FEEDING FOR MILK COMPOSITION[1]

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INTRODUCTION

Dietary factors can greatly affect the composition of milk of dairy cows, and nutrition offers the most effective means of rapidly altering the composition of milk. Among milk components (fat, protein, lactose, minerals, and vitamins), fat and protein are the two most subjected to changes due to dietary manipulation.

Increasing fat concentration in milk has always been a major goal when feeding lactating dairy cows. In certain milk markets, a premium is paid for contents of milk components. In California, fat receives the major emphasis when premiums are paid to producers; therefore, maximizing output of fat corrected milk per cow will determine most of the profitability of the dairy operation. In 1998, after El Niño affected weather conditions in California and other states of the USA, and milk production dropped 5 to 10%, the producer price for milk fat reached an all time high of $7.50/kg. To deal with situations such as that, nutritionists and veterinarians should formulate diets to maximize production of milk and milk fat.

In recent years, research has rediscovered the importance of milk in human nutrition. It has been shown that certain fatty acids in milk act as anticarcinogenic agents (Cook, 1999; Parodi, 1999; Parodi, 1997). The presence of anticancer components in milk fat such as butyric acid and conjugated linoleic acid has inspired researchers to investigate nutritional approaches to manipulate the fatty acid profile of milk fat.

Diet can also affect the protein content of milk. Although changes in milk protein are not as dramatic as those observed for milk fat, increases in 0.05 to 0.15 percentage units in milk protein might be observed when the energy and protein content of the diet is manipulated. Perhaps, even more important than increasing the crude protein content of milk is increasing the

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concentration and yield of casein. In areas where the N content of milk is analyzed as true protein, increasing urea-N in milk does not result in increased content of milk protein. In addition, in places where milk is marketed for cheese manufacturing, a special attention should be given to feeding dairy cows in order to optimize milk output of true protein. This paper will discuss some of the nutritional factors that impact fat and protein concentration in milk.

Abbreviation key: CLA = Conjugated linoleic acid; **DCAD** = Dietary cation-anion difference; **DM** = Dry matter; **NE_L** = Net energy for lactation; **NDF** = Neutral detergent fiber; **NFC** = Nonfibrous carbohydrates; **RUP** = Rumen-undegradable protein; **TMR** = Total mixed ration; **VFA** = Volatile fatty acids.

MILK FAT

General understanding of dietary factors that impact milk fat content have greatly progressed in the last 5 years. Several theories have been suggested to explain the variations in milk fat caused by dietary manipulation. Traditionally, decreases in milk fat content have been attributed to decreases in availability of precursors for fatty acid synthesis in the mammary gland.

Milk fat is composed by two major groups of fatty acids. About 50 to 70% of the milk fatty acids are those with long carbon chains (equal to or greater than 16 C). The other 30 to 50% are fatty acids with less than 14 C (short and medium chain fatty acids). Long chain fatty acids are originated from dietary sources or from mobilization of adipose tissue and they are taken up by the mammary cells and incorporated into milk fat. The short chain fatty acids are synthesized *de novo* by the mammary gland from precursors such as acetate and butyrate. Generally, when milk fat is depressed, the fatty acids that decrease are those with less than 16 C (Palmquist et al., 1993). This indicates that milk fat suppression is characterized by a decrease in *de novo* synthesis by the mammary gland.

Low milk fat syndrome has been recognized for many years, but the exact mechanism is still unclear. Several theories have been postulated to explain why dietary factors affect fat content in milk (Erdman, 1999). Fat deficiency, acetate deficiency, butyrate insufficiency, Vit. B_{12} deficiency, and the glucogenic theory (insulin theory) have all been postulated as possible causes for decreased fat concentration in milk when cows are fed high grain diets or diets that are high in fat. However, only recently (Erdman, 1999) a more detailed explanation has been given

for the mechanism of milk fat depression. Erdman (1999) reviewed data from several studies, primarily from University of Maryland and Cornell University, and observed that the suppression of fat content in milk is probably related to the amount of *trans* fatty acids synthesized in the rumen, absorbed by the small intestine and incorporated into milk fat.

