

**THE EFFECT OF FEEDING LACTOSE IN THE FORM OF WHEY
PERMEATE ON THE PRODUCTIVITY OF LACTATING DAIRY
CATTLE**

Final Report

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Acronyms and abbreviations

°C – degrees Celsius

°F – degrees Fahrenheit

α - alpha

β - beta

BOD - Biological Oxygen Demand refers to the amount of oxygen that would be consumed if all the organics in one liter of water were oxidized by bacteria and protozoa (ReVelle and ReVelle, 1988).

BV – biological value

BW – body weight

C – carbon

CP – crude protein

CPE – crude protein equivalent

CPM – Cornell-Penn State-Miner Institute ration balancing program

d – day

dl – deciliter (1/10th of a liter; 100 milliliters)

DM – dry matter

DMI – dry matter intake

FACW – fermented ammoniated condensed whey

g – gram

h – hour

H – hydrogen

HIV – human immunodeficiency virus

HWPS – hydrolyzed whey permeate syrup

K – potassium

kg – kilogram

lbs – pounds

mg – milligram

N – nitrogen

Na – sodium

NDF – neutral detergent fiber

NH₃ – ammonia

NPN – non-protein nitrogen

NSC – non-structural carbohydrate

OH – hydroxy group

OM – organic matter

P – phosphorus

PUN – plasma urea nitrogen

RNA-N – ribonucleic acid nitrogen

SBM – soy bean meal

TMR – total mixed ration

VFA – volatile fatty acids

WPC – Whey Protein Concentrate

Executive Summary

Introduction

The dairy and cheese industry has long treated whey as a matter of waste disposal. Means of disposal have included use as a feed supplement for swine and cattle and land application as a fertilizer. For cheese manufacturers it was a legitimate, least cost means of disposal, for farmers whey was an inexpensive nutrient input. Though with improved understanding of the nutritional value of whey, specifically whey proteins, coupled with new extraction technologies, whey has become more valuable for feed and food markets. The remaining lactose and minerals after harvest of the whey proteins have unfortunately not found a substantial large-scale industrial use. Individual cheese plants struggle to decide how to handle their whey, and to find a suitable and consistent market.

One such example is the Agrimark-Cabot owned McCadam Cheese plant of Chateaugay, New York. This plant manufactures a concentrated whey permeate product that is between 35-40% solids, 3-5% protein and 85% lactose on a dry matter (DM) basis. Approximately 100,000 lbs of this wet 40% solids product are produced per day, amounting to nearly two 50,000 lb truckloads of whey permeate per day needing to be managed. Currently, Agrimark is accepting much of this permeate at its Middlebury, Vermont plant where it can be dried down to powder and handled as a dry product. The market for dry lactose is limited and the expense of drying is prohibitive of possible uses. Finding a sustainable market for the 40% solids product would be a viable alternative. Consideration of using this product as a dairy feed is currently being undertaken.

The objective of this project was to evaluate the feasibility of utilizing concentrated whey permeate as a dairy feed energy supplement. The first aspect was to determine lactose utilization in the rumen through an *in vitro* continuous culture fermentation trial at the Rumen Fermentation Profiling Lab at West Virginia University. Dr. Will Hoover and Tammy Miller-Webster manage this facility and lead the field of *in vitro* research examining microbial growth and fermentation parameters of various feed ingredients with their continuous culture fermentation system. Analysis of microbial growth and protein yields as well as rate of rumen fermentation of carbohydrate sources is critical to understanding the nutritional applications of feed ingredients such as lactose. Understanding whether lactose is a viable substitute for corn grain or molasses in promoting microbial growth is of great importance if lactose is to be valued as a nutritional supplement. The second aspect of this project was to determine the stability of the 40% solids whey permeate in terms of its storage life and special handling considerations that a feed mill or commercial dairy operation would need to consider. Lastly, a literature review of whey, whey permeate and, specifically, lactose is provided as a review of previous research and a source of information as to the nutritional use of lactose in dairy rations and the possible means of improving the nutritional value of whey permeate.

Rumen Fermentation Profiling Lab *in vitro* continuous culture fermentation trial

The fermentation trial examined effects of molasses (sucrose) versus 30-40% solids whey permeate (lactose) as compared to a control ration on nutrient digestibilities, pH, volatile fatty acid (VFA) profile, nitrogen partitioning, microbial growth and efficiencies and rate of sugar fermentation. The diets were formulated for a high production lactating dairy cow. The control

ration had a sugar level of 3.4% DM basis while the whey and molasses rations were 7.1 and 7.2% respectively. These percentages were equivalent to feeding 6 lbs of whey permeate and 3.5 lbs of molasses per cow. Feeding schedule, liquid dilution rate and solids retention time were held constant, mimicking a high production dairy cow.

Digestion of acid detergent fiber (ADF) was significantly greater with the whey permeate compared to either the molasses or control ration. Digestion of neutral detergent fiber (NDF) tended to be greater with the whey though not statistically significant. This is in agreement with previous research that also saw improved fiber digestion with whey/lactose. No difference between whey and molasses was observed for the digestibility of DM, organic matter (OM), non-structural carbohydrates (NSC) or total carbohydrate. Though both whey and molasses improved digestion of OM, ADF, NSC and total carbohydrate compared to the control ration. There was no significant difference between whey and molasses on pH. Whey did affect VFA profiles by increasing butyric and decreasing acetate versus molasses ($P < 0.05$). Both the whey and molasses reduced the acetate: propionate ratio compared to the control diet (3.05, 3.15, 3.42) ($P < 0.05$). This also is in agreement with the published research findings, (see literature review).

With regards to nitrogen (N) partitioning, compared to the control diet, whey permeate decreased protein digestion while molasses, increased protein digestion. Microbial N yield was significantly greater with the molasses ration versus the whey diet ($P < 0.05$). The efficiency of microbial N created per unit of digested DM, OM and total carbohydrate were all significantly greater for the molasses diet compared to the permeate diet. The differences were primarily due to the decreased efficiencies observed with the permeate supplementation compared to the control diet. Molasses did not appear to have a benefit in microbial N efficiency compared to the control ration. However, the whey diet resulted in significantly more total VFAs produced per unit of microbial N produced than the control or molasses diet. This may suggest that lactose could supply more energy in the form of VFAs to the rumen as opposed to production of microbial proteins. Secondly, it could be the result of a population shift of the microflora resulting in an increase in species that generate more VFAs in relation to microbial protein. This notion is supported by the greater ribonucleic acid-nitrogen (RNA-N) found with the whey diet compared to molasses and control rations. This difference in RNA-N may be indicating a shift in the population profile of the microbes in the fermenters. As mentioned in the literature review, not all species of rumen microbes are able to ferment lactose. This theoretical change in species populations may require special nutritional considerations when formulating diets to maximize the benefits of feeding lactose.

Sugar digestion rates were similar between sucrose and lactose in the fermenters. Nearly 80% of both sugars was digested within 1 hour, most of which (75%) occurred in the first 30 minutes. The remaining 20% of lactose, however, fermented more slowly than sucrose as evidenced by the pulse dose data. Rates of digestion for both lactose and molasses for the first hour were about 175%, as calculated based on the 24 hour digestion of the potentially digestible fraction. Though, clearly the 20% of lactose and sucrose that was not digested in the first 30 minutes, digested at much slower rates, 13% and 32% per hour respectively. Questions can be asked of whether the system became saturated with sugar resulting in a quick initial burst of digestion followed by a slower phase after 30 minutes, or if enough available N was present to support 100% sugar digestion within the first hour. This fraction of lactose that takes longer to ferment raises issues of potential washout from the rumen and hindgut fermentation. Hindgut

fermentation results in less efficient energy utilization and the potential for hemorrhagic bowel syndrome.

Hoover and Miller-Webster conclude that lactose (whey permeate) is less effective than sucrose (molasses) at promoting microbial growth, at least in the continuous culture fermenter system. Though indications are that lactose may improve fiber digestion and result in microbial population shifts that have yet to be accounted for and may require special nutrient considerations. If the fiber-digesting bacteria are benefited by lactose, peptides need to be made available in order to meet their requirements and to maximize their ability to ferment lactose and fiber. Traditional soluble protein and non-protein nitrogen (NPN) ration values may be inappropriate benchmarks when supplementing lactose.

Field observations of feeding whey permeate

Whey permeate and dried lactose have been fed and in some cases continue to be fed to mature lactating dairy cows on commercial farms in upstate New York and Vermont. Conversations with a professional feed representative indicate that lactose does function well for dairy cows. Observations and recommendations presented here are purely testimonial as no designed research has been conducted on any of these farms, including Miner Institute, which has fed pure dry lactose to milking cows. Feeding a dry, whey permeate product at 0.33-0.50 lb/cow to close-up dry cows appears to help minimize ketosis and help in milk production. Previous attempts at using sucrose, as molasses appeared not to have as great an effect on aiding against ketosis.

Another area farm had fed a 20% solids whey permeate product to lactating cows in the total mixed ration (TMR) for nearly 3-4 years. The inclusion rate was 12-13 lb as fed, or about 2.5 lbs of DM. At 85% lactose, that equates to just over 2 lbs of sugar (lactose) added to the TMR. Information on the total sugar of the ration is unavailable but believed to have been around 5-6% of DM. The primary concerns with the product centered more on handling rather than nutrition. In one case, two elevated storage tanks were used to feed from through a “boom” pipe with drilled holes to drizzle the permeate directly onto the TMR in the mixer wagon. Issues of lactose crystallization and settling were alleviated with manual agitation. Mold was not a serious nutritional issue as it tended to float rather than be fed out. Cleaning was indicated to need improvement. Overall, the thought was that “for the price” (cost of trucking) the whey permeate was an effective energy source that helped maintain herd production at nearly 90 lbs/cow.

Stability trial

Stability of the 40% solids whey permeate produced at the McCadam plant in Chateaugay, New York was investigated using samples collected from three different batches on three separate days. Samples were obtained warm from the plant and analyzed for pH and bacterial growth over 5 days of storage at either 77°F or 86.5°F. The pH levels increased from initial readings of 5.4 to 5.8 on average after 5 days, at both storage temperatures. These results were not expected considering whole whey becomes more acidic during storage, dropping from pH of 6.8 to 5.4 in some cases. Dr. Karen Smith of the Wisconsin Center for Dairy Research explains the pH rise of the permeate as the result of the type of microorganisms surviving the processing of the various types of cheese produced. Different cheeses result in varied microfloral in the whey in spite of the similar nutritional analyses of the whey. Some bacteria can survive in the concentrated permeate that do not produce acids. The presence of yeasts are another possible explanation for

the rising pH observed in this trial. Yeasts can ferment some acids and thereby neutralize the acidity. Dr. Smith mentioned that with some surface ripened cheeses it is common to smell a hint of ammonia, indicating a basic pH environment.

Bacterial growth was negligible for the first 2 days of sample incubation. At day 2, significant microbial growth was observed which increased through day 5. Temperature had a tremendous effect as nearly 4 times the growth was observed in the samples stored at 86.5°F versus those at 77°F. Dr. Smith mentioned that the risk of bacterial growth is greatest at temperatures above 70°F for 35-50% solids permeate and dramatically increases with each 10°F rise in temperature. Unfortunately, at 70°F, lactose in a 40% solids permeate will crystallize and precipitate from solution, though agitation should easily resuspend the lactose particles. This is what was observed at Miner Institute. At room temperature, 60-70°F, the lactose in the 40% solids permeate will crystallize and settle out of solution. Upon swirling, the mass of lactose could be resuspended only until agitation was stopped, where upon the lactose again fell out of solution. However, if allowed to cool, as happened when samples were refrigerated at 4°F, boiling and severe agitation were required to resolublize the lactose.

Volatile fatty acid analyses corroborated the findings with pH, in that lactose was not being fermented during the 5 days of incubation. Apparently the microorganisms that were present did not produce lactate or acetate as an end product. The levels of acetic and lactic acid did not change significantly throughout storage or differ as a result of temperature. Acetic acid levels remained at 12.0 µMol/mL on average while lactic acid levels were about 52.7 µMol/mL on average over the 5 days.

Concerns about use of preservatives during short-term storage of the permeate are minimal. Dr. Smith indicated that cleanliness of equipment is the primary means of controlling microbial growth and contamination. Rapid turnover of the product will prevent stagnation that would foster growth. Also, controlling temperature during storage, at a maximum of 70°F, will minimize microbial growth. Means of preservation are limited in effectiveness as there is no single treatment that can control all microbes. Use of some preservatives such as formaldehyde are no longer acceptable in the dairy feed industry. Peroxide use will not inhibit the numerous microbes that are catalase positive. Overall, the added expense of trying to preserve whey permeate is cost prohibitive.

Based on this initial examination of concentrated whey permeate, it appears that the product will remain stable over a period of 2-5 days. Temperature will be an important condition to monitor with its effects on bacterial growth and lactose crystallization. Higher temperatures will help keep the lactose in solution, but at the risk of increase bacterial growth. Cleanliness of handling equipment will be critical in maintaining product stability.

Alternative uses of lactose, concentrated whey permeate

Much of the literature indicates the value of lactose as an initial, base nutrient from which value added products could be produced. With respect to the dairy industry, lactose in concentrated whey permeate is a potential source of energy. The primary shortcoming is the lack of nitrogen in the form of protein or NPN. Technologies exist to increase the protein value of whey permeate, such as ammoniation and growth of yeasts and single celled organisms in the permeate. When asked why these technologies have not been pursued of late, Dr. Smith responded that corn is cheap in the Midwest and that there was little economic incentive to pursue these technologies. Secondly, the Wisconsin cheese industry is still dominated by smaller sized operations that cannot afford to invest in technologies of whey and permeate treatment that a larger corporation could afford. Much of the whey in the Midwest is used in milk replacers for the veal and dairy industry. It is interesting how geographic location influences the development and use of technology. Again, the value of whey proteins exemplified. Another concern about fermenting whey and permeate, is that the type of cheese the whey was produced from will influence subsequent fermentations when trying to promote desired microbial growth.

