds with high RUP values are low in one or more essential AA. As a result, a deficiency of one AA could be exacerbated by feeding a RUP source low in that AA.

Combinations of several RUP proteins that are complementary to each other could overcome this problem. Ferguson et al. (1994) reported on a research study involving 35 herds and 7000 cows. Cows were supplemented with a marine-animal protein blend to attain a similar protein level as unsupplemented cows. Nineteen of the 35 herds had a positive response (79% of cows> where cows averaged 1.22 kg more milk per cow per day. In early lactation cows only, 26 of 35 herds responded (95% of cows) with an average increase of 2.64 kg more milk per day.

Amino acid supplementation

Free AA are generally not recommended as supplements in ruminant diets because they are degraded rapidly in the rumen. However, free lysine-HCl has been used with estimates of ruminal bypass ranging from zero to 20% of dose. Thus, chemical alteration or physical protection are required to protect an AA from rumen degradation and to increase the supply of that specific AA to the duodenum. A balance must be achieved so that AA protected from ruminal degradation are still available for intestinal absorption. In addition, these compounds should be stable. Pelleting and even over-mixing can cause breakdown of the protective coating. Extensive exposure to silage-based TMR can weaken the protective coating of pH sensitive RPAA.

Various analogs of AA have been tested for resistance to ruminal degradation (Ayoade et al., 1982). One of the more tested AA derivatives is 2-hydroxy-4-methyl thio butanoic acid (HMTBA). HMTBA is a source ef methionine (Met) activity in all production animals. Because HMTBA differs in its chemical structure from L-Methionine (L-Met), it is recognized differently by rumen microbes. The HMTBA contains a hydroxyl group rather than an amino group. Because of this, HMTBA is and behaves as an organic acid. Rumen bacteria possess highly

efficient uptake systems to scavenge amino acids such as L-Met. Conversely, as an organic acid, HMTBA is recognized as a fermentation endproduct similar to other organic acids of similar size. Additionally, because it is a relatively reduced acid (compared to lactate which is more oxidized), only a selective group of microbes are capable of extracting energy by fermenting it. Patterson and Kung (1988) showed rumen microorganisms have higher affinity for DL-Met than HMTBA resulting in faster rate ef degradation for DL-Met than HMTBA. By 12 hours, virtually all DL-Met was degraded whereas 80% of HMTBA still remained.

Koenig et al (1998) studied the kinetics of HMTBA degradation in the rumen and found that ruminal escape of HMTBA was approximately 50% with 40% of the dose being recovered in the small intestine. Ruminal bypass of HMTBA was a function of ruminal liquid turnover rate and was not affected by dosage rate (Koenig et al., 2002). The role of the passage rate of the liquid digesta on the availability of HMTBA was further verified using continuous culture fermenters (Vazquez-Anon et al., 2001). Unlike HMTBA, ruminally protected sources of DL-Methionine (RPMet) are insoluble and leave the rumen with the solids. Once the DL-Met is released from its protective coating, it can only be absorbed by active transport at selective sites in the small intestine. Conversely HMTBA is absorbed by diffusion across the rumen, omasum and intestinal wall and into the blood stream.

As an organic acid, HMTBA can be absorbed across the intestinal epithelium via diffusion instead of via an active transport system like DL-Met. As HMTBA is absorbed along the digestive tract, approximately 30 % of it is converted to L-Met and used by the gut tissue to synthesize protein (Lobley et al. 2001, 2006). Of the remaining HMTBA that is not metabolized by the gut and enters the portal blood system, 25% is converted to L-Met in the liver. The remaining HMTBA that leaves the liver and reaches peripheral tissue is rapidly converted to L-Met in most tissues of the body where it is metabolized. Productive tissues such as mammary gland and muscle are able to remove HMTBA from circulation, converted to L-Met and use it, with the exception of the kidney. The kidney has a high capacity for HMTBA conversion and low L-Met usage for protein synthesis and therefore, secretes L-Met back into circulation (Lobley et al. 2001, 2006).

In ruminants, measurements of plasma free Met concentrations have been used to evaluate the efficacy of several Met sources that contain the racemic mixture of D- and L-Met. However, this methodology is not valid when evaluating HMTBA. The L-Met derived from HMTBA is synthesized and used at the tissue level and a very small portion of the L-Met will be secreted into circulation. In fact, the liver takes up a very high proportion of the L-Met that enters the organ where it is metabolized (Lapierre, 2006). In the case of rumen protected sources of DL-Met, elevation of plasma Met concentration reflects the accumulation of D-Met in plasma prior to its conversion to L-Met by the tissues (Vazquez-Anon et al., 2001) and not its bioavalability. Measurement of plasma Met concentration is not an appropriate tool to evaluate different sources of Met due to differences in the contribution of D- and L-Met and sites of absorption and metabolism.

Lactation Responses to Supplemental Amino Acids

Feeding for milk protein components has been viewed as a matter of balancing for metabolizable lysine and methionine delivery and by trying to achieve the ideal 3:1 lysine:methionine ratio, milk protein components can be maximized. However, newer information on the efficiency of amino acid utilization as well as a re-examination of the literature on amino acid supplementation indicates that our focus on lysine:methionine ratios may need to be re-examined. Because amino acids, particularly methionine have roles in the body other than as building blocks.