Trans fatty acids are normal metabolic intermediates found in the rumen. They are synthesized during the process of biohydrogenation of unsaturated fatty acids by rumen microbes (Jenkins, 1993). Because hydrogenation of unsaturated fatty acids may be incomplete, and also because the rumen does not maintain digesta flow with constant lipid composition in all circumstances, the flow of trans fatty acids to the duodenum may increase under certain feeding conditions.

Data from Cornell University (Griinari et al., 1997) have shown that duodenal infusion of a mixture of conjugated linoleic acid (CLA – C18:2 *cis*-9 *trans*-11 and C18:2 *trans-10 cis*-12) caused a curvilinear depression in milk fat concentration. Because more *trans* fatty acids were available in the small intestine for absorption, the content of milk CLA was also increased. In addition to absorption and incorporation of *trans* fatty acids into milk fat, the mammary gland has the ability to desaturate fatty acids. The presence of the stearate desaturate enzyme in mammary tissues allows the conversion of stearic acid to oleic acid (Bauman et al., 1998). Therefore, milk trans fatty acids can originate from absorption of these fatty acids by the digestive tract, and also by desaturation of fatty acids by the mammary gland.

Erdman (1999) showed a negative relationship (r = -0.53) between milk *trans* fatty acid content and milk fat content. As the concentrations of trans fatty acids in the milk increased, the content of fat in milk decreased. Preliminary data from Piperova et al. (1998) showed that high grain high unsaturated fat diets increased milk *trans* fatty acid content and decreased the activity of the enzymes acetyl CoA carboxylase and fatty acid synthase. These diets also decreased the abundance of mRNA for acetyl CoA carboxylase (Piperova et al., 1998). These data suggest that *trans* fatty acids not only depress the activity of lipogenic enzymes, but they also inhibit gene transcription for acetyl CoA carboxylase. The effects of polyunsaturated fatty acids on regulation of gene expression have also been demonstrated in lipogenic and non-lipogenic tissues of other animal species (Sessler and Ntambi, 1998).

Depression in milk fat is caused by a decrease in short and medium chain fatty acid content in milk (Palmquist et al., 1993). These fatty acids originate from *de novo* synthesis by the mammary gland. Therefore, it is possible that the mechanism of milk fat suppression is mediated by *trans* fatty acids decreasing the *de novo* synthesis of short and medium chain fatty acids through the inhibition of activity and the decrease in abundance of lipogenic enzymes in the mammary gland.

Carbohydrate Source and Milk Fat

The two major carbohydrate fractions of dairy cattle diets are the neutral detergent fiber (NDF) and the nonfibrous carbohydrate (NFC). NDF comprises the fibrous material that is slowly digested, partially available for the microbes in the rumen and large intestine. NFC are composed by starch, pectin, β-glucan, fructans, sugars, and organic acids. The components of the NFC fraction are rapidly digested by the rumen microflora, and some of them (starch, sugars, and fructans) can be digested by mammalian enzymes in the small intestine.

When the fiber (NDF) content of the ration declines, starch (NFC) generally increases. Ruminal bacteria that ferment starch produce large amounts of propionate and, when more fermentable energy is available in the rumen, the molar concentration of volatile fatty acids (**VFA**) increases. This increase in VFA concentration causes a drop in rumen pH and an increase in rumen osmolarity. Such changes in rumen environment favor the synthesis of *trans* fatty acids. Kalscheur et al. (1997) observed that cows fed a low forage diet without supplemental buffer had lower rumen pH, greater flow of *trans* fatty acids to the duodenum, greater concentration of milk *trans* fatty acids and lower milk fat content than those fed a low forage diet supplemented with buffer. However, in the same study (Kauscheur et al., 1997), only decreasing the amount of forage was not sufficient to increase milk *trans* fatty acid content and decrease milk fat synthesis. It is possible that the less dramatic decline in rumen pH in cows fed the low forage diet with additional buffer, compared with those fed the same diet, but without buffer, might have not been low enough to favor the synthesis of *trans* fatty acids in the rumen. Although the data from Kalscheur (1997) indicate that lowering rumen pH decreases milk fat, extensive reviews of the literature have shown contradictory results.