Phase I: Review of the literature of feeding whey, whey permeate and lactose to dairy cattle

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Introduction

Whey is the liquid portion of milk that separates from the curd during cheese manufacturing. Until recently, whey has been considered a waste product by the cheese industry simply requiring disposal. Methods of disposal have included land spreading as fertilizer, dumping into rivers and use as an animal feed (Kosikowski 1979). Because of the high nutrient levels in whey in the forms of proteins, sugars and minerals, whey has a high biological oxygen demand (BOD) value and can be a potent pollutant. With increasing concerns for the environment, greater understanding of the nutrient value of whey and the advent of technologies to capture those nutrients, whey itself is no longer simply a waste product.

As a means of disposal, whey has been used as an animal feed, particularly for pigs and cattle. Consumption by cattle has been documented at up to 200 lbs (91 kg), or about 25 gallons per day. It is said that 100 lbs of whey is equivalent to 7.5 lbs of 12% crude protein (CP) grain on a dry matter (DM) basis. Animals consuming large quantities of whey, however, will increase urine output significantly and require greater attention to manure management and more bedding materials. Increases in manure output with free choice whey consumption can approach 200% (Modler 1987, Welch 1973).

Using liquid whey as an animal feed has other drawbacks. Whey is corrosive and requires plastic, stainless steel, glass or fiberglass storage and handling equipment. It ferments quickly with rapid reduction in pH and has been shown to promote tooth erosion and sore gums in cattle consuming large quantities (Welch 1973). The shelf-life of liquid whey is only about 2 days under ambient conditions as pH can drop considerably, from 6.3 to 4.4 after 1 day at 20°C (Crawshaw 2001). Means of extending the shelf-life include pasteurization or the addition of preservatives such as formaldehyde, acetic acid and benzoic acid. Unfortunately, these means only slightly increase the stability of the product. Consideration must also be given to animal health and legal restrictions when deciding on a means of preservation (Modler 1987, Schingoethe 1990).

Field spreading of whey as fertilizer has been noted as a use for whey. Characteristics such as a high carbon to nitrogen ratio (20:1), a good source of nitrogen (N), phosphorous (P) and potassium (K), a slow release of N from whey proteins and a lack of toxic compounds make it a good fertilizer. The lactose in whey is fermented by soil microbes which produce gums that bind soil particles together, thereby improving soil texture, reducing run off and erosion while increasing percolation rate (Modler 1987). Because of this, whey has been said to be a useful soil amendment.

Fertilizer uses and animal feeding of whole whey, however, are short-term, limited solutions to the disposal of whey on a small scale. Industry wide solutions on how best to utilize the nutrients in whey and minimize its disposal are required for the dairy industry. Through greater understanding of the nutrients in whey and the creation of new technologies on how to harvest and utilize those nutrients, whey will no longer be an issue of disposal but rather a valuable resource. The objective of this review is to provide an overview of whey, through characterizing the product and its components, reviewing past nutritional applications and listing some potential future uses. The primary focus of this paper is to cover the use of concentrated whey permeate and more specifically lactose as a potential dairy feed.

Options for handling whey

There are basically three options for handling whey; either as an unprocessed, condensed or dried product. The primary issue of processing whey is the cost of component separation, drying and the value added to the processed whey versus transport costs of the larger fluid volume of unprocessed whey. Unprocessed whey incurs the least input costs of heating and cooling equipment, but has the most limited shelf life and least revenue generation. Condensed whey requires inputs of heating, cooling, evaporation or reverse osmosis equipment that decreases fluid volume. There is little added value to the resulting concentrate other than decreasing shipping costs. It is this low value product that this project is trying to determine the viability of its use as a dairy supplement. Dry whey product incurs the greatest input costs of preparation as well as bagging and storage costs. The finished product does have a longer shelf life of 6-12 months (Whey Technical Fact Sheet).

Types of whey product and their nutrient content

There are primarily two types of whey resulting from the production of different types of cheeses. They are sweet whey and acid whey. Sweet whey results from the production of hard cheeses, such as cheddar and mozzarella, and accounts for nearly 80% of total whey production. It has a relatively high pH of around 6.0 and lower ash content than acid whey. Acid whey is from the production of fresh or soft cheeses such as cream and cottage cheeses. A third type of whey is also produced but of small consequence, and that is salt whey, which is the drippings from the salted curds. Salt whey can be up to 10% salts, and not advisable as an animal feed source.

Whey can be processed through various filtration technologies such as microfiltration, ultrafiltration, nanofiltration, or reverse osmosis which all separate molecules out of solution by size. This is how the whey proteins are harvested from whole whey. The fluid that passes through the filter is then called permeate, and depending on what nutrient is filtered out and to what extent, various whey products result, including whey permeate, delactosed whey and demineralized whey. The retentates of filtration are numerous and include whey protein concentrate and whey protein isolates, but will not be included in this discussion. Please note Nielsen 1992 for a review of filtration technologies. Nutrient profiles of the types of whey are listed in Table 1. See Appendix 1 for additional nutrient analyses of processed whey and milk co-products including whey, whey concentrate, whey permeate and delactosed whey.

Whey permeate is deproteinised whey which primarily consists of lactose, minerals and water. The high value whey proteins are filtered out for isolation, resulting in a very low protein product. In its natural state, whey permeate will have only 5% DM, though it can be concentrated to between 18-45% DM. The high DM product is also referred to as concentrated whey permeate. This is the product of interest currently under investigation at Miner Institute. Appendix 2 lists the nutrient analyses of concentrated whey permeate produced at the McCadam cheese plant in Chateaugay, NY.

Delactosed whey, or more aptly named reduced lactose whey, is whey that has had some lactose removed. The resulting product is still 55% lactose on a DM basis, down from the unprocessed whey which is 75% lactose on a DM basis. Delactosed whey is proportionally greater in protein and minerals, which can improve its feed value, particularly for swine. Delactosed whey is

typically 38-43% DM of which 20-24% is protein. Sodium (Na) content is around 2.4%, which puts limitations on the amount that can be fed before exceeding animal requirements and tolerances for Na.

Other terms used for processed whey products include whey protein concentrate (WPC), which is the retentate after filtration of whey and demineralized whey (Table 1). Milk permeate is the liquid fraction obtained through the ultrafiltration of milk which concentrates the milk solids and minimizes the whey resulting from cheese manufacturing. Milk permeate is high in lactose, with very little protein if any.

Table 1. Composition of various types of whey. From Yang and Silva, 1995.

Whey Type	Composition					
	Lactose	Protein	Lactic Acid	Ash	Salt	Fat
	(% dry basis)					
Sweet whey	74-81	12.8-15.2	1.8-2.2	8.0	2.5	1.0
Acid whey	65-80	9.9-15.5	7-10	7.0-19.4	2.5	1.0
Sweet whey permeate	86.0	0.2	2.4	8.8	NA	<0.1
Acid whey permeate	74	0.3	7.5	9.7	NA	<0.1
Delactose whey*	<60	16-24	NA	11-27	NA	0.2-0.4
Demineralized whey	<85	10-24	NA	<7	NA	0.2-0.4

*Industrial term for mother liquor resulting from lactose crystallization.

Available nutrients from whey

Whey proteins are of high biological quality and therefore high economic value. These proteins include β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulins and some casein residues. These proteins are of high nutritional value based on their amino acid profiles, digestibility and metabolic use as measured by the Protein Efficiency Ratio and Net Protein Utilization assays (Forsum 1974). Compared to the biological value (BV) of whole eggs, valued at 100, WPC valued at 104; beef protein, 80; casein, 77 and soy protein, 61. Interestingly, casein, the predominant protein in cheese production is nutritionally inferior to whey proteins. The amino acid profile of whey proteins compared to human amino acid requirements is valued at 123 in the Protein Digestibility Corrected Amino Acid Score; egg whites are valued at 100, and soy at 94. Clearly, whey proteins are valuable food and nutritional supplements with many uses including infant formulas. There are also immunoglobulins in whey, such as lactoferrin, which has antimicrobial and antiviral applications that are used in the treatment of herpes, influenza, HIV and hepatitis (US Dairy Export Council 2002).

It is little wonder that animals fed whey proteins have shown positive production responses. Growing-finishing pigs that were fed dried whey as the source of 50% of their dietary CP had a 36% increase in weight gains compared to pigs fed a soybean meal (SBM) diet (C'eslak, D.G. et al. 1986, as cited by; Schingoethe 1990). Whey proteins used in milk replacers have resulted in similar growth responses and N utilization compared to casein proteins from skim milk (Schingoethe 1990). As for ruminant nutrition, whey proteins are a significant source of

branched chain amino acids, commonly referred to as isoacids. These isoacids are essential factors for microbial growth, especially the fiber-digesting bacteria (Schingoethe 1976).

Clearly, whey proteins have significant biological value. The harvesting of these proteins, however, results in a whey product that is primarily lactose and minerals. Unfortunately, the amount of lactose produced from whey exceeds the commercial demand for lactose (Yang and Silva 1995). Statistics for 1992-1993 indicate that about 27 million tonnes of liquid whey were produced per year as a by-product of the cheese industry. This equates to nearly 1.3 million tonnes of whey lactose (Yang and Silva 1995). According to the U.S. Dairy Export Council, over 25% of the world's whey and lactose is produced in approximately 200 whey plants in the United States (Langan 2003). Data for 2001 indicates that 470 million pounds of lactose were manufactured from whey from U.S. cheese manufacturing, and 726 million pounds from European cheese production. The market price for lactose over the past decades has ranged between \$0.10-0.30/lb and was around \$0.20 in 2001 (Elliot et al. 2001). Lactose is clearly not a high value co-product recovered from whey.

The primary uses of whey products of economic significance are for the human food market. These include dried whey powder, lactose and whey protein (Figure 1). Most of these nutrients are harvested from sweet whey. Only about 10% of acid whey is processed to a marketable product. As noted earlier separation of the whey proteins, leaves the lower value whey permeate and lactose after filtration (Yang and Silva 1995). This product now requires a means of utilization rather than disposal.

Whey Product Distribution in US

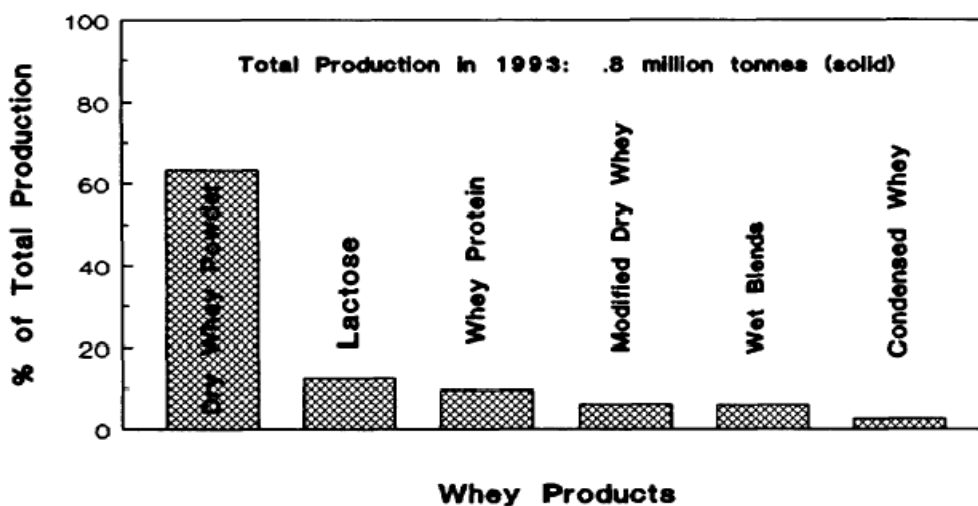


Figure 1. Distribution of whey products as a percentage of total production (Yang and Silva 1995).

Whey chemistry: Lactose

Lactose is a disaccharide which yields D-glucose and D-galactose upon hydrolysis. It occurs in two natural crystalline forms that differ in their steric configuration of the H and OH around the C-1 of glucose (Figure 2). The two forms are: α -monohydrate lactose, the usual crystalline form

obtained from cheese whey or aqueous lactose solutions upon concentration to supersaturation at $< 93.5^{\circ}\text{C}$, and the anhydrous β -lactose. The α and β forms can occur in solution together resulting in what is referred to as an “amorphous glass mixture”. (Holsinger 1988).

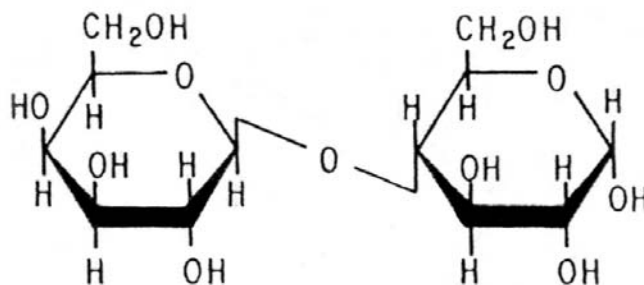


Figure 2. Lactose molecule: galactose and glucose.