In current ration formulation strategies for amino acids in dairy cows, the focus has been on balancing for lysine and methionine requirements. The basic principle is to try and meet the cows metabolizable protein (MP) requirement and to ensure that lysine (as a percent of MP) is 7.2 and methionine (as a percent of MP) is 2.4. Given that it is very difficult to formulate diets to deliver 7.2% lysine without a commercial source of rumen protected lysine, practical recommendations are to try and maximize metabolizable lysine (at least 6.6% of MP) and to maintain a lysine:methionine ratio of 3.1:1 (expressed as a percent of MP). This premise is based on the extensive efforts of Rulquin and Schwab (Schwab and Boucher, 2005) which has become the basis of the NRC (2001) recommendations.

These recommendations are based on the response in milk protein percentage achieved when different levels of lysine and methionine were supplemented and is a function of the amino acid profile of milk protein. However, Lapierre et al (2006) have demonstrated that the "requirement" as defined by NRC (2001) may actually be a function of the changing efficiency of amino acid utilization. Additionally, the evaluation of lysine and methionine requirements has only focused on milk protein and ignores the impact of amino acids, particularly methionine on milk fat production and other metabolic pathways that are not associated with proteogenic activity. While a lysine:methionine ratio of 3:1 at the the mammary gland may be optimal for milk protein synthesis, the efficiency of methionine utilization is controlled at the liver and lysine is controlled at peripheral tissues such as the mammary gland (Lapierre et al 2006). Therefore it is easy to see why trying to optimize dietary or metabolizable concentrations of amino acids does not always result in the response we anticipate in terms of milk protein concentrations. Weekes et. al. (2006) showed that under severe imbalances of AA, milk fat concentration increased dramatically with only small changes in milk protein concentration. For example, low lysine: or histadine:methionine ratios resulted in milk protein: fat ratio dropping from 0.84 to 0.58 and 0.50 respectively.

We recently conducted a literature survey to investigate the impact of HMTBA, and rumen protected Met (RPMet) sources on milk yield and components (Rode and Vazquez-Anon, 2004). Fifty-eight and sixty-one studies were identified in the literature where rumen protected DL-Met (RPMet) and HMTBA were fed compared a control diet. When the milk yield and components response over the control was evaluated, differences were found between Met sources. HMTBA supplementation improved milk yield (1.39%), milk protein (2.9%) and fat yield (4.99%). Whereas RPMet supplementation reduced milk yield (-0.85%) and the milk protein (1.49%) and fat yield (2.24%) response was numerically lower than HMTBA. The higher milk and component yield response of HMTBA over RPMet might be partially explained by its effect on

microbial protein synthesis. Microbial protein is largely used as gluconeogenic precursor and consequently lactose synthesis and as amino acid supply for protein synthesis at the mammary gland. On the other hand, RPMet improved milk protein percentage more than HMTBA, mostly due to the reduction in milk yield response. Both Met sources improved milk fat percentage similarly indicating Met has a lipogenic effect.

Several mechanisms have been postulated by which Met or HMTBA could improve milk fat secretion. Systemically, Met may improve hepatic lipid secretion and thereby improve milk fat synthesis at the mammary gland. Alternatively, Met may have an effect at the level of the mammary gland by ameliorating the effect that trans fatty acids have in blocking de novo milk fat synthesis. Ruminal effects can not been ruled out but seem unlikely in that HMTBA would have to be accelerating the rate of ruminal lipid biohydrogenation and there has been no evidence of this effect.

Non-nutritional Factors Affecting Milk Composition

Nutrition is probably the fastest and most efficient way by which milk composition can be manipulated. However, several other physiological and environmental factors will affect milk composition.

Genetics offer the only real alternative to nutrition as an effective means of manipulating milk composition. Genetic selection can alter milk composition in almost any direction. The most efficient selection would be for altered fat:protein ratio, primarily by changing fat concentration (Gibson 1989). Selection for higher concentrations of components will have a depressing effect on total milk and protein yield. This is particularly the case if selection is for fat concentration (Gibson 1989). Therefore, farmers should make selection decisions on the basis of component yield rather than component concentration.

Other factors affect milk composition, but are virtually impossible to manipulate. These factors must be considered for their ability to interfere with dietary regimes designed to alter milk composition. Milk fat and SNF percentage decline with age of cow and length of lactation. Mastitis generally decreases lactose and casein percentage while increasing total and whey protein content. Seasonal variations will occur in milk composition, although it is difficult to separate the effects of nutrition and environment. In Ontario, fat and protein percentage declined in summer, corresponding with higher milk yields and spring-calying cows (Burton et al. 1986). Environmental factors that have been identified as affecting milk composition include photoperiod, heat stress, humidity and cold stress. Cows milked at 12/12 hour intervals produce more milk (3%) but unequal milk intervals generally result in greater fat (5%) and SNF (4%) percentage.

Conclusions

When dairy farmers are paid for their milk on the basis of milk components, there is always impetus in altering milk component production. Of the three major milk components, lactose is most constant and subject to only minor variations due to feeding. The concepts of feeding for (or against) milk fat content has changed considerably in the last decade. Milk fat depression is

now known to be a function of the trans fatty acid production in the rumen and the bacteria involved in their metabolism. The success in manipulating milk fat content will depend upon our ability to take into account dietary changes that affect the rumen environment. Feeding for milk protein components has been viewed as a matter of balancing for metabolizable lysine and methionine delivery and by trying to achieve the ideal 3:1 lysine:methionine ratio, milk protein components can be maximized. However, newer information on the efficiency of amino acid utilization as well as a re-examination of the literature on amino acid supplementation indicates that our focus on lysine:methionine ratios may need to be re-examined. Because amino acids, particularly methionine have roles in the body other than as building blocks for protein, it is possible to use this information to alter milk fat as well as milk protein composition.

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