Erdman (1988) observed no relationship between rumen pH and milk fat content. Armentano and Pereira (1997) observed a weak relationship (r^2 = 0.06 to 0.11) between rumen

pH and milk fat for diets adjusted for a common nonforage NDF basis. Contrary to Erdman (1988) and Armentano and Pereira (1997), Allen (1997) observed a linear relationship between mean rumen pH and milk fat content ($r^2 = 0.39$) for 90 observations from 23 studies reported in the literature. Regardless of what the relationship between rumen pH and milk fat content might be, diets that favor a more acid rumen environment due to replacement of forage with grain favor a decrease in milk fat content. Decreasing the amount of forage, the amount of NDF, or the physically effectiveness of NDF by reducing particle size of fibrous material, will all impact rumen environment and promote milk fat suppression.

The NRC (1989) recommends that diets for lactating dairy cows should contain a minimum of 25 to 28% of the total DM as NDF, and it is suggested that 75% of that NDF should be from a forage source. Because NDF is a measure of chemical fiber and it does not take into consideration its effectiveness to stimulate rumination, cud chewing and rumen fill, a novel approach has been suggested to meet the fiber requirements of dairy cows (Mertens, 1997). When the physical effectiveness of NDF was regressed against milk fat percentage, Mertens (1997) established that a minimum of 20% of the diet DM should be physically effective NDF in order to maintain a milk fat test of 3.5% in Holstein cows. Many factors can confound these results and not only physically effective NDF will determine the fat content in milk, but this is an initial step to better formulate diets that promote better rumen health and yields of milk components. Because physical effectiveness of NDF contained in different feedstuffs is variable and difficult to be measured in the field, an estimation of physically effective NDF in the diet could be generated by computer models based on information on feed quality and diet mixing routine. The Cornell-Penn-Miner model (CPM-Dairy) utilizes Mertens (1997) data to describe the fiber requirements of dairy cows. General guidelines for providing adequate fiber in the ration to maintain adequate milk fat content include a total NDF between 28 to 33%, forage NDF of at least 60 to 70% of that, and physically effective NDF (as calculated by Mertens) between 21 and 23%.

Because the fiber content of the ration of dairy cows is inversely related to its energy content (NRC, 1989), lactating dairy cows are fed diets with minimum amounts of NDF to assure adequate rumen function and milk fat synthesis. Generally, replacing NDF with NFC leads to increased energy content of the diet, increased milk production, and decreased milk fat content. However, it is possible to maintain high levels of milk production with a reasonable

concentration of milk fat. This strategy would lead to greater yields of fat/cow/lactation. Information from the Dairy Herd Improvement records of California dairies that utilize total mixed rations (**TMR**) with 28 to 32% NDF and 38 to 41% NFC demonstrates that it is possible to achieve average herd productions of 35 to 40 kg/d (11,000 to 13,000 kg/lactation) with greater than 3.5% milk fat content (Santos, unpublished information).

Increasing the amount of ruminally fermentable carbohydrate in the diet also influences milk fat content. Processing of cereal grains improves starch digestion in the rumen, increases total rumen VFA concentration, mainly because of an increase in propionate, and it decreases mean rumen pH (Santos, 2000). Steam-flaking of corn and sorghum grains decreased milk fat content by about 0.2% units when compared with less extensive processing methods (cracking, dry-rolling, and steam-rolling), but the total daily fat yield was similar (Santos, 2000; Theurer et al., 1999). Therefore, increasing the amount of fermentable energy in the diet might decrease milk fat content, but not necessarily milk fat yield. In situations where milk production is increased, total daily fat yield might actually be improved.

Dietary Protein and Milk Fat

The impact of dietary protein on milk fat content is not clear. Dietary protein is manipulated to improve milk production, maximize DM intake, improve milk protein synthesis, and reduce N wastage. However, little is known regarding protein feeding to manipulate milk fat content. Protein may supply specific amino acids that provide precursors for the synthesis of branched chain fatty acids. Also, protein degradation within the rumen may supply ammonia-N for fiber digesting bacteria, which favors acetate synthesis. Furthermore, protein degradation within the forestomachs may buffer the rumen environment due to ammonia release.