The α -hydrate can appear in a variety of crystalline shapes depending on the conditions of crystallization, the most familiar are the prism and tomahawk shapes. The crystals are hard, not very soluble and feel gritty or sand like. These α -lactose crystals account for the sandy defect in some ice creams, condensed milk and cheese spreads. Prevention of crystallization can be aided with gelatin, or marine and vegetable gums that help inhibit crystal formation. Lactose crystal formation results as a function of temperature and concentration of lactose in solution. As a solution becomes saturated to the point of supersaturation, as with whey permeate, decreasing temperatures cause lactose molecules to aggregate, forming crystals that grow and eventually fall out of solution resulting in sand like particles. The crystallization process is increased with low pH (<1) and also by methanol and ethanol. The presence of sucrose in solution will significantly decrease lactose solubility. Calcium chloride acts to increase the rate of lactose crystallization as well. The degree of saturation, in conjunction with solution temperature, will determine the point at which lactose crystallization will occur (see Figure 3). There exists a metastable range as a function of temperature and concentration in which crystallization will not readily occur. Though speaking with Ray Dyke, vice-president of technology for Agrimark/Cabot Cheese, lactose crystallization of a 30% solids solution will occur at $55\text{-}60^{\circ}\text{C}$ ($130\text{-}140^{\circ}\text{F}$). Precipitation of a 20% solids permeate occurs at 10°C (50°F). Above that area is the labile range of higher supersaturation where crystallization readily occurs. The apparent break point for a 40% lactose solution is around 35°C (95°F). Note, however, the discrepancy in temperatures of crystallization indicated in Figure 3 versus that quoted by Ray Dyke of Cabot, speaking from personal experience with their product. This may be the result of actual field observations versus rigidly controlled lab experimental conditions. Given the field observation of a 30% solids permeate crystallizing at 55°C , handling and long distance trucking of a 40% solids whey permeate may be more difficult. Data regarding the temperature drop of a truck load of concentrated whey permeate traveling long distances in cold weather need to be obtained.

Crystallization depends on many factors, not just temperature. The presence of a specific seed surface will encourage crystal formation. Crystallized lactose residues in storage vessels will act to precipitate incoming lactose in solution. Storage and handling equipment will require thorough cleaning to remove any lactose crystals that may act as seed surfaces that can initiate crystallization of fresh product in solution. Crystallized lactose is known to plug, wear and rupture stainless steel pipes, (conversations with Dr. Karen Smith at the Wisconsin Center for Dairy Research). Crystallization of lactose around the perimeter of tanks during transit could

pose handling problems and act as seed material to initiate crystallization of incoming fresh product. Clean-up would not be easy as attempts of resolubilizing crystalline lactose in the lab at Miner Institute have required boiling and vigorous agitation.

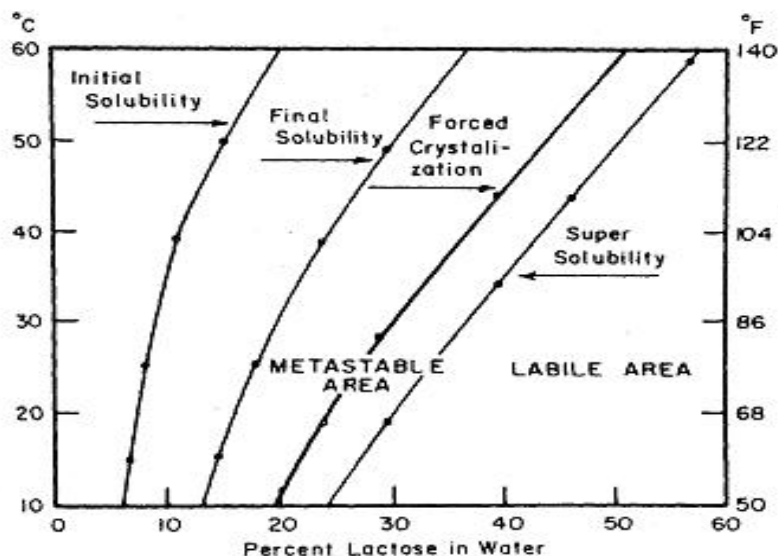


Figure 3. Lactose solubility curves. Fundamentals of Dairy Chemistry

The rate of supersaturation, either through cooling rate or evaporation rate of drying the solution, will affect crystallization as will mechanical agitation. The lines of Figure 3 are not precise quantitative values but rather conceptual markers indicating regions of supersolubility. Points to consider concerning crystallization are: 1. neither crystal growth, nor nucleation (crystal initiation) can occur in the unsaturated region, 2. crystal growth can occur in both the metastable and labile areas, 3. nucleation can occur in the metastable area only if seeds (centers for crystal growth) are added, 4. spontaneous nucleation can take place in the labile area without the addition of seeding materials (Fundamentals of Dairy Chemistry). Table 2 indicates how sucrose in solutions can lower the temperature at which lactose will begin to crystallize. This table is more relevant for the food industry, ice cream and condensed milk products for instance, but also may have application when blending whey/lactose solutions with molasses products.

Table 2. Relative Solubility of Lactose in Sucrose Solutions*

Solution	Temperature (°C)					
	25	40	50	60	80	85
40% sucrose	74.5	76.7	75.5	81.9	89.4	80.5
50% sucrose	63.0	64.8	64.9	71.9	76.7	73.0
60% sucrose	50.9	53.5	53.3	57.8	70.2	66.4
70% sucrose	42.1	44.3	43.3	54.3	63.9	62.7

*Percentage of lactose solubility in distilled water at the same temperature.
Fundamentals of Dairy Chemistry

Feeding of whey and whey products to non-ruminants

Considerable research has been conducted over the years feeding liquid whey and various whey products to swine. Schingoethe (1990) notes research that involved feeding whey or whey

permeate, both as liquid or dried powder to pigs from 20-90 kg in weight. Weight gains and feed-to-gain ratios were not different when whey was fed at a rate of 25% of dietary energy, or up to 20% of the diet. Though when permeate was fed at 40-60% of dietary energy, there was a decrease in weight gains and feed efficiency versus the control diet. Schingoethe (1990) notes other research that found reduced weight gains and feed efficiencies when whey permeate was fed at more than 20% of the diet. For swine, it would appear that 20% of the diet as whey permeate is the upper limit for nutritional efficiencies. Research with whey protein concentrates fed at 33% of ration DM has shown good growth in weanling and growing pigs. Though when fed a 70% of the diet DM, weight gains were reduced (Schingoethe 1990). It appears that the upper limit for feeding pigs whey protein concentrate would be around 33% of dietary DM.

Research conducted feeding dried whey to nonruminants showed increased weight gains and feed efficiencies in poultry, swine, horses and rats. Improvements in protein digestibility, N retention, fat digestion and mineral absorption were observed with feeding dry whey or lactose (Schingoethe 1976). Recommendations for poultry are 3-4% of the diet as dry whey. Lactose fed at >20% of the diet, decreased chick growth. It should be noted that mature hens are less tolerant than chicks to lactose. Swine can be fed up to 15-20% dry whey and rats can consume up to 20-30% of their diet as lactose with no ill effects (Schingoethe 1976).

Dairy feed applications

With the focus of this report on the application of whey permeate/lactose as a dairy feed supplement, a fundamental review of ruminant nutrition is presented here. The concepts of protein and sugar utilization by rumen microorganisms are critical for determining the most efficient use of sugar in the dairy diet.

Ruminant nutrition basics

Optimizing production of a growing ruminant or lactating dairy cow involves maximizing the growth and health of rumen microorganisms. Supplying the proper levels and variety of carbohydrate and protein/nitrogen sources to the rumen is critical in maximizing microbial growth and function. Rumen microorganisms ferment carbohydrates in the form of sugars, starch, soluble fiber, hemicellulose and cellulose to volatile fatty acids (VFA). These VFA's, primarily acetate, butyrate, propionate and lactate, are the energy units absorbed by the rumen wall and utilized by ruminant tissues. In order for the microorganisms to most efficiently ferment the carbohydrate sources, the proper types of nitrogen sources need to be made available. Typically this is in the form of proteins, peptides and non-protein nitrogen (NPN), such as urea and ammonia. In the same respect, to make the most efficient use of N sources for creation of microbial protein, the correct amounts and types of carbohydrate need to be supplied to the rumen microorganisms. Relative rates of degradation of N sources and carbohydrate sources need to be considered when trying to synchronize the availability of each nutrient for microbial use.

Depending on the physiological status of the animal, dry matter intake (DMI) and other feed and environmental factors, the rate of digestion and passage of feed through the rumen and gastrointestinal tract will vary. Some of the protein and carbohydrates will escape rumen degradation and appear in the small intestines in their original form. The primary source of

protein provided to the small intestine is the bacterial mass flowing from the rumen. The amino acid profile of rumen microflora is optimal for the amino acid requirements of growth and milk production of dairy animals (NRC 2001). It is therefore advantageous to maximize the amount of microbial mass flowing to the lower gut of the cow/ruminant in order to maximize the quality of the nutrients supplied to the animal.

Efforts to maximize milk production and the microbial mass or the microbial protein generated in the rumen have lately focused on carbohydrate sources. Sugar has prominence as a chief carbohydrate source to achieve greater microbial protein yields and milk production. Hoover and Miller Webster 2000, provide an excellent review of sugars and their efficacy in maximizing rumen fermentation. Typical dairy diets contain between 1.5-3.0% sugar on a DM basis. By increasing the level of dietary sugar, it has been theorized that there will be noticeable improvements in rumen fermentation resulting in improved animal production. Ideas put forth for the benefits include: increasing microbial growth through the increased level of rapidly available carbohydrate (sugar); improved efficiency of nitrogen utilization of soluble protein and NPN; or possibly altering VFA production resulting in improved milk production or milk composition (Hoover and Miller Webster 2000).

The primary sugar sources of interest have been molasses (sucrose) and whey products (lactose). Other by-product feeds that also contain relatively high levels of sugar, include: beet and citrus pulp, almond hulls and various fruit and vegetable co-products (Hoover and Miller Webster 2000).

Sugar Effects on Rumen Function

The fermentation of sugars results in decreased rumen pH with an increase in lactic acid. (Cullen 1986 as cited by Hoover Miller Webster 2000). Upon feeding 2.2 lb of sucrose to male Friesian cattle, Khalili and Huhtanen (1991) found rumen pH to be decreased and lactic acid production was tripled. When the same amount of sucrose was fed with buffers that prevented the drop in pH, lactic acid levels did not increase. The negative effect of sugar supplementation on rumen fermentation is the drop in pH. The decrease in pH can result in depressed fiber digestion. Huhtanen and Khalili (1992) found that supplementing sucrose decreased the activity of both cellulolytic and hemicellulolytic enzymes when pH was allowed to drop, but not when pH was maintained. If the pH level can be maintained, fiber digestion is not greatly affected with increased sugar levels (Hoover, 1986).

The effect of dietary sugar levels on VFA proportions is considerably varied. Sugar fed at less than 15% of DM showed little effect on VFA ratios (Hoover and Miller Webster 2000). When fed at greater than 15% of DM, there is a tendency for butyrate levels to increase. When a variety of sugars were fed to steers at 14% of DM, acetate levels were reduced, butyrate levels increased and there was little effect on propionate. Though in the case of dairy cattle, effects on VFA changes of 15% sugar rations may be secondary considering the possible reduction in pH and decreased fiber digestion observed at 4-5% dietary sugar, unless proper buffers are also fed.

Sugar and nitrogen metabolism

Rumen microbes use sugars and NPN to create microbial proteins. When soluble protein or NPN is in excess of microbial needs, ammonia is absorbed across the rumen wall and

metabolized by the animal. Often times excess ruminal NPN results in excretion of excessive amounts of urea. Not only is this a metabolic expense to the animal and economic waste of nutrient, but also creates an environmental burden. Balancing available carbohydrates to the soluble protein and NPN levels in the rumen allow for maximal microbial growth and minimal nutrient expense to the cow and losses to the environment. Ruminal ammonia levels have been reduced in nearly all studies where sugar has been added to the diet (Hoover and Miller-Webster, 2000). A study by Giduck and Fontenot (1987) showed reductions in ruminal ammonia concentrations with the addition of carbohydrate, lactose having the greatest effect over starch, sucrose and glucose. Sugar was fed at nearly 36% of the diet DM. The reduction of ruminal ammonia levels indicates that either NPN sources were in short supply or that carbohydrate sources were in oversupply to maximize microbial growth. Increased microbial N efficiency has the potential to minimize N losses but not necessarily increase microbial yield. It should be noted though that an increase in microbial efficiency (quantity of microbes grown per unit of carbohydrate fermented) differs from a greater microbial mass resulting from greater amount of fermentable carbohydrate (Hoover and Miller Webster, 2000). Indications are that increases in microbial yield are the result of adequately supplying the limiting nutrient, in this case, rapidly available carbohydrate in the form of sugar. Data presented by Hoover and Miller-Webster (2000) indicate microbial efficiency to be about 25% (g microbial N/kg organic matter digested), ranging between 20-30% across diets that contained sugar at 3-19% of the diet DM. A review of limited data indicates that the optimal ratio of available sugar to soluble protein, for microbial growth is when sugar is in excess of soluble protein by more than 2:1 (Hoover and Miller Webster 2000).

Fermentable carbohydrates in whey products

The readily fermentable lactose in whey has been shown to increase microbial protein synthesis and to increase the use of ruminally degradable protein such as urea (Stock et al. 1986; Windschitl and Schingoethe 1984). If diets were limited in degradable protein, no microbial response was seen with supplemented lactose (Stock et al 1986).

High producing early lactation dairy cows often times increase milk production when fed proteins that escape ruminal degradation. In comparison, production can be decreased when rapidly degradable NPN sources are fed in excess, such as urea. Excessive ruminal ammonia levels will metabolically tax the animal and diminish productivity as a result of the energy required to detoxify and excrete the excess N. Though, when cows fed urea were also fed dried whole whey, production increased compared to a urea-corn grain diet and was similar to a corn-soybean meal supplemented diet (Casper 1986). Whether the benefits were from the lactose being utilized by the microbes in conjunction with the NPN or there was a benefit due to the whey proteins in the whole dried product is uncertain. Both the urea and dried whey are sources of highly rumen degradable proteins. It may also reason that the rate of digestion of the carbohydrate available from corn grain was better synchronized with the availability of the whey and SBM peptides and proteins compared to the more rapidly available urea NPN. Lambs and steers showed greater weight gains with urea and dried delactosed whey compared to diets containing urea and sucrose (Stock et. al. 1986 II.). This suggests that either the lactose in whey is used more efficiently with urea or that some other compounds in the delactosed whey promoted the response. Considering the high biological value of the SBM and delactosed whey peptides and amino acids, it may reason that the noted responses were to these proteins rather than the sugar content of the whey product.