In an extensive review of the literature, Santos et al. (1998) observed that replacing soybean meal (a protein source of high rumen degradability) with different sources of high rumen-undegradable protein (**RUP**), in most cases, decreased milk fat content. In 29 comparisons between soybean meal and a high RUP soybean meal supplement, milk fat content was not affected in 28 cases, and increased in only 1 case. When soybean meal was replaced by fish meal, milk fat content decreased in 16 of the 28 comparisons, was not affected in 11, and increased in only one. It is likely that the high polyunsaturated fatty content of fish meal was responsible for the milk fat depression. When different combinations of animal byproducts,

brewers or distillers grains, corn gluten meal, or blends of animal and vegetable proteins replaced soybean meal, milk fat content was generally unaffected. In their review (Santos et al., 1998), 129 comparisons from 89 lactation trials were evaluated, and replacing a rumen-degradable protein source with a high RUP source decreased milk fat in 21 cases, had no effect in 99, and increased in 9 cases. The high RUP source that most consistently decreased milk fat content was fish meal. Therefore, it is unlikely that manipulating protein nutrition is an efficient method to alter milk fat, but when protein sources with high polyunsaturated fatty acid content are added to the diet, milk fat concentration might decrease.

Dietary Fat and Milk Fat

Feeding fat to lactating dairy cows has become a common practice. The net energy for lactation (**NE_L**) of fat sources is 2.5 to 3.0 fold higher than that of carbohydrates (NE_L of corn is 1.9 to 2.2 Mcal/kg and the NE_L of tallow is 6.0 Mcal/kg). Fat can increase the energy density of the diet, improve the energetic efficiency of milk production, reduce dustiness of the diet, and improve reproductive parameters. However, incorporation of supplemental fat to dairy cattle diets can present challenges. It is well know that certain fat sources can impact rumen metabolism, decrease fiber digestibility, reduce microbial protein synthesis, decrease DM intake, and impact milk fat and protein synthesis.

Increasing the amount of supplemental fat from 1 to 5% of the diet DM decreased linearly the *de novo* synthesis of short chain fatty acids in milk (Grummer, 1991). Also, fat feeding can alter not only the fat content in milk but also the fatty acid profile of milk fat (Parodi, 1999; Bauman et al., 1998; Palmquist et al., 1993). When Palmquist et al. (1993) compared the effects of a low and a high fat diet, increasing the amount of fat decreased the concentration of C8 to C15 fatty acids, but had no effect on long chain fatty acids.

Addition of unsaturated fatty acids to the diet of dairy cows increases the synthesis of *trans* fatty acids in the rumen, which has been shown to depress milk fat content (Chouinard et al., 1999, Erdman, 1999, Giesy et al., 1999). The decrease in milk fat seems to be associated with an increase in milk *trans* fatty acid content (Erdman, 1999). *Trans* fatty acids have the capability to alter gene transcription (Cook, 1999; Parodi, 1999; Piperova et al., 1998; Parodi, 1997), which leads to a depression on the lipogenic activity of the mammary gland (Piperova et al., 1998).

Therefore, when feeding fat to dairy cows, it is important to be aware that different fat sources might have different effects on milk fat content and also on milk fatty acid profile.

The relationships between fat source and forage level and source is also important. Cows fed high forage diets or diets high in alfalfa tend to be less affected by the negative impacts of supplemental fat rumen metabolism. Such diets offer more binding sites for fatty acids to be adsorbed, which reduces their availability to interact with the rumen microflora. Also, high forage diets or diets based on alfalfa hay have a faster rumen turnover rate of the liquid phase, which may remove fatty acids at a faster rate from the rumen, decreasing their availability for complete hydrogenation. An additional hypothesis is that high forage diets or diets based on alfalfa increase rumen buffering and prevent a drop in pH, which seems to be important for *trans* fatty acid synthesis.

Jenkins (unpublished data, 1998) has suggested the following method to calculate the amount of free fat to be included in the diet (% of the diet DM) in order to prevent problems with fiber digestibility and milk fat suppression:

Supplemental fat (%) = (6 x % diet ADF) / % unsaturation of the fat supplement

E.g. Diet: 20% ADF

Supplemental fat: 50% unsaturated fatty acids

The total amount of supplemental fat suggested would be: (6 x 20)/50 = 2.4% units. Others have suggested that the total amount of fat fed should be equal to the amount of fat produced in milk (Palmquist, 1998). Regardless of the criteria used for fat supplementation, nutritionists and veterinarians should avoid high fat diets (> 6%). These guidelines are especially important when the supplemental fat is high in unsaturated fatty acids and the basal diet is low in forage, or when the forage is mostly corn silage.

In recent years, scientists have rediscovered the importance of milk fat on human nutrition. Parodi (1999, 1997) reviewed the importance of certain components in milk fat as anticarcinogenic agents. Because CLA may be capable of regulating gene expression, may have a role on cancer prevention, may help weight loss, and may improve the immune system (Cook, 1999; Parodi, 1999, Parodi, 1997), feeding strategies that increase the amount of CLA in milk might become attractive.

A preliminary report by Giesy et al. (1999) examined the effects of feeding calcium salts of CLA to early lactation dairy cows. Treatments began at 2 weeks postpartum and continued until about 80 days in milk. Cows received either calcium salts of long chain fatty acids or calcium salts of CLA (provided 50 g of CLA). Cows supplemented with calcium salts of CLA had significantly lower milk fat content throughout the experimental period, but most of the depression started after 3 weeks of treatment. Because cows supplemented with calcium salts of CLA had lower milk fat content, less energy was required for milk synthesis, which tended to improve energy balance and milk production. In markets where quota systems are based on milk fat yield, such feeding strategies should be considered. In addition, studies where CLA was abomasaly infused in dairy cows (Chouinard et al., 1999), milk CLA content increased by as much as 10 fold. Therefore, supplementing dairy cows with fat sources rich in certain fatty acids might be used to reduce milk fat content and to increase CLA in milk.

Buffers and Milk Fat

Buffers are salts that are soluble in an aqueous solution, with a pKa similar to the pH of the aqueous system where it is present. They cause resistance to changes in pH of the media where they are solubilized when an acid or base is added. Sodium bicarbonate, sodium sesquicarbonate, and potassium bicarbonate are examples of buffers used in dairy cattle diets. Agents such as magnesium oxide and limestone are not considered buffers because of their low solubility in the rumen environment, but they act as alkalinizing agents, which help to maintain rumen pH and milk fat test.

In ruminants, most of the buffering salts that reach the rumen originate from the saliva. Estimates of total flow of saliva in lactating dairy cows consuming 18 to 20 kg/d of DM and producing 29 kg of milk/d were about 220 L/d (12 L/kg of DM ingested) (Erdman, 1988). Saliva of cattle contains 125 mEq/L of HCO_3^-. Erdman (1988) estimated that the total $NaHCO_3$ equivalent produced per day by a cow consuming diets with 30%, 50% and 70% forage was 3,418 g/d, 3,517 g/d and 3,617 g/d, respectively. These figures indicate that decreasing the amount of forage in the diet from 70 to 30% decreases the daily flow of salivary $NaHCO_3$ by 200 g, which is equivalent to adding 1% $NaHCO_3$ to the diet of a cow consuming 20 kg/d of DM.

Using similar figures as described by Erdman (1988), the amount of $NaHCO_3$ produced for every kg of NDF ingested in a diet that contains 35% NDF would be about 500 g. Assuming

that these figures represent what happens to a lactating dairy cow, every 1% unit drop in NDF content in the ration would be the buffer equivalent to adding 0.5% of $NaHCO_3$ to the diet. This implies that even when $NaHCO_3$ represents 1% of the diet DM, it supplies the equivalent in buffer of 2% units of NDF.

A summary of several studies that looked at the effects of buffer addition to the diets of dairy cows was compiled by Erdman (1988). When $NaHCO_3$ was added to diets with a high forage content, milk fat content was increased by only 0.1% unit. In the same review, when $NaHCO_3$ was added to diets with low forage content (< 30% forage), the positive effect of $NaHCO_3$ on milk fat concentration was higher (0.3% units). When different forage sources were compared, the addition of $NaHCO_3$ was effective in increasing milk fat content in corn silage diets, but had little or no effect when the forage was either alfalfa hay or haylage, or a combination of alfalfa and corn silages (Erdman, 1988). Although most of the data does not strongly support the use of buffers to improve animal performance when lactating dairy cows are fed high alfalfa diets, studies at the University of Arizona with processed grains observed an improvement in DM intake and feed efficiency when dairy cows fed high alfalfa high starch diets were supplemented with 1% $NaHCO_3$ (Santos, 2000).