Whey Research

Liquid whey and liquid permeate have long been fed to cattle. It is well known that cattle will consume whey ad libitum. Though acclimation to whey may take 1-2 weeks. It is recommended to force the animals to consume the whey by withholding water until the animals are freely drinking the whey. Once adapted, free choice water should be made available. The animals will continue to consume the whey, generally at a rate of 2/3 of their liquid consumption, the remainder as clear water. Issues of bloat can be avoided by ensuring continuous access to whey under ad lib feeding conditions. In this manner fluctuating intake levels are avoided. Also, ensuring forage fiber consumption of 6 lbs of hay or as a minimum of 10% of ration DM should be adequate to prevent bloat. The use of ionophores to prevent feed lot grain bloat is common. Whether ionophores have the same effect on helping with whey bloat is unclear.

Dairy plant wash water, with very dilute milk residues and solids has also been fed to cattle, sheep and swine with no harmful effects. Though this dilute product is not a desirable feed since it contains very few nutrients (Schingoethe 1990)

Research in the 1970's with lactating dairy cows showed that production could be maintained when adding whole whey to the diet. Anderson et al. 1974, fed lactating dairy cows whey as the only source of liquid, which supplied 29% of the diet DM, and found that cows maintained milk production at 21 kg. Welch 1973, found a slight improvement in milk production in a similar study. When whey was the only source of liquid, cows consumed about 90 kg/d. When water was provided but restricted, whey consumption was 60-75 kg/d (Anderson et al. 1974), and when water was allowed ad lib, whey intake was 64-78 kg/d. Individual whey intakes will vary and consumption will vary seasonally (Anderson et al. 1974, Welch 1973). Dry matter intake tends to remain constant, for each kg of whey DM consumed there will be an equal reduction in solids DM consumed from forage or concentrate. Palatability of whey may be reduced as the acid content increased. After 36 hours at ambient temperatures, whey consumption was reduced, though pH values of the whey were not reported (Welch 1973).

Windschitl and Schingoethe (1984) fed dried whole whey to two fistulated Holstein cows and measured microbial protein synthesis. In this study, dried whole whey was fed at 38% of the ration DM. Whole whey is a significant source of sugar and soluble nitrogen for microbial protein synthesis. In support of other research, Windschitl and Schingoethe (1984) found that feeding high levels of dried whey resulted in an increase in ruminal butyrate levels while propionate and ammonia levels were all significantly decreased. The whey diet resulted in higher ruminal pH than did the control diet. The drop in ruminal NH₃ levels was significant, inspite of the higher level of soluble N supplied by the whey diet (60% vs. 39% of dietary N) compared to the control diet. This suggests that there was greater utilization of the soluble N sources by the rumen microbes with the whey. The dried whey diet also resulted in cows having greater rumen fluid volume (33.9 and 39.2 liters/day for control and dried whey) and a greater fluid dilution rate (10.2 and 12.8%/h). A possible explanation for the increased fluid volume and dilution rate is the effect of the high mineral content of the dried whey, particularly sodium (Na). The Na content of the whey diet was approximately 0.52% of the DM and only 0.13% for the control diet. The higher Na levels could have resulted in greater water consumption by these animals, though water intake was not measured in this trial. Greater water consumption would result in greater rumen fluid volume. Secondly, the osmotic pressures due to the higher

mineral content could have resulted in the greater fluid volume in attempts to maintain isotonic gradient between the ruminal and interstitial tissues. Regardless of the cause, fluid dilution rates were greater for the dried whey diet. Infusion of mineral salts into the rumen and the feeding of additional salts in the diet has been shown to increase rumen fluid dilution rates (Rogers and Davis 1982; Rogers et al. 1982; Rogers et al. 1979). Though not significant, bacterial N as a percentage of total ruminal N tended to be higher for the whey diet, while protozoal N tended to be lower. Combining the bacterial and protozoal data, 72 and 74% of the total ruminal N was from microbial N for the control and whey diets respectively. Combined with the increased rumen fluid volume and turnover rate, there appeared to be more N carried to the cows lower gut for digestion when fed dried whey. The fast liquid turnover rate in the rumen promotes the growth of microbes, such as the bacteria, with faster growth rates. Those with slower growth rates, such as protozoa, tend to be washed out of the rumen (Chalupa 1979, as cited by Windschitl and Schingoethe 1984). This may benefit the cow, in that, protozoa prey upon rumen bacteria. Protozoa are also less efficient at synthesizing protein than bacteria. Therefore, by maximizing bacterial growth and minimizing protozoal growth, N utilization within the rumen can be improved, benefiting the host animal. The results of this trial indicate that feeding dried whole whey, with its sugar and soluble N sources, at 38% of the diet DM, increased microbial protein synthesis and increased the ruminal fluid dilution rate.

Boukila et al. (1995) fed wethers, castrated male sheep, ammoniated whey permeate in place of molasses and urea and found no difference in DM digestibility or rumen fermentation. Though DMI and digestible energy intake were increased on the whey diets. The ruminal VFA concentrations were similar across diets. These results support the notion of replacing molasses with whey permeate and seeing no negative effects on rumen function

Whey protein concentrate (WPC)

In further evidence to the nutritional value of whey proteins to ruminants, 1% formaldehyde treated WPC fed to dairy cows producing 60 lbs of milk/d showed a 7% increase in milk production compared to untreated WPC (Muller et al. 1975). Milk-fat percentage increased from 3.10 to 3.42, while milk protein percentage dropped significantly from 3.14 to 3.09. Making the whey proteins by pass rumen degradation through treatment with formaldehyde improved production.

Calves and young stock on whey

Bayat et al. (2003) fed whole whey to 3-6 month old Holstein steers and found similar growth and feed conversion efficiencies when whey replaced 1/3 –2/3 of the concentrate in the diet. Diets consisted of alfalfa hay at 0.7% of animal bodyweight (BW) plus grain supplement or replacement of grain with varying amounts of whey. Plasma urea nitrogen (PUN) levels were significantly lower in the whey diets than the control diet (14.9, 18.6 versus 25.2 mg/dl). There also was a trend toward improved fiber digestion with the higher whey diet compared to the control. This may be in support of the notion that the small peptides in the whole whey are more beneficial to the fiber digesting bacteria than the lactose is to the sugar fermenters.

As noted, much of the nutritional benefit of whey and whey products centers around the protein content. The feeding value of deproteinated whey products such as concentrated whey permeate then rests on the sugar content as a fermentable energy source. Research has been conducted on

fermenting and ammoniating whey in order to increase the CP content. This process has shown some success in steers that had similar growth rates and feed utilization compared to a SBM supplement (Schingoethe et al. 1988). Ideas for improving the feeding value of whey permeate and other uses for lactose are presented later in this paper.

Lactose effects on rumen parameters and VFA profile.

It is quite well documented that lactose has been shown to increase ruminal levels of butyrate and some of the minor VFAs while decreasing acetate and propionate levels (Schingoethe 1990). Effects on ruminal VFA profiles may indicate changes in the populations of microorganisms in the rumen. The ability to ferment lactose by most rumen bacteria is limited (Hungate 1966). Considering this raises significant nutritional questions: 1. at what level of feeding lactose do significant quantities by pass the rumen, 2. with by pass lactose, will intestinal lactase activity be induced, 3. will improvements in ration digestibility and mineral absorption be realized as with nonruminants, 4. or will poor lower gastrointestinal digestion of lactose result in scours/diarrhea? (Schingoethe 1976).

Whey permeate research

Hussain and Miller 1998b, using wethers/sheep replaced corn starch with varying amounts of whey permeate, lactose or sucrose, and measured rumen effects. Whey permeate (85% lactose) was substituted for corn starch in the diet at levels ranging from 0.625-5.00% of the diet. The maximum amount of whey permeate fed was 50 g (5% of the diet). Sucrose or lactose were also substituted for equal amounts of corn starch in the diets at 10 g each. Compared with the corn starch diet, rumen pH and VFA concentrations were unchanged by the permeate and lactose. There was a decrease in the population of rumen protozoa with the permeate. Sucrose showed significantly lower total tract DM, organic matter (OM), neutral detergent fiber (NDF) and nitrogen digestion compared to lactose. There were no microbial yield differences by sugar versus the corn starch control diet. The authors concluded that substituting whey permeate for corn starch at levels less than 5% of total diet showed no negative effects on rumen metabolism. Though, replacing starch with sugars, in this case, whey permeate, lactose and sucrose, did decrease the population of rumen protozoa. Interesting to note the drop in rumen protozoa, which may indicate population shift of the rumen microflora that could have resulted from effects of fluid dilution rate as discussed earlier.

In a second report, Hussain and Miller (1998a) compared rumen parameters between substituting either sucrose, lactose or whey permeate (85% lactose) for starch in wethers. Supplementation levels were isoenergetic at levels equivalent to 50 g of sucrose. Lactose and whey permeate resulted in less of a rumen pH drop, a greater reduction of ruminal NH_3 and decreased VFA levels compared to the starch and sucrose treatments. The lactose and whey permeate diets resulted in a significant drop in rumen protozoa versus the starch and sucrose diets. The sucrose diets resulted in a drop in protozoal numbers but not nearly as great as the lactose and permeate treatments. Total tract digestion of nutrients was not effected by treatment. Lactose and whey permeate did show less OM and NDF flow at the duodenum possibly indicating better rumen fiber digestion, while microbial N flow was increased. Replacing 50 g of starch with lactose or whey permeate, equivalent to nearly 5% of the diet consisting of dry hay and concentrate, improved rumen metabolism and microbial nitrogen output. Yet the issue of rumen parameters in wethers versus high producing dairy cows has yet to be verified.

Lactose Supplementation

There is limited information concerning the effects of feeding lactose to dairy cattle. Much of the research conducted has been with whey products that still contained the whey proteins. Cows fed 2.1-3.0 kg/d of lactose in the form of dried whey or delactosed whey maintained milk production similar to control cows (Huber et al. 1967). Though this research was conducted years ago and milk production was only about 20-23 kg/cow/d. Other research showed slight milk production decreases when 3.5 kg lactose/d was fed, from 24.2 to 18.9 kg/cow (Bowman and Huber 1967) and with 4.3-6.0 kg lactose/d (Huber et al. 1967). Only slight reductions in milk were observed with 4 kg of lactose/d (Anderson et al. 1974). Based on these past research projects, it appears that milk production may be reduced when lactose is fed at 3-4 kg/d or as 20-30% of the ration DM, when fed as either lactose, dried whey or partially delactosed whey. The question remains as to the whole nutritional status of these cattle, their rumen available N sources and DMI and milk production levels. Whether these findings are still applicable with current improvements in ration balancing and today's high producing dairy cow is debatable. Schingoethe (1976) had previously found whey and lactose supplementation to not affect DMI with a trend toward decreasing milk production. More recent research conducted at West Virginia University showed lactose to significantly increase intake and milk true protein content, but did not increase milk volume (Hoover and Miller-Webster 2003).

Wing et al. 1988, examined replacing some of the corn grain in lactating dairy cattle diets with various types of liquid sugar supplement. The control diet consisted of cottonseed hulls as the only forage source. To the control diet was added either 6% citrus molasses (43% sugar), 3% Masonex (54% sugar) or 3% Flambeau (45% sugar). All treatments increased DMI. Only marginal increases in milk production were observed, with a range of daily milk yields of 22.7-25.0 kg (50-55 lbs). Only the cane molasses increased milk production without increasing DMI. Morales et al. (1989) varied the levels and types of forage fed with and without substitution of molasses for corn grain. This study allowed for observing the effects of various sugar:starch ratios and total sugar and starch:soluble protein levels. The overall conclusion was that production responses were related to DMI differences rather than to sugar:starch ratios or the sugar:soluble protein ratio.

Casper and Schingoethe (1989) tried varying the ratios of sugar and starch to soluble nitrogen in dairy cow rations. Diets varied from 29-40% soluble protein, using urea or SBM as the N source. Feeding corn as the carbohydrate source resulted in higher milk production than feeding barley or dried whey. The whey diet resulted in increased DMI, but a significant 1-5 lb drop in production compared to the corn only diet. Though, the sugar level in the corn plus whey diet was 14% of DM compared to only 3% for both the corn and barley diets. Interestingly, on the SBM diet, the difference in milk production between the corn and corn plus whey was only 0.4 kg; 32.7 to 32.3 kg (72 vs 71 lbs); with the urea diet the difference was 2.2 kg (5 lbs) of milk between the corn versus corn plus whey diet; 32.7 to 30.5 (72 vs 67 lbs). These data suggest the need for special consideration in formulating whey/lactose supplemented diets to ensure sufficient peptide sources for the microbes that may be able to ferment lactose most efficiently. The fiber digesting bacteria require peptides and SBM is a good source. Urea only supplies free NPN which in the case of whey and lactose supplementation, may not be the N source required.

McCormick et al. 2001, conducted an in vitro trial comparing sucrose to lactose from dried whey at 0, 2.5 and 5.0% of ration DM. The diets consisted of 50% ryegrass and about 40% ground corn, plus 7% SBM. Sucrose was shown to decrease ammonia levels at the 5% inclusion rate, while lactose had no effect. These results are in contrast with those obtained by Windschitl and Schingoethe 1984 as discussed previously and Chamberlain et al. 1993. Chamberlain et al. 1993 fed sheep a grass silage diet with either sucrose or lactose supplementation. In that trial, 18% lactose in the ration decreased rumen NH₃ levels, increased propionate and butyrate and decreased acetate.

Ordway et al. (2002), fed Holstein dairy cows, pre- and post-partum, sucrose at 2.7% of the diet DM. By analysis, the control diet was 6.6% sugar and the treatment diet was 8.8% sugar. Soluble protein levels were 32.0% and 32.4% respectively. Supplementing the sugar for ground corn showed no benefit as there were no differences in DMI or milk production. Blood glucose levels were significantly higher prepartum with the sugar diet, 66.3 vs. 69.3 mg/dl. The high level of sugar in the control diet, however, may have masked the possible effects of the additional sugar in the treatment diet. Most high production dairy diets have sugar levels of 1.5-3.0% of DM (Hoover and Miller Webster 2000).

Limits on lactose feeding to growing ruminants are not known. Research in the 1970's showed acceptable growth rates of steers and heifers consuming up to 30% of their DMI as liquid whey (Anderson et al. 1974, Welch 1973). Lynch et al. 1975, showed good animal growth and meat quality of steers fed 57% of their DM intake as whey.

Fermented ammoniated condensed whey (FACW)

One of the nutritional limitations of condensed whey is the low protein content after most of the casein and whey proteins have been removed. Attempts have been made to increase the CP level with NPN sources. Whey, typically sweet whey, has been fermented with *Lactobacillus bulgaricus* and bubbled with ammonia prior to evaporation to 60% solids. The resulting product is 45% crude protein equivalent (CPE), and 37% ammonium lactate. The product is said to handle easily, blend well and remain fairly stable (Juengst 1979). This product can be used to supply 8% of the dietary CP for dairy cattle (Coton 1980). This may be a feasible option for improving the nutrient value of whey permeate as well.

Feeding trials with beef cattle showed similar growth rates of yearling steers fed FACW or SBM, which both resulted in better growth rates compared to the control and urea supplemented groups (respective gains were 1.34, 1.42, 1.17, and 1.29 kg/d) (Juengst 1979). Dairy cows fed similar diets comparing FACW to SBM and urea supplementation had similar milk production during a 12 week feeding trial, though production data was not presented. Toxicity research with feedlot steers ruminally infused with FACW indicates that ammonia is released more slowly than urea in the rumen. Acute toxicity with intraruminal infusion of 200-300 mg urea-N/kg BW was observed within 30-60 minutes. Toxicity from the FACW ammonia (400 mg N/kg BW) was observed at 3.0-3.5 hours. It was concluded that the ammonia in FACW is released and absorbed more slowly than free urea in the rumen, showing potential as a slow release NPN source (Juengst 1979). The lactate portion of the FACW was shown to increase the propionate:acetate ratio, suggesting that lactate is used more efficiently than sugar or starch in the rumen. Lactate does not ferment to methane. Approximately 10% of sugar and starch is fermented to methane. It is concluded that FACW has potential as a dairy supplement based on

the increased CPE value, the slower release of NPN as compared with urea and the improved availability of energy in the form of lactate. Given current environmental concerns about nutrient management and N accounting, FACW appears to hold promise for a method of improving the nutritional quality of whey permeate.

Similarly, lactosyl urea offers the same opportunity as a source of NPN in ruminant diets. Lactose is reacted with urea to form lactosyl urea. It hydrolyzes more slowly than urea in the rumen providing a more controlled release for microbial usage thereby improving N utilization in comparison to urea.

Alternative Uses for Whey Permeate

Lactose can be used as a binder for powders and pellets. In the steel industry, lactose has been used to form iron ore pellets from iron fines that need to be held together while entering a blast furnace. Its properties of low solubility, high melting point and high heat of combustion make it a useful binding agent. This should be beneficial in forming grain pellets.

Potable ethanol can be derived from whey, permeate and lactose. Though whey derived ethanol (gasohol) has proven to be uneconomical. Vinegar can also be derived from lactose. (Morrissey 1985)

Whey and whey permeate have been used as a media for the production of food grade yeast (Shay and Wegner 1986). Yeast has also been used to decrease the solids content of waste whey, thereby decreasing the BOD levels. The result is a 65% protein whey-yeast byproduct which has been successfully fed to pigs (Ajeanl 1979).

Hydrolyzed whey permeate syrup (HWPS)

Hydrolyzed lactose whey syrups are produced by splitting the lactose molecule into galactose and glucose. By doing so, the quality of the lactose sugar for use in the food and feed industry is improved. Crystallization is diminished, the sugars are nearly as sweet as sucrose, and lactose intolerant individuals can digest the hydrolyzed product. This procedure has not been widely adapted due to competition from other sweeteners, primarily corn sweeteners. The resulting HWPS shows good functionality in many human food applications, including confections, bakery, frozen desserts, infant formula, powdered foods and spices and brewing (Coton 1980, Ogunrinola 1988)

There are a myriad of products that can be derived through the fermentation of whey. These include single-cell protein by-products, methane, alcohols, organic acids, vitamins and biopolymers. The primary hurdle to this product is the low level of organic N in whey, which do not meet the requirements of many microorganisms for industrial purposes, as well as the rumen. Supplementation with N sources is possible. Demineralization is sometimes required to reduce the sodium chloride levels for microbial growth. Also, lactose is not always the preferred carbohydrate source of microbes. Therefore hydrolysis to glucose and galactose can help.

Currently there is a surplus of whey for the number and amount of uses for it. Unfortunately, the goal is still to keep it out of the waste stream. Lactose hydrolysis technology improves its usability but the amounts still are in excess of market demand. Since lactose is an organic

compound, the potential exists for creating a niche in the “organic” market. In 1995, lactose was valued at \$0.40/kg, which could increase.

The use of whey permeate and lactose in lick blocks is also a consideration. Deproteinized whey that was 0.6% N, 70-72% lactose and 12% ash was treated with ammonia or urea to raise N levels to 1.7% and formed into solid blocks. These feed blocks were fed to growing calves. Growth was similar for calves consuming the blocks versus control calves. Growth rate and feed efficiency was lower for the calves on the unsupplemented blocks versus the urea treated blocks. Calves consumed between 15-24% of their DMI from the blocks (Lynch et al. 1974). This again exemplifies the improved nutritional value of treating whey permeate to increase its N content and the possibilities of creating feed blocks for range and pasture operations of both growing and lactating cattle.

The issue of economy of scale exists for many of the smaller cheese manufacturing plants that do not process enough whey to offset the costs of substantial investment in technologies to process whey into value added products. The larger manufacturers will take the risk of investing in new technologies while the smaller processors will have to find niche solutions for whey and lactose dispersal (Yang and Silva 1995).

For those interested in reading more about new technologies for processing and utilizing lactose, Elliot et al. (2001) is an excellent review. Lactose shows promise as a platform compound which can be processed into higher value products such as glycols (ethylene, and propylene) for recyclable plastics and sorbitol, a sugar substitute.

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Appendix 1. Milk Processing Co-products nutrient analyses.

Apart from DM, all values are expressed on DM basis

Feed material	Whey	Whey Concentrate	Whey Permeate	Delactosed Whey	Yoghurt	Ice Cream
Maximum allowance ruminants*	15	15	15	15	NA	NA
Maximum allowance pigs*	20	25	20	25	20	10
Dry matter %	5-7	30-50	18, 25 or 45	38-45	5-9	12-15
Ruminant ME (estimated) MJ/kg	13.5	13.5	11.6	11.2	NA	NA
Ruminant FME (estimated) MJ/kg	11.4†	13.2	11.5	10.8	NA	NA
Pig DE (estimated) MJ/kg	16.5	16.5	15.0	13.8	14.7-16.8	20-21
Crude protein %	13-15	12.4	3.8	24	18-34	9-10
Oil (B) %	1.0	1.4	0.2	1.2	1.5-8.0	20-25
Total sugars %	75	75	83	55-60	50-75	60
Ash %	10.0	7.4	11.0	16	5	4
NDF %	0.0	0.0	0.0	0.0	0.0-0.7	Trace
Calcium %	1.00	0.84	0.86	2.1	0.6-1.3	0.34
Phosphorus %	0.75	0.72	0.66	1.4	0.6-1.0	0.26
Magnesium %	0.08	0.07	0.07	0.26		0.03
Sodium %	1.0-1.3	0.69	1.00	1.9	0.35-0.65	0.26
Potassium %	1.20	1.00	2.10	4.9		0.41
Lysine %	0.94	0.94	0.18	2.06	0.8-1.6	0.63
Methionine %	0.24	0.24	0.03	0.53	0.2-0.4	0.16
Cystine %	0.24	0.24	0.04	0.63	0.2-0.4	0.16
Threonine %	0.70	0.70	0.14	1.60	0.6-1.2	0.47
Isoleucine %	0.66	0.66	0.17	1.58	0.56-1.12	0.44
Tryptophan %	0.21	0.21	0.03	0.42	0.18-0.36	0.14
pH	3.25-4.0					

Sources: Taymix; Wheyfeed; WPSA, 1992; Ling, 1956; McCance & Widdowson, 1991; NDC, 2000; NRC, 1998

*The maximum allowances are expressed as % of dietary dry matter. They aim to represent sensible limits in practical rations. Other dietary factors, such as the sodium content of other feeds, may necessitate a lowering of the suggested amounts. The pig allowances refer to fattening pigs and higher amounts may be possible in sow diets. The ruminant allowances refer to dairy cows and higher amounts may be possible in beef cattle diets.

† The FME value includes an adjustment for the lactic acid concentration

Appendix 2. Agri-mark cheese plant concentrated whey permeate nutrient profile.

Solids	35 – 40%
Following values on 4% moisture basis	
Protein	3 – 5% *
Lactose	83 – 85%
Ash	8.4 – 9.0 %
Calcium	0.68%
Phosphorus	0.92%
Magnesium	0.11%
Potassium	2.50%
Sodium	0.97%
Iron	2 ppm
Zinc	<1ppm
Copper	<1ppm
Manganese	<1ppm
Molybdeum	<1ppm
Sulfer	0.06%
Chloride	1.95%
pH	5.5 – 6.0

* Proteins mostly urea and other NPN compounds, some peptides

Values provided by Agrimark/Cabot and Dairy One analyses of samples provided to Miner Institute for the CPM Feedbank.

Phase II: Evaluation of the stability of whey permeate under warm storage conditions

Final Report

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Objective

The objective of this trial was to test the stability of concentrated whey permeate (~40% solids) over a five day period at elevated temperature and humidity conditions. Warm temperature and high humidity conditions were chosen to simulate summer-like storage conditions. Microbial growth and fermentation of the permeate was examined under these conditions. Analyses included bacterial growth counts, pH measurements and VFA profiles.

Methods and Materials

Samples of 40% solids permeate were collected from three different batches of permeate production at the McCadam cheese plant in Chateaugay, NY. Samples were taken on three separate days. The first sample, taken on 16 September 2003, was obtained from the bulk storage tank. This permeate sample was 41% solids resulting from the production of Muenster cheese (batch 1). The second sample, taken on 17 September 2003, was obtained as an “in line”, 42% solids whey permeate from the production of Jalapeno Pepper Jack cheese (batch 2). The third sample, taken on 19 September 2003, was obtained as an “in line”, 42% solids whey permeate from the production of Yellow Cheddar cheese (batch 3). The whey permeate samples were collected warm and transported back to Miner Institute for stability testing. Samples were evaluated for stability at two conditions over a five-day period: an elevated temperature and humidity (87°F, 85% RH) and an ambient temperature and humidity (77°F, ~65%RH). A subsample was also placed in a sterile bottle and left undisturbed for visual observations of bacterial growth and lactose crystallization.

For stability testing, 200 mL of permeate was placed into sterile tissue culture flasks and stored in an 87°F Stericult incubator at 85% relative humidity. A second sample was stored in 1L Erlenmeyer flasks at room temperature (77°F) and ambient humidity which was approximately 65%. Samples were shaken to resuspend solids and aliquots were taken on six consecutive days (days 0 through day 5). Bacterial growth counts were performed following the standard bacterial plate count method (Atherton and Newlander, 1977). Aliquots were diluted and plated in duplicate on pour plates in plate count agar, and incubated for 48 hours at 90°F. Following incubation, duplicate colony counts were averaged and bacterial concentrations were calculated. The same undiluted aliquots used for bacterial growth determination were used to determine pH and VFA concentration. The pH was determined at subsampling on a Beckman Φ 71 pH meter at room temperature. The remainder of the sample was refrigerated for later VFA analysis on a Varian 3800 gas chromatograph using a 6% carbowax column.

Results and Discussion

Microbial growth and pH

Whey permeate from batch 3 was visually different from batches 1 and 2 at the time of collection. This batch had a brighter yellow color as compared to a more golden color of the other batches (Figure 1). This was likely a result of the coloring agents used to produce the Yellow Cheddar cheese. Upon sampling, this batch appeared to have less crystallization on day 0 than batches 1 and 2. It is not believed that sampling “in line” versus bulk tank sample would account for this difference. “In line” samples were taken mid stream between whey

concentration and the storage tank. Upon setting undisturbed for five days, the lactose crystallization in batch 3 occurred on the fluid surface and also on the sides of the glass container (Figure 2) while crystallization in batches 1 and 2 occurred at the bottom of the glass container.