Addition of $KHCO_3$ or K_2CO_3 to the diets of dairy cows resulted in improvements on milk fat content similar to those observed for $NaHCO_3$ (Erdman, 1988). The increases in milk fat ranged from 0.1 to 0.45% units.

Recent data from Kalscheur et al. (1997) support the use of buffers to improve milk fat test. They observed that cows fed a low forage diet had higher rumen pH, lower rumen concentration of trans fatty acids, lower milk trans fatty acid content, and higher milk fat content when supplemented with buffers, compared with those that did not receive any buffer in the diet. Because lactating dairy cows respond to diets high in Na and K, addition of buffers that contain Na and K should not only benefit the rumen environment, but overall animal performance. There is sufficient indication that lactating diets should contain between 0.3 and 0.5% Na, 1.5 and 1.7% K, and a high dietary cation-anion difference (**DCAD**). In most situations, when alfalfa is the major forage source, K requirements can be met, but sodium needs to be supplemented. In these cases, $NaHCO_3$ is added at 0.7 to 1.0% of the diet DM. When corn silage is the major forage,

supplemental K is probably required and either $KHCO_3$ or K_2CO_3 can be used. The DCAD of lactating diets should be between 350 to 400 mEq/kg.

MILK PROTEIN

Milk protein is the most valuable of all the milk components. Enhancing the content of true protein in milk improves cheese yields and increases the efficiency of nitrogen utilization by dairy cows. Dietary factors that impact milk protein content are not clearly known. In most cases, diet impacts milk protein yield, rather than protein concentration in milk (Theurer et al., 1995).

Until recently, protein in milk in the USA was calculated by measuring the amount of N in milk and multiplying that by 6.39. Because milk protein is comprised of 15.65% N, an equation was developed to derive crude protein from the total N concentration in milk (100/15.65 = 6.39. Crude protein in milk = N content x 6.39). However, such calculation is no longer used. Starting on May 1st of 2000, protein in milk is being measured as total true protein, which excludes non-protein N. Therefore, nutritional factors that affect milk N content should now improve the true protein (casein) content, and not urea-N.

Dietary Protein and Milk Protein

Ruminally synthesized microbial protein supplies more than 50% of the absorbable amino acids for lactating dairy cows. Microbial protein is considered to be a consistent source of high quality protein (Chandler, 1989), and milk protein concentration in response to dietary protein is dependent upon the amino acid profile of the absorbable protein. Therefore, maximizing microbial protein synthesis is the key step to improve milk protein synthesis.

The effect of dietary protein source and milk protein concentration was reviewed by Santos et al. (1998) and Theurer et al. (1995). Theurer et al. (1995) suggested that increasing the amount of dietary protein within a constant dietary energy level has little effect on milk protein synthesis. Whenever dietary protein level increases milk protein yield, the effect seems to be associated with an increase in milk yield.

Ruminants have very low efficiency of dietary N conversion into milk protein. A high producing Holstein dairy cow consuming 25 kg of DM containing 18% crude protein (2.9% N in the diet DM) and producing 40 kg of milk with 3.3% crude protein (0.52% N in milk), utilizes

dietary N to convert into milk N with an efficiency of 28.7%. Such low efficiencies of N utilization for milk protein synthesis have been reported (Kennelly et al., 2000; Santos, 2000). Emery (cited by Theurer et al. 1995) suggested that for every percentage unit increase in dietary crude protein, milk protein content increased by 0.2 g/kg ($r^2 = 0.35$). This implies that increasing dietary crude protein from 16 to 19% would increase milk protein content by 0.06% units. In addition, it is not known whether this increase represents true protein or non-protein nitrogen. Therefore, increasing dietary protein is not a successful or efficient method to manipulate protein content of milk.

Schwab (1996) and Rulquin et al. (1995) have characterized the two most limiting amino acids for milk protein synthesis and their optimum amounts in the duodenal content of dairy cows. When soybean meal was replaced by a high RUP source, the flow of nonammonia N was not affected, but microbial N was reduced by 35 g/d ($P < 0.001$) (Santos et al. 1998). In addition to that, the intestinal flow of lysine was dramatically decreased when the high RUP supplement was corn gluten meal. It is well known that microbial protein has a well balanced amino acid profile relative to the milk amino acid profile (Chandler, 1989). If microbial protein synthesis is compromised, milk protein synthesis and yield might suffer.