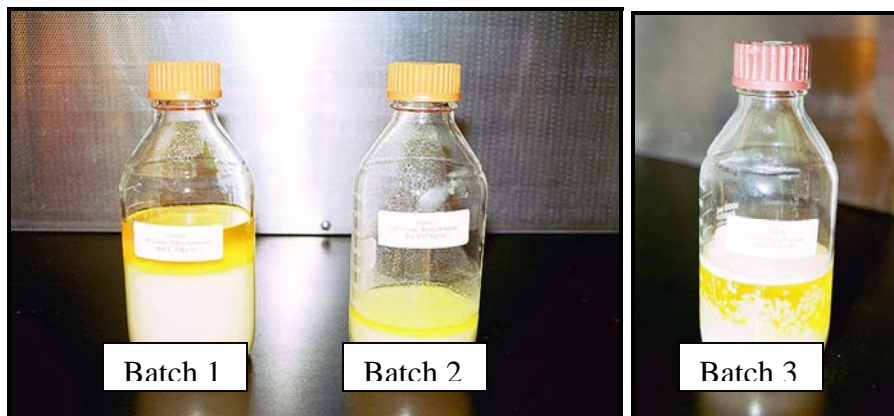


Figure 1. Undisturbed samples from three batches of whey permeate tested on day 5.



Figure 2. Growth of lactose crystals on the surface of batch 3 (day 5).

Bacterial growth counts by batch over the five days of storage are presented in Table 1. On day zero, batch 3 had a statistically higher bacterial concentration as compared to batches 1 and 2 ($P < 0.001$). Growth counts between batches varied considerably. However, by day four differences in bacterial growth were not statistically significant ($P = 0.102$).

Table 1. The effect of batch on bacterial count (CFU/mL) by day of storage.

Batch	Day of storage					
	0	1	2	3	4	5
1	4.8×10^{1b}	2.4×10^{3c}	8.2×10^6_b	4.1×10^7_b	9.2×10^7	1.4×10^8
2	1.5×10^{1c}	6.5×10^4_b	2.0×10^7_a	7.1×10^7_a	1.2×10^8	1.5×10^8
3	7.2×10^{2a}	5.4×10^{5a}	1.0×10^7_b	3.4×10^7_b	6.3×10^7	9.4×10^7
Std Err	6.0×10^0	9.4×10^3	2.2×10^6	9.3×10^6	1.6×10^7	1.9×10^7
<i>P</i> -value	<0.001	<0.001	0.018	0.063	0.102	0.135

^{a,b,c}Means in columns without common superscripts are different ($P < 0.1$).

When looking at storage temperature, bacterial growth at 87°F was significantly greater than that at 77°F over all five days regardless of batch (Table 2). This was to be expected as bacteria do have higher rates of growth at increased temperatures. In general, all batches held at the elevated temperature conditions showed an increase in bacterial growth starting on day 1 as compared to the 77°F storage. Counts continued to rise over the remainder of the five days (Figure 3).

Batches held at ambient temperature did not show a significant increase in bacterial counts until day 2 and the counts continued to increase over the remainder of the five days. At the end of the five day period, the bacterial counts in the batches held at the elevated temperature and humidity were approximately five-times those in the batches held at room temperature. This would suggest the critical nature of temperature when this product is stored for longer than two days. As noted by Dr. Karen Smith of the Wisconsin Center for Dairy Research, bacterial growth can be limited by reducing temperature however lactose crystallization increases significantly below 70°F. This poses storage concerns of crystallization verses contamination. Depending on rate of usage, storage temperatures could be increased to reduce crystallization at the risk of increased microbial growth. Though batch samples were taken from permeate of different cheese types, there is not enough evidence to suggest cheese type relates to storage life.

Table 2. The effect of storage temperature on bacterial count (CFU/mL) by day.

Storage Condition	Day of storage					
	0	1	2	3	4	5
87°F	2.6×10^2	4.0×10^5	2.6×10^7	9.1×10^7	1.6×10^8	2.2×10^8
77°F	2.6×10^2	6.5×10^2	2.0×10^5	6.9×10^6	2.7×10^7	4.6×10^7
Std Err	4.9×10^0	7.7×10^3	1.8×10^6	7.6×10^6	1.3×10^7	1.6×10^7
<i>P</i> -value	1.000	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 3 graphically displays the geometric increase in bacterial growth under the 87°F verses 77°F storage temperatures. Bacterial growth differed by batch but whether this is natural variation within the permeate or attributable to cheese type is unclear.

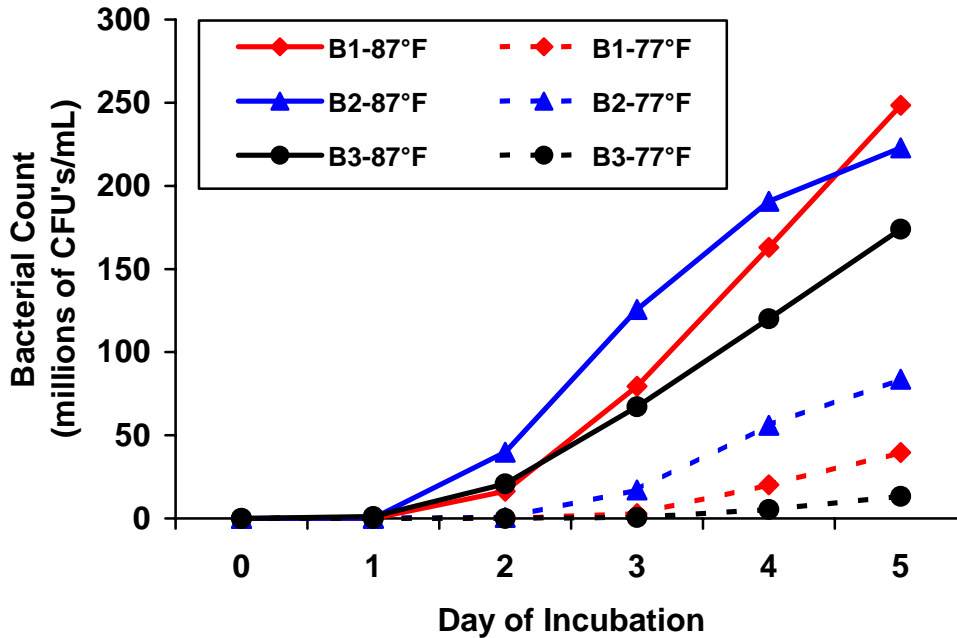


Figure 3. Bacterial counts from three batches of concentrated whey permeate held at 87°F and 77°F over a five-day period.

The pH's of each batch of permeate over the five day storage period showed a minimal increase. On average initial day 0 pH was 5.54 and at day five, 5.78 (Table 3). The pH values were very consistent between batches and over the five days. Unlike whole whey fed to livestock where pH can drop significantly to values of 4, under the sterile storage conditions used for this testing pH remained constant.

Table 3. The effect of batch on pH by day of storage.

Batch	Day of storage					
	0	1	2	3	4	5
1	5.522 ^b	5.642 ^b	5.657 ^b	5.701 ^b	5.749 ^b	5.767 ^b
2	5.582 ^a	5.651 ^a	5.699 ^a	5.768 ^a	5.811 ^a	5.808 ^a
3	5.516 ^b	5.602 ^c	5.657 ^b	5.679 ^c	5.723 ^c	5.750 ^b
Std Err	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
P-value	<0.001	<0.001	0.001	<0.001	<0.001	0.028

^{a,b,c}Means in columns without common superscripts are different ($P < 0.1$).

Temperature did not effect pH as shown in Table 4. After five days of storage, there was no significant difference in pH between the 87°F and 77°F storage temperatures ($P=0.296$).

Table 4. The effect of storage temperature on pH by day.

Storage Condition	Day of storage					
	0	1	2	3	4	5
87°F	5.540	5.630	5.677	5.726	5.769	5.768
77°F	5.540	5.634	5.665	5.706	5.752	5.782
Std Err	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P-value	1.000	0.152	0.084	0.046	0.122	0.296

Figure 4 displays pH values for each batch of permeate by temperature over the five days of storage.

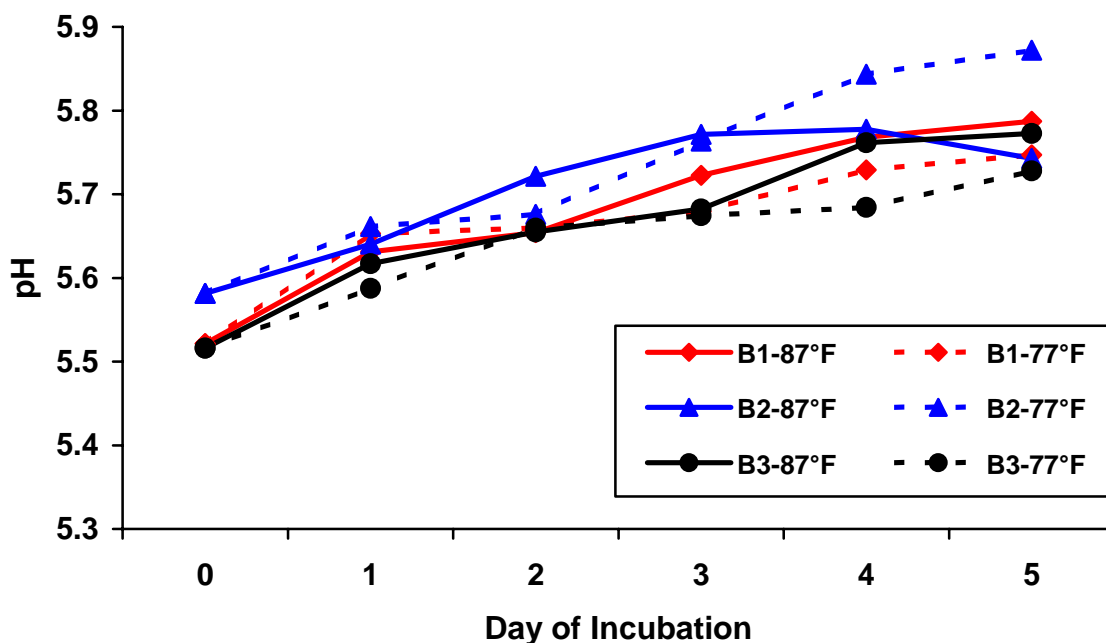


Figure 4. pH from three batches of concentrated whey permeate held at 87°F and 77°F over a five day period.

Interestingly, pH tended to increase over time rather than decrease. Bacterial growth increased, however this did not result in increased acid formation. As noted by Dr. Karen Smith not all bacteria produce acids as products of fermentation. In this case it would appear that bacteria may have been metabolizing acidic compounds resulting in a rise in pH.

VFA Analysis

Volatile fatty acid data indicated considerable variation between batches and across the five days of storage. Acetic acid levels tended to increase over time within batch nearly doubling over the five days (Table 5).

Table 5. The effect of batch on Acetic acid levels ($\mu\text{Mol/mL}$).

Batch	Day of storage					
	0	1	2	3	4	5
1	11.07 ^a	11.38 ^a	18.49 ^a	18.61 ^a	20.49	19.34 ^a
2	7.03 ^b	7.14 ^b	7.30 ^b	7.09 ^b	12.14	9.68 ^b
3	6.66 ^b	6.79 ^b	6.37 ^b	4.26 ^b	16.03	25.25 ^c
Std Err	0.59	0.94	1.25	2.13	2.35	1.82
<i>P</i> -value	0.003	0.025	<0.001	0.007	0.151	0.003

^{a,b,c}Means in columns without common superscripts are different ($P < 0.1$).

As noted in Table 6, acetic acid levels did not differ as a result of temperature.

Table 6. The effect of storage temperature on Acetic acid levels ($\mu\text{Mol/mL}$).

Storage Condition	Day of storage					
	0	1	2	3	4	5
87°F	8.25	9.49	10.93	11.34	12.81	17.84
77°F	8.25	7.38	10.51	8.63	19.63	18.34
Std Err	0.48	0.77	1.02	1.74	1.93	1.48
<i>P</i> -value	1.000	0.101	0.781	0.312	0.041	0.820

Figure 5 displays variation in acetic acid levels for the three batches over the five days of storage.

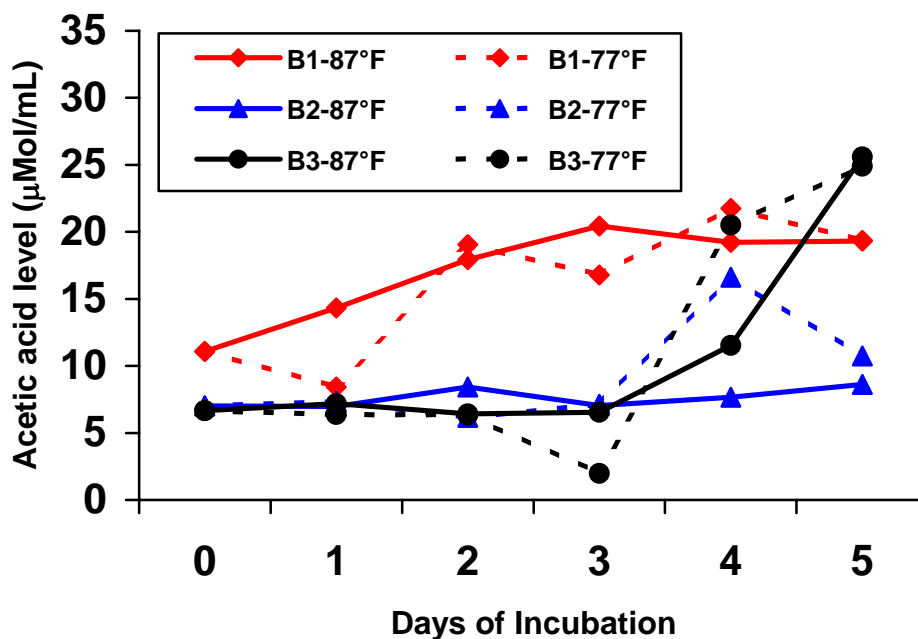


Figure 5. Acetic acid levels from three batches of concentrated whey permeate held at 87°F and 77°F over the five days of storage.

Lactic acid analyses showed slight increases in lactate levels over the five days of storage (Table 7).

Table 7. The effect of batch on Lactic acid levels ($\mu\text{Mol/mL}$).

Batch	Day of storage					
	0	1	2	3	4	5
1	49.35 ^a	64.18	68.84 ^a	74.74 ^a	60.93	78.62 ^a
2	46.45 ^{a,b}	54.80	58.66 ^a	47.93 ^b	53.87	55.91 ^b
3	39.87 ^b	35.40	31.56 ^b	19.83 ^c	51.84	58.48 ^b
Std Err	2.41	8.63	4.38	5.73	11.32	5.18
<i>P</i> -value	0.077	0.132	0.002	0.002	0.978	0.040

^{a,b,c}Means in columns without common superscripts are different ($P < 0.1$).

There was little effect of temperature on lactate formation though at day five differences were significant ($P = 0.02$) (Table 8).

Table 8. The effect of storage temperature on Lactic acid levels ($\mu\text{Mol/mL}$).

Storage Condition	Day of storage					
	0	1	2	3	4	5
87°F	45.22	51.53	43.81	44.65	45.19	54.99
77°F	45.22	51.39	62.23	50.36	65.91	73.68
Std Err	1.97	7.05	3.573	4.68	9.26	4.23
<i>P</i> -value	1.000	0.990	0.011	0.422	0.201	0.020

Figure 6 displays variation in lactic acid levels for the three batches over the five days of storage.

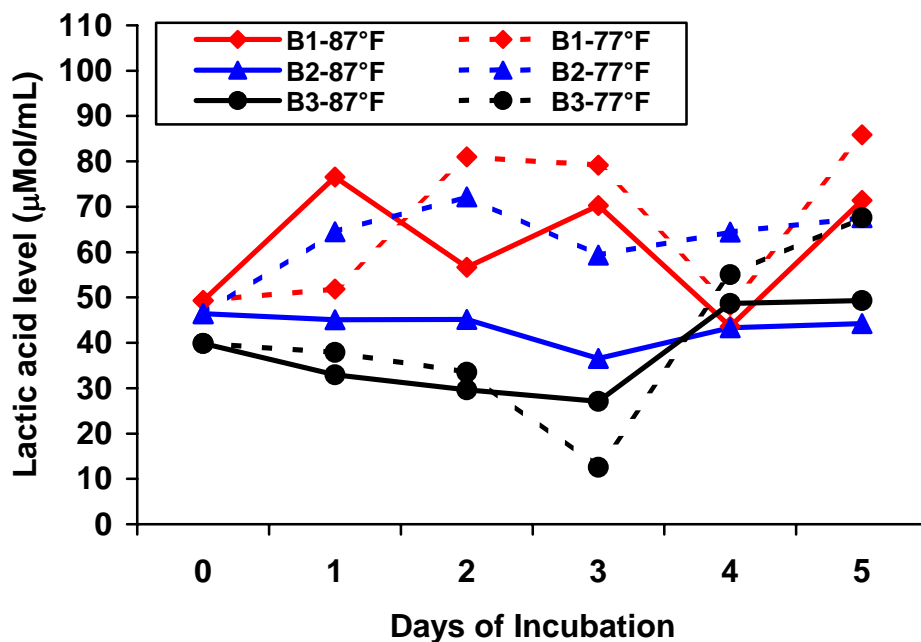


Figure 6. Lactic acid levels from three batches of concentrated whey permeate held at 87°F and 77°F over the five days of storage.

It appears that in spite of increased microbial growth over time and with increased temperature permeate acidity measured by pH and levels of acetic and lactic acid are relatively stable. This may be a result of the type of bacteria present in these samples not being acid forming bacteria. It was believed that pH would drop as a result of the fermentation of lactose however that was not observed.

Conclusion

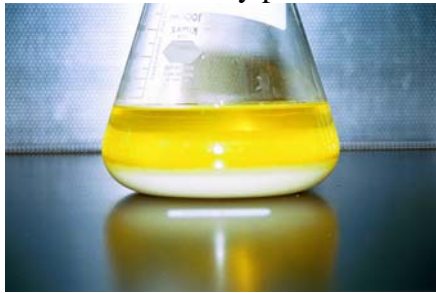
Product stability is certainly affected by storage temperature and time. Considerations as to product storage and usage rate need to be invoked when determining storage requirements. Storage of concentrated whey permeate at 77°F for a two day period can help limit bacterial growth without drastically increasing lactose crystallization. The lack in change of pH, acetate and lactate levels over the five days of storage indicate product stability. Whether this was unique to these samples is unknown. It would take replicated trials of permeate from many batches and cheese types to determine differences in stability in production runs. It is apparent that concentrated whey permeate at 40% solids is a stable product when stored in controlled environments.

Reference

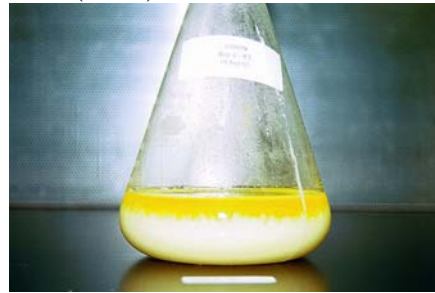
Atherton, Henry V. and J. A. Newlander. Chemistry and Testing of Dairy Products, 4th Edition. Pg. 272. AVI Publishing Company, Westport, CT. 1977.

Appendix 1

Concentrated whey permeate stored at room temperature (77°F).



Day 1



Day 2



Day 3



Day 4



Day 5

Concentrated whey permeate stored at 87°F.



Day 1



Day 2



Day 3



Day 4



Day 5

Phase III: The Effects of Addition on Sugar Sources to Lactating Cow Diets on Microbial Growth and Efficiency in Continous Culture of Rumen Contents

Final Report

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**Submitted to:
Miner Agricultural Research Institute**

August 26, 2003

Objectives

To examine the effects of sugar source (molasses and whey permeate) on microbial growth and metabolism in continuous culture of rumen microbes.

Procedures

Diet composition and analyses are shown in Table 1, where the diets are identified as Control - (no added sugar), Whey (whey permeate) and Molasses. A portion of the starch in the control diet was removed and replaced with sugar from either whey permeate or molasses. Analysis of the individual sugar sources is in Appendix Table 1.

The three diets were fermented in quadruplicate under the following conditions:

Feed Amount	100 g DM/d
Feeding schedule	4x /day at 6 hour intervals
Fermentation temperature	39° C
Liquid dilution rate	13%/hr
Solids retention time	22 hr
PH	Recorded at 2 hour intervals

Statistical Analyses - Data were analyzed using the General Linear Model Procedures of SAS.

Statistical contrasts included:

1. C vs Whey and Molasses
2. Whey vs Molasses

On day 6 of the 12-day fermentation period, samples were taken directly from the continuous culture vessels to determine rates of sugar disappearance following a dosing of the sugar sources. Fermenters were fed 50 grams DM of the basal control, whey and molasses diets. Two of the fermenters receiving the sugar diets were given an additional 15 grams of sugar from either whey permeate or molasses. Sample intervals were at 0, .5, 1, 2, 4, 8 and 24 hours after dosing.

Table 1. Diet Composition and Analyses (% Dry Matter Basis)

Ingredient	Control	Whey	Molasses
Haylage	20.32	20.69	20.71
Corn Silage	30.34	30.90	30.93
Ground Corn	19.77	13.43	13.44
Soy Hulls	6.82	6.94	6.95
Whole Cottonseed	8.61	8.77	8.78
Soy 48	10.95	11.16	11.45
Blood meal	1.26	1.29	1.29
Whey Permeate	----	4.86	----
Molasses	----	----	4.49
Megalac Plus	0.73	0.75	0.75
Urea	0.09	0.09	0.09
Dicalcium:Phosphate	0.37	0.38	0.38
Salt	0.37	0.38	0.38
ADE Mix	0.11	0.11	0.11
Limestone	0.19	0.19	0.19
Vitamin E	0.06	0.06	0.06
Analyses			
Crude Protein	17.91	17.86	17.63
Soluble Protein, % CP	27.87	32.99	30.77
Neutral Detergent Fiber	33.53	34.34	32.88
Acid Detergent Fiber	21.87	22.89	23.31
Nonstructural Carbohydrate ¹	31.57	29.60	31.89
Starch	28.16	22.52	24.65
Sugar	3.41	7.08	7.24
Ether Extract	4.35	4.29	4.56
Ash	5.75	6.09	5.96
Calculated Non-Fiber Carbohydrate ²	38.46	37.42	38.97

¹Nonstructural carbohydrate = true starch + sugar

²Non-fiber carbohydrate calculated as: $100 - (\text{CP} + \text{NDF} + \text{Ash} + \text{Ether Extract})$

Results

Digestion coefficients are shown in Table 2. Addition of both sugar sources increased digestion of ADF significantly over that of the control diet, with whey resulting in significantly greater digestion than molasses. Digestion of NDF was similarly increased by the whey but not the molasses diet. Digestion of the NSC fraction was increased over the control by both sugars ($P = 0.01$), with no difference between the sugar sources. Total grams of carbohydrate digested (sugar, starch and NDF) was equally increased over that of the control ($P = 0.05$) by both sources of sugar.

Table 2. Nutrient Digestibilities, %

Item	Diets			<i>P</i> -value	
	Control	Whey	Mol	C vs W+M	W vs M
Dry Matter	69.0	70.0	67.7	0.95	0.52
Organic Matter	47.0	50.0	50.2	0.04	0.88
Acid Detergent Fiber	44.3	54.8	47.1	0.03	0.03
Neutral Detergent Fiber	35.0	40.6	35.4	0.26	0.11
Non-Structural Carbohydrate	81.5	86.5	85.6	0.01	0.55
Total Carbohydrate Digested (g/day)	37.5	39.5	38.9	0.05	0.48

Table 3 shows the average pH and volatile fatty acid (VFA) production and molar ratio responses to the diets. The average pH was maintained at normal levels across all diets, and did not differ due to treatments. As shown in Figure 1, the pH after feeding was not reduced to levels lower than 6.15 at any time. Total VFA production did not differ due to the diets. Both sugar sources tended ($P = 0.13$) to increase the production of propionic acid over that of the control, but only lactose caused significant reduction in acetate production compared to either the control or the molasses treatment. Both sugar sources also increased production of butyrate ($P = 0.02$) with whey having a significantly greater response in increasing butyrate than did molasses ($P = 0.06$). The iso-acids of butyrate and valerate were significantly reduced by both treatments relative to the control, while both treatments tended to increase valerate production ($P = 0.11$). Both sugar sources tended to decrease the molar ratio of acetic acid ($P = 0.009$), with lactose causing a significant reduction in acetic acid ratio compared to molasses. Molar ratio of propionic acid tended to increase ($P = 0.10$) with the addition of both sugar sources.

Table 3. Volatile Fatty Acid Production, Molar Ratios and Fermentation pH

Item	Diets			<i>P</i> -value	
	Control	Whey	Mol	C vs W+M	W vs M
Average pH	6.25	6.28	6.31	0.42	0.56
VFA mmoles/day					
Total	388.5	396.8	401.2	0.35	0.73
Acetic	252.4	239.5	252.5	0.31	0.099
Propionic	74.0	78.6	80.4	0.13	0.64
Butyric	42.2	58.9	48.5	0.02	0.06
Isobutyric	3.98	3.56	3.56	0.11	0.98
Valeric	12.3	13.2	13.0	0.11	0.68
Isovaleric	3.61	3.04	3.18	0.006	0.36
VFA, Molar %					
Acetic	64.98	60.36	62.97	0.0099	0.04
Propionic	19.04	19.81	20.03	0.1006	0.69
Butyric	10.86	14.83	12.07	0.02	0.02
Isobutyric	1.03	0.90	0.89	0.07	0.93
Valeric	3.17	3.33	3.24	0.38	0.59
Isovaleric	0.93	0.76	0.79	0.006	0.52
A-P Ratio	3.42	3.05	3.15	0.02	0.45

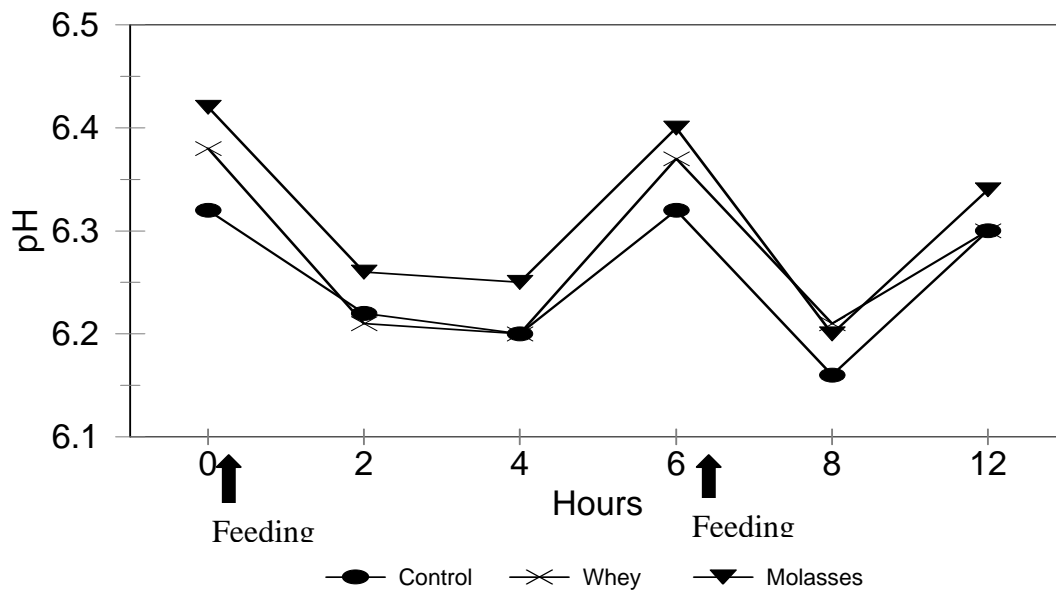


Figure 1. Fermentation pH as Affected by Diet.

Nitrogen partitioning is presented in Table 4. Compared to the control diet, whey decreased, and molasses increased protein digestion, resulting in a significantly ($P = 0.05$) greater protein digestion for the molasses diet compared to whey. Microbial N was increased and by-pass N decreased by the molasses diet compared to the whey diet. This resulted in no significant change in NAN flow. Microbial efficiencies based on microbial N produced per unit of digested DM, OM, and carbohydrates all were significantly greater for the molasses diet when compared to the whey diet. Compared to the control responses, it appears that these differences are most closely associated with decreases in efficiency due to lactose rather than increases due to molasses. Similarly digested feed N converted to microbial N was greater for the molasses compared to the whey diet, primarily as a result of the lower efficiency on the whey diet. The significantly lower microbial N produced per kg of carbohydrate digested on the whey diet is supported by the greater amount of VFA produced per kg of microbial N produced on that diet. In contrast, VFA/kg microbial N was lower for the molasses diet than for the control diet, resulting in a significant higher VFA/kg microbial N on the whey diet.

Table 4. Nitrogen Partitioning, Microbial Growth and Microbial Efficiency

Item	Diets			<i>P</i> -value	
	Control	Whey	Mol	C vs W+M	W vs M
C.Protein digested, %	58.3	53.0	64.3	0.93	0.05
Non-ammonia N, g/day	3.00	2.95	2.97	0.15	0.55
Ammonia-N, mg/dl	5.73	6.37	5.78	0.65	0.50
By-pass N, g/day	1.34	1.50	1.13	0.84	0.04
Microbial N, g/day	1.66	1.46	1.84	0.93	0.03
Efficiencies:					
Mic N/kg DMD ¹	24.05	20.77	27.18	0.94	0.001
Mic.N/kg OMD ²	37.43	31.03	39.00	0.27	0.01
Mic.N/kg CHOD ³	44.25	36.83	47.36	0.45	0.01
Feed N ⁴	89.09	86.17	90.92	0.63	0.008
TVFA/ kg CHOD ⁵	10.37	10.04	10.32	0.56	0.47
TVFA/kg Mic N ⁶	234.8	274.6	218.6	0.45	0.02

¹Microbial N produced/kg dry matter digested.

²Microbial N produced/kg organic matter digested.

³Microbial N produced/kg carbohydrate digested.

⁴Digested Feed N converted to microbial N, %.

⁵Moles VFA produced/kg carbohydrate digested.

⁶Moles VFA produced/kg Microbial N produced.

Table 5 shows the composition of the microbes. As indicated by the significant RNA-N difference between the whey and molasses diets, whey may have had an effect on the microbial species cultured in the fermenters.

Table 5. Effects of Treatments on the Composition of the Microbes

Item	Diets			<i>P</i> -value	
	Control	Whey	Mol	C vs W+M	W vs M
Nitrogen, %	8.58	8.44	8.43	0.68	0.99
Ash, %	12.93	11.54	12.38	0.71	0.78
RNA-N, % Total N	12.47	13.29	12.57	0.07	0.02

Disappearance of pulse doses of sugar at 0 time, corrected for losses due to flow, are shown in Table 6. By 0.5 hours post-dosing, 55 - 75% of the sugar had been digested. This disappearance was followed by a considerably slower loss over time out to 24 hr post-dosing. Compared to the control, which had no added sugar than that found in the ingredients, the diets containing about 3.5% added sugar had 71% of the total sugar fermented in the first half-hour, while 55% of the control diet sugar disappeared. When the added sugar was increased to about 15 g from either whey or molasses, disappearances increased to 74 - 75% of the added sugar,

Table 6. Sugar Digestion, % Digested

Hours after Dose	Diets				
	Control	Whey	Mol	Whey+Sug	Mol + Sug
0.50	55.00	71.63	71.05	75.41	74.29
1.0	61.45	79.84	78.27	80.01	79.87
2.0	64.07	80.81	79.81	78.23	77.63
4.0	68.49	83.52	83.42	75.82	88.58
8.0	73.78	86.21	86.26	89.75	94.17
24.0	75.08	86.79	86.84	96.92	96.16
Digestion Rate:					
0 - 1 hr ¹	170.6	252.5	231.6	174.6	177.6
0 - 8 hr ²	34.8	39.5	41.3	12.8	32.0

¹Digestion Rate calculated using 24 hr digestion as the potentially digestible fraction.

kd = Natural Log (LN) of (Potentially digestible Fraction/(Pot dig. fraction - 1hr dig))/1 hr

² Digestion Rate determined from regression lines shown in Figure 3.

with no difference due to sources. Between 1 and 4 hr, disappearance of sugars from the base and sugar-added diets was extremely small, as shown in Figure 2. From 4 - 24 hr, disappearance was increased only slightly for the base diets. Disappearance of sugar from the sugar added diets varied considerably during this period, however, with the molasses diet having a greater disappearance than the whey diet.

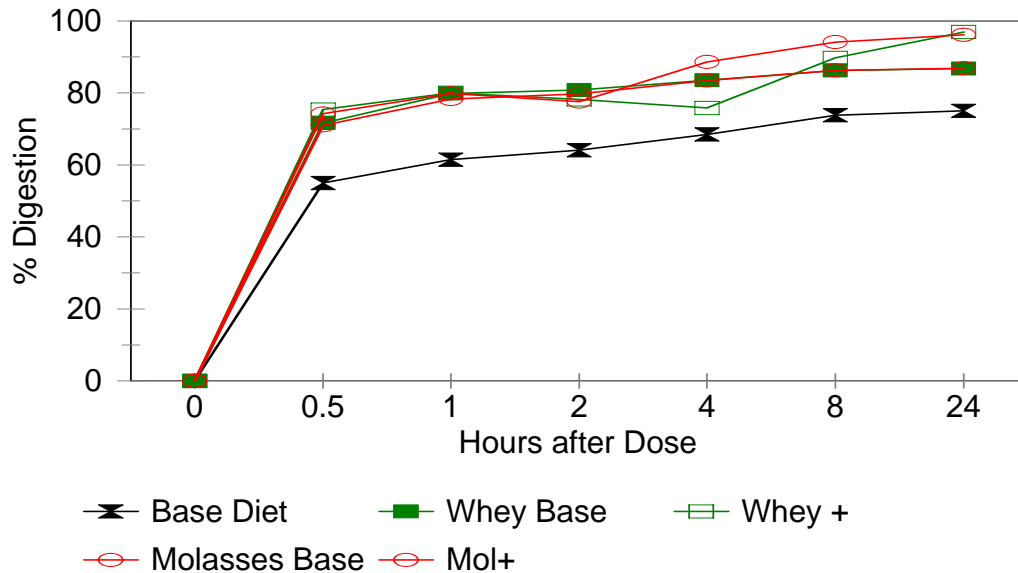


Figure 2. Sugar Digestion over time

Using the 24 hour disappearance as the potentially digestible fraction, rates were calculated for the 0 to 1 hr time period (using the Van Soest one time-point formula) and for the 1 to 8 hr time period using the linear regressions of the natural logs of the sugar disappearing. These values are shown in Table 6, while the plots of the ln values, along with the regression formulae, are shown in Figure 3. In Table 6, the rates for the sugar added treatments, 174.6 and 177.6 %/hr for whey and molasses, respectively, did not differ. Between 1 and 8 hr, however, the rate of disappearance for molasses sugar was nearly three times that of lactose.

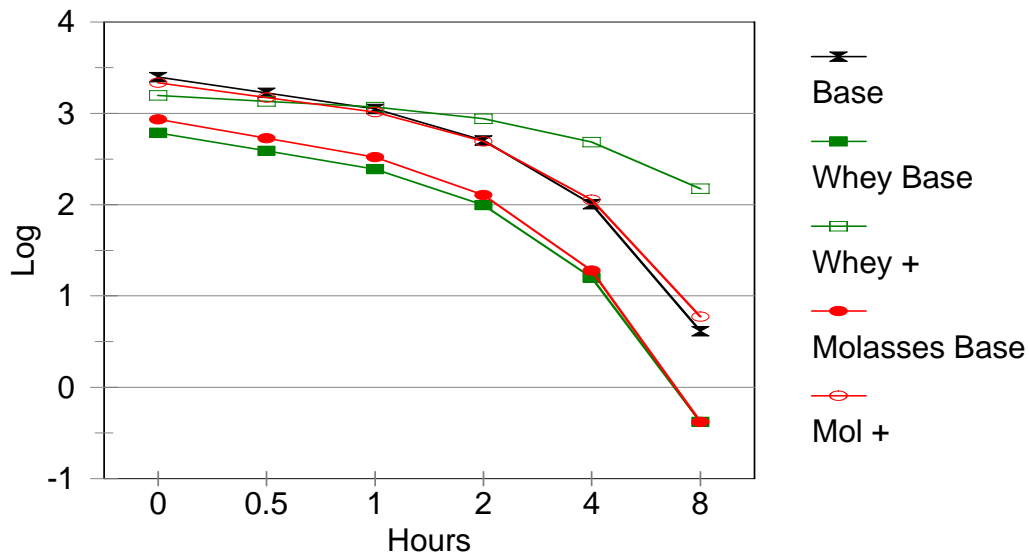


Figure 3. Regression of the log of the potentially digestible Fraction remaining

Based on 24 hr as the potentially dig fraction

Regression Line: Base Diet (no added sugar)

$$y = 3.3971 + (-.3479 * x)$$

Regression Line: Whey Diet (without dose)

$$y = 2.7847 + (-.3954 * x)$$

Regression Line: Whey Diet+ (+dose of 15.94 grams of Whey sugar)

$$y = 3.1953 + (-.1275 * x)$$

Regression Line: Molasses Diet (without dose)

$$y = 2.9324 + (-.4138 * x)$$

Regression Line: Molasses Diet+ (+dose of 15.07 grams of Molasses sugar)

$$y = 3.3323 + (-.3198 * x)$$

Discussion and Conclusions

In this study, molasses was superior to whey as a carbohydrate source for growth of rumen microbes. Although whey supported greater fiber digestion than did molasses, the increased digestion did not translate into improved microbial growth or microbial efficiency. On the contrary, microbial efficiencies for the control diet and for the molasses diet were similar and both higher than that of the whey diet. Since the molasses and control diets probably had similar sources of sugars (glucose and sucrose) it appears that rumen microbes may have a limited capacity to utilize lactose for microbial growth in spite of apparently being capable of rapid lactose hydrolysis.

The microbial response to molasses suggests a practical advantage to replacing 14 - 15% of the ration starch with molasses or similar sugar sources. This is supported by the modest increases in microbial efficiencies for the molasses diet compared to the control, and confirmed further by the higher level of microbial N produced and the decrease in VFA/g microbial N.

The rate of disappearance study confirmed some of our speculations and *blew the heck out of others (Dr. Hoover's words)*. Confirmed was the very rapid initial rate of sugar fermentation followed by a slower rate, as seen in batch culture. Also tentatively confirmed was the speculation that the batch cultures performed previously may have compromised the initial rate data because the concentration of microbes at 0 time was lower than seen in the in vivo or continuous culture situation. In batch cultures, 1 to 2 hours was required to achieve the plateau in rate of fermentation of sucrose-based sugar sources, while in continuous culture this was accomplished in 0.5 to 1.0 hr. Digestion of lactose in whey required 2 - 4 hrs to reach plateau in batch cultures, while in continuous culture only 0.5 to 1 hr was required. It was speculated from the batch culture data that lactose, because of the slower release rate, may be less effective in promoting microbial growth due to wash-out in a dynamic system. Since, in this study, 80% of the lactose was digested by 1 hr., unavailability was not likely the cause of the low microbial growth seen for lactose. Because the availability of the residual 20% of the lactose was much slower than that of sucrose, it still can be speculated that availability of lactose may partially explain the low microbial growth, but this is doubtful. It appears that lactose is simply less effective than sucrose in promoting microbial growth.

A general indication from this study, based on the molasses data, is that the initial fermentation of sugar, although extremely fast, is not more rapid than can be used for microbial growth. A further speculation is that the initial, rapid uptake may repress hydrolytic enzymes, or, more likely, inhibit uptake of sugar for a period, explaining the plateau in sugar disappearance.

Finally, this approach to determining the rates of fermentation of sugar and other rapidly soluble carbohydrate appears to be useful in that it avoids the problems seen in both batch and in situ methods. It has the potential to serve as standard for the development of quick, simple methods for determining rates, such as batch cultures or enzymatic procedures.

From the results of this study it can be concluded that:

1. Molasses, at 3 - 5% of the diet DM, is more effective than a similar amount of whey in promoting microbial growth.
2. There is an initial, rapid rate of fermentation of both lactose and sucrose, followed by a slow rate.
3. Approximately 80% of the digestion of both lactose and sucrose occurred within 1 hour after dosing, most of that within the first 0.5 hr.
4. The technique developed in this study appears to have the potential to serve as a standard for development of other methods for rate determinations.

Appendix 1. Nutrient Composition of Sugar Products (%DM)

Component	Whey Permeate	Molasses
Dry Matter	32.61	72.48
CP	5.77	1.88
Sol. Protein (%CP)	90.66	71.31
NSC ¹	82.41	93.17
Starch	1.15	11.34
Sugar	81.26	81.83
Ash	8.11	7.11

¹Nonstructural carbohydrate = true starch + sugar