Santos et al. (1998) reviewed 136 comparisons between soybean meal and a high RUP source from 88 performance studies with lactating dairy cows. In 129 comparisons where milk protein concentration was available, replacing soybean meal with a high RUP source decreased milk protein % in 28 cases, had no effect in 95, and increased in only 6 cases. In the same review (Santos et al., 1998), partial replacement of a source of true protein with urea decreased milk protein content in 5 comparisons, had no effect in 17, and increased in 1 comparison.

Data from these studies suggest that simply replacing a source of rumen degradable protein with a high RUP supplement does not consistently alter milk crude protein content. When formulating diets for lactating dairy cows to maximize milk protein content and yield, careful analysis of the supply of N for optimum rumen fermentation is important. If the rumen environment lacks ammonia N, microbial protein synthesis is compromised and milk protein concentration might decrease. Diets that supply 11 to 13% of DM as degradable protein and a RUP with good amino acid profile that complements that of microbial protein should maximize the amino acid supply for milk protein synthesis.

Dietary Amino Acids and Milk Protein

The ability of the mammary cell to extract amino acids from the blood and the metabolism of these amino acids within the mammary cells are the major factors affecting protein synthesis in the mammary gland.

Considerable progress has been made in the area of amino acid nutrition of dairy cows. Several studies have identified lysine and methionine as the two most limiting amino acids for milk protein synthesis in most lactating dairy diets (Schwab, 1996; Rulquin et al. 1995). Studies at the University of New Hampshire (Schwab, 1996) have suggested that methionine and lysine should represent 5 and 15% of the total essential amino acids flowing to the duodenum, respectively. Rulquin et al. (1995) suggested a slightly different approach. Their research looked at the ratio of methionine and lysine relative to the total amount of metabolizable protein. They suggested that these two amino acids should comprise 2.5 and 7.2% of the metabolizable protein, respectively. Formulating diets to achieve such ratios is almost impossible with conventional feedstuffs. Only with the addition of protected amino acids can diets be formulated with high amounts of methionine and lysine flowing to the duodenum of dairy cows.

Rulquin et al. (1995) reviewed 121 studies (78 with rumen-protected amino acids, and 43 with post-ruminal infusions of amino acids) that focused on the effects of methionine and lysine supplementation on milk protein yield. The overall result from all those studies was an increase of about 29 g/d more milk protein for cows supplemented with amino acids. Most of that response was due to an increase in milk protein concentration (1.1 g/kg), since no response in milk yield was observed. More recently, Garthwaite et al. (1998) reviewed a more limited data set and observed similar increases in milk protein concentration (1.5 g/kg) when cows were supplemented with protected methionine and lysine postpartum.

Supplementing dairy cows with ruminally protected lysine and methionine may present challenges due to the cost of such ingredients and the lack of consistent response. In studies where either lysine or methionine was fed in excess, or their ratios were unbalanced, milk production decreased (Garthwaite et al, 1998). Utilizing dynamic models that take into consideration the amino acid profile of the protein flowing to the duodenum of dairy cows may facilitate a more accurate utilization of amino acids in diets of lactating dairy cows to improve milk protein synthesis.

Dietary Energy and Milk Protein

Of all the dietary nutrients that impact milk protein synthesis, energy is by far the most important. Increasing energy intake of dairy cows increases yields and concentration of milk protein (Theurer et al. 1995; Spöndly, 1986). However, not all dietary energy sources are capable of increasing milk protein. Generally, dietary fat decreases milk protein concentration (Wu and Huber, 1994), although energy intake is increased. Therefore, to impact protein concentration in milk, the increase in dietary energy intake should come from a ruminally fermentable energy source.

Diets that supply more fermentable energy generally increase NE_L intake and microbial protein synthesis. These diets are often formulated by increasing forage digestibility, adding more grain to the diet, increasing the amount of NFC, and improving rumen digestibility of the NFC. Studies at the University of Arizona (Santos, 2000; Theurer et al., 1999) have shown that more extensive processing of corn and sorghum grains consistently increase rumen and total tract digestion of starch, which alters the supply of nutrients to the digestive tract, and the uptake of nutrients by the mammary gland.

In a summary of 19 lactation studies involving steam-processing of corn and sorghum grains, Theurer et al. (1999) observed that steam-flaking increased milk protein content 0.07% units, and milk protein yield by 80 to 100 g/d, when compared with dry-rolling or steam-rolling of sorghum and corn grains.

Diets that provide more fermentable energy increase microbial protein synthesis and the molar concentration of propionate in the rumen (Santos, 2000; Theurer et al., 1999). Microbes provide protein with well balanced amino acid profile (Chandler, 1989). The higher molar concentration of propionate favors greater output of glucose by the splanchnic tissue, which increases plasma insulin (Santos, 2000). Several studies at Cornell University (Mackle and Bauman, 1998) have suggested that endocrine control of milk protein synthesis may be regulated by energy substrates. Utilizing an euglycemic-hyperinsulinemic clamp technique, Cornell researchers have been able to demonstrate the importance of insulin as a mediator of protein synthesis in the mammary gland (Mackle and Bauman, 1998). When intravenous infusion of insulin was combined with duodenal infusion of branched chain amino acids, a remarkable increase in milk protein production was observed.

When cows were fed diets with more ruminally degradable starch, plasma insulin increased by 20% (Santos, 2000). Also, Theurer et al. (1999) observed that cows fed diets with more rumen degradable starch had increased uptake of amino acid N by the mammary gland. Therefore, it is suggested that the mechanisms by which increasing fermentable energy in the diet enhances milk protein synthesis to be:

- Increased supply of limiting amino acids provided by the greater flow of microbial protein;
- Greater molar concentration of propionate in the rumen;
- Increased output of glucose by the splanchnic tissues;
- Higher levels of plasma insulin; and
- Greater uptake of amino acid N by the mammary gland.

Because feeding more ruminally degradable starch increases yields of milk and milk protein, cows fed diets with steam-flaked sorghum were more efficient in utilizing dietary protein to produce milk protein (Santos, 2000).

In conclusion, when formulating diets for lactating dairy cows to maximize milk protein production, veterinarians and nutritionists have to evaluate the supply of ruminally fermentable energy in the diet. There is an opportunity to improve milk protein content and yield by synchronizing the availability of energy and protein in the rumen.

Dietary Fat and Milk Protein

Wu and Huber (1994) reviewed the relationship between dietary fat supplementation and milk protein concentration in lactating dairy cows. They (Wu and Huber, 1994) showed that supplementing dairy cows with fat generally increased milk production, but milk protein concentration dropped. A curvilinear relationship between dietary fat and milk protein concentration was observed. However, dietary fat was responsible for only 24% of the variation in milk protein concentration. Because casein is the N fraction most affected by dietary fat, when milk is marketed for cheese production, the response to fat feeding should be evaluated with criteria.

Casein is synthesized *de novo* by the mammary gland. Amino acids are taken up by the mammary cells from the bloodstream, metabolized within the mammary cells, and utilized for synthesis of milk protein. When availability of amino acids is decreased, mammary uptake is compromised, or intracellular metabolism is altered, milk protein synthesis can be compromised.

When cows are fed fat, the energetic efficiency of milk synthesis is increased. Cows fed high fat diets required less liters of blood per kg of milk produced (Cant et al., 1993). Because mammary uptake of amino acids is dependent upon amino acid concentration in the blood and blood flow to the mammary gland, these data suggest that the decrease in blood flow per volume of milk produced would limit the uptake of amino acids for milk protein synthesis.

Recently, researchers from University of Idaho (Moloi et al., 1998) reviewed 161 treatment means from 1,378 cows utilized in studies where inert fat was supplemented in the diet. Their analysis revealed that increasing dietary fat decreased milk protein concentration, but the effect was more dramatic in diets with low RUP content (< 6% RUP). They suggested that the RUP content of diets that contain rumen inert fat should be increased to more than 6% to overcome a possible decrease in milk protein content and yield. Wu and Huber (1994) suggested that more extensive processing of cereal grains, to increase rumen availability of starch, overcomes milk protein depression caused by diets supplemented with fat. These data suggest that availability of amino acids or uptake by the mammary gland is reduced when fat is supplemented in the diet. Therefore, formulating diets with supplemental fat might require additional fermentable energy to support greater synthesis of microbial protein, or an increase in the post-ruminal supply of a protein source with a well balanced amino acid profile.